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EXPRESSION OF NUCLEAR-CYTOPLASMIC INTERACTIONS ON QUANTITATIVELY INHERITED TRAITS FROM INTERSPECIFIC MATINGS OF OAT SPECIES (AVENA SATIVA L. AND A. STERILIS L.)

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Expression of nuclear-cytoplasmic interactions on quantitatively inherited traits from interspecific matings of oat (*Avena sativa* L. and *A. sterilis* L.)

by

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GENERAL INTRODUCTION

The development of cytoplasmic substitution lines through reciprocal matings is possible in plant species that exhibit maternal inheritance of the cytoplasm. Genetic lines with the same nuclear genomes in reciprocal cytoplasms often express differences for agronomic and physiological traits. These differences can be attributed to cytoplasmic effects. Hermesen (1968) suggested that all traits are affected by a cytoplasmic component. For example, if a population with nuclear-cytoplasmic male sterility were to lose the male-fertile cytoplasm, then the population would appear to be one of genetic male sterility (Charlesworth and Ganders, 1979; Clark, 1984).

Kihara (1973) introduced the term nuclear-cytoplasmic heterosis to describe the superior performance of cytoplasmic substitution lines (nuclear-cytoplasmic hybrids) relative to the performance of sib lines in the original cytoplasm. Kihara (1980) also developed nuclear-cytoplasmic hybrids that exhibit nuclear-cytoplasmic heterosis for grain yield in both spring and winter wheat varieties.

A phenomeon akin to nuclear-cytoplasmic heterosis was reported by Robertson and Frey (1984) for cytoplasmic isopopulations of oats. Robertson (1980) created 20 isopopulations in the BC_0, BC_1, and BC_2 generations from reciprocal matings of two Cornbelt oat varieties (Avena sativa L.) and five A. sterilis L. accessions. The grain yield average of five BC_2 populations with A. sterilis cytoplasm from crosses involving 'CI 9170' was greater than the counterpart average in A. sativa cytoplasm by 8.6%. One of the five BC_2 populations with A. sterilis

cytoplasm exhibited 8.6% greater grain yield than the recurrent parent. These results suggest that the expression of nuclear-cytoplasmic heterosis in oats may be realized in as few as two backcross generations; although the extent of the phenomenon from matings of A. sativa and A. sterilis is unknown.

Robertson (1980) used 20 lines per population to evaluate cytoplasmic effects, although fewer lines may have produced similar results. Analyses of Robertson's results (Robertson and Frey, 1984) indicated both direct cytoplasmic effects and nuclear-cytoplasmic interaction effects influenced seven traits. There were no significant interactions of cytoplasms with backcross generations but there were significant interactions of cytoplasms with matings for grain yield and heading date. Therefore, Robertson and Frey (1984) suggested that superior grain yields and later heading dates of the isopopulations with A. sterilis cytoplasm may have been influenced by interactions of the A. sterilis cytoplasm with A. sativa nuclear genes. All other traits showed significant cytoplasm by backcross generation interactions, which the authors cited as evidence for plasmagenes interacting with nuclear genes from both parents. Although Robertson and Frey (1984) suggested the sources of variability, they did not model their results.

The general objective of the studies reported herein is to investigate the nature of nuclear-cytoplasmic interactions from matings of A. sativa and A. sterilis on agronomic traits such as grain yield, straw yield, biomass, harvest index, heading date, height, vegetative growth rate and unit straw weight. Specific objectives are:

1. To investigate the ability of existing genetic models to describe and predict generation means from four of Robertson's (1980) matings.
2. Propose an alternative model based upon biological evidence and derive quantitative genetic variances and covariances for a theoretical population exhibiting nuclear-cytoplasmic interactions.
3. Determine the minimum number of random segregating lines per population necessary to evaluate isopopulations in the BC_2 generation.
4. Investigate the extent of nuclear-cytoplasmic interactions and heterosis among 80 BC_2F_2 isopopulations from reciprocal matings of four A. sativa Cornbelt varieties with 10 A. sterilis accessions.

LITERATURE REVIEW

Extranuclear Effects

Inheritance of extranuclear effects has been known since early studies of foliar variegation by Bauer, 1909 and Correns, 1909, cited in Kirk and Tilney-Bassett (1967), and it can be classified as dauermodification, maternal inheritance, or cytoplasmic inheritance (Caspari, 1948). Endosperm effects represent a fourth category applicable to many plant species.

Dauermodification is described as environmentally induced changes which are transmitted maternally through a number of generations. Viral infections that are transmitted from mother to offspring in animals are examples of this type of extranuclear inheritance.

Caspari (1948) described maternal inheritance as the maternal transmission of a substance which is manifested in the offspring and gradually disappears during the growth and development of the individual. A more appropriate term to describe such phenomena is maternal effects; maternal inheritance is best used as a descriptor of heredity. Models with maternal effects have been described by Mather and Jinks (1982) and Willham (1963), who developed a quantitative theory to describe the impact of maternal effects on genetic variability.

The endosperm is, in effect, a nurse organism to germinating embryos. The endosperm is related to both the maternal parent, from which it receives two-thirds of its triploid nuclear genome, and the paternal parent of a developing seedling. Van Aarde (unpublished manuscript) has developed a quantitative theory to describe the impact of endosperm effects on genetic variability.

Cytoplasmic inheritance is used to describe the inheritance of cytoplasmic effects (Caspari, 1948). The discovery of DNA in the organelles of the cytoplasm (Beale and Knowles, 1978; Gillham, 1978) provided evidence that cytoplasmic effects have a genetic basis. To date, a quantitative genetic theory which describes the influence of cytoplasmic effects on genetic variability has not been developed.

Many studies have been conducted on maternally inherited effects through the use of reciprocal mating designs (Ashri, 1964; Ellsworth and Peloquin, 1972; Miksch, 1980; Ruebenbauer, 1962). Caspari (1948) and Bhan (1964) indicate that differences from reciprocal matings can be ascribed to cytoplasmic sources only if the effects remain in the offspring obtained from repeated backcrosses to the paternal parent. Indeed, the development of cytoplasmic substitution lines in wheat (Triticum and Aegilops spp.) by Kihara (1951) has been a powerful tool for studying cytoplasmic effects on nuclear genomes. Robertson and Frey (1984) used the "isopopulation method" (Burton, 1966) for comparing two alternative cytoplasms in oats (Avena spp.). In this method, a set of F_2-derived lines from a backcross generation of a mating are compared with another set of F_2-derived lines from the same backcross generation of the reciprocal mating. Thus, assuming that the cytoplasm does not affect segregation in the nuclear genome, the two sets of lines are expected to have equivalent samples of nuclear genes. Caspari (1948) proposed that loss of an effect in successive generations is likely a dauermodification. However, as Robertson and Frey (1984) indicated, loss of an effect with successive backcrosses also may be related to the loss of specific nuclear-cytoplasmic interactions.

Function and Structure of the Cytoplasm

The cytoplasm consists of subcellular organelles imbedded in an amorphous matrix. The fine structure of the cytoplasmic matrix has been studied in recent years because it is responsible for both intra- and intercellular transport of metabolites (Marx, 1983). However, very little is known about its genetic regulation or its impact on the cytoplasmic genome.

The discovery of DNA in the cytoplasmic organelles in the early 1960s (Gillham, 1978) increased the frequency of studies on the cytoplasm and its hypothesized plasmagenes (Caspari, 1948). In the last 20 years, emerging biotechnologies have enhanced studies of subcellular phenomena. These studies have identified encoding sequences for specific cytoplasmic polypeptides and elucidated the inheritance, replication, and structure of the cytoplasmic genome.

Many quantitative traits of economic importance are end-products of metabolic processes that occur in cytoplasmic organelles. For example, vegetative biomass measures the end product of photosynthesis, which occurs in the chloroplasts, and respiration, which occurs in the mitochondria. Many cytoplasmic functions are regulated by enzymes encoded in both nuclear and cytoplasmic DNA. The "light harvesting complex" of the chloroplasts contains two types of chlorophyll, a and b, that are composed of polypeptides encoded by nuclear-genes (Dunsmuir et al., 1983a). The ATP synthetase complex responsible for electron transport across membranes of the chloroplast and for conversion of ADP to ATP has four of its sub-units encoded by the chloroplast genome

(Huttly and Gray, 1984). The reverse process, controlled by the ATPase complex of the mitochondrion has four of its sub-units encoded in the mitochondrial genome (Borst et al., 1983). The code for Cytochrome F of wheat has been located within the chloroplast and sequenced for wheat (Willey et al., 1984).

The most studied enzyme of photorespiration is ribulose-1,5-bisphosphate carboxylase (Rubisco). Rubisco is a bifunctional enzyme that is composed of eight copies of two nonidentical sub-units. The larger sub-unit is encoded in the chloroplast (Bowman et al., 1981) and the smaller sub-unit is encoded in the nucleus (Cashmore et al., 1978; Highfield and Ellis, 1978) by a multigenic family (Dunsmuir et al., 1983b). The composition, site of translation, and transport of the small sub-unit to the chloroplast also have been studied (Cashmore et al., 1978; Chua and Schmidt, 1978; Corruzi et al., 1984; Highfield and Ellis, 1978; and Roy, 1982).

The cytoplasmic genome of plants consists primarily of DNA in the chloroplasts and mitochondria (Beale and Knowles, 1978). The numbers of chloroplasts and mitochondria per cell are variable and depend upon the state of cellular differentiation (Butterfass, 1979; Grun, 1976). Generally, organelles tend toward a homogenous state within the cell. This tendency occurs in species that exhibit uniparental inheritance (Michael, 1978; Conde et al., 1979) and species that exhibit biparental inheritance (Metzlaff et al., 1981; Michaelis, 1958; Tilney-Bassett, 1975) of the cytoplasm. A tendency toward homogenous lines also occurs in artificial systems designed to create heteroplasmic conditions; e.g.,

plant cells subjected to mutagenic agents (Hagemann, 1976), somatic hybridization (Izhar, 1980), and cytoplast-protoplast fusion (Maliga et al., 1982). Unlike chromosomes, replication and transmission of organelles are independent of nuclear control (Birkey, 1983). Michaelis (1958) proposed that the process of apportioning organelles to daughter cells during mitosis is stochastic. Thus, it is probable that the daughter cells will not receive equal distributions of organelles. Birky (1983) agreed with Michaelis and added that replication of organelles also was stochastic. For example, an organelle might reproduce itself 10 times or not at all during the interphase of mitosis. Birkey (1983) also indicated that the phenomenon of uniparental inheritance perpetuates the homoplasmic condition within maternal lines of descent.

The structure of the chloroplast (cp) genome is known. Each chloroplast contains 10 to 60 complete copies of its genome and each copy consists of a single, circular, double-stranded molecule of DNA (Gillham, 1978; Grun, 1976). The structure of the mitochondrial (mt) genome is still being investigated. Early studies by Kolodner and Tewari (1972) on mtDNA from pea leaves indicated that the mt-genome consisted of single, circular, double-stranded molecules of DNA. However, according to Leaver and Gray (1982) and authors cited therein, maize (Zea mays L.) mitochondria have yielded various sizes of circular and linear mtDNA. In addition, electrophoretic patterns on restriction endonuclease digests of mtDNA from a single sample are heterogenous in numerous plant species. Leaver and Gray (1982) suggest that the

source of the heterogenous pieces of mtDNA are intra- rather than inter-mitochondrial. Levings et al. (1979) proposed that heterogeneous pieces of mtDNA may be a manifestation of a genome organized into different sized "chromosomes." An alternative hypothesis currently supported by most investigators (Leaver et al., 1983; Levings, 1983; Lonsdale et al., 1983a) is that the genome exists as a single, large, circular, duplex molecule of DNA from which smaller molecules arise by various mechanisms. If this latter hypothesis is accepted, the structure of the cytoplasmic genome can be viewed as two populations of polyploid organelles with a basic chromosome number of one.

Cytoplasmic Diversity

Maternal inheritance

Most higher plant species exhibit uniparental inheritance of cytoplasmic effects through the maternal parent, although exceptions occur in species of Secale, Solanum and Oenethera (Gillham, 1978). Mechanisms responsible for uniparental maternal inheritance include exclusion of paternal organelles from the male gamete during spermatogenesis, loss of parental plastids from the motile sperm, exclusion of paternal plastids during fertilization, and degradation of paternal plastids within the zygote (Sears, 1980). Regardless of the mechanism, the result is that little cytoplasmic diversity is expected within maternal lines of descent.

As previously noted, maternal inheritance of cytoplasm does not imply that differences between individuals from reciprocal matings are

due to cytoplasmic effects. The literature is replete with studies on reciprocal effects; see Robertson (1980) for a comprehensive review. It is the intent of this review to concentrate on studies of traits that show the effects of cytoplasmic diversity.

Cytoplasmic diversity, expressed as nuclear-cytoplasmic interaction effects on numerous agronomic, qualitative, and physiological traits, has been studied best through the development of cytoplasmic substitution lines. The trait studied most, because of its commercial implications, is nuclear-cytoplasmic male sterility.

Male sterility

Nuclear-cytoplasmic (NC) male sterility in maize was first reported by Rhoades (1931). Since Rhoades' discovery, three NC-male sterility inducing cytoplasms have been identified and are classified as S, C, and T (Beckett, 1971). The commercial usefulness of each was assessed by Duvick (1972). NC-male sterlity first was reported in sorghum, Sorghum bicolor (L.) Moench., by Stevens and Holland (1954) and has been necessary for commercial production of hybrid sorghum. Initial identification and classification of male sterility cytoplasms of sorghum were pursued primarily through the Sorghum Conversion Program conducted by Schertz and Ritchey (1978). Recently, the Sorghum Genetic Resources Unit at ICRISAT (International Crops Research Institute for Semi-Arid Tropics), Hyderabad, India, has identified different cytoplasms responsible for NC-male sterility (ICRISAT, 1983). NC-male sterility also is known in other crop species. Unlike corn and sorghum, NC-male sterility in other crop species was induced by plant breeders

through creation of cytoplasmic substitution lines; examples include wheat, Triticum and Aegilops spp. (Kihara, 1951), barley, Hordeum spp. (Schooler, 1967), tobacco, Nicotiana spp. (Clayton, 1950), and cotton, Gossypium spp. (Meyer and Meyer, 1961; Richmond and Kohel, 1961). Until recently, none of the induced systems were utilized for commercial hybrid production because the hybrids often were undesirable for quality and agronomic traits (Kihara and Tsunewaki, 1964).

Quantitative traits

The impact of NC-male sterile lines on hybrid performance in maize has been studied primarily for the T-cytoplasm. Duvick (1958) produced normal and T-cytoplasm forms of six hybrids. He did not include male sterile forms with restoration genes. He evaluated these in three locations at six sowing densities. At high densities, the T-cytoplasm hybrids had a yield advantage but a disadvantage at low densities. Stringfield (1958) found that T-cytoplasm hybrids with restorer alleles were higher yielding than their normal counterparts. Noble and Russell (1963) included restoration genes in both normal and T-cytoplasm forms of hybrids and found that hybrids with T-cytoplasms gave reduced yields but differences were not universally significant. Russell and Marquez-Sanchez (1966) also observed reduced yields in some hybrids with T-cytoplasm. After the southern corn leaf blight race T epiphytotic of 1970, hybrid corn has been produced by using mechanical detasseling and the C cytoplasm. The S NC-male sterility inducing systems exhibit incomplete and unstable male sterility (Duvick and Noble, 1978).

Quinby (1970) and Atkins and Kern (1972) contrasted the influence of normal and NC-male sterile lines on hybrid performance in sorghum and found no significant differences in grain yield. Kern (1969) noted that such studies in sorghum are academic because there are no alternatives to producing hybrid sorghum on a commercial scale.

As with hybrid seed corn production prior to 1970, most (97%) grain sorghum grown in the U.S. is produced using a single cytoplasm known as 'milo' cytoplasm (Harvey, 1977). An insect or disease capable of attacking this cytoplasm would be more devastating to the sorghum seed industry than southern corn leaf blight was to the seed corn industry because there is no other method for producing commercial hybrids. Thus, there has been considerable effort in sorghum to assess hybrid performance in different NC-male sterile inducing cytoplasms. Ross and Kofoid (1979) compared six 'KS' cytoplasms with the milo cytoplasm. Hybrids from one, KS37, consistently yielded better than hybrids in the milo cytoplasm. Other hybrids with KS cytoplasms gave mixed performances relative to hybrids with milo cytoplasm. Lenz and Atkins (1981) found no differences in hybrid performance between hybrids with KS cytoplasms and milo cytoplasm. Thus, these two studies indicate that as many as six alternative cytoplasms are available to diversify the cytoplasmic genomes used in hybrid seed production of sorghum. The effect of cytoplasm on flowering (Barikar and Balaich, 1977) and seed viability (Rao and Fleming, 1978) also have been noted in isogenic inbred lines of sorghum and maize.

Nuclear-cytoplasmic effects have been described in several crop species by using cytoplasmic substitution lines per se. The development of alloplasmic wheat lines by Kihara (1951) provided genetic materials, i.e., those with cytoplasmic diversity, for studies on heading date, number of spikes, plant height, biomass, number of spikelets, seed fertility, and grain yield (Kinoshita et al., 1979; Mukai and Tsunewaki, 1975; Tsunewaki, 1980; Kihara, 1980).

Qualitative traits

The effect of Helminthosporium maydis Nisikado and Miyari, race T, on maize plants with different cytoplasms provides a striking example of cytoplasmic diversity. Cytoplasm T is also more susceptible to yellow leaf blight caused by Phyllosticta spp. (Ayers et al., 1970). Washington and Maan (1974) reported differential responses of cytoplasmic substitution lines of three wheat varieties to three races of wheat stem rust, Puccinnia recondita (Rob). Cytoplasm from Gossypium harkenessi improved resistance to bacterial blight in cotton, G. hirsutum caused by Xanthomonas malvacearum E. F. Sm. (Mayhill and Davis, 1978).

Resistance to some antibiotics has been shown to be regulated by the cytoplasm (Cseplo and Maliga, 1984; Maliga et al., 1982; Menczel et al., 1983). In addition, Menczel et al. (1983) showed that cytoplasmic resistance to streptomycin was associated with nuclear-cytoplasmic male sterility in regenerated plants from irradiated cell line cultures of fused protoplasts from tobacco species (N. tabacum and N. plumbaginifolia). However, resistance to another antibiotic, lincomycin,

in regenerated tobacco plants from cell lines of fused protoplasts as not associated with male sterility.

The emerging biotechnologies have permitted the study of cytoplasmic effects on specific metabolic products. For example, seed proteins of the endosperm, exhibit various electrophoretic patterns depending upon the cytoplasm of the alloplasmic line (Yamada et al., 1980). Beta-amylase, which is encoded in the nucleus, shows different specific activities in various alloplasmic lines (Li et al., 1980). Respiratory and photosynthetic rates both exhibit differential responses to cytoplasmic substitution (Iwanaga et al., 1978). Variant forms of Rubisco's large sub-unit have been detected (Hirai and Tsunewaki, 1981), but variability in Rubisco has not been related to variability in photosynthesis or photorespiration (Tomarchio et al., 1983). Different cytoplasms also produce variable mitochondrial protein products (Forde and Leaver, 1980).

Data on cytoplasmic diversity in Triticum and related genera has been used in phylogenetic studies of wheat (Endo and Tsunewaki, 1975; Mukai and Tsunewaki, 1975; Ohtsuka and Otsuka, 1981; Tsuji and Tsunewaki, 1976) by applying cluster analyses to quantitative and qualitative traits exhibited by alloplasmic lines. The results often have confirmed nuclear genome analyses (Kihara, 1929; Riley et al., 1958) but have provided conflicting evidence on the origin of the B genome.

Direct evidence

Despite evidence for cytoplasmic diversity from measurements on quantitative and qualitative traits, it was electrophoretic patterns

of endonuclease restricted cpDNA and mtDNA that confirmed genomic diversity of cytoplasms. Vedel et al. (1976) first showed that an endonuclease restriction enzyme EcoR1 when applied to isolated chloroplast DNAs from higher plants produced distinctive electrophoretic patterns. Later, Vedel and Quetier (1978) and Vedel et al. (1981) used EcoR1 to elucidate the genomic relationships in wheat cytoplasms. Vedel et al. (1982) also confirmed strict maternal inheritance of cpDNA and mtDNA in Triticale. Tsunewaki and Ogihara (1983), who used other enzymes to analyze the chloroplast-genomic relationships in wheat, confirmed and expanded the work of Vedel et al. (1981). Electrophoretic patterns of numerous restriction endonucleases have been investigated for most major crop species (Hansen and Macker, 1984; Hatfield et al., 1985; Kung et al., 1982; Levings et al., 1979; Sisson et al., 1978) including oats (Rines et al., unpublished manuscript).

Because each endonuclease restriction enzyme produces a unique electrophoretic pattern, Nei and Li (1979) developed a multivariate cluster analysis to categorize similar patterns. Thus, the dendrograms produced for the expression of plant traits can be compared with those produced for electrophoretic patterns (Terachi et al., 1984). Most taxonomic descriptions which use electrophoretic patterns of endonuclease restriction digests of cpDNA and mtDNA are based upon an assumption that there is little diversity within maternal lines of descent.

Origin

If there is no diversity among maternal lines of descent, a question arises concerning the origin of diverse cytoplasms within species, such as maize and the diverse cytoplasms of the closely related genera, Triticum and Aegilops. The biological mechanisms responsible for cytoplasmic and nuclear diversity are probably similar, although mutation rates may differ. For example, Avise et al. (1979) demonstrated that silent nucleic acid substitutions in the mitochondria of mice occur six times as often as substitutions in nuclear genes, but the rate of substitutions causing amino acid changes in gene products appeared to be the same for nuclear and mitochondrial genomes (Brown et al., 1979). In addition, a cpDNA sequence associated with the large sub-unit of Rubisco has been detected in the mitochondrial genome of some maize lines (Lonsdale, 1983b).

Theoretical models to explain the evolution and maintenance of cytoplasmic diversity have been proposed. A model of nuclear-cytoplasmic male sterility with biallelic restoration and two cytoplasm types, male fertile and male sterile, was investigated by Charlesworth and Ganders (1979). Their model showed that if the nonrestorer allele was eliminated, both cytoplasm types could be maintained in the population. Conversely, if the nonrestorer alleles remained in the population, the male-sterile cytoplasm would become fixed, and the population would be indistinguishable from one with genetic male sterility. In the model of Charlesworth and Ganders (1979), polymorphisms at both nuclear and cytoplasmic loci for male sterility would not be maintained in a random

mating population. Clark (1984) showed similar results for viability; i.e., polymorphisms at nuclear and cytoplasmic loci for viability would not be maintained. Delanney et al. (1981) used the model of Charlesworth and Ganders for a self-pollinated population of hermaphrodites, but allowed for different female fertilities: i.e., male sterility was pleiotropic. Nontrivial equilibria could be maintained for nuclear and cytoplasmic diversity if the fertility of nuclear-cytoplasmic male sterile cytoplasms was greater than the fertility of normal fertile individuals. Gregorius and Ross (1984) and Ross and Gregorius (1985) have described the minimum sufficiency conditions for fitness needed to maintain both nuclear and cytoplasmic diversity in a random mating population. The conditions include sexual asymmetry for fertility and negatively frequency-dependent fitness (Gregorius and Ross, 1984). An implication of their results is that nuclear-cytoplasmic interactions are needed to establish the phenomenon of gynodioecy (Ross and Gregorious, 1985). Thus, given certain constraints, nuclear-cytoplasmic interactions fit established tenets of evolutionary theory.

Explanation of Dissertation Format

This dissertation consists of four sections. Section I investigates the ability of models with maternal effects (Mather and Jinks, 1982) to describe grain yield of parental and backcross generations from reciprocal matings of CI 9170 with four A. sterilis accessions. Section II develops an alternative theoretical model, based upon biological evidence to describe traits influenced by nuclear-cytoplasmic

interactions. Variance and covariance components of this model are derived for a theoretical population and the estimation of these components via a reciprocal mating design is described. Section III estimates the minimum sample size necessary to describe variability among cytoplasmic isopopulations in the BC_2 generation of oats. Section IV investigates the extent of nuclear cytoplasmic heterosis among 76 cytoplasmic isopopulations of oats for seven traits. General conclusions follow Section IV.

Each section is in a form of a complete manuscript that will be submitted to a professional journal. Sections I and III will be submitted to Crop Science, Section II will be submitted to Theoretical and Applied Genetics, and Section IV will be submitted to Euphytica. References cited in the General Introduction and General Conclusions sections are listed in Additional References Cited, which follows the General Conclusions section. This "alternative format" is authorized on page six of the 1985 edition of the Iowa State University Graduate College Thesis Manual.

SECTION I. GENETIC MODELLING OF NUCLEAR-CYTOPLASMIC HETEROSIS IN OATS

ABSTRACT

A phenomenon akin to nuclear-cytoplasmic heterosis (NC-heterosis) was observed for grain yield in a cytoplasmic isopopulation of oat (Avena sativa L.) lines with A. sterilis L. cytoplasm. An accurate modelling of the genetic effects responsible for the phenomenon would provide the plant breeder with a predictive tool for screening a large number of matings.

Models developed by Mather and Jinks adequately described generation means from three of four matings involving A. sterilis accessions and 'CI 9170'. In all three, the best model included parameters for additive nuclear effects, cytoplasmic effects, and an interaction of additive nuclear and cytoplasmic effects. In two mating systems, an added parameter describing epistatic nuclear effects was needed for a good fit. The predicted grain yields of advanced backcross generations and NC-hybrids were calculated by using the best fitting model for each mating system. Data from advanced backcross generations tested in 12 environments indicated that the best fitting model did not predict grain yield of advanced backcross generations.

Additional Keywords: Nuclear-cytoplasmic hybrids, reciprocal backcross populations, cytoplasmic isopopulations, cytoplasmic substitution lines

INTRODUCTION

A study in 1909 conducted by Correns (Beale and Knowles, 1978) showed cytoplasmic influences on plant variegation. Later, Malinowski (Ruebenbauer, 1962) reported height differences between reciprocal hybrids of two Petunia species, and Ruebenbauer (1962) presented a theoretical explanation for these differences. The discovery of cytoplasmic male sterility in maize (Zea maize L.) and sorghum (Sorghum bicolor Moench.) led to numerous studies on cytoplasmic effects in hybrids (Atkins and Kern, 1972; Duvick, 1958; Ross and Kofoid, 1979; Russell and Marquez-Sanchez, 1966). Kihara and Tsunewaki (1964) working with wheat species (Triticum and Aegilops spp.) used the term cytoplasmic heterosis to describe the added vigor of agronomic traits in cytoplasmic substitution lines. Kihara (1973) referred to cytoplasmic substitution lines as nuclear-cytoplasmic hybrids (NC-hybrids) and proposed the term nuclear-cytoplasmic heterosis (NC-heterosis) to describe superior yields of NC-hybrids (Kihara, 1979). Kihara (1980) has identified a few wheat NC-hybrids that exhibit heterosis, but the phenomenon is not universal.

A phenomenon akin to NC-heterosis was reported by Robertson and Frey (1984) in oats (Avena spp.). They evaluated the BC_0F_4, BC_1F_4, and BC_2F_4 populations of segregates from reciprocal matings of 'CI 9170' (A. sativa L.) and four A. sterilis L. accessions. A BC_2F_4 population of segregates with A. sterilis (PI 317757) cytoplasm yielded significantly more than the recurrent parent, CI 9170. They suggested

that cytoplasmic effects and/or interactions of the A. sterilis cytoplasm with the nuclear genome from CI 9170 was responsible for the heterotic response.

Accurate genetic modelling of the nuclear-cytoplasm system would predict heterosis in NC-hybrids and could be used to screen specific matings for potential to exhibit NC-heterosis. I used data from the BC_0, BC_1, and BC_2 backcross generations from reciprocal matings of true-breeding lines created by Robertson (1980) to test the ability of Mather and Jinks (1982) genetic models to describe the generation means. The best fitting model for each mating was used to predict grain yield in the BC_3, BC_4, and BC_∞ generations. Data from BC_3 and BC_4 bulk populations derived by additional backcrossing onto four high yielding BC_2F_2 lines were used to assess the predictive ability of the best fitting model.

MATERIALS AND METHODS

Average grain yield of BC_0F_4, BC_1F_4, and BC_2F_4 cytoplasmic isopopulations were obtained from experiments conducted by Robertson (1980). The mating scheme followed by Robertson (1980) in creating these isopopulations is shown in Figure 1.

Means of isopopulations were described using the genetic models and generation means analyses of Mather and Jinks (1982). This technique attempts to describe generation means in terms of "net" genetic effects. Coefficients of net effects are derived from the expected segregation patterns at a single locus. The genetic effects for a single locus model are m±a, for the homozygotes. Mather and Jinks (1982) extended the model to multiple genes by assuming there are k different genes in two true-breeding lines. Within a line, k' genes will be a+ and k-k' will be a-, and the difference between the two true-breeding lines is:

$$P_1 - P_2 = 2r_a \sum_k a_{\pm} \; ; \qquad (1)$$

where r_a is a coefficient of gene distribution between the two lines (Mather and Jinks, 1982). The coefficient of gene distribution can take on a value of unity if one parent has all a+ alleles and a value of zero if the a+ alleles are evenly distributed between the parents. The coefficient, r_a, cannot be estimated from generation means and is a convenience to facilitate algebraic manipulation of additive effects for the whole nuclear genome; i.e., the net additive effects are assumed to segregate similar to an effect under the control of a single locus.

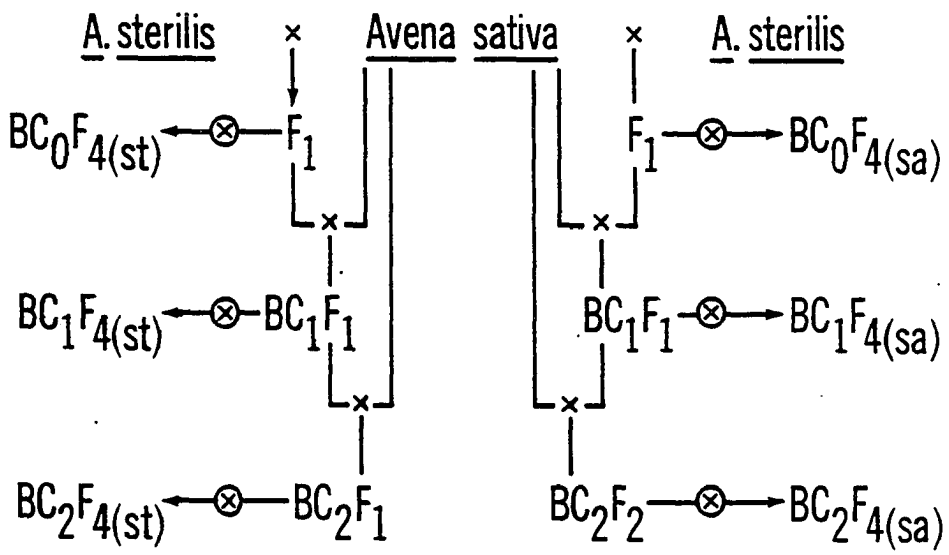

Figure 1. Generalized flow chart for development of reciprocal backcross populations

Formula (1) can be set equal to 2α, which is equivalent to 2[d] in the notation of Mather and Jinks (1982). Thus, the net effect expressed by either homozygote is $\pm\alpha$. Coefficients of α for populations obtained from matings and backcrosses of the homozygous parents are equal to the difference in frequencies of homozygotes in the populations. Dominance effects would have little influence on the populations created by Robertson (1980) because the frequency of heterozygous loci would be only 1/8, 1/16, and 1/32, respectively, for the BC_0F_4, BC_1F_4, and BC_2F_4 populations.

Cytoplasmic effects were modelled such that two true breeding parents with cytoplasm t and u have cytoplasmic effect of +c and -c, respectively. Because the cytoplasmic integrity is maintained through maternal inheritance in oats (Beale and Knowles, 1978), cytoplasmic effects need not be expanded into additive and dominance components. Therefore, the difference between cytoplasmic effects of two true breeding lines is equal to $2r_cC$; where $r_c = 0$ if the two lines have the same cytoplasm, or $r_c = 1$ if they do not. To keep the notation consistent, let $2r_cC = 2\gamma$. The use of γ to describe cytoplasmic effects is merely a specification of one of the possible effects that might arise from reciprocal matings. Mather and Jinks (1982) developed parameters for the more general reciprocal or maternal effects.

Because coefficients of α are calculated from the expected frequencies of segregates at a single locus, the coefficients of parameters describing interaction effects (e.g., $\alpha\gamma$, $\alpha\alpha$, $\alpha\alpha\gamma$, etc.) are calculated as the products of coefficients of the corresponding simple effects

(Mather and Jinks, 1982). Thus, the interaction effects are also parameters that describe net effects. Coefficients for μ, α, γ, and parameters describing interaction effects for parental and backcross generations are presented in Table 1. Mather and Jinks (1982) suggest that models which include only parameters for simple effects should be used to describe the data initially. If the simple model does not fit the data (i.e., a significant X^2 statistic produced by the joint scaling technique of Cavalli (1952)), then other models which include simple effects, epistatic nuclear effects, and nuclear-cytoplasmic interaction effects should be tested to fit. Among models which fit the data for a pair of reciprocal matings, a "best" model was defined to be the one with the fewest parameters.

If a model with a good fit accurately identifies the genetic effects responsible for the generation means, the model should be able to predict the yield of NC-hybrids. With this in mind, the four highest yielding F_2-derived lines of the BC_2 population from 'PI 317757' x 'CI 9170' were backcrossed to CI 9170 to form BC_3 and BC_4 bulk populations. Each of these superior F_2-derived lines (denoted D623-13, D623-15, D623-17, D623-18) and their respective BC_3 and BC_4 bulk populations were evaluated for grain yield in 12 environments: four locations with two replications in each of three years. The results of these yield tests were compared with modelled predictions for the BC_3 and BC_4 from PI 317757 x CI 9170.

Table 1. Coefficients of genetic parameters that describe additive nuclear (α), cytoplasmic (γ), and nuclear-cytoplasmic interaction ($\alpha\gamma$, $\alpha\alpha$, $\alpha\alpha\gamma$) effects for parental and reciprocal backcross generations

Generation[a]	Effect					
	μ	α	γ	$\alpha\gamma$	$\alpha\alpha$	$\alpha\alpha\gamma$
$P_{1(sa)}$	1	1	1	1	1	1
$P_{2(st)}$	1	-1	-1	1	1	-1
$BC_0F_{4(sa)}$	1	0	1	0	0	0
$BC_0F_{4(st)}$	1	0	-1	0	0	0
$BC_1F_{4(sa)}$	1	$\frac{1}{2}$	1	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{4}$
$BC_1F_{4(st)}$	1	$\frac{1}{2}$	-1	$-\frac{1}{2}$	$\frac{1}{4}$	$-\frac{1}{4}$
$BC_2F_{4(sa)}$	1	3/4	1	3/4	9/16	9/16

[a]Cytoplasm for the parental, P, and backcross, BC, generations is indicated by (sa) for A. sativa L. and (st) for A. sterilis.

RESULTS

Mean grain yield ± one standard deviation for isopopulations and parents are presented in Figure 2. Mean yields of the four A. sterilis accessions were one-third to one-half that of CI 9170. Mean yields of all four BC_0 populations with A. sativa cytoplasm were lower than the mid-parent values, whereas mean yields of three of four BC_0 populations with A. sterilis cytoplasm were greater than their respective mid-parent values. All BC_1 and BC_2 isopopulations, irrespective of cytoplasm, yielded above the mid-parent values. The mean yield of isopopulations with A. sterilis cytoplasm exceeded their counterparts with A. sativa cytoplasm in nine of 12 mating-generation combinations; for eight, the superiority was significant. The mean yield of three populations, all with A. sterilis cytoplasm, exceeded the yield of CI 9170 (i.e, the BC_2 from PI 317757 x CI 9170, BC_2 from 'PI 317982' x CI 9170, and BC_1 from 'PI 217512' x CI 9170), but only the BC_2 from PI 317757 x CI 9170 was significantly greater.

Estimates of the parameters for the best fitting model for each mating and its respective backcross isopopulations are presented in Table 2. A model which fit the generation means was found for three of four matings. For all three, the best model included additive nuclear (α), cytoplasmic (γ), and additive nuclear by cytoplasmic effects ($\alpha\gamma$). Epistatic nuclear effects ($\alpha\alpha$) were included in the best models for matings of CI 9170 with PI 317757 and PI 317982. No model fit the generation means from the mating of CI 9170 x PI 217512. Sixty-six percent of the lack of fit was due to the deviation of the

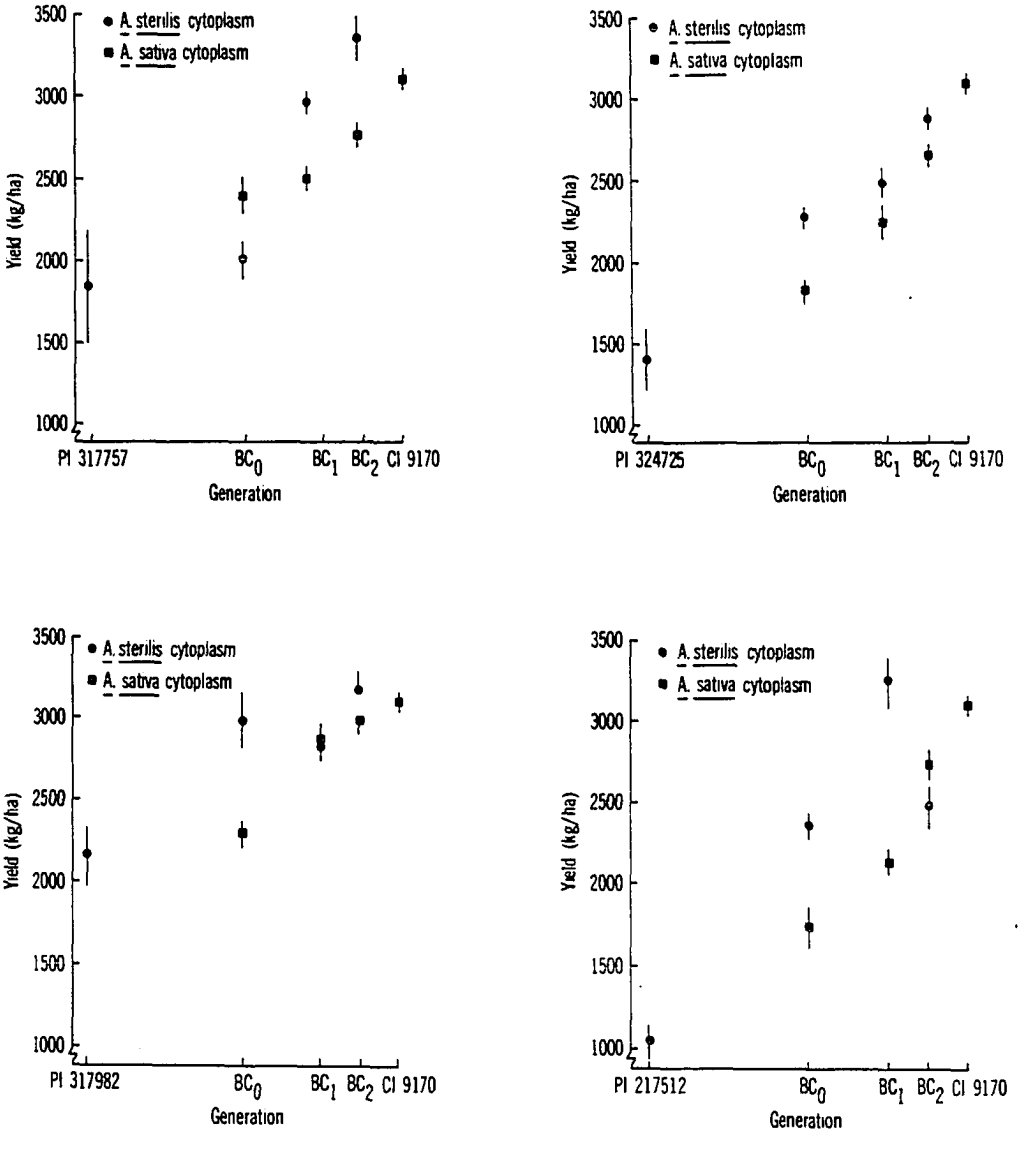

Figure 2. Mean grain yields (■,●) and standard deviations (|) of parental and backcross populations for crosses of CI 9170 mated reciprocally to four A. sterilis accessions

Table 2. Estimates and standard deviations of genetic parameters from the best fitting model for generation means from reciprocal crosses of CI 9170 mated with four A. sterilis accessions; where best model is defined as one with the lowest significant X^2 statistic (PI 217512) or as one with significant estimates of parameters among models with nonsignificant X^2 statistics (PI 317757, PI 317982, and PI 324725)

A. sterilis	Estimated parameters						
	μ	α	γ	αγ	αα	ααγ	X^2
PI 317757	2232 ± 70	514 ± 176	157 ± 87	-683 ± 166	888 ± 230		1.63
PI 317982	2604 ± 64	740 ± 105	-261 ± 79	347 ± 151	-308 ± 196		4.62
PI 324725	2010 ± 44	1039 ± 69	-232 ± 44	248 ± 69			6.10
PI 217512	2101 ± 69	719 ± 225	-361 ± 69	-122 ± 225	89 ± 208	692 ± 208	19.35**

observed mean from the predicted value for the BC_1 isopopulation with A. sterilis cytoplasm (Figure 2); i.e., the BC_1 was a "peak" or "optional" backcross generation.

The predicted grain yields for the BC_3, BC_4, and BC_∞ in A. sterilis cytoplasm were calculated for the three matings with means that were described by the models (Table 3). The predicted grain yield of the NC-hybrid (BC_∞) derived from PI 317757 x CI 9170 was the greatest of the three. The predicted grain yields for the BC_3 and BC_4 of this mating was 3802 kg/ha and 3977 kg/ha, respectively. Empirical grain yields of the BC_3 and BC_4 populations were obtained by backcrossing the four highest yielding F_2-derived lines from the BC_2 of this mating to CI 9170. Grain yields averaged over 12 environments are shown in Figure 3. Further backcrossing of these F_2-derived lines gave BC_3 and BC_4 populations with very different grain yields than those predicted for these isopopulations. Whereas the predicted grain yield for BC_3 and BC_4 were greater than the BC_2, the actual yields showed a rather sharp negative regression toward CI 9170. If the performance of the BC_3 and BC_4 bulk populations from the four superior BC_2F_2 lines is indicative of the performance to expect from backcross bulk populations in general, then the BC_2 would be the optimal backcross generation for the mating.

Table 3. Predicted grain yield (kg ha^{-1}) and predicted NC-heterosis (kg ha^{-1}) of the BC_3, BC_4, and BC_∞ (NC-hybrid) from matings of CI 9170 with three A. sterilis accessions

A. sterilis accession	$\hat{BC}_3$	$\hat{BC}_4$	$\hat{BC}_\infty$	Predicted NC-heterosis[a]
PI 317757	3802	3977	4160	1052
PI 317982	2973	2962	2950	-172
PI 324725	2934	2983	3033	-32

[a]Predicted NC-heterosis = Predicted ($BC_{\infty(st)}$) - Predicted CI 9170.

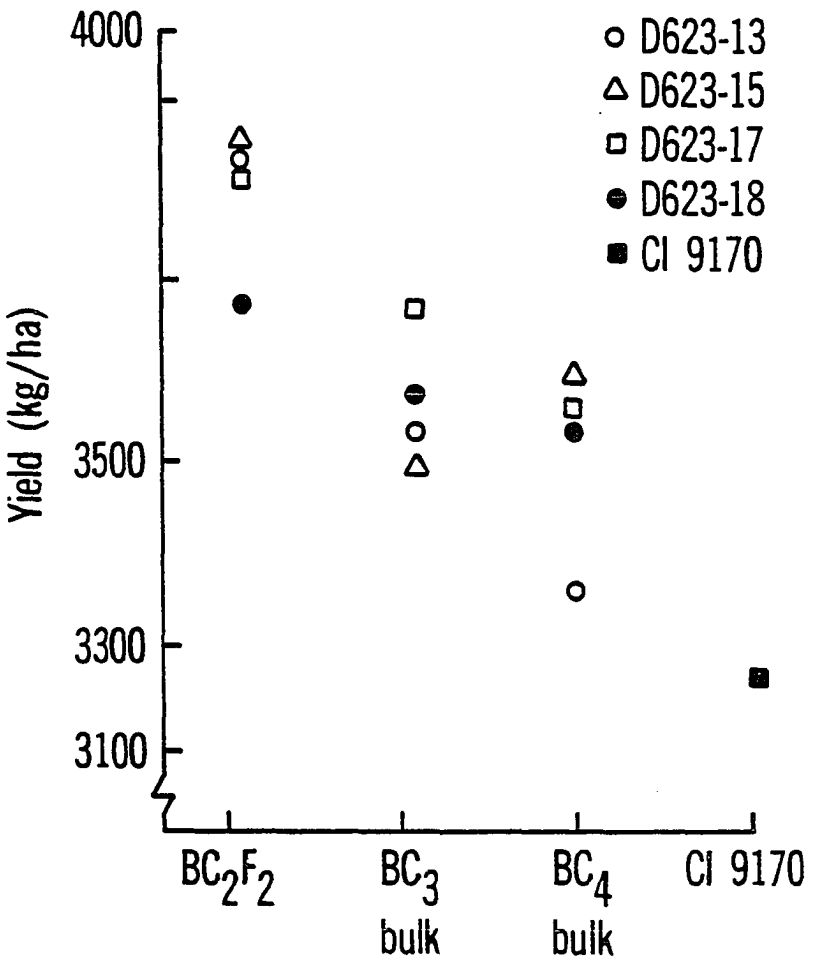

Figure 3. Mean grain yield of four BC_2F_2-derived lines, their associated BC_3 and BC_4 bulk populations, and the recurrent parent CI 9170 evaluated in three years at four locations

DISCUSSION

Research on male sterile cytoplasms of corn and sorghum indicate nuclear-cytoplasmic interactions may influence hybrid vigor (Atkins and Kern, 1972; Ross and Kofoid, 1979; Russell and Marquez-Sanchez, 1966). Kihara (1980) found NC-heterosis in NC-hybrids of wheat, and Robertson (1980) showed NC-heterosis could occur in any backcross generation of oats. These studies also showed that NC-heterosis is not a general phenomenon.

Robertson and Frey (1984) suggested that superior grain yield of backcross generations in A. sterilis cytoplasms could be due to cytoplasms per se, an interaction of A. sativa nuclear genes with A. sterilis cytoplasms, an interaction of A. sterilis nuclear genes with A. sterilis cytoplasms, and/or the second order interaction of A. sterilis and A. sativa nuclear genes with A. sterilis cytoplasms. The Mather and Jinks models which fitted the generation means for three of four matings showed that the second order interactions were not significant. The models used combined A. sterilis nuclear by cytoplasmic interaction effects with A. sativa nuclear by cytoplasmic interaction effects into a single parameter ($\alpha\gamma$), which describes the net effect of all nuclear by cytoplasmic interactions. Estimates of ($\alpha\gamma$) were significant in all mating systems.

No model could adequately describe the inheritance responsible for the generation means for the matings of PI 217512 and CI 9170. Recall that the BC_1 population in the A. sterilis cytoplasm from these parents deviated from that predicted by a fully parameterized model. Failure to

describe the generation means from these parents shows that the Mather and Jinks models of net effects may not accurately describe the underlying genetic effects. One possible reason is that the interaction, $\alpha\gamma$, lumps the effects of A. sativa nuclear by cytoplasm and A. sterilis nuclear by cytoplasm interactions. Because cytoplasmic DNA constitutes only 2% of the total plant genome (Beale and Knowles, 1978), perhaps the beneficial nuclear-cytoplasmic interaction effect may involve as little as 2% of the nuclear genome. Therefore, under random assortment of the nuclear genome, there is a nonzero probability that beneficial A. sterilis nuclear by A. sterilis cytoplasmic interaction effects would remain through several backcrosses to the A. sativa parent. There is also a nonzero probability that beneficial interactions could be eliminated in one or two backcross, which could explain the situation in the second backcross generation of the mating PI 217512 x CI 9170.

Models that accurately describe the inheritance patterns in reciprocal crosses are needed for correct estimation of generation means and for making accurate predictions of means from anticipated matings. Since the genetic models did not describe generation means for all mating pairs, it is not surprising that the model that best described generation means from PI 317757 x CI 9170 did not predict the observed trend in BC_3 and BC_4 bulk populations of the mating. However, one should be careful in drawing inferences about average grain yield from the BC_3 and BC_4 isopopulations of the mating PI 317757 x CI 9170 because the BC_3 and BC_4 bulk populations were obtained from selected BC_2F_2 lines. In stating that the BC_2 of this cross is an optimal backcross generation,

I have assumed that all BC_2F_2 lines will produce BC_3 and BC_4 bulk populations with yields similar to the four best BC_2F_2 lines. Given that the models did not describe generation means that exhibit a peak generation, the models may not describe generation means that include advanced backcross generations from the cross PI 317757 x CI 9170. Perhaps an alternative model which does not combine nuclear-cytoplasmic generations into a single parameter would be more flexible and this should be investigated for ability to describe mating systems with backcross generations that exhibit peaks.

Mean yields of backcross generations from PI 217512 x CI 9170 and PI 317757 x CI 9170 also show that NC-heterosis can occur in any backcross generation. The concept of an optimal number of backcross generations for matings involving an unadapted nuclear genome has been studied theoretically by Bailey (1977) and Dudley (1982). Lawrence (1974), using empirical data, showed that the BC_2 to BC_4 were the optimal generations for deriving superior lines from A. sativa by A. sterilis matings. However, none of these studies have considered reciprocal cytoplasmic effects. Given an assumption of Mendelism for nuclear genes, no model that is dependent upon data-based estimates of its genetic parameters can predict the optimal backcross generation for a specific mating when nuclear-cytoplasmic interactions are important.

CONCLUSIONS

The genetic models of Mather and Jinks (1982) showed the existence of significant genetic parameters that describe reciprocal generation means found by Robertson (1980). However, the genetic models were unable to describe the generation means where the BC_1 exhibited a performance that was higher than the BC_0 and BC_2. This occurs because the models used herein combine all nuclear-cytoplasmic interactions into a single parameter of net effects. An alternative model which does not combine all nuclear-cytoplasmic effects into a single parameter may be flexible enough to describe generation means when a mating system has an intermediate backcross generation that shows peak performance. Further, no model that depends upon data-based estimates of parameters can predict the optimal backcross generation for a specific mating.

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SECTION II. A THEORETICAL MODEL FOR QUANTITATIVELY INHERITED TRAITS INFLUENCED BY NUCLEAR-CYTOPLASMIC INTERACTIONS

ABSTRACT

Cytoplasmic inheritance is distinct from other types of non-mendelian inheritance. Cytoplasms of crop species affect quantitatively inherited traits that are measurable end products of photosynthesis and respiration, e.g., biomass and grain yield. Numerous enzymes involved in regulating photosynthesis and respiration are encoded by both cytoplasmic and nuclear genes.

The cytoplasm contains two organellar genomes: the chloroplast genome and the mitochondrial genome. Unlike the nuclear genome, the cytoplasmic genomes consist of single, circular, double stranded molecules of DNA. In many crop species, the cytoplasmic genomes are transmitted solely through the maternal parent and exhibit little variability within maternal lines of descent. These biological features were used to genetically model the genotypic value of an individual. Genetic variances and covariances were derived for a random mating population. However, clear estimates of some variance components cannot be obtained through the use of reciprocal mating designs if cytoplasms are inherited solely through the maternal parent.

Keywords: cytoplasmic genome, extranuclear inheritance, reciprocal mating designs

INTRODUCTION

Caspari (1948) classified the different types of nonmendelian inheritance as maternal inheritance, dauermodification, and cytoplasmic inheritance. The phenomenon of maternal inheritance led to the development of a quantitative theory for traits influenced by maternal effects in animals by Willham (1963). Just as maternal inheritance led to the concept of maternal effects, so too has cytoplasmic inheritance led to the concept of cytoplasmic effects. Evidence for cytoplasmic effects on quantitative traits of plants has been adequate since Kihara (1951) developed alloplasmic wheat (Triticum aestivum L.) lines. The effects of cytoplasms involve both direct influence and interactions of cytoplasms with nuclear effects (Ashri, 1964; Duvick, 1958; Robertson and Frey, 1984). Hermeson (1968) suggested that every nuclear effect has a cytoplasmic component.

Many quantitative traits rely on metabolic pathways that are controlled by both nuclear and cytoplasmic genes (Beale and Knowles, 1978). For example, vegetative biomass is a measure of CO_2 assimilation which is regulated by photosynthesis and respiration. Both processes are jointly controlled by nuclear and cytoplasmic genes (Iwanaga et al., 1978). Most organelle enzymes are constructed from polypeptides encoded in both the organelle and nuclear genomes (Borst et al., 1983). For example, ribulose-1,5-bisphosphate carboxylase, an enzyme responsible for carbon fixation in bundle sheath cells of maize (Zea mays L.), consists of eight copies of two nonidentical subunits. The larger subunit

is coded by chloroplast DNA (Bowman et al., 1981) and the smaller one is encoded in the nucleus (Highfield and Ellis, 1978; Cashmore et al., 1978) by a multigenic family (Dunsmuir et al., 1983).

To date, a theory for quantitative traits influenced by cytoplasmic effects has not been developed; however, with certain assumptions, models for maternal or reciprocal effects (Mather and Jinks, 1982) can describe cytoplasmic effects. For example, Beavis and Frey (1985) attempted to describe the nuclear and cytoplasmic effects on grain yield means of backcross populations from reciprocal matings by using the models of Mather and Jinks (1982). Their results indicated, however, that the models do not always fit the data. They suggested that a model based upon biological phenomena might better describe the underlying genetic effects of cytoplasms for quantitative traits.

Most higher plant species exhibit uniparental inheritance of cytoplasmic organelles through the maternal parent, although exceptions occur in Secale, Solanum, and Oenethera (Gillham, 1978). Developments in biotechnology have allowed molecular geneticists to confirm the inheritance of organellar DNA. Electrophoretic patterns of endonucleic restriction digests of chloroplast and mitochondrial DNA have been used to describe the inheritance of organelle DNA in numerous plant species (Conde et al., 1979; Hatfield et al., 1985; Sisson et al., 1978; Tsunewaki and Ogihara, 1983; Vedel et al., 1976). Although there are numerous mechanisms responsible for uniparental inheritance (Sears, 1980), the important feature is that the cytoplasmic genome from the paternal parent is eliminated from the zygote.

This manuscript proposes a quantitative genetic model for traits influenced by cytoplasmic genes which exhibit strict maternal inheritance, develops variance/covariance components for a random mating population, and considers the use of a reciprocal mating design for the estimation of the components.

ASSUMPTIONS

Cytoplasmic Genome

The cytoplasmic genome consists primarily of DNA in the chloroplasts (cpDNA) and mitochondria (mtDNA) (Beale and Knowles, 1978). The number of chloroplasts and mitochondria per cell is variable and depends upon the differentiated state of the cell (Butterfass, 1979). Organelles in both biparental and maternal inherited cytoplasms tend toward a homogenous state within the cell (Michael, 1978; Sears, 1980) and, unlike chromosomes, replication and transmission of orgenelles are independent of nuclear control (Birky, 1983). Michaelis (1958) proposed that the partitioning of organelles during mitosis is stochastic; i.e., there is a positive probability that daughter cells will not receive equal quantities of organelles. Birky (1983) also indicated that the uniparental inheritance perpetuates the homoplasmic condition within maternal lines of descent. Thus, it can be assumed that plant species exhibiting uniparental inheritance of the cytoplasm consist of individuals with cells that contain genetically homogenous chloroplast and mitochondrial populations.

Each chloroplast contains from 10 to 60 complete copies of its genome (Grun, 1976), and each copy consists of a single, circular, double-stranded DNA molecule (Gillham, 1978). The structure of the mitochondrial genome is unknown, but Kolodner and Tewari (1972), working on mtDNA from pea (Pisum sativum L.) leaves, indicated that the mitochondrial genome also consists of single, circular, double-stranded molecules

of DNA. However, according to Leaver and Gray (1982) and authors cited therein, maize (Zea mays L.) mitochondria contain circular and linear DNA of various sizes. Also, electrophoretic patterns of restriction endonuclease digests of mtDNA from a single sample show heterogeneity in many plant species. Leaver and Gray (1982) suggest that the heterogenous pieces of mtDNA may be a manifestation of a genome organized into different sized "chromosomes." An alternative hypothesis currently supported by most investigators (Leaver et al., 1983; Levings, 1983; Lonsdale et al., 1983) is that the genome exists as a single, large, circular, duplex molecule of DNA from which smaller molecules arise. Given this hypothesis, the structure of the cytoplasmic genome can be viewed as two populations of polyploid organelles where the basic chromosome number is one. If the polyploid organelles are homozygous for all loci, then they are effectively haploid and all genes may be considered linked.

In summary, I assumed that the cytoplasm is maternally inherited, consists of two homogenous populations of polyploid (x=1) organelles, each of which is homozygous, and that cytoplasmic genes appear completely linked.

Population Model

Consider a hypothetical metric trait possessed by diploid individuals in an infinite random mating population. Assume that diverse cytoplasms exist within the population, but not within maternal lines of descent, and assume, at least temporarily, no environmental influence.

Let the genotypic value be under the control of two genetic loci: one located in the nuclear genome and the other in the cytoplasmic genome. Because the cytoplasmic genome of plant species consists of two distinct genomes, arbitrarily assign the cytoplasmic locus to either the chloroplast or mitochondrial genome. At each locus, it is possible to have an arbitrary number of alleles. Denote the nuclear alleles, A_1, A_2, A_3, ... A_k and the cytoplasmic alleles C_1, C_2, C_3, ... C_m. Each of these alleles have arbitrary allelic frequencies in the population of p_1, p_2, ... p_k such that $\sum_i P_i = 1$ for the nuclear genes and q_1, q_2, ... q_m such that $\sum_k q_k = 1$ for the cytoplasmic genes. The genotype representing the nuclear locus of an individual in the population may be denoted A_iA_j. A genotype representing the cytoplasmic genome for the same individual may be denoted C_t. Thus, the genotype of an individual may be fully expressed as $A_iA_jC_t$. With Mendelian inheritance of the nuclear genes and strict maternal inheritance of the cytoplasmic genes, the probability of selecting $A_iA_jC_t$ is $p_ip_jq_t$. Following the algebra of Kempthorne (1957), the genotypic value of this individual may be denoted Y_{ijt} and can be modelled as

$$Y_{ijt} = \mu + \alpha^n_i + \alpha^n_j + \gamma^c_t + \delta^n_{ij} + (\alpha^n\gamma^c)_{it} + (\alpha^n\gamma^c)_{jt} + (\delta^n\gamma^c)_{ijt}. \quad (1)$$

In (1), μ is the population mean and is calculated as the sum of the product of gene frequencies by genotypic values; i.e.,

$$\mu = \sum_i \sum_j \sum_t p_ip_jq_tY_{ijt}$$

α_i^n and α_j^n are the additive effects of alleles i and j at the nuclear locus and are calculated as average deviations from the population mean; i.e.,

$$\alpha_i^n = \sum_j \sum_t p_j q_t Y_{ijt} - \mu \text{ , and}$$

$$\alpha_j^n = \sum_i \sum_t p_i q_t Y_{ijt} - \mu \text{ .}$$

γ_t^c is the additive effect of allele t at the cytoplasmic locus; i.e.,

$$\gamma_t^c = \sum_i \sum_j p_i p_j Y_{ijt} - \mu \text{ .}$$

δ_{ij}^n is the dominance deviation attributed to the nuclear locus and is calculated as an average deviation from the population mean after it is adjusted for additive effects from the nuclear locus; i.e.,

$$\delta_{ij}^n = \sum_t q_t Y_{ijt} - (\mu + \alpha_i^n + \alpha_j^n) \text{ .}$$

Due to the assumption of a homozygous organelle, no dominance deviation needs to be defined for the cytoplasmic locus. The three nuclear-cytoplasmic interaction effects can be defined in a manner similar to the nuclear dominance effects; i.e.,

$$(\alpha^n\gamma^c)_{it} = \sum_j p_j Y_{ijt} - (\mu + \alpha_i^n + \gamma_t^c) \text{ ,}$$

$$(\alpha^n\gamma^c)_{jt} = \sum_i p_i Y_{ijt} - (\mu + \alpha_j^n - \gamma_t^c) \text{ , and}$$

$$(\delta^n\gamma^c)_{ijt} = Y_{ijt} - (\mu + \alpha_i^n + \alpha_j^n + \gamma_t^c + \delta_{ij}^n + (\alpha^n\gamma^c)_{it} + (\alpha^n\gamma^c)_{jt}).$$

Some important algebraic properties resulting from these definitions are:

1. $\sum_i p_i \alpha_i^n = \sum_t q_t \gamma_t^c = 0$,

2. $\sum_i \sum_j p_i p_j \delta_{ij}^n = 0$,

3. $\sum_i \sum_t p_i q_t (\alpha^n \gamma^c)_{it} = 0$,

4. $\sum_i \sum_j \sum_t p_i p_j q_t (\delta^n \gamma^c)_{i,t} = 0$.

RESULTS

Variances

By utilizing the properties developed in the assumptions section, the genotypic variance for a random mating population can be partitioned into independent components:

$$\sigma_g^2 = \sum_i \sum_j \sum_t p_i p_j q_t (Y_{ijt} - Y_{...})^2$$

$$= 2\sum_i p_i (\alpha_i^n)^2 + \sum_t q_i (\gamma_t^c)^2 + \sum_i \sum_j p_i p_j (\delta_{ij}^n)^2 + 2\sum_i \sum_t p_i q_t (\alpha^n \gamma^c)_{it}^2$$

$$+ \sum_i \sum_j \sum_t p_i p_j q_t (\delta^n \gamma^c)_{ijt}^2 . \qquad (2)$$

Notice that two terms describe variability from the additive effects at the nuclear locus and one term describes the variability attributable to additive effects at the cytoplasmic locus. One term describes variability derived from effects of dominance deviations at the nuclear locus, but no analogous term is associated with the cytoplasmic locus. Also, the epistatic components consist of three terms, two associated with interlocus variability attributed to additive by additive effects and one associated with the interlocus variability attributeable to the additive by dominance effects. The result is similar to the more general two locus model with arbitrary epistasis and no linkage (Kempthorne, 1957) and may be written as:

$$\sigma_g^2 = \sigma_{A_n}^2 + \sigma_{A_c}^2 + \sigma_{D_n}^2 + \sigma_{(A_1A_2)_{nc}}^2 + \sigma_{(DA)_{nc}}^2 . \qquad (3)$$

Consider a case of two unlinked nuclear loci with arbitrary epistasis and one cytoplasmic locus. Again, the algebra of Kempthorne (1957) is utilized to determine the genotypic variance for a random mating population:

$$\sigma^2_g = \sum_i \sum_j \sum_k \sum_\ell \sum_t p_i^1 p_j^1 p_k^2 p_\ell^2 q_t (Y_{ij,k_\ell,t} - Y_{...})_2 \ ,$$

where the superscript above p is used to designate a nuclear locus with $\sum_k p_k^2 = 1$. Therefore, the algebraic properties which apply to the first nuclear locus also apply to the second, and the decomposed genetic variance may be written as:

$$\begin{aligned}\sigma^2_g = {} & \sigma^2_{A_{n_1}} + \sigma^2_{A_{n_2}} + \sigma^2_{D_{n_1}} + \sigma^2_{D_{n_2}} + \sigma^2_{AA_{n_1n_2}} + \sigma^2_{AD_{n_1n_2}} + \sigma^2_{DD_{n_1n_2}} \\ & \sigma^2_{A_c} + \sigma^2_{AA_{n_1c}} + \sigma^2_{AA_{n_2c}} + \sigma^2_{AD_{cn_1}} + \sigma^2_{AD_{cn_2}} + \\ & + \sigma^2_{AAA_{n_1n_2c}} + \sigma^2_{ADA_{n_1n_2c}} + \sigma^2_{DDA_{n_1n_2c}} \ . \end{aligned} \qquad (4)$$

Note that the decomposition consists of the same terms derived by Kempthorne (1957) for a two-locus model with arbitrary epistasis; where the subscripts describe the source and the locus (loci) responsible for the effects. For example, A_{n_1} refers to the additive effects from the first nuclear locus and $AD_{n_1n_2}$ refers to the sum of the additive by dominance epistatic effects from the nuclear loci. The decomposition also includes a term that describes variability at the cytoplasmic locus, $\sigma^2_{A_c}$, and seven terms that describe variability due to nuclear by

cytoplasmic interactions. For example, $\sigma^2_{(AAA)_{n_1 n_2 c}}$ describes the variability due to additive by additive nuclear epistasis interacting with cytoplasmic effects.

The model obtained by increasing the number of cytoplasmic loci that affect a trait can be obtained by reconsidering the structure, replication, and inheritance of the cytoplasmic genomes. Recall, we assumed that each copy of organellar DNA is an exact replica of its sisters. Because x=1, organellar loci can be considered to be completely linked. Also consider that at least one copy of the chloroplast and mitochondrial genomes will be passed on to all daughter cells. Therefore, the integrity of the cytoplasmic genome will remain constant in a maternally inherited cytoplasm, if no mutations occur (Birky, 1983). Therefore, the results given in (4) apply for two nuclear loci and any number of cytoplasmic loci and can be extended to any number of nuclear loci as well.

Covariances

The algebra of Kempthorne (1957) also can be utilized to calculate the covariance between individuals for a trait influenced by nuclear and cytoplasmic genes. Consider two individuals, X and Y, drawn from an infinite random mating population with genotypes denoted $X = A_i A_j C_t$ and $Y = A_k A_l C_u$. Setting the population mean equal to zero, the genotypic values for X and Y may be modelled as:

$$X_{ijt} = \alpha^n_i + \alpha^n_j + \gamma^c_t + \delta^n_{ij} + (\alpha^n\gamma^c)_{it} + (\alpha^n\gamma^c)_{jt} + (\delta^n\gamma^c)_{ijt} \text{ and}$$

$$Y_{k\ell u} = \alpha^n_k + \alpha^n_\ell + \gamma^c_u + \delta^n_{k\ell} + (\alpha^n\gamma^c)_{ku} + (\alpha^n\gamma^c)_{\ell u} + (\delta^n\gamma^c)_{k\ell u} .$$

Thus, the Cov(X,Y) is $E(X_{ijt}\ Y_{k\ell u})$ which equals

$$E[(\alpha^n_i + \alpha^n_j + \gamma^c_t + \delta^n_{ij})(\alpha^n_k + \alpha^n_\ell + \gamma^c_u + \delta^n_{k\ell})] +$$

$$E[((\alpha\gamma)_{it} + (\alpha\gamma)_{jt})((\alpha^n\gamma^c)_{ku} + (\alpha\gamma)_{\ell u})] + E[(\delta^n\gamma^c)_{ijt}(\delta^n\gamma^c)_{k\ell u}] .$$

The four previous algebraic properties and an additional property, $E(\alpha_{i_x}\gamma_{u_y}) = 0$, make Cov (X,Y) equal to

$$(P_{ik} + P_{i\ell} + P_{jk} + P_{j\ell})\tfrac{1}{2}\sigma^2_{A_n} + P_{tu}\sigma^2_{A_c} + (P_{ik,j\ell} + P_{i\ell,jk})\sigma^2_{D_n} +$$

$$P_{tu} \cdot (P_{ik} + P_{i\ell} + P_{jk} + P_{j\ell})\tfrac{1}{2}\sigma^2_{(AA)_{nc}} +$$

$$P_{tu} \cdot (P_{ik,j\ell} + P_{i\ell,jk})\tfrac{1}{2}\sigma^2_{(DA)_{nc}} ,$$

where P_{ik} is the probability that A_i is identical by descent to A_k, and $P_{ik,j\ell}$ is the joint probability that A_i is identical by descent to A_k and A_j is identical by descent to A_l. Notice that P_{tu} is the probability that X and Y have the same cytoplasmic genes by descent. In species that exhibit strict maternal inheritance of cytoplasmic genes,

$P(C_t = C_u \mid X \text{ and } Y \text{ are full sibs}) = P(C_t = C_u) \mid X \text{ and } Y \text{ are maternal half sibs}) = 1$, and

$P(C_t = C_u \mid X \text{ and } Y \text{ are reciprocal full sibs}) = P(C_t = C_u \mid X \text{ and } Y \text{ are paternal half sibs}) =$.

By utilizing Malecot's coefficient of parentage (r_{xy}), Kempthorne's U_{xy} to denote $(P_{ik,j\ell} + P_{i\ell,jk})$, and C_{xy} to denote P_{tu},

$$\text{Cov}(X,Y) = 2 \cdot r_{xy} \cdot \sigma^2_{A_n} + C_{xy} \cdot \sigma^2_{A_c} + U_{xy} \cdot \sigma^2_{D_n} + 2 \cdot r_{xy} \cdot C_{xy} \cdot \sigma^2_{(AA)_{nc}}$$

$$+ U_{xy} \cdot C_{xy} \cdot \sigma^2_{(DA)_{nc}} . \qquad (5)$$

The model may be extended to any number of nuclear and cytoplasmic loci. Again, by making use of Kempthorne's results, and assuming that all cytoplasmic loci are completely linked and that strict maternal inheritances of cytoplasms occurs, Cov(X,Y) is given by

$$\sum_{n} \sum_{n_2} (2r_{xy})^n (U_{xy})^{n_2} \sigma^2_{A^{n_D} n_2} + C_{xy} \sigma^2_{A_c} +$$

$$C_{xy} \sum_{n_1} \sum_{n_2} (2r_{xy})^{n_1} (U_{xy})^{n_2} \sigma^2_{(AD)_{n_1 n_2 A_c}} , \qquad (6)$$

where n_1 is the total number of nuclear loci involved in the interaction of additive effects and n_2 is the total number of nuclear loci involved in the interaction of dominance effects.

Estimation of Parameters

Under certain assumptions, model (1) provides biological interpretations for parameters generated from statistical analyses of reciprocal crosses. Yates (1947) was the first to analyze data from a complete set of reciprocal crosses. Griffing (1956) considered such an analysis as one of four possible diallel methods. Cockerham (1963) related variance components of reciprocal crosses generated by the complete

diallel and North Carolina Design II with reciprocals of Comstock and Robinson (1948) but presented no biological interpretation for reciprocal or maternal effects. Cockerham and Weir (1977) considered the relationships among three models used to describe reciprocal effects generated by quadratic analyses. One of the models, a "bio model," considers nuclear and extranuclear effects from both parents.

My model (1) appears to be a special case of the "bio model" which, in the notation of Cockerham and Weir (1977), is

$$G_{ij} = n_l + n_j + t_{ij} + m_i + p_j + k_{ij} \ . \qquad (c)$$

G_{ij} is the coded genotypic mean of effects attributable to maternal parent i and paternal parent j; where n and t refer to nuclear effects, and m, p, and k refer to simple and epistatic extranuclear effects. The relationship between our model (1) and model (c) of Cockerham and Weir (1977) can be shown as follows. If a maternal parent, $A_iA_jC_t$, and a paternal parent, $A_vA_wC_u$, are mated, the array of full sib offspring from the mating is

$$\tfrac{1}{4}[A_iA_vC_t + A_iA_wC_t + A_jA_vC_t + A_jA_wC_t) \ . \qquad (7)$$

Thus, if Cockerham and Weir's (i) is replaced by (ijt) and (j) is replaced by (vwu), the coded genotypic mean, G_{ij}, becomes

$$\begin{aligned} G_{(ijt),(vwu)} &= n_{ij} + n_{vw} + t_{ij,vw} + m_{ijt} + p_{vwu} + k_{(ijt)(vwu)} \\ &= (\tfrac{1}{2}(\alpha^n_l + \alpha^n_j)) + (\tfrac{1}{2}(\alpha^n_v + \alpha^n_w)) + \tfrac{1}{4}(\delta^n_{lv} + \delta^n_{lw} + \delta^n_{jv} + \delta^n_{jw}) \\ &\quad + (\gamma^c_t + \tfrac{1}{2}(\alpha^n\gamma^c)_{it} + \tfrac{1}{2}(\alpha^n\gamma^c)_{jt}) \\ &\quad + (\tfrac{1}{2}(\alpha^n\gamma^c)_{vt} + \tfrac{1}{2}(\alpha^n\gamma^c)_{wt} + \tfrac{1}{4}(\delta^n\gamma^c)_{ivt} \end{aligned}$$

$$+ \tfrac{1}{4}(\delta^n\gamma^c)_{iwt} + \tfrac{1}{4}(\delta^n\gamma^c)_{jvt} + \tfrac{1}{4}(\delta^n\gamma^c)_{jwt}) \ . \qquad (8)$$

By (7), $A_iA_vC_u$, $A_iA_wC_u$, $A_jA_vC_u$, and $A_jA_wC_u$ do not exist. Therefore, the components of variance are

$$\sigma^2_n = \tfrac{1}{4}\sum\sum p_ip_j(\alpha_i + \alpha_j)^2 = \tfrac{1}{4}\sigma^2_{A_n} ,$$

$$\sigma^2_t = \tfrac{1}{4}\sum_i\sum_j p_ip_j(\delta^n_{ij})^2 = \tfrac{1}{4}\alpha^2_{D_n} ,$$

$$\sigma^2_m = \sum_t q_t(\gamma^c_t)^2 + \tfrac{1}{4}\sum_i\sum_t p_iq_t(\alpha^n\gamma^c)^2_{it} + \tfrac{1}{4}\sum_j\sum_t p_iq_t(\alpha^n\gamma^c)^2_{jt}$$

$$= \sigma^2_{A_c} + \tfrac{1}{4}\sigma^2_{(AA)_{nc}} ,$$

$$\sigma^2_p = 0 ,$$

$$\sigma^2_k = \tfrac{1}{4}\sum_i\sum_t p_iq_t(\alpha^n\gamma^c)^2_{it} + \tfrac{1}{4}\sum_j\sum_t p_iq_t(\alpha^n\gamma^c)^2_{jt}$$

$$+ \tfrac{1}{4}\sum_i\sum_j\sum_t p_ip_jq_t(\delta^n\gamma^c)^2_{ijt} = \tfrac{1}{4}(\sigma^2_{(AA)_{nc}} + \sigma^2_{(DA)_{nc}}) \ .$$

Covariances for specific relationships investigated by Cockerham and Weir (1977) are determined for model (1) using the general formula for covariances, (5) and the coefficients of Table 1. Results of covariances for Cockerham and Weir's model (c) and our model (1) are summarized in Table 2. The analysis of variance table for a reciprocal mating design (Table 3 from Cockerham and Weir) can be recast in our notation (Table 3). It is obvious that model (1) will only produce estimates of genetic variance components for

$$\sigma^2_{A_n}, \ \sigma^2_{D_n}, \ \sigma^2_{A_c} + \tfrac{1}{4}\sigma^2_{(AA)_{nc}}, \text{ and } \sigma^2_{(AA)_{nc}} + \sigma^2_{(DD)_{nc}}$$

from a reciprocal mating design.

Table 1. Values of Malecot's coefficient of parentage r_{xy}, Kempthorne's U_{xy}, and the cytoplasmic coefficient C_{xy} for relationships obtained from reciprocal mating designs

Relationship	Cockerham-Weir[a] notation	$2r_{xy}$	U_{xy}	C_{xy}
full sibs	ϱ_f	½	¼	1
reciprocal full sibs	ϱ_{rf}	½	¼	0
maternal half sibs	ϱ_m	¼	0	1
paternal half sibs	ϱ_p	¼	0	0
reciprocal half sibs	ϱ_r	¼	0	0

[a] Cockerham and Weir (1977).

Table 2. Covariances of related individuals obtained from a reciprocal mating design

Relationship[a]	Covariance	
	Model (1)	Model (c)[a]
ϱ_f	$\frac{1}{2}\sigma^2_{A_n} + \frac{1}{4}\sigma^2_{D_n} + \sigma^2_{A_c} + \frac{1}{2}\sigma^2_{AA_{nc}} + \frac{1}{4}\sigma^2_{DA_{nc}}$	$\sigma^2_M + \sigma^2_P + \sigma^2_{MP}$
ϱ_{rf}	$\frac{1}{2}\sigma^2_{A_n} + \frac{1}{4}\sigma^2_{D_n}$	$2\sigma^2_n + \sigma^2_t$
ϱ_m	$\frac{1}{4}\sigma^2_{A_n} + \sigma^2_{A_c} + \frac{1}{4}\sigma^2_{AA_{nc}}$	σ^2_M
ϱ_p	$\frac{1}{4}\sigma^2_{A_n}$	σ^2_P
ϱ_r	$\frac{1}{4}\sigma^2_{A_n}$	σ^2_n

[a]Notation of Cockerham and Weir (1977).

Table 3. Expected mean squares for indicated sources of variability in an analysis of variance of offspring from a reciprocal mating design

Source	df	Expected mean squares	
		Model (1)	Model (c)[a]
General	$2(N-1)$	$\sigma^2 + \frac{U}{4}(\sigma^2_{A_n}) + \frac{U}{2}\sigma^2_{D_n} + \frac{UN}{2}(\sigma^2_{A_c} + \frac{1}{4}\sigma^2_{AA_{nc}})$	$\sigma^2 + U\sigma^2_k + 2U\sigma^2_t + \frac{UN}{2}(\sigma^2_m + \sigma^2_p) + 2UN\sigma^2_n$
Specific	$(N-1)^2$	$\sigma^2 + \frac{U}{4}(\sigma^2_{A_n}) + \frac{U}{2}\sigma^2_{D_n}$	$\sigma^2 + U\sigma^2_k + 2U\sigma^2_t$
Reciprocal General	$2(N-1)$	$\sigma^2 + \frac{U}{4}(\sigma^2_{(AA)_{nc}} + \sigma^2_{(DA)_{nc}}) + \frac{UN}{2}(\sigma^2_{A_c} + \frac{1}{4}\sigma^2_{(AA)_{nc}})$	$\sigma^2 + U\sigma^2_k + \frac{UN}{2}(\sigma^2_m + \sigma^2_p)$
Reciprocal Specific	$(N-1)^2$	$\sigma^2 + \frac{U}{4}(\sigma^2_{(AA)_{nc}} + \sigma^2_{(DA)_{nc}})$	$\sigma^2 + U\sigma^2_k$
Error	$2N^2(U-1)$	σ^2	σ^2
Total	$2N^2U-2$		

[a]In the notation of Cockerham and Weir (1977).

DISCUSSION

A quantitative genetic model was developed for traits influenced by cytoplasmic genes which are strictly maternally inherited based upon biological features of the cytoplasmic genome. Application of the model will depend, in part, upon the biological validity of the assumptions. For cytoplasms that are maternally inherited, it was assumed that each organellar genome is homoplasmic, that each organelle was polyploid with a basic chromosome number of one (x=1), and that each organelle was homozygous at all loci.

There is abundant evidence that both the chloroplasts and mitochondria of a cell tend toward a homoplasmic state; i.e., that a population of chloroplasts or mitochondria within each cell tends to be homogenous (Birky, 1983). This tendency is best shown in species which exhibit bi-parental cytoplasmic inheritance but also has been shown to occur in studies on protoplast fusion (Izhar, 1980) and cytoplasm-protoplast fusion (Maliga et al., 1982). Work on cytoplast fusion among Nicotiana spp. (Maliga et al., 1982; Cseplo and Maliga, 1984) indicated that heterogenous populations could be maintained through plant regeneration and a generation of seed production. Nevertheless, the tendency toward a homoplasmic condition was evident. Indeed, taxonomic studies on cytoplasmic genomes which utilize electrophoretic patterns of endonuclease restricted cpDNA and/or mtDNA rely on the assumption that the organellar genomes are homogenous within maternal lines of descent. Thus, it is

not likely that a heteroplasmic condition needs to be considered in a genetic model for maternally inherited cytoplasmic effects.

As noted in the introduction, the structure of the genome in both the chloroplast and mitochondrion is hypothesized to consist of many double stranded, circular molecules of DNA. Each molecule is thought to code for the same genes. Thus, I assumed that each chloroplast and mitochondrion is a polyploid (x=1) organelle. There is a possibility that the mitochondrial genome contains more than one "chromosome" (Leaver and Gray, 1982). If this alternative structure is true, then the possibilities of interorganellar recombination and segregation would have to be investigated. To date, fusion between organelles has not been oberved (Birky, 1983). Of course, the impact of inter-organellar recombination and segregation on the model is negligible given that the cytoplasm consists of homogenous organelles that are homozygous. If recombination events occur between heterozygous organelles, then it is unlikely that the integrity of the cytoplasm will be maintained under the relaxed controls of replication and segregation described by Birky (1983), and none of my assumptions about the cytoplasm would hold.

To my knowledge, the heterozygosity of organelles has not been tested, and the technology needed to investigate intra-organellar heterozygosity may not exist. A heterozygous, homoplasmic, cellular condition of the mitochondrial genome might explain observed heterogenous restriction endonuclease digests of mtDNA (Leaver and Gray, 1982). If the organelles are heterozygous, then the cytoplasmic effect (γ)

may be a composite of additive, dominance, and interactions of additive and dominance, genetic effects. Nuclear by cytoplasmic interaction effects in (1) also would be more complex. However, if the integrity of the cytoplasm is maintained, i.e., a homoplasmic condition is maintained, from one generation to the next, then the individual component effects of a heterozygous cytoplasm would not be detected and the model could still be applied.

My population assumptions are the usual ones for a diploid species except that I also assumed that multiple cytoplasmic alleles existed in the random mating population. Theoretical models describing the origination, evolution, and equilibrium conditions of cytoplasmic gynodieocy (male sterility) have been proposed (Charlesworth and Ganders, 1979; Clark, 1984; Gregorius and Ross, 1984; Ross and Gregorius, 1985). All of these studies were developed on simple models with two cytoplasm types (e.g., male sterile and male fertile) and can be generalized to cover multiple cytoplasmic types. Of course, under the condition of complete linkage, multiple alleles are equivalent to multiple cytoplasms. In addition, cytoplasmic variability in the form of cytoplasmic gynodioecy has been observed in natural random mating populations (Ganders, 1978) and is well-utilized in plant species that are economically important (Duvick, 1965; Quinby, 1970). Therefore, the assumption of multiple cytoplasmic alleles is valid and the resulting model can be applied to some random mating populations.

In order to apply model (1) to populations derived from reciprocal matings, it must be assumed that the only source of extranuclear effects

are cytoplasmic. For most plant breeding experiments where careful field husbandry is practiced, maternal effects will be minimized. However, for those species where a triploid endosperm affects growth and development of the seedling, reciprocal effects cannot be ascribed solely to the cytoplasm. Even in the absence of endosperm effects, the use of a reciprocal mating design will not provide unique estimates of the cytoplasmic variance components for species that experience uniparental maternal inheritance of cytoplasms. Our results indicate that the estimate of general reciprocal effects will be an amalgam of cytoplasmic variability and additive nuclear by cytoplasmic variability. The reason is revealed in equation (8). Cockerham and Weir's (1977) extranuclear maternal effects include cytoplasmic effects and additive nuclear by cytoplasmic interaction effects from the maternal parent. From a biological perspective, it is not possible to separate cytoplasmic effects from additive nuclear by cytoplasmic interactions because both are inherited as a unit in species that exhibit strict maternal inheritance of the cytoplasm. There is little biological evidence for pure cytoplasmic effects; products of cytoplasmic genes combine with the products of nuclear genes to produce functional enzymes. Also from a plant breeding perspective, confounding $\sigma^2_{A_c}$ with $\sigma^2_{AA_{nc}}$ will have little impact on estimates of heritability or genetic gain.

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SECTION III. THREE METHODS FOR DETERMINING THE MINIMUM NUMBER OF SEGREGATING LINES NEEDED TO DETECT SIGNIFICANT VARIABILITY AMONG CYTOPLASMIC ISOPOPULATIONS OF OATS

INTRODUCTION

Plant breeders who conduct research on germplasm introgression have an interest in identifying superior populations from wide crosses. Superior populations are defined as those that exhibit above average performance and/or large genetic variability. Most populations derived from wide crosses perform poorly relative to the adapted germplasm, although exceptional populations occur occasionally. Because exceptional populations are identified infrequently in germplasm introgression studies, the researcher must evaluate as many populations as possible. Identification of superior populations can be accomplished only through field evaluation, which is expensive. Due to the cost of field evaluation, the number of plots available per experiment is restricted. Therefore, in order to evaluate a large number of populations with a fixed number of plots, it is important to use the minimum number of lines needed to represent a population. The minimum sample size of a population depends upon the objective of the researcher. As noted above, criteria may be the mean performance of a population, the variability exhibited by a population, or both.

The small grains research project at Iowa State University has been involved in introgression of *Avena sterilis* L. genes into cultivated oats (*A. sativa* L.) since ca. 1970 (Frey, 1986). Robertson and Frey (1984) studied the effects of introgression of both cytoplasmic and nuclear *A. sterilis* genes and identified one heterotic isopopulation from 10 reciprocal pairs. Robertson (1980) also identified cytoplasmic effects as a significant source of variability in five of 10

pairs of isopopulations. The sample size used by Robertson was 20 F_2-derived lines per population. However, Frey (Dept. of Agronomy, Iowa State University, Ames, IA, personal communication) felt that 20 F_2-derived lines was too large a sample size. Since future studies involving introgression of nuclear and cytoplasmic genes from A. sterilis are planned, it was felt that an estimate of the minimum sample size to detect significant variability was needed. Therefore, the primary objective of this study was to determine the minimum number of F_2 lines per population needed to detect significant variability among populations. The methods used, results obtained, and impact upon related results are presented and discussed herein.

MATERIALS AND METHODS

Empirical Data

The minimum number of lines needed to detect significant differences among populations was estimated by three methods. All three utilize empirical data to estimate a minimum sample size. We used grain yield data obtained from 20 BC_2 isopopulations created by Robertson (1980) from 10 paired reciprocal crosses involving all possible matings among two Avena sativa L. and five A. sterilis L. parents. Robertson evaluated 20 random F_2 lines from each of these populations in hill plots replicated three times at each of two locations in 1979. The linear model used to describe grain yield was:

$$Y_{ijkl} = \mu + L_i + R_{ij} + P_k + G_{kl} + (LP)_{ik} + (LG)_{ikl} + e_{ijkl} \quad ; \qquad (1)$$

where

Y_{ijkl} = yield of the lth F_2-derived line from the kth population evaluated in the jth replicate at the ith location;

μ = an average effect for the experiment;

L_i = the effect of the ith location;

R_{ij} = effect of the jth replicate within the ith location;

P_k = effect of the kth population;

G_{kl} = effect of the lth F_2 line within the kth population; and

e_{ijkl} = experimental error of the lth F_2-derived line from the kth population evaluated in the jth replicate at the ith location.

It should be pointed out that $P_k = C_t + M_u + (CM)_{tu}$; where C_t is the effect of the tth cytoplasm, and M_u is the effect of the uth pair of parents (mating). The F_2-derived lines within populations were obtained randomly. All other identified sources of variability, i.e., populations, cytoplasms, matings, and locations, were considered to be fixed. The analysis of variance for Robertson's data is shown in Table 1. The interaction of cytoplasms with matings were significant, so cytoplasmic differences were investigated for each mating using an "LSD statistic." The hypothesis of no cytoplasmic differences was rejected ($\alpha = 0.05$) for matings involving five pairs of parents: 'PI 324725' and 'Otee,' 'PI 317982' and Otee, 'PI 317757' and Otee, 'PI 324819' and 'CI 9170,' and 'PI 317757' and CI 9170 (Robertson, 1980).

Method I

This first method for estimating minimal sample size is an intuitive approach. The ratio of MS_{popn} to $MS_{(lines/popn)}$ provides the F statistic for testing the null hypothesis of no population difference. Consider first MS_{popn}. The data set from Robertson (1980) is balanced and described by a linear model with independent components. Therefore, the estimate of sums of squared deviations due to populations should be independent of the number of lines within populations; i.e., Robertson's estimate of the variability among populations (θ_p^2 in Table 1) should be a best unbiased estimater. Next, consider the denominator of the F statistic, $MS_{(lines/popn)}$. The maximum sums of squared deviations due

Table 1. Analysis of variance of 20 BC_2F_2-derived lines from 10 reciprocal crosses of two Avena sativa L. and varieties and five A. sterilis L. accessions evaluated for grain yield in three replications at two locations in 1979 (adapted from Robertson, 1980)

Source	df	MS	E(MS)[a]	$\hat{MS}$
Location	2			
Rep/location	4			
Population	19	M3	$\sigma^2 + r\sigma^2_{L\ell/p} + rL\ell\Theta^2_p$	690.9**
Lines/populations	380	M2	$\sigma^2 + r\sigma^2_{L\ell/p}$	133.6**
Location x population	19		$\sigma^2 + r\sigma^2_{L\ell/p} + rg\sigma^2_{Lp}$	171.1**
Location x lines/ population	380		$\sigma^2 + r\sigma^2_{L\ell/p}$	31.2
Error	1596	M1	σ^2	24.9

[a] $\Theta^2_p = \sum_{k=1}^{20} (p_k - \bar{p})^2/19$.

to lines within populations will result if all 20 lines per population are included in the calculation. If one line per population is removed, the sums of squared deviations due to lines within populations will either decrease or be unaffected by its removal. Likewise, the removal of two lines, or three lines, or four lines, ..., or 18 lines will either decrease or have no effect on the sums of squared deviations. If it is assumed that the removal of 1 to 18 lines per population will not affect the sums of squared deviations for populations, then the estimate of $MS_{(lines/popn)}$ will be maximized for decreasing numbers of lines per

population. The estimated F statistic under these assumptions will be a function of the form $\frac{mx+k}{mx}$; where $k = \theta_p^2$, m = slope of line, and x = number of lines per population.

Method II

The second method used to estimate the minimum sample size can be referred to as a Monte Carlo method (McCracken, 1955). In this method, the analysis of variance was determined on a random sample of lines from each of Robertson's populations. Sample sizes of five to 10 random lines were obtained. Each sample size was repeated 10 times using a unique seed for the random number generator, "Uniform," provided by SAS (1982). The minimum sample size was determined to be the one in which the null hypothesis of no population effects was rejected at $\alpha = 0.05$ for all 10 repeated samples.

Method III

Odeh and Fox (1975) presented a methodology for determining the minimum sample size based upon the use of the test statistic of a null hypothesis for a parameter of a general linear model. The methodology requires that the researcher state the null hypothesis and fix the probability of committing a type I error. This probability is usually denoted as α. The alternative hypothesis also needs to be stated and the power function, denoted π, needs to be fixed. π is equal to $1-\beta$; where β is the probability of committing a type II error. A third quantity known as the noncentrality parameter, denoted Φ, also needs to be estimated from experimental results.

For purposes of this study, α was set equal to 0.05 and 0.01 and β was set equal to 0.01, 0.05, 0.1, and 0.2. For the test of no population differences, the third parameter, Φ, is equal to

$$\left[\frac{g \cdot r \cdot \ell \theta_p^2 \left(\frac{p-1}{p}\right)^{\frac{1}{2}}}{\sigma_e^2 + r\ell\sigma^2_{\text{lines/popn}}}\right] \qquad (2)$$

where all variables were estimated from the data supplied by Robertson (1980) (Table 1).

Evaluation of Estimates

The minimum sample size for each of the three methods was compared and a mid-range value, denoted g_m, was accepted as an estimate of the minimum number of lines. The effect of g_m on the sources of variability within populations was investigated by repeated sampling of g_m random lines per population. The sampling was repeated 10 times. An analysis of variance for the complete model, (1), was calculated for each repetition. Estimates from these 10 repeats were used to construct an LSD statistic for testing cytoplasmic effects in pairs of BC_2 isopopulations.

RESULTS

The estimated F statistic for the null hypothesis of no variability among populations calculated by method I is plotted against the number of lines per population in Figure 1. Also plotted are the values of the F statistic for $\alpha = 0.05$ and $\alpha = 0.01$. Most plant breeders would reject the null hypothesis for values of F_c which are equal to or above the plot of $F_{0.05}$. The minimum number of lines needed to arrive at such a conclusion is determined from the intersection of F_c and $F_{0.05}$; which for these data is nine lines per population.

Results from method II indicated that the minimum number of lines necessary to reject the null hypothesis ($\alpha = 0.05$) in all 10 sample lines was seven (Table 2). In method III, both α and β were fixed prior to determining the minimum sample size. The results of method III are given in Table 3. As might be expected, small values of α and β will produce large (conservative) estimates of the minimum sample size. For example, the minimum sample size needed to detect significant variability with $\alpha = 0.01$ among populations is 13 when the alternative hypothesis also is rejected ($\beta = 0.01$). It is apparent that method III provided the widest range of estimates of minimum sample size.

From the range of values provided by the three methods, 10 lines is a moderate estimate of minimum sample size. The impact of $g_m = 10$ on Robertson's inferences about sources of population variability are shown in Table 4. Of 10 analyses on random samples of 10 lines per population, six rejected the hypothesis of no variability among

Figure 1. Plots of F statistics vs. number of segregating lines per population from 20 BC_2 populations (Robertson, 1980). F_c are the values calculated under assumptions that a 20-line estimate of sums of squares for lines within populations is a maximum, that the estimate of variability among populations is independent of sample size and equal to the value obtained from Robertson's data. $F_{.01}$ and $F_{.05}$ are tabulated values (Vogler and Norton, 1957) for V_1 = 19 degrees of freedom and V_2 = (20 x number of segregating lines - 1) degrees of freedom

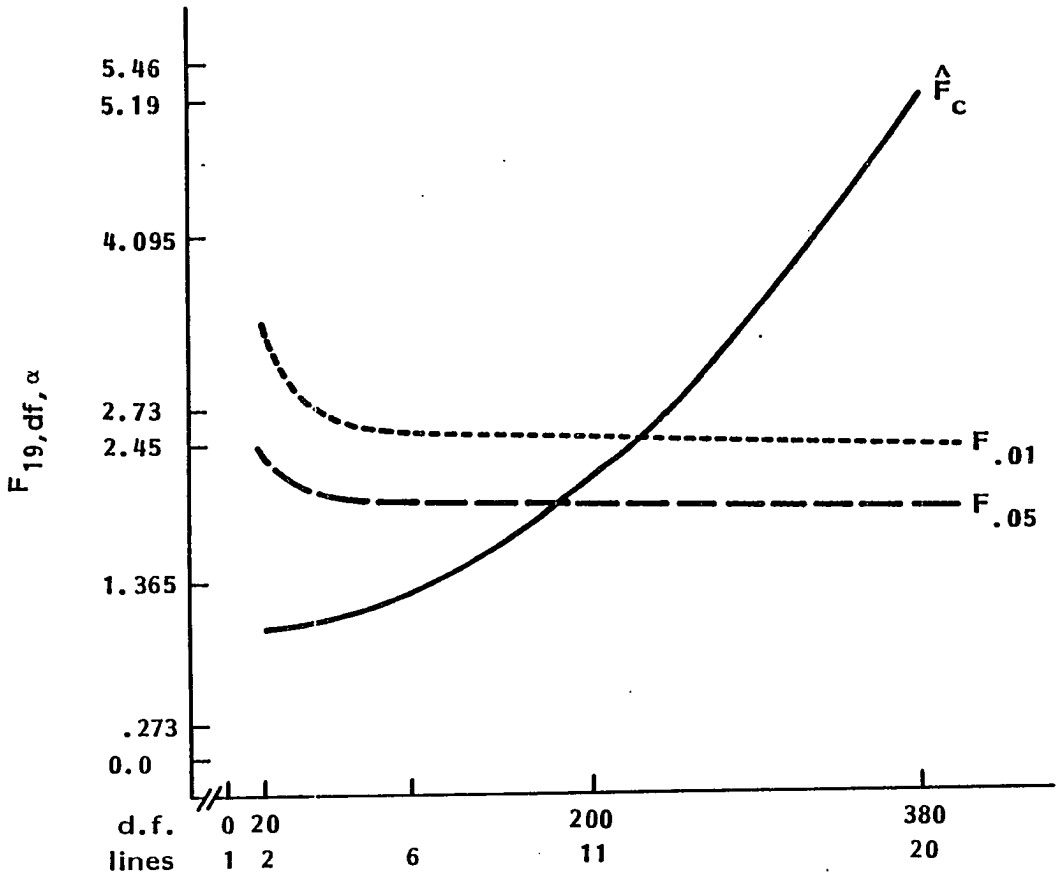
5.46
5.19
4.095
2.73
2.45
1.365
.273
0.0
$F_{19,df,\alpha}$
$\hat{F}_c$
$F_{.01}$
$F_{.05}$
d.f.
0 20
200
380
lines
1 2
6
11
20

Table 2. Influence of number of segregating lines, n, on number of significant mean squares (α = 0.05, 0.01) for the population source of variability from 10 repeated samplings of n BC_2F_2-derived lines per population

Sample size=n	Number of significant mean squares (α=0.05)	(α=0.01)
5	8	4
6	8	6
7	10	7
8	10	8
9	10	8
10	10	10

Table 3. Effect of fixing β and α on minimum sample size, for $\hat{\Phi} = \sqrt{(0.198)g}$. Φ was estimated by the function $(grI\Theta_p^2\sigma^2+rL\sigma_\ell^2/p)^{\frac{1}{2}}$; where g is number of lines, r = 3, L = 2, and the estimate of $\Theta_p^2/\sigma^2+rL\sigma_\ell^2/p$ was 0.66 for Robertson's 20 BC_2 isopopulations (1980)

β	Minimum sample size (α=0.05)	(α=0.01)
0.2	7	9
0.1	8	10
0.05	10	11
0.01	12	13

Table 4. Effect of sampling 10 random lines on estimates of mean squares for the cytoplasm, mating, and cytoplasm by mating interaction sources of population variability. MS_i refers to the estimated mean square for the i = 1, 2, 3, ... 10 sampling of 10 lines. MS_R refers to the mean square estimated from 20 lines (Robertson, 1980)

Source of variability	MS_1	MS_2	MS_3	MS_4	MS_5	MS_6	MS_7	MS_8	MS_9	MS_{10}	MS_R
Population	499.8	427.9	497.0	469.3	421.1	393.4	288.9	576.2	283.7	329.8	690.9
Cytoplasm	122.2	434.4	581.0	1200.0	603.5	855.1	782.5	391.0	779.2	22.4	1215.5
Mating	1221.4	481.2	712.2	496.7	318.9	272.0	260.0	697.9	242.8	478.1	751.4
Cytoplasm by Mating	373.4	373.8	273.0	360.8	503.0	463.6	262.9	475.0	269.6	215.9	572.1
Lines/ Populations	140.9	120.8	120.6	143.4	147.9	131.0	129.6	141.5	116.6	131.6	133.6

cytoplasms, 10 rejected the hypothesis of no variability among matings, and nine rejected the null hypothesis of no variability in cytoplasm by mating interactions at $\alpha = 0.05$. Thus, in only six of these random samples of $g_m = 10$ would the results of Robertson be repeated.

Recall that the interaction effects of cytoplasms by matings is the important feature in the analysis of these data; i.e., the decision to investigate cytoplasmic differences by mating was based upon rejection of the null hypothesis for the interaction effect, cytoplasm by mating. In nine of 10 repeats, such a decision would have been made.

The results of investigating cytoplasmic differences between isopopulations of each mating for 20 lines and each of 10 repeats of 10 lines are given in Table 5. None of the results obtained for the reduced sample size would have been identical to those of Robertson (1980) for all 10 matings. Only two of 10 repeats of 10 lines produced the same result as that obtained with 20 BC_2F_2 lines from matings of PI 324725 and Otee. Six of 10, three of 10, nine of 10, and eight of 10 repeats produced the same results as did 20 lines from the respective matings involving PI 317982 and Otee, PI 317757 and Otee, PI 324819 and CI 9170, and PI 317757 and CI 9170. Three of 10 repeats detected differences in cytoplasmic isopopulations for the mating of PI 217512 with CI 9170. The null hypothesis was not rejected for these populations when 20 BC_2F_2 lines were used.

Table 5. Effect of sampling 10 random lines on yield means (g/plot) for 10 repeated samplings of 20 BC_2F_2-derived lines from 10 A. sativa x A. sterilis matings in either A. sativa or A. sterilis cytoplasm. The estimated mean in A. sativa cytoplasm is referred to as (sa) and the estimated mean in A. sterilis cytoplasm is referred to as (st) for each sample number. S_i; i = 1, 2, 3, ... 10

A. sterilis parent	A. sativa parent	Sample number									
		S1		S2		S3		S4		S5	
		A_{st}	A_{sa}	A_{st}	A_{sa}	A_{st}	A_{sa}	A_{st}	A_{sa}	A_{st}	A_{sa}
324725	Otee	27.6	23.9	27.9	24.7	26.0	23.4	27.0	23.0	27.6	23.5
217512		28.1	27.1	25.7	28.1	26.6	27.1	24.0	28.3	24.8	27.2
317982		33.0	27.6	31.5	26.9	31.7	27.3	31.7	28.8	31.3	28.4
324819		27.4	26.7	30.4	28.8	28.3	28.1	30.8	27.4	27.1	27.0
317757		27.5	31.9	27.4	32.6	27.8	31.3	28.3	29.7	26.1	32.0
324725	CI 9170	26.5	24.1	32.6	24.8	27.2	23.9	26.0	24.3	26.1	24.0
217512		22.0	26.4	22.1	24.6	20.7	23.7	24.4	23.9	24.6	26.0
317982		29.3	27.5	28.9	28.5	29.8	28.3	29.2	27.8	29.1	27.2
324819		26.7	22.1	29.8	24.4	27.4	22.7	31.4	22.7	29.8	23.9
317757		31.6	27.1	30.0	26.0	30.2	25.9	28.6	25.6	32.3	24.6
lsd 0.05		4.25		3.93		3.93		4.28		4.35	
lsd 0.01		5.59		5.17		5.17		5.63		5.72	

Table 5. Continued

A. sterilis parent	A. sativa parent	Sample number									
		S6		S7		S8		S9		S10	
		A_{st}	A_{sa}	A_{st}	A_{sa}	A_{st}	A_{sa}	A_{st}	A_{sa}	A_{st}	A_{sa}
324725	Otee	26.8	23.6	27.9	23.2	25.0	25.0	28.2	24.4	26.1	24.5
217512		26.6	28.6	28.8	26.3	27.6	27.9	27.1	25.8	25.3	28.7
317982		30.3	26.0	31.3	26.6	32.8	28.4	31.5	29.2	29.3	29.3
324819		27.2	28.0	27.0	29.7	30.9	28.6	27.5	29.0	27.8	29.4
317757		26.2	29.7	26.5	29.0	26.1	29.7	27.6	29.8	28.2	30.3
324725	CI 9170	28.9	25.8	27.2	24.7	26.0	25.6	26.8	25.0	26.0	24.8
217512		21.8	25.9	25.2	25.7	20.2	25.9	24.9	26.8	22.1	25.0
317982		29.9	27.3	29.8	28.5	29.4	28.7	28.2	27.6	29.7	27.1
324819		30.5	23.7	26.7	24.2	29.0	22.8	30.6	24.6	27.4	23.3
317757		31.8	25.1	30.8	25.1	32.9	26.0	31.1	25.1	29.6	26.4
		4.10		4.07		4.26		3.86		4.10	
		5.39		5.36		5.60		5.08		5.39	

DISCUSSION

Minimum sample sizes from methods I and II suggest that inferences about variability among populations should not change with as few as seven segregating lines. Notice that neither method I nor method II calculates the number of lines based upon the probability of error in accepting the alternative hypothesis, β. Beta can be obtained from the tables of Odeh and Fox (1975) for fixed levels of α, estimated values of Φ, and sample size. For example, the probability (β) of failing to detect variability when the null is rejected ($\alpha = 0.05$) with sample sizes of seven and nine would be 0.2 and less than 0.1, respectively (Table 3).

The method of Odeh and Fox (1975), referred to as method III, does consider the effect of β on calculating the minimum sample size. It is the only method of the three which considers all parameters that influence estimates of minimum sample size. The use of method III assumes that β can be determined. Unfortunately, most plant breeders have little experience with the significance of β. I arbitrarily chose values of α and β that gave a moderate estimate of sample size to illustrate the effect of this criterion on the other results obtained by Robertson (1980).

With a sample size of 10 lines per population, I detected significant mean squares for the cytoplasm by mating source of variability in only nine of 10 samples. Thus, there is a positive, albeit small, probability that the reduced sample size of 10 would not produce the same results that Robertson (1980) produced with 20 lines per population.

As noted, none of our investigations of specific cytoplasm by mating interactions with g_m = 10 produced the same results as Robertson (1980). However, the reduced sample size consistently identified two isopopulations that he identified as superior.

From the results, it is apparent that the criterion used to decide minimum sample size is critical. If the goal of reducing the sample size was to repeat the analysis of variance on sources of population variability, then the estimate of cytoplasm variability, not population variability, should have been used. If it were important to repeat the results of comparisons of isopopulations within matings, then the pair of isopopulations with the smallest significant lsd statistic should have been used to reduce sample size.

Our original intent was to determine the minimum sample size needed to detect significant variability among cytoplasmic isopopulations in the BC_2 generation created by Robertson (1980). The results will be used in future studies on the introgression of A. sterilis nuclear and cytoplasmic genes. From model (1), it is obvious that the inference base consists of the BC_2 cytoplasmic isopopulations. Because matings produced by Robertson (1980) were a small selected sample of all possible A. sativa and A. sterilis matings, the value of g_m = 10 should be viewed as merely a "ball park" figure to be applied to BC_2 cytoplasmic isopopulations from other A. sterilis by A. sativa matings.

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SECTION IV. EXPRESSION OF NUCLEAR-CYTOPLASMIC INTERACTIONS AND HETEROSIS IN QUANTITATIVE TRAITS OF OATS (*Avena* spp.)

ABSTRACT

Nuclear and cytoplasmic genes from 10 diverse Avena sterilis L. accessions were introgressed into four Corn Belt oat (A. sativa L.) varieties. Grain yield, straw yield, harvest index, heading date, height, unit straw weight, and vegetative growth index were evaluated in the BC_2 generation of 76 cytoplasmic isopopulations. Relative to the recurrent parent 'Tippecanoe,' all seven traits improved with introgression of A. sterilis germplasm, whereas all seven traits were inferior in BC_2 populations of 'Ogle.' BC_2 populations of the other two oat varieties, 'CI 9170' and 'CI 9268' gave mixed responses relative to the recurrent parents.

The phenomena of nuclear-cytoplasmic interaction effects and nuclear-cytoplasmic heterosis also were investigated. Where significant differences were expressed between cytoplasmic isopopulations, those with A. sterilis cytoplasm were usually superior; although no trait exhibited consistent cytoplasmic effects across matings. Thus, all seven traits were influenced by significant nuclear-cytoplasmic interactions. Nuclear-cytoplasmic heterosis for grain yield was observed in only two of 38 isopopulations with A. sterilis cytoplasm; both were from matings involving Tippecanoe. Nuclear-cytoplasmic heterosis among the remaining traits was observed in four to eight of the 38 isopopulations with A. sterilis cytoplasm.

INTRODUCTION

Cytoplasmic effects in plants are involved in the expression of male sterility (Stephens and Holland, 1954; Duvick, 1965), heading date (Barikar and Balaich, 1977; Kinoshita et al., 1979), plant height, biomass, grain yield (Tsunewaki, 1980) and seed viability (Rao and Fleming, 1978; Yamada et al., 1980). Through the use of cytoplasmic substitution lines of wheat (Triticum spp. and Aegilops spp.), Kihara (1980) identified "nuclear-cytoplasmic" hybrids that exhibited nuclear-cytoplasmic heterosis for grain yield. A phenomenon akin to nuclear-cytoplasmic heterosis was reported by Robertson and Frey (1984) in a BC_2 cytoplasmic isopopulation of oats (Avena sativa L.).

Although differences among cytoplasmic substitution lines often are attributed to differences in cytoplasms, the cause is more likely an interaction of nuclear and cytoplasmic factors (Hermeson, 1968). Data from Robertson and Frey (1984) support this hypothesis; none of the seven traits they studied showed a consistent cytoplasmic effect across matings and backcross generations.

Robertson and Frey (1984) were the first to investigate the nature of nuclear-cytoplasmic interactions from the introgression of A. sterilis L. nuclear and cytoplasmic genes into cultivated oats. They studied reciprocal matings of two A. sativa cultivars and five A. sterilis accessions. This represented a small sample of both Cornbelt oat varieties and A. sterilis accessions, so the extent of nuclear-cytoplasmic heterosis among matings of A. sativa and A. sterilis is still unknown.

This study was conducted to investigate the extent of nuclear-cytoplasmic interaction effects and nuclear-cytoplasmic heterosis from a larger matrix of interspecific matings of Cornbelt oat varieties and accessions of A. sterilis.

MATERIALS AND METHODS

Materials

The four A. sativa cultivars used as recurrent parents were developed from breeding programs in three different states (Table 1). 'CI 9170' and 'CI 9268' are lines developed from the A. sterilis introgression program at Iowa State University; 'Ogle' was developed at the University of Illinois; and 'Tippecanoe' was developed at Purdue University.

To insure that A. sterilis accessions used as donor parents were diverse, I selected them based upon the results of cluster analyses of 15 agronomic traits measured on 457 plant introductions (PI) of A. sterilis (Rezai, 1977). Rezai (1977) grouped PIs by country and geographic region. The 10 A. sterilis accessions used and their origin are given in Table 1.

A. sativa and A. sterilis parents were mated with reciprocals according to a North Carolina Design II (Comstock and Robinson, 1948) to give 80 hybrids. The hybrids were backcrossed twice to their respective A. sativa parents (Figure 1) to give 40 pairs of cytoplasmic isopopulations. An isopopulation is represented by BC_2F_2-derived lines from one reciprocal cross of a mating. Isopopulations from the crosses Ogle x 'PI 412267' and 'PI 324780' x Tippecanoe were lost during development of the BC_2 generation so these two matings were eliminated from the study. All crosses were made in the greenhouse, and BC_2F_2 seeds were space-planted in the field. The bulk seed from each plant was used to establish a BC_2F_2-derived line in the F_3.

Table 1. Name and origin of four Avena sativa L. cultivars and 10 A. sterilis accessions used as parents in a North Carolina II mating scheme with reciprocals

Parental line	Origin
A. sativa	
CI 9170	Iowa
CI 9268	Iowa
Ogle	Illinois
Tippecanoe	Indiana
A. sterilis	
PI 318253	Northern Israel
PI 324740	Italy
PI 324716	Greece
PI 324780	Libya
PI 412267	Morocco
PI 309033	Southern Israel
PI 411560	Ethiopia
PI 412578	Turkey
PI 411816	Iran
PI 411976	Iraq

Beavis and Frey (1985a) found that a random sample of seven to 13 F_2-derived lines were needed to detect significant variability between cytoplasmic isopopulations in the BC_2 generation. I chose a conservative estimate and utilized 12 random BC_2F_2-derived lines to represent each isopopulation, except from two crosses: only 11 lines were available from 'PI 309033' x Tippecanoe and only eight lines were available from CI 9170 x 'PI 411976.' Thus, the experiment consisted of 38 matings of two BC_2 isopopulations each. Each isopopulation was represented by 12 F_2-derived lines except for the two cases noted. I also entered each A. sativa parent 10 times to serve as checks.

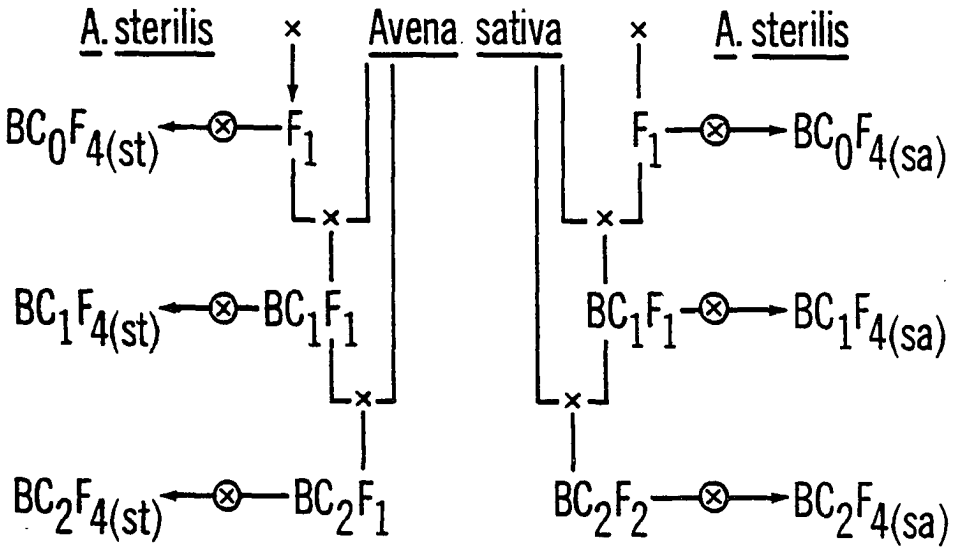

Figure 1. Generalized flow chart for development of reciprocal backcross cytoplasmic isopopulations

Field Evaluation

In 1984, the 931 F_2-derived lines in the F_3 and 40 parental checks were evaluated in a randomized complete block design with three replicates at each of three locations. The experiments were sown on April 19, April 20, and April 25 at the Agronomy Field Research Center near Ames, Iowa, the Clarion-Webster Research Center near Kanawha, Iowa, and the Northwest Research Center near Sutherland, Iowa, respectively. The soil at the Ames and Kanawha sites is a clay loam of the Clarion-Webster association and at Sutherland it is a silty clay loam of the Galva-Sac association. Soybeans occupied the experimental fields at all three sites in the year prior to the study. Fertilizer applications were 33.6 kg N and 51.5 kg P_2O_5 and K_2O at the Ames site; 33.6 kg N, 7 kg each of P_2O_5 and K_2O at the Kanawha site; and 16.8 kg N, 67.2 kg P_2O_5, and 7 kg K_2O at the Sutherland site. A plot was a hill sown with 30 seeds, and hills were spaced 30.5 cm apart in perpendicular directions. Two rows of border hills were sown around each replicate to provide competition for peripheral plots. A systemic fungicide, Bayleton, was applied to the plots at anthesis to eradicate crown rust (Puccinia coronata Cda. avena Frazier and Led.) and preclude other foliar diseases.

Seven traits were measured or computed on a plot basis. Heading date was recorded as the number of days from sowing until 50% of the panicles were fully emerged. Plant height was recorded as the distance (cm) from ground level to the tip of the tallest panicle. When mature, the plants in a plot were cut at ground level, dried, and weighed to obtain biological yield (kg ha^{-1}). Subsequently, the plants from a

plot were threshed and grain yield was recorded (kg ha^{-1}). Straw yield was calculated by subtracting grain from biological yield, and harvest index was calculated as the ratio of grain to biological yield and expressed as a percentage. Vegetative growth index was calculated as the ratio of straw yield to heading date, and unit straw weight was computed as the ratio of straw yield to height. Plant height and unit straw weight were determined from two replicates at Ames, heading date and vegetative growth rate were determined from three replicates at Ames, and other traits were measured on all nine replicates.

Statistical Analyses

Because two isopopulations had fewer than 12 lines, sums of squared deviations for lines within populations and lines within population by locations were initially computed by isopopulation using SAS (1982), and later the degrees of freedom and sum of squares for populations were combined to obtain mean squares for these sources of variability. Population marginal means (Searle et al., 1980) were used by SAS (1982) to calculate sums of squares for locations, replications within locations, populations, and populations x locations sources of variability. The residual sum of squares was computed by subtraction.

Comparisons of cytoplasms for individual matings and over matings were tested for significant differences by calculating a t statistic (Snedecor and Cochran, 1980):

$$t_c = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{2S^2/0}} \qquad (1)$$

where

$\bar{X}_1$ = mean value for population(s) in A. sativa cytoplasm;

$\bar{X}_2$ = mean value for population(s) in A. sterilis cytoplasm;

S^2 = mean square for lines within populations; and

0 = number of observations per mean.

Significance of t_c was determined by comparison with $t_{\alpha,df}$; where

$t_{\alpha,df}$ = tabular t-value at the α probability level; and

df = degrees of freedom associated with S^2.

The A. sativa parental checks were compared with: 1) BC_2F_2 isopopulations pooled across parents, matings, and cytoplasms; 2) cytoplasms pooled across parents and matings; 3) BC_2F_2 isopopulations pooled by parent across matings and cytoplasms; 4) cytoplasms pooled by parent across matings; and 5) BC_2F_2 cytoplasmic isopopulations. These comparisons were tested using a t statistic (Snedecor and Cochran, 1980):

$$t'_c = \frac{\bar{X}_1 - \bar{X}_1}{\sqrt{S_1^2/n_1 + S_2^2/n_2}} \qquad (2)$$

where

$\bar{X}_1$ = mean value for BC_2F_2 isopopulation(s);

$\bar{X}_2$ = mean value for parental check(s);

S_1^2 = mean square for lines within BC_2F_2 populations;

S_2^2 = appropriate mean square for testing variability among parental varieties (Carmer et al., 1969);

n_1 = number of observations in the mean of the BC_2F_2 isopopulation(s); and

n_2 = the number of observations in the mean of the parental variety(ies).

Significance of t'_c was determined by comparison with $t'_{\alpha,df'}$; where $t'_{\alpha,df'}$ = tabular t-value at the α probability level, and from Satherwaite (1946):

$$df' = \left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)^2 \Big/ \left(\frac{1}{df_1}\frac{S_1^2}{n_1} + \frac{1}{df_2}\frac{S_2^2}{n_2}\right) .$$

RESULTS

When pooled over all crosses, the BC_2F_2-derived lines had greater straw yield, later heading dates, and were taller than the averaged values from the parental checks (Table 2). When pooled over crosses, the A. sterilis cytoplasm gave significantly higher grain yield and harvest index, earlier heading date, and shorter plant height than did A. sativa cytoplasm (Table 3). Relative to recurrent parents (Table 2), the BC_2F_2-derived lines with A. sterilis cytoplasm (Table 3) were later, taller, and produced more straw; however, they produced less grain than the A. sativa parents.

Means of A. sativa parents and their respective BC_2F_2 progenies pooled over matings for seven traits are given in Table 4. Mean grain yields of BC_2 isopopulations from CI 9170 and Ogle were significantly lower than their respective recurrent parents, but the reverse was observed for Tippecanoe and its BC_2 isopopulations. For three cases, CI 9170, CI 9268, and Tippecanoe, the BC_2 populations had greater straw yield than their respective recurrent parents. BC_2 isopopulations from Ogle produced less straw, were taller, and had lower unit straw weight than the recurrent parent. Vegetative growth rate was improved by introgression of A. sterilis genes into CI 9268 and Tippecanoe. All traits had significantly larger values for the BC_2 populations of Tippecanoe than for the recurrent parent.

Means of BC_2 isopopulations with A. sativa and A. sterilis cytoplasms pooled by recurrent parent over crosses for seven traits are given in Table 5. Grain yield and harvest index were greater in

Table 2. Means of A. sativa (recurrent) parents and BC_2F_2-derived lines for grain yield, straw yield, harvest index, heading date, plant height, unit straw weight, and vegetative growth index

Trait	Populations	
	Parents	BC_2F_2-derived lines
Grain yield (kg ha^{-1})	3473	3424
Straw yield (kg ha^{-1})	3932	4091**
Harvest index (%)	46.82	45.64
Heading date (days)	66.14	66.51*
Plant height (cm)	97.27	100.58**
Unit straw weight (g cm^{-2})	0.465	0.469
Vegetative growth index (kg ha^{-1} day^{-1})	71.70	73.21

**,* Significance at the 1% and 5% probability levels, respectively, for the mean of the comparison.

Table 3. Means for grain yield, straw yield harvest index, heading date, plant height, unit straw weight, and vegetative growth index over all matings for F_2-derived lines with A. sativa and A. sterilis cytoplasms

Trait	Number of replicates	Cytoplasm	
		A. sativa	A. sterilis
Grain yield (kg ha^{-1})	9	3395	3452**
Straw yield (kg ha^{-1})	9	4079	4103
Harvest index (%)	9	45.53	45.75*
Heading date (days)	3	66.6*	66.48
Plant height (cm)	2	100.89**	100.18
Unit straw weight (g cm^{-1})	2	0.466	0.471
Vegetative growth index (kg ha^{01} day^{-1})	3	73.00	73.42

**,* Significance at the 1% and 5% probability levels, respectively, for the larger mean of the comparison.

Table 4. Means for A. sativa parents and their F_2-derived lines pooled over matings and cytoplasms for seven traits

Trait	CI 9170		CI 9268		Ogle		Tippecanoe	
	A. sativa parent	BC_2 isopopulation	A. sativa parent	BC_2 isopopulation	A. sativa parent	BC_2 isopopulation	A. sativa parent	BC_2 isopopulation
Grain yield (kg ha^{-1})	3430**	3129	3554	3599	4270**	3667	2686	3298**
Straw yield (kg ha^{-1})	3313	3471*	4091	4458**	4635**	4263	3570	4174**
Harvest index (%)	49.67**	47.46	46.31**	44.63	48.33**	46.23	43.00	44.22*
Heading date (days)	62.83	63.88**	68.9	69.27	67.03	67.52	65.7	66.24*
Plant height (cm)	96.7	98.83**	98.45	101.209**	96.8	100.27**	97.15	100.93**
Unit straw weight (g cm^{-1})	0.401	0.417	0.465	0.490	0.578**	0.481	0.415	0.487**
Vegetative growth index (kg ha^{-1} day^{-1})	65.36	66.22	70.41	76.22*	85.89	79.07	64.82	76.43**

**,* Indicate that the means for an A. sativa parent and its B_2F_2-derived lines differ at the 1% and 5% probability levels, respectively.

Table 5. Means of seven traits pooled by parent over matings for BC_2F_2 isopopulations with A. sativa and A. sterilis cytoplasms pooled by parent over matings

Trait	Cytoplasm	Parents			
		CI 9170	CI 9268	Ogle	Tippecanoe
Grain yield (kg ha^{-1})	A. sativa	3217*	3629*	3608	3225
	A. sterilis	3148	3569	3757**	3360**
Straw yield (kg ha^{-1})	A. sativa	3441	4453	4275	4145
	A. sterilis	3499	4462	4301	4210*
Harvest index (%)	A. sativa	48.40*	44.94*	45.82	43.90
	A. sterilis	47.52	44.32	46.56**	44.39*
Heading date (days)	A. sativa	63.81	68.64*	67.74	66.24
	A. sterilis	63.95	68.19	67.52	66.27
Plant height (cm)	A. sativa	98.88	102.58*	101.09**	100.98
	A. sterilis	98.79	101.59	99.92	100.90
Unit straw weight (g cm^{-1})	A. sativa	0.407	0.493	0.480	0.484
	A. sterilis	0.427**	0.488	0.488	0.490
Vegetative growth index (kg ha^{-1} day^{-1})	A. sativa	65.25	76.54	73.96	75.79
	A. sterilis	67.08**	75.90	74.71	76.86

**,* Significance at the 1% and 5% probability levels, respectively.

A. sativa cytoplasm from CI 9170 and CI 9268 matings, whereas the reverse was observed in matings of Ogle and Tippecanoe. There was no mean cytoplasmic effect on heading date except with CI 9268. A. sterilis cytoplasms had higher average values for unit straw weight and vegetative growth index for matings of CI 9170.

Despite these general trends, significant nuclear-cytoplasmic interactions occurred for all traits. The numbers of matings exhibiting significant differences between cytoplasmic isopopulations are summarized by recurrent parent for each trait in Table 6. A consistent cytoplasmic effect was exhibited for heading date when the A. sterilis parent, 'PI 411560,' was involved in the mating. This cytoplasm consistently produced earlier heading dates in all matings.

Of the matings that exhibited significant differences between cytoplasmic isopopulations, the numbers with superior performance in A. sterilis cytoplasm are given in Table 7. (Superior is defined for all traits except heading date as having the larger numeric value.) In matings involving CI 9170, there were 22 traits-mating combinations where the means of cytoplasmic isopopulations were significantly different and 17 of these favored the A. sterilis cytoplasm. In CI 9268 matings, 30 trait-mating combinations showed significant differences between cytoplasmic populations, and 14 were superior in A. sterilis cytoplasm. In Ogle and Tippecanoe matings, 32 and 30 trait-matings, respectively, showed significant differences between isopopulations, and 20 and 19 of these were superior in A. sterilis cytoplasm.

Table 6. Numbers of matings that exhibit significant differences between A. sativa and A. sterilis isopopulations summarized by recurrent parent

Trait	Recurrent parent			
	CI 9170	CI 9268	Ogle	Tippecanoe
Grain yield	2	1	3	3
Straw yield	2	3	4	4
Harvest index	3	4	3	2
Heading date	6	5	7	7
Height	4	7	4	5
Unit straw weight	2	5	6	4
Vegetative growth index	3	5	4	5

Table 7. Number of matings that exhibited significant superior performance in A. sterilis cytoplasm summarized by recurrent parent

Trait	Recurrent parent			
	CI 9170	CI 9268	Ogle	Tippecanoe
Grain yield	2	0	3	2
Straw yield	2	2	3	2
Harvest index	2	1	3	2
Heading date	3	4	4	5
Height	3	3	1	3
Unit straw weight	2	2	4	2
Vegetative growth index	3	3	2	3

Numbers of isopopulations with A. sterilis cytoplasm that exhibited nuclear-cytoplasmic heterosis summarized by trait and recurrent parent are given in Table 8. Only two isopopulations, both from Tippecanoe matings, expressed nuclear-cytoplasmic heterosis for both grain yield and harvest index. This phenomenon occurred for straw yield, unit straw weight, and vegetative growth index in isopopulations of some matings with every recurrent parent except Ogle. It was for only two trait-mating combinations when Ogle was a parent, whereas A. sterilis isopopulations from Tippecanoe matings exhibited nuclear-cytoplasmic heterosis for all traits.

Table 8. Numbers of isopopulations with A. sterilis cytoplasm that exhibited significant nuclear-cytoplasmic heterosis relative to the A. sativa recurrent parent

Trait	Recurrent parent			
	CI 9170	CI 9268	Ogle	Tippecanoe
Grain yield	0	0	0	2
Straw yield	3	3	0	2
Harvest index	0	0	0	4
Heading date	0	5	1	2
Height	1	3	1	3
Unit straw weight	1	2	0	2
Vegetative growth index	1	3	0	3

DISCUSSION

Robertson (1980) found that expression of nuclear-cytoplasmic interactions for all traits occurred most frequently in the BC_0 and BC_1 of interspecific oat matings. However, the greatest frequency of nuclear-cytoplasmic heterosis was observed in the BC_2. Lawrence (1974) showed that the BC_2 to BC_4 were optimal generations for selecting good lines after introgression of nuclear genes from A. sterilis. Beavis and Frey (1985b) reasoned that a few A. sterilis nuclear genes could interact with the A. sterilis cytoplasm to produce large nuclear-cytoplasmic interaction effects, and that these few genes could be lost easily in a backcrossing program. Therefore, to minimize the chance of losing favorable nuclear-cytoplasmic interaction effects and to use the optimum number of backcrosses for obtaining desirable lines (Lawrence, 1974), I investigated nuclear-cytoplasmic interaction effects and nuclear-cytoplasmic heterosis in the BC_2 generation.

Because Robertson and Frey (1984) investigated cytoplasmic isopopulations from only a few parental combinations, the extent of nuclear-cytoplasmic interactions and nuclear-cytoplasmic heterosis among matings of A. sativa and A. sterilis was unassessed. In this study, the A. sterilis parents were sampled from diverse sources, and the sampling represents a single stage cluster procedure (Cochran, 1977) of the A. sterilis world germplasm collection. A. sterilis accessions used by Robertson (1980) were not classified by Rezai (1977), although their geographic origins are known. The four A. sativa parents I used

were selected to represent Cornbelt oat varieties developed in three different plant breeding programs. Thus, information from this study should assess how Corn Belt oat varieties of different origin respond to introgression of A. sterilis nuclear and cytoplasmic genes.

My results were similar to those of Robertson and Frey (1984) in that significant cytoplasm x mating interactions were observed for all traits. No A. sterilis cytoplasm gave consistently superior or inferior performance relative to its A. sativa cytoplasm counterpart for any trait except heading date; i.e., isopopulations with cytoplasm from PI 411560 consistently exhibited earlier heading dates than their counterparts in A. sativa cytoplasm. Except for these four matings, there was no evidence for a cytoplasmic effect per se. That is, all cytoplasmic effects were expressed through an interaction with nuclear sources. Thus, the assertion by Hermesen (1968) that all traits are affected by nuclear-cytoplasmic interactions is supported by my results. Recent work in molecular genetics also supports this hypothesis, in that most enzymes encoded by cytoplasmic DNA contain polypeptides encoded by nuclear DNA (Borst et al., 1983).

No A. sterilis accession that I used was common with any used by Robertson and Frey (1984), but both studies included CI 9170 as a recurrent parent. Robertson and Frey (1984) found that the average grain yield of isopopulations in A. sterilis cytoplasm was 8% greater than the mean for A. sativa isopopulations. This result was caused primarily by two of the five isopopulations, which exceeded their A. sativa counterparts by 17% each. My study, like Robertson and Frey (1984), also

found two CI 9170 isopopulations with A. sterilis cytoplasm that exceeded their A. sativa counterparts, one by 26% and one by 11%. However, when averaged over all matings, the A. sterilis cytoplasm had 2% less grain yield than the A. sativa cytoplasms; although no A. sterilis isopopulation yielded significantly less than its A. sativa counterpart.

Relative to CI 9170, on average, the BC_2 isopopulations in A. sterilis cytoplasm created by Robertson (1980) yielded 6.6% less and those I created yielded 8.2% less than the recurrent parent. Robertson (1980) found one A. sterilis isopopulation that yielded more than CI 9170 by 8%, and I also found one A. sterilis isopopulation that yielded more than CI 9170, however, by only 1.5%. This isopopulation was from PI 412267 x CI 9170 and it yielded more than its counterpart in A. sativa cytoplasm by 6.7%. The mean grain yield of this isopopulation was not considered to be due to nuclear-cytoplasmic heterosis because its superiorities to both recurrent parent and cytoplasmic reciprocal were not statistically significant. The results from my study and those of Robertson and Frey (1984) show nuclear-cytoplasmic heterosis for grain yield from matings of CI 9170 and A. sterilis accessions is rarely observed in the BC_2 generation.

Grain yield in A. sterilis cytoplasm from the cross PI 412267 x CI 9268 exceeded the recurrent parent by 8%. This is the same A. sterilis accession that was responsible for superior yield when mated to CI 9170. However, the isopopulation from PI 412267 x CI 9268 did not exceed its counterpart in A. sativa cytoplasm; therefore, the response was due to beneficial A. sterilis nuclear genes. Because nuclear genes from

PI 412267 provided beneficial effects for grain yield when combined with both Iowa cultivars, this accession may be a good source for genetic improvement in the A. sterilis introgression program.

Mean grain yield of isopopulations in A. sterilis cytoplasm from Ogle matings was greater than that of their A. sativa counterparts by 4.1%, but in only three were the A. sterilis isopopulations significantly better. No isopopulation in either cytoplasm yielded more than Ogle; on the contrary, grain yield was significantly less than Ogle for most isopopulations. Apparently, the effects of A. sterilis genes for grain yield were detrimental when introgressed into Ogle; although the detrimental effect of A. sterilis genes in A. sterilis cytoplasms tended to be less than in A. sativa cytoplasms. This illustrates a situation where detrimental effects of A. sterilis nuclear genes are either offset by beneficial interactions with A. sterilis cytoplasms or intensified by detrimental interactions with A. sativa cytoplasms.

Isopopulations in A. sterilis cytoplasm developed from Tippecanoe, on average, yielded 4.1% more than counterpart isopopulations in A. sativa cytoplasm. Most isopopulations developed from Tippecanoe matings yielded more than the recurrent parent, and the two with A. sterilis cytoplasm from PI 324740 and PI 318253 exhibited nuclear-cytoplasmic heterosis for grain yield. These two isopopulations exceeded the recurrent parent by 29% and 38%, respectively. The isopopulation from the cross PI 318253 x Tippecanoe produced a grain yield that was greater than either CI 9170 or CI 9168, both of which exceeded Tippecanoe. These

two heterotic populations from Tippecanoe matings illustrate a situation where grain yield was enhanced by interactions of A. sterilis nuclear genes with A. sterilis cytoplasmic genes.

In general, the grain yield response to introgression of A. sterilis genes into the germplasm of Corn Belt oat varieties produced variable results. Introgression caused decreased yield in Ogle and CI 9170 matings, increased yield in Tippecanoe matings, and had no effect for matings with CI 9268. The general response was modified by A. sterilis cytoplasm and in some specific crosses the response was improved significantly by A. sterilis cytoplasmic genes. Robertson (1980) found one BC_2 cytoplasmic isopopulation in 10 that exhibited nuclear-cytoplasmic heterosis for grain yield; I found two in 38.

For the traits straw yield, harvest index, heading date, plant height, unit straw weight, and vegetative growth index, Robertson (1980) found one or two of 10 cytoplasmic isopopulations that exhibited nuclear cytoplasmic heterosis. For these same traits, I found nuclear-cytoplasmic heterosis in four to eight of 38 cytoplasmic isopopulations. Thus, depending upon the trait, nuclear-cytoplasmic heterosis occurs in about 5 to 20% of cytoplasmic isopopulations in the BC_2 generation from matings of Corn Belt varieties with A. sterilis accessions.

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GENERAL CONCLUSIONS

Cytoplasmic inheritance of variegation in plants was reported by Correns, 1909, cited in Kirk and Tilney-Bassett 1967, and it represents an influence of the cytoplasm upon a plant trait. From a biological perspective, cytoplasmic effects are probably due to interactions of nuclear and cytoplasmic factors. For example, certain enzymes involved in plant photosynthesis and respiration are encoded by DNA located within both the cytoplasm and nucleus. The interaction of nuclear genes with cytoplasm has been studied primarily in the context of cytoplasmic male sterility systems. Kihara (1980) described a nuclear-cytoplasmic interaction he called nuclear-cytoplasmic heterosis and suggested that nuclear-cytoplasmic heterosis in cytoplasmic substitution lines could give significant improvement in grain yield of self-pollinated crops. A phenomenon akin to nuclear-cytoplasmic heterosis was observed by Robertson (1980) in a cytoplasmic isopopulation of oats (Avena sativa L.) with A. sterilis L. cytoplasm. Robertson and Frey (1984) discussed the possible genetics responsible for the phenomenon but did not genetically model their results.

I attempted to fit the genetic models of Mather and Jinks (1982) to generation means for grain yield from four matings that Robertson (1980) created, and the results are reported in Section I. For the matings that fit the data, none had a significant second order interaction effect of A. sterilis by A. sativa nuclear genes by cytoplasms, but all included significant nuclear-cytoplasmic interaction

effects. No model was able to describe generation means from one mating that exhibited maximum grain yield in an intermediate (BC_1) backcross isopopulation with A. sterilis cytoplasm. No model could predict grain yield in advanced backcross generations. The inability of the models to describe grain yield in all matings was attributed to the use of assumptions that simplified algebraic manipulations, but were erroneous for the inheritance involved. It was suggested that an alternative model based upon biological evidence might better describe and predict backcross generation means from reciprocal matings. I also indicated that models dependent upon data-based estimates of parameters probably would not consistently predict the result of further backcrossing where nuclear-cytoplasmic interactions occurred.

Lewontin (1977) suggested that findings in molecular genetics could not be ignored by quantitative geneticists and that the discoveries from emerging biotechnologies would either be compatible with previously established theory or new theories would have to be developed. In Section II, a theoretical model, such as that suggested in Section I, was developed. I assumed that the cytoplasmic genome was maternally inherited and consisted of two homogenous populations of homozygous polyploid organelles. My assumptions about the population structure of nuclear genes were the usual ones (i.e., infinite, random mating, and an arbitrary number of alleles at each locus). The algebra of Kempthorne (1957) was sufficiently robust to derive the model and theoretical variance/covariance components. Thus, for this model a previously established algebra permitted the incorporation of findings about the cytoplasmic genome from molecular genetics.

My model appeared to be a special case of one developed by Cockerham and Weir (1977). Their model gives unconfounded estimates of extranuclear variance components from reciprocal mating designs if the extranuclear effects are inherited from both parents. My model assumes cytoplasmic effects are inherited solely through the maternal parent. The result of reciprocal mating designs will estimate cytoplasmic variability that is confounded with a variance component for additive nuclear by cytoplasmic interactions. Thus, a reciprocal mating design cannot be used to test whether or not cytoplasmic variability exists independently of nuclear-cytoplasmic variability; although from an applied perspective, estimates of heritability will be unaffected.

Section III is a description of methods that can be used to determine the minimum number of random lines needed to represent cytoplasmic isopopulations. Also discussed is the impact of choosing a minimum sample size on different analyses. Two methods, referred to as the intuitive and Monte Carlo techniques, do not utilize the minimal sufficient theoretical parameters needed to determine sample size. The third method is a procedure developed by Odeh and Fox (1975) based upon theoretical considerations. If the estimated minimum sample size obtained by the intuitive or Monte Carlo techniques had been used, then the results from the third method indicate that there is a high probability (β=0.2) that the significant variability among isopopulations reported by Robertson and Frey (1984) would not have been detected.

The experimental component of this study, reported in Section IV, includes the effects of introgressing nuclear and cytoplasmic genes from

A. sterilis into Cornbelt oat varieties (A. sativa) and the phenomena of nuclear-cytoplasmic interactions and heterosis exhibited in the BC_2 generation from these matings. In general, A. sterilis genes improved the performance of all traits in 'Tippecanoe,' had little influence on 'CI 9268,' were slightly detrimental to 'CI 9170,' and very detrimental to the performance of all traits in 'Ogle.' These general trends were modified by cytoplasms and where differences in cytoplasms existed the A. sterilis cytoplasm generally provided superiority. No significant cytoplasmic effects per se were observed and all traits exhibited significant nuclear-cytoplasmic interactions. Nuclear-cytoplasmic heterosis was detected for all traits in 5 to 20% of the isopopulations with A. sterilis cytoplasm.

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