AN ABSTRACT OF THE THESIS OF

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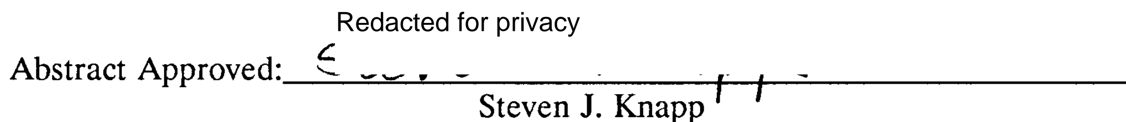
Title: Cytology, Inbreeding Depression and Heterosis of *Cuphea lanceolata* Ait.



Cuphea lanceolata Ait. is an undomesticated capric acid rich species of the genus *Cuphea*. Genomic affinity has not been described among different forms of this species. The breeding behavior of this species is still unknown. We investigated the meiotic pairing of F_1s between two extreme forms of *C. lanceolata*, *C. lanceolata f. silenoides* (LNS-43) and *C. lanceolata f. typica* (LNT-78), to assess genomic affinity. For describing the breeding behavior, we studied the effects of inbreeding within a *C. lanceolata* synthetic population and heterosis of single-cross F_1s. F_1s of LNS-43 x LNT-78, and LNT-78 x LNS-43 were found completely male sterile. Only 1.2 and 1.0% viable pollens were found in F_1s of LNS-43 x LNT-78, and LNT-78 x LNS-43, respectively. No meiotic irregularities were observed within LNS-43 and LNT-78 *per se*. Many meiotic irregularities were found among LNS-43 x LNT-78, and LNT-78 x LNT-43 F_1 PMCs. Univalents were observed within nearly every PMC of F_1s. Mean chromosome associations were 3.7 bivalents and 4.6 univalents per cell for LNS-43 x LNT-78 and 2.4 bivalents and 7.3 univalents per cell for LNT-78 x

LNS-43. An unequal chromosomal distribution of 7:5 at anaphase I was observed in 78.6 and 75% of cells of LNS-43 x LNT-78 and LNT-78 x LNS-43, respectively. The estimates of arm pairing frequency, genomic affinity index, and arm affinity index were 0.4, 0.6 and 0.5, respectively, for LNS-43 x LNT-78; and 0.3, 0.4 and 0.4, respectively for LNT-78 x LNS-43. We did not find any evidence for the presence of pairing control gene. Chromosome pairing data of F_1s and their parents suggest that genomes of *C. lanceolata f. silenoides* and *C. lanceolata f. typica* diverged remarkably. The mean plant height, biomass plot^{-1}, seed yield plot^{-1}, seed oil percentage, and 500-seed weight decreased as inbreeding increased. Biomass plot^{-1} and seed yield plot^{-1} were more severely affected than other traits. Linear decrease was predominant among lines and traits with the increase of inbreeding. Significant mid-parental heterosis was observed in every single-cross F_1s for every trait. Mean mid-parental heterosis for plant height, biomass plot^{-1}, seed yield plot^{-1}, seed oil percentage, and 500-seed weight were 70.7, 103.7, 216.8, 76.0, and 57.3%, respectively. Non-additivity was found important for the expression of heterosis as indicated by significant mean heterosis for every trait. LN-96 was found to be a good general combiner. The line effect of LN-98 was greatest for every trait measured. This evidence indicates that considerable potential exists for exploiting the hybrid vigor of *C. lanceolata*.

Cytology, Inbreeding Depression, and Heterosis of *Cuphea lanceolata* Ait.

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In dedication to:

Samirah, my daughter
Nargis, my wife
Rizia, my mother
and
the departed soul of my father

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CYTOLOGY, INBREEDING DEPRESSION, AND HETEROSIS OF *CUPHEA LANCEOLATA* AIT.

INTRODUCTION

Cuphea is the largest genus of the family Lythraceae comprising approximately 260 species. Several species of this genus have been domesticated as a new, temperate, annual oil seed crop because the seed oil of *Cuphea* is an excellent natural source of medium chain fatty acids (MCFA) such as caprylic ($C_{8:0}$), capric ($C_{10:0}$), lauric ($C_{12:0}$) and myristic ($C_{14:0}$) acids (Graham et al., 1981; Wolf et al., 1983; Graham, 1988). Lauric acid has excellent physical characteristics, and is used widely in manufacturing soap and detergents (Young, 1983; Thompson, 1984; Arkcoll, 1988). It is also used in cooking fats and shortening (Young, 1983; Arkcoll, 1988). Caprylic and capric acids have medicinal value. They are used to treat gallstones, epilepsy, and disorders of lipid metabolism, and as a source of rapidly absorbed, high-energy fuel for critically ill patients (Bach and Babayan, 1982; Babayan, 1987). MCFAs may also be used as nontoxic, antimicrobial preservatives in food and other perishables (Kabara, 1984).

Lauric acid is exclusively derived from tropical coconut (*Cocos nucifera* L.) and palm (*Elaeis guinensis* Jacq.) kernel oils, and from petrochemicals (Arkcoll, 1988). Tropical storms, drought, disease, and pests cause coconut oil yield to fluctuate (Young, 1983; Arkcoll, 1988). A temperate natural source of

MCFAs is thus desirable to reduce the dependency on coconut and palm oils. *Cuphea* was found as potential alternate source of MCFAs in a USDA germplasm screening program (Earle et al., 1960). Although several annual species have the growing potential in temperate climate, seed shattering impedes their domestication (Hirsinger and Knowles, 1984; Knapp, 1990).

Cuphea lanceolata is one of the capric acid rich members of the genus *Cuphea* and is native to the central plateau of Mexico. The typical capric acid percentage of seed oil of *C. lanceolata* is 83.5 (Graham, 1988). *C. lanceolata per se* is not being domesticated because it requires insect pollination, and effective pollinators have not been found. It has, however, become an important source of germplasm for breeding *C. viscosissima*, because the *C. lanceolata* germplasm pool has many useful variations, and crosses between certain populations of *C. lanceolata* and *C. viscosissima* are fertile and cytogenetically normal (Ronis et al., 1990; Brandt, 1991). Although *C. lanceolata* and *C. viscosissima* are closely related and have $2n=2x=12$ chromosomes, their floral morphology and mating systems are quite different. *C. lanceolata* is strongly allogamous and autosterile, whereas *C. viscosissima* is strongly autogamous and autofertile. Both species are self-compatible, however.

The phylogeny of the genus *Cuphea* is still speculative, and taxonomic changes have been suggested at all levels (Graham, 1988). Detailed cytological investigations have just begun and most of them are limited to the species

level. Two extremes in variation were observed in early collections of *C. lanceolata* (Graham, 1963). Graham (1988) lists the forms as *C. lanceolata f. silenoides*, and *C. lanceolata f. typica* which were incorrectly cited as varieties by Koehne. She noticed a continuum of floral characteristics between these forms. However, no taxonomic justification exists to classify these forms as different species because they share other morphological characteristics in addition to the floral morphology.

Interspecific hybridization has been proposed as a means for exploiting between species diversity in *Cuphea*; however, most of the interspecific F_1 hybrids obtained thus far are sterile (Lorey and Röbbelen, 1984; Gathman and Ray, 1987; Ray et al., 1988; 1989), with the exception of interspecific hybrids between *C. viscosissima* and *C. lanceolata* f. *silenoides* (Ronis, 1990; Brandt, 1991). Both *C. viscosissima* and *C. lanceolata* f. *silenoides* are undergoing domestication and have a significant role in the commercialization of *Cuphea.*

An understanding of genomic affinity of the parents is important for genetic manipulation. Kimber (1984) reviewed methods of genomic identification, such as comparison of nucleotide sequences, chromosome pairing, morphologies, chromosome banding, karyotype, DNA hybridization, DNA amount, protein electrophoresis, immunological techniques, and restriction enzyme analysis that could be useful for assessing phylogenic relationships. Although chromosome pairing does not represent a full comparison of total DNA or entire nucleotide sequences, it provides a

comparison along the entire chromosome and a great amount of DNA at a time and still is the most reliable method for assessing genomic affinity (Kimber et al., 1981; Kimber, 1983; Kimber, 1984). Chromosome pairing and hybrid fertility provide a direct measure of phylogenic relationships among taxa (Menzel and Martin, 1970; Jensen, 1989).

The number, distribution and position of chiasmata indicate the efficiency of synapsis and determine the extent of recombination in segregating populations (Jackson, 1984). Chiasma formation in the paired segments determines the type of configuration observed at diakinesis or metaphase I (Zadoo, 1989). Based on number and distribution of chiasmata, models have been developed to estimate expected configurations at metaphase I. Tests can be made for deviations from normal pairing in species and species hybrids, and for the presence of pairing control gene heterozygotes (Jackson, 1984; Jackson, 1989; Shang et al., 1989) using random and non-random models, respectively. In the non-random model, chiasmata distribution among bivalents is not normal, each bivalent forms at least one chiasma. In the random model, chiasmata are allocated randomly among homologous and homeologous chromosomes.

Self-fertilization in natural cross-pollinated species results in inbreeding depression. Recessive alleles that would remain undetected in heterozygotes are exposed as homozygotes in inbreeding. The exposed recessive alleles are then subject to natural and artificial selection, and a reduction in fitness of

inbred lines occur (Burton et al., 1978). Selection against deleterious genes within and among inbreds enhances population improvement. The trends of the inbreeding depression indicate the nature of gene action controlling the expression of traits. Linear decrease indicates additivity and non-linear trends indicate non-additivity.

Heterosis or hybrid vigor is the superiority of a hybrid over the mean value of its parents. The amount of heterosis exhibited by hybrids depends largely on the genetic divergence of the parental population from which the inbreds have been developed. Abundant heterosis indicates that the parental lines are more genetically diverse (Ghaderi et al., 1984; Mungoma and Pollok, 1988; Ordás, 1991). Heterosis will always be proportional to genetic diversity if heterosis is caused only by positive dominance effects (Eberhart and Gardner, 1966). Diallel-cross analysis provides a basis for preliminary information on heterotic patterns among crosses (Gardner and Eberhart, 1966; Eberhart and Gardner, 1966; Hallauer and Miranda, 1981). This mating design has also been used to gain an understanding of the nature of gene action (Hayman, 1954a; Hayman, 1954b; Hayman, 1957; Griffings, 1956; Gardner and Eberhart, 1966). As *C. lanceolata* is allogamous, we presume that it is subject to inbreeding depression and may exhibit significant heterosis. There is, however, no information on the breeding behavior and nature of gene action for *C. lanceolata*.

Our objectives were to assess phylogenic relationships between two races

of *C. lanceolata, C. lanceotata f. silenoides* and *C. lanceolata f. typica*, and to describe breeding behavior of *C. lanceolata*. The first chapter reports the meiotic pairing analysis of F_1s between *C. lanceolata f. silenoides* and *C. lanceolata f. typica*. Sterility found in the F_1 generation motivated us to investigate meiosis. The second paper reports our estimates of the effects of inbreeding within a *C. lanceolata* synthetic population and heterosis of single-cross F_1s. Nature of gene action involved for heterotic response was also described.

Chapter I

MEIOTIC ANALYSIS OF F_1S BETWEEN *CUPHEA LANCEOLATA F. SILENOIDES* AND *CUPHEA LANCEOLATA F. TYPICA*

ABSTRACT

Cuphea lanceolata f. silenoides and *C. lanceolata f. typica* are the two extreme forms of *C. lanceolata*, an undomesticated capric acid rich species of the genus *Cuphea*. Genomic affinity has not yet been described between these forms. We investigated meiotic pairing of F_1s between *C. lanceolata f. silenoides* (LNS-43) and *C. lanceolata f. typica* (LNT-78). F_1s of LNS-43 x LNT-78 and LNT-78 x LNS-43 were completely male sterile; no seed was produced upon extensive selfing. Only 1.2 and 1.0% viable pollen were found in LNS-43 x LNT-78 and LNT-78 x LNS-43, respectively. No meiotic irregularities were observed within LNS-43 and LNT-78. Meiotic irregularities were found among LNS-43 x LNT-78 and LNT-78 x LNS-43 F_1 PMCs. The common irregularities were formation of univalents, reduced chiasmata frequency, unequal distribution of chromosomes, and laggardness. Mean chromosome associations were 3.7 bivalents and 4.6 univalents per cell for LNS-43 x LNT-78 and 2.4 bivalents and 7.3 univalents per cell for LNT-78 x LNS-43. Mean numbers of chiasmata per cell were 4.8 and 3.3 in LNS-43 x LNT-78 and LNT-78 x LNS-43, respectively. An unequal chromosomal distribution of 7:5 at anaphase I was observed in 78.6 and 75% PMCs of LNS-43 x LNT-78 and LNT-78 x LNS-43, respectively. The estimates of arm pairing frequency, genomic affinity index, and arm affinity index were 0.4, 0.6, and 0.5, respectively, for LNS-43 x LNT-78 and 0.3, 0.4, and 0.4, respectively, for LNT-78 x LNS-43. We did not

find any evidence for the presence of pairing control gene. The chromosome pairing data of F_1 hybrids and their parents suggest that genomes of *C. lanceolata f. silenoides* and *C. lanceolata f. typica* diverged markedly.

INTRODUCTION

Several species of *Cuphea* are being domesticated as new, temperate, annual oil seed crops because the seed oil of *Cuphea* contains remarkably high amounts of medium-chain fatty acids (MCFA) such as caprylic ($C_{8:0}$), capric ($C_{10:0}$), lauric ($C_{12:0}$), and myristic ($C_{14:0}$) acids (Graham et al., 1981; Wolf et al., 1983; Graham, 1988). Lauric acid is mainly used in manufacturing soap and surfactants (Arkcoll, 1988). Caprylic and capric acids are used to treat gallstones, epilepsy, and disorders of lipid metabolism, and as a source of rapidly absorbed, high-energy fuel for critically ill patients (Bach and Babayan, 1982; Babayan, 1987). The primary commercial sources of MCFAs are coconut (*Cocos nucifera* L.) and palm kernel (*Elaeis guinensis* Jacq.) oils (Arkcoll, 1988). Tropical storms, drought, diseases, and pests cause coconut oil yield to fluctuate (Young, 1983; Arkcoll, 1988). Thus, a temperate oil seed crop is desirable as an alternate source of MCFAs to stabilize the global market and to ensure constant supply of MCFAs. *Cuphea* was identified as a possible alternative source of MCFA oil seeds in a USDA germplasm screening program (Earle et al., 1960).

Cuphea lanceolata has become an important source of germplasm for breeding C. viscosissima because *C. lanceolata* germplasm has much useful variation, and crosses between certain populations of *C. lanceolata* and *C. viscosissima* are fertile and cytogenetically normal (Ronis et al., 1990; Brandt,

1991). *C. lanceolata* is native to the central plateau of Mexico and has a chromosome number of 2n=2x=12 (Graham, 1988). Its seed oil is very rich in capric acid, which comprises 83.5% of the seed oil content (Graham, 1988).

The phylogeny of *Cuphea* is still speculative and taxonomic changes have been suggested at all levels (Graham, 1988). Detailed cytological investigations have just begun, and most of them are limited to the species level. Two extremes in variation were observed in early collections of *C. lanceolata* (Graham, 1963). Graham (1988) lists these forms as *C. lanceolata f. silenoides*, and *C. lanceolata f. typica*, which were incorrectly cited as varieties by Kohene. She noticed a continuum of floral characteristics between these forms. However, no taxonomic justification exists to classify these forms as different species because they share other morphological characteristics in addition to the floral morphology.

Interspecific hybridization has been proposed as a means for exploiting between species diversity in *Cuphea*; however, most of the interspecific F_1 hybrids made thus far are sterile (Lorey and Röbbelen, 1984; Gathman and Ray, 1987; Ray et al., 1988; 1989), and most of them have been made between species which lack characteristics essential for commercialization, e.g., seed yield. A major exception is interspecific hybrids between *C. viscosissima* and *C. lanceolata* f. *silenoides* (Ronis et al., 1990; Brandt, 1991). Both of these species are undergoing domestication and have a significant role in the commercialization of *Cuphea*.

The options for sterile F_1 interspecific hybrid production are to backcross to either parent species or to make fertile amphidiploids by colchicine doubling; however, neither of these options are particularly useful. Only limited backcrossing has been done. Even under fairly good circumstances, extensive recombination between genomes may not take place, and target transgressive segregates may not be recovered. Amphidiploids are not especially useful since no intergenome recombination takes place and the amphidiploid is fully homozygous.

An understanding of genomic affinity of the parents is important for genetic manipulation. Kimber (1984) reviewed methods for assessing phylogenic relationships such as comparison of nucleotide sequences, chromosome pairing, morphologies, chromosome banding, karyotype, DNA hybridization, DNA amount, protein electrophoresis, immunological techniques and restriction enzyme analysis. Although chromosome pairing does not represent a full comparison of total DNA or entire nucleotide sequences, it provides a comparison along the entire chromosome and the greatest amount of DNA at a time, and still is the most reliable method for assessing genomic affinity (Kimber et al., 1981; Kimber, 1983; Kimber, 1984). Chromosome pairing and hybrid fertility provide direct measures of phylogenic relationships among taxa (Menzel and Martin, 1970; Jensen, 1989).

The number, distribution, and position of chiasmata indicate the efficiency of synapsis and determine the extent of recombination in segregating

populations (Jackson, 1984). Chiasma formation in the paired segments of the chromosomes determines the type of configuration observed at diakinesis or metaphase I (Zadoo, 1989). Based on number and distribution of chiasmata, models have been developed to estimate expected configurations at metaphase I. In non-random model, chiasmata distribution among bivalents is not random in a normal diploid because normal synapsis is expected, each bivalent forms at least one chiasma, and univalents are rare and unpredictable (Jackson, 1982; Jackson and Hauber, 1982; Jackson, 1984; Shang et al., 1989). According to the random model, chiasmata are allocated randomly among homologous and homeologous chromosomes. Tests can be made for deviations from normal pairing in species and species hybrids, and for the presence of pairing control gene heterozygotes (Jackson, 1984; Jackson, 1989; Shang et al., 1989). The phenotypic expression of such genes is the production of univalents.

We investigated meiosis within *C. lanceolata f. silenoides x C. lanceolata f. typica* F_1s to estimate their genomic affinities. F_1s between these races are completely male sterile (unpublished data). No cytological data are available to explain the genomic relationship of these populations and to explain the causes of sterility of F_1s. In this paper we report the genomic affinity of the two forms, *C. lanceolata f. silenoides* and *C. lanceolata f. typica*, through the meiotic pairing analysis of the F_1s and present a probable cytological explanation for the observed male sterility.

MATERIALS AND METHODS

F_1s were made between *C. lanceolata f. silenoides* (LNS-43) and *C. lanceolata f. typica* (LNT-78) by crossing randomly chosen plants from each population. The origins of these populations have been described (Knapp and Tagliani, 1990). Crosses were made by emasculating female plants, which were pollinated one to two days later. F_1 seed was harvested from 20 plants within each forms; however, only 14 LNS-43 x LNT-78 and 6 LNT-78 x LNS-43 plants were grown to maturity for cytological analysis. F_1 plants from each interform cross were grown in the greenhouse from May, 1989 to June, 1991.

Because *C. lanceolata* requires an insect vector for pollination, we manually self-pollinated every F_1 plant within the LNS-43 x LNT-78 and LNT-78 x LNS-43 populations to check for fertility and seed set. In addition, fresh pollen grains were harvested from every plant and bulked. These were stained with I_2KI and examined under a light microscope (Stanley and Linsken, 1974; McCommon and Honma, 1983; Jensen, 1989). The percentage of stainable pollen grains was estimated by counting ~1000 grains from each bulk sample. Dark staining pollen was presumed to be viable.

Flower buds were harvested from 20, 16, 14, and six plants of LNS-43, LNT-78, LNS-43 x LNT-78, and LNT-78 x LNS-43, respectively. They were immediately fixed in modified Carnoy's solution (4 parts chloroform : 3 parts ethanol : 1 part acetic acid) for 24 hours and then transferred to fresh Carnoy's

solution for an additional 24 hours (Gathman and Ray, 1987). The fixed flower buds were removed from the Carnoy's solution, transferred to 70% ethanol, and stored at 4°C. Pollen mother cells (PMCs) were prepared by using a standard acetocarmine squash method. Squashed PMCs were observed with phase contrast at 400x magnification. Two buds from each plant were examined. Four metaphase I/diakinesis cells were observed for each sample. The total number of bivalents, open and closed bivalents, and number of univalents were recorded for each cell. Four anaphase I cells were observed for each sample to estimate chromosome distributions.

Several parameters were used to estimate meiotic affinities of the *C. lanceolata* forms. The probability of association (PA) was estimated by using, (number of closed bivalents)/[number of closed bivalents + number of open bivalents + 2(univalents)] x 1/(Probability of chiasmata formation)^2 (Driscoll et al., 1979). The probability of chiasma formation between a pair of chromosome arms (PCF) was estimated by using 2(number of closed bivalents)/[2(number of closed bivalents) + number of open bivalents] (Driscoll et al., 1979). The number of chiasmata (xta) was estimated by counting the number of open and closed bivalents and assuming that open and closed bivalents have one and two chiasmata, respectively (Driscoll et al., 1979). Arm pairing frequency (APF) was estimated by dividing the number of chiasmata per cell by the available chromosome arms (Kimber et al., 1981). The genome affinity index (GAI) was estimated by dividing the number of

bivalents per cell by the base chromosome number (Menzel and Martin, 1970). The arm affinity index (AAI) was estimated by dividing the APF for the hybrid by the mean APF of the parents (Gathman and Ray, 1987).

We estimated the expected meiotic configuration frequencies for non-random (Jackson, 1984; 1989) and random (Shang et al., 1989) chiasma distribution models. The non-random model assumes normal pairing and a non-random distribution of chiasmata (Jackson, 1984; 1989). For the non-random model, the expected number of closed bivalents (bivalents with two chiasmata) is (xta - n)m, and the expected number of open bivalents (bivalents with one chiasmata) is n - (xta - n)m where n is the number of bivalents under normal pairing, xta is the number of chiasmata per cell, and m is the number of cells.

The random model assumes random pairing and a random distribution of chiasmata (Shang et al., 1989). For the random model, the expected number of closed bivalents is p^2mn, the expected number of open bivalents is $2p(1 - p)mn$, and the expected number of univalents is $2(1 - p)^2mn$ where p (chiasmata coefficient) is the mean number of chiasmata per bivalent divided by 2, the maximum number of chiasmata. The goodness-of-fit of chiasmata data obtained from the populations to the non-random and random models was tested using χ^2-statistics.

RESULTS

Fertilization rates were much greater in the LNS-43 x LNT-78 F_1 cross than in the reciprocal (LNT-78 x LNS-43) F_1 cross. The latter yielded far less seed per manual pollination than the former. F_1 plants from the cross between *C. lanceolata* forms were completely sterile–no seed was produced upon extensive self-pollination of LNS-43 x LNT-78 and LNT-78 x LNS-43 F_1 plants. The percentage of viable LNS-43 x LNT-78 or LNT-78 x LNS-43 F_1 pollen was 1.2 and 1.0%, respectively (Figure 1). The percentage of viable pollen within LNS-43 and LNT-78 was 93.0 and 96.8%, respectively (Figure 1).

No meiotic irregularities were observed within LNS-43 and LNT-78 *per se*. Bivalents were observed at diakinesis within 100% of the PMCs sampled from these populations (Table 1 and Figure 2). Loosely synapsed open bivalents predominated in LNS-43 PMCs, whereas tightly synapsed closed bivalents predominated in LNT-78 PMCs (Table 1 and Figure 2).

Many meiotic irregularities were observed among LNS-43 x LNT-78 F_1 and LNT-78 x LNS-43 F_1 PMCs. Bivalents and univalents were observed at diakinesis in these populations (Table 1 and Figure 2). Univalents were observed within nearly every PMC (Table 1 and Figure 2). The number of bivalents within hybrids ranged from zero to six. Three bivalents were observed in 57.1 and 58.3% of the PMCs of LNS-43 x LNT-78 and LNT-78 x LNS-43, respectively. Open bivalents were much more frequent than closed

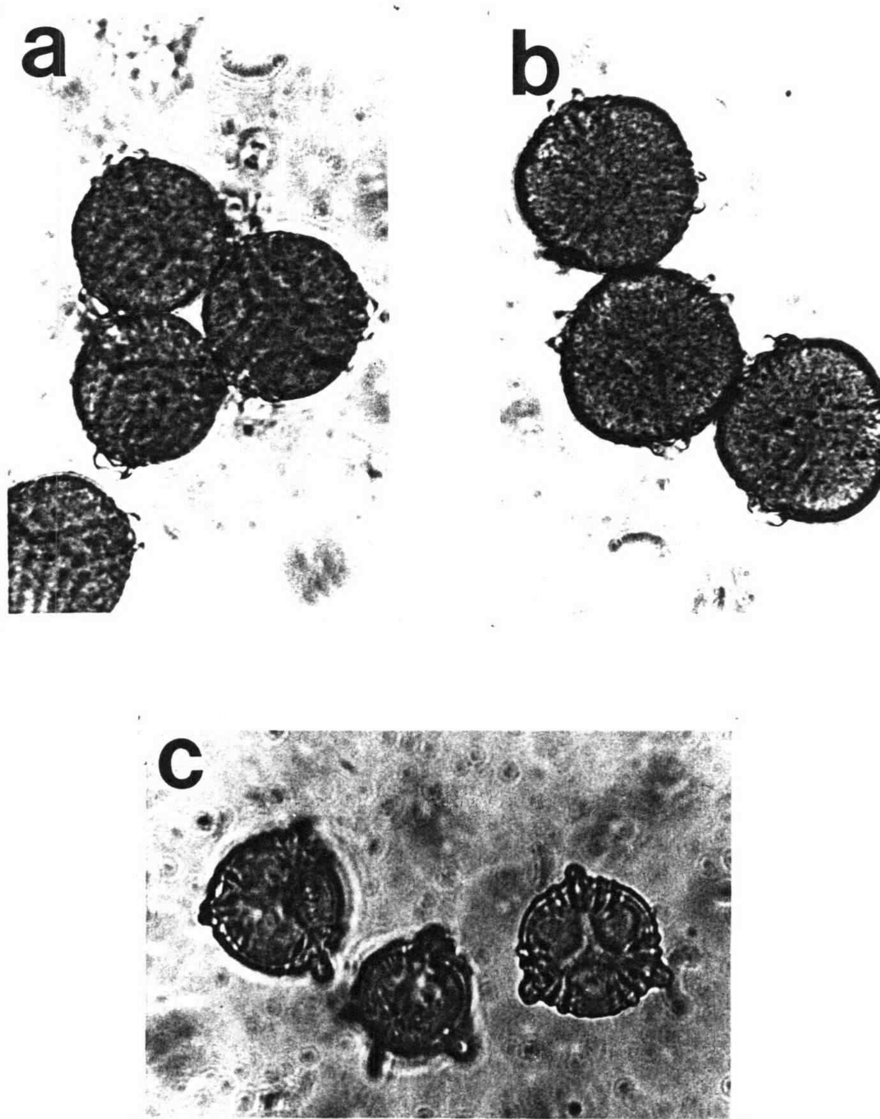

Figure 1. Staining of pollen in I_2KI. (a) *C. lanceolata f. silenoides*, (b) *C. lanceolata f. typica*, (c) *C. lanceolata f. silenoides x C. lanceolata f. typica.*

Table 1. Mean number of open and closed bivalents, and univalents at metaphase I/Diakinesis within PMCs of LNS-43, LNT-78, and their hybrids.

Population	Open Bivalents $Cell^{-1}$	Closed Bivalents $Cell^{-1}$	Univalents $Cell^{-1}$	Number of PMCs
LNS-43	3.75±0.43	2.25±0.43	0.00	160
LNT-78	1.88±0.49	4.13±0.49	0.00	128
LNS-43 x LNT-78	2.61±1.48	1.11±1.12	4.57±2.45	112
LNT-78 x LNS-43	1.42±0.96	0.92±0.77	7.33±1.91	48

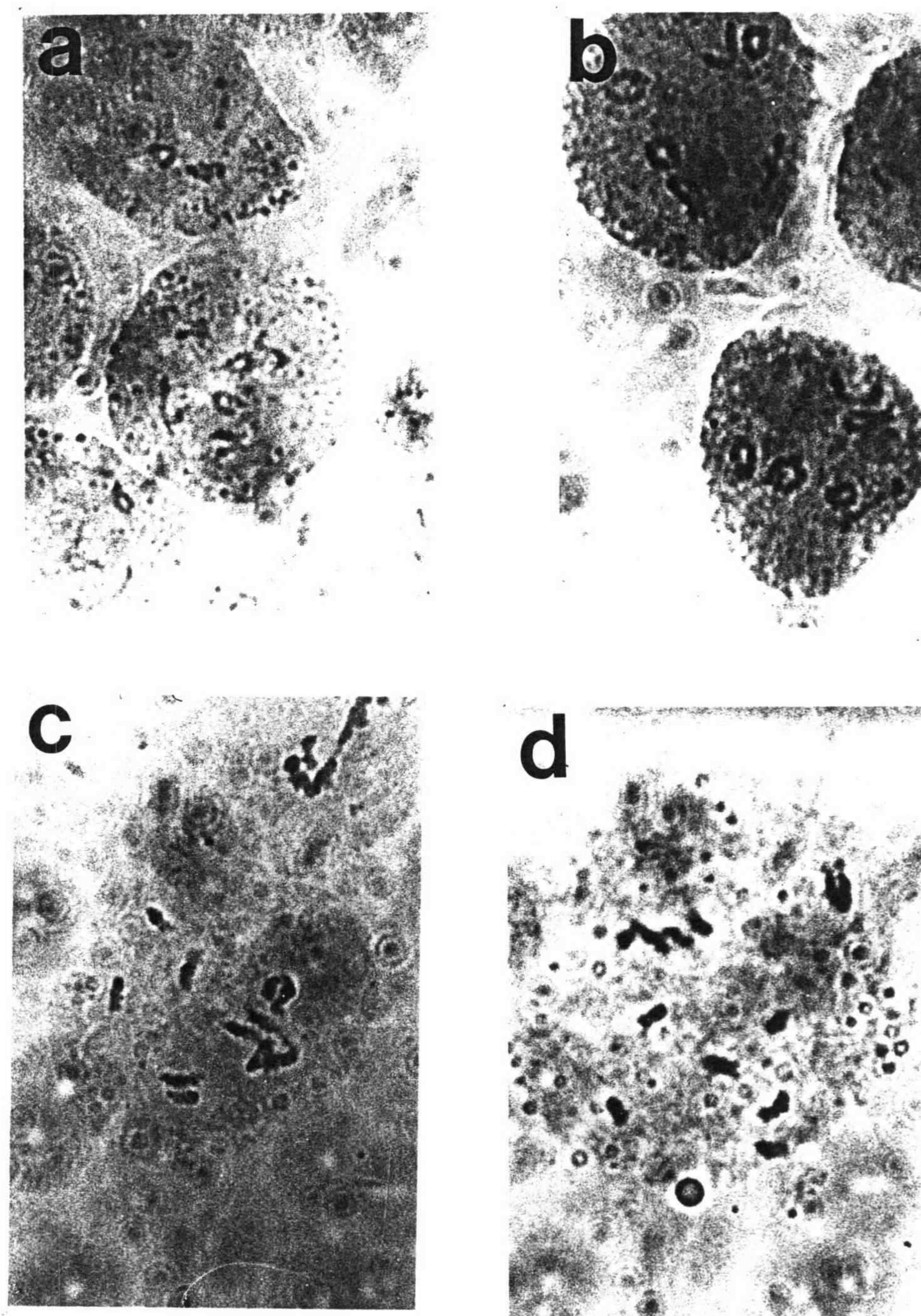

Figure 2. Diakinesis/Metaphase I. (a) *C. lanceolata f. silenoides*, 4 open and 2 closed bivalents; (b) *C. lanceolata f. typica*, 2 open and 4 closed bivalents; (c) *C. lanceotata f. silenoides x C. lanceolata f. typica*, 1 closed and 2 open bivalents, and 6 univalents; (d) *C. lanceolata f. typica x C. lanceolata f. silenoides*, 1 closed bivalent and six univalents.

bivalents (Table 1). Six univalents were frequently observed in the hybrids, but up to 12 univalents were observed in some PMCs.

Even though cytogenetic abnormalities were equally frequent in LNS-43 x LNT-78 and LNT-78 x LNS-43, there were differences in reciprocal crosses, and the severity of the abnormalities was greater in the LNT-78 x LNS-43 population. The bivalent distribution for LNS-43 x LNT-78 was much wider than for LNT-78 x LNS-43 (Figure 3). The number of bivalents for LNS-43 x LNT-78 ranged from 2 to 6, whereas for LNT-78 x LNS-43 it ranged from 0 to 3 (Figure 3). The univalent distributions for LNS-43 x LNT-78 and LNT-78 x LNS-43 were left- and right-skewed, respectively, and overlapped at 6 and 8 univalents (Figure 4). The number of univalents for LNS-43 x LNT-78 ranged from 0 to 8, whereas LNT-78 x LNS-43 ranged from 6 to 12 (Figure 4). The goodness-of-fit of the LNS-43 and LNT-78 data to the non-random pairing model was excellent, whereas it very poorly fit the random pairing model (Table 2). The fit of the hybrid data to the random pairing model was likewise very poor (Table 2). We did not test the fitting of the F_1s data to the non-random model because chiasmata coefficients of the hybrids were less than 0.5 that resulted in negative expected number of closed bivalents and χ^2-statistics. In hybrids, observed number of open bivalents were less than the expected; however, univalents and closed bivalents were more in number than expected. The distribution of open bivalents was wider in hybrids than in parents (Table 3).

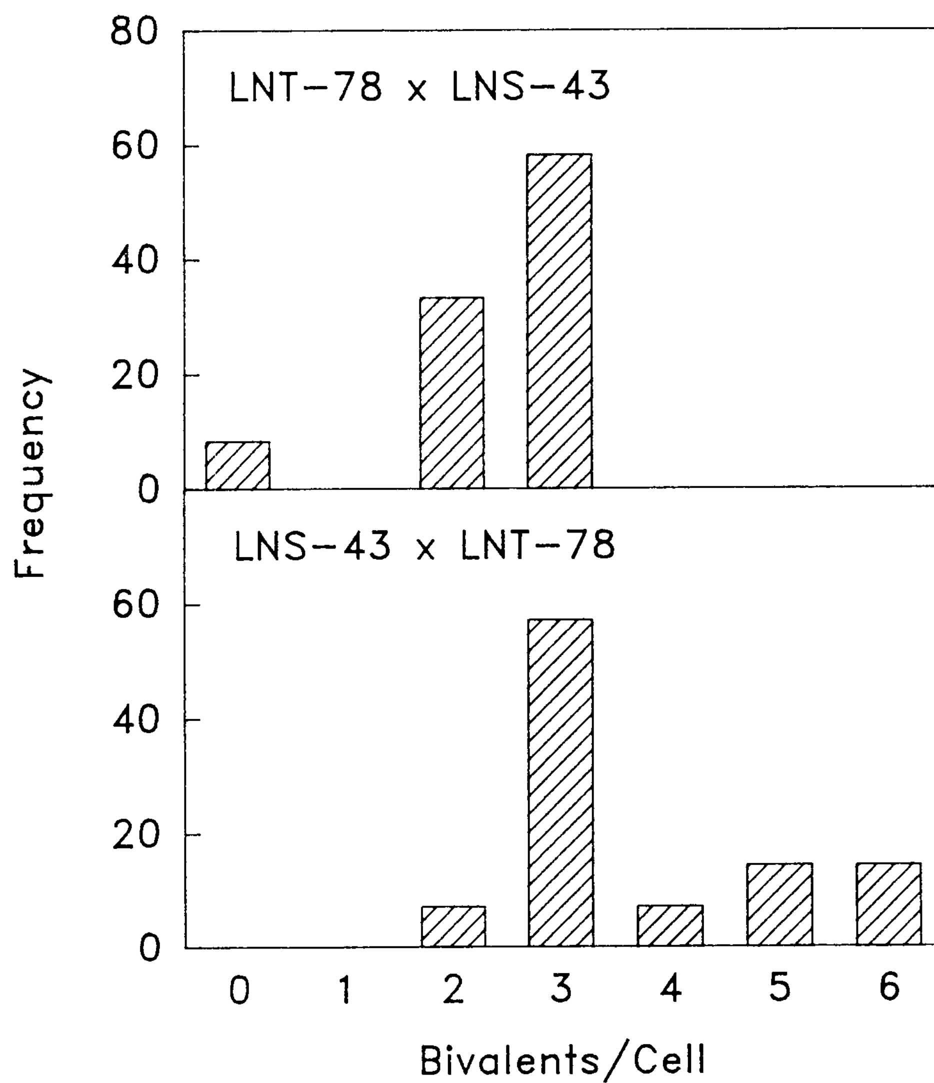

Figure 3. Frequency distribution of number of bivalents cell^{-1}

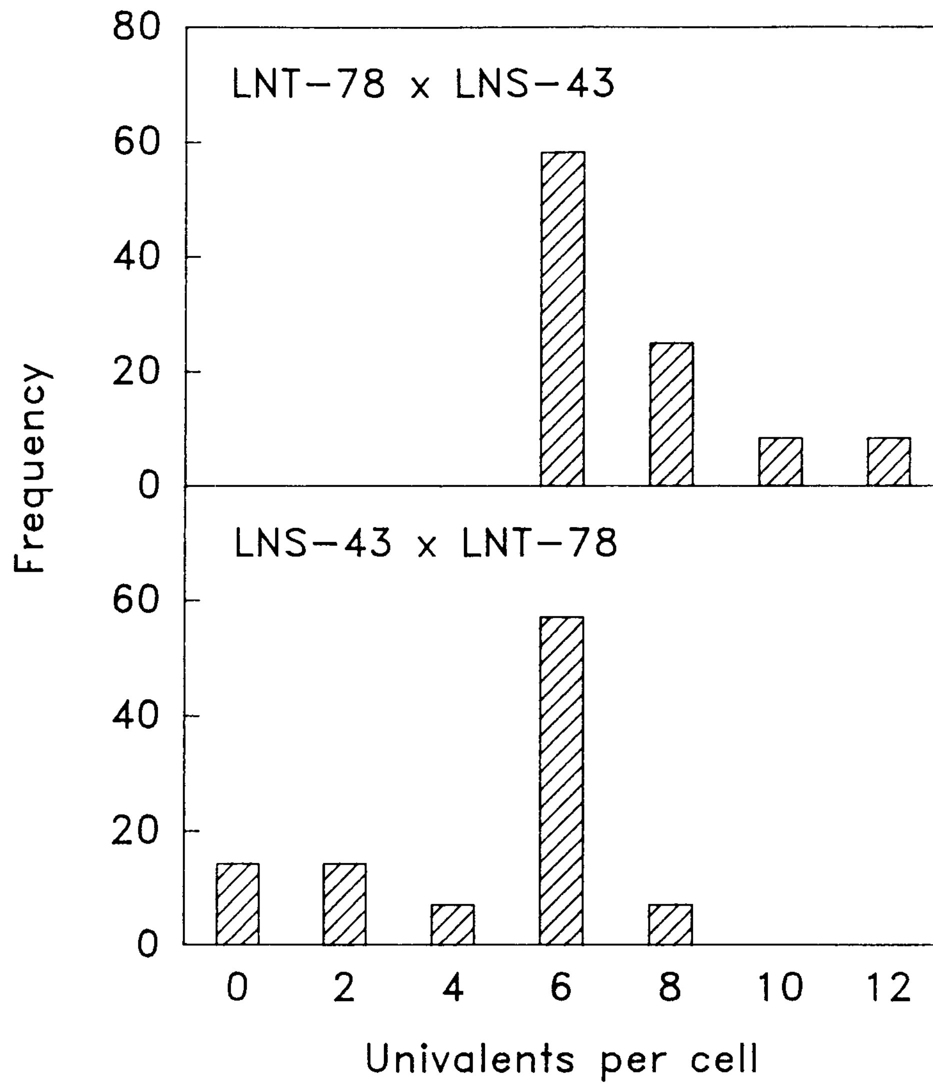

Figure 4. Frequency distribution of number of univalents $cell^{-1}$

Table 2. Observed and expected numbers of bivalents and univalents for the non-random and random chiasmata distribution models statistics for testing the goodness-of-fit of the expected numbers for these models to the observed numbers for LNS-43 and LNT-78 and their F1s.

Population	P[1]	Number[2]	Closed Bivalents	Open Bivalents	Univalents	χ^2	$Pr > \chi^2$
LNS-43	0.863	O	360.0	600.0	0.0		
		E(NR)	360.0	600.0	0.0	0.0	1.0
		E(R)	453.8	412.5	187.5	292.1	<0.001
LNT-78	0.844	O	528.0	240.0	0.0		
		E(NR)	528.6	239.4	0.0	0.0	0.964
		E(R)	527.3	202.1	37.3	45.1	<0.001

Table 2. Continued.

Population	P[1]	Number[2]	Closed Bivalents	Open Bivalents	Univalents	χ^2	$Pr>\chi^2$
LNS-43 x LNT-78	0.402	O	124.0	292.0	512.0		
		E(R)	108.4	323.0	481.1	7.2	0.027
LNT-78 x LNS-43	0.271	O	44	68.0	352.0		
		E(R)	21.12	113.7	306.3	50.1	<0.001

[1]p (chiasmata coefficient) is the mean number of chiasmata per bivalent divided by 2.

[2]O is the observed number, E(NR) is the expected number for the non-random model, and E(R) is the expected number for the random model.

Normal chromosome disjunction was observed at anaphase I within the LNS-43 and LNT-78 populations, whereas unequal disjunction and laggards were observed within every PMC from the hybrid populations (Table 4 and Figure 5). The most frequent anaphase I distribution was 7:5:0 (Pole 1 : Pole 2 : Laggards)–78.6 and 75.0% of the PMCs of LNS-43 x LNT-78 and LNT-78 x LNS-43, respectively, had this chromosome distribution (Table 4 and Figure 5). Besides unequal disjunction and laggardness, hybrid chromosomes often clumped at anaphase I (Figure 5).

The estimates of probability of associations and probability of chiasmata formation for the hybrid populations ranged from 0.27 to 0.38 and 0.14 to 0.47, and were significantly less than those for the parent populations (Table 5). Every measure of genome affinity was lower in the hybrids than in the parent populations (Table 5). The mean GAI estimates for the hybrids and the parents, for example, were 0.51 and 1.00, respectively (Table 5).

Table 3. Open bivalent frequencies within LNS-43 and LNT-78, and their hybrids.

	Number of open bivalents						
Population	0	1	2	3	4	5	6
LNS-43	0	0	0	40	120	0	0
LNT-78	0	24	96	8	0	0	0
LNS-43 x LNT-78	16	12	12	40	24	8	0
LNT-78 x LNS-43	12	8	24	4	0	0	0

Table 4. Anaphase I chromosome distribution for LNS-43 and LNT-78, and their F_1s.

Population	Pole1:Pole2:Laggard	Percentage
LNS-43	6:6:0	100.0
LNT-78	6:6:0	100.0
LNS-43 x LNT-78	7:5:0	78.6
	6:5:1	3.6
	6:4:2	3.6
	5:5:2	3.6
	4:4:4	3.6
	3:3:6	7.1
LNT-78 x LNS-43	7:5:0	75.0
	5:4:3	16.7
	4:3:5	8.3

Table 5. Estimates of the parameters of the meiotic affinity of LNS-43 and LNT-78, and their F_1s.

Population	PA[1]	PCF[2]	xta[3]	APF[4]	GAI[5]	AAI[6]
LNS-43	1.3	0.5	8.3	0.7	1.0	1.0
LNT-78	1.0	0.8	10.1	0.8	1.0	1.0
LNS-43 x LNT-78	0.3	0.4	4.8	0.4	0.6	0.5
LNT-78 x LNS-43	0.1	0.5	3.3	0.3	0.4	0.4

[1]PA is the probability of association (PA)

[2]PCF is the probability of chiasmata formation between a pair of chromosome arms

[3]xta is the number of chiasmata (xta)

[4]APF is the arm pairing frequency (APF)

[5]GAI is the genome affinity index (GAI)

[6]AAI is the arm affinity index (AAI)

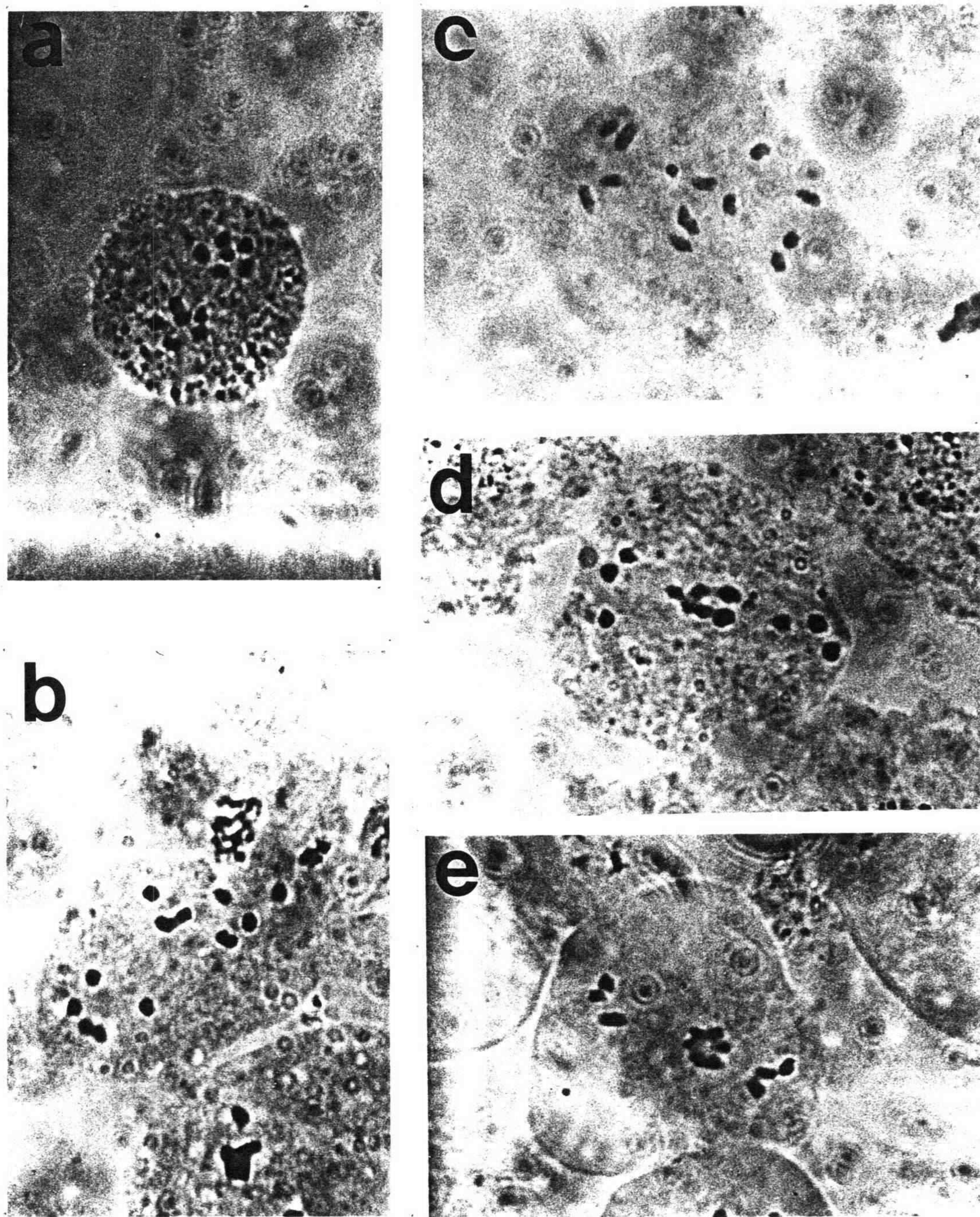

Figure 5. Chromosome distribution at anaphase I. (a) *C. lanceolata f. silenoides*, 6:6:0; (b) *C. lanceolata f. silenoides x C. lanceolata f. typica*, 7:5:0; (c) *C. lanceolata f. typica x C. lanceolata f. silenoides*, 4:3:5 laggards; (d) *C. lanceolata f. silenoides x C. lanceolata f. typica*, 3:3:6 laggards; (e) *C. lanceolata f. silenoides x C. lanceolata f. typica*, 3:3:6 clumped.

DISCUSSION

The *C. lanceolata* forms examined in this study were not taxonomically different enough to be recognized as separate species (Graham, 1988), but F_1 progeny between them were sterile, had shrunken and deformed anthers, and meiosis did not proceed normally within them. Although 1.0% of the pollen of the interspecific hybrid stained and seemed normal, no seed were produced upon self-pollination of the F_1. It could be that stainable pollen grains were not yet fully degraded in the samples assayed.

Meiosis was normal within *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typica per se*–six bivalents were observed at metaphase I and chromosome disjunction and migration was normal in 100% of the PMCs examined (Table 4). Open bivalents were more prevalent in *C. lanceolata* f. *silenoides*, whereas closed bivalents were more prevalent in *C. lanceolata* f. *typica* (Table 1). This might be a consequence of centromere location differences between *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typica* (Zadoo, 1989), but this has yet to be determined. The chromosomes of *C. lanceolata* f. *silenoides* might be submetacentric or subacrocentric, whereas the chromosomes of *C. lanceolata* f. *typica* might be metacentric. Because chiasmata form less frequently in the short arms of submetacentric and subacrocentric chromosomes, these chromosome types have a high probability of forming open bivalents at metaphase I (Zadoo, 1989); however, systematic interference across the

centromere can lead to a high frequency of open bivalents within all chromosome types (Lavania, 1986).

Meiosis was abnormal in 100% of the PMCs of the interform hybrids (Tables 1-5). Migration of the chromosomes to the equatorial plate and chromosome disjunction was not synchronized in the interform hybrids. This might be a function of differences in cell cycle durations. The cytogenetic abnormalities observed within the interform hybrids may be a consequence of asynapsis (chromosome pairing failures at prophase), desynapsis (premature separation of chromosome pairs), or incomplete homology between chromosomes which leads to pairing failures. The distributions of open bivalents within the forms and hybrids (Table 3) show that chiasmata failed to form in both arms of at least one of the chromosome pairs in the hybrid–univalents were generated in the hybrid at the expense of open bivalents.

The observed meiotic configurations of the interform hybrids did not fit the random chromosome pairing model (Shang et al., 1989); so evidence for a pairing control gene was weak. We presume that consistent and regular pairing took place between homeologous chromosomes in the interform hybrids, rather than random pairing between heterologous chromosomes. These conclusions may be overly simplistic because the random model excludes the formation of univalents as a consequence of asynapsis.

PA, PCF, and xta estimates for the hybrids were quite low (Table 5),

and are indicative of a lack of homology between the genomes of *C. lanceolata* f. *silenoides and C. lanceolata* f. *typica*. The high frequency of univalents we observed within the interform hybrids is explained by a lack of genome homology and decreased chiasmata formation. Chromosome morphology *per se* might play a role, since short-armed chromosomes sometimes separate prematurely and lead to univalents (Kostoff, 1940).

The genomes of *C. lanceolata* f. *silenoides* (LNS-43) and *C. lanceolata* f. *typica* (LNT-78) seem to have diverged quite markedly. APF, GAI, and AAI estimates for the interform hybrids were much lower than those of the parental forms (Table 5). The presence of 12 univalents within some PMCs and greatly decreased chiasmata frequencies are indicative of chromosome structural divergence between *C. lanceolata* f. *silenoides and C. lanceolata* f. *typica*, at least for the limited sample of germplasm we examined.

The scope of this study was limited by a lack of germplasm of both forms. Since we only had one population of each of the forms, and other populations have not yet been collected from the wild, it is not feasible to extrapolate our results to *C. lanceolata per se*--the sterility and cytogenetic abnormalities we observed might be extraordinary for forms within this species. Furthermore, the morphological and cytogenetic distinctness of different forms may not be great for some natural populations (Graham 1989). Because *C. lanceolata* has a major role in the domestication and commercialization of *Cuphea*, it is essential to gain a more thorough understanding of the diversity

within this species and of the accessibility of this diversity for plant breeding. Fertile interspecific hybrids between *C. lanceolata* f. *silenoides* and *C. viscosissima* have been made and have become important for breeding *Cuphea* (Brandt, 1991). But hybrids between *C. lanceolata* f. *typica* and *C. viscosissima* are sterile (Brandt, 1991). Populations which can serve as bridges between the extreme forms of *C. lanceolata* might be found as additional germplasm. These could be extremely useful for breeding *C. lanceolata per se* and for introgressing genes from divergent *C. lanceolata* f. *typica* populations to *C. viscosissima.*

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Chapter II

INBREEDING DEPRESSION AND HETEROSIS IN *CUPHEA LANCEOLATA* AIT.

ABSTRACT

Cuphea lanceolata Ait. is one of the capric acid rich species of the genus *Cuphea*. It is allogamous and autosterile, but self compatible. In this paper we report inbreeding within a synthetic population and heterosis of single-cross F_1 hybrids of *C. lanceolata f. silenoides*. Mean plant height, biomass plot^{-1}, seed yield plot^{-1}, seed oil percentage, and 500-seed weight decreased as inbreeding increased. Biomass plot^{-1} and seed yield plot^{-1} were more severely affected than other traits, and there were significant differences among line sources. Significant mid-parent heterosis was observed in every single-cross F_1 hybrid for plant height, biomass plot^{-1}, seed yield plot^{-1}, seed oil percentage, and 500-seed weight. Heterosis was greatest for seed yield plot^{-1}, whereas it was least for 500-seed weight. Mean mid-parent heterosis for plant height, biomass plot^{-1}, seed yield plot^{-1}, seed oil percentage, and 500-seed weight were 70.7, 103.7, 216.8, 76.0, and 57.3%, respectively. Mean heterosis was significant for every trait. The line effect of the inbred line LN98 was greatest for every trait. The best general combiner was the inbred line LN96. This evidence suggests that considerable potential exists for exploiting hybrid vigor of *C. lanceolata* through the use of synthetics or F_1s; however, no mechanism presently exists for producing F_1 hybrid *Cuphea* seed.

INTRODUCTION

Many *Cuphea* species are excellent natural sources of medium-chain fatty acids (MCFAs), but none have been fully domesticated (Graham et al., 1981; Wolf et al., 1983; Graham, 1988; Graham, 1989). The main interests are focusing on caprylic ($C_{8:0}$), capric ($C_{10:0}$), and lauric ($C_{12:0}$) acids. Lauric acid is widely used to manufacture soaps and detergents (Young, 1983; Thompson, 1984; Arkcoll, 1988); it is also used as a cooking fat and shortening (Young, 1983; Arkcoll, 1988). Capric and caprylic acids have medicinal uses. They are used to treat gallstones, epilepsy, and lipid disorders (Bach and Babayan, 1982; Babayan, 1987).

Lauric acid is exclusively derived from coconut (*Cocos nucifera* L.) and palm (*Elaeis guinensis* Jacq.) kernel oils, and from petrochemicals (Young, 1983; Thompson, 1984). A temperate natural source of MCFAs is desirable to reduce the dependency on coconut and palm oils. *Cuphea* was found as potential alternate source of MCFAs (Earle et al., 1960). Although several annual species can be grown in temperate climates, seed shattering impedes their domestication (Hirsinger and Knowles, 1984; Knapp, 1990).

C. lanceolata Ait. and *C. viscosissima* Jacq. are closely related and have $2n=2x=12$ chromosomes, but their floral morphology and mating systems are quite different. The floral tubes of *C. lanceolata* are much larger than those of *C. viscosissima*. The flowers of *C. lanceolata* are protandrous. Stamens

dehisce, and exert before the stigma becomes receptive. At anthesis the stigma of *C. viscosissima* passes through dehiscing anthers, thereby enabling fertilization without vectors. *C. lanceolata* is strongly allogamous and autosterile, whereas *C. viscosissima* is strongly autogamous and autofertile. Both species are self-compatible. Outcrossing rates between 61 and 94% have been estimated for *C. lanceolata* (Knapp et al., 1991). *C. lanceolata per se* is not being domesticated because it requires insect pollination and effective pollinators have not been found; however, *C. lanceolata* has become an important source of germplasm for breeding *C. viscosissima* because the *C. lanceolata* germplasm pool has much useful genetic variation, and crosses between certain populations of *C. lanceolata* and *C. viscosissima* are fertile and cytogenetically normal (Ronis et al., 1990; Brandt, 1991).

Mating between individuals that are more closely related than random chance results in inbreeding depression. Self fertilization is the most extreme form of inbreeding. Recessive alleles are exposed as homozygotes due to inbreeding. The exposed recessive alleles are then subjected to natural and artificial selection, and a reduction of fitness of inbred lines occurs (Burton et al., 1978). Selection against the deleterious recessive alleles within and among inbreds enhances population improvement. Inbreeding depression increased with the increase of the number of segregating loci affecting a trait (Falconer, 1981; Ziehe and Roberds, 1989). The trends of the inbreeding depression indicate the nature of gene action controlling the expression of traits. Linear

decrease indicates additivity; non-linear trends indicate non-additivity. Information on inbreeding effects is also useful in assessing strategies for genetic manipulation (Kenna et al., 1991).

Heterosis or hybrid vigor is the superiority of a hybrid over its parents. It is more frequent and more intense in open pollinated plants. It can be a measure of maximum potentiality of different traits of the crop plants. The amount of heterosis exhibited by hybrids depends largely on the genetic divergence of the parental population from which the inbreds have been developed. Abundant heterosis indicates that the parental lines are more genetically diverse (Ghaderi et al., 1984; Mungoma and Pollok, 1988; Ordás, 1991). Heterosis will always be proportional to genetic diversity if heterosis is caused only by positive dominance effects (Eberhart and Gardner, 1966). Diallel-cross analysis provides a basis for preliminary information on heterotic patterns among crosses (Gardner and Eberhart, 1966; Eberhart and Gardner, 1966; Hallauer and Miranda, 1981). It has also been used to gain an understanding of the nature of gene action (Hayman, 1954a; Hayman, 1954b; Hayman, 1957; Griffings, 1956; Gardner and Eberhart, 1966). However, there is no information on the breeding behavior and nature of gene action for the new oil seed crop, *C. lanceolata.*

Because *C. lanceolata* is allogamous, we presume that it is subject to inbreeding depression and may exhibit significant heterosis. To test this, we estimated the effects of inbreeding within a *C. lanceolata* synthetic population

and heterosis of single-cross F_1s. The specific purposes of this paper were to determine the inbreeding effects for several lines, to estimate the heterotic response of single-cross F_1s, and to delineate the nature of gene action involved for the heterotic response.

MATERIALS AND METHODS

Experimental Procedures

Experiments were conducted in 1989 and 1990 in adjacent nurseries near Corvallis, Oregon to examine the effects of inbreeding and heterosis in *C. lanceolata* f. *silenoides* Ait.. Experiments were planted on May 15, 1989 at the Lewis Brown Farm, and on June 6, 1990 at the East Farm. The soil type of both experimental farms belongs to the Chehalis series which is characterized by dark brown silt loam. Seeds were planted directly by hand into a 1.0 m long single row at a depth of 1.0 cm, and 1.25 m between row space was maintained. We seeded 40 seeds in each row. Irrigation was applied by sprinkler irrigation soon after planting to keep the soil moist until germination. Thereafter, irrigation was applied once a week throughout the growing season to maintain available moisture required for good plant growth. Herbicides Balan (at the rate of 2.2 kg active ingredient hectare^{-1}) and Roundup (at the rate of 0.6 kg active ingredient hectare^{-1}) were applied at East and Lewis Brown farms, respectively, before planting. We controlled weeds mechanically in the standing crop mechanically by hoeing whenever necessary. Fertilizers were applied at the rate of 53.8 kg N and 67.2 kg P_2O_5 hectare^{-1} at Lewis Brown farm during land preparation. No fertilizer was applied at East Farm.

The experiments were harvested on September 19, 1989 at Lewis Brown and on September 29, 1990 at East Farm. Plants harvested from individual

plots were dried completely in a dryer for one week at a temperature of 120^0C. Seeds were threshed and cleaned mechanically. Data were collected on plant height (cm), biomass plot^{-1}, seed yield plot^{-1}, seed oil percentage, and 500-seed weight. Only plant height data were collected from the field. We used Nuclear Magnetic Resonance (NMR) to estimate the seed oil percentage.

Plant Materials and Analysis for the Inbreeding Effects Experiment

S_1 and S_2 lines were derived by single seed descent from 10 randomly selected S_0 individuals from the LN43 population. The LN43 population was synthesized by intermating 24 populations of *C. lanceolata* (Knapp 1990). We used bulk seed of LN43 for the S_0 line. A randomized complete block experimental design with four complete blocks was used. Within each block, the S_0 line was repeated 10 times so that orthogonal polynomials could be used to estimate the linear and quadratic effects of inbreeding for each group of lines. This means we had one S_0 plot for every pair of S_1 and S_2 lines derived from a common S_0 individual for a total of 30 plots within one complete block.

The expected inbreeding coefficients for S_0, S_1, and S_2 lines were 0.0, 0.5, and 0.75, respectively (Crow and Kimura 1970). These coefficients were used as quantitative factor levels in an analysis of variance to test the effects of inbreeding. Coefficients of orthogonal polynomials for estimating linear and quadratic effects of inbreeding were found using the SAS (SAS, 1987) function ORTHPOL–since the inbreeding coefficients were not equally spaced, these

coefficients are not presented anywhere. Linear and quadratic coefficients for factor levels with the 0.0, 0.5, and 0.75 spacing were -0.771, 0.154, and 0.617, and 0.267, -0.802, and 0.535, respectively.

Linear and quadratic effects of inbreeding were estimated by using

$-0.771\bar{y}_0 + 0.154\bar{y}_1 + 0.617\bar{y}_2$, and

$0.267\bar{y}_0 - 0.802\bar{y}_1 + 0.535\bar{y}_2$

where $\bar{y}_0$, $\bar{y}_1$, and $\bar{y}_2$ are estimates of the means of the S_0, S_1, and S_2 lines where the S_1 and S_2 lines trace to a common S_0 individual. Because each S_0 line was repeated for each of the 10 pairs of S_1 and S_2 lines, estimates of inbreeding effects were orthogonal.

Plant Materials and Analysis for the Heterosis Experiment

All possible single cross F_1 hybrids, excluding reciprocals, were made among five S_5 inbred lines of *C. lanceolata* f. *silenoides*. The specific lines used were LN95, LN96, LN97, LN98, and LN99. This gave us a partial diallel without reciprocals; however, a more elaborate treatment design was used so that we could get orthogonal estimates of mid-parent heterosis effects. The parents and F_1 hybrids were grown in a randomized complete block experiment design. Instead of planting the parents once within each complete block, as is done in an ordinary diallel, we planted four plots of each parent within each complete block–once for each time they were used in a particular hybrid. So within a complete block we had $(5^2 - 5)/2 = 10$ F_1 hybrid plots and 20 parent

line plots, for a total of 30 plots. This gave us the data needed to get orthogonal estimates of mid-parent heterosis effects, which were estimated by $\{[\bar{y}_{F1} - (\bar{y}_{P1} + \bar{y}_{P2})]/\ \bar{y}_{MP}\}$ where $\bar{y}_{F1}$, $\bar{y}_{P1}$, and $\bar{y}_{P2}$ are means of the F_1, parent 1, and parent 2, respectively. Orthogonal contrasts were used to test the hypothesis of no mid-parent heterotic effect for each F_1 hybrid.

In addition to estimating mid-parent heterosis from the analysis of variance, the sums of squares among crosses were partitioned into mean, line, and specific heterotic effects using the diallel analysis II estimators of Gardner and Eberhart (1966), where the effects of entries (inbred lines and F_1 hybrids) are fixed and the effects of years are random. The analysis was done using means of entries (Gardner and Eberhart 1966). The effects of entries were partitioned into the effects of inbred lines and heterotic effects. Heterotic effects were further partitioned into mean, line, and specific heterotic effects (Gardner and Eberhart 1966). Hypotheses about the effects of entries and years were tested using the entry x environment mean square. Hypotheses about the effects of entry x environment interaction were tested using the error mean square.

RESULTS AND DISCUSSION

Effects of Inbreeding

The means of every trait decreased as the inbreeding coefficient increased (Table 6). The effect of inbreeding was not equal across the traits. Seed yield and biomass were more severely affected by inbreeding than the other traits. The percentage decrease in the means of S_2 lines relative to S_0 lines ranged from 23.3 to 78.1%, 18.6 to 74.8%, 20.8 to 79.2, 45.4 to 92.9%, and 39.8 to 100% for plant height, biomass $plot^{-1}$, seed yield $plot^{-1}$, seed oil percentage, and 500-seed weight, respectively (Table 6).

The effects of inbreeding were significant for every trait and lineage (Tables 7a and 7b). Very strong linear inbreeding effects were observed. Quadratic effects were far less important for most traits and were rarely significant (Tables 7a and 7b). Line x year interaction effects were significant, but this was a consequence of differences in the magnitudes of the effects between years, and not a consequence of changes of in the ranks of S_0, S_1, and S_2 line means (Tables 6, 7a, and 7b).

Inbreeding depression is a function of gene frequency, directional dominance, and the number of segregating loci affecting a trait (Falconer, 1981), and a consequence of the increased frequency of homozygous recessive deleterious loci (Allard, 1960; Hallauer and Sears, 1973). A linear decrease is expected with a decrease in heterozygosity because the mean of a trait is

Table 6. Least square means across years for S_0, S_1, and S_2 lines and mean percentage changes for S_1 and S_2 lines relative to S_0 lines of *C. lanceolata* grown in 1989 and 1990 at Corvallis, Oregon.

Lines		Plant height (cm)		Biomass (g plot^{-1})		Seed yield (g plot^{-1})		Oil percentage		500-seed weight (g)	
		Mean	% S_0	Mean	% S_0	Mean	% S_0	Mean	% S_0	Mean	% S_0
LN-43	S_0	116.9		870.2		69.0		31.1		1.7	
LN-162	S_1	93.1	79.7	674.2	77.5	44.5	64.5	28.9	93.1	1.6	97.6
LN-172	S_2	46.0	39.4	426.9	49.1	24.4	35.3	14.1	45.4	0.7	43.4
LN-43	S_0	118.6		951.8		64.7		30.5		1.8	
LN-163	S_1	99.6	84.0	735.8	77.3	46.4	71.7	27.4	89.7	1.6	91.0
LN-173	S_2	89.9	75.8	582.8	61.2	36.0	55.6	27.0	88.6	1.7	94.4
LN-43	S_0	114.3		823.9		58.4		30.3		1.8	
LN-164	S_1	93.9	82.2	632.7	76.8	38.7	66.4	27.4	90.4	1.7	93.4
LN-174	S_2	77.8	68.1	336.3	40.8	24.2	41.4	18.5	61.0	1.1	62.4

Table 6. continued

Lines		Plant height (cm)		Biomass (g plot^{-1})		Seed yield (g plot^{-1})		Oil percentage		500-seed weight (g)	
		Mean	% S_0	Mean	% S_0	Mean	% S_0	Mean	% S_0	Mean	% S_0
LN-43	S_0	118.8		857.6		67.2		30.5		1.7	
LN-165	S_1	105.8	89.1	791.8	92.3	54.2	80.6	29.8	97.5	1.8	100.1
LN-175	S_2	27.6	23.3	159.6	18.6	14.0	20.8	14.1	46.3	0.7	39.9
LN-43	S_0	113.8		916.6		64.8		30.3		1.7	
LN-166	S_1	100.6	88.5	682.3	74.4	50.6	78.1	28.4	93.9	1.5	90.5
LN-176	S_2	88.8	78.1	429.5	46.9	30.1	46.4	27.7	91.3	1.4	83.9
LN-43	S_0	119.8		893.0		64.5		29.8		1.8	
LN-167	S_1	104.6	87.4	773.0	86.6	58.4	90.6	27.3	91.6	1.6	90.9
LN-177	S_2	77.6	64.8	474.4	53.1	28.5	44.2	18.9	63.5	1.2	67.4

Table 6. continued

Lines		Plant height (cm)		Biomass (g $plot^{-1}$)		Seed yield (g $plot^{-1}$)		Oil percentage		500-seed weight (g)	
		Mean	% S_0	Mean	% S_0	Mean	% S_0	Mean	% S_0	Mean	% S_0
LN-43	S_0	121.4		913.4		71.3		30.1		1.6	
LN-168	S_1	101.9	83.9	711.4	77.9	50.6	71.0	29.6	98.3	1.7	103.7
LN-178	S_2	88.1	72.6	612.9	67.1	38.1	53.4	27.2	90.4	1.6	
LN-43	S_0	112.3		829.0		56.2		29.5		1.7	
LN-169	S_1	99.1	88.3	778.8	93.9	56.9	101.4	28.2	95.7	1.6	96.4
LN-179	S_2	82.3	73.3	620.4	74.8	44.5	79.2	27.4	92.9	1.6	98.2
LN-43	S_0	122.4		1,010.5		78.9		31.9		1.8	
LN-170	S_1	91.7	74.9	786.1	77.8	53.3	67.6	29.4	92.2	1.8	96.2
LN-180	S_2	86.5	70.6	582.2	57.6	48.6	61.6	28.2	88.4	1.7	91.3

Table 6. continued

Lines		Plant height (cm)		Biomass (g $plot^{-1}$)		Seed yield (g $plot^{-1}$)		Oil percentage		500-seed weight (g)	
		Mean	% S_0	Mean	% S_0	Mean	% S_0	Mean	% S_0	Mean	% S_0
LN-43	S_0	115.0		934.7		73.0		30.2		1.7	
LN-171	S_1	94.4	82.1	563.9	60.3	44.3	60.7	27.2	90.1	1.5	86.2
LN-181	S_2	84.9	73.8	576.0	61.6	49.7	68.0	26.4	87.3	1.4	81.6

Table 7a. Analysis of variance of plant height and biomass for S_0, S_1, and S_2 lines of *C. lanceolata* grown in 1989 and 1990 at Corvallis, Oregon.

Sources of variation	df	Plant height: Mean square	Plant height: Pr>F	Biomass: Mean square	Biomass: Pr>F
Year	1	43,805.2	<0.001	11,344,915.5	<0.001
Block	3	123.1	0.529	3,415.2	0.954
Line	29	3,300.00	<0.001	277,645.1	<0.001
Line 1					
Linear(L)	1	16,326.6	<0.001	670,989.2	<0.001
Quadratic(Q)	1	2,813.7	<0.001	50,256.6	0.203
Line 2					
L	1	2,858.9	<0.001	456,717.2	<0.001
Q	1	0.1	0.979	4,079.4	0.717
Line 3					
L	1	4,415.4	<0.001	731,761.2	<0.001
Q	1	70.8	0.515	80,671.8	0.108
Line 4					
L	1	25,508.6	<0.001	1,461,900.7	<0.001
Q	1	11,201.6	<0.001	784,079.9	<0.001
Line 5					
L	1	2,284.7	<0.001	852,403.5	<0.001
Q	1	62.1	0.545	40,266.9	0.255

Table 7a. Continued.

Sources of variation	df	Plant height		Biomass	
		Mean square	Pr>F	Mean square	Pr>F
Line 6					
L	1	5,379.1	<0.001	513,773.8	<0.001
Q	1	755.6	0.034	113821.6	0.057
Line 7					
L	1	4,030.8	<0.001	341,128.5	0.001
Q	1	36.2	0.641	12.1	0.984
Line 8					
L	1	2,827.1	<0.001	124,858.9	0.046
Q	1	212.9	0.259	35,510.4	0.285
Line 9					
L	1	4,955.5	<0.001	611,624.2	<0.001
Q	1	199.5	0.275	16,542.0	0.465
Line 10					
L	1	3,546.1	<0.001	580,394.1	<0.001
Q	29	0.9	0.941	77,015.1	0.116
Year x Line	29	528.1	<0.001	54,121.4	0.016
Error	160	166.0		30,877.7	

Table 7b. Analysis of variance of seed yield, oil percentage and 500-seed weight for S_0, S_1, and S_2 lines of *C. lanceolata* grown in 1989 and 1990 at Corvallis, Oregon.

Sources of variation	df	Seed yield Mean square	Seed yield Pr>F	Oil percentage Mean square	Oil percentage Pr>F	500-seed weight Mean square	500-seed weight Pr>F
Year	1	7,288.2	<0.001	218.6	<0.001	1.7	<0.001
Block	3	44.3	0.929	4.2	0.538	0.1	0.072
Line	29	1,783.8	<0.001	148.2	<0.001	0.6	<0.001
Line 1							
Linear(L)	1	7,133.1	<0.001	845.1	<0.001	2.5	<0.001
Quadratic(Q)	1	141.2	0.488	427.3	<0.001	1.7	<0.001
Line 2							
L	1	2,829.2	0.002	46.8	0.005	0.1	0.190
Q	1	3.3	0.916	2.9	0.478	0.0	0.255
Line 3							
L	1	3,905.1	<0.001	400.6	<0.001	1.3	<0.001
Q	1	45.7	0.693	110.8	<0.001	0.5	<0.001
Line 4							
L	1	9,146.6	<0.001	785.2	<0.001	3.0	<0.001
Q	1	2,477.5	0.004	508.0	<0.001	2.9	<0.001
Line 5							
L	1	4,197.9	<0.001	27.3	0.031	0.3	0.002
Q	1	395.5	0.246	0.1	0.929	0.0	0.809

Table 7b. Continued.

Sources of variation	df	Seed yield Mean square	Seed yield Pr>F	Oil percentage Mean square	Oil percentage Pr>F	500-seed weight Mean square	500-seed weight Pr>F
Line 6							
L	1	3,588.7	<0.001	335.8	<0.001	1.0	<0.001
Q	1	1,440.4	0.028	101.3	<0.001	0.2	0.005
Line 7							
L	1	4,090.7	<0.001	25.2	0.038	0.0	0.924
Q	1	11.2	0.845	10.5	0.179	0.0	0.399
Line 8							
L	1	335.2	0.285	14.7	0.112	0.0	0.686
Q	1	328.9	0.290	0.1	0.919	0.0	0.668
Line 9							
L	1	3,504.6	<0.001	48.3	0.004	0.1	0.103
Q	1	126.9	0.511	0.0	0.985	0.0	0.685
Line 10							
L	1	2,657.1	0.003	60.3	0.001	0.4	<0.001
Q	1	772.1	0.106	0.8	0.705	0.0	0.753
Year x Line	29	564.3	0.106	112.2	<0.001	0.5	<0.001
Error	160	291.7		5.7		0.0	

proportional to the decrease in heterozygosity, regardless of the number of alleles or degree of dominance at each locus. A quadratic response to increased inbreeding might be the evidence for epistasis (Wright, 1922). *C. lanceolata* has a fairly substantial genetic load as evidenced by the rapid decrease in the means of most of the traits, especially seed yield, plant height, and biomass (Table 6). Hallauer and Miranda (1981) and Rai et al. (1985) observed that seed weight was less affected by inbreeding than seed yield in maize. We observed this trend in *Cuphea* as well–500-seed weight was less affected by inbreeding than the other traits (Tables 6, 7a, and 7b).

Given that *C. lanceolata* is a strongly allogamous species (Knapp et al. 1991) and open-pollinated populations of this species are extremely heterozygous for most marker loci (Knapp and Tagliani, 1989; Webb et al. 1991), it is not surprizing that the means of fitness of the traits are depressed by inbreeding within previously noninbred populations. *C. lanceolata* responds much like maize (Meghji et al. 1984; Lamkey and Smith, 1987; Rodriguez and Hallauer, 1988; Horner et al., 1989; Walters et al., 1991) and other allogamous species, e.g., alfalfa (El-Nahrawy and Bingham, 1989), gamagrass (Kenna et al., 1991) and pearl millet (Rai et al., 1985) to inbreeding. But like in maize, some inbred lines of *C. lanceolata* are more vigorous than others. Many lines of *C. lanceolata*, for example, have been lost while developing inbred lines through continuous self-pollination as a consequence of fixing lethal and deleterious genes. Like in *Cuphea*, significant inbreeding depression has been observed for

seed yield, plant height, and oil percentage in *Brassica napus* (Schuster and Michael, 1976; Meng and Liu, 1986; Brandle and McVetty, 1988). We observed significant decreases in oil percentage upon inbreeding. Many lines of *C. lanceolata* lost one third to one half of their oil upon inbreeding to S_2, which is typical of the decreases observed in *B. napus*.

Heterosis

Significant mid-parent heterosis was observed for every F_1 hybrid for every trait (Table 8). Heterosis was greatest for seed yield and biomass. Mid-parent heterosis for seed yield and biomass ranged from 112.7 to 879.4% , and from 68.0 to 197.7%, respectively (Table 8). Heterosis was less prominent for 500-seed weight (Table 8), which is consistent with what we observed with inbreeding (Tables 6, 7a, and 7b), but heterosis for 500-seed weight was nevertheless significant for every F_1 hybrid.

Mid-parent heterosis was especially great for F_1 hybrids between LN-96 and the other inbreds, and between LN-99 and the other inbreds. The mean heterosis percentage for F_1 hybrids with LN-96 or LN-99 for seed yield were 459.9 and 395.3%, respectively, which is roughly 200 to 250% more than the mean heterosis percentage for all F_1 hybrids. Percentage of heterosis can be somewhat deceiving–the mid-parent mean for seed yield for LN-96 and LN-99 was far lower than for other pairs of inbred lines. LN-96 was so severely depressed that many of the plots failed to yield any seed. The estimate of heterosis percentage for seed yield of LN-96 x LN-99 was quite high as 879.4% (Table 8), but this reflects the severe depression of this line as much as anything. The mean seed yield of the LN-96 x LN-99 F_1 hybrid, for example, was the lowest observed.

Inbred line *per se* and F_1s performance are important to identify useful lines for improvement of parents of single crosses (Dudley, 1984; 1987; Gallias,

Table 8. Means of mid-parents ($\bar{y}_{MP}$) and F_1s ($\bar{y}_{F1}$) and percent mid-parent heterosis of *C. lanceolata* in 1989 and 1990 at Corvallis, Oregon. Mean squres and P-values for the test of the hypothesis of no mid-parent heterosis are listed for each F_1s.

F_1 hybrid	$\bar{y}_{MP}$	$\bar{y}_{F1}$	Heterosis (%)	Mean square	Pr>F
			Plant height (cm)		
LN-95 x LN-96	51.6	118.1	129.0	22,971.9	<0.001
x LN-97	66.2	100.5	51.8	6,099.3	<0.001
x LN-98	66.6	108.4	62.8	9,324.2	<0.001
x LN-99	69.7	116.5	67.1	11,030.8	<0.001
LN-96 x LN-97	58.9	103.8	76.3	10,451.2	<0.001
x LN-98	53.8	104.5	94.2	13,702.5	<0.001
x LN-99	48.6	112.8	132.2	21,375.5	<0.001
LN-97 x LN-98	69.7	99.5	42.8	4,740.2	<0.001
x LN-99	70.4	102.8	45.9	5,568.5	<0.001
LN98 x LN-99	74.8	109.0	45.7	6,069.0	<0.001
Mean	63.0	107.6	70.7	7,420.5	<0.001
			Biomass (g plot^{-1})		
LN-95 x LN-96	365.5	1,027.7	181.2	2,274,090.7	<0.001
x LN-97	471.0	791.5	68.0	532,647.5	<0.001
x LN-98	489.0	822.8	68.3	594,266.4	<0.001
x LN-99	480.5	855.4	78.0	708,805.2	<0.001

Table 8. continued

F_1 hybrid	$\bar{y}_{MP}$	$\bar{y}_{F1}$	Hetero-sis (%)	Mean square	Pr>F
			Biomass (g plot^{-1})		
LN-96 x LN-97	478.4	973.8	103.6	1,273,032.1	<0.001
x LN-98	420.9	1,091.7	159.4	239,9961.4	<0.001
x LN-99	363.4	1,081.9	197.7	2,677,408.1	<0.001
LN-97 x LN-98	545.0	958.0	75.8	909,852.8	<0.001
x LN-99	531.6	922.5	73.5	815,055.2	<0.001
LN-98 x LN-99	537.3	1,011.9	88.3	1,168,441.4	<0.001
Mean	468.3	953.7	103.7	853,777.2	<0.001
			Seed yield (g plot^{-1})		
LN-95 x LN-96	12.5	81.8	554.7	24,891.0	<0.001
x ln-97	31.0	87.6	182.8	16,617.3	<0.001
x LN-98	37.5	79.7	112.7	9,516.7	<0.001
x LN-99	18.2	77.8	327.2	17,888.5	<0.001
LN-96 x LN-97	28.8	77.2	168.1	12,160.3	<0.001
x LN-98	25.7	86.8	237.5	19,891.3	<0.001
x LN-99	7.9	77.0	879.4	24,782.7	<0.001
LN-97 x LN-98	46.7	112.3	140.2	22,900.6	<0.001
x LN-99	28.8	88.1	206.0	18,770.8	<0.001
LN-98 x LN-99	35.7	96.0	168.8	18,849.0	<0.001
Mean	27.3	86.4	216.8	12,049.3	<0.001

Table 8. Continued.

F_1 hybrid	$\bar{y}_{MP}$	$\bar{y}_{F1}$	Heterosis (%)	Mean square	Pr>F
			Seed oil percentage		
LN-95 x LN-96	12.3	27.6	124.5	1,212.7	<0.001
x LN-97	20.3	29.5	45.6	443.0	<0.001
x LN-98	17.8	29.8	67.5	767.8	<0.001
x LN-99	16.8	28.7	71.1	719.2	<0.001
LN-96 x LN-97	14.5	26.1	80.7	706.8	<0.001
x LN-98	10.5	27.3	159.5	1,505.5	<0.001
x LN-99	6.6	26.6	300.8	2,062.7	<0.001
LN-97 x LN-98	20.8	28.9	38.8	347.4	<0.001
x LN-99	20.0	27.5	37.6	300.9	<0.001
LN-98 x LN-99	19.8	28.6	43.9	393.0	<0.001
Mean	15.9	28.1	76.0	549.9	<0.001
			500-seed weight (g)		
LN-95 x LN-96	0.8	1.6	102.5	3.6	<0.001
x LN-97	1.36	1.8	28.7	0.8	0.010
x LN-98	1.26	1.8	41.3	1.4	<0.001
x LN-99	1.25	1.8	47.2	1.8	<0.001
LN-96 x LN-97	0.95	1.6	69.5	2.2	<0.001
x LN-98	0.75	1.7	122.7	4.5	<0.001
x LN-99	0.49	1.7	253.1	7.9	<0.001
LN-97 x LN-98	1.43	1.7	21.0	0.5	0.043
x LN-99	1.31	1.7	29.8	0.8	0.008
LN-98 x LN-99	1.34	1.8	35.1	1.1	0.002
Mean	1.1	1.7	57.3	1.6	<0.001

1988). Simple correlation coefficients between the mean of F_1 hybrids and the mean of the parents were -0.34, -0.47, 0.72, 0.68, and 0.57 for plant height, biomass plot^{-1}, seed yield plot^{-1}, seed oil percentage, and 500-seed weight, respectively. The negative correlation coefficients for plant height and biomass plot^{-1} are insignificant, however. A positive correlation between line *per se* and number of favorable alleles in F_1 hybrid performance has been observed in maize (Zanoni and Dudley, 1989). Very poor inbreds do not necessarily make outstanding hybrids. What is more important is the diversity between lines. There seems to be significant diversity between the set of inbreds we used, but this only taps a very limited part of the diversity of this species. Only one population of *C. lanceolata* has been collected from the wild thus far (Knapp, 1990; Knapp and Tagliani, 1990). This wild population is *C. lanceolata* f. *typica*, which is different from the source populations of the *C. lanceolata* f. *silenoides* inbreds reported in this paper. These inbreds came from open-pollinated populations of *C. lanceolata* f. *silenoides* collected by G. Röbbelen from specimens held in European botanical gardens. Unfortunately, hybrids between *C. lanceolata* f. *silenoides* and *C. lanceolata* f. *typica* are sterile (unpublished data). How much of the uncollected diversity of *C. lanceolata* we can exploit remains to be seen.

Mean heterosis explained most of the differences attributed to heterosis (Tables 9a and 9b). Mean heterosis was significant for every trait (Tables 9a and 9b). Line and specific heterosis was not significant for any trait (Tables 9a

Table 9a. Fixed effects diallel analysis of variance of *C. lanceolata* inbred lines and F_1s grown in 1989 and 1990 at Corvallis, Oregon. Entry sums of squares were partitioned into different heterotic effects using the diallel analysis II estimates of Gardner and Eberhart (1966).

		Plant height		Biomass	
Sources of variation	df	Mean square	Pr>F	Mean square	Pr>F
Year	1	3,924.5	<0.001	3,733,791.8	<0.001
Block	3	19.4	0.887	8,498.2	0.825
Entries	14	608.9	0.186	71,282.9	0.205
Lines	4	87.8	0.917	14,769.8	0.204
Heterosis	10	817.3	0.088	93,888.2	0.237
Mean	1	7,420.5	<0.001	853,777.2	0.004
Line	4	176.7	0.757	18,832.0	0.511
Specific	5	9.1	1.000	1,955.4	1.000
Year x Entries	14	374.0	<0.001	32,153.5	0.339
Error	87	91.4		28,282.1	

Table 9b. Fixed effects diallel analysis of variance of *C. lanceolata* inbred lines and F_1 hybrids grown in 1989 and 1990 at Corvallis, Oregon. Entry sums of squares were partitioned into different heterotic effects using the diallel analysis II estimates of Gardner and Eberhart (1966).

Sources of variation	df	Seed yield		Oil percentage		500-seed weight	
		Mean square	Pr>F	Mean square	Pr>F	Mean square	Pr>F
Year	1	7,247.2	<0.001	6.6	0.248	0.3	<0.001
Block	3	363.2	0.492	5.0	0.380	0.0	0.911
Entries	14	1,070.5	0.110	61.4	0.046	0.2	0.205
Lines	4	622.3	0.378	55.4	0.111	0.2	0.204
Heterosis	10	1,249.8	0.077	63.8	0.048	0.2	0.237
Mean	1	12,049.3	<0.001	549.9	<0.001	1.6	0.004
Line	4	21.9	0.997	21.8	0.490	0.1	0.511
Specific	5	72.2	0.983	0.1	1.000	0.0	1.00
Year x Entries	14	547.5	0.275	24.2	<0.001	0.1	<0.001
Error	87	448.4		4.9		0.0	

and 9b). This outcome is typical of outcrossing species, e.g., maize (Mungoma and Pollak, 1988; Crossa et al., 1990; Mišević, 1990; Ordás, 1991), and rapeseed (Grant and Beversdorf, 1985; Lefort-Buson et al., 1987).

The positive effects of LN-98 were greater than those of other lines for every trait except oil percentage, with LN-97 a close second (Table 10). The seed yields of these lines were greater than those of the other lines, which is another example of the strong positive correlation we observed for seed yield and other traits between line *per se* and hybrid performance.

Heterosis and F_1 hybrids of *C. lanceolata* have obvious economic, agronomic, and commercial value, but a mechanism has not yet been developed for F_1 hybrid seed production of this species. Cytoplasmic-genic male-sterility has not been discovered within *C. lanceolata* or within the genus. Gametocides might be useful for the short term (Hirose, 1969; Pike and Peterson, 1969; Beyers et al., 1972; Tolla and Peterson, 1979; Rudich, 1980), but using chimeric genes to engineer genetic-male sterility for F_1 hybrid seed production is becoming increasingly attractive (Mariani et al. 1990). Fertility restoration is a problem with engineered male-sterility, but methods are being examined and advances should be forthcoming (Mariani et al., 1990).

Another problem with exploiting *C. lanceolata per se* is a lack of suitable commercial pollinators. *C. lanceolata* is an entomophagous species which must be pollinated by insects. Although honeybees are occasional pollinators, they are not effective, practical, or economical for large scale commercial

Table 10. Line (v_j), line heterotic (h_j) and mean heterotic ($\bar{h}$) effects for different traits.

Lines	Plant height		Biomass		Seed yield		Oil percentage		500-seed weight	
	v_j	h_j	v_j	h_j	v_j	h_j	v_j	h_j	v_j	h_j
LN-95	-3.5	6.1	-107.0	-52.3	-10.3	-1.1	1.1	0.6	0.1	-0.0
LN-96	-21.1	13.5	-108.4	174.3	-24.6	4.7	-14.4	5.6	-1.0	0.4
LN-97	3.2	-9.6	77.6	-95.1	16.1	-1.6	7.3	-3.7	0.4	-0.3
LN-98	14.2	-10.1	126.3	-40.0	28.3	-4.4	6.0	-2.2	0.5	-0.2
LN-99	7.2	-0.0	11.6	13.2	-9.5	2.5	0.0	-0.3	-0.1	0.1
$\bar{h}$		47.2		506.1		60.1		12.8		0.7

production of *Cuphea*. Nevertheless, *C. lanceolata* has been extensively used to breed autofertile lines and populations through the use of interspecific hybrids with *C. viscosissima* (unpublished data). The ultimate way to exploit the diversity of *C. lanceolata* is to use these lines to create autofertile F_1 hybrids.

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