INTERSPECIFIC HYBRIDIZATION AND AMPHIDIPLOIDY BETWEEN *CUCURBITA MOSCHATA* DUCH. EX POIR AND *CUCURBITA FOETIDISSIMA* HBK

by

EL Awad Mohamed Ali EL Fahal

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN HORTICULTURE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 7 7

THE UNIVERSITY OF ARIZONA

GRADUATE COLLEGE

I hereby recommend that this dissertation prepared under my direction by EL Awad Mohamed Ali EL Fahal

entitled Interspecefic Hybridization and Amphidiploidy between _Cucurbita moschata_ Duch. Ex Poir and _Cucurbita foetidissima_ HBK

be accepted as fulfilling the dissertation requirement for the degree of Doctor of Philosophy

William P. Bemis — Dissertation Director

June 30, 1977 — Date

As members of the Final Examination Committee, we certify that we have read this dissertation and agree that it may be presented for final defense.

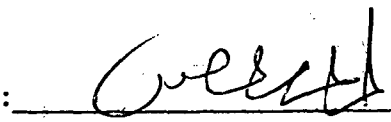
[signature]	30 June 77
[signature]	6 July 77
[signature]	1 July 77

Final approval and acceptance of this dissertation is contingent on the candidate's adequate performance and defense thereof at the final oral examination.

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: 

ACKNOWLEDGMENTS

The author wishes to thank the Democratic Republic of the Sudan for sponsoring his education and the Department of Horticulture for providing the facilities and equipment to carry out the research program.

The author wishes to extend sincere appreciation to his major professor, Dr. William P. Bemis. His constructive guidance and willingness to share knowledge enormously aided the author during the research program and especially at the time this manuscript was being prepared.

The author is most grateful to Dr. Robert E. Foster and Dr. Norman F. Oebker for their support as members of the major committee, and to Dr. Kaoru Matsuda and Dr. James W. O'Leary for their support as members of the minor committee. Additional appreciation is extended to Dr. Stanley M. Alcorn and Dr. Merritt R. Nelson, from the Department of Plant Pathology, for the examination of plant and soil samples and their suggestions during the research study.

Finally, the author manifests his gratitude to his wife, Badria, daughter, Nahid, and parents for their constant encouragement in the culmination of this degree.

TABLE OF CONTENTS

TABLE OF CONTENTS--Continued.

LIST OF ILLUSTRATIONS

LIST OF TABLES

ABSTRACT

This study was conducted to create a fertile amphidiploid between two selected cultivars of Cucurbita moschata and C. foetidissima and to formulate an hypothesis for the genetics involved in the male sterility of previous amphidiploids made from these two species.

Two methods were described to create the amphidiploid. One was the hybridization of the diploid parental species and then the chromosome number of the hybrid was doubled to create the amphidiploid. The second way was to double the chromosome number of the parental species before hybridizing them to create the amphidiploid.

Seeds and seedlings of both species were treated with 0.2 and 0.4% aqueous solution of colchicine in order to double the chromosome number. Male buds used for cytological analysis were fixed in Carnoy's solution and stained in Snow's alcoholic hydrochloric acid-carmine mixture.

Cross pollinations were made by hand. Different concentrations of plant growth hormones were applied to the stigma just before pollination, to improve fruit set.

Forty-three reciprocal cross pollinations were made but without any successful fruit set. This was due to collapse of the styler end of the fruits 5 or more days after pollination. By using plant growth hormones, 9 fruits out of 56 cross pollinations were set and reached maturity. None of these nine fruits contained any seed.

Based on limited data from the crosses of these two species, a genetic model was worked out to explain the genetics of male sterility in certain interspecific crosses in the genus Cucurbita. The data showed that the male sterility is not due to cytoplasmic or cytotoplasmic nuclear factors. From the segregation ratio, it could only be explained on a nuclear-genic basis. It is hypothesized the C. moschata cv. 'Butternut' (MM) and C. foetidissima (FF) have the carrier alleles ms^m and ms^f. Genic male sterility results when the two carrier alleles are in various combinations. The presence of the non carrier allele Ms found in C. moschata from Puerto Rico (M^1M^1) and other primitive cultivars of C. moschata restore male fertility. It was concluded that, probably only one pair of chromosomes and only one single locus is involved in the male sterility.

The way in which the amphidiploid is made is very important from a breeding standpoint with regards to how much heterozygosity will be transmitted to the amphidiploid. If the chromosomes of the hybrid are doubled to create the amphidiploid, the amphidiploid may have a high degree of true breeding permanent heterozygosity. If the chromosome number of the parents is doubled, and then crossed to create the amphidiploid, a high percentage of segregational heterozygosity and a low percentage of permanent heterozygosity will be transmitted to the amphidiploid depending on the amount of heterozygosity of the parents and the partition of the eight chromatids of the first meiotic division.

In tetraploids, the frequency of segregating homozygotes from heterozygotes is very low, and requires a large population of F_2

progeny if the desired phenotype is homozygous recessive. This becomes more complicated when two or more loci are considered.

CHAPTER 1

INTRODUCTION

For years botanists of different disciplines have been fascinated with the different species of Cucurbita. The morphologists find them well suited for studies of structure and of the developmental relationship of cells and organs. The geneticist and plant breeder see in them an almost limitless variation in fruit characters and marked, but less obvious variation in characters of seeds and vegetative organs (Whitaker and Bohn, 1950).

Investigations on interspecific hybridization have been helpful to understand the relationships existing among the various species by studying the chromosome pairing in the F_1 hybrids. Cytogenetic studies of such hybrids have provided some understanding of the origin of cultivated species. Interspecific hybridization studies have also indicated the possibility of the transference of desirable characters such as resistance to pests and disease from wild to cultivated species.

Many species do not cross readily and some cross with difficulty after numerous trials. The hybrid seed so obtained will have to be germinated very carefully under special conditions. The surviving F_1 diploid plants so obtained often produce inviable gametes because of chromosome imbalance resulting from failure of complete synapsis. This is referred to as chromosomal sterility. This problem of chromosomal sterility is usually overcome by doubling the chromosome number of the

allodiploid or the parental species with a mitotic poisoner such as colchicine. The resulting amphidiploid now contains chromosomes, each of which has a homologous partner, thus eliminating any problem which may arise due to chromosome imbalance. Occasionally sterility is found in these hybrids. This type of sterility is not a result of the failure of normal chromosome pairing, but rather it is of genic nature.

Chromosomal sterility is manifested by the abortion of gametes, however the anthers remain intact and dehiscence is noted at anthesis, whereas species and interspecific hybrids of Cucurbita which are genically male sterile show a completely aborted androecium at anthesis.

Interspecific genic male sterility is found in hybrids between C. moschata cv. 'Butternut' and the wild species C. foetidissima. Allodiploid, alloautotriploid and amphidiploid hybrids between these two species all show complete androecial collapse at flower maturity. By introducing a genome from a second cultivar of C. moschata into the hybrid, genic fertility is restored (Bemis, 1970).

The present study was designed to create a fertile amphidiploid between two selected cultivars of C. moschata and C. foetidissima and to formulate an hypothesis for the genetics involved in the male sterility of previous amphidiploids made from these two species.

CHAPTER 2

REVIEW OF LITERATURE

Origin and Characteristics

Cucurbita, the genus which includes the squashes, gourds, and pumpkins, has been associated with man for centuries. It is of new world origin with the center of distribution being either in the south central region of Mexico or the northern portion of central America. The early species of the genus were mesophytic and therefore were indigenous to tropical and semi-tropical areas. The genus spread northward up through the United States and southward through South America to Argentina. In its northward expansion some of the mesophytic species gave rise to xerophytic species which were capable of adapting to the desert regions of northern Mexico and southwestern United States (Whitaker and Davis, 1962, p. 81).

There are 27 named species comprising the genus. Within the genus are found annual and perennial species, those which are well adapted to tropical and sub-tropical regions (mesophytes), and those which are most suited to desert or semi-desert areas (xerophytes). Five cultivated species are included in the genus.

Whitaker and Davis (1962) characterize the genus as follows: Monoecious, annual or perennial, scandent herbs having fibrous, tuberous or tuberculate roots; stems long running or short and bushy, more or less prickly, angled or furrowed, often rooting at the nodes; tendrils

branched; leaves simple alternate, shallowly to deeply lobed, occasionally palmately compound or nearly so; flowers large, solitary, showy, creamy-white to deep orange-yellow, having both calyx and corolla campanulate; anthers free, usually connivent into a long twisted body, the filament partly free; pistilate flowers short pedunculate; pistil oblong or discoid, unilocular with three to five placentae; style thick, the stigmas three each two lobed; fruit a pepo, fleshy or fibrous; seeds numerous flat, smooth, occasionally checked, with or without raised margins, white, tawny, tan, buff, or black.

The taxonomic characteristics of the species used in this study have been described in both Bailey (1943) and Whitaker and Davis (1962). These characteristics as well as other significant information pertinent to each species is as follows:

Cucurbita moschata Duch. ex. Poir was well distributed over a wide area covering both North America and South America as early as pre-Columbian times. It is indigenous to Central America. The first record of seed was found in Huaca Prieta, Peru, dating back to 4000-3000 B. C. Presently C. Moschata has been found in Mexico southward into Guatemala and Panama in Central America, and through Columbia and Venezuela in South America (Whitaker and Davis, 1962). The botanical characteristics of this species are:

> Monoecious, annual, running vines; foliage soft hairy, not harsh or prickly, the leaves shallowly lobed, often with white spots along the veins, calyx tube of staminate flowers short or lacking, the lobes often foliaceous; corolla with

> widely spreading, mostly reflexed lobes; peduncle smoothly angled, expanded or flared at the fruit attachment; fruit variable, usually large, globular, cylindrical, or flattened; seeds with a thin or ragged margin, scalloped or shredded in appearance, the margin more deeply colored than the body of the seed which is 16-20 mm long (Whitaker and Davis, 1962, p. 102).

Cucurbita foetidissima HBK has been classified in a number of genera. In 1817 the name of Cucurbita foetidissima was postulated by Humboldt, Bonpland, and Kunth. The plant was first made known botanically to North America in 1820 by James as Cucumis perennis. Asa Gray transferred it in 1852 as Cucurbita perennis, and under this name it remained until in 1881 when Cogniaux restored the name Cucurbita foetidissima of Humboldt, Bonpland, and Kunth. The natural habitat of C. foetidissima is the hard and waste dry land, prairies, old washes from Nebraska, Missouri, Kansas to Colorado, Utah, Nevada, Texas, New Mexico to California and to Mexico as far south as Guanajuato. The well known, long-lived dull green or gray harsh plant often produces colonies yielding abundant crops year after year (Bailey, 1943). The characteristics of this species were reported by Bailey (1943, p. 313) as follows:

> Variable coarse rough perennial, eventually forming large persisting colonies, plant commonly but not always strong-smelling; root fusiform, perpendicular, and sometimes 2m deep and 25 cm thick; axis angled and cornered, muriculate and sometimes thinly pubescent; tendrils stout but remaining short, the branches mostly coiled into a head and the plant mostly confined ground or covering wastes; leaves diverse, gray-green to cinereous mostly triangular-ovate, 15-20 cm long and two-thirds as broad at base, openly cordate to nearly truncate,

apex acute and usually mucronate, sometimes angled, in the United States commonly not lobed but in Mexico sometimes prominently lobed; margins sinuate-denticulate to practically entire, upper surface very veiny and stiffly pubescent and roughly muriculate; staminate flowers large, 10-12 cm long, corolla ribbed and veiny, rough pubescent, lobes broad and apiculate; calyx-tube 15-20 cm long; pistillate flowers 9-10 cm long, ovary pubescent, corolla pubescent, much ribbed and veined; calyx lobes narrow, 1 cm long more or less; pepo slightly oblong--globular, 6-7 cm high and commonly a little broader, weighing 6-7 ounces when fresh, 3-loculed, dull light green marked with 5 or 6 main cream-white stripes and a few intermediate ones and many narrow mottlings; peduncle angled, expanded at attachment; seeds small, oblong-ovate, 12 mm long, 6-7 mm broad, immarginate, edges obtuse.

All species of Cucurbita contain 2N = 40 chromosomes (Whitaker and Davis, 1962, p. 102).

Passmore (1930) studying microsporogenesis in C. pepo, found 20 bivalents at metaphas 1 of meiosis. She also verified earlier work done by Castetter (1926) on C. maxima, showing it to contain 20 bivalents as well.

Yamane (1950) and Ruttle (1931) showed that C. moschata, C. maxima and C. pepo all contained 2N = 40 chromosomes and Hayase (1951) working with the two species C. moschata and C. maxima confirmed they indeed contained 20 (N) chromosomes.

Chromosomes numbers of three wild species and one domestic species were recorded by McKay (1931). Cucurbita digitata, C. palmata and C. foetidissima were all found to contain 2N = 40 chromosomes. In addition, the chromosome number of the Fig-leaf or Malabar gourd C. ficifolia was reported to be 2N = 40.

Finding that both wild xerophytes and mesophytes as well as the cultivated species all contain the diploid number of 40 chromosomes indicates how closely associated the species members are. This has led several workers to examine the feasibility of making interspecific hybrids and both xerophyte-xerophyte and xerophyte-mesophyte interspecific hybrids have been successfully produced. In addition some domestic-wild species crosses have been tried.

Interspecific Hybrids Involving the Wild Species

Interspecific hybrids and compatibilities involving several wild species have been extensively studied by Bemis and Nelson (1963). Interspecific crosses were made between two xerophytic species from Southwestern United States, C. palmata and C. digitata, and a third, C. cylindrata which is indigenous to Baja, Mexico. Each species is cross compatible producing fertile F_1 hybrids. A fourth xerophytic species, C. foetidissima, was found to be completely incompatible with the other three xerophytes since no viable embryos were ever produced in attempts to cross the species. Four mesophytes were also used in this study. Cucurbita sororia and C. radicans were totally cross compatible, yielding fertile F_1 plants. Cucurbita Lundelliana was not compatible with either C. sororia or C. radicans. Results from this study indicate that C. palmata, C. digitata and C. cylindrata are all closely related species, C. foetidissima is more distant from these three xerophytes and that the four wild mesophytes are quite distinct from all of the four xerophytes.

Groff and Bemis (1967b) reported the bivalent frequency of the hybrids between C. cylindrata, C. palmata and C. digitata. Their findings indicate that nearly 100% of the cells showed 20II, with the occasional presence of univalents and multivalents which were no more frequent than in each diploid species. This strong evidence of absolute homology between the chromosomes of these species suggests close evolutionary ties. There was no loss in fertility of the hybrids either in the F_1 or other generations and the hybrids were often more vigorous than their parents.

Interspecific Hybrids Involving the Cultivated Species

Bailey (1929) described a successful interspecific hybrid cross between C. pepo (female) and C. moschata (male) which yielded a few F_1 seed. These viable seeds were grown out and successive generations were produced. The work done by Castetter (1930) showed that this cross was accomplished in both directions, but reasonably fertile F_1 and F_2 and later successive generations came about only when C. pepo was used as the female parent. The F_1 hybrid, C. pepo x C. moschata was however reported to be completely self sterile in another study (Whitaker and Bohn, 1950).

Another series of crosses involving C. pepo and C. maxima has been attempted by several investigators. Castetter (1930) showed that the hybrid with C. pepo as the female parent was reasonably fertile and a large F_2 population was grown out. The reciprocal cross however, yielded 100% self sterile F_1 hybrids. Weiling (1959) reported on this

hybrid in a rather extensive study of meiosis in the F_1 population. He observed that the C. pepo x C. maxima hybrid had a mean of 12.9 univalents per each metaphase configuration whereas the C. maxima x C. pepo hybrid had a mean of only 7.3 univalents per cell. This was evidence that several chromosomes from each species had the ability to pair during meiosis. Although the F_1 with C. maxima as the female, was totally self sterile, it produced more synapsed chromosomes according to Weiling. The percent normal quartets (4 microspores, 0 micronuclei) observed in the C. maxima - C. pepo cross was higher than the reciprocal hybrid, 61.1 ± 0.25 vs. 43.5 ± 0.19. Since the C. pepo - C. maxima cross produced more univalent it seemed probably that there would have been more cells with micronuclei than in the reciprocal cross.

In studying the amphidiploid between C. maxima and C. pepo, Whitaker and Bohn (1950) showed it to be nearly 100% sterile. The sterility was not a result of a chromosome imbalance since each chromosome had a suitable pairing partner, but rather a result of some genic disturbance.

An interspecific hybrid which has been studied by numerous workers is that involving the cross C. maxima x C. moschata and the reciprocal. Table 1 is a summary of the work done by some of these individuals. As indicated by Table 1 there is only slight chance that the F_1 will be productive enough to yield progeny. Greater success in producing F_1 plants was achieved when C. maxima was the male parent in a backcross.

Bemis and Nelson (1963) showed that viable seed, producing F_1 plants was possible when either C. maxima or C. moschata was used as the

Table 1. Compatibility Studies on the Interspecific Hybrid between C. maxima and C. moschata.

Cross		F_1 Self Fertility	Microsporogenesis: Number of Univalents	Microsporogenesis: Normal Quartets	Microsporogenesis: % Viable Pollen	Backcross Parent: Max*	Backcross Parent: Mos*	Author
Max x Mos	(2N)	Completely Sterile				Success		Castetter (1930)
Max x Mos	(2N)	Completely Sterile		Many abort		Success (F_1 as)		Whitaker (1933)
Max x Mos	(2N)	Nearly Sterile	36-38	5-6/PMC**	1.0-5.0	___	___	Pearson, Hopp and Bohn (1951)
Mos x Max	(2N)	Nearly Sterile	36-38	___	___	Success	Very Slight	Pearson et al. (1951)
Max x Mos	(2N)		0-2		10.2			
Mos x Max	(2N)		0-2		0.2			Yamane (1953)
Max x Mos	(2N)		9.8	35.6%				Weiling (1959)
Max x Mos	(4N)	Slightly Fertile				Cross Sterile	Cross Sterile	Whitaker and Bohn (1950)

* Max and Mos refer to Cucurbita maxima and C. moschata respectively.
** PMC - pollen mother cell.

female parent. The F_1 seed development using C. maxima as the female parent achieved 91% of the normal diploid where as F_1 seed using C. moschata as the female parent attained only 69% of normal development.

Whitaker and Bohn (1950) reported on the amphidiploid between these two species and showed that the fertility of the F_1 increased over that of the allodiploid but complete sterility resulted when the amphidiploid was crossed with either diploid parent.

Other crosses involving the compatability of the cultivated Cucurbita have been somewhat successful. Grebenscikov (1965) studied the crosses C. ficifolia x C. maxima and C. ficifolia x C. pepo and found both F_1s to be 100% sterile. Another investigation of meiosis in the F_1 hybrid between C. maxima and C. ficifolia showed a mean of 23.6 univalents per cell with about 37.0% normal quartets (Weiling, 1959). He also examined the meiotic process in the C. mixta x C. pepo F_1 and showed it to be nearly normal. The mean number of univalents per PMC was 1.4.

With respect to the 5 cultivated species, there is a great degree of homology between chromosomes from one species to another. One perennial, C. ficifolia, is the most far removed of the species. Cucurbita moschata appears to be more centrally related to the other annual species (Whitaker and Davis, 1962, p. 110). Why these species are incompatible is not known. It was suggested that complete pollen tube growth is inhibited in certain species crosses (Hayase, 1950). Whitaker and Davis (1962, p. 114), citing unpublished work of Bohn on crosses involving the annual species, suggested that malfunction of endosperm leads to embryo starvation and subsequent abortion.

To this point evidence has clearly shown that (1) there is a distant similarity between the annual cultivated species based on compatibility and viability studies, (2) there is an extremely close relationship between three xerophytes, C. palmata, C. digitata and C. cylindrata, and the fourth xerophytic species, C. foetidissima, is not compatible with these three xerophytes nor are several of the wild mesophytes.

Interspecific Hybrids Involving the Domestic and Wild Species

Several reports on the successes of wild-domestic interspecific hybridization in Cucurbita have been cited. Perhaps the most widely reaching series of crosses involve C. lundelliana, the Peten gourd. Whitaker (1956) described the successful crosses involving C. lundelliana and the five cultivated species. Table 2 shows a summary of his data. The first three crosses showed good self and backcross fertility. These examples indicate the chromosome similarities between some cultivated and wild species. It appears that C. lundelliana is perhaps a close evolutionary link between the domestic and wild species.

The cross between C. moschata and C. lundelliana has been investigated because of the high degree of fertility in the F_1 and all other generations. Whitaker (1959) extensively studied F_1, F_2 and backcross (BC) generations. The mean pollen fertility for F_1, F_2, BC mos. and BC lund. was 42%, 39%, 48% and 76% respectively. Restoration of pollen fertility seems to be maternally affected based on the backcross variation from one parent to the other. In another study involving C. moschata and C. lundelliana, under normal conditions a mean of less than

Table 2. Crosses between C. lundelliana and Some of the Cultivated Species of Cucurbita.

Cross*	Metaphase I Configuration	F_1 Fertility	Backcross Successful to	Stainable Pollen(%)
Lund. x Mos.	19-20 bivalents	good	either parent	42
Lund. x Max.	19-20 bivalents	good	either parent	17
Lund. x Fic.	19-20 bivalents	good	either parent	15
Lund. x Pepo	many seed needing embryo culture			
Lund. x Mix.	many seed needing embryo culture			

*Lund. - C. lundelliana, Mos. - C. moschata, Fic. - C. ficifolia, Pepo - C. pepo, Mis. - C. mixta.

one univalent per pollen mother cell (PMC) was observed, demonstrating the tremendous affinity between the two different genomes (Groff and Bemis, 1967a).

Whitaker (1962) reported on the interspecific hybrid between C. maxima and C. lundelliana in great detail. He found that there was much male sterility associated with the BC to the C. maxima parent. Mean pollen fertility for F_1, F_2, BC lund. and BC max. was 17.6%, 28.4%, 65.9% and 5.2% respectively. Similar results were obtained by Grebenscikov (1965). He also found that the hybrid between C. sororia and C. pepo yielded fertile F_1, F_2 and BC progeny.

Other wild-domestic crosses have been attempted. Bemis (1963) made a series of several crosses involving C. moschata with C. foetidissima, C. palmata, and C. digitata. Each cross was successful and with the aid of embryo culture, F_1 plants were produced which were 100% male and female sterile.

Grebenscikov (1958) first reported on the cross C. moschata x C. foetidissima. The F_1 was very vigorous once it started growing, but the plants were totally sterile and the male flowers showed complete anther abortion. In the same cross, Bemis (1970) extensively examined the allodiploid, alloautotriploid and amphidiploid. He showed chromosome configurations of 8 II and 40 II for the allodiploid and amphidiploid respectively. The amphidiploid, although it showed normal pairing, was completely male sterile, indicating a genetic block to normal pollen development. This plant was female fertile and was used as the female to create alloautotriploids. Two cultivars of C. moschata were used as the pollen source. One of the cultivars from Puerto

Rico restored male fertility, that is the anthers were functional and pollen was dehisced in the alloautotriploid. The other cultivar 'Butternut,' which was the original C. moschata parent in the hybrid, failed to restore fertility, the anthers totally collapsed at dehiscence. Groff and Bemis (1967a) found that there was a range of 2-10 microcytes per each quartet configuration with 34.5% of the cells having the normal quartet of microspores. They further observed that several cells had only 2 microcytes, a phenomenon associated with nuclear division into two masses in place of normal meiosis. This took place only in sterile material.

The fact that highly successful interspecies crosses can be made between domestic and wild species lends itself to genetic manipulation and an increase in genetic diversity of the cultivated species. As Rhodes (1959) pointed out, it is easier to transfer genes from one cultivated species to another using C. lundelliana in a multispecies cross. Cucurbita lundelliana has many horticulturally desirable characteristics including powdery mildew resistance, perennial growth habit and ease of fruit set. Highly successful crosses such as(C. lundelliana x C. moschata)x(C. mixta x C. moschata) yielded fertile offspring possessing characteristics from all three parents. One fact that these crosses were accomplished is most surely due to increase in the genetic diversity of the gametes. Wall and York (1960) studied the cross involving C. pepo and C. moschata and found a tremendous increase in viable seed production using the double cross (F_1 cultivar of hybrid C. moschata x F_1 cultivar of hybrid C. pepo). They explained that to be due to successful gene combinations.

Genetic or Genic Interspecific Incompatibility

Stephens (1946) has described the genetics of "Corky," a type of incompatibility between two species of cotton. Interspecific hybrids between certain strains of Gossypium hirsutum and G. barbadense are abnormal. The abnormal hybrids have a characteristic bushy habit owing to their shortened internodes and excess production of lateral branches. Leaves are inrolled and exhibit a yellowish mottling; and stem, petiole, and leaf midribs tend to be covered with a thick layer of cork. Such 'Corky' types are more or less female sterile.

Based on genetic data from many crosses and populations, Stephens formulated an hypothesis of one locus and three alleles. Both species of Gossypium carry a common allele "CK" which is described as a non-carrier allele. This allele conditions a normal plant regardless of the second allele. Each species also has a recessive or carrier allele. Corky symptoms depend on the interaction of complementary carrier alleles, ck^h carried by the hirsutum parent and ck^b carried by the barbadense parent. True breeding corky types cannot therefore be isolated. The diagrams in Fig. 1 show the genetic hypothesis of corky.

A second example of genetic interspecific incompatibility is that of Bemis and Kedar (1961) who were studying interspecific Phaseolus hybrids. These authors were not aware of Stephens' publication in 1946 and yet developed an hypothesis identical to that of Stephens.

The interspecific incompatibility between Phaseolus vulgaris and P. coccineus was manifested by abnormal seedling development which was lethal in one case and sublethal in another. The sublethal pheno-

GOSSYPIUM HIRSUTUM

Non-carrier CkCk

H-carrier $ck^h ck^h$

GOSSYPIUM BARBADENSE

Non-carrier CkCk

B-carrier $ck^b ck^b$

H-carrier $ck^h ck^h$ x B-carrier $ck^b ck^b \longrightarrow ck^h ck^b$

normal x normal $\longrightarrow$ corky

Non-carrier CkCk x Corky $ck^h ck^b \longrightarrow Ckck^h : Ckck^b$

normal x corky $\longrightarrow$ normal

(a) $Ckck^h$ x $ck^h ck^b \longrightarrow Ckck^h : Ckck^b : ck^h ck^h : ck^h ck^b$

$Ckck^b$ x $ck^h ck^b \longrightarrow Ckck^h : Ckck^b : ck^b ck^b : ck^h ck^b$

normal x corky $\longrightarrow$ 3 normal : 1 corky

Data from 13 crosses:	normal	corky
Total	164	55
Expected (3:1)	164.25	54.75

(b) $Ckck^h$ x $ck^h ck^h \longrightarrow Ckck^h : ck^h ck^h$

normal x normal $\longrightarrow$ normal

$Ckck^b$ x $ck^h ck^h \longrightarrow Ckck^h : ck^b ck^h$

normal x normal $\longrightarrow$ normal : corky

Data from 57 crosses:	Normal (no-seg.)	seg. corky & normal
Total	32	25
Expected (1:1)	28.5	28.5 (X^2 = .86)

Fig. 1. Genetics of Corky (Stephens, 1946).

type was capable of producing pollen but was incapable of producing fruit or seed.

When the P. vulgaris parent was the cultivar 'Blue Lake' the entire hybrid population was of a B-type dwarf. This type of abnormal seedling was essentially lethal in that it rarely survived through the development of the first trifoliate leaf, and never past the development of the third trifoliate leaf. The leaves were smooth but restricted in size, and even though the seedling remained alive for several weeks, the cotyledons remained unabsorbed.

When the cultivars 'Tendergreen,' 'Light Red Kidney' or 'White Marrow' were used as P. vulgaris parent, the hybrid populations were all of a T-type dwarf. The leaves of these hybrids were restricted in size, rugose, and stippled with brown spots. The stems were also stippled. The cotyledons were absorbed and growth continued in a restricted manner. This type of morphologically abnormal plant produced flowers that were normal in size and containing viable pollen. Growth has been maintained for over a year with continued flowering but fruit and seed were never produced. A fifth P. vulgaris cultivar 'Canocel' produced only normal hybrids when crossed with P. coccineus. All intraspecies crosses among the five P. vulgaris cultivars produced only normal progeny.

The genetic results of additional crosses were explained by an hypothesis identical to the one proposed by Stephens (1946) except in the case of Phaseolus two independent loci were involved instead of one. The interesting segregation was the interspecific hybrid population of the F_1 (Tendergreen x Blue Lake) when used as P. vulgaris parent.

This hybrid population was made up of four phenotypes in a 1:1:1:1 ratio. The phenotypes were B-type dwarfs, T-type dwarfs, TB-type dwarfs showing the characteristics of both B and T-type dwarfs and normal seedlings. The genetic system is summarized as follows in Figures 2 and 3.

Genic Sterility

The distinction between genic and chromosomal sterility was made by Dobzhansky (1951, p. 215). He stated that genic factors may be responsible for abortion even though each chromosome has an identical pairing partner. In addition, normal development may proceed to the point of the production of a mature gametophyte, but then suddenly the gametophyte collapses, rendering the plant sterile. As Stebbins (1971, p. 123) pointed out, there is no simple mechanism to explain the operation of male sterility. It is a highly complex phenomenon not necessarily resulting from the action of one deficient gene. It is more likely a result of the interaction between two or more loci which may even be located on different chromosomes. Stebbins described the mode of action of genic sterility by demonstrating the makeup of complex enzyme systems. Given that a gene is altered (mutation), its product and the enzyme subunits will also be changed, rendering it incapable of forming necessary multimeric association with other subunits. This alteration may make the enzyme complex useless, which in turn could lead to sterility if its function was vital to organismic development.

In hybrids, genetic disruption which affects developmental process of the zygote, embryo or young organism leads to hybrid inviability whereas if the gametes or gametophyte are affected, hybrid sterility results (Stebbins, 1958).

P. vulgaris x *P. coccineus*	Seedlings		
	Normal	T-dwarf	B-dwarf
Blue Lake (B.L.) x Scarlet Runner (S.R.)	-	-	46
Tendergreen (Tend.) x " "	-	88	-
Light Red Kidney (L.R.K.) x "	-	18	-
White marrow (W.M.) x "	-	20	-
Canocel (Can.) x "	9	-	-

P. vulgaris x *P. coccineus*	Normal	T-dwarf	B-dwarf	TB-dwarf
(Tend. x B.L.) F_1 x S.R.	28	26	28	29
(L.R.K. x B.L.) F_1 x "	10	9	9	4
(B.L .x W.M.) F_1 x "	8	4	11	4
Total	46	39	48	37
Expected 1:1:1:1	42.5	42.5	42.5	42.5

$X^2 = 2.00$ P = .50-.70

Fig. 2. Interspecific Seedling Abnormalities (*Phaseolus*)

P. Vulgaris	genotype	P. coccineus	genotype
B. L.	TT $b^V b^V$	S. R.	$t^C t^C b^C b^C$
Tend., L.R.K., W.M.	$t^V t^V$BB		
Can.	TT BB		
(B.L. & Tend) F_1	$Tt^V Bb^V$		

Phenotype	genotype
Normal	Any combination other than $t^V t^C$ or $b^V b^V$
B-dwarf	T-$b^V b^V$
T-dwarf	$t^V t^C$B-
TB-dwarf	$t^V t^C b^V b^C$

P. vulgaris x P. coccineus	Normal	T-dwarf	B-dwarf	TB-dwarf
(Tend. x B.L.) F_1 x S.R.	28	26	28	29
Tt^V Bb^V x $t^C t^C b^C b^C$	$Tt^C Bb^C$	$t^V t^C Bb^C$	$Tt^C b^V b^C$	$t^V t^C b^V b^C$

Fig. 3. Two Loci, Three Allele Hypothesis.

Genic sterility can also result from an upset in the various meiotic steps leading to the formation of the mature gametophyte. Sometimes meiosis is interrupted or completely lacking as Groff and Bemis (1967a) noted in sterile interspecific hybrids of Cucurbita.

Male sterility is associated with abnormal tapetal behavior. Tapetal cells in some cases of genic male sterility may degenerate prematurely. Rick (1948) reported this occurrence in some male sterile tomato mutants. The tapetum can also persist and actually enlarge, crushing developing microspores. Artschwager (1947) noted this enlargement in male sterile sugar beets. Tapetal expansion into the anther cavity was observed by Zenkteler (1962) in cytoplasmic-genetic male sterile carrots. Francis and Bemis (1970) noted this enlargement of the tapetum in a male sterile Cucurbita mutant.

Microspores sometimes abort before the formation of mature pollen. This phenomenon was noted by Bohn and Whitaker (1949) in a male sterile muskmelon mutant. Mature pollen can form with subsequent abortion and the anthers may abort before anthesis. Francis and Bemis (1970) reported anther collapse in the genic male sterile mutant of Cucurbita maxima. This was also observed in another mutant of Cucurbita by Shifriss (1945).

Gabelman (1956) noted that male sterility can manifest itself in one of three ways: (1) as pollen sterility due to the abortion of pollen, (2) as staminal sterility due to destruction, malformation or total absence of stamens, and (3) as functional pollen sterility due to failure of anther dehiscence.

Chromosomal Sterility

Chromosomal sterility results from structural differences between potentially homologous chromosomes from two different parents. These structural dissimilarities may be of such magnitude that there is little or no pairing between homologous chromosomes or there may be complete pairing if the structural differences between the two homologues are minutes. The minute differences between homologous chromosomes have been termed cryptic structural hybridity by Stebbins (1945).

Evidence for cryptic structural differences has been shown. Stebbins (1971, p. 119) pointed out that paired chromosomes containing cytological markers such as knobs and constrictions show these markers in different positions from one homologue to another, that pachytene analysis has revealed small bulges and folds between paired chromosomes. Preferential pairing which is greater affinity of a chromosome for an exact homologue also has been used to support the evidence for these cryptic differences. By doubling chromosomes, if the resulting amphidiploid has become fertile, even though the diploid showed 100% bivalent formation, then cryptic structural hybridity most probably was the cause of sterility. An example shown by Newton and Pellew (1929) involved the hybrid between Primula verticullata and P. floribunda. The diploid showed mostly bivalent association and yet it was completely sterile, whereas the allotetraploid which also had mostly bivalents, was completely fertile. The explanation is that there was much structural hybridity between paired chromosomes from the two different

species. With mostly bivalents formed, there was crossing over and chromosomes segregation led to genetically imbalanced gametes which subsequently aborted. In the allotetraploid each chromosome had a partner that was structurally identical thus bivalent separation led to no imbalance and therefore fertility was restored.

Homoeologues may be incapable of pairing. An example of this extreme involves the intergeneric cross Brassica oleracea and Raphanus sativus. The diploid hybrid showed a total lack of synapsis with almost 100% univalent formation (Darlington, 1958, p. 41). From this it has been shown that the presence of univalents or a loose chromosome association does not necessarily preclude structural dissimilarity. In fact, the lack of chromosome association may be of genic nature.

Polyploidy

Organisms containing two or more genomes from the same species have been referred to as autoploids whereas those with genomes from two or more different species are called alloploids.

Diploidy

Individuals which contain two genomes derived from the same species are known as autodiploids or simply diploids. Diploid organisms maintain a high degree of fertility because each paternal chromosome has a maternal homologous partner. The meiotic process is normal insofar as only bivalents are produced.

Diploid individuals resulting from interspecific or intergeneric crosses have been referred to as alloploids. Very frequently, allodiploids are sterile because of many factors. One of the more obvious factors involves the failure of total bivalent formation. The cause could be genic, but most certainly chromosomal sterility due to the vast differences of the two parental genomes, is also responsible. These homologues may no longer be capable of pairing resulting in univalent formation. The production of univalents leads to sterility because of random chromosome distribution to one pole or the other at anaphase I, creating a genic imbalance. The production of gametes with duplications and deficiencies in great numbers is usually not tolerated.

Very often homeologous chromosome pair, at least initially but a failure of chiasma formation may lead to premature separation and yielding univalents at metaphase I.

Dutt and Roy (1971) investigated interspecific hybrids of Luffa. They studied crosses involving three species of n = 13, Luffa acutangula (A), L. graveolens (G) and L. echinata (E). In the hybrid AG they found the mean of bivalents to be 12.3 whereas EG and GE showed total bivalent means of 6.88 and 9.40 respectively. The chromosome distribution in cells which both received the same number of chromosomes was quite similar between the different crosses. In the AG and EG crosses, 76% and 72% of the cells respectively showed equal chromosome distribution to each pole. With only 0.65 univalents per each PMC in the AG cross, it was apparent that the two species were closely related. There must have been a great deal of cryptic structural hybridity or genic disturbance because there was no stainable pollen.

In another study, Dunford (1971) examined interspecific hybrids between 9 species of Grindelia (n = 6). He found that all of the species but Grindelia squarrosa were closely related to each other. The hybrids showed a percent stainable pollen range of 65-99. All the hybrids studied had 6 bivalents except those involving G. squarrosa as one parent. When this species was involved in hybrid combination, the range of stainable pollen was 34%-48%, and a configuration of 4 II and 1 IV was observed at metaphase I.

Pollen fertility is normally very low in interspecific diploids. Species crosses in zinnia reported by Ramalingam, Sree and Raman (1971) showed only 6.6% pollen fertility whereas both parents had greater than 90% pollen fertility. Also a common occurrence, that of micronuclei, was observed in over 80% of the cells. Unusual microspore numbers were observed by Abdel-Hameed (1972) in the hybrid between Clarkia lussensis and C. amoena spp. Huntiana. He found 4-13 microspores per PMC. Of the cells scored, 52.7% contained 6 microspores whereas only 11.1% showed the normal 4 microspore quartet.

Triploidy

Three distinct types of triploids are known: (1) autotriploids are those which contain 3 genomes from the same species, (2) alloautotriploids which contain 2 genomes from one species and a third genome from another species, and (3) trispecies and triple haploids which contain one genome from each of three different species. Only the latter two will be discussed.

Alloautotriploids would be expected to produce bivalents and univalents. Two genomes from one species would pair and the third genome from the other species would lack a homologous partner. If however the two species were closely related, then latent homology might still be present, enabling homeologous association to occur. Bemis (1970) showed a photomicrograph of metaphase I in the alloautotriploid between Cucurbita moschata and C. foetidissima. The configuration was 20 II and 20 I.

Alloautotriploids do not always give pairing configurations to fit the above pattern. There is strong evidence for homeologous association. Chen and Gibson (1970) compared the pairing between two alloautotriploid hybrids. One hybrid involved the cross Trifolium nigrescens (N), 2N = 16 x T. repens (R), 4N = 32. Trifolium repens is a natural tetraploid. The second hybrid was the cross between the induced autotetraploid T. occidentale (O), 4N = 32 and T. nigrescens. Mean III, II and I frequencies for the two crosses (N) x (R) and (N) x (O) were 4.27, 3.73, 3.73 and 5.69, 2.31, 2.31 respectively. From these data it was observed that T. nigrescens and T. occidentale were closely related species since over two thirds of their chromosomes were involved in trivalent association. Examination of the newly synthesized tetraploid revealed almost 60% quadrivalent formation whereas the natural tetraploid showed almost no quadrivalent. This is indicative of diploidization of autotetraploids through their evolution.

Another investigation by Rao, Khan and Khan (1971) revealed that triploids of Solanum were more vigorous than either of their parents, however they were highly sterile with a percent pollen

fertility of only 0.6. The presence of trivalents indicated some similarities between the two species but for the most part they were closely related. The triploid had lagging chromosomes at anaphase I and anaphase II and there were often more than 4 nuclei present at telophase II.

Trispecific and trigeneric crosses have been made although they are extremely rare. Hybrids containing three different species usually are sterile but occasionally they can possess fertility great enough to produce functional progeny. Groff and Bemis (1967a), reporting on a trispecific hybrid, (C. moschata x C. lundelliana) (2N = 40) x C. foetidissima (2N = 40) found it to be completely sterile. The study of chromosome pairing in this hybrid revealed a high number of univalents, 27.6 ± 5.3 at metaphase I, indicating that a mean of only 6.2 bivalents was present.

Another study by Sree Rangasamy, Devasahayam and Raman (1971) involved the progeny of a trispecific cross in Pennisetum. The triple species hybrid was produced by crossing P. squamulatum (2N = 48) x the amphidiploid (P. lyphoides x P. purpurem) (2N = 48). Chromosome homology and fertility of the hybrid suggested close ancestral ties between the three species.

It is therefore possible to create trispecies hybrids which in some cases are functional. With progeny being produced, this opens up another avenue in which new genetic material can be introduced into cultivated plants.

Tetraploidy

Autotetraploids contain 4 genomes from the same species whereas allotetraploids or amphidiploids possess 2 genomes each from two different species.

In newly induced autotetraploids, multivalent associations are frequently present. Since each chromosome is represented by 4 homologues, one would expect a high frequency of quadrivalents as well as bivalents. It is also probable that an occasional trivalent and univalent would be present. Stebbins (1947) explained that sterility in autotetraploids was most likely due to an irregular chromosome distribution caused by meiotic abnormalities of a physiological nature under genetic control and was probably not a result of the unequal segregation of multivalents.

According to Myers (1945), three major meiotic irregularities were observed in the autotetraploid Lolium perenne (n = 7, (1) uneven quadrivalent disjuntion producing chromosome distributions of 13-15, and 12-16, (2) incomplete disjunction of quadrivalent members resulting in lagging and dividing univalent chromosomes and, (3) high number of univalents which either tend to lag and divide precociously or remain in the cytoplasm. From his data, multivalent frequency was unreliable criterion to indicate the stability and fertility of the autotetraploid. Variation in multivalent frequency did not affect fertility. The frequency of univalents and laggers however, was closely correlated to the formation of micronuclei.

Jauhar (1970) noted a gradual shift to bivalent formation after successive generations of selfing the newly induced autotetraploid of Pennisetum typhoides (n = 7). The initial generation of tetraploidy showed a mean of 4.15 IV and after six generations the mean dropped to 1.76 IV. In addition pollen fertility increased from 37.5% to 68.5%. There was a strong indication that the remaining quadrivalents were only loosely connected. It was also suggested that genetic factors played a role in the conversion of high quadrivalent to low quadrivalent formation.

Allotetraploids which contain pairing partners for each chromosome would be expected to behave like diploids, hence the term amphidiploids has been frequently used. If, however, two species were closely related, the resulting amphidiploid between the two species might show other than bivalents at metaphase I.

Rajasekaran (1970) attempted the hybridization of Solanum inducum and S. melongena. He reported that in spite of normal meiosis, the F_1 hybrid was partly sterile with 48.9% stainable pollen. Its sterility is attributed to cryptic structural differences. The amphidiploid (S. indicum-melongena) obtained through colchicine treatment was fully fertile and it is classified as a segmental allopolyploid. Progenies from backcross and open pollination segregated widely combining perenniality and cluster bearing habit of the wild parent and fruit shape and size of the cultivars. This was said to offer prospects for selection of economic types in eggplant breeding.

Rangasamy and Kadambavanasundaram (1974) reported on the above mentioned cross and found that F_1 was sterile. The hybrid sterility was analyzed based upon cytological, morphological and genetical behavior of F_1, amphidiploid, F_2 and backcross progenies. The hybrid sterility might be due to the non-synchronization of chromosomes at the metaphase plate and controlled by genic factors. The non-reduction in chiasma frequency in the hybrid and the occurrence of free recombination in the hybrid derivatives precludes the possibilities of segmental differences between the parental chromosomes. The resulting amphidiploid of the hybrid (S. inducum x SM.34) did not restore fertility and this formed a very concrete proof in supporting the genic cause for the hybrid sterility. The occurrence of petaloid anthers in some of the male sterile segregants in the F_2 melongena backcross progenies also revealed that it was due to diplontic sterility. The fertility levels of the F_2 and backcross progenies ranged from 0-89% with a continuous variation indicating the sterility must be governed by major genes with modifiers involved. There are indications which revealed that male and ovule sterility in this species cross must be governed by different independent non-allelic genes or gene complexes.

A comparative analysis of micro- and macrosporogenesis, development of the male and female gametophytes in an amphidiploid of N. tabacum L. was carried out by Bannykova and Ovsyannykova (1975). In the given amphidiploid about 80% of pollen grains were normal. During the investigation of the female gametophyte some deviations from the normal development and constitution were found. Twelve percent of

the embryo sac degenerated at the 2nd and 4th nuclear stages. Non-coincidence in the rate of development of male and female gametophytes was observed. The delayed rates of development of a female gametophyte led to the break in correlation between the function of a pollen tube, style and embryo sac. The lowered fertility of amphidiploid is determined, first of all, by abnormalities in macrosporogenesis and the development of female gametophytes, as the percentage of normally formed pollen grains is sufficiently high that normal pollination and fertilization could occur.

Khasha and Sadasivaiah (1971) gave a detailed report on the genomic relationship between Hordeum vulgare and H. bulbosum. Several interspecific crosses and levels of polyploidy were studied by these authors. The diploid H. vulgare had a mean of 7 II whereas the autotetraploid had a mean of 4.67 II and 4.64 IV. Similar results were obtained with the diploid and autotetraploid H. bulbosum plants. The F_1 interspecific hybrid revealed a mean frequency of I and II to be 3.92 and 5.04 respectively. The alloautotriploid containing two genomes of H. bulbosum and one genome of H. vulgare showed mostly univalents and bivalents in about equal numbers but trivalents were also observed, indicating some homology between the genomes of the two species. In fact only 19.3% of the cells of the alloautotriploid showed 7 II and 7 I where as 73% of the cells had from 1-5 III.

Although much homology existed between the chromosomes of the two species, most of the hybrids were quite unstable. Allodiploids and amphidiploids were very rare and when these crosses were made in either direction, the resulting progeny usually lacked the chromosomes

from H. bulbosum. Similarly, the cross between the H. vulgare autotetraploid and the H. bulbosum diploid resulted in H. vulgare progeny. The only chromosomally stable hybrid was that produced by the cross between the autotetraploid H. bulbosum and the diploid H. vulgare. The mechanism involved in the selective elimination of H. bulbosum chromosomes must be stabilized when two genomes of H. bulbosum and one of H. vulgare are present.

It can be concluded that both genic and chromosomal factors affect chromosome pairing. The appearance of 100% bivalents does not necessarily assure fertility nor does the presence of multivalents and univalents necessarily reduce fertility.

CHAPTER 3

MATERIALS AND METHODS (AMPHIDIPLOIDY)

Materials

The source of plant material used in this study consisted of one wild species, C. foetidissima, and five cultivars of C. moschata which do not cause genic male sterility with C. foetidissima.

Colchicine Treatment

Colchicine has been used for years as an effective chemical agent capable of inducing chromosome doubling in somatic cells. It can be applied as an aqueous solution or in a paste mixed with a substance such as lanolin.

Francis (1973) reported that the most successful time of treatment in Cucurbita is at the seed or seedling stage using a concentration of 0.2 and 0.4% respectively. Treatment of the apical portion of a mature stem was never successful, most probably because the colchicine never completely penetrated the tightly compacted cell layers which surround the meristematic region.

Seed Treatment

Seeds from both species were presoaked in water for 24 hours to initiate the early processes of germination. Then the seeds were soaked in a .2% aqueous solution of colchicine for 24 hours, thoroughly rinsed in water. When germinated, the seedlings were transferred to

glass vials filled with pyrex glass wool and watered with a nutrient solution (Randolph and Cox, 1943). The vials were then placed in a growth chamber set at 30°C and 35°C night and day temperatures respectively. After the second leaf stage the seedlings were freed from the glass wool, transferred to peat pots containing sterilized soil mix, returned to the chamber until established after which they were transferred to the ground bed of the greenhouse.

Seedling Treatment

Seeds were soaked in water in petri dishes until germinated, transferred to glass vials filled with pyrex wool, transferred to the growth chamber set at 30°C night and 35°C day temperatures and watered with a nutrient solution. When the cotyledons expanded and the plumule was barely visible, the nutrient solution was drained from the vials and they were inverted on a tray such that the cotyledons and growing point were immersed in a 0.4% colchicine solution for 14 hours. The seedlings were then rinsed in water for 24 hours and transferred to peat pots containing a sterilized soil mix, returned to the growth chamber until they showed active growth after which they were transferred to the ground bed of the greenhouse.

Cultural Aspects

All plant material was grown in a plastic greenhouse of the Department of Plant Sciences, located at The University of Arizona Campbell Avenue Farm. Three plants were grown per each six foot of ground bed space. Twine was suspended from wires to be used for the vertical support of the growing vines.

White flies and spider mites were the main insect problems. The white flies were controlled with Cygon and Malathion while the spider mites were controlled with Kelthane. Powdery mildew was not a persistent disease problem and whenver it appeared, it was controlled by spraying with Morestan.

Stomatal Analysis

The time between treating seeds and seedlings with colchicine and chromosome verification of a successful treatment often took four months or longer. Colchicine is a potent poison which greatly affects plant development so consequently, the seedlings were very slow to develop. A faster method of verification is to measure stomate length. The length of a stomate is considerably greater in plants whose chromosomes have been doubled. This method was studied and proved quite successful by Francis and Bemis (1974).

Cytological Method

Male buds for the study of meiosis and verification of autotetraploids were collected from each plant between 6:00 and 8:30 a.m. The collected buds were killed and fixed in a freshly prepared Carnoy's solution made of 3 parts 95% ethanol and 1 part glacial acetic acid. Occasionally, excessive amounts of lipid material inhibited the analysis, so chloroform was added to the killing solution. The proportion of 95% ethanol-glacial acetic acid-chloroform used was 65-20-15 respectively. After the buds were in Carnoy's fixative for at least 24 hours, they were transferred to 70% ethanol. Following 2-3 rinses in this solution, they were placed in Snow's carmine containing a few drops of

ferric citrate (Snow, 1963). The buds were removed from this solution after a period of at least 48 hours and they were then washed in 70% ethanol several times and stored in same until analyzed.

Bud material was crushed on a slide containing a drop of aceto-carmine stain, then cleaned off and a cover slip placed over the remaining material. Alternate heating and pressure was employed until the meiotic stages were made as distinct as possible. If the cytoplasm remained dark after heating, a few drops of 45% acetic acid was placed on the slide next to the cover slip and reheated, with the process being repeated until the desired degree of destaining was reached.

Plant Growth Substances

Cross pollinations were made by hand early in the morning. Fruit set was almost a failure on both parental species. Since plant growth hormones are well known for improving fruit set, a concentration of 50, 100 and 150 ppm of each indole acetic acid, gibberellic acid and kinetin was applied with a fine brush to the stigma just before pollination. The fruits were kept on the vines for 35 days after pollination to allow morphological seed development, and for an additional 30 days after harvest for after-ripening or physiological seed development.

CHAPTER 4

RESULTS

In order to verify the effectiveness of colchicine to double the chromosome number, Stomate analysis was done according to Francis and Bemis method (1974). Six seed treated plants showed a mean increase of 21% over the diploids while six seedling treated plants showed an increase of 53%. From all the plants one was cytological verified as being an autotetraploid by counting the metaphase chromosomes. This was the C. moschata cultivar used in the crosses with autetraploid C. foetidissima.

The production of a fertile amphidiploid using selected cultivars of Cucurbita moschata and C. foetidissima was attempted but unsuccessfully. No data were obtained from this study due to the several problems described below.

During the growing season of 1975 only one C. moschata cultivar produced flowers. The diploid and autotetraploid C. foetidissima did not flower at all. Girdling of the branches and cutting down irrigation intervals were ineffective in enforcing flowering. Consequently no crosses were ever made in that season. Attempts to investigate why the plants did not flower were not made, but limited light intensity in the greenhouse might be a probable cause.

In preparation for the 1976 season, the autotetraploids of both species were propagated asexually and transferred to a section of the greenhouse where there was better light. In this section of the greenhouse the autotetraploid C. foetidissima produced normal male and female

flowers but not a single flower in the section where it was originally planted. In contrast, C. moschata produced normal female and male flowers but the male flowers never reached anthesis, but where it was originally planted both male and female flowers reached anthesis.

The first thing observed was the great non-synchrony in the flowering of the two species which made it impossible to make any pollinations during the first month of the flowering period, but selfing and sib-crossing of C. foetidissima and C. moschata was accomplished.

Later 43 reciprocal cross pollinations were made but without any successful fruit set. Five days after pollination or even later, and after normal fruit development, the fruits started to collapse from the styler end. Soid and fruit samples were examined by plant pathologists and no pathogenic organism was isolated. It was indicated that it might be a genetic or a physiological disorder.

As indicated in the Materials and Methods section, different plant growth hormones were used to aid fruit set. By using this technique, 9 fruits (6 on C. foetidissima and 3 on C. moschata) out of 56 cross pollinations, were set and reached maturity. Of the 3 fruits on C. moschata parent, one was normal and two showed some collapsing of the styler end. None of these nine fruits contained any seed. The series of photographs on the following pages (Fig. 4) illustrate the above mentioned abnormalities and seedlessness.

Thus since not enough data were collected from this study, limited data from previous studies will be used to develop a genetic hypothesis to explain male sterility in certain interspecific crosses of Cucurbita and the possibility of creating a fertile amphidiploid as presented in the Discussion section.

Fig. 4. Various Morphological Characteristics of the Autotetraploid C. moschata, a Primitive Cultivar from Brazil.

a. Normal male and female flowers of C. moschata.

b. A vine of C. moschata showing tip burn of terminal buds, leaves, and female flowers.

c. A normal C. moschata fruit 4 days after cross pollination.

d. C. moschata fruit showing the early stages of styler collapse.

e. An advanced stage of styler collapse of C. moschata fruit.

f. Mature fruits of C. moschata. The fruit on the left is normal for the fruit shape of this cultivar and the two on the right show varying degrees of styler collapse.

g. A seedless fruit from pollination of C. moschata with C. foetidissima.

h. Seedless fruits from reciprocal cross pollination of C. moschata and C. foetidissima.

(a)

(b)

(c)

(d)

Fig. 4. Various Morphological Characteristics of the Autotraploid C. moschata, a Primitive Cultivar from Brazil.

(e)

(f)

(g)

(h)

Fig. 4. Continued.

CHAPTER 5

DISCUSSION

Male Sterility

Sterility is characterized by nonfunctional gametes. It is caused by chromosomal aberrations, gene action or cytoplasmic influences that cause abortion or modification of entire flowers, stamens, or pistils, or that upset the development of pollen, embryo sac, embryo, or endosperm. The particular type of sterility of concern in this study is the type in which the male gametes are rendered nonfunctional as a result of the effects of genes or cytoplasmic factors or by the combined effects of these two factors.

The primary objective of this section is to develop an hypothesis as to which of the above mentioned factors is causing male sterility in certain interspecific crosses in the genus Cucurbita. The hypothesis will be based on limited data reported by Bemis (1970).

In the genomic diagram below, each letter refers to a single genome of that species, for example, the C. foetidissima diploid has the code (FF) whereas the tetraploid of this species which contains 4 genomes would have the code (FFFF). The species used and their genomic content are as shown below.

Cucurbita moschata 'Butternut' = MM

Cucurbita moschata from Puerto Rico = M^1M^1

Cucurbita foetidissima (wild species) = FF

MM x FF ⟶ MF male sterile

MF doubled ⟶ MMFF male sterile

MMFF x MM ⟶ MMF male sterile

x FF ⟶ MFF male sterile

x M^1M^1 ⟶ MM^1F male fertile

x MM^1 ⟶ 12 MMF : 15 MM^1F

male sterile male fertile

Initially MM was pollinated by FF. The resulting MF seed had to be cultured using the embryo culture of Randolph and Cox (1943) modified by the addition of manganese. Seedlings that were started from culture were allowed to develop until the cotyledons expanded and the plumule was barely visible. The seedlings were then submerged in a 0.4% aqueous solution of colchicine for 12-24 hours at 30°C. The resulting amphidiploid (MMFF) was used as the female parent to produce the 4 alloautotriploids. Note that the amphidiploid when pollinated by the MM^1 diploid, produced alloautotriploid progeny which segregated for male sterility and male fertility in a 1:1 ratio.

Now it remains to examine these data to find out whether the male sterility in Cucurbita is caused by genetic, cytoplasmic or cytoplasmic genetic factors.

Cytoplasmic Male Sterility

If the male sterility in Cucurbita is due to cytoplasmic factors, a pattern similar to that found in Mirablis jalapa, the four o'clock plant, can be developed (Correns, 1909). In this plant, a

certain variety had branches that produce either green, white, or variegated leaves. In crosses between flowers of these branches, the offspring are all green if the maternal parent is a flower from a green branch. Furthermore such offspring remain green throughout subsequent generations as long as the maternal plant is green. Similarly, as long as the maternal parent is from a white branch, the offspring are all white. If the maternal parent is mixed green and white or variegated, then the offspring is similarly variegated. But what was found in Cucurbita, and as shown in the genomic diagram, could not be explained by cytoplasmic factors. This is because when the amphidiploid MMFF was pollinated by MM all the progeny were male sterile and when pollinated by MM^1 ($MM \times M^1M^1$) the progeny segregated into male sterile and male fertile in equal ratio in spite of the fact that both the maternal (MMFF) and paternal parents (MM and MM^1) have the same cytoplasm. It is worth mentioning here that in all the diploid crosses, MM was used as the maternal parent, and when FF was used as the maternal parent no hybrid was created.

Cytoplasmic-genetic Male Sterility

Since all Cucurbita parents used in these studies are self fertile and no male sterile plants were found in their progeny, it can be assumed that they are homozygous recessive for the male fertility gene(s). If there is an interaction between the genes and the cytoplasm, different results would be expected from the crosses MMFF x MM and MMFF x FF in which the alloautotriploids (MMF and MFF) have the same cytoplasm, but both the alloautotriploids were completely male sterile.

This indicates that the male sterility in Cucurbita is not due to cytoplasmic-genetic factors as opposed to what was found in Sorghum (Stephens and Holland, 1954). In Sorghum, F_1 from 'Milo' and 'Kafir' was fertile. Repeated backcrosses of the F_1 to 'Kafir' segregated into male sterile and male fertile in unequal ratios and by BC_5 the progeny were almost completely male sterile. On the other hand male sterile plants from the F_2 and BC when backcrossed to 'Milo' all the progeny were male fertile.

Genic Male Sterility

If male sterility in Cucurbita is caused by genetic factors alone, a genetic pattern can be developed which is identical to that resulting in the incompatibility in Gossypium (Stephens, 1946) and Phaseolus (Bemis and Kedar, 1961). The genetic system describing genic male sterility in Cucurbita could be presented as follows:

MM	=	$ms^m\ ms^m$	male fertile
FF	=	$ms^f\ ms^f$	" "
M^1M^1	=	Ms Ms	" "
MF	=	$ms^m\ ms^f$	" sterile
MMFF	=	$ms^m ms^m\ m^f ms^f$	" sterile
MM^1F	=	$ms^m MS ms^f$	" fertile

MMFF x MM^1 MMF = $ms^m ms^m\ ms^f$ } segregate 1 : 1 sterile fertile

MM^1F = $ms^m MS\ ms^f$ }

Cucurbita moschata cv. 'Butternut' (MM) and C. foetidissima (FF) have the carrier alleles ms^m and ms^f. Genic male sterility results

when the two carrier alleles are in various combinations as shown. The presence of the non-carrier allele Ms found in C. moschata from Puerto Rico (M^1M^1) and in other primitive cultivars of C. moschata from Brazil, Cuba, San Cristobal, Mexico, Tehancan, Mexico and Virgin Islands also restores male fertility.

In conclusion it could be hypothesized on the basis of the limited data available, that the male sterility found in the hybrids of C. moschata and C. foetidissima is not caused by cytoplasmic or cytoplasmic-genetic factors. Since MM^1 is heterozygous for the male fertility gene and the segregation into 1:1 ratio, it could only be explained on genetic basis and it is probably that only one pair of chromosomes and only one single locus are involved in the male sterility. It could also be concluded that the male fertility genes are widespread in Cucurbita.

Amphidiploidy

The fertility of interspecific hybrids is affected by the failure of chromosomal pairing during meiosis. If the chromosome pairs fail to synapse at metaphase I of meiosis, the meiotic process becomes highly irregular resulting in a high frequency of aneuploid gametes causing sterility. The degree of fertility in the interspecific hybrid is dependant upon the amount of chromosomal pairing. Hybrid sterility that results from abnormal segregation of chromosomes due to failure of synapsing at meiosis is called segregational sterility (Stebbins, 1970).

Segregational sterility can be partly or completely overcome by doubling the chromosome number of the hybrid thereby creating complete sets of homologues or in other words to create amphidiploids.

Amphidiploids can be created in one or two ways. The first way is to cross the parental diploid species and then double the chromosome number of the hybrid to create the amphidiploid. The second way is to double the chromosome number of the parents or in other words to create the autotetraploids and then make the cross to give the amphidiploid. Whether the amphidiploid is made one way or the other, is very important from a breeding standpoint, as to how much heterozyogosity will be transmitted to the amphidiploid. The following paragraphs will show the two ways by which the amphidiploid can be created.

1. The first way is to hybridize the diploid parental species and then double the chromosome number of the hybrid to create the amphidiploid. Genomically this can be presented in this way:

$$MM \times FF \longrightarrow MF \text{ (Doubled)} \longrightarrow MMFF$$

[Thus homologous chromosomes M and M of Cucurbita moschata, in the amphidiploid MMFF, are very homozygous, having been derived by doubling from M of the hybrid (MF)]. The same is true for chromosomes F and F of C. foetidissima but the more homoeologous chromosomes of the two sets, M and F are heterozygous, as genetically different as were the two original species from which the hybrid was derived. Therefore, the amphidiploid created this way may have a high degree of true breeding permanent heterozygosity.

2. The second way is to double the chromosomes of the parental species and then cross these to create the amphidiploid. This can be presented in this way.

$$\begin{array}{l} MM \text{ (doubled)} \longrightarrow MMMM \\ FF \text{ (doubled)} \longrightarrow FFFF \end{array} \quad x \rightarrow MMFF$$

In these tetraploids, the meiotic process is more complicated than that of diploids since it involves the partition of the eight chromatids of the first meiotic division into four pairs, each pair corresponding to one of the four gametes produces by each sporocyte. In an autotetraploid five genotypes, AAAA (quadruplex), AAAa (triplex), AAaa (duplex), Aaaa (simplex) and aaaa (nulliplex), are possible at each locus. These zygotic combinations arise from the fusion of any two of three different types of gametes, AA, Aa and aa, whose relative frequency, for a given genotype, depends on the cytological events of meiosis. Table 3 shows the gametic ratios expected for each genotype from the two types of partition of chromatids for a single locus, i.e., random chromosome and random chromatid assortment.

Table 3. Gametic Ratios Expected.

Genotype	Random Chromosome Assortment	Random Chromatid Assortment
AAAA	all AA	all AA
AAAa	1AA:1Aa	15AA:12Aa:1aa
AAaa	1AA:4Aa:1aa	3AA: 8Aa:3aa
Aaaa	1Aa:1aa	1AA:12Aa:15aa
aaaa	all aa	all aa

Based on these gametic ratios and the genotype of the diploid parent (whether homozygous or heterozygous for a particular locus), it remains to show how much heterozygosity is transmitted to the amphidiploid. This can be presented as below putting in mind that there are three kinds of heterozygosity:

1. Permanent heterozygosity (AAa^1a^1 or aaA^1A^1) in which the homologues are homozygous but different.

2. Segregational heterozygosity (AAA^1a^1 or aaA^1a^1) in which one pair of homologues is homozygous and the other pair is heterozygous.

3. Segregational heterozygosity (AaA^1a^1) in which both pairs of homologues are heterozygous.

If both parents are homozygous dominant (AAAA) or recessive ($a^1a^1a^1a^1$), the amphidiploid will be permanently homozygous. On the other hand, if one parent is homozygous dominant and the other is homozygous recessive, the amphidiploid will be permanently heterozygous ($AAAA \times a^1a^1a^1a^1 \longrightarrow AAa^1a^1$ or a^1a^1AA).

In the above mentioned cases, each parent produces one kind of gamete under chromosome or chromatid segregation since they are homozygous. But if one parent is homozygous dominant (AAAA or $A^1A^1A^1A^1$) or recessive (aaaa or $a^1a^1a^1a^1$) and the second parent is heterozygous ($A^1A^1a^1a^1$ or AAaa), 83.4% heterozygosity for one pair of homologues will be transmitted to the amphidiploid under chromosome segregation. This includes 16.7% permanent heterozygosity (AAa^1a^1 or aaA^1A^1) and 66.7% segregational heterozygosity (AAA^1a^1 or aaA^1a^1). Under chromatid segregation, the percentage of permanent and segregational heterozygosity is 21.4 and 57.1 respectively. If both parents are heterozygous,

94.4% heterozygosity will be transmitted to the amphidiploid under chromosomal segregation. This includes 5.5% permanent heterozygosity and 89.9% segregational heterozygosity. Under chromatid segregation the percentages are 88.5, 11.6 and 76.9 for total, permanent and segregational heterozygosity respectively. It is worth mentioning that in this latter case, the segregational heterozygosity is contributed by two genotypes. One genotype is heterozygous for one pair of homologues (AAA^1a^1) and the other is heterozygous for both pairs of homologues ($Aa\ A^1a^1$). Although these genotypes occur in equal frequency, $Aa\ A^1a^1$ contributes twice the heterozygosity as $AA\ A^1a^1$.

From the above discussion it is clear that in tetraploids, the frequency of homozygotes is very low, and requires raising a large population of F_2 in order to obtain and select the recessive phenotype. This becomes more complicated when two or more loci are considered. As an example, if we consider two unlinked loci and suppose that one species is AA bb and the other is aa BB and the desired amphidiploid is aaaa bbbb, it becomes a bit difficult to obtain such a multiple recessive type from hybrids. From these species the dihybrid F_1 is AAaa BBbb. When this hybrid is selfed the frequency of the double recessive phenotype is 1 in 1296 on the basis of chromosome assortment and 81 in 37416 (1 in 462) on the basis of chromatid segregation.

Summary and Conclusion

The presence of genic male sterility in Cucurbita is noted by the total collapse of the androecium at anthesis. Chromosomal sterility

on the other hand is manifested by the abortion of the gametes, however the anthers remain intact and dehiscence is noted at anthesis.

Six cultivars of Cucurbita moschata were crossed with a wild species, Cucurbita foetidissima, to create a fertile amphidiploid and to study the inheritance of male sterility found in interspecific crosses made from these two species.

The creation of the amphidiploid was attempted in two ways. The first way was to cross the parental diploid species and then the chromosome number of the hybrid was doubled by treatment with colchicine. The second way was to create autotetraploids of the parental species which were then crossed to create the amphidiploid. Whether the amphidiploid is made one way or the other, is very important from a breeding standpoint, as to how much heterozygosity will be transmitted to the amphidiploid.

Forty-three reciprocal cross pollinations were made but without any successful fruit set. This was due to collapse of the styler end of the fruits 5 or more days after pollination. By using plant growth hormones, 9 fruits out of 56 cross pollinations were set and reached maturity. None of these nine fruits contained any seed.

Data from previous studies were used to develop an hypothesis to explain the genetics of male sterility of different hybrid combinations made from these two species. It was hypothesized that C. moschata cv. 'Butternut' (MM) and C. foetidissima (FF) have the carrier alleles ms^m and ms^f. Genic male sterility results when the two carrier alleles are in various combinations. The presence of the non-carrier allele MS

found in C. moschata from Puerto Rico (M^1M^1) and other primitive cultivars of C. moschata from different regions restore male fertility. It is probable that only one pair of chromosomes and only one single locus are involved in the male sterility. This clearly shows that male fertility genes are widespread in Cucurbita and that a single locus will be a barrier to interspecific hybridization.

The way in which the amphidiploid was made and depending on the amount of heterozyosity of the parents and the way in which the eight chromatids were partitioned during meiosis is important from a breeding standpoint. It was shown how much heterozygosity will be transmitted to the amphidiploid. The distinction was made between permanent and segregational heterozygosity.

LIST OF REFERENCES

Abdel-Hameed, F. (1972), "Chromosome abnormalities in F_1 diploid hybrids between Clarkia lussensis and C. amoena ssp. Huntiana," Genetica 43(1): 3-24.

Artschwager, E. (1947), "Pollen degeneration in male sterile sugar beets with special reference to the tapetal plasmodium," J. Agr. Res. 74: 191-197.

Bailey, L. H. (1929), "The domesticated cucurbitas," Gentes Herbarum 2(2): 63-115.

Bailey, L. H. (1943), "Species of Cucurbita," Gentes Herbarum 6(5): 267-322.

Bannykova, V. P., and O. V. Ovsyannykova (1975), "Causes of decreased fertility of amphidiploids of the genus Nicotiana," DOPV Akad Nauk ukr Rsr B Heol Heofiz Khim Biol. 8: 760-762.

Bemis, W. P. (1963), "Interspecific hybridization in Cucurbita. II C. moschata Poir X xerophytic species of Cucurbita. J. Hered.

Bemis, W. P. (1970), "Polyploid hybrids from the cross Cucurbita moschata Poir X C. foetidissima H.B.K. 1970."J. Am. Soc. Hort. Sci. 95(5): 529-531.

Bemis, W. P., and N. Kedar (1961), "Inheritance of morphological abnormalities in seedlings of two species of phaseolus," Journ. Hered. 52: 171-178.

Bemis, W. P., and J. M. Nelson (1963), "Interspecific hybridization within the Genus Cucurbita. I, fruit set, seed and embryo development," J. of the Ariz. Acad. of Sci. 2(3): 104-107.

Bohn, G. W., and T. W. Whitaker (1949), "A gene for male sterility in the muskmelon," Proc. Am. Soc. Hort. Sci. 53: 309-314.

Castetter, E. F. (1926), "Cytological studies in the Cucurbitaceae. I. Microsporogenesis in Cucurbita maxima," Am. J. Bot. 13: 1-10.

Castetter, E. F. (1930), "Species crosses in the genus Cucurbita," Am. J. Bot. 17: 41-57.

Chen, C. C., and P. B. Gibson (1970), "Chromosome pairing in two interspecific hybrids of Trifolium," Can. J. Gen. and Cyt. 12: 790-794.

Correns, C. (1909), "Vererbungsversuche mit blass (gelb) grünen und bunt blättrigen Sippen bei Mirabilis, Urtica, and Lunaria," Zeit. Induk. Abst. u. Verebung. 1: 291-329.

Darlington, C. D. (1958), The Evolution of Genetic Systems, Basic Books, Inc. Publishers, New York, 265 pp.

Dobzhansky, T. (1951), Genetics and the Origin of the Species, Columbia University Press, New York, 365 pp.

Dunford, M. P. (1971), "Chromosome characteristics of Texas species of Grindelia (Compositae) and some interspecific hybrids," The W. W. Naturalist, 16(2): 131-142.

Dutt, B., and R. P. Roy (1971), "Cytogenetic investigations in Cucurbitaceae. I. Interspecific hybridization in Luffa," Genetica 42: 139-156.

Francis, R. R. (1973), "Genic and chromosomal sterility in some species and interspecific hybrids of the genus Cucurbita," Ph. d. dissertation, The University of Arizona, Tucson.

Francis, R. R., and W. P. Bemis (1970), "A cytomorphological study of male sterility in a mutant of Cucurbita maxima Duch.," Econ. Bot. 24(3):325-332.

Francis, R. R., and W. P. Bemis (1974), "Stomatal size in induced polyploids in Cucurbita," Hort. Sci. 9(2): 138.

Gabelman, W. H. (1956), "Male sterility in vegetable breeding," in Genetics in Plant Breeding, Brookhaven Symp. Biol., No. 9, p. 113-122.

Grebenscikov, I. (1958), "Uber zwei Cucurbita - Artkreuzungen," Zuchter 28: 233-237.

Grebenscikov, I. (1965), "Notulae Cucurbitologicae VI. Uber einege Artkreuzungen in der Gattung Cucurbita," Kulturpflanze 13: 145-161.

Groff, D., and W. P. Bemis (1967a), "Meiotic irregularities in Cucurbita species hybrids," J. Hered. 58(3): 109-111.

Groff, D., and W. P. Bemis (1967b), "Bivalent frequency in interspecific hybrids from three xerophytic species of Cucurbita," The S. W. Naturalist 12(4): 365-368.

Hayase, H. (1950), "The pollen tube growth in interspecific hybrids," Jap. J. Genet. 25(5/6): 181-189. Abstract only.

Hayase, H. (1951), "On the chromosome number, polysotamy and persistent nucleoli in the cultivated species of Cucurbita," Jap. J. Genet. 26(1/2): 41-51. Abstract only.

Jauhar, P. P. (1970), "Chromosome behavior and fertility of the raw and evolved synthetic tetraploids of pearl millet, Pennisetum typhoides Staff et Hubb," Genetica 41: 407-424.

Khasha, K.J., and R. S. Sadasivaiah (1971), "Genome relationship between Hordeum vulgare L. and H. bulbosum L.," Chromosoma 35(3): 267-287.

McKay, J. W. (1931), "Chromosome studies in the Cucurbitaceae," Univ. of Cal. Publications in Bot. 16(9): 339-350.

Myers, W. M. (1945), "Meiosis in autotetraploid Lolium perenne in relation to chromosome behavior in autotetraploids," Bot. Gaz. 106: 304-316.

Newton, W. C. F., and C. Pellew (1929), "Primula kewensis and its derivatives," J. Genet. 20: 405-467.

Pearson, O. H., R. Hopp, and G. W. Bohn (1951), "Notes on species crosses in Cucurbita," Proc. Am. Soc. Hort. Sci. 57: 310-322.

Passmore, S. F. (1930), "Microsporogenesis in the Cucurbitacea," Bot. Gaz. 90: 213-223.

Rajasekaran, S. (1970), "Cytogenetic studies of the F1 hybrid Solanum indicum L. x S. melongena and its amphidiploid," Euphytica. 19: 217-224.

Ramalingam, R. S., S. R. Sree, and V. S. Raman (1971). "The cytology of an interspecific hybrid in zinnia," Cytologia 36(3): 522-528.

Rangasamy, P., and M. Kadambavanasundaram (1974), "A cytogenetic analysis of sterility in interspecefic hybrid Solanum indicum x S. melongena Linn.," Cytologia (Tokyo) 39(4): 645-654.

Randolph, L. F., and L. G. Cox (1943), "Factors influencing the germination of iris seed and the relation of inhibiting substances to embryo dormancy," Proc. Am. Soc. Hort. Sci. 43: 284-306.

Rao, R., R. Khan, and A. H. Khan (1971), "Structural hybridity and genetic systems of Solanum nigrum complex," Bot. Mag. (Tokyo) 84(995): 335-338.

Rhodes, A. M. (1959), "Species hybridization and interspecific gene transfer in the genus Cucurbita," Proc. Am. Soc. Hort. Sci 74: 546-551.

Rick, C. M. (1948), "Genetics and development of nine male sterile tomato mutants," Hilgardia 18: 599-633.

Ruttle, M. L. (1931), Technical bulletin of the New York Agricultural Experimental Station, Number 168. 12 pp.

Shifriss, O. (1945), "Male sterility and albino seedlings in cucurbita," J. Hered. 36: 47-52.

Snow, R. (1963), "Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparations," Stain Tech. 38: 9-13.

Sree Rangasamy, S. R., P. Devasahayam and V. W. Raman (1971), "Cytogenetical evolution of the progenies of a trispecific hybrid in Pennisetum," Carvologia 24(1): 19-26.

Stebbins, G. L. (1945), "The cytological analysis of species hybrids," Bot. Rev. 11: 463.

Stebbins, G. L. (1947), "Types of polyploids. Their classification and significance," Adv. in Genet. 1: 403-430.

Stebbins, G. L. (1958), "The inviability, weakness and sterility of interspecific hybrids," Adv. in Genet. 9: 147-215.

Stebbins, G. L. (1970), "Essays in evolution and genetics in honor of Theodosius Dobzhansky (Pgs. 173-208).

Stebbins, G. L. (1971), Chromosome Evolution in Higher Plants, Addison-Wesley Publishing Co., Reading, Mass, 216 pp.

Stephens, J. C., and R. F. Holland (1954), "Cytoplasmic male sterility for hybrid sorghum seed production," Agron. Jour. 46: 20-23.

Stephens, S. G. (1946), "The genetics of 'Corky' I. The new world alleles and their possible role as an interspecific isolating mechanism," Jour. Genetics. 47: 150-161.

Wall, J. R., and T. L. York (1960), "Gametic diversity as an aid to interspecific hybridization in Phaseolus and in Cucurbita," Proc. Am. Soc. Hort. Sci. 75: 419-428.

Weiling, F. (1959), "Genomanalytische untersuchungen bei Kurbis (Cucurbita L.)," Der Zuchter 29(4): 161-169.

Whitaker, T. W. (1933), "Cytological and phylogenetic studies in the Cucurbitaceae," Bot. Gaz. 94: 780-790.

Whitaker, T. W. (1956), "The origin of the cultivated Cucurbita," The Am. Naturalist, 90: 171-176.

Whitaker, T. W. (1959), "An interspecific cross in Cucurbita (C. Lundelliana Bailey x C. moschata Duch.)," Madrono:15(1): 4-13.

Whitaker, T. W. (1962), "An interspecific cross in Cucurbita: C. lundelliana Bailey x C. maxima Duchesne," Euphytica 11: 273-281.

Whitaker, T. W., and G. W. Bohn (1950), "The taxonomy, genetics, production and uses of the cultivated species of Cucurbita," Econ. Bot. 4(1): 52-81.

Whitaker, T. W., and G. N. Davis (1962), Cucurbita, Interscience Publishers, Inc., New York, 249 pp.

Yamane, Y. (1950), "Chromosome numbers in the genus Cucurbita," Zeiken Ziho 4: 72-74.

Yamane, Y. (1953), "Studies on species hybrids in the genus Cucurbita. II F_1 hybrids between C. moschata and C. maxima," Seiken Ziho 6: 112-116.

Zenkteler, M. (1962), "Microsporogenesis and tapetal development in normal and male sterile carrots (Daucus carrota)," Am. J. Bot. 49: 341-348.