Evaluation of Anther-derived, Inbred, and Hybrid Populations of *Streptocarpus* X *hybridus* Voss

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EVALUATION OF ANTHER-DERIVED, INBRED, AND HYBRID

POPULATIONS OF

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(ABSTRACT)

The potential of inbreeding and selective hybridization, along with anther culture, for increasing homogeneity with 2 early flowering cultivars of *Streptocarpus* X *hybridus* was examined. A 'Concorde' and 'Mixed Nymph' genotype expressed inbreeding depression within 2 generations, characterized by a reduction in corolla, leaf, and peduncle size, as well as an increase in floral deformities (flower part fusions). Inbred populations contained individuals which resembled the parent cultivars for several morphological traits. Such could be selected by cluster analysis of important traits, for the development of inbreeding depression resistant lines for hybrid production of more uniform, seed-propagated *Streptocarpus* cultivars. Reciprocal differences were observed among hybrids of first generation inbreds, suggesting that female parent selection is critical

for certain ornamental traits such as corolla size and peduncle length.

Plants were regenerated from anther-derived callus of 'Concorde' and 'Mixed Nymph'. The plants were found to be of somatic cell origin, but exhibited somaclonal variation for overall morphology. Variation for leaf variegation and morphology, days to flowering, flowers per peduncle, peduncle length and leaf area were observed among progeny of anther-derived plants, indicating that heritable somaclonal variation in *Streptocarpus* may be exploited for increased genetic diversity for cultivar development.

DEDICATION

To my father

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CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

The Gesneriaceae, a family in the subclass Asteridae and the order Scrophulariales, consists of approximately 125 genera containing over 2000 species (Kimmins, 1980). They are distributed in mainly tropical regions of America, Spain, Asia, and Africa. About 300 species of gesneriads have been cultivated, but only a few have gained commercial importance. *Saintpaulia ionantha* (African violet) and *Sinningia speciosa* (Florist's gloxinia) are the most widely cultivated gesneriads; however, *Episcia* hybrids, miniature *Sinningia*, and hybrid *Streptocarpus* are becoming increasingly popular as ornamental plants (Kimmins, 1980).

The genus *Streptocarpus*, the largest African plant genus, is divided in two groups according to overall morphology. The *Streptocarpella* subgenus contains caulescent type plants, while the *Streptocarpus* subgenus consists of acaulescent types. The 132 species of the genus are generally distributed throughout Southeastern Africa including Madagasgar and Central West Africa (Hilliard and Burtt, 1971). Though found over a large range, *Streptocarpus* occupy similar habitats characterized by sufficient summer rains, a dry winter, partial shade, and good drainage. They are mostly forest dwellers, growing on banks, stream sides, rock outcrops, and tree outcrops (Hilliard and Burtt, 1971). In cultivation, *Streptocarpus* require day temperatures below 30°C and night temperatures of 16-18°C for proper growth and flowering (Hammer, 1980). Light intensities of 15-20 Klx should be provided. The *Streptocarpus* cultivars grown today are complex hybrids developed by interbreeding among several acaulescent species.

IN VITRO STUDIES

Gesneriads can easily be asexually propagated by regeneration of many adventitious shoots from leaf cuttings. These adventitious shoots are thought to arise from single epidermal cells (Broertjes, 1969; Geier, 1983). Because of the regenerative potential of gesneriad leaves, it is expected that they would be responsive to *in vitro* systems.

Streptocarpus

Streptocarpus cultivars are generally asexually propagated by leaf cuttings of stock plants. Leaves are cut longitudinally on each side of the midrib. The remaining lamina halves are placed horizontially in the propagation medium (Marston, 1964). Plantlets emerge from the leaf in 6-8 weeks and mature in another 4-5 months. This method is undesirable because of variable response over different environments and seasons. Hence, *in vitro* methods of propagation have been investigated (Table 1). Depending on photoperiod and medium components, both floral and vegetative buds have been successfully regenerated from leaf discs (Appelgren and Heide, 1972; Handro, 1977; Simmonds, 1982). Cultured floral parts have provided a good system for large scale *in vitro* propagation. Pedicel segments taken just prior to anthesis can produce up to 300 plantlets within 5-6 months (Raman, 1977). Corolla explants have produced callus and subsequent buds, with a mature flower producing up to 150 plantlets (Peck and Cummings, 1984).

Other Gesneriads

Studies concerned with rapid clonal multiplication, especially of the most commercially important Gesneriads, have been prevalent (Table 2). *Episcia cupreata*, one of the most popular cultivated *Episcia* species, has been regenerated from leaf sections (Johnson, 1978a) and shoot tips (Bilkey and McCown, 1979). *Sinningia speciosa* has been propagated *in vitro* from shoot apices (Haramaki, 1971), and peduncle and leaf sections (Johnson, 1978b). Several researchers have successfully micropropagated *Saintpaulia ionantha* from petiole sections and leaf sections. Recent studies have investigated the use of *Saintpaulia ionantha* as a model for non-enzymatic protoplast isolation; protoplasts and microplasts have been isolated mechanically from petiole callus (Bilkey and Cocking, 1982; Bilkey *et al.*, 1982) with subsequent growth of protoplast-derived callus. Floral parts, i.e., sepals, petals, and ovaries have been induced to form callus and adventitious shoots in culture (Vasquez and Short, 1978).

Anther Culture

Anther culture is the isolation and culturing on a nutrient medium under aseptic conditions of individual anthers from an immature flower in order to induce embryogenesis from microspores (androgenesis). The goal of anther culture is to generate haploid plants from the microspores. Haploid plants have long been of interest to plant breeders as a source of desirable mutants and homozygous, pure breeding lines after chromosome doubling (Maheshwari *et al.*, 1980). Inbred lines could be developed in a single generation, much faster than traditional inbreeding. Spontaneous production of haploids as well as other techniques for haploid production prior to anther culture, such as distant hybridization, delayed pollination, application of irradiated pollen, temperature shock, and hormone treatment have all been unreliable sources of haploid plants for most species (Bhojwani, 1983).

The production of haploids via anther culture was first successful with a common Solanaceous weed, *Datura innoxia* (Guha and Maheshwari, 1966). Since then, anther culture has been successful for approximately 153 species from 52 genera and 23 families of monocots and dicots (Maheshwari *et al.*, 1980). Many factors effect androgenesis, such as genotype of the plant, physiological state of the plant, pollen stage, culture medium, *in vitro* environmental conditions, and pretreatment of the anthers. Extensive discussions of these factors can be found in several recent review papers and chapters (Maheshwari *et al.*, 1980, 1982; Bajaj, 1982; Bhojwani, 1983)

Anther Culture of the Gesneriaceae

Anther culture of *Streptocarpus* X *hybridus* has not been reported in the literature. Haploid plants have been regenerated from cultured anthers of *Saintpaulia ionantha* (Table 3), initally by Hughes *et al.* (1975). Anthers with pollen at the first mitotic division of microsporogenesis were cultured on Blaydes' (1966) medium with 5 mg/l IAA and 0.5 mg/l kinetin. About 18% of the anthers produced from 1-5 plantlets directly. This low production of haploids is typical of most species responsive to anther culture. Several researchers have used Hughes' methods with similar results (Smith *et al.*, 1981; Norris *et al.*, 1982; Bhaskaran *et al.*, 1983); haploid regenerants have been found to be reduced in size (Smith *et al.*, 1981) with phenotypic variation for leaf margin, indentation, shape (Norris *et al.*, 1982), and enzyme activity (Bhaskaran, 1983). Weatherhead *et al.* (1982) have reported an increased production of haploids by indirect embryogenesis (callus intermediate). Anthers with pollen at the late uninucleate stage were cultured on Murashige and Skoog (1962) basal medium (MS) with 1 mg/l NAA and 0.5 mg/l BAP. A similar percent of anthers (15.5%) produced callus which, after isolation, produced 50-200 haploid plants per anther. The disadvantages of indirect embryogenesis through callus are genetic instability and a reduced percent of haploids (Table 3).

Haploids have also been regenerated from anthers of *Digitalis purpurea* (foxglove), a Schrophulariaceous species in the same order as the Gesneriaceae (Corduan and Spix, 1975). Anthers produced callus on Nitsch and Nitsch (1969) medium with 5 mg/l 2,4-D and plantlets were regenerated on the same medium without 2,4-D. Most of the regenerants were normal diploids; however, some haploids were produced.

Inbred Lines from Anther Culture

The development of inbred lines from anther culture has been much slower than the success of the procedure itself. Inbred lines from doubled-haploids have been developed with one of the most responsive crops to anther culture, *Nicotiana tabacum* (Arcia *et al.*, 1978; Deaton *et al.*, 1982; Kasperbauer *et al.*, 1983). Doubled haploid lines of *Nicotiana tabacum* L. were compared to inbred lines derived through many generations of inbreeding. Reduction in yield and vigor was seen in doubled haploid lines (Arcia *et al.*, 1978; Kasperbauer *et al.*, 1982), whereas doubled haploids were found to be similar to inbred lines for 26 of 35 characteristics (Deaton *et al.*, 1982). Inbred lines of *Secale cereale* X *S. vavilovii* hybrids derived through anther culture were compared to inbred lines derived by self-pollination. Certain anther derived lines were comparable to selected inbred lines for important characteristics; however, inbreeding was more effective in producing many vigorous inbred lines (Friedt *et al.*, 1983).

ORIGIN AND GENETICS OF STREPTOCARPUS X HYBRIDUS

Origin

The subgenus *Streptocarpus* contains acaulescent species of two forms, rosulate and unifoliate. The rosulate types consist of a rosette of leaves, whereas the unifoliate types consist of a single large leaf which develops by continual expansion of one cotyledon (Hilliard and Burtt, 1971). Each leaf produces a determinate number of floral meristems at the base of the petiole which bloom in succession, and then the leaf ceases to produce flowers.

The cultivars of *Streptocarpus* grown today have originated from a complex hybridization of many species (Brown, 1973). *Streptocarpus rexii*, a blue-flowering rosette species, was one of the first species introduced into cultivation in England around 1825. About 50 years later, *Streptocarpus parviflorus*, a white flowered rosette species, along with *S. polyanthus*, *S. saundersii*, and *S. dunnii* which are blue flowered, lilac flowered, and red flowered unifoliate species, respectively, were introduced into cultivation. At the Royal Botanic Garden in Kew, England, *S. parviflorus* was crossed to *S. dunnii* to produce *S.* X *wattsonii*, a rosy purple flowering rosette hybrid. Also, *S. rexii* was crossed with *S. dunnii* to produce *S.* X *kewensis*, a mauve-purple flowering rosette hybrid. The following year, these hybrids along with *S. parviflorus* and *S. rexii* were all intercrossed. It is thought that other species such as *S. polyanthus*, *S. saundersii*, *S. wendlandii*, and *S. fanniniae* were also involved in the production of the hybrid mixture. In 1896 Voss named this diverse hybrid mixture *Streptocarpus* X *hybridus*, which is the source of all current large flowered cultivars. Currently grown cultivars most closely resemble *S. rexii* because of constant selection pressures towards that type.

'Concorde' cultivar

The cultivar 'Concorde' is an F_1 hybrid which is produced by the parthenogenic seed formation (pseudogamy) on a tetraploid selection after pollination with irradiated pollen (personal communication, C. Hopka). Hence, it is not a true F_1 hybrid derived from inbred lines. It segregates for flower color with a range including shades of blue, mauve, and white. Although not completely homozygous, it is one of the most homogeneous *Streptocarpus* X *hybridus* cultivars as determined by cluster analysis for several morphological traits (Veilleux and Lyons, 1984).

'Mixed Nymph' cultivar

A selection of *S.* X *hybridus*, 'Merton Blue', was crossed as the female with *S. johannis* to produce the superior hybrid 'Constant Nymph' (Brown, 1973). 'Constant Nymph' exhibits functional male sterility (non-dehiscent anthers) due to the *Da* allele in the completely dominant or heterozygous condition (Zeven, 1972). The cultivar 'Mixed Nymph' is a mixture of mutations derived from 'Constant Nymph'

(Broetjes, 1969), and consists of a range of flower colors from dark purple to light pink, as well as shades of mauve, magenta, blue, and red. Although not as homogeneous as 'Concorde', it is more homogeneous than most cultivars as determined by cluster analysis (Veilleux and Lyons, 1984).

Genetics

The diploid chromosome numbers of the subgenera are 32 for *Streptocarpus* and 30 for *Streptocarpella*. This effectively prevents hybridization between the two subgenera. Genetic analysis of *Streptocarpus* has been primarily through the efforts of two different research groups.

F. Oehlkers conducted extensive studies on cytoplasmic inheritance in *Streptocarpus* (Oehlkers, 1964). Utilizing interspecific crosses and backcrosses, he determined that there are 4 different cytoplasms within the genus and designated these as Class 1, 2, 3, and 4. He demonstrated that certain nuclear genes were expressed phenotypically only when combined with foreign cytoplasm. Differences were seen in sex-abnormalities (reduction of stamens), choripetaly, flower size, pollen sterility, and patches of yellow in the corolla throat. The species which have been used to develop commercially grown *Streptocarpus* cultivars are of 2 cytoplasmic classes which show no abnormalities when crossed in either direction.

W. J. C. Lawrence, in a series of papers, investigated the genetics and inheritance of flower colors and patterns among *Streptocarpus* (Lawrence *et al*., 1939; Lawrence, 1957; Lawrence and Sturgess, 1957). He determined that 7 flower color phenotypes are produced by the interaction of 9 genes which are independent and segregate in Mendelian ratios. Five of these genes are important in determining the flower color phenotypes, while the other four modify the intensity of each color. The *V* gene produces anthocyanins in the flower stems and leaves, while the *F* gene, in the presence of *V*, produces anthocyanins in the flowers. Genes *O*, *R*, and *D* modify the chemical structure of the pigments to yield the different flower colors. The following are the 7 phenotypes produced from the interaction of the 5 described genes (Lawrence and Sturgess, 1957):

*V_F_O_R_D_*	blue
*V_F_ooR_D_*	magenta
*V_F_oorrD_*	pink
V_F_O___dd	mauve
V_F_ooR_dd	rose
V_F_oorrdd	salmon
vvF________	white
V_ff_______	white.

Flower color patterns are controlled by 7 genes, four of which are linked and form a supergene. The following is a list of the seven genes and their phenotypic effect (Lawrence, 1957):

F	- necessary for anthocyanin production in the flowers
I	- intensifies the color
B	- produces an anthocyanin blotch in the corolla tube
H	- produces anthocyanin in the capitate pistil hair stalks
L	- controls anthocyanin lines in the corolla throat
Y_1, Y_2	- complementary genes producing a yellow stripe in the corolla.

Genes *F*, *I*, *B*, and *H* form the supergene, which has only three genotypes found in cultivated *Streptocarpus*: *FiBH*, *fibH*, and *FIbh*, which correspond to the flower patterns found on *S. rexii*, *S. parviflorus*, and *S. dunnii* respectively, the species involved in the early hybridization which produced *Streptocarpus* X *hybridus* Voss. Gene Y_1 is linked to the supergene, and gene Y_2 is linked to *L*. Inheritance of the patterns is Mendelian (Lawrence, 1957). The inheritance of the yellow stripe also has been investigated by Oehlkers (1966, 1967), who observed a cytoplasmic effect in reciprocal crosses.

Lawrence (1947) identified two complementary sublethal genes in *S. rexii*, *L* and *E*, which produce abnormal foliage and often death when the two dominant alleles occur in the same plant.

Streptocarpus Breeding

Because of their history of hybridization, *Streptocarpus* X *hybridus* cultivars show considerable variation when sexually propagated (Hammer, 1976; Schmidt and Erikson, 1981; Veilleux and Lyons, 1984). Greenhouse growers desiring uniform cultivars must therefore rely upon asexual propagation, generally leaf cuttings of desirable genotypes. Mutation breeding of asexual cultivars has been employed to develop new cultivars. Irradiation of leaves by X-radiation has led to the release of Nymph types with many flower colors (Broetjes, 1969). Further mutation breeding of 'Constant Nymph' yielded a mutant that flowered throughout the year, rather than flowering only during the spring and summer (Davies and Hedley, 1975).

Inbreeding depression has been reported in *Streptocarpus*. 'Constant Nymph' showed an increase in variation in the first inbred generation, and a significant loss of vigor after two (Schmidt and Erikson, 1981) and three generations (Hammer, 1976). Inbreeding increases homozygosity, and the expression of deleterious recessive traits in the early generations, leading to inbreeding depression (Briggs and Knowles, 1967). Inbreeding depression is the reduction in yield, smaller plant organs, and overall loss of vigor observed after continued self-pollination. The presence or extent of inbreeding depression depends upon the species; in general, cross-pollinating species are more sensitive to inbreeding depression than self-pollinating ones. There are some cross-pollinating species, such as the cucurbits, which show little or no deleterious response to inbreeding.

Heterosis, or hybrid vigor, is the increase in size, vigor, yield, insect and disease resistance, and environmental tolerance seen in hybrids of genetically different parents (Briggs and Knowles, 1967). The genetic basis for heterosis and inbreeding depression is uncertain; however, maximum heterozygosity, dominance, and epistasis are possible reasons for heterosis (Briggs and Knowles, 1967). Heterosis has been observed among cultivated *Streptocarpus* hybrids (Beuttel, 1940) and also natural hybrids in the wild (Hilliard and Burtt, 1971).

Traditional breeding and selection of *Streptocarpus* X *hybridus* has yielded improved cultivars (Anon., 1972; Brown, 1973). Recent ideotype breeding has produced new cultivars selected for floriferousness, compactness, flower color, and uniformity (Hopka and Beck, 1984).

Table 1. *In vitro* studies with *Streptocarpus*

Explant	Morphogenic response	Medium[a]	Reference
leaf tissue	callus	LS + 10^{-5} M IAA, 10^{-6} M KIN	Inoue *et al.*, 1984
leaf discs	floral and vegetative buds depending on photoperiod	K, N & N + 100 mg/l IAA + 1500 mg/l adenine	Handro, 1977
leaf discs	adventitious shoots, roots	S & S, + various hormones	Appelgren and Heide, 1972
leaf sections	adventitious shoots	N + 0.1 mg/l IAA, 0.1 mg/l KIN	Geier, 1983
leaf sections	adventitious shoots	LS + 0.2 mg/l IAA, 1.0 mg/l BAP	Grunewaldt, 1977
leaf segments	floral and vegetative buds	MS + IAA (veg.), BAP (floral)	Simmonds, 1982
pedicel segments	callus, roots, adventitious shoots	MS + 1.0 mg/l IAA & 1.0 mg/l BAP (roots), 1.0 mg/l BAP (shoots)	Raman, 1977
corolla	callus, roots, adventitious buds	MS + 125 mg/l adenine, 100 mg/l L-tyrosine, 1.0 mg/l NAA, 5.0 mg/l BAP	Peck and Cummings, 1983

[a]K = Knop's macroelements (Gautheret, 1959)
LS = Linsmaier and Skoog (1965)
MS = Murashige and Skoog (1962)
N = Nitsch (1969)
N & N = Nitsch and Nitsch (1965)
S & S = Schott and Schraudolf (1967)

Table 2. *In vitro* studies (excluding anther cultures) with other Gesneriads.

Species	Explant	Morphogenic response	Medium[a]	Reference
Chirita sinensis	leaf pieces	adventitious shoots	--	Allen and Mott, 1975
Episcia cupreata	leaf pieces	callus, adv. shoots	MS + 0.2 mg/l IAA, 0.2 mg/l KIN	Johnson, 1978
	leaf pieces, shoot tips	callus, adv. shoots	MS + 0.1 mg/l NAA, 0.5 mg/l BAP	Bilkey and McCown, 1979
E. X *wilsonii*	"	"	"	"
Kohleria spp.	leaf sections	adventitious shoots	N + 0.5 mg/l IAA, 1.0 mg/l KIN	Geier, 1983
Nautilocalyx lynchii	epidermal layers	roots, adv. shoots	MS + 1 μM zeatin, 1 μM IAA (roots); + 1 μM zeatin (sh.)	Venerloo *et al.*, 1983
Saintpaulia ionantha	leaf sections	adventitious shoots	MS + 2.0 mg/l IAA, 0.08 mg/l BAP	Cooke, 1977
	"	"	N + 0.5 mg/l IAA, 0.5 mg/l KIN	Geier, 1983
	"	"	LS + 0.8 mg/l IAA, 0.4 mg/l BAP	Grunewaldt, 1977
	"	"	MS + 5.4 μM NAA, 22 μM BAP, 308 μM adenine sulfate	Start and Cummings, 1976

[a]B-5 = Gamborg *et al.* (1968)
LS = Linsmaier and Skoog (1965)
MS = Murashige and Skoog (1962)
N = Nitsch (1969)

Table 2. Con't

Species	Explant	Morphogenic response	Medium[a]	Reference
	petiole sections	adventitious shoots	MS + 0.1 mg/l NAA, 0.01 mg/l BAP	Bilkey *et al.*, 1978
	"	adventitious shoots	LS + 0.2 mg/l IAA or 0.1 mg/l 2,4-D, 0.4 mg/l BAP	Grunewaldt, 1976
	"	"	MS + 0.5 mg/l NAA, 0.5 mg/l KIN	Harvey and Knap, 1977
	"	roots, adv. shoots	0.01-1 ppm NAA(ro) 0.01-1 ppm BAP(sh)	Jungnickel, 1976
	"	callus	MS + 1 mg/l 2,4-D	Bilkey and Cocking, 1982
	"	callus	B-5 + 0.1 mg/l NAA,	Bilkey *et al.*, 1982
	sub-epidermal tissue	adventitious shoots	B-5 + 0.1 mg/l NAA, 0.5 mg/l BAP	Bilkey and Cocking, 1981
	floral parts	callus, roots, adv. shoots	MS + 2.0 mg/l NAA, 0.2 mg/l BAP (c); 1.0 mg/l NAA, 1.0 mg/l BAP (s); 0.5 mg/l NAA, 1.0 mg/l KIN (r)	Vasquez and Short, 1978
Sinningia hybrida	peduncle sections	adventitious shoots	MS + 0.2 mg/l NAA, 1.0-2.0 mg/l BAP	Bigot, 1974
S. speciosa	shoot apex	adventitious shoots	MS + 0.1 mg/l IAA, 30 mg/l KIN	Haramaki, 1971
	leaf sections	adventitious shoots	MS + 1.7 mg/l IAA, 0.5 mg/l KIN	Johnson, 1978

[a]B-5 = Gamborg *et al.* (1968)
LS = Linsmaier and Skoog (1965)
MS = Murashige and Skoog (1962)
N = Nitsch (1969)

Table 3. *Saintpaulia ionantha* anther culture reports.

Reference	microspore stage	Medium[a]	% of anthers producing plantlets	no. of plantlets per anther	% of tested regenerants haploid
Hughes *et al.*, 1975	first mitotic division	B + 5.0 mg/l IAA, 0.5 mg/l KIN	18.0	1-5	100
Smith *et al.*, 1981	"	"	30.0	1-5	100
Norris *et al.*, 1982	"	"	10.0	11	100
Bhaskaran *et al.*, 1983	"	"	--	--	0[b]
Weatherhead *et al.*,[c] 1982	late uninucleate stage	MS + 1.0 mg/l NAA, 0.5 mg/l BAP	15.5	50-200	91

[a]B = Blaydes (1966)
MS = Murashige and Skoog (1962)

[b]all regenerants spontaneously doubled their chromosome number by endomitosis

[c]only report of indirect embryogenesis (callus intermediate) rather than direct embryogenesis

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CHAPTER 2

EVALUATION OF ANTHER-DERIVED *STREPTOCARPUS* X *HYBRIDUS* AND THEIR PROGENY

ABSTRACT

Regeneration of plants through indirect embrogenesis was obtained on MS basal medium with 2.0 mg/l NAA and 0.5 mg/l BAP or 0.5 mg/l NAA and 1.1 mg/l BAP in anther cultures of *Streptocarpus* X *hybridus*. All regenerated plants were diploid and of somatic cell origin as determined by chromosome counts, flower color and morphological similarity with the anther donor plants, and flower color segregation among progeny of anther-derived plants. Somaclonal variation expressed as deformed and dwarfed transient morphology was observed in 2 anther-derived plants. Variation was observed in progeny of 5 anther-derived plants from the same anther donor plant for days to flowering, flowers per peduncle, peduncle length, and leaf area. Additional morphological variation including leaf variegation among seedlings and twisted or inverted leaves on mature plants was also observed in progeny of anther-derived plants. Variation generated by either anther culture or plant regeneration from callus may be instrumental in future *Streptocarpus* improvement.

INTRODUCTION

The possibility of developing inbred lines for use in hybrid seed production of *Streptocarpus* X *hybridus* could be realized through production of haploid plants by androgenesis followed by chromosome doubling. Successful production of haploids in closely related *Saintpaulia ionantha* (Hughes, *et al*. 1975; Weatherhead, *et al*. 1982) is encouraging for the application of anther culture to *Streptocarpus*. Rapid inbreeding depression of *Streptocarpus* cultivars (Hammer, 1976; Schmidt and Erikson, 1981) would necessitate the evaluation of many inferior genotypes over several generations by traditional inbreeding for development of pure lines. If sufficient numbers of genotypes could be regenerated by anther culture, superior individuals could be selected in a single generation.

The objectives of the present research were: 1) to regenerate plants of *Streptocarpus* by anther culture, and 2) to determine the origin (i.e., pollen grain or somatic cell) of anther-derived plants.

MATERIALS AND METHODS

Experiment 1

First generation inbred selections of *Streptocarpus* X *hybridus* 'Concorde' (3042) and 'Mixed Nymph' (1171) were grown under ambient conditions (May - September, 1984) in a fan and pad cooled greenhouse, 22°C day, 18°C night. Fifty percent shading was provided, and plants were fertilized weekly with 200 ppm N from 20N-8.8P-16.6K (20-20-20 water soluble). Immature buds (3-7 mm) were harvested and disinfested in 70% ethanol for 15 sec, then in 5.25% sodium hypochlorite with a drop of Tween-20® surfactant for 5 min, followed by 3 rinses in sterile, distilled water. Anthers were dissected from the bud (2 per bud), leaving the filament attached to prevent wound callus formation. Anthers containing microspores at the late uninucleate stage of pollen development ('Concorde' anther width=1.2-2.0 mm; 'Mixed Nymph' anther width=1.2-2.5 mm) were plated (Figure 1a) on Murashige and Skoog (1962) salts and vitamins (MS) with 30 g/l sucrose, 7 g/l agar (Phytagar®), 100 mg/l myo-inositol, 2.0 mg/l α-napthalene acetic acid (NAA), and 0.5 mg/l N^6-benzyladenine (BAP). This medium was found to yield the most response from anthers of several *Streptocarpus* X *hybridus* genotypes (Jensen, unpublished). Six anthers were placed in each 60 x 15 mm petri dish containing 7 ml medium and incubated at 24-26°C under 54 µE s^{-1} m^{-2} of fluorescent light, 16 hr photoperiod.

Cultures were transferred monthly to fresh medium of the same composition or with growth regulators adjusted to 0.01 mg/l NAA and 0.1 mg/l BAP to encourage shoot formation. Calli emerging directly from the anthers, rather than the filament, were transferred to 25 x 150 mm culture tubes containing 25 ml medium as above. Regenerating plantlets were isolated and placed either on MS basal medium for rooting or were removed from culture if sufficient roots were already present. Rooted plantlets were removed from the culture tubes, their roots rinsed in distilled water to remove any residual medium, and placed in cell packs containing 1 sphagnum peatmoss:1 perlite:1 vermiculite (BX-Promix®) by volume that was dampened and autoclaved for 20 min at 15 psi. The potted plantlets were sealed in a plastic bag under two 40 W fluorescent tubes and one 40 W incandescent bulb (60-75 µE s^{-1} m^{-2}, 16 hr photoperiod) and maintained at 20°C day and night. The plastic bag was opened for gradually increasing durations to acclimate the plantlets. Soil moisture was maintained by saturation with distilled water every 3-4 days. Once acclimation was complete (3-6 weeks), plantlets were transferred to the greenhouse and transplanted into 10 cm square plastic pots and maintained as above.

Chromosome counts were taken on young root tips derived from leaf cuttings under intermittent mist. The roots were pretreated for 3-4h (18°C) in 0.002 M 8-hydroxyquinoline and fixed in 3:1 ethanol:acetic acid overnight. Fixed roots were hydrolyzed in 1 N HCl for 10 min at 60°C, and then stained in leuco-basic fuchsin for 15-30 minutes. Squash preparations were observed in acetocarmine (Karp *et al.*, 1982).

Self-set seed was harvested from 5 anther-derived plants (3-40, 4b-8, 4b-11, 4b-15, 4b-23) and was sown on BX-Promix® in closed plastic Plantcons™ (Flow Laboratories, McLean, VA) placed 10 cm beneath two 40 W fluorescent tubes and one 40 W incandescent bulb (60-75 µE s^{-1} m^{-2}, 16 hr photoperiod, 20°C day and night). Seedlings emerged within 2-4 weeks. Soil moisture was maintained by saturation with a gentle mist from a hand sprayer (biweekly while Plantcon™ tops were in place and every 2-3 days after the tops were removed). Plantcon™ tops were removed 50 days after sowing, and plants were transferred to the greenhouse 1 week later (March, 1985) and transplanted into 10 cm square plastic pots. Plants were fertilized as above. Fifty percent shading of natural irradiance was provided. Twenty-one plants of each family were arranged in a randomized complete block design consisting of 3 blocks (different benches in the same greenhouse section), 14 plots/block [two families from anther-derived plants 4b-8 and 4b-15 were subdivided into 3 populations (randomly selected, variegated, and green)] and 7 plants/plot. The following data were taken at first flower anthesis: days to first flower, leaf length and maximum width of the leaf subtending the open flower, peduncle length, corolla width and length (taken on completely expanded corollas 4-5 days after anthesis). Leaf area was estimated by the product of leaf length and leaf width. The number of flowers on 10 peduncles per plant was recorded 30 weeks after seed was sown. Statistical analyses were by SAS ANOVA.

Experiment 2

Twenty-five plants of 'Concorde' selection (2-11) and 'Mixed Nymph' selection (6-11) were grown under ambient greenhouse conditions (September - June, 1984) in a fan and pad cooled greenhouse, 22°C day, 18°C night. The same culture and regeneration procedures were used as in Experiment 1 unless otherwise noted. Buds were disinfested in 5.25% sodium hypoclorite for 30 minutes. Five anthers were cultured per plate on the following 4 bilayer media (7 ml solid, 1.5 ml liquid):

1 = MS + 10µM NAA (1.9 mg/l) + 2 µM BAP (0.5 mg/l)

2 = MS + 2.5µM NAA (0.5 mg/l) + 5 µM BAP (1.1 mg/l)

3 = MS + 5µM NAA (0.9 mg/l) + 5 µM BAP (1.1 mg/l)

4 = MS + 2 µM BAP (0.5 mg/l) + 0.5% activated charcoal. Anthers of each genotype were randomly placed in plates of the above media to yield 27 replications per genotype.

The following abbreviations will be used to designate each population in futher discussions:

R = anther-derived plants of 'Concorde' genotype 2-11 and/or 'Mixed Nymph' genotype 6-11

R_1 = progeny after self-pollination of 5 anther-derived 'Concorde' plants (3-40, 4b-8, 4b-11, 4b-15, and 4b-23).

RESULTS

Experiment 1

Within 3 weeks of culturing, responsive anthers began to swell and turn brown, often accompanied by filament elongation (Figure 1b). Eight weeks after culture, calli protruded through walls of responsive anthers and occasionally differentiated into roots, and/or less frequently shoots (Figure 1c). Calli-root masses produced shoots readily when transferred to shoot induction medium. Of the 40 anthers cultured of both 'Concorde' and 'Mixed Nymph', 2 and 3 anthers produced 35 and 25 plantlets (Figure 1d), respectively, within 6 months.

All R exhibited flower color identical to the anther donor plant and were similar for overall morphology. Two 'Concorde' regenerants were very small, with abnormal flowers and foliage, but reverted to normal morphology within 5 months. Attempts to self-pollinate these plants were unsuccessful. Chromosome counts of the 2 abnormal 'Concorde' R and a few randomly chosen R indicated a diploid complement ($2n$=32). Because of uncertain identity of the exact 'Mixed Nymph' anther donor plant, furthur studies were restricted to 'Concorde' R and R_1.

After self-pollination of 5 randomly selected R, R_1 families contained from 7 to 45 % variegated seedlings (Figure

2a), a phenomenon that was not observed in progeny of first inbred plants not cycled through an *in vitro* system (Table 1). As R_1 matured, the variegation was less apparent with white patches confined to the leaf tip at first flower anthesis (Figure 2b). Analysis of variance for morphological traits (days to flowering, corolla width and length, flowers per peduncle, peduncle length and leaf area) among plots of randomly selected, variegated, and green plants within 2 families of R_1 revealed no consistent effect of variegation during the seedling stage on subsequent morphological characteristics measured at flowering. Within one of the families, variegated seedlings flowered later than the rest. In subsequent analyses of quantitative data, only the randomly selected seedling plots of these 2 families were used.

Significant differences for days to flowering, flowers per peduncle, peduncle length, and leaf area were observed among the 5 R_1 families (Table 2). Flower abnormalities [floral part fusions (Figure 3), increases in floral part number, and deformities] were observed in over half of the R_1; however, plants exhibiting these abnormalities also had normal flowers. Among R_1, plants with twisted, inverted leaves, or plants exhibiting leaves with extended petioles were commonly observed.

Flower color segregation occurred in all R_1 families. A dominant allele (*V* and *F*) at two of the 5 loci that control flower color are required for expression of blue flowers (Lawrence and Sturgess 1957). Hence, a *V_F_* genotype was temporarily assigned to 'Concorde' 3042. Heterozygosity at both loci would have resulted in 9 blue:7 white flowered progeny on self-pollination whereas heterozygosity at only one of the 2 loci would have resulted in 3 blue:1 white flowered progeny. χ^2 analysis revealed a 3:1 ratio of blue:white flower color segregation in all families (Table 3).

Experiment 2

Many contaminated cultures occured randomly with respect to media type. All plantlet regeneration was by indirect embryogenesis as in experiment 1. Both 'Concorde' and 'Mixed Nymph' genotypes formed callus; however, plantlet formation was restricted to anther-derived callus of 'Mixed Nymph'. Most regenerated plantlets were derived from anthers cultured on medium 2 (Table 4). All regenerants appeared similar in morphology to the anther donor plant.

Table 1. Percentage of variegated seedlings after self-pollination of 5 anther-derived *Streptocarpus* X *hybridus* Concorde (3042) genotypes.

Genotype	Percentage variegated seedlings[z]
3-40	25
4b-8	38
4b-11	11
4b-15	45
4b-23	7

[z]Scored from 100 randomly chosen seedlings.

Table 2. Means for various morphological characteristics of progeny of 5 **Streptocarpus × hybridus** Concorde (3042) anther-derived plants. Each family consisted of 21 plants.

Family	Days to flowering	Corolla width (cm)	Corolla length (cm)	Flowers per peduncle[z] (cm)	Peduncle length (cm)	Leaf area[y] (cm^2)
3-40	145d[x]	5.8a	6.9a	1.6a	10.1a	67.8ab
4b-8	148bcd	5.5a	6.8a	1.4cd	8.4c	57.8b
4b-11	146d	5.7a	6.7a	1.4cd	9.5ab	70.6a
4b-15	152a	5.6a	6.8a	1.5abc	9.3abc	73.2a
4b-23	146cd	5.7a	6.8a	1.4bcd	9.2abc	69.9a

[z] Mean of 10 peduncles.

[y] Estimated by the product of leaf length and leaf width.

[x] Mean separation within columns by LSD test, 5% level.

Table 3. Flower color segregation among progeny of 5 *Streptocarpus* X *hybridus* Concorde (3042) anther-derived plants. Each family consisted of 21 plants.

Family	Blue:white	χ^2 for 3:1	P
3-40	15:6	0.14	.50-.75
4b-8	14:7	0.78	.25-.50
4b-11	16:5	0.02	.75-.90
4b-15	17:4	0.30	.50-.75
4b-23	14:7	0.78	.25-.50
Total	76:29	0.38	.50-.75

Table 4. Response of 135 anthers cultured of both *Streptocarpus* X *hybridus* Concorde 2-11 and Mixed Nymph 6-11 on 4 media varying for NAA and BAP concentrations.

Cultivar	Media	No. of anthers producing callus	No. of regenerated plants
Concorde	1[z]	2	0
	2	2	0
	3	2	0
	4	0	0
Mixed Nymph	1	5	4
	2	2	32
	3	0	0
	4	0	0

[z]1= MS + 10 µM NAA (1.9 mg/l) + 2 µM BAP (0.5 mg/l).
2= MS + 2.5 µM NAA (0.5 mg/l) + 5 µM BAP (1.1 mg/l).
3= MS + 5 µM NAA (0.9 mg/l) + 5 µM BAP (1.1 mg/l).
4= MS + 2 µM NAA (0.5 mg/l) + 0.5% activated charcoal.

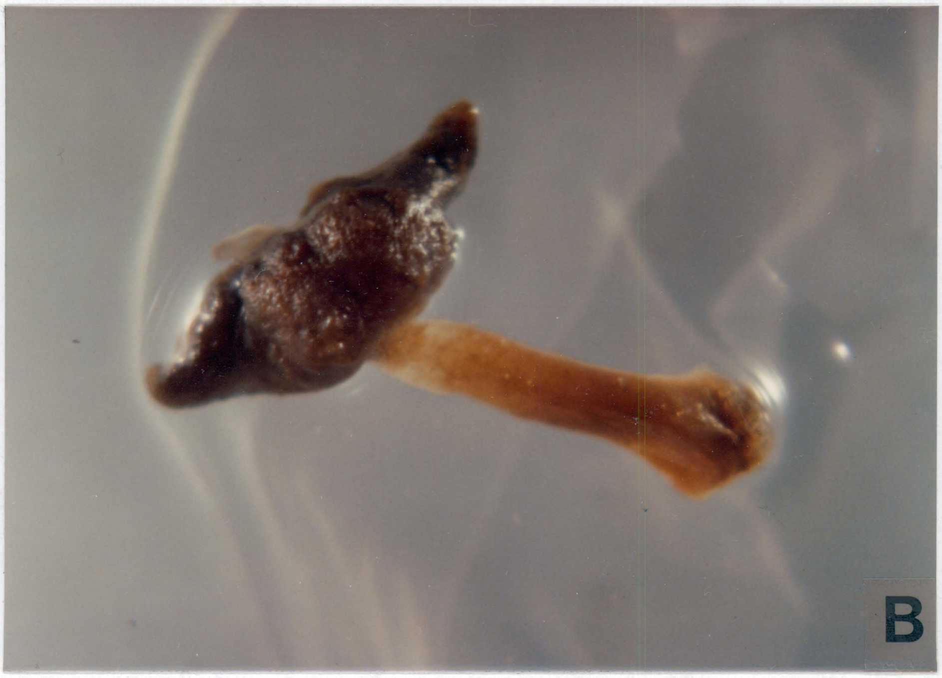

Figure 1. *In vitro* stages of *Streptocarpus* X *hybridus* anther culture. A. anther 1 day after culturing. B. responsive anther swelling with filiment elongation. C. calli protruding from anther, differentiating into roots and shoots. D. isolated plantlet on MS basal medium.

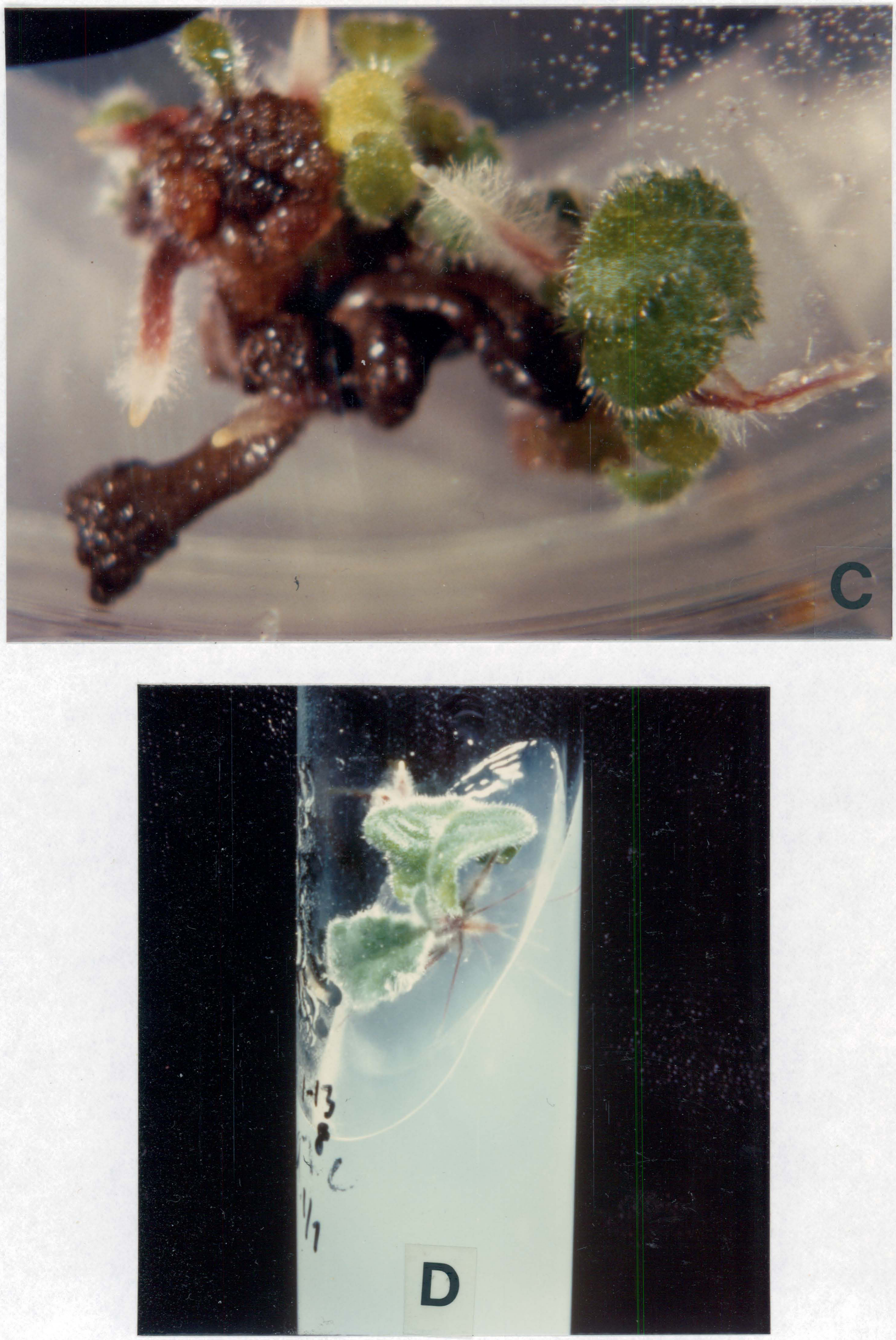

Figure 1. Continued

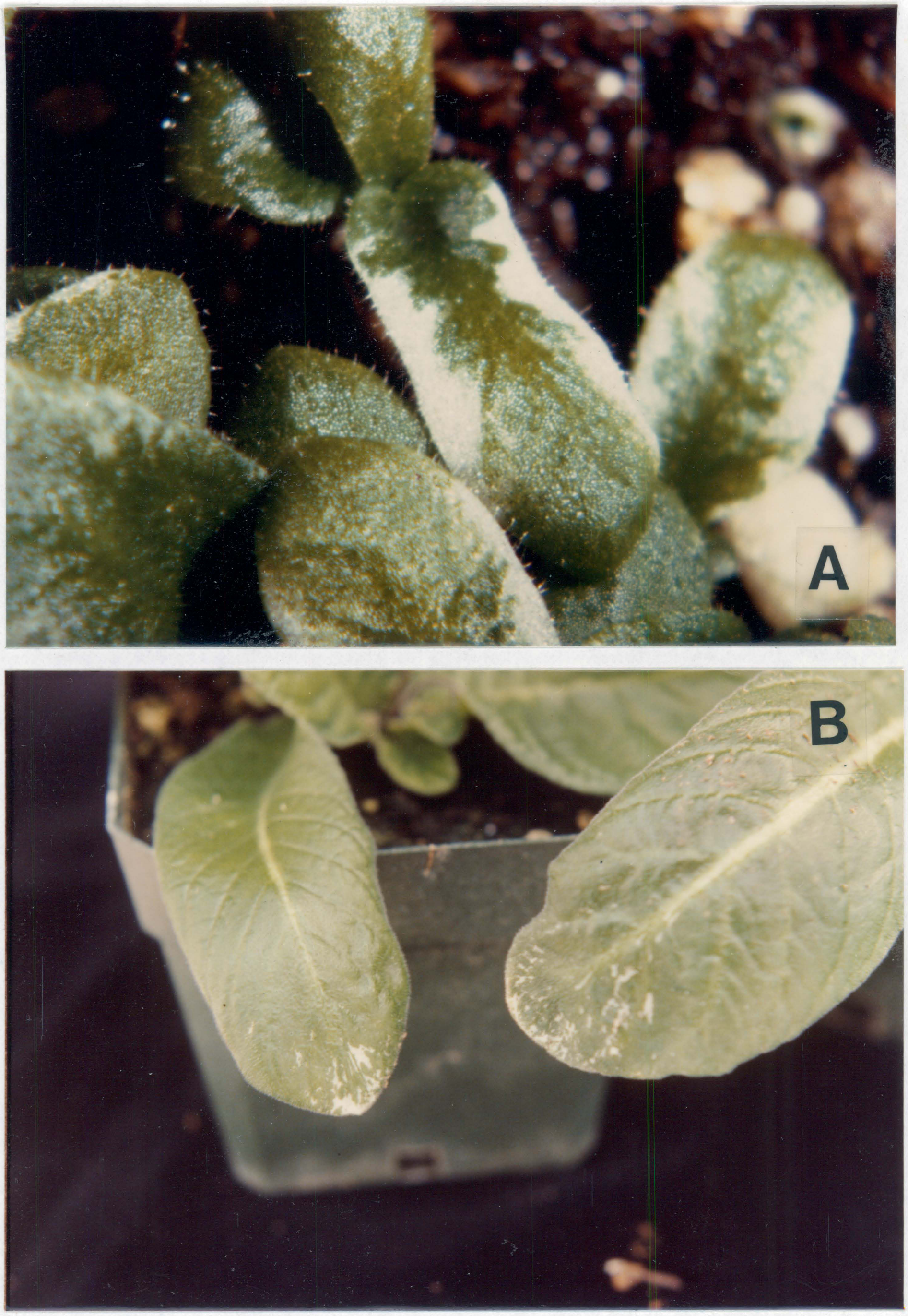

Figure 2. Leaf variegation seen among *Streptocarpus* x *hybridus* Concorde R_1. A. typical variegation observed on seedlings. B. typical variegation observed on plants at first flower anthesis.

Figure 3. Pistil fusions observed commonly among *Streptocarpus* X *hybridus* Concorde R_1. Normal pistil at right with increasing fusion from right to left until 2 joined but unfused pistils.

DISCUSSION

Somatic cell origin of R from 'Concorde' inbred selection 3042 was confirmed by chromosome counts, phenotypic similarity to the anther donor plant, and flower color segregation of R_1. Although anther-derived plantlets were of somatic origin, some variation was observed among R in the 2 deformed and dwarfed plants previously noted. Variation among R_1 included flower part fusion, multiple or deformed flower parts, abnormal leaves, leaf variegation, and significant differences among 5 families for days to flowering, flowers per peduncle, peduncle length, and leaf area. Somaclonal variation likely occurred during the culture phase, and can perhaps be attributable to indirect embryogenesis. This variation has possibly been enhanced after a sexual cycle. Except for genes controlling flower color (for which somaclonal variation was not observed), little is known of the inheritance of morphological characteristics in *Streptocarpus*. The characteristics for which somaclonal variation was observed were mostly quantitative and likely controlled by several genes. Such unexpected variability has been attributed to modifications of repetitive DNA sequences controlling gene regulation during development (Marx, 1984). Their modification has been associated with the stress induced by the culture conditions. Alternatively, mobilization of transposable elements has been suggested as a source of somaclonal variation (Larkin *et al.*, 1984), particularly regarding unstable mutations, such as the two highly abnormal phenotypes observed among 'Concorde' R.

Little of the variation observed among R or R_1 would be considered horticulturally desirable. Leaf variegation, which was very apparent in R_1 as seedlings, was almost without exception, inconspicuous by first flower anthesis. The flower abnormalities, which have also been observed infrequently among first and second inbred generations of 'Concorde' and 'Mixed Nymph', are particularly undesirable.

Heritable somaclonal variation has been reported for only a few crops (Larkin *et al.*, 1984; Earle, 1985). In order to exploit somaclonal variation for plant improvement, it must be heritable over sexual generations or transferred through asexual propagation and must occur for economically important characteristics (Earle, 1985). Somaclonal variation was observed among R as well as among R_1; however, desirable variation was not observed for *Streptocarpus* X *hybridus* in the present study. The future improvement of *Streptocarpus* cultivars through somaclonal variation is possible, as somaclonal variation, albeit undesirable, has been identified.

Although haploids have been regenerated from *Saintpaulia ionantha*, only diploids of somatic origin were regenerated by anther culture of *Streptocarpus* in the present studies. Because R from 'Mixed Nymph' in Experiment 2 appear uniform and normal and regenerated under conditions similar to those in Experiment 1, it is expected that they are also of somatic origin. Most tissue culture procedures are very genotype specific, requiring considerable manipulation for different species or different genotypes within species. The repeatability of *in vitro* responses is often difficult, as indicated by the change in cultivar response between the 2 experiments. Successful regeneration of *Streptocarpus* is not precluded. Species such as *Streptocarpus*, with limited *in vitro* studies, often require repeated trial and error to a attain the desired *in vitro* response. Further investigation of factors which affect androgenesis in *Streptocarpus* must be undertaken before haploids can be regenerated by anther culture.

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CHAPTER 3

EVALUATION OF INBRED AND HYBRID POPULATIONS OF

STREPTOCARPUS X *HYBRIDUS*

ABSTRACT

Early generation inbreeding in one selection of early flowering, seed-propagated *Streptocarpus* X *hybridus* 'Concorde' and 'Mixed Nymph' was characterized by smaller flowers and leaves, shorter peduncles, and increased frequencies of deformed flowers. Multivariate cluster analysis revealed exceptional genotypes which resembled the parent cultivar within the generally inferior inbred populations. Although a loss of vigor was seen in early inbred generations of 'Concorde' and 'Mixed Nymph', populations did not increase significantly in homogeneity as determined by coefficients of variation. Reciprocal hybrids between selections of 'Concorde' and 'Mixed Nymph' and their first inbred populations, though apparently vigorous, were not statistically superior to parental lines. Reciprocal differences were observed for corolla width and length, and peduncle length between hybrids derived by crosses between selections among the first inbred generation of each cultivar. Development

of superior seed propagated *Streptocarpus* cultivars may be achieved through further inbreeding and selective hybridization.

INTRODUCTION

Cultivar development in *Streptocarpus* X *hybridus*, a complex species of hybrid origin, has routinely been by selection of superior genotypes for asexual propagation (Anon., 1972; Brown, 1973; Hopka and Beck, 1984). Mutation breeding has also been effective as a means of inducing changes, e.g., for flower color, in already popular cultivars (Broetjes, 1969; Davies and Hedley, 1975). Little research has been reported on the development of inbred parents for sexually propagated hybrids which might be sufficiently homogeneous for important horticultural traits such as small leaf size, long peduncles, and early flowering. The variability for these characteristics exhibited by most currently available seed-propagated cultivars indicates a need for more uniform hybrid cultivars (Veilleux and Lyons, 1984).

The objectives of the present report were: 1) to observe the response to 2 generations of inbreeding in 2 early-flowering cultivars of seed-propagated *Streptocarpus*, and 2) to compare horticultural characteristics and uniformity of hybrids between selected genotypes representing each cultivar as well as hybrids between first generation inbreds selected from the self-pollinated progeny of single genotypes representing each cultivar.

MATERIALS AND METHODS

Seed of *S.* X *hybridus* 'Concorde' and 'Mixed Nymph' was obtained from Bodger Seed Ltd., El Monte, CA 91734 and Park Seed Company, Greenwood, SC 29647, respectively. Representative genotypes (2-11 and 6-11 from 'Concorde' and 'Mixed Nymph', respectively) were self-pollinated to generate I_1 seed. Early flowering, vigorous genotypes (2-11-3 and 6-11-2) within each I_1 family were selected and self-pollinated to generate I_2 seed. Reciprocal hybrid populations were also developed between 2-11 and 6-11, and additionally between 2-11-3 and 6-11-2. The following codes will be used to designate each population:

C	=	'Concorde' population
C_1	=	First inbred population of 'Concorde' derived from self-pollination of selection 2-11
C_2	=	Second inbred population of 'Concorde' derived from self-pollination of selection 2-11-3
N	=	'Mixed Nymph' population
N_1	=	First inbred population of 'Mixed Nymph' derived from self-pollination of selection 6-11
N_2	=	Second inbred population of 'Mixed Nymph'

derived from self-pollination of selection 6-11-2

C x N = 2-11 x 6-11

N x C = 6-11 x 2-11

C_1 x N_1 = 2-11-3 x 6-11-2

N_1 x C_1 = 6-11-2 x 2-11-3.

The ten seed lots listed above were sown on a medium composed of 1 sphagnum peatmoss:1 perlite:1 vermiculite (BX-Promix®) by volume in plastic PlantCons™ (Flow Laboratories, McLean, VA). The soil mix was dampened and autoclaved for 20 min at 15 psi to eliminate algae and microorganisms which compete with or inhibit seed germination of the fine *Streptocarpus* seed. After planting, the seed was placed under two 40 W fluorescent tubes and one 40 W incandescent bulb providing 60-75 $\mu E\ s^{-1}\ m^{-2}$. Soil moisture was maintained by saturation with a gentle mist of distilled water from a hand sprayer at approximately biweekly intervals. Seeds germinated within 2-4 weeks and the PlantCon™ tops were removed 6 weeks after sowing to acclimate the seedlings. One week later, they were transferred to the greenhouse (January, 1985), transplanted into 10 cm square plastic pots, and arranged in a randomized complete block design consisting of 5 blocks, 10 plots/block, and 5 plants/plot. Each block occupied a separate bench in the same greenhouse section. Missing plots occurred in some populations due to death of weak seedings and/or loss due to cold damage. Plants were fertilized weekly with 200 ppm N from 20N-8.8P-16.6K (20-20-20 water soluble). After March 8, the plants were grown under 50% natural irradiance. The following data were recorded at first flower anthesis: corolla width and length (taken on fully expanded corollas 4-5 days after anthesis), peduncle length, leaf length and width for the leaf subtending the flowering peduncle. Leaf area was estimated by the product of leaf length and leaf width. Statistical analyses were performed using SAS GLM for analysis of variance and SAS FASTCLUS for cluster analysis.

RESULTS

Evaluation of cultivars and the first 2 inbred generations

Means for morphological traits (flowers per peduncle, corolla width and length, peduncle length, and leaf area) of C were significantly greater than those for C_1 and C_2 (Table 1). C also flowered sooner than C_1 and C_2. C_2 was similar to C_1, except for a significant increase in leaf area and decrease in days to flowering and corolla width. N_1 was similar to N for corolla width and length, and flowers per peduncle; however, significant increases were seen in days to flowering, peduncle length, and leaf area (Table 1). N_2 differed from N_1 by a reduction in corolla width and length, peduncle length, and leaf area, as well as an increase in flowers per peduncle.

Cultivars and their inbred generations were further differentiated by cluster analysis. C, C_1, and C_2 were separated based on days to flowering, corolla width and length, peduncle length, and leaf area, and grouped into clusters consisting of similar plants for these traits. A cubic clustering criterion (CCC) of 12.72 was obtained for this analysis. The CCC value represents how well the data is separated into a predetermined number of clusters through minimization of within cluster variation and maximization of between cluster variation. A CCC value greater than 3 is indicative of good clustering. Each of the 3 clusters consisted of the majority of genotypes from one of the generations. C genotypes, though predominantly found in cluster 2, were distributed in all 3 clusters, whereas C_1 and C_2 genotypes were distributed in only 1 and 2 clusters respectively (Table 2). N, N_1, and N_2 were also separated into 3 groups by cluster analysis (CCC = 4.35). One cluster was composed of the majority of N and N_1, the second cluster was composed mostly of N_2, and the third cluster consisted of 2 plants which were very abnormal for the measured traits (Table 3). Means for days to flowering, corolla width and length, peduncle length and leaf area of plants in each cluster from both cluster analyses characterized the morphology of the plants in each cluster (Table 4). Two dimensional representations of the clustering of each cultivar and its inbreds using the first 2 variables from the canonical discriminant analysis show the distribution of each cluster and its constituents (Figures 1 and 2).

Evaluation of cultivars and reciprocal hybrids

C had significantly larger corollas, longer peduncles, and larger leaves than N and is earlier to flower (Table 5). Means for days to flowering and leaf area for C x N and N x C were significantly different from and intermediate between parental cultivar means. Corolla width and length were similar to N, whereas peduncle length was similar to C. No reciprocal differences for C x N vs. N x C were seen for any of the measured traits.

C_1 x N_1 and N_1 x C_1 were similar to C with regard to days to flowering, and N with regard to flowers per peduncle. Leaf area was different from and intermediate between parental values. Reciprocal differences were observed for corolla width and length, with means closer to or equal to the means of the female parent. Transgressive segregation was observed for peduncle length in the C_1 x N_1 hybrid and for corolla width in the N_1 x C_1 hybrid when compared to means for C_1 and N_1. Coefficients of variation (cv) did not indicate increased homogeneity for hybrids over parental cultivars for most traits measured.

Table 1. Means and coefficients of variation (cv) for several morphological characteristics of Streptocarpus X hybridus Concorde and Mixed Nymph and their first 2 inbred generations.

Genotype	Days to flowering	Corolla width (cm)	Corolla length (cm)	Flowers per peduncle[z]	Peduncle length (cm)	Leaf area[y] (cm^2)
	mean cv	mean cv	mean cv	mean cv	mean cv	mean cv
Concorde	152d[x](6.6)	7.2a(11.5)	7.9a(7.4)	1.8b(25.0)	11.0a(19.9)	158.3a(23.7)
C_1	178b (8.2)	6.0b(13.5)	7.1b(6.6)	1.5c(16.1)	8.9b(22.0)	86.1d(33.5)
C_2	165c(11.6)	4.6d(10.5)	6.7c(4.9)	1.6c(13.1)	8.1b(22.7)	133.4b(22.9)
M. Nymph	175b (8.0)	6.5b(11.2)	6.8c(7.9)	2.0b(26.8)	8.9b(19.0)	102.8c(35.7)
N_1	195a (5.8)	6.2b(8.7)	6.5c(6.5)	1.9b(28.0)	9.8a(24.9)	124.4b(24.9)
N_2	200a (3.7)	5.2c(13.3)	5.9d(7.1)	2.4a(24.5)	8.7b(20.2)	81.5d(43.7)

[z] Mean of 10 peduncles.

[y] Estimated by the product of leaf length and leaf width.

[x] Mean separation within columns by Student-Newman-Keuls' test, 5% level.

Table 2. Distribution of genotypes of *Streptocarpus* x *hybridus* Concorde and its first 2 inbred generations into 3 clusters as defined in Table 4.

Cluster	Generation			
	C	C_1	C_2	Total no. of genotypes per cluster
1	2	0	18	20
2	19	0	0	19
3	4	20	7	31
Total no. of genotypes per generation	25	20	25	70

Table 3. Distribution of *Streptocarpus* x *hybridus* Mixed Nymph and its first 2 inbred generations into 3 clusters as defined in Table 4.

Cluster	Generation			
	N	N_1	N_2	Total no. of genotypes per cluster
1	3	2	12	17
2	18	19	3	40
3	1	1	0	2
Total no. of genotypes per generation	22	22	15	59

Table 4. Cluster means for various morphological traits of 2 populations (Concorde and Mixed Nymph) of Streptocarpus X hybridus from independent analyses by SAS FASTCLUS. Each population consisted of approximately equal numbers of the named cultivar as well as first and second generation inbred lines of that cultivar.

Population	Cluster	No. of genotypes in cluster	Days to flowering	Corolla width (cm)	Corolla length (cm)	Peduncle length (cm)	Leaf area[z] (cm^2)
Concorde	1	20	156	4.7	6.7	8.4	137.3
	2	19	152	7.4	8.2	11.5	168.7
	3	31	178	5.8	7.0	14.0	96.0
M. Nymph	1	17	198	5.1	5.7	8.3	72.9
	2	40	185	6.5	6.7	9.2	115.2
	3	2	202	6.0	6.8	15.3	183.4

[z]Estimated by the product of leaf length and leaf width.

Table 5. Means and coefficients of variation (cv) for several morphological characteristics of **Streptocarpus** × **hybridus** Concorde, Mixed Nymph, and reciprocal hybrids of a) a single selection of each cultivar (C × N and N × C), and b) a single selection of the first inbred generation of selections in a) ($C_1 \times N_1$ and $N_1 \times C_1$).

Genotype	Days to flowering		Corolla width (cm)		Corolla length (cm)		Flowers per peduncle[z]		Peduncle length (cm)		Leaf area[y] (cm^2)	
	mean	cv	mean	cv	mean	cv	mean	cv	mean	cv	mean	cv
Concorde	152c[x]	(6.60)	7.2a	(11.51)	7.9a	(7.40)	1.8b	(25.01)	11.0b	(19.89)	158.3a	(23.67)
M. Nymph	175a	(7.97)	6.5b	(11.21)	6.8cd	(7.93)	2.0ab	(26.78)	8.9c	(19.03)	102.8c	(36.66)
C × N	159b	(7.13)	6.1b	(10.12)	7.0c	(8.33)	2.1ab	(24.89)	10.7b	(20.84)	119.0b	(26.46)
N × C	165b	(6.23)	6.1b	(11.08)	7.2c	(8.06)	2.1ab	(31.64)	11.0b	(25.88)	133.8b	(35.07)
$C_1 \times N_1$	152c	(9.65)	6.2b	(8.33)	7.5b	(5.69)	2.1ab	(26.71)	14.2a	(18.11)	137.1b	(21.66)
$N_1 \times C_1$	150c	(9.06)	5.2c	(10.79)	6.6d	(6.19)	2.4a	(24.21)	8.3c	(20.78)	134.6b	(25.58)

[z] Mean of 10 peduncles.

[y] Estimated by the product of leaf length and leaf width.

[x] Mean separation within columns by Student-Newman-Keuls' test, 5% level.

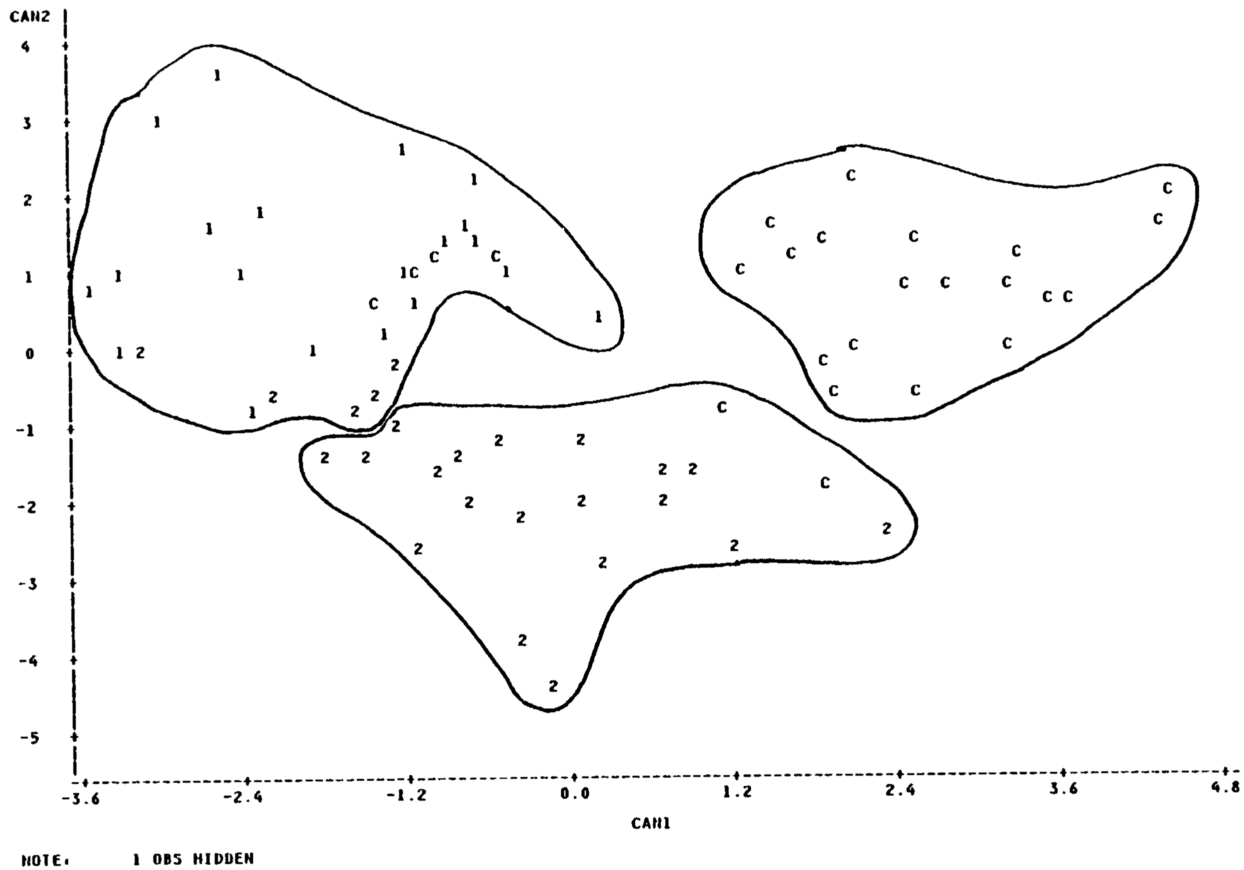

Figure 1. Two-dimensional representation of divergence of *Streptocarpus* X *hybridus* Concorde and its first 2 inbred generations (C, 1, and 2 respectively) using the first 2 variables from canonical discriminant analysis. The 3 contours represent the Concorde clusters described in Table 4.

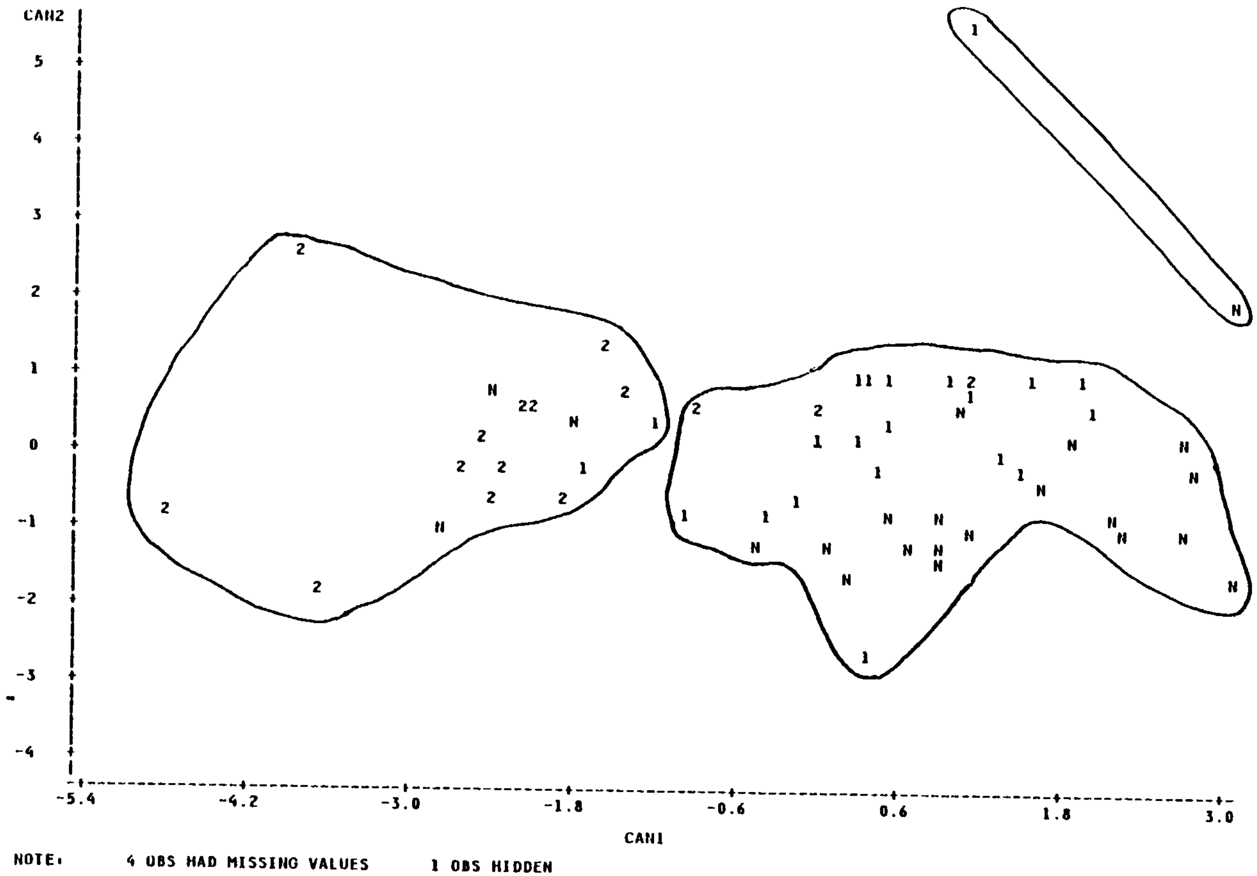

Figure 2. Two-dimensional representation of divergence of *Streptocarpus* X *hybridus* Mixed Nymph and its first 2 inbred generations (N, 1, and 2 respectively) using the first 2 variables from canonical discriminant analysis. The 3 contours represent the Mixed Nymph clusters described in Table 4.

DISCUSSION

Effect of inbreeding

Inbreeding depression occurred in both cultivar selections, and was expressed as a reduction in plant organ size (corolla and leaf) as well as delayed first flower anthesis. An increased frequency of floral abnormalities (fused or deformed corollas) was also observed in inbred populations, especially among C_2 and N_2. This supports previous reports of a significant loss of vigor on inbreeding of *S*. X *hybridus* 'Constant Nymph' (Hammer, 1976; Schmidt and Erikson, 1981). The more rapid response to inbreeding of C over N could reflect the genetic composition of the two cultivars. C is reportedly an F_1 hybrid whereas N has undergone significant breeding and selection since the initial interspecific hybridization used to develop the Nymph types (Brown, 1973). Hence, greater homozygosity has probably been attained within 2 generations of inbreeding for N than for C. However, inbred populations of each cultivar were represented by progeny of only a single genotype; therefore, generalizations about inbreeding effects upon the cultivars are limited

Potential value of inbreds

Selected genotypes within the inbred populations varied substantially for important horticultural characteristics including early flowering, increased flowers per peduncle, and reduced foliage. Multivariate cluster analysis can be effectively used to select individuals from inbred populations which most resemble the cultivar type. In Figs. 1 and 2, certain C_1, C_2, N_1, and N_2 individuals have been grouped in the same cluster as or are very close to individuals representing the parent cultivar, and thus would be similar to plants of that cultivar for all the measured morphological traits. Selection of the rare inbreds similar to the parent cultivar as identified in the cluster analysis could result in the development of inbred lines more resistant to inbreeding depression. Likewise, cultivar and inbred genotypes characteboted by different traits could be selected and utilized for further breeding. Flower color genes, as identified by Lawrence and Sturgess (1957) could be manipulated to develop inbred lines which yield either multicolored or single colored families in hybrid combinations.

Evaluation of reciprocal hybrids

Significant increases in homogeneity were not seen within C_1 x N_1 or N_1 x C_1 over C, N, C x N or N x C. This indicates that C_1 and N_1 were still fairly heterogeneous after 1 generation of inbreeding and did not yield more uniform hybrids than the original cultivars or cultivar hybrids. Hybrid populations were more uniform for flower color than the parent cultivars. Early generations of inbreeding often increase rather than decrease the expression of previously hidden recessive alleles (Briggs and Knowles, 1967). Increased variation has been observed in early inbred generations of *S.* X *hybridus* 'Constant Nymph' (Hammer, 1976). Further inbreeding would be required to develop sufficiently homozygous lines for uniform hybrid production.

Reciprocal differences were observed for C_1 x N_1 vs. N_1 x C_1, but not between C x N vs. N x C. Different classes of cytoplasm have been found among *Streptocarpus* species (Oehlkers, 1964). Inbreeding may have caused the expression of recessive extranuclear genes that effect inheritance of certain traits. Because reciprocal differences have been observed to occur after inbreeding, the choice of the female parent could be critical in yielding the desired hybrids from inbred lines of *Streptocarpus*.

Conclusion

Within 2 generations, selected genotypes of *S.* X *hybridus* 'Concorde' and 'Mixed Nymph' suffered inbreeding depression characterized by reduced plant organ size and increased floral deformities. Hybrids of first inbred selections were vigorous; however, heterosis was not statistically detected because of the highly heterogeneous nature of the first inbred generation populations.

The development of more homogeneous, seed-propagated *S.* X *hybridus* cultivars via selection of superior inbred lines for hybrid production seems possible. In order to achieve this goal, the effect of further inbreeding on vigor of several genotypes would have to be investigated. Inbred populations should be sufficiently large to detect superior genotypes. Cluster analysis may be useful to identify inbred genotypes within variable inbred populations with characteristics similar or dissimilar to the parental cultivar.

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