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Sex Expression and Sex Ratios in Intra- and Interspecific Hybrid Families of Salix L.

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Summary

Artificial hybridization studies with *Salix amygdaloides* ANDERSS., *S. bebbiana* SARG., *S. discolor* MUHL., *S. eriocephala* MICHX., *S. exigua* NUTT., *S. lucida* MUHL., *S. pellita* MUHL. and *S. petiolaris* SMITH led to observations on deviations from the 1:1 sex ratio expected in dioecious species. Female biased sex-ratios were observed in several controlled intraspecific crosses of *S. eriocephala* and *S. petiolaris*, in controlled interspecific crosses of *S. pellita* × *exigua*, *S. petiolaris* × *eriocephala*, *S. petiolaris* × *exigua* and in open-pollinated families of *S. discolor*, *S. eriocephala*, *S. petiolaris*, and *S. pellita* × *discolor*. Pollen certation may explain female biased sex ratios in intraspecific families. Biased sex ratios in interspecific hybrid families may also be explained by genetic incompatibility between sex determining genes within the hybrid genome. Cytoplasmic interactions are implicated in male biased sex ratios observed in interspecific hybrids in which *S. exigua* was the pistillate parent. Hermaphrodite plants were observed in several controlled intraspecific crosses of *S. amygdaloides*, *S. exigua*, and *S. lucida*. Most hermaphrodite plants reverted to full male sex expression with age, suggesting that the genetic mechanism responsible for the suppression of female sex expression in the male genotype is labile and under a greater degree of environmental control. The evidence presented suggests that males are the heterogametic sex.

Key words: Sex expression, set ratios, sex determination, hermaphrodite, hybridization, pollen certation.

Zusammenfassung

Künstliche Hybridisierung mit *Salix amygdaloides* ANDERSS., *S. bebbiana* SARG., *S. discolor* MUHL., *S. eriocephala* MICHX., *S. exigua* NUTT., *S. lucida* MUHL., *S. pellita* MUHL. and *S. petiolaris* SMITH führte zu Abweichungen des in diözischen Arten erwarteten 1:1 Geschlechtsverhältnisses. Weiblich beeinflußte Geschlechtsverhältnisse wurden in einigen kontrollierten interspezifischen Kreuzungen von *S. eriocephala* und *S. petiolaris*, in kontrollierten interspezifischen Kreuzungen von *S. pellita* × *discolor*, *S. bebbiana* × *petiolaris*, *S. eriocephala* × *exi-*

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gua, S. petiolaris × *eriocephala, S. petiolaris* × *exigua* und in frei-abgeblühten Familien von *S. discolor, S. eriocephala, S. petiolaris* und *S. pellita* × *S. discolor* beobachtet. Genotypbedingte Pollenkonkurrenz könnte das weiblich beeinflußte Geschlechtsverhältnis in intraspezifischen Hybridfamilien erklären, und das in interspezifischen Hybridfamilien beeinflußte Geschlechtsverhältnis könnte durch genetische Inkompatibilitäten zwischen geschlechtsbestimmenden Genen innerhalb des Hybridgenoms erklärt werden. Zytoplasmatische Interaktionen sind in männlich beeinflußten Geschlechtsverhältnissen von Bedeutung, wenn *S. exigua* der weibliche Elter war. Hermaphrodite Pflanzen wurden in einigen kontrollierten intraspezifischen Kreuzungen von *S. amygdaloides, S. exigua* and *S. lucida* beobachtet. Die meisten hermaphroditen Pflanzen bildeten mit dem Alter den männlichen Sexualtyp aus. Dies läßt vermuten, daß der genetische Mechanismus, der das weibliche Geschlecht im männlichen Genotyp bedingt, labil ist und stärker durch die Umwelt kontrolliert wird. Die dargelegten Befunde legen nahe, daß das männliche das heterogametische Geschlecht ist.

Introduction

The family *Salicaceae contains* 3 genera (*Populus* L., *Salix* L. and *Chosenia* Nakai) and is an entirely dioecious family. Dioecy occurs in only about 4% of all flowering plant species (Richards, 1986), but dioecious species are widely spread over taxonomic groups appearing in 75% of flowering plant families (Lewis, 1942). The dioecious condition has probably evolved from either hermaphrodite or monoecious ancestors (Charlesworth and Charlesworth, 1978).

The existance of a heteromorphic pair of chromosomes in the Salicaceae has not been confirmed (Westergaard, 1958; Grant and Mitton, 1979), and the small size and large number (n = 19) of chromosomes in this family make them difficult to distinguish morphologically. In most sexually dimorphic species for which the sex determination mechanism has been verified, sex is genetically determined at conception through the mechanism of either male or female heterogamety (Westergaard, 1958; Karlin and Lester, 1986). This results in plant populations with a primary sex ratio (from seed) of approximately equal numbers of males and females.

The only report of deviations in the primary sex ratio in willows comes from artificial crosses involving *S. cinerea* L. in which Heribert-Nilsson (1918) observed a significant female bias. In a study of sex ratios in natural populations of the arctic willows, *Salix herbacea* L. and *S. polaris* Wahlenb., Crawford and Balfour (1983) found that 59% of individuals sampled from both species were female. Deviations from the expected sex ratio in natural populations are often a function of sex related ecological adaptations which lead to differential mortality between the sexes (Freeman *et al.*, 1976; Grant and Mitton, 1979; Crawford and Balfour, 1983).

Sex expression in plants is expected to be labile and under some degree of environmental control (Charnov and Bull, 1977), especially among species living in patchy environments where sex expression may be adapted to maximize the relative genetic fitness of individuals (Freeman *et al.*, 1981). Such lability in sex expression has been observed in many dioecious species (Lester, 1963; Stettler, 1971; Freeman *et al.*, 1976; Conn and Blum, 1981; Horowitz and Dulberger, 1983; Primack and McCall, 1986).

A study of reproductive barriers between some North American willow species provided an opportunity to observe sex expression in the progenies of controlled crosses and from open pollinated families (Mosseler, 1987). The present study attempts to relate these observations to probable sex determination mechanisms in *Salix*, the possibility of pollen certation, impairment of the genetic mechanism determining sex in interspecific hybrids and the evolution of dioecy in *Salix*.

Materials and Methods

Most of the parent clones used in hybridization were collected from natural populations within a 100 km radius of Toronto, Ontario, Canada (lat. 44°, long. 80°). Several of the parent plants from *S. petiolaris* and *S. lucida* and the 2 pistillate plants of *S. pellita* used in this study originated in northern Ontario (Mosseler, 1987). The parent plants were established as clones in a common garden from which open pollinated seed was collected.

Artificial hybridization was carried out in 1983 and 1984 with the *Salix* species listed in *Table 1*. Dormant flower branches of these species were forced to root and flower simultaneously in small pots. Male and female plants were housed in separate glasshouse compartments maintained at a temperature of 20° C + 5° C with a relative humidity of between 40 and 50 percent during artificial pollination and seed maturation. Controlled pollinations were carried out by removing male catkins at anthesis and depositing large amounts of fresh pollen directly onto the receptive female flowers which were pollinated as soon as the lobes of the stigma were fully reflexed. Each controlled cross involved a single pollen parent — no pollen mixtures were used.

Seeds were geminated in plastic Petri dishes containing moist silica sand. The young germinants were transplanted to individual pots and raised in a glasshouse for 3 to 4 months prior to establishment in two separate nursery tests planted in May (test 1) and August (test 2), 1984, respectively. The plants were established in a randomized complete block design consisting of 6 replications of 4 tree plots with a triple border row of surrounding plants. The spacing between plants was 1 m × 1 m. The planting sites were silty-clay loam hilltops on former agricultural land. Since these sites were too dry for most willow species to attain their optimum growth, supplemental irrigation was applied during extended dry periods during mid-summer.

The controlled crosses listed in *Table 2* that were established in nursery test 1, were made in 1983 and were ob-

Table 1. — Taxonomic classification of *Salix* species studied[1])

Taxonomical classification				Number of parental clones studied	
Subgenus	Section	Species	Acronymn	female	male
Salix	Humboldtiana	S. amygdaloides Anderss.	SAM	4	3
	Salicaster	S. lucida Muhl.	LUC	7	7
	Longifoliae	S. exigua Nutt.	INT	5	6
Vetrix	Cordatae	S. eriocephala Michx.	ERIO	7	10
	Vetrix	S. bebbiana Sarg.	BEBB	5	2
		S. discolor Muhl.	DIS	6	9
		S. petiolaris Smith	PET	6	8
	Vimen	S. pellita Anderss.	SPEL	2	0

[1]) According to Dorn (1976).

Table 2. — Number of female, male, hermaphrodite and non-flowering plants observed among full-sib *Salix* families.

Full-sib *Salix* families (female x male)	Number of surviving plants	Percent of surviving progeny: Females %	Males %	Bi-sexual %	Non-flowering %	x^2 sex ratio
BEBB291 x ERIO292^^	8	25	12.5		62.5	
BEBB291 x INT287^^	12	16.7			83.3	
BEBB304 x PET281^^	32	100				32.00***
ERIO 16 x ERIO 24^	42	50	50			
ERIO 19 x ERIO 23^	24	41.7	58.3			0.66
ERIO 21 x ERIO 23^	32	50	46.9		3.1	0.32
ERIO 28 x ERIO 24^	38	36.8	63.2			2.63
ERIO 39 x ERIO 25^	38	57.9	42.1			0.95
ERIO 16 x ERIO263^^	33	42.4	57.6			0.76
ERIO 16 x ERIO276^^	33	45.4	54.6			0.27
ERIO 16 x ERIO292^^	33	42.4	57.6			0.76
ERIO 16 x ERIO307^^	33	54.6	45.4			0.27
ERIO269 x ERIO263^^	33	93.9	6.1			25.48***
ERIO269 x ERIO292^^	33	100				33.00***
ERIO 28 x DIS 22^	30	20.0	36.7		43.3	
ERIO269 x INT 287^^	29	13.8			86.2	
LUC 5 x LUC 24^	30	50	50			
LUC 5 x LUC 43^	32	62.5	31.3	3.1	3.1	3.33
LUC 17 x LUC 43^	30	53.3	46.7			0.13
LUC 32 x LUC 69^	24	45.8	54.2			0.17
LUC 68 x LUC 69^	32	50.0	46.9	3.1		0.03
LUC 70 x LUC 64^	37	48.6	51.4			0.03
LUC 70 x LUC 69^	38	55.3	44.7			0.42
LUC 68 x LUC 43^^	33	39.4	51.5		9.1	0.53
LUC 68 x LUC 64^^	33	48.5	27.3	24.2		1.96
LUC251 x LUC 64^^	33	51.5	30.3	6.1	12.1	1.81
LUC251 x LUC 69^^	33	63.6	36.4			2.45
LUC317 x LUC 43^^	33	45.4	51.5		3.0	0.12
LUC317 x LUC 69^^	33	42.4	54.5		3.0	0.50
INT 61 x INT 63^	49	28.6	40.8		30.6	1.06
INT 31 x INT 66^	24	33.3	37.5	12.5	16.7	0.58
INT 27 x INT 66^^	32	65.6	34.4			3.12
INT 27 x INT287^^	33	39.4	42.4		18.2	0.04
INT 27 x INT293^^	33	39.4	45.4	3.0	12.1	0.14
INT 61 x INT 66^^	33	33.3	57.6		9.1	2.13
INT 61 x INT287^^	33	30.3	36.4	9.1	24.2	0.18
INT 61 x INT293^^	32	12.5	21.9	3.1	62.5	
INT 62 x INT 42^^	33	48.5	48.5		3.0	
INT 62 x INT 66^^	33	57.6	42.4			0.76
INT 62 x INT293^^	24	54.2	25.0		20.8	2.58
INT 62 x ERIO 57^	32		59.4		40.6	19.0***
INT 17 x PET 49^	8		75		25	
INT 27 x PET 55^	16		87.5		12.5	14.0***
INT 31 x PET 55^	18		77.8		23.2	14.0***
INT 62 x PET 55^	17		64.7		35.3	11.0***
INT 27 x DIS277^^	27	22.2	22.2		55.6	
INT 27 x ERIO276^^	31		61.3	3.2	35.5	19.0***
INT 61 x ERIO263^^	3		33.3		66.7	
INT 61 x ERIO307^^	14				100	
INT 62 x DIS261^^	4		75		25	
INT 62 x DIS277^^	6	16.7	50		33.3	
INT 62 x ERIO276^^	7		14.3	28.6	57.1	
SPEL 1 x DIS 13^	41	41.5	58.5			1.20
SPEL 1 x DIS 23^	37	51.4	35.1		13.5	1.12
SPEL 2 x DIS 8^	41	87.8			12.2	36.0***
SPEL 2 x DIS 23^	40	100				40.0***
PET 36 x PET 33^	24	100				24.0***
PET311 x PET298^^	32	100				32.0***
PET311 x PET323^^	33	100				33.0***
PET 36 x ERIO 25^	33	93.9			6.1	31.0***
PET 4 x ERIO 65^	23	52.2	12.5	34.8		5.40*
PET 16 x ERIO 57^	28	28.6	60.7	10.7		4.84*
PET 53 x INT 42^	15	73.3			26.7	11.0**
PET311 x ERIO263^^	37	29.7			70.2	
PET311 x ERIO276^^	7	100				
PET311 x ERIO292^^	30	26.7			73.3	
PET311 x ERIO294^^	23	69.6	4.3		26.1	13.2***
PET311 x ERIO307^^	9	100				
PET311 x INT 287^^	6	50			50	
PET311 x INT 293^^	3	100				
SAM 44 x SAM282^^	33	24.2		15.2	60.1	
SAM 44 x SAM315^^	33	15.2		3.0	81.8	
SAM259 x SAM282^^	31	6.4	6.4		87.1	
SAM259 x SAM315^^	33	9.1	12.1	3.0	75.8	
SAM272 x SAM282^^	33	18.2	27.3	12.1	42.4	

^ Families established in May 1984 (test 1)

^^ Families established in August 1984 (test 2)

* Significant at $0.01 < P \leq 0.05$

** Significant at $0.001 < P \leq 0.01$

*** Significant at $P < 0.001$

Table 3. — Number of female, male, hermaphrodite and non-flowering plants observed among open-pollinated *Salix* families established in August 1984 (test 2).

Pistillate parent	Number of surviving plants	Percent of surviving plants: Females %	Males %	Bi-sexual %	Non-flowering %	x^2 sex ratio
DIS 14	33	60.6	36.4	3.0		2.0
DIS 40	29	89.6	7.7	3.4		20.6***
DIS 41	33	60.6	36.4		3.0	2.0
DIS 58	32	78.1	12.5	3.1	6.2	15.2***
ERIO 16	33	51.5	48.5			0.03
ERIO 21	33	69.6	31.3			5.12*
ERIO 28	33	51.5	48.5			0.03
ERIO 39	33	100				33.0***
PET 36	33	87.9	6.1		6.1	23.5***
SPEL 1	33	78.8	15.2		6.1	14.2***

* Significant at $0.01 < P \leq 0.05$

*** Significant at $P < 0.001$

served for flowering and sex expression in 1985 after one full growing season, and again in 1987 to determine if sex expression changed with time. The controlled crosses listed in *Table 2* that were made in 1984 were established in nursery test 2, together with the open-pollinated families listed in *Table 3*. The plants in nursery test 2 were observed for sex expression in 1986 after plants had grown for 1.5 growing seasons under natural conditions. Sex expression in hermaphrodite plants from both tests were observed again in 1987 by forcing dormant branches to flower under glasshouse conditions. The number of males, females, hermaphrodite and non-flowering plants in each family were noted. The significance of deviations from the expected 1:1 ratio of staminate to pistillate plants was determined by the Chi-squared test (SOKAL and ROHLF, 1981).

Results

A high proportion of progeny from controlled crosses of *S. amygdaloides* failed to flower after their first growing season in the nursery tests *(Table 2)*. This species is a tree-forming willow and may require more time to reach sexual maturity. Among the progeny that did flower, a high proportion of hermaphrodite plants was observed. In the following year these hermaphrodite plants produced only male catkins.

Poor rootability of the dormant flower branches of *S. bebbiana* and *S. discolor* resulted in heavy mortality, preventing seed maturation in most artificial crossing attempts. The unusually vigorous hybrid family of *S. bebbiana* 304[1]) × *petiolaris* 281 (*Table 2*) produced only pistillate plants and two of the open pollinated families of *S. discolor* (*Table 3*) that were collected from a common garden produced highly significant deviations in favour of female progeny (at $P < 0.001$). The three hermaphrodite plants observed from these open pollinated families in 1986 produced only female flowers in 1987.

Most intraspecific families of *S. eriocephala* (*Tables 2* and *3*) did not deviate significantly from the expected 1:1 ratio of female to male plants. The notable exceptions were the controlled intraspecific crosses involving *S. eriocephala* 269 and the open pollinated family, *S. eriocephala* 39 (*Table 3*) which produced only female plants. No hermaphrodite plants were observed from controlled intraspecific

[1]) The number following species names refers the specific parent clone used in breeding.

crosses of *S. eriocephala* but several hermaphrodite plants were observed among interspecific hybrid families of *S. exigua* × *eriocephala* (*Table 2*).

Intraspecific families of *S. exigua* (*Table 2*) were among the first to reach sexual maturity often producing flowers after several months of growth from seed and did not deviate significantly from the expected 1:1 sex ratio but did produce a high proportion (18.4% over all families) of non-flowering plants as well as several hermaphrodite plants. Many of the catkins of hermaphrodite plants were entirely staminate with the proportion of hermaphrodite catkins appearing to decrease as plants aged. Hermaphrodite catkins consisted of a mixture of staminate and pistillate flowers. No hermaphrodite flowers were observed on *S. exigua* catkins. Among 359 progeny from intraspecific crosses of *S. exigua* (*Tables 2* and *3*), 8 plants produced hermaphrodite catkins during their first year of flowering. When observed in the following year (1987), 4 of these 8 plants produced only male flowers whereas the remaining 4 plants continued to produce a small proportion of hermaphrodite catkins.

Interspecific hybrid families of *S. exigua* × *eriocephala* and *S. exigua* × *petiolaris* (*Table 2*) failed to produce any sexually mature pistillate plants after 2 years growth. High proportions of the hybrid progeny from these interspecific crosses grew poorly and failed to produce flowers. Only the more vigorous interspecific hybrids from these families flowered, producing infertile male plants. Both male and female plants were observed in progeny from *S. exigua* × *discolor* crosses but a male bias was evident in 2 of these crosses (*Table 2*).

None of the controlled intraspecific families of *S. lucida* (*Table 2*) deviated significantly from the expected 1:1 sex ratio. Hermaphrodite plants appeared relatively frequently in *S. lucida*. Within hermaphrodite catkins both male and hermaphrodite flowers were observed. The stamens of hermaphrodite flowers were attached at the base of the pistil (stipe). Successful self-pollinations carried out within hermaphrodite catkins revealed that both male and female flower parts were functional. Only one of the 12 hermaphrodite plants observed (in 1985 and 1986), produced hermaphrodite catkins when observed in 1987. The remaining 11 plants produced only male flowers.

The controlled crosses between *S. pellita* × *discolor* (*Table 2*), in which *S. pellita* 1 (SPEL 1) was used as the female parent did not deviate significantly from the expected sex ratio, whereas the open pollinated seed collected from ramets of SPEL 1 established in the common garden resulted in a highly significant deviation in favour of female progeny (*Table 3*). These open pollinated progeny were morphologically similar to artificially produced interspecific hybrids between *S. pellita* × *discolor*, indicating that the pollen parents were clones of *S. discolor* established in the same common garden. No staminate plants of *S. pellita* were present in the common garden (or in the Toronto area which is outside the natural range of this species). Controlled interspecific crosses with *S. pellita* 2 (SPEL 2) produced families with a highly significant female bias (*Table 2*).

Most of the controlled intra- and interspecific crosses of *S. petiolaris* (*Table 2*) and the open pollinated family PET 36 (*Table 3*) had significant female biases. Females outnumbered males by 118 to 2 and by 119 to 21 in intra- and interspecific families, respectively. No hermaphrodite plants were observed among the progeny of this species.

Discussion

Female biased primary sex ratios in willow species can be explained by the occurance of agamospermy, certation or a breakdown in the genetic mechanisms governing sex determination. Agamospermy was suspected but never confirmed by Ikeno (1922) in crosses between several Asian willow species. Species prone to agamospermy would be more likely to produce seed apomictically in response to interspecific pollination (Gustafsson, 1946, 1947), but controlled interspecific pollinations in *Salix* rarely produced progeny with maternal morphological types, with the exception of several plants from crosses in which *S. petiolaris* (clone 311) was used as the pistillate parent. Most controlled interspecific crosses with *S. petiolaris* resulted in the production of progeny with intermediate morpholocal traits.

The phenomenon of pollen certation, first described by Correns (1928 — cited in Conn and Blum, 1981), may explain the highly significant female bias observed in some controlled crosses. Correns found that sex-ratios in the dioecious species *Rumex acetosa* and *Silene alba* could be manipulated by controlling the amount of pollen applied to the stigma. Under high pollen densities, female biased sex-ratios would result, while under lower pollen densities progeny sex-ratios would return to equality. In *Rumex* species, sex is determined by X (female) and Y (male) chromosomes in which the Y chromosomes are partially inert (Smith, 1963; Zuk, 1970). The inertness of the Y chromosome weakens Y carrying pollen resulting in pollen competition within the pistil which favours X-determined pollen tubes and leads to the predominance of female plants among progeny (Conn and Blum, 1981). The high variability in pollen tube growth rates within controlled crosses observed by fluorescent light microscopy indicated strong competition between pollen tubes within the style (Mosseler, 1987).

Lewis (1942) suggested that pollen certation in nature would provide a useful feedback mechanism through which dioecious species might be able to adjust population sex ratios in the presence of an overabundance of pollen (staminate plants) by producing an excess of female plants. As Lloyd (1974) has pointet out, such a mechanism is not directly selected for, but may be a consequence of gamete competition based on differences associated with sex chromosomes such as those discribed by Smith (1963), Mulcahy (1967), Zuk (1970) and Conn and Blum (1981). Pollen certation could have a selective value in adjusting population sex ratios but this should not be interpreted as an argument for 'group selection' at the level of the sporophyte although the ultimate effect may be similar. Any influence of pollen certation on population sex ratios will always be constrained by the selective advantages conferred upon the less frequent sex (Shaw and Mohler, 1953).

Some open pollinated willow families produced highly significant deviations in favour of female progeny (*Table 3*). Although it would appear unlikely that such deviations from the expected sex-ratio could result from pollen certation under natural conditions, such a possibility cannot be discounted, especially under the conditions of heavy natural pollination that prevailed in the densely spaced (1 m × 2 m/plant) common garden from which open-pollinated seed was collected.

Natural populations of willows such as *S. petiolaris* sometimes occur at very high plant densities relative to congeneric competitors like *S. amygdaloides* and *S. lucida*.

The latter two species also have between 30 to 40 ovules per locule (MOSSELER, unpublished data) making them less susceptible to the effects of certation under conditions of heavy pollination than *S. petiolaris* which has only 6 or 7 ovules per locule (G. W. ARGUS, pers. comm.). Although no data on sex ratios in natural populations of *S. petiolaris* are available (nor has the effect of varying pollen densities on sex ratio been tested), a female bias might enhance the competitive ability of *S. petiolaris* relative to competing willow species which may have a higher capacity for seed production (MOSSELER, 1987).

The sex determining mechanism(s) in *Salix* species may be similar to that found in *Silene alba* by MULCAHY (1967) or that of *Rumex hastatulus* (CONN and BLUM, 1981). If certation were the cause of the female bias observed in some families, this would suggest that female willow plants are probably the homogametic sex. Hermaphrodite plants of *S. exigua, S. amygdaloides,* and *S. lucida* produce predominantly male flowers and normally revert to a fully male sex expression with age, indicating that the male genotype has a labile phenotype and is therefore probably the heterogamete in which the female suppression mechanism is malfunctioning. If hermaphrodite plants are considered to be inconstant males then the 2:1:1 ratio between females, males and hermaphrodites observed in the cross LUC68 × LUC64 (*Tables 2*) becomes the 1 female to 1 male sex ratio usually observed in *S. lucida.* All but one of the hermaphrodite plants from this cross became fully staminate in 1987, suggesting the presence of a weak (recessive) female suppressor gene that results in the incomplete expression of maleness. In *Populus trichocarpa* TORR. and GRAY., and related hybrids the situation appears to be reversed with female plants showing variation in sex expression while male plants remain constant (STETTLER, 1971).

Polyploidy in *S. lucida* (2n = 76) apparently has no effect on the 1:1 sex ratio. The relationship between male and female determining chromosome(s)/gene(s) in polyploid willows may be similar to that found by WESTERGAARD (1958, p. 253) in *Melandrium* (*Silene*) polyploids, where the presence of a single Y chromosome can suppress the effects of three X chromosomes and 4 sets of autosomes.

The only crosses showing strong male biased sex ratios were those from interspecific crosses in which *S. exigua* was the pistillate parent. Interspecific crosses between *S. exigua* × *eriocephala* and *S. exigua* × *petiolaris* produced only male offspring or inferior non-flowering progeny, suggesting a possible negative interaction between either cytoplasmic factors or the female determining chromosome/genes of *S. exigua* and the female determining chromosome/genes of *S. eriocephala* and *S. petiolaris* that renders female hybrids sterile and/or inviable. In reciprocal species crosses such as *S. petiolaris* 53 × *exigua* 42 (*Table 2*) only female offpring or inferior non-flowering progeny were produced, suggesting that cytoplasmic interactions may be involved in sex expression or sex determination rather than genetic incompatibility between nuclear sex chromosomes in interspecific crosses involving pistillate plants of *S. exigua.*

Male and female sterility in interspecific hybrids between *Drosophila species* has been related to genetic incompatibility between sex chromosomes from different species and also from negative cytoplasmic interactions (COYNE, 1984; ORR, 1987). While female biased sex ratios in intraspecific *Salix* crosses may be most easily explained by pollen certation effects, biased sex ratios from controlled interspecific hybrid families may be explained both by certation effects or by a breakdown in the genetic mechanism of sex determination. Further investigations into linkages between sex determination and viability, and the presence of cytoplasmic interactions in sex determination are needed to explain these results in *Salix* hybrids.

Under male heterogamety, the male determining genes may function by suppressing the expression of femaleness (CHARLESWORTH and CHARLESWORTH, 1978; KARLIN and LESTER, 1986). According to CHARLESWORTH and CHARLESWORTH (1978), dioecy evolved from a hermaphrodite or monoecious ancestral condition via an intermediate condition consisting of populations of both hermaphrodite and male sterile (gynodioecious) plants. The reversion of hermaphrodites to males and in particular the 2:1:1 ratio between females, males and hermaphrodites observed in the cross LUC68 × LUC64 (*Table 2*), suggests that dioecy in willows may have evolved via gynodioecy following the establishment of a dominant mutation leading to complete female sterility in heterogamete males.

Hermaphrodite plants were most prevalent in progeny from species of subgenus *Salix* (*Table 2*). Hermaphrodites were also observed by the senior author in natural populations of *S. amygdaloides* and *S. exigua* (subgenus *Salix*) in southern Ontario. With the exception of *S. discolor* (a tetraploid species with n = 38), in which 3 out of 127 open-pollinated progeny produced hermaphrodite catkins (*Table 3*), no hermaphrodites were observed in seedling families from other species of the subgenus *Vetrix.* The predominance of hermaphrodites in subgenus *Salix* supports the reductionist view that willows probably evolved from a hermaphrodite condition to one of dioecy (LEWIS, 1942; WESTERGAARD, 1958), and the view that subgenus *Salix* contains the most primitive of the extant species within the genus *Salix* (DORN, 1976). The greater stability of the dioecious condition in subgenus *Vetrix supports* their more advanced phylogenetic position within the genus.

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Site and Age Effects on Genotypic Control of Juvenile Juglans Nigra L. Tree Height

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Summary

Trends in heritabilities for tree height and coefficients of genetic prediction from three black walnut progeny tests were compared from establishment through age 13. Although the trend in genetic variance components was relatively uniform from location to location, the patterns of variation for heritabilities and other variance components differed, underscoring the site sensitivity of the species. It is concluded that black walnut progeny tests should employ single-tree plots or noncontiguous family plots for more precise estimation of genetic and environmental parameters.

Key words: Coefficients of genetic prediction, genotype × environment interactions, genotype × block interactions, heritability.

Annotation

Compares genetic variance components for height in three black walnut progeny tests. Concludes that differences in heritability patterns resulted from site sensitivity of black walnut.

Although the amount of genetic improvement that can be achieved in black walnut from selection has already been estimated, most estimates are either based on data from very young trees (KUCERA *et al.*, 1974) or on data from only one outplanting location (RINK, 1984). Because estimates of genetic variation in other tree species change with age and are also affected by outplanting site conditions (FRANKLIN, 1979; NAMKOONG and CONKLE, 1976; NAMKOONG *et al.*, 1972), estimates of genetic variance and gain for black walnut are needed from different sites and from trees of different ages. Such estimates are of particular value due to the extreme site sensitivity of black walnut. The objective of this paper is to compare age-related changes in black walnut variance components, heritabilities and coefficients of genetic prediction from progeny tests at three outplanting locations.

Methods

Height measurements from three open-pollinated progeny tests of stand-grown trees in southern Illinois were used in this study. For two of the progeny tests seed was collected in 1969, cleaned, stratified overwinter, germinated, and outplanted in the spring of 1970 as germinating nuts at a depth of 5 cm. These two progeny tests were established on an upland sideslope (the University Farm plantation) and a narrow floodplain site (the Union County plantation). The University Farm plantation is located at 89.2° W., 37.7° N, elevation 152 m, in Jackson County on a Hosmer silt loam previously used as agricultural cropland. The Union County progeny test is located at 89.4° W, 37.5° N, elevation 134 m, on Haymond and Elsah silt loams that had been in fescue sod since 1965. The third progeny test (Pleasant Valley plantation) was established with 1-0 seedlings in spring 1973 on a Haymond silt loam in a wide floodplain of Sexton Creek, Alexander County, Illinois (89.3° W, 37.3° N, elevation 146 m) on an abandoned pasture. Weed control at all three progeny tests consisted of strip-spraying a simazine, dalapon, 2,4-D mix prior to outplanting and spot-spraying for 3 years thereafter. At age 13 the trees averaged 5.0, 4.9, and 5.2 m at Union County, University Farm, and Pleasant Valley, respectively.

All three progeny tests were designed to be converted to seedling seed orchards at a subsequent age; seedlings