deli (clone G), falls in the lower group of instability indices constantly, whereas no other clone performs similarly. (2) The sum total of random individual directionality makes for non-directional clones, except for the Laos (clone N) teak which shows significant right-sidedness. Within a clone, at least one tree out of three in every clone has positive (left sided directionality) and at least one has negative directionality. (3) The study of instability indices at different height (3 zones) shows that vein-distance variability is higher in top and lower zones than in the middle zone. The Nilambur (clone A) teak gives an increasing order of vein distance variabilities from the top to the bottom zone, and in Kurseong (clone M) teak, the variability is relatively uniform over all zones. (4) Indices of developmental instabilities are positively interrelated. (5) Indices of developmental instability is correlated with other vegetative characters like leaf length, leaf breadth and bole-length.

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The Genetic Consequences of Mass Selection for Growth Rate in Engelmann Spruce

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Summary

The genetic consequences of mass selection for growth rate in Engelmann spruce, *Picea engelmannii*, were studied with 4 enzyme polymorphisms. Super seedlings and their controls were chosen from a nursery and established in two plantings in the Rio Grande National Forest in Colorado. Super seedlings differed from controls in allelic frequencies at 1 of the 4 loci, and at one of the plantings, super seedlings had a higher proportion of highly heterozygous genotypes. The mass selection may have favored highly heterozygous genotypes, but the genetic consequences of this selection were slight.

Selection has been reported to produce excesses of heterozygotes in the mature stands of several species of conifers. The excesses in heterozygotes are relevant to both the controversy concerning the form of selection in conifers, and the question of the utility of protein genotypes in artificial breeding programs.

Key words: Protein polymorphism, heterozygosity, growth rate, Engelmann spruce.

Introduction

Natural populations of coniferous forest trees have moderate levels of heritability for growth rate. For example, the broad sense heritabilities of growth rates in southern pines are typically 40% to 80%, and the narrow sense heritabilities typically 10% to 50% (Dorman, 1976). Foresters may capitalize on these high levels of genetic variation by realizing substantial genetic gains with just a single round of mass selection. In these selection programs, plus trees are chosen on the basis of form and growth rate, and these selected trees are utilized as the parents for the next generation. This relatively simple program of selection has achieved excellent genetic gains in loblolly pine (Zobel, 1971), slash pine (Goddard *et al.*, 1973), and Monterey pine (Shelbourne, 1974), but there has been little or no response to selection in Scots pine (Nilsson, 1968) and jack pine (Canavera, 1969, 1975).

In addition to describing patterns of variation at polygenic characters in provenance studies, forest geneticists use electrophoretic analyses to estimate levels of genetic variation and to describe patterns of geographic variation of protein loci (Mitton, 1983; Guries, 1984). These studies have demonstrated that conifers are the most genetically variable group of species (Hamrick *et al.*, 1979).

The consequences of mass selection upon the variability of proteins are unknown, yet mass selection for growth may influence protein variability, for several recent studies have reported relationships within populations between protein heterozygosity and components of growth. Growth rate is positively correlated with individual heterozygosity in quaking aspen (Mitton and Grant, 1980), pitch pine (Ledig *et al.*, 1983; Bush, Smouse and Ledig, 1987), and knobcone pine (Strauss, 1986), but not in Douglas-fir (Bongarten *et al.*, 1985). The variability of growth rate is associated with individual heterozygosity in lodgepole pine (Knowles and Grant, 1981; Linhart and Mitton, 1985), and Douglas-fir (Bongarten *et al.*, 1985). For consideration of genetic conservation and response to selection, we need to know

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if mass selection programs are selective with respect to protein polymorphisms.

This study examines genetic variation in control and plus seedlings of Engelmann spruce, *Picea engelmannii* Parry, chosen for growth rate. Super seedlings and matched control seedlings were chosen from a nursery and planted into orchards established in natural localities. The super seedlings continued to exhibit superior growth three years after outplanting. The allelic and genotypic distributions of super and control seedlings were compared using 4 highly polymorphic enzyme polymorphisms. We report that the effects of mass selection practiced by forest geneticists resemble the recurrent changes within the life cycle produced by natural selection. These similar patterns of response to selection lead us to discuss the nature of selection in conifers.

Materials and Methods

Nursery Selections

Super seedlings were selected by R. M. Jeffers, Regional Geneticist, and C. D. McAninch, USDA Forest Service, Reforestation Specialist, at the Mt. Sopris Nursery in west-central Colorado, November 5 to 7, 1980. They were selected visually as the tallest seedlings within a seed source. Each selection had to have a single terminal shoot and no observable damage. A total of 500 three-year-old super seedlings from 12 seed sources, all of Rio Grande National Forest origin, were selected from nursery production beds. Selections were made from several seed sources and elevations to insure a diversity of geographic localities and elevations.

Average total height varied among seed sources from 11.0 to 18.0 cm. Average total height of super seedlings from those same seed sources ranged from 31.8 cm to 42.2 cm. Individual selections ranged from 27.5 cm to 58.0 cm. Most of the selected super seedlings had third-year terminal shoot heights between 15.0 cm and 30.0 cm. For each super seedling, a control seedling was chosen from the seedlings within a circle of radius 30 cm. In comparison to controls, the average height of super seedlings ranged from 114% to 226%.

Two plantings of Engelmann spruce super seedlings and their matched controls were established in the Rio Grande National Forest, in south-central Colorado in May of 1981. The first planting was established at California Gulch in the Saguache Ranger District and the second planting was established at Neff Mountain in the Conejos Ranger District. The Neff Mountain planting is located on a more productive site about 65 miles south of the California Gulch planting.

The total height of super seedlings continued to exceed that of controls during the first 3 years of growth in the field (Jeffers and Anderson, 1982).

Needle samples were taken from control and super seedlings at California Gulch and Neff Mountain in August, 1984. Needle samples were prepared for electrophoresis by the method of Mitton *et al.* (1979). The enzymes utilized in this study were UDP glucose pyrophosphorylase (Udp), phosphoglucomutase (Pgm), glycerate dehydrogenase (Gdh), and isocitrate dehydrogenase (Idh). Udp and Pgm were scored from the the histidine pH 7.0 buffer of Pitel and Cheliak (1984), and Gdh and Idh were scored from the lithium hydroxide pH 7.2 buffer of O'Malley *et al.* (1979).

The inbreeding coefficient, F, was calculated from genotypic distributions, with F = 1 - O/E, where O was the observed proportion of heterozygotes, and E was the expected proportion of heterozygotes. Individual heterozygo-

Table 1. — Genetic variation in Engelmann spruce with different seedling growth rates.

Site	Enzyme	Group	N	Genotype			P(2) ± SE	F	H	X^2_a	X^2_b	X^2_c
				22	23	33						
California Gulch												
	Udp	Super	86	39	36	11	.66 ± .04	.06	.42	.35	.25	.31
		Control	56	24	23	9	.63 ± .04	.12	.41	.74		
	Pgm	Super	73	21	38	14	.55 ± .04	-.05	.52	.19	.22	.23
		Control	54	14	28	12	.52 ± .05	-.04	.52	.08		
	Gdh	Super	79	46	29	4	.76 ± .03	-.02	.37	.04	.30	.73
		Control	57	32	20	5	.74 ± .04	.10	.35	.52		
	Idh	Super	82	3	25	54	.19 ± .03	.00	.30	.00	2.45	2.08
		Control	55	1	11	43	.12 ± .03	.04	.20	.09		
Neff Mountain												
	Udp	Super	96	36	52	8	.65 ± .03	-.18	.54	3.25	.00	4.21
		Control	64	29	25	10	.65 ± .04	.14	.39	1.31		
	Pgm	Super	93	28	54	11	.59 ± .04	-.20	.58	3.77	.39	1.69
		Control	63	19	32	12	.55 ± .04	-.03	.51	.05		
	Gdh	Super	96	68	26	2	.84 ± .03	-.03	.27	.07	.00	.10
		Control	65	47	16	2	.85 ± .03	.05	.25	.05		
	Idh	Super	96	8	32	56	.25 ± .03	.11	.33	1.18	6.5*	7.26*
		Control	64	0	17	47	.13 ± .03	-.15	.26	.37		

Note: X^2_a tests the fit of observed to expected equilibrium frequencies. X^2_b tests homogeneity of allelic frequency between super seedlings and their controls. X^2_c tests the homogeneity of genotypic frequencies between super seedlings and their controls. * = $P < .05$; other values are not significant. 22, 23, and 33 are genotypes bearing alleles 2 and 3, where 2 is the faster migrating allele. P is the frequency of allele 2, and SE is the standard error of the allelic frequency. F is the fixation index, and H is the proportion of heterozygous genotypes.

Table 2. — Genetic variation in Engelmann spruce differing in growth rate.

Pooled Samples			Genotype								
Enzyme	Group	N	22	23	33	P(2) ± SE	F	H	X^2_a	X^2_b	X^2_c
Udp	Super	182	75	88	19	.65 ± .02	-.07	.48	.84	.09	2.94
	Control	120	53	48	19	.64 ± .03	.13	.40	2.03		
Pgm	Super	166	49	92	25	.57 ± .03	-.13	.55	2.89	.64	1.44
	Control	117	33	60	24	.54 ± .03	-.03	.51	.12		
Gdh	Super	175	114	55	6	.81 ± .02	-.02	.31	.04	.16	.96
	Control	122	79	36	7	.80 ± .02	.09	.30	1.09		
Idh	Super	178	11	57	110	.22 ± .02	.07	.32	.94	8.75**	8.86*
	Control	119	1	28	90	.13 ± .02	-.07	.24	.55		

Note: X^2_a tests the fit of observed genotypic frequencies to expected equilibrium frequencies. X^2_h tests homogeneity of allelic frequencies between super seedlings and their controls, X^2_c tests homogeneity of genotypic frequencies between super seedlings and their controls. * = $P < .05$, ** = $P < .01$; other values are not significant: 22, 23, and 33 are genotypes bearing alleles 2 and 3, where 2 is the faster migrating allele. P is the frequency of allele 2, and SE is the standard error of the allelic frequency. F is the fixation index, and H is the proportion of heterozygous genotypes.

Table 3. — The distribution of individual heterozygosity in groups of Engelmann spruce differing in growth rate.

		# Heterozygous Loci									
Site	Group	0	1	2	3	4	N	X^2	H	S^2_H	HH
California Gulch	Super	10	26	21	15	0	72	0.29	1.57	.95	.21
	Control	4	26	14	9	0	53		1.53	.75	.17
Neff Mountain	Super	14	26	27	24	2	93	4.03*	1.72	1.16	.28
	Control	10	21	23	8	1	63		1.51	.93	.14
Pooled	Super	25	51	48	39	2	165	3.57	1.62	1.18	.25
	Control	14	47	37	17	1	116		1.52	.84	.16

Note: X^2 reports a row by columns test of independence between super and control seedlings in which heterozygosity classes have been pooled to low (0—2) and high (3—4). * = $P < .05$. HH is the proportion of individuals highly heterozygous (3—4). H is the mean of the distribution of individuals heterozygosity.

sity (Mitton and Pierce, 1980) is the number of loci for which an individual is heterozygous. In this study, which utilized 4 polymorphic loci, the domain of individual heterozygosity was 0 to 4.

Results

Three alleles were segregating at the loci coding for Udp and Pgm, while two alleles were segregating at Idh and Gdh. At both Udp and Pgm, the fastest allele was rare, with frequencies of less than 0.02. To simplify the analyses and presentation of data, these rare alleles were pooled with the alleles of most similar electrophoretic mobility, and presented as P (2) in *tables 1* and 2.

Allelic frequencies were similar in super seedlings and their controls. In 8 tests of heterogeneity, significant differences were observed only for Idh at Neff Mountain (*Table 1*). Allelic frequencies were also similar in the two plantations.

The fit of observed to expected genotypic distributions was tested for each locus, at each plantation, for both control and super seedlings (*Table 1*). In each case, the genotypic distributions exhibit a satisfactory fit to the expected distributions. Comparisons of the F values between super seedlings and their controls generally revealed lower values of F, or higher heterozygosity relative to expectations, in the super seedlings; the only exception to this statement was Idh at Neff Mountain. Heterozygosity is identical in one of the comparisons, but in the remaining 7 of 8 comparisons, super seedlings have higher absolute levels of heterozygosity. Tests of heterogeneity of genotypic frequencies revealed one significant difference. At Neff Mountain, Idh allelic frequencies differ between groups, and this difference is reflected in genotypic frequencies as well.

Comparisons of pooled samples of super seedlings and control seedlings (*Table 2*) show the same patterns seen in *Table 1*. At Udp, Pgm, and Gdh, super seedlings have lower values of F than do controls, while Idh has the opposite pattern. At each locus, the absolute value of the proportion of heterozygous genotypes is higher in super seedlings than in controls. Allelic frequencies and genotypic frequencies differ between super seedlings and controls at Idh, but allelic frequencies at the remaining loci are similar.

The means for individual heterozygosity are consistently higher in super seedlings than in control seedlings, but these differences do not reach statistical significance (*Table 3*). When the distributions of individual heterozygosity are pooled to two classes, low (0—2 loci heterozygous) and high (3—4), the percentage of highly heterozygous individuals is consistently higher in super seedlings, and at the plantation at Neff Mountain the difference is statistically significant.

Discussion

There is controversy over the nature of natural selection in conifers. Natural selection may act primarily to remove selfed and other inbred individuals from populations (Ledig, Guries and Bonefeld, 1983), or alternatively, selection may simply remove the most homozygous individuals from populations, regardless of whether they are produced by selfing or by outcrossing (Mitton and Grant, 1984). There is a possibility to distinguish between these alternatives, but first they will be described in greater detail.

Selection against inbred individuals

Inbreeding depression may be pronounced in forest trees; studies with numerous species have documented the decrease in proportion of filled seed, seed size, seed germinability, viability and growth rate with increasing levels of inbreeding (Sorensen, 1969; Franklin, 1972; Sorensen and Miles, 1982). Conifers are predominantly outcrossed, with a small proportion of seed produced by selfing and/or mating with related individuals. Given this dramatic range of variation produced by the mating system, natural selection is presumed to eliminate selfed and inbred individuals, leaving outcrossed individuals.

A clear example of selection against inbred individuals has been reported for yellow poplar, *Liriodendron tulipifera* (Brotschol, Roberds and Namkoong, 1986). Like many forest trees, yellow poplar reproduces with a mixed mating system, so that some offspring are selfed and others are outcrossed. The mating system is variable, with the proportion of outcrossed seed estimated to be 55% and 86% in two localities. Yet when the genotypes of mature trees are examined, their genotypic frequencies meet Hardy-Weinberg expectations. Selfed individuals, abundant when seed are shed, disappear as a cohort ages. Numerous other observations in the literature are consistent with this model of selection (e.g. Tigerstedt *et al.*, 1982; Phillips and Brown, 1977; Farris and Mitton, 1984).

Selection against highly homozygous individuals

Computer simulation of additive, multiplicative, and epistatic models of fitness determination have revealed a general relationship between fitness and heterozygosity (Ginzburg, 1979; Turelli and Ginzburg, 1983). For loci whose variation is maintained by balancing selection, fitness increases with the number of heterozygous loci (Turrelli and Ginzburg, 1983). Given this general conclusion, one model of natural selection can be constructed with truncation selection of threshhold selection (Wills, 1981), in which the most homozygous individuals are eliminated first, and the majority of reproductive success is captured by highly heterozygous genotypes.

Discriminating between hypotheses

It is difficult to discriminate between these alternative hypotheses, but some insight into the problem might be gained by examining the distributions of genotypes surviving selection. If selection acts only to remove inbred individuals, then selection would simply decrease values of F until they reached zero. On the other hand, if the axis of individual heterozygosity is truncated by natural selection, values of F may be positive, zero, or negative, depending on where the threshhold falls. The important point is that selection against inbred individuals cannot produce negative values of F (excesses of heterozygotes), while selection against homozygous individuals may. A simple simulation will illustrate this point.

Consider species with mixed mating systems, in which some of the seed are produced by outcrossing to unrelated individuals, and some of the seed are produced by selfing. The degree of overlap of the distributions of heterozygosity of outcrossed and selfed genotypes is examined with computer simulation (*Figure 1*). For this demonstration, each polymorphic locus has 2 alleles, all allelic frequencies equal 0.5, and loci are distributed independently of one another. The mean and variance of individual heterozygosity are

$$H = 2pqL$$

and

$$S^2_H = [(L-H)\cdot H]/L$$

where p and q are allelic frequencies, and L is the number of polymorphic loci. The distribution of heterozygosity in outcrossed progeny was generated empirically, using the assumptions of independent assortment of loci and equilibrium genotypic frequencies with random mat-

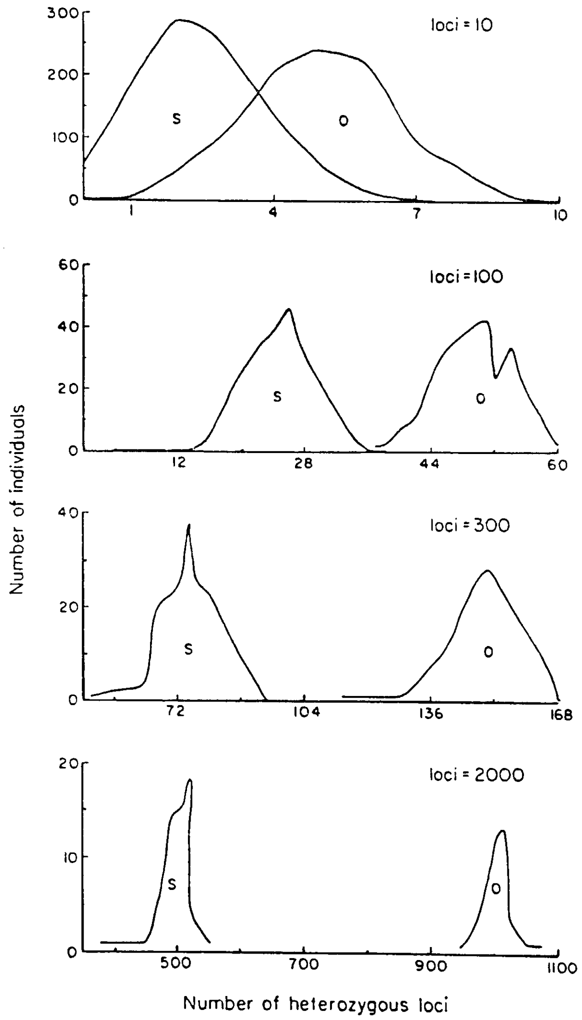

Figure 1. — The distribution of individual heterozygosity in selfed (s) and outcrossed (o) genotypes. There are 2 alleles at each polymorphic locus, and all allelic frequencies are equal to 0.5. The distributions are generated by computer simulation. When only 10 polymorphic loci are available, the distributions of heterozygosity of outcrossed and selfed genotypes overlap broadly. But as the number of polymorphic loci increases, the distributions become more and more distinct.

ing. As each outcrossed genotype was produced, a selfed genotype was produced from it in the following way. Homozygous loci in the outcrossed genotype were simply transferred to the selfed genotype. Then a random number generator was used to determine whether heterozygous loci in the outcrossed genotype would be heterozygous or homozygous in the selfed genotype; each probability was equal to 0.5. The mean individual heterozygosity of selfed progeny was half that of the outcrossed (*Figure 1*); selfing decreases heterozygosity by 50%.

This exercise clearly demonstrates that the degree of the difference between outcrossed and selfed progeny is a function of the number of loci under consideration (*Figure 1*). When we consider only 10 polymorphic loci, the two distributions overlap broadly, and from this narrow perspective, selfed and outcrossed progeny can not be discriminated. However, the discrimination of selfed and outcrossed genotypes increases with the number of polymorphic loci. For example, when the simulation is run with 2000 polymorphic loci, outcrossed and selfed genotypes are grandly disparate. In reality, the levels of heterozygosity in selfed and outcrossed genotypes do not overlap.

Now if selection merely removes selfed individuals, the distribution to the left in *Figure 1* would disappear, leaving only outcrossed individuals, and yielding an F of 0.0. If, on the other hand, selection ranks individuals by individual heterozygosity and removes the most homozygous individuals first, the value of F would reflect the intensity of selection. When the threshhold was in the range of selfed individuals, the value of F would be positive. But when the threshhold was in the range of outcrossed individuals, then the remaining individuals would yield a negative F.

The inbreeding reflected in values of F measured in the field may be produced both by selfing and by mating between related individuals (RITLAND, 1984). The majority of the inbreeding appears to be due to selfing (SHAW and ALLARD, 1982a), although there is evidence of family substructuring of populations in some species (LINHART *et al.*, 1981). By analogy to the example presented above, selection against midly inbred individuals, the progeny of matings between related individuals, cannot produce negative values of F.

Excess heterozygosity in conifers

Selection has been reported to produce excesses of heterozygosity in the mature stands of several species of conifers (*Table 4*). Several of these estimates are accompanied with either quantitative estimates of the mating system or estimates of genotypic frequencies of seedlings, documenting increases of heterozygosity as cohorts age and suffer mortality.

Selection acts within the life cycle of ponderosa pine, *Pinus ponderosa,* modifying genotypic frequencies (MITTON *et al.*, 1981; FARRIS and MITTON, 1984). In populations with low density, about 20% of the seed are produced by selfing, and genotypes in seeds exhibit deficiencies of heterozygotes (F = .17). But seedling mortality removes predominantly homozygotes, moving the population to HARDY-WEINBERG expectations (F = −.03, FARRIS and MITTON, 1984). Seeds collected in a stand with typical density are predominantly outcrossed, and their genotypes meet HARDY-WEINBERG expectations (F = .02). But the mature trees in that stand have excesses of heterozygotes (F = −.108 ± .054, MITTON *et al.*, 1981; FARRIS and MITTON, 1984; *Table 4*). While the first case of selection is consistent with, and perhaps most parsimoniously explained by selection against inbred genotypes, some other model of selection must be invoked to explain the second case of selection.

Douglas-fir, *Pseudotsuga menziesii,* reproduces with a small proportion of seed set by selfing, so that seeds and seedlings exhibit a value of F of approximately .05 (SHAW and ALLARD, 1982a, b). But selection within the life cycle produces a systematic pattern of excess heterozygosity in

Table 4. — Values of the inbreeding coefficient, F_{is}, in mature stands of conifers.

Species	Site	N	Polymorphic loci	F_{is}	S.E.	Reference
Picea mariana	Newfoundland	218	13	−.065	± .017^a	Yeh et al. 1986
Pinus radiata		262	17			Plessas and Strauss 1986
	Ano Nuevo			−.118		
	Cambria			−.150		
	Monterey			−.089		
				−.119	± .018^b	
Pinus ponderosa	Boulder Canyon	218	7	−.108	± .054^a	Linhart et al. 1981
Pseudotsuga menziesii		185	11			Shaw and Allard 1982b
	Springfield 1			−.020		
	Springfield 2			−.030		
	Springfield 3			−.005		
	Springfield 4			.000		
	Longview 1			−.017		
	Longview 3			−.058		
	Longview 4			−.047		
	Longview 5			−.038		
				−.027	± .007^b	
Abies balsamea	Mt. Moosilauke (1464 m)	25	8	−.149	± .033^a	Neale and Adams 1985
Pinus banksiana	Alberta	30	4	−.064	± .020^a	Cheliak et al. 1985

Note: N is the number of mature trees in the sample. Standard errors with superscript a are calculated across loci, while those marked with superscript b are calculated across localities.

mature trees (*Table 4*; Shaw and Allard, 1982b). Jack pine, *Pinus banksiana,* has a remarkably variable mating system, with a mean of 88% of seed produced by outcrossing, but mature trees have excess heterozygosity (*Table 4*; Cheliak *et al.*, 1985). There is also evidence suggesting that seed viability increases with heterozygosity in jack pine (Cheliak *et al.*, 1985). Estimates of outcrossing in black spruce, *Picea mariana,* are slightly but not significantly below 1.0, yet F values in mature trees are negative (Boyle and Morgenstern, 1986; Yeh *et al.*, 1986; *Table 4*). The value of F decreases monotonically with age in Monterey pine, changing from .091 in embryos to −.119 ± 0.018 in 14 to 17 year old trees Plessas and Strauss, 1987; *Table 4*).

The mating system of balsam fir, *Abies balsamea,* varies among sites along an elevational gradient in New Hampshire (Neale and Adams, 1985). There is nearly perfect outcrossing at some sites, but above tree line about 22% of the seed are produced by selfing. Although the expected value of F with this degree of inbreeding is .125, the observed value in mature trees is −0.149 ± 0.033 (Neale and Adams, 1985; *Table 4*).

A mixed mating system produces a very heterogeneous assortment of progeny in a genetically variable population (*Figure 1*). Some proportion of seed is produced by either selfing or breeding among related individuals, and deficiencies of heterozygotes from Hardy-Weinberg expectations are expected and typically seen in seeds and young seedlings. In the examples summarized above, heterozygote deficiencies in seeds are contrasted with excesses of heterozygotes in mature trees. Because selection against inbred genotypes cannot produce excesses of heterozygotes (Shaw and Allard, 1982b), selection must favor heterozygous genotypes, even within the pool of outcrossed individuals.

Protein polymorphisms and selective breeding

Associations between protein genotype and growth rates in forest trees suggested the possibility that protein polymorphisms would be useful to tree breeders (Mitton, 1983). After describing a taxonomically widespread pattern of increasing growth rate with heterozygosity, Mitton and Grant (1984) proposed that it would be financially beneficial for tree breeders to incorporate enzyme polymorphisms into programs of tree improvement. Bongarten *et al.* (1985) examined the relationships between protein genotype and growth in full-sib progeny tests of Douglas-fir, and concluded that isozyme heterozygosity was of little value as a selection criterion. Most recently, Bush and Smouse (1987) used the adaptive-distance analysis of Smouse (1986) to examine relationships between enzyme genotype and growth in pitch pine, *Pinus rigida.* They found evidence that some enzymes play a role in determining growth rate of pitch pine, but they did not speculate on the value of these relationships to tree breeders.

Unfortunately, the results of this report on the genetic consequences of mass selection are equivocal. Super seedlings consistently had higher observed heterozygosities than controls (*Table 1*), and at one of the plantations, super seedlings had a significantly higher proportion of highly heterozygous individuals (*Table 3*). Although the mean value of F was positive in controls and negative in super seedlings (0.028 ± 0.034 and −0.040 ± 0.038, respectively, *Table 1*), these values are not significantly different. These results support little optimism that protein heterozygosity would be useful for plant breeders. Yet selection in natural populations often does produce excesses of heterozygotes (*Table 4*), and the mass selection studied here moved the population toward an excess of heterozygotes. The utility of enzyme genotypes to tree breeders remains unresolved.

The controversy over the form of selection in conifers is of general interest as a problem in genetics and evolution, but it is of special interest to plant and animal breeders. For if selection simply removes inbred genotypes, then selective breeding systems must guard against inbreeding. But if fitness increases regularly with heterozygosity, breeding systems should be designed to preserve variability and enhance heterozygosity. The utility of protein polymorphisms to selective breeders depends upon the outcome of this controversy. If the objective is to identify and eliminate selfed genotypes from bulked seeds, a handful of polymorphic loci will not efficiently discriminate selfed and outcrossed seed (*Figure 1*). But if the objective is to identify or produce highly heterozygous individuals, protein polymorphisms could be useful.

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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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