A Comparison of Isozyme and Quantitative Genetic Variation in *Pinus contorta* ssp. *latifolia* by F_{ST}

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ABSTRACT

We employed *F*-statistics to analyze quantitative and isozyme variation among five populations of *Pinus contorta* ssp. *latifolia*, a wind-pollinated outcrossing conifer with wide and continuous distribution in west North America. Estimates of population differentiation (F_{ST}) for six quantitative traits were compared with the overall estimate of the differentiation (F^*_{ST}) from 19 isozymes that tested neutral to examine whether similar evolutionary processes were involved in morphological and isozyme differentiation. While the F_{ST} estimates for specific gravity, stem diameter, stem height and branch length were significantly greater than the F^*_{ST} estimate, as judged from the 95% confidence intervals by bootstrapping, the F_{ST} estimates for branch angle and branch diameter were indistinguishable from the F^*_{ST} estimate. Differentiation in stem height and stem diameter might reflect the inherent adaptation of the populations for rapid growth to escape suppression by neighboring plants during establishment and to regional differences in photoperiod, precipitation and temperature. In contrast, divergences in wood specific gravity and branch length might be correlated responses to population differentiation in stem growth. Possible bias in the estimation of F_{ST} due to Hardy-Weinberg disequilibrium ($F_{IS} \neq 0$), linkage disequilibrium, maternal effects and nonadditive genetic effects was discussed with special reference to *P. contorta* ssp. *latifolia*.

GENETIC differentiation among natural populations in a plant or animal species arises from two major mechanisms, random drift and selection. LANDE (1976, 1977) proposed a test for the selective divergence of population means by comparing the observed additive genetic variance between populations with that expected from random drift. The computation of the expected variance, however, requires the estimation of unknown parameters such as the rate of mutation, time since divergence and effective population size (LYNCH 1988). Thus, in the absence of detailed historical information on the populations under study, it is difficult to obtain reliable estimates of these parameters.

Alternatively, when assessment of population differentiation for quantitative traits (F_{ST}) is accompanied by a similar assessment for gene markers such as isozymes (F^*_{ST}), testing for the selective divergence can be achieved by comparing F_{ST} estimates with the overall estimate of F^*_{ST} from all neutral genes (WRIGHT 1951, 1965; FELSENSTEIN 1986; ROGERS 1986; LANDE 1992; SPITZE 1993). Divergent selection may be invoked as a cause for the observed differentiation when F_{ST} is significantly greater than F^*_{ST}. If F_{ST} is in the same magnitude of F^*_{ST} or is significantly less than F^*_{ST}, the hypothesis that the among-population variance is due to random drift cannot be rejected or convergent selection may be invoked as a cause for the reduced differentiation. Making such a comparison, PROUT and BARKER (1993) and SPITZE (1993) were recently able to show that divergent selection was responsible for the among-population differentiation observed for body size in *Drosophila buzzatii* and *Daphnia obtusa*. SPITZE (1993) also analyzed other quantitative traits in *D. obtusa* and showed that while population divergence for relative fitness might be impeded by convergent selection, estimates of divergence for clutch size, reproductive age and growth rate were indistinguishable from the neutral expectation, suggesting that random drift could not be excluded as the cause for the observed divergence.

Except for a recent attempt to make the same comparison in an annual outcrossing plant, *Clarkia dudleyana* (PODOLSKY and HOLTSFORD 1995), comparisons of quantitative and isozyme variation in plant populations have been described only heuristically (*e.g.*, HAMRICK 1983, p. 343–345). In range-wide surveys of natural variation for many plant species, particularly those for long-lived woody plants, one major impediment to making the necessary comparison is lack of estimates of the required genetic variances because it is costly to test a large number of families over long periods (NAMKOONG and KANG 1990). Consequently, isozyme studies in woody plants generally have not been accompanied by parallel studies of genetic variation in quantitative traits known to contribute to fitness.

In the present study, we describe a comparison be-

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tween estimates of population differentiation for quantitative traits and the corresponding overall estimate for isozymes in *Pinus contorta* ssp. *latifolia* from British Columbia. *P. contorta* ssp. *latifolia* is a wind-pollinated outcrossing conifer with wide and continuous distribution in west North America (CRITCHFIELD 1957, 1980). A number of ecologically desirable attributes (*e.g.*, serotinous cones, regular and abundant seed production, effective seed dispersal and rapid juvenile growth) make *P. contorta* ssp. *latifolia* an aggressive pioneer in a variety of site and climatic conditions (CRITCHFIELD 1980). In particular, release of the seeds from serotinous cones following fire leads to the rapid establishment of even-aged stands with dense stocking. Thus, many of the present-day forests of *P. contorta* ssp. *latifolia* are thought to be of fire origin. Surveys of genetic variation of *P. contorta* ssp. *latifolia* have shown population differences for a number of morphological and biochemical traits (*e.g.*, CRITCHFIELD 1957; YEH and LAYTON 1979; WHEELER and GURIES 1982; YANG and YEH 1993) as well as mtDNA markers (DONG and WAGNER 1993). Our objective is to examine if the population divergence observed for quantitative traits and for isozymes are caused by similar evolutionary processes in this conifer.

MATERIALS AND METHODS

Quantitative traits: The material used in this study was part of a range-wide *P. contorta* population survey in western North America administrated by the British Columbia Ministry of Forests (ILLINGWORTH 1975). The five study populations were originated from the five relatively uniform zones that were randomly chosen among a variety of zones each characterized by unique physiography, climate, soil and vegetation in the province of British Columbia (MEIDINGER and POJAR 1991). In particular, these five zones are located in four of the five physiographic regions in the province (Figure 1): zone #1 from Columbia Mountains and Southern Rockies, zone #3 from Great Plains, zone #4 from Northern and Central Plateaus and Mountains, and zones #2 and #5 from Interior Plateau. The sampling zone for each population was an irregular ellipse spanning ~1.5–2 degrees of latitude and longitude (Figure 1). The experiment was carried out at the British Columbia Ministry of Forests Red Rock Nursery (latitude 53° 46′ N, longitude 122° 42′ W, altitude 620 meters). The experiment design and trait measurements were detailed in YANCHUK (1986). Eighteen families were sampled from zone #1 and 27 families from each of the remaining four zones. Actual field layout of the experiment was a completely randomized design with hierarchical structure with families plots randomly assigned to populations. Up to 12 open-pollinated progenies per family were evaluated at age 10 at a 10′ × 10′ spacing between trees. We analyzed six quantitative traits measured for all five populations: wood specific gravity, stem diameter, stem height, branch angle, branch diameter and branch length.

Analysis of variance and calculations of variance components were carried out using the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_{ij} + \epsilon_{ijk},$$

where y_{ijk} = the phenotypic value on the kth progeny of the jth family (parent tree) in the ith location (local population);

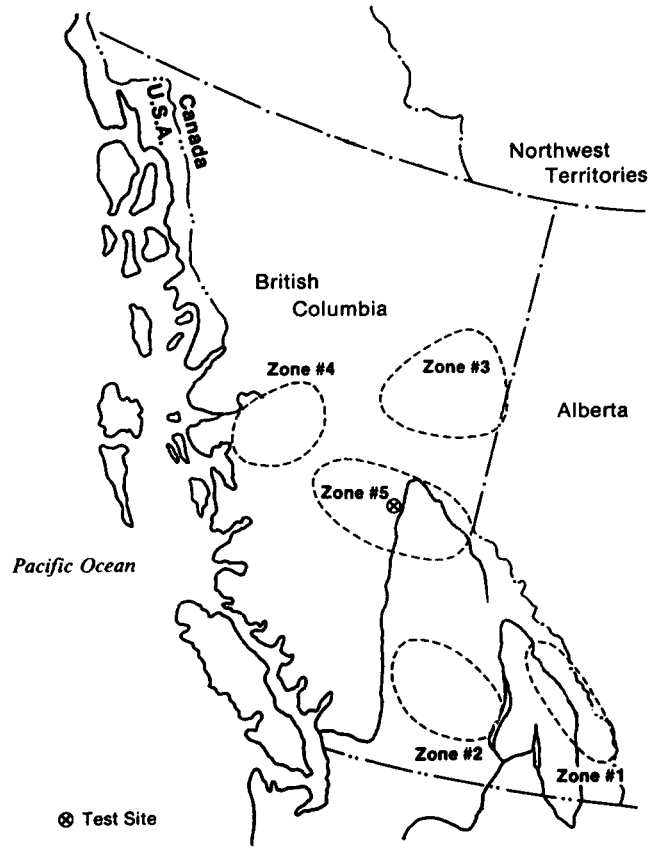

FIGURE 1.—Location of *P. contorta* ssp. *latifolia* populations and the test site for studying quantitative genetic variation.

μ = the overall mean; α_i = the ith population effect; β_{ij} = the jth family effect within the ith population; ϵ_{ijk} = the residual representing variability within families. All effects except for μ were considered random and unrelated, with means of zero and variances of V_α, V_β and V_ϵ, respectively. Direct F-test could not be made for every source of variation because the data set was unbalanced. SATTERTHWAITE's (1946) approximation, therefore, synthesized the approximate error term for each source (MILLIKEN and JOHNSON 1984).

WRIGHT (1965) showed how his *F*-statistics (fixation indices) could partition the total additive genetic variance (σ_T^2) into the among-population (σ_B^2) and the within-population (σ_W^2) components: $\sigma_T^2 = (1 + F_{IT})\sigma_A^2$, $\sigma_B^2 = 2F_{ST}\sigma_A^2$ and $\sigma_W^2 = (1 + F_{IS})(1 - F_{ST})\sigma_A^2$, where σ_A^2 is the additive genetic variance in the Hardy-Weinberg and linkage equilibrium population (FALCONER 1981). The relationship among *F*-statistics is well known: $(1 - F_{IT}) = (1 - F_{ST})(1 - F_{IS})$, where F_{IS} and F_{IT} measure the deviations from random mating in local and total population, respectively, and F_{ST} measures the level of genetic divergence among local populations. Assuming the variance component for open-pollinated (half-sib) families to estimate one-quarter of the additive genetic variance (NAMKOONG 1966; FALCONER 1981), we have

$$V_\alpha = \sigma_B^2 = 2F_{ST}\sigma_A^2$$

$$V_\beta = \frac{1}{4}\sigma_W^2 = \frac{1}{4}(1 + F_{IS})(1 - F_{ST})\sigma_A^2$$

$$V_\epsilon = \frac{3}{4}\sigma_W^2 + \sigma_r^2 = \frac{3}{4}(1 + F_{IS})(1 - F_{ST})\sigma_A^2 + \sigma_r^2, \quad (1)$$

where σ_r^2 is the variance due to nonadditive genetic and envi-

ronmental effects. The relationship between variance components and *F*-statistics in (1) suggests that without estimation of σ_A^2, it is not possible to estimate both F_{IS} and F_{ST} from the estimates of variance components. One possible solution to this problem is to impose restrictions (assumptions) on F_{IS} or F_{ST} so that either one is estimable. Since our primary interest is to estimate population differentiation (F_{ST}) in *P. contorta* ssp. *latifolia,* we assume that local populations are in Hardy-Weinberg equilibrium ($F_{IS} = 0$). With $F_{IS} = 0$, we have $\sigma_W^2 = (1 - F_{ST})\sigma A^2$, $\sigma_B^2 = 2F_{ST}\sigma_A^2$, and the well-known result, $F_{ST} = \sigma B^2/(\sigma_B^2 + 2\sigma_W^2)$. Therefore, population genetic differentiation (F_{ST}) can be estimated using estimates of variance components, V_α and V_β (*cf.* Equation 1):

$$\hat{F}_{ST} = \frac{\hat{\sigma}_B^2}{\hat{\sigma}_B^2 + 2\hat{\sigma}_W^2} = \frac{\hat{V}_\alpha}{\hat{V}_\alpha + 8\hat{V}_\beta}. \quad (2)$$

Empirical distributions of variance components and F_{ST}-statistic (variance ratio) were obtained by bootstrapping (EFRON 1982). Since there was a family structure within populations, we adopted ARVESEN and SCHMITZ's (1970) data subsetting strategy for the bootstrap resampling. Each bootstrap sample was drawn from the data by sampling (with replacement) 126 times from the 126 families (subsets). The estimates of variance components and F_{ST} were computed directly from the data and from each of 1000 bootstrap samples. From the bootstrap distribution, we found an approximate 95% confidence interval for each of the six traits examined.

Isozymes: The material used here was part of the seed collection for a range-wide survey of isozyme variation in western North America (YANG and YEH 1993). This collection was a subset of the original collection (ILLINGWORTH 1975) chosen to cover as much of the natural range of the species as possible. To ensure the sampling of the same populations as those for the quantitative genetic analysis, it was necessary to amalgamate seed collections from several populations considered in YANG and YEH (1993). The trees sampled from each population for isozyme assay were not necessarily the same as those for the quantitative genetic analysis. However, since both sets of trees represented random samples from each of the five populations, the bias stemming from such sampling error would be negligible.

Electrophoretic analysis of maternal haploid tissues (megagametophytes) (*i.e.,* protein extraction, horizontal starch-gel electrophoresis and scoring) was previously described (YANG and YEH 1993). Here it suffices to note that 19 isozyme loci were scored (*Aat-1, Aat-2, Aco, Adh, Aph, Dia-2, Dia-3, Gdh, G6p, Idh, Mdh-1, Mdh-2, Mdh-3, Mdh-4, Me, Pgi, Pgm, 6Pg-1, 6Pg-2*) and were each polymorphic in at least one population.

Before computing F_{ST}^*, we carried out the Ewens-Watterson test for neutrality (MANLY 1985) for each of the 19 loci. Results showed that all 19 loci fit the hypothesis of neutrality. In a different electrophoretic survey of the same subspecies, YEH and LAYTON (1979) found a similar result from a comparison of the observed variance of heterozygosity with its expectation under a neutral model. Following WEIR (1990, p. 145–152) and YANG and YEH (1993), we conducted an analysis of variance of allele frequencies (COCKERHAM 1969) for each of the 19 loci and partitioned the total variance over the five populations into the among (*MSA*) and within (*MSW*) components. Thus, the overall estimate of population differentiation (F_{ST}^*) from all 19 loci was computed as:

$$\hat{F}_{ST}^* = \frac{\sum_{j=1}^{19}\sum_{i=1}^{r}(MSA_{ji} - MSW_{ji})}{\sum_{j=1}^{19}\sum_{i=1}^{r}[MSA_{ji} + (n_c - 1)MSW_{ji}]}, \quad (3)$$

where MSA_{ji} and MSW_{ji} were, respectively, the among- and within-population mean squares for the ith allele at the jth locus, r was the number of alleles at the jth locus and n_c was the weighted number of haplotypes over the populations. A 95% confidence interval for F_{ST}^* was obtained by bootstrap resampling (EFRON 1982; WEIR 1990) of individual loci 1000 times. Specifically, we computed $\hat{F}_{ST}^*$ using the mean squares from each of 1000 bootstrap samples and found the approximate confidence limits from the empirical distribution of $\hat{F}_{ST}^*$.

RESULTS

Quantitative genetic variation: The analysis of variance showed that there was significant variation among populations for all traits except branch angle. Significant differences among families within populations were also found for all six traits. Accordingly, with the exception of among-population variance component for branch angle, variance components among populations and among families were significant as the respective lower confidence limits by bootstrapping were greater than zero (Table 1). Values of $\hat{F}_{ST}$ varied considerably from 0.6% for branch angle to 19.5% for stem height but were significantly greater than zero, except the $\hat{F}_{ST}$ value for branch angle.

Our test for the significance of variance components and F_{ST} was based on the estimation of confidence limits from 1000 bootstrap sample estimates of relevant parameters. To determine if 1000 bootstrap samples would give an accurate estimate of confidence intervals, we obtained 10,000 bootstrap samples for all six traits and then computed their 95% confidence intervals. The estimates of confidence intervals from both sets of samples were essentially the same. For example, the confidence interval estimated from 10,000 bootstrap samples (−0.008, 0.028) for branch angle, was the same as that from 1000 samples (Table 1). Therefore, 1000 bootstrap samples were sufficient to provide accurate estimates of confidence intervals even for very skewed distributions. This also agrees with WEIR's (1990) recommendation of 1000 bootstrap samples for constructing confidence intervals for the estimators.

Inference about F_{ST}: Isozyme variation among populations accounted for only a small fraction of the total variation, and the majority resided within populations (Table 2). Locus-specific estimates of F_{ST} ranged from 0.0001 for *Idh* to 0.0648 for *G6p*. The overall estimate from all 19 isozyme loci was 0.0190. Since the 19 isozyme loci, according to the Ewens-Watterson test, fit the hypothesis of neutrality, we employed the overall estimate of population differentiation for isozymes as a null hypothesis ($F_{ST}^* = 0.0190$) to test for interpopulation divergence (F_{ST}) for quantitative traits. Judging from bootstrap confidence intervals for F_{ST} and F_{ST}^*, the estimates of F_{ST} for specific gravity, stem diameter, stem height and branch length were significantly >0.0190, while those for branch angle and branch diameter were indistinguishable from 0.0190 (Tables 1 and 2).

TABLE 1

Estimates of variances among populations ($\hat{V}_\alpha$), among families ($\hat{V}_\beta$) and within families ($\hat{V}_\epsilon$) and population differentiation ($\hat{F}_{ST}$) for six quantitative traits of *P. contorta* ssp. *latifolia*

Trait	$\hat{V}_\alpha$	$\hat{V}_\beta$	$\hat{V}_\epsilon$	$\hat{F}_{ST}$	L[a]	U[a]
Specific gravity	1.004*	0.818*	6.192	0.133	0.054	0.178
Diameter	0.232*	0.146*	0.725	0.166	0.088	0.221
Height	0.142*	0.073*	0.244	0.195	0.114	0.265
Branch angle	0.332	6.903*	46.002	0.006	−0.008	0.028
Branch diameter	0.879*	1.802*	13.381	0.057	0.014	0.101
Branch length	151.810*	99.030*	437.346	0.161	0.085	0.214

* Indicates variance component significantly greater than zero, according to a 95% confidence interval based on 1000 bootstrap samples.

[a]L and U are, respectively, the lower and upper confidence limits for a 95% confidence interval for F_{ST} based on 1000 bootstrap samples.

DISCUSSION

The organization of genetic variability: Our analysis of isozyme data corroborates previous findings that over 90% of the isozyme variation in *P. contorta* ssp. *latifolia* resided within local populations (*e.g.*, YEH and LAYTON 1979; WHEELER and GURIES 1982; DANCIK and YEH 1983) even though each of the five populations in our analysis covers relatively large geographic areas. Near complete outcrossing (EPPERSON and ALLARD 1984; PERRY and DANCIK 1986) and extensive gene exchange between populations (EPPERSON and ALLARD 1989; R.-C. YANG and F. C. YEH, unpublished results) might in part be responsible for the low isozyme differentiation in *P. contorta* ssp. *latifolia*. Given the consistent result of vast amounts of isozyme variation residing within populations in this conifer, it is our opinion that future research should focus on elucidating the patterns of genetic variation within populations of *P. contorta* ssp. *latifolia*. Since both spatial and temporal patterns of genetic variation within populations would affect the level of inbreeding, such a research should be directed primarily toward studying spatial and temporal dynamics of mating systems in this conifer. While changes in the level of inbreeding may affect our estimates of F_{ST}, the effect is small in most cases (see Equation 4 and Table 3 below for detail).

Estimates of population differentiation for six quantitative traits in *P. contorta* ssp. *latifolia* varied considerably, from near zero for branch angle to 20% for stem height (Table 1). WHEELER and GURIES (1982) employed principal component analysis to apportion the phenotypic variation in 12 cone and seed traits to the among-subspecies, among-populations and within-population components in *P. contorta* Dougl. Their analysis showed that ~19% of morphological variation in cone and seed traits was organized among populations within subspecies *P. contorta* ssp. *latifolia*. While comparable to our estimates in magnitude, such an estimate of morphological differentiation from a summary statistic does not have the same genetic interpretation as ours. In addition, their estimate could be inflated by environmental effect. For a population consisting of half-sib families, for example, one-quarter of the within-family variance is due to the environmental effect.

Comparison of F_{ST} estimated from quantitative traits and F^*_{ST} from isozymes enables us to examine whether similar evolutionary processes were involved in morphological and isozyme differentiation in *P. contorta* ssp. *latifolia*. Divergent selection may be invoked as a cause for the observed differentiation when F_{ST} is significantly greater than F^*_{ST}. If F_{ST} is in the same magnitude of F^*_{ST} or is significantly less than F^*_{ST}, the hypothesis that

TABLE 2

Estimates of among-population ($\hat{\sigma}^{2a}_B$), within-population ($\hat{\sigma}^{2a}_W$) and population differentiation ($\hat{F}^*_{ST}$) for 19 enzyme loci in *P. contorta* spp. *latifolia*

Enzyme locus	$\hat{\sigma}^2_B$ (×1000)	$\hat{\sigma}^2_W$ (×1000)	$\hat{F}^*_{ST}$
Aat-1	1.313	168.83	0.0077
Aat-2	1.547	157.76	0.0097
Aco	5.994	518.85	0.0114
Adh	1.633	497.21	0.0032
Aph	2.395	626.68	0.0194
Dia-2	2.012	168.34	0.0118
Dia-3	0.026	4.18	0.0062
Gdh	0.002	4.40	0.0002
G6p	2.710	471.96	0.0648
Idh	0.002	22.22	0.0001
Mdh-1	0.042	11.14	0.0038
Mdh-2	0.076	29.14	0.0026
Mdh-3	6.709	477.51	0.0139
Mdh-4	0.420	153.93	0.0027
Me	4.757	280.50	0.0167
Pgi	0.183	46.77	0.0039
Pgm	0.562	50.47	0.0110
6Pg-1	2.258	107.01	0.0207
6Pg-2	0.342	35.63	0.0095
Overall	72.973	3836.52	0.0190
95% bootstrap confidence interval:			0.0087–0.0320

[a]$\hat{\sigma}^2_B = (MSA - MSW)/n_c$ and $\hat{\sigma}^2_W = MSW$, where *MSA* and *MSW* are, respectively, mean squares among and within populations, and n_c is the weighted number of haplotypes over populations.

TABLE 3

Estimated F'_{ST} in the presence of inbreeding or assortative mating within subpopulations (F_{IS})

	F_{ST}									
F_{IS}	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
−1.0	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
−0.8	0.3571	0.5556	0.6818	0.7692	0.8333	0.8824	0.9211	0.9524	0.9783	1.0000
−0.6	0.2174	0.3846	0.5172	0.6250	0.7143	0.7895	0.8537	0.9091	0.9574	1.0000
−0.4	0.1563	0.2941	0.4167	0.5263	0.6250	0.7143	0.7955	0.8696	0.9375	1.0000
−0.2	0.1220	0.2381	0.3488	0.4545	0.5556	0.6522	0.7447	0.8333	0.9184	1.0000
0.0	**0.1000**	**0.2000**	**0.3000**	**0.4000**	**0.5000**	**0.6000**	**0.7000**	**0.8000**	**0.9000**	**1.0000**
0.2	0.0847	0.1724	0.2632	0.3571	0.4545	0.5556	0.6604	0.7692	0.8824	1.0000
0.4	0.0735	0.1515	0.2344	0.3226	0.4167	0.5172	0.6250	0.7407	0.8654	1.0000
0.6	0.0649	0.1351	0.2113	0.2941	0.3846	0.4839	0.5932	0.7143	0.8491	1.0000
0.8	0.0581	0.1220	0.1923	0.2703	0.3571	0.4545	0.5645	0.6897	0.8333	1.0000
1.0	0.0526	0.1111	0.1765	0.2500	0.3333	0.4286	0.5385	0.6667	0.8182	1.0000

the among-population variance is due to random drift cannot be rejected or convergent selection may be invoked as a cause for the reduced differentiation. Such comparison was also made in three recent studies for isozymes with quantitative traits in *D. buzzatii* (PROUT and BARKER 1993), *D. obtusa* (SPITZE 1993) and *C. dudleyana* (PODOLSKY and HOLTSFORD 1995). Our comparison (Tables 1 and 2) suggests that while the patterns of differentiation for branch angle and branch diameter are indistinguishable from that expected under the neutral hypothesis, population differentiations for specific gravity, stem diameter, stem height and branch length are likely attributable to some form(s) of selection.

From the ecological perspective, stem growth traits, such as stem height and stem diameter, are probably under selection in *P. contorta* ssp. *latifolia,* because individuals must grow rapidly to escape suppression by their neighboring plants. As a pioneer species, *P. contorta* ssp. *latifolia* is capable of occupying quickly a site where natural or man-caused disturbances have created a condition of full sunlight due to the large reserve supply of seeds in serotinous cones, low shade tolerance and rapid juvenile growth. Given its occurrence on a variety of site and climatic conditions (CRITCHFIELD 1980), it is not surprising that this conifer has experienced varying selection pressures for fast and vigorous growth, most likely induced by regional patterns of precipitation, temperature and day-length, etc., resulting in unique (differentiated) populations adapted to their own environments. Judging from the mean values of stem height and diameter (data not presented), southern populations grew more rapidly than the three northern populations. YANCHUK (1986) also found a significant (<0.01) negative correlation between stem height and latitude of populations. Such a clinal pattern probably reflects the inherent adaptation of *P. contorta* ssp. *latifolia* to regional differences in photoperiod, precipitation and temperature. For example, southern sources continued shoot elongation for 2 or 3 weeks longer than northern sources in the same plantation (YANCHUK 1986).

Selection for fast growth leads to increased production of juvenile wood with low wood density (*i.e.,* low specific gravity) because a fast-growing tree does not have the reserve photosynthate necessary to produce thick secondary cell walls (ZOBEL and VAN BUIJTENEN 1989, ch. 5). This expected relationship was supported by our finding that the fast growing southern populations exhibited lower specific gravity than the slow growing northern populations (data not presented). Strong clinal pattern for specific gravity has also been noted in other conifers (*e.g.,* GODDARD and STRICKLAND 1962; LEDIG *et al.* 1975). We also computed genetic correlations between growth traits and wood specific gravity for individual populations and all populations combined. Our overall estimates of genetic correlations between specific gravity and growth traits were all highly negative (< -0.5). Further, while clinal patterns were evident for individual traits, they were not observed for the covariance structure (additive genetic covariance matrices for individual populations not presented). Therefore, there is a need for a comparative analysis of population differentiation at the single-trait and multitrait levels in *P. contorta* ssp. *latifolia.*

Branch length could be an integral part of a tree in maintaining a balanced structure for stem growth. This is consistent with the pattern of genetic correlations between branch length and stem growth in *P. contorta* ssp. *latifolia.* The overall estimates of genetic correlation estimates were high, at 0.837 for branch length-stem diameter and 0.864 for branch length-stem height. Corresponding estimates of genetic correlations for the five populations were also high. In view of these high genetic correlations, it is possible that branch length has no "real" selective values *per se* but is indirectly responding to divergent selection imposed on the stem growth. Should this scenario be true, genetic hitchhik-

ing may have contributed to the development of association between genes controlling stem growth and branch length.

Issues in estimating population differentiation for quantitative traits: We assumed that local populations are panmictic ($F_{IS} = 0$) when estimating population differentiation (F_{ST}) for quantitative traits in *P. contorta* ssp. *latifolia*. Using inferred parental tree genotypes from megagametophyte haplotypes, we conducted χ^2 testing for $F_{IS} = 0$ at 19 isozyme loci in five populations. Out of 95 (19×5) tests, only five showed a significant ($P < 0.05$) deviation from Hardy-Weinberg equilibrium. The average of estimates of F_{IS} for all populations was -0.042, suggesting only about a 4% excess of heterozygotes relative to Hardy-Weinberg expectations. In a different survey of isozyme variation from other portion of natural range of *P. contorta* ssp. *latifolia* (DANCIK and YEH 1983), a 3% deficiency of heterozygotes was observed. In view of these results, the assumption of $F_{IS} = 0$ appears reasonable in *P. contorta* ssp. *latifolia*. Nevertheless, Hardy-Weinberg disequilibrium ($F_{IS} \neq 0$) may occur in local populations due to many factors including the relatedness of seed and pollen parents (NAMKOONG 1966), restricted gene dispersal within populations, selection arising from inbreeding depression and strong disassortative mating coupled with selection as in a balanced lethal or self-sterile population (WRIGHT 1965; MITCHELL-OLDS and RUTLEDGE 1986). When local populations are in Hardy-Weinberg disequilibrium, the estimate of F_{ST} (F'_{ST}) has the expression:

$$F'_{ST} = \frac{\sigma_B^2}{\sigma_T^2 + \sigma_W^2} = \frac{\sigma_B^2}{\sigma_B^2 + 2\sigma_W^2} = \frac{F_{ST}}{1 + F_{IS}(1 - F_{ST})}. \quad (4)$$

This expression enables us to examine the sensitivity of F_{ST} estimates to variation in F_{IS}. Table 3 presents the F'_{ST} estimates for given values of F_{IS} and F_{ST}. F'_{ST} is smaller and greater than F_{ST} when there is inbreeding ($F_{IS} > 0$) and avoidance of mating between relatives ($F_{IS} < 0$), respectively. It is evident that unless there is very strong disassortative mating ($F_{IS} \rightarrow -1$), our estimates of F_{ST} are in fact conservative estimates of population differentiation for quantitative traits. Thus, even moderate deviation of F_{IS} from zero would be unlikely to change our conclusion that growth traits are under divergent selection in *P. contorta* ssp. *latifolia*.

The assumption of linkage equilibrium may not be warranted if severe inbreeding depression occurs in *P. contorta* ssp. *latifolia*. Many conifers are known to express varying levels of inbreeding depression in seed and growth traits (SORENSEN 1982). When there is strong inbreeding depression, adult plants may be in linkage disequilibrium even though they may be close to Hardy-Weinberg proportions (R. LANDE, personal communication). Therefore, natural selection arising from inbreeding depression, coupled with many other nonselective forces, may be responsible for prevalence of linkage disequilibria between isozyme loci in *P. contorta* ssp. *latifolia* (YANG and YEH 1993). However, it remains to be investigated how linkage disequilibrium affects the partitioning of the total variance (σ_T^2) and thus biases estimation of population differentiation for quantitative traits.

Maternal effects could potentially bias our estimates of F_{ST} because they would increase the expected resemblance (*i.e.*, covariance) between seed parent and its progeny or between progeny of the same seed parent. While maternal effects are often considered important in plant species, they are difficult to detect (MITCHELL-OLDS and RUTLEDGE 1986). In conifer species, seed weight and seedling emergency rate have been considered as two major maternal or preconditioning factors (BONNER 1988), but their effects on population and family differentiation are most easily detected at the seedling stage, before age three (*e.g.*, KRIEBEL *et al.* 1972). In *P. contorta* ssp. *latifolia*, seed weight and seedling emergency rate did not correlate with seedling growth traits (X. WU and F. C. YEH, unpublished), suggesting little maternal effect for growth traits in this conifer.

An additional bias may result if some family members are full-sibs instead of half-sibs (M. LYNCH, personal communication). Presence of full-sibs would bias upward the estimate of additive genetic variance and would include a portion of the dominance genetic variance in the partitioning of σ_T^2. For true half-sib structure to exist in our study of 12 seedlings per family, at least 12 unrelated trees would have to contribute as the male parents. We estimated *F*-statistics at each of 19 isozyme loci from the inferred parental tree genotypes. Averages of estimates of F_{IS}, F_{IT} and F_{ST} over the 19 loci were -0.042, -0.019 and 0.022, respectively. Following QUELLER and GOODNIGHT (1989), we computed the coefficient of within-family relatedness (r) as $r = 2F_{ST}/(1 + F_{IT}) = 0.045$. If many family members were full-sibs, we would expect the r value near 0.5. Consequently, the open-pollinated progenies in our study are probably half-sibs.

Presence of nonadditive genetic effects might be another source of bias in estimating F_{ST}. In the presence of dominance, for example, it is not possible to make an unique partitioning of σT^2 into σ_B^2 and σW^2 (ROBERTSON 1952; WRIGHT 1952). Consequently, population differentiation (F_{ST}) cannot be estimated without defining new genetic parameters concerning the population structure. While nonadditive effects have not been reported for *P. contorta* ssp. *latifolia*, they have been demonstrated in other conifers (*e.g.*, GERHOLD and PARK 1986; YEH and HEAMAN 1987), but their variances are smaller relative to additive genetic variance. In *Pseudotsuga menziesii*, for example, dominance genetic variance was about 18% of additive genetic variance (YEH and HEAMAN 1987). Thus, small nonadditive effects may be of little consequence in estimating F_{ST}.

Implications: Knowledge pertaining to population differentiation is important in the management of plant genetic resources (BROWN 1978). In this study of *P. contorta* ssp. *latifolia* from British Columbia, we found three distinct patterns of among-population differentiation that were trait specific. First, population differentiation in stem height and diameter likely reflected adaptive response of the populations to spatial and temporal variability during tree growth and development. Second, specific gravity and branch length may have no "real" selective values *per se* but are indirectly responding to divergent selection imposed on the stem growth. Third, nonselective forces such as genetic drift and gene flow probably played significant roles in governing the level of population differentiation in branch angle, branch diameter and isozymes. Similar findings have been recently reported in *D. buzzatii* (PROUT and BARKER 1993), *D. obtusa* (SPITZE 1993) and *C. dudleyana* (PODOLSKY and HOLTSFORD 1995). Given our results, recommendations for genetic conservation strategies in *P. contorta* ssp. *latifolia* probably should not be based on a small set of traits, be they isozymes or quantitative traits. Instead, we suggest integrating traits whose expression is modulated mainly by environmental factors (*i.e.*, growth, phenology, cold and drought hardiness) to reveal adaptation with traits (*i.e.*, isozymes) regulated primarily by genetic drift and gene flow for discerning the forces that shape the variation pattern in this conifer.

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