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Observations on the ecology and evolution of quaking aspen, *Populus tremuloides*, in the Colorado Front Range

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been the focus of debate (Moss, 1938; Ellison, 1943; Larson, 1944; Marr, 1961; Graham, Harrison, and Westell, 1963).

Aspen clones presently covering hillsides in the west may be truly ancient. Reports in the literature estimate that aspen clones may live thousands of years (Cottam, 1954; Barnes, 1975), but this estimate is difficult or perhaps currently impossible to verify quantitatively. It is certain that these clones can live for centuries.

Sexual reproduction each generation mixes genes and genotypes, and may erase some of the record of natural selection. Recent studies of heterocyclic daphnia (Hebert, Ward, and Gibson, 1972; Hebert, 1974a,b) have utilized periods of parthenogenic reproduction that accumulate fitness differentials between genotypes to detect the influence of natural selection. In this study, we have used the same principle by studying a predominantly asexually reproducing population to detect the action of natural selection. We have investigated growth rate, age, sex, and protein polymorphism in 106 clones in the Front Range of Colorado to identify genetic differentiation with respect to elevation and sex, to test for correlations among genetic loci, to partition variance of growth rate within and between clones, and to test for effects of heterozygosity on growth rate and growth rate variation.

MATERIALS AND METHODS—Core samples from 5 ramets of each of 106 clones were taken in an area covering approximately 500 sq km just west of Boulder, Colorado. There is no guarantee that a stand of quaking aspen is a single clone, for clones can abut or even interdigitate. Therefore, our identification of clones for sampling purposes was conservative and pragmatic. The five largest stems within close proximity (usually within 10 m) of one another were chosen for leaf and core samples. In order to sample ramets from a single clone only we looked at such characteristics as branching habit, bark color, and sex (Grant and Mitton, 1979). An adjacent clone was defined as a stand of trees separated by at least 400 m of terrain lacking ramets of aspen, or separated by some impassable ecological barrier (e.g., rock outcropping). The elevational distribution of the sampling area was from 1,700–3,200 m, the approximate local limits of aspen.

The elevation of each clone was estimated with an altimeter. Growth rings (Grant and Mitton, 1979) were counted to age each ramet, and were measured to provide an estimate of the mean and variance of the growth rate. The sex of each clone was identified, and a sample

of leaves was taken for electrophoretic analysis in order to compare genotype to the morphological and physiological characters.

Tissue was prepared for electrophoresis in the following way. Three to six leaves from a single ramet were ground to a fine powder in a mortar and pestle under liquid nitrogen. Just as the liquid nitrogen evaporated, a 5% solution of polyvinylpyrrolidone in .18 M phosphate buffer pH 7.0 was added with a pipette, and the constituents mixed to prepare a slurry. This material was not allowed to warm to room temperature; best results were obtained when the ground material partially froze the buffer. The slurry was then placed into test tubes and centrifuged for a few minutes at low speed to pack down the cellular debris. Wicks of double thickness Whatman No. 1 filter paper were used to absorb the supernatant and apply it to horizontal starch gels. The buffer system is that described in Mitton et al. (1977). Gels were stained for phosphohexose isomerase, peroxidase, and glutamate dehydrogenase by the methods in Shaw and Prasad (1970). These are the only polymorphic enzyme systems presently resolvable. Heterozygosity was scored simply as the number of loci heterozygous (0–3).

Heterogeneity of gene frequencies was tested with the chi-square test of Workman and Niswander (1970). The association between genotype and aspects of growth rate were tested with multiple regression, and associations among age, growth rate, elevation and sex were studied both with simple and partial correlations. This approach allows examination of dependent variable responses to independent variables with the effects of other correlated independent variables held constant.

RESULTS—Clones studied here were polymorphic for phosphohexose isomerase (PHI, 4 alleles), peroxidase (PER, 4 alleles), and glutamate dehydrogenase (GDH, 2 alleles). Our genetic interpretations of the banding patterns are supported by 1) appropriate patterns of variation (monomeric, dimeric) for each locus, and 2) genotypic frequency fits to Hardy-Weinberg expectations. The PHI and PER polymorphisms were condensed to 2 alleles by pooling rare alleles (frequency less than .10) with more common alleles displaying similar mobilities in order to keep sample sizes large enough for statistical testing.

Complete data for age, elevation, genotype, and growth rate were taken for 98 clones. For each clone, a mean growth rate and standard deviation of growth rate was estimated, and the correlation between them was .94 ($P <$

TABLE 1. Multiple regression of mean growth rate in quaking aspen

	Coefficients	Probability
Multiple R	.606	$P < .001$
Independent variables		
Age	-.152	$P < .001$
Heterozygosity	1.637	$P < .05$
Sex	1.425	$P > .20$
Elevation	-.017	$P < .01$

.001). Mean growth rate was used as the dependent variable in the multiple regression analysis while age of the clone (oldest ramet), heterozygosity, sex, and elevation were employed as independent variables. Mean growth rate was found to be very highly correlated with variation in growth rate, so that all statistical relationships reported for mean growth rate hold equally well for year to year variation in growth rate as well. The multiple regression explains 37% of the variance in growth rate of

clones ($P < .001$, Table 1). There is no effect of sex with the effects of other independent variables held constant. Mean growth rate decreases both with age of oldest standing ramet ($P < .001$) and elevation ($P < .01$). The mean growth rate significantly increases with increasing heterozygosity ($P < .05$).

Analyses of variation in the three enzymatic loci revealed different patterns. For each locus, the sample of clones was first contrasted by elevation, and then by sex. The sampling midpoint elevation of 2,750 m was used to partition the clones into high and low groups. For each locus and for each elevational group, there is good agreement between observed genotypic frequencies and those expected under assumptions of the Hardy-Weinberg Law. No differences in allelic frequencies were noted among the elevational groups (Table 2), a result contrasting strongly with the differentiation observed between sexes. Allelic frequencies are significantly different in males and females for PHI. For PER, the genotypic distributions

TABLE 2. Comparisons of allelic frequencies by elevation and by sex for three enzymatic loci in quaking aspen. Numbers enclosed in parentheses are the expected numbers under Hardy-Weinberg assumptions. N = sample size, $f(1)$ = frequency of allele 1, SE = standard error of the allelic frequency, χ^2_a tests the fit of observed to expected genotypic proportions, and χ^2_b tests heterogeneity of allelic frequencies

expected genotype proportions, and χ^2 tests heterogeneity by elevation										
2A Comparison by elevation			N	f(1)	SE	χ^2_a	P	χ^2_b	P	
Locus	Genotype									
GDH-high	11	12	22	54	.093	.028	0.1	> .5	0.9	> .5
	1	8	45							
GDH-low	2	9	37	48	.135	.035	0.5	> .5		
	(0.5)	(9.1)	(44.5)							
PHI-high	3	20	31	54	.241	.041	0.0	> .5	0.5	> .5
	(0.9)	(11.2)	(35.9)							
PHI-low	2	15	31	48	.198	.041	0.0	> .5		
	(3.1)	(19.7)	(31.1)							
PER high	4	13	37	54	.194	.038	1.0	> .5	0.2	> .5
	(1.9)	(15.2)	(30.9)							
PER low	3	15	30	48	.219	.042	0.1	> .5		
	(2.0)	(16.9)	(35.0)							
	3	15	30	48	.219	.042	0.1	> .5		
	(2.3)	(16.4)	(29.3)							
2B Comparison by sex			N	f(1)	SE	χ^2_a	P	χ^2_b	P	
Genotype										
GDH-male	2	9	45	56	.116	.030	0.6	> .5	0.2	> .5
	(0.8)	(11.5)	(43.8)							
GDH-female	2	9	36	47	.138	.036	0.5	> .5		
	(0.9)	(11.2)	(34.9)							
PHI Male	5	21	30	56	.277	.042	0.2	> .5	4.9	< .05
	(4.3)	(22.4)	(29.3)							
PHI Female	0	14	33	47	.149	.036	0.4	> .5		
	(1.0)	(11.9)	(34.0)							
PER Male	6	10	40	56	.196	.038	3.7	< .05	0.1	> .5
	(2.2)	(17.7)	(36.2)							
PER Female	1	18	28	47	.213	.042	0.4	> .5		
	(2.1)	(15.7)	(29.1)							

of males is significant, for the expected, for the genotypes. The fit of this locus is significant, a few more heterozygotes, though the allelic frequencies are different between sexes. The test of independence of frequencies in the sexes are different, either allelic frequencies or GDH locus.

Additional analyses acting upon the data can be obtained by testing the fit of the genotypic frequencies (Thomson, 1974). A measure of the contribution of nonrandom mating to the heterogeneity of linkage disequilibrium is not different between the joint distributions of GDH, PHI, and PER. The calculated frequencies of the loci within the sexes. Although age disequilibrium is not significant, the action of natural selection on the single genotype can be generated by which differentiation. Koehn (1974) it is difficult to distinguish between a distribution equilibrium) is significant, differentiation of genotypes is significant, selection of genotypes is significant. Individuals of the same genotype have different characters, as allozymes, have a subunit determine growth rate of ramets of clones. Our sampling method allowed components of variation in growth rate year to year, ramets of clones (barring chance in the clone, and soil, sun, the variation

of males is significantly different than the expected, for there is a deficiency of heterozygotes. The fit of the genotypic distribution for this locus is satisfactory in females: there are a few more heterozygotes than expected. Although the allelic frequencies of PER are not different between the sexes, a row by columns test of independence of the observed genotypic frequencies indicates that genotypic frequencies are different ($\chi^2 = 7.2$, $P < .05$). No differences between the sexes were noted for either allelic or genotypic frequencies at the GDH locus.

Additional evidence of non-random forces acting upon the genetics of populations may be obtained by analyzing the joint distribution of the genotypes of different loci (Lewontin, 1974). A measure of the non-random distribution of non-homologous alleles is the parameter of linkage disequilibrium (Geiringer, 1944; Thomson, 1977; Clegg, 1978). This parameter is not different from zero when calculated for the joint distributions of PER and GDH or PHI and GDH, but it is significantly different when calculated for PER and PHI. These are also the loci which are differentiated between the sexes. Although a significant value of the linkage disequilibrium parameter may indicate the action of natural selection above the level of the single gene (Lewontin, 1974), it may also be generated by the pooling of populations which differ in frequencies for those loci (Mitton, Koehn, and Prout, 1973). For these data, it is difficult to discern whether the non-random distribution of genotypes (linkage disequilibrium) is simply caused by the independent differentiation of two loci in the sexes, or whether differentiation of two loci is apparent because selection acts differently upon the joint protein genotypes in the sexes.

Individual ramets within a clone share the same genotype, and hence are identical for characters determined solely by genes, such as allozyme phenotypes. But characters that have a substantial proportion of their variance determined by environmental factors (e.g., growth rate) could differ markedly between ramets of a clone.

Our sampling design of ring width measurement allows partitioning of the variance into components (Table 3). The largest component of variance (54%) is that within ramets—growth rates of aspen vary considerably from year to year for individual stems. Although the ramets of a clone are genotypically identical (barring somatic mutation), 14% of the variance in growth rates is among ramets of a clone, and is due to microsite differences in soil, sunlight, water availability, etc.; 33% of the variance in growth rates is among clones

TABLE 3. Apportionment of variance of growth rate within and among ramets of male and female clones of quaking aspen, *Populus tremuloides*

Variance component	Variance	Percent
Between years, within ramets. within clones, within sexes	43.56	53.85
Between ramets, within clones. within sexes	10.94	13.52
Between clones, within sexes	26.52	32.78
Between sexes	-.13	-.16

of the same sex. The proportion of the variance among sexes is approximately zero.

A rough estimate of heritability of variance of growth rate may be generated from these data. Both the variation within ramets across years and the variation among ramets of a clone are strictly environmental. Variation among clones has two components; they differ genetically and occur in a diverse array of environments. Variation among the sexes is, once again, both genetic and environmental, but this component of variance approaches zero, and may be ignored for this rough estimate. Interactions may well occur between genetic and environmental portions of these variance components, but we cannot estimate them from these data, so we must assume, for these calculations, that they approach zero. If the strictly environmental components of variance are subtracted from the total phenotypic variance, we are left with the among clones within sexes component, which should have both genetic and environmental portions. If we utilize this component to estimate an upper bound to the broad sense heritability of growth rate ($h^2 = \text{variance among clones/total phenotypic variance}$), the upper bound is 32.6%.

A generalization commonly held concerning broad sense heritability is that characters with a strong influence on fitness have low levels of heritability—an optimum phenotype is chosen by artificial or natural selection, and the majority of genetic variation influencing that character has been eliminated (Falconer, 1960). This generalization is compatible with our data, which suggest an upper bound of heritability of variance of growth rate to be about 33%. It is our belief that characteristics of growth are important in the determination of fitness.

Cores from several ramets of each clone provide minimum estimates of clone age only, but such ages do have substantial biological significance indicating, for example, how recently fires and avalanches have perturbed the forest. The age distribution for clones is given in Fig. 1, and correlations between age and

Fig. 1. The distribution of age from ramets of 106 clones of quaking aspen.

elevation, diameter of the largest ramet, and sex of the clone are presented in Table 4. Ages of ramets increase slightly with elevation, but the true ages of the clones need not necessarily have the same pattern. In both male and female clones, ring width tends to be greater in ramets with larger diameter. The correlation between ring width and tree diameter may be standardized by removing the effects of age, elevation and sex. The resulting partial correlation, .221 ($P < .001$) suggests something unusual about the growth of aspen ramets. Ring width decreases with both elevation and age. Correlations between age and diameter are positive in both the sexes, but the correlation is more than twice as strong in females as it is in males ($r = .397$ vs. $r = .188$). This may be because females are generally more common at lower elevations where age and diameter may be more highly correlated.

Some species of dioecious trees switch sex from year to year (Vasek, 1966). In order to find out if sex was fixed in aspen, we returned

to most of the clones a second year and to some of the clones for a third year to determine whether sex was fixed or labile. All clones retained the same sex from year to year.

During the first check of the constancy of the sex of aspen clones, we noted an inconsistency in our records of the sex of clone 15, approximately 15 km north of Nederland on Highway 72. This stand had initially been marked as a male, but in the second year there were female catkins. A closer inspection revealed both male and female catkins, and possibly a dimorphism in bark color. This seemed like an opportunity to document the overlap of clones, so a comprehensive study of this stand was initiated. Every ramet with a diameter greater than one inch was marked with a number and mapped by the method of Rohlf and Archie (1978). Leaf samples were collected from each ramet, and the genotype of each ramet was determined for each of the enzyme loci. By sex determination alone, we knew that there were at least two clones in the stand, while three polymorphic protein loci can provide the resolution to identify dozens of clones (Hancock and Wilson, 1976). In fact, the additional markers did not identify any further heterogeneity, indicating that there are only two clones at this specific site. The sex of the ramets could not be used to map precisely the interdigitation of clones, for not all ramets produced catkins, but the genotypic data may be used to delimit the area of overlap (Fig. 2). This particular stand includes an area occupied solely by the female, an area occupied solely by the male, and an area of overlap. Bark color provided some discrimination of these clones, but it is not totally reliable. Large female ramets have greenish bark and large male ramets have whiteish bark, but small ramets appear to be greenish whether they are male or female. Both clones are homozygous for the second

TABLE 4. Correlations among variables for male and female clones of quaking aspen. N = Sample size. Each data point consists of the width of an annual growth increment, the age and diameter of the ramet from which it was taken, and the elevation of the clone. All correlations are statistically significant

Males $N = 10,254$					
diameter	1.000				
elevation	-.072	1.000			
ring width	.310	-.135	1.000		
age	.188	.109	-.170	1.000	
	diameter	elevation	ring width	age	
Females $N = 7,734$					
diameter	1.000				
elevation	-.207	1.000			
ring width	.163	-.138	1.000		
age	.397	.054	-.202	1.000	
	diameter	elevation	ring width	age

Fig. 2. Interdigitation of clones of aspen. The genotype of each ramet (•) has been identified for three protein loci. See text for further explanation.

allele of peroxidase, but the female is heterozygous for the remaining loci, and the male is homozygous for all three of these markers.

Clones of aspen that differ genetically and overlap in space provide a natural experiment to test for genetic influences upon growth rate. To describe growth rates, cores were taken from approximately 20 ramets of each clone, and ring widths were measured to estimate the mean and variance of growth rates. The means were not different, but the variances were. The more heterozygous female clone had the greater variance ($P < .05$) in growth rate.

DISCUSSION—The results of this study indicate a significant positive relationship between heterozygosity and mean growth rate. We know from biochemical studies that heterozygous individuals may have greater phys-

iological versatility than their homozygous counterparts (e.g., Hochachka and Somero, 1973; Fincham, 1972; Johnson, 1976), but we are not yet in a position to specify the mechanistic causes of the observed higher growth rate of heterozygotes. Our data do suggest a role of the test loci, or perhaps other closely linked loci, in a trait (differential growth rate) which undoubtedly affects fitness. We believe the effect is probably from the observed loci since it is not sufficient to postulate that loci closely linked to the ones observed be involved in the growth rate. There would also have to be linkage disequilibrium between the enzymatic loci and the linked loci to produce the observed result. Linkage disequilibrium is certainly found in natural populations, but it is rarely of sufficient magnitude to produce the effect considered here (Clegg, 1978).

e. Each data
which it was

1.000
age

1.000
age

The results of the multiple regression indicate that the effect of a single additional heterozygous locus is detectable. There are precedents for this observation (Mitton, 1978; Eanes, 1978; Singh and Zouros, 1978), but it is nevertheless remarkable that a significant relationship between heterozygosity and growth rate could be detected by utilizing only three loci, notwithstanding theoretical arguments (Kimura and Ohta, 1971) and some empirical data (Fuerst, Chakraborty, and Nei, 1977) that suggest that protein polymorphisms have little or no impact upon fitness. It is difficult to image how heterozygosity at a single locus can influence the phenotype of an organism polymorphic for hundreds or thousands of loci (Lewontin, 1974). However, since we did detect such an effect, it is probable that natural selection could also.

In this study, the measure of growth rate has been based upon the width of annual growth rings of several ramets per clone. This measure is easily obtained, and is valuable as a measure of adaptation or vigor, but annual rings do not indicate the true age of the clone, nor do they tell anything about the relative apportionment of energy to asexual reproduction, sexual reproduction, or growth of the root stock. It may well be that in terms of understanding the long term viability of a clone, energy channeled into sexual reproduction or invested in the root system may be more informative than age or growth rate in standing ramets. Growth rates measured from standing ramets indicate both genotypic and environmental effects, and, at least partially, reflect the degree to which a clone is adapted to its specific environment.

We observed an interesting contrast between the relationships among age, diameter, and ring width in aspen versus ponderosa pine. Within a stand of ponderosa pine, diameter is a good predictor of age, which could vary from a few to several hundred years. In contrast, the distribution of age of ramets of aspen has relatively low variance in our sample (Fig. 1), and age could not be accurately predicted from diameter (Table 4). The life expectancy of an aspen ramet in the Colorado Front Range is 50–70 years at best. The ages of the oldest standing ramets may simply reflect pressure from herbivores, diseases, competition from conifers, or stress imposed by the physical components of the environment. Ring width generally decreases with age of a tree (Fritts, 1976), yet there is a positive partial correlation between diameter and ring width in this sample ($r = .221$, $P < .001$). Within a ramet, ring width decreases with age, but perhaps some trees simply grow much faster than others, but have similar longevities in the Front Range

environment. The correlation between these variables may be a consequence of the dynamics of an early successional species.

There are certainly some problems associated with working with clones in the field. In this species, it is difficult to determine the extent of a clone (but see Barnes, 1969), for they may overlap and the root system could conceivably extend far beyond presence of ramets. We were able to document overlap of clones here (Fig. 2), but we have not by any means delimited the extent of all clones.

To balance the disadvantages of working with clones, there are some distinct advantages. Ramets provide replicates of an individual and regeneration by asexual reproduction changes a long-lived perennial to a perennial with exceedingly long generation times. Intensities of natural selection that might not be detected over several decades may be immediately apparent if natural selection is allowed to work for several centuries or millenia. The time spans involved here can only be guessed at, but geographic variation in sex ratio (Grant and Mitton, 1979) and differentiation of allelic frequencies between the sexes (Table 2) is now apparent.

Our finding that allelic frequencies were independent of elevation stands in marked contrast to previous studies of other Front Range taxa (Grant and Mitton, 1977; Mitton et al., 1977). The elevational transect studied encompasses a wide range of environmental conditions and we expected to find corresponding genetic differentiation.

The contrasting finding (significant allelic and genotypic differentiation between the sexes) is consistent with data which indicate that proportions of male and female clones vary with elevation, probably as a result of differential mortality (Grant and Mitton, 1979). Whatever the mechanisms, natural selection is apparently influencing the sexes differentially.

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