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PHENETIC AFFINITIES IN SMALL POPULATIONS OF NEW YORK ASPENS

Charles M. Harrison[1] and F. A. Valentine[2]

According to Anderson (1962) much of the variation in wild populations of plants is attributable to occasional introgressive hybridization. Introgression generally has been associated with disturbed habitats occupied by two or more species that normally are separated by some mechanism of sexual isolation. Barnes (1961) found considerably more introgression of *Populus tremuloides* Michx. and P. *grandidentata* Michx. on disturbed sites (abandoned farmlands) in southeastern Michigan than in natural stands in the forested region of northern Michigan. Heimburger (1936) reported natural hybrids to be scarce in eastern Canada, whereas Pauley (1956) found them to be not uncommon in Massachusetts. Since the native aspens, especially P. *tremuloides*, are among the most common pioneer tree species on the extensive abandoned farmlands of the Lake States and northeastern United States, introgression should result in much greater variation in these species and be a major force in their evolution. It conceivably could lead to a breakdown of the species barrier and result in a single polymorphic aspen species in eastern North America.

The present study was undertaken to determine the reliability of large numbers of phenotypic characteristics in distinguishing between trembling (P. *tremuloides*) and bigtooth aspen (P. *grandidentata*) and to compare putative interspecific hybrids with the two parent species on the basis of traits deemed reliable. Methods of numerical taxonomy (Sokal and Sneath, 1963) have been employed in this preliminary study of small populations in central New York. The reliability of each trait is expressed quantitatively by a statistic termed the Character State Distance, which is based upon the difference between the population means of the two species and the within-species variations. It is a modification of Crovello's distance statistic (1968). A second statistic, the Affinity Index, measures the phenetic or phenotypic relationship of an individual tree to the two aspen species. It differs from most hybrid indices (Anderson, 1949; Sibley, 1954; Goodman, 1967) in that the contribution of each trait is weighted by the reliability of that trait in distinguishing between the two species, i.e., by the Character State Distance. The validity of this method of numerical taxonomy is established by the separation of trembling and bigtooth trees from unrelated stands into two well defined groups. The Affinity Indices of putative natural hybrids and known interspecific hybrids fall between those of the two parent species, but their values indicate a much greater phenetic affinity to trembling than to bigtooth aspen.

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MATERIALS AND METHODS

Three natural stands with trembling and bigtooth aspen were chosen for this study. They are located (1) along Otisco Road, approximately 16 miles SSW of Syracuse, New York, the main study area; (2) near Marcellus, New York, about 9 miles west of Syracuse; and (3) near Camden, New York, about 35 miles NNE of Syracuse. The number of trees of each species selected in each stand and the putative hybrids are given in table 1, with the sex indicated where this could be determined. The trees in the main study area randomly sampled to serve as the "reference populations" for the two aspen species, ranged in age from 19 to 30 years except for one 59-year-old bigtooth aspen. Additional trees were analyzed for certain traits at Heiberg Forest, Tully, New York; Petawawa Experimental Forest, Chalk River, Ontario; and known interspecific hybrids produced by Dr. Ian Brown (unpublished results).

Table 1.--Trembling and bigtooth aspen and putative interspecific hybrid trees studied.

Origin	Number of trees by sex:									Total
	Trembling			Bigtooth			Hybrids			
	M	F	U[1]	M	F	U	M	F	U	
Reference Stand:										
Otisco Road	2	2	1	4		2		2		13
Unrelated Stands:										
Marcellus	1		1	1		2	1		1	7
Camden	1		1	1		1			1	5
Heiberg Forest			2							2
Petawawa			1			1			1	3
F_1 Hybrids									4	4
Totals	4	2	6	6	0	6	1	2	7	34

[1] Sex unknown.

The procedures followed in the field collections; the laboratory sampling, analyses, and measurements; and field classifications for the forty-five traits analyzed are summarized in table 2. Branches for mature leaf and bud traits were collected in October and dried in plant presses. Branches for floral and young leaf traits were collected in February and immediately forced in the greenhouse.

Table 2.--Field collections, sampling, classification, and measurement procedures for the forty-five traits analyzed in the Otisco Road, Marcellus, and Camden trees.

Traits	Units	Field collection: No. branches	Field collection: Position in tree	Field collection: Portion of branch	Laboratory analysis or field classification: Organ or tree part	Laboratory analysis or field classification: No. per tree	Laboratory analysis or field classification: Sampling procedures
Blade length	cm	2	Middle crown	Terminal & 4 outer 1st-order lateral increments	Mature leaf	20	2nd & 5th nodes
" width	cm						
" L/W	Ratio						
" widest point	Code 1-3						
Blade apex angle	o						
" base "	o						
" dentation one side	No.						
Petiole length	cm						
Terminal bud:							
Length	cm	2	Middle crown	Terminal & 4 outer 1st-order lateral increments	Terminal buds	10	
Widest diam.	cm						
L/Widest diam.	Ratio						
Widest pt.	Code 1-3						
[1]Pubescence amount	Code 1-4						
Lateral bud:							
Divergence angle	sine	2	Middle crown	Terminal increment	Lateral buds	10	5 outer nodes
plus same 5 traits for terminal buds							
Young leaf:							
[2]Thickness	Meter units	2	Upper crown	Terminal & 1st-order laterals	Young leaf	20	2nd & 5th nodes
[1]Pubesc. amt.	Code 1-5						
[3]Pubesc. type	Code 1-6						
Floral:							
Length	cm	2	Upper crown	Ibid	Floral catkins	20	Random
Flowers along one side	No.						
Length	cm				Floral bracts	50	Random mature flowers
Width	cm						
No. clefts	No.						
Depth deepest cleft	cm						
No. stamens	No.				Flowers	50	Random mature flowers
[4]Pigmentation anther or stigma	Code 3-29						

TABLE 2 (concl.)

Traits	Units	Field collection: No. branches	Field collection: Position in tree	Field collection: Portion of branch	Laboratory analysis or field classification: Organ or tree part	Laboratory analysis or field classification: No. per tree	Laboratory analysis or field classification: Sampling procedures
Length, branch	cm	4	Middle crown	Main branch	Annual length growth	12	1960, 64 & 68 increments
Av. diam. midpoint	cm					12	
68 diam. mid. point	cm					4	
68 L/DM	Ratio					4	
Av. internode length	cm					12	
Survival	%	4	Middle crown	Main branch	Lateral branches	4	No. 1st-order laterals 1964 increment divided by no. nodes
Frequency	No.					24	[5]Lat. branches 1962-67 increments
Fall color changes:							
Earliness of color	Code 1-5	-	----	--------	Entire crown	1	% colored in top, middle & bottom third on Oct.10, 17, & 24
Rapidity of color change	Code 1-5	-	----	--------	Entire crown	1	Greatest change in one week compared to three weeks

[1] Coded from glabrous with the lowest code value (1) to dense pubescence.

[2] Amount of light from an American Optical Co. Model 651 Illuminator at its lowest intensity transmitted through a leaf two inches away that was held flush to a Microsix L exposure meter.

[3] Based upon Benson's types (1957), coded by length, diameter, and stiffness from long, thin, and soft to short, thick, and stiff.

[4] Coded according to Maertz and Paul (1930) Color Plate Nos. 49-53 and the row and column numbers.

[5] The total number of branches per annual increment is based upon (1) the number of first-order laterals, (2) the number of second-order laterals on the 4 outermost first-order laterals, and (3) the number of third-order laterals on the 3 outermost second-order laterals on the 4 outermost first-order laterals. A regression of number of laterals (Y) on age of the length increment (X) was used to estimate x/y .

Catkins and flowers were sampled when pollen was shed or the stigma was receptive as determined by successful pollination. Catkins were preserved in FAA solution[3] for later measurements for most traits. Freshly harvested materials, however, were used for flower pigmentation and young leaf thickness and pubescence. Branches extending in approximately the four cardinal directions were collected in April and stored at 39° F in a coldroom for twig and branching traits. Fall color observations were made on weekly trips in October. Detailed descriptions of the classification and coding for traits, where this was employed, are given by Harrison (1971).

Statistical Methods

Two statistics have been developed: the Character State Distance (D_o), a measure of the reliability of each trait in discriminating between the two aspen species, and the Affinity Index (I), an estimate of the resemblance of a given individual to the two species.

The equation for Character State Distance is shown in table 3. It was developed in an attempt to modify Crovello's (1968) equation in which CSD is equal to the hypotenuse of a right triangle, with the two sides the difference between the means and the difference between the ranges of the groups being compared.

Table 3.--Character state distance (D_c).

Equation and Discrimination Ratings

$$D_c = \frac{(\bar{x}_L - \bar{x}_S) + [(\bar{x}_L - s_L) - (\bar{x}_S + s_S)]}{(\bar{x}_L - \bar{x}_S)}$$

$$= 2 - \frac{(s_L + s_S)}{(\bar{x}_L - \bar{x}_S)}$$

Where: $\bar{x}_L$ = larger value of species' means

$\bar{x}_S$ = smaller value of species' means

s_L = standard deviation for species with the larger mean

s_S = standard deviation for species with the smaller mean

Trait rating	Second term	D_c
Prime	$(\bar{x}_L - \bar{x}_S) > (s_L + s_S)$	>1
Some value	$\frac{1}{2}(s_L + s_S) < (\bar{x}_L - \bar{x}_S) \le (s_L + s_S)$	$0 < D_c \le 1$
No value	$\frac{1}{2}(s_L + s_S) \ge (\bar{x}_L - \bar{x}_S)$	0 or < 0

[3] 18parts 50% ethyl alcohol to 1 part glacial acetic acid to 1 part 40% formaldehyde.

The first equation in table 3 shows that the Character State Distance is a direct function of two characteristics of the two populations: (1) the difference in "Central tendency," as expressed by the arithmetic means ($X_L - X_s$); and (2) the difference in "dispersion," by the degree of overlap or nonoverlap of their variation, expressed as the difference between the points of inflection of the frequency distribution curves (assuming normal distribution) one standard deviation from the mean $[(X_{L}, - s_L) - (X_s + s_s)]$. The sum of these two differences forms the numerator oT the first equation. The term in the denominator "standardizes" the value by making the maximum contribution of each of the two population parameters equal to 1. Therefore, the maximum Character State Distance is 2.0 and would occur only if the means differ (the magnitude is not important) and if there is no variation in either population. The latter condition is not likely, so that D_c is expected to be less than 2.0.

The second equation for D_c, derived by algebraic manipulation, is the "working equation." The relative sizes of the numerator and denominator of the second term determine the reliability of the trait in discriminating between the two species. It is obvious that D_e can vary from +2.0 to $-\Phi$. Our ratings for the reliabilities of traits based upon D_e are given at the bottom of table 3.

The Affinity Index equation is given in table 4 along with an example to illustrate how the equation is applied to determine the contribution of the trait, leaf dentation, to the Affinity Index of tree T6. The Affinity Index equation provides for a quantitative expression of the over-all phenotypic similarity of an unknown tree to two reference populations. In this study, the Otisco Road populations of trembling and bigtooth aspen serve as the reference groups. The numerical value of the index is the summation of the affinity of each trait in the unknown tree to that in the reference populations (the quantity between the brackets) weighted by the reliability of the trait in distinguishing .he reference groups (D_o), which is then multiplied by a correction term ($2/\varepsilon D_c$) to give a standardized value whose size is independent of the number of traits analyzed. The limits of the index are + 2.0 for trembling aspen affinity and - 2.0 for bigtooth aspen affinity.

The weighted contribution of each trait to the index is determined by the portion of the equation between the braces and the three conditions given below the equation. The affinity of a particular trait to one of the two species is the quantity between the brackets. The absolute value calculated for the part between the parallel lines is signed (see Condition 1) to designate species affinity, with a "+" if it more closely resembles the trembling reference population and a "-", the bigtooth reference population. The numerical value is a quotient, with the dividend the difference between the mean value of the trait in the unknown tree (xi) and the midpoint value between the reference populations' means, i.e. 1/2(3, + xs), and the divisor the difference between the value in the expected distribution of means for the species it more closely resembles two standard errors from the mean closest to the midpoint of the population's mean, i.e. ($XL - 2sx_L$) or ($Xs + 2sx_s$) (see Condition 2) minus the midpoint value. The absolute value of this quotient is given a limit of 1.0 (*see* Condition 3) so that traits with values larger than it - 2851 or smaller than $xs + 2sx_s$ will not make a disproportionate contribution to the index.

Table 4.--Affinity index equation (I).

$$I = \frac{\sum \left\{ D_c \left[\pm \left| \frac{\bar{x}_i - \frac{1}{2}(\bar{x}_L + \bar{x}_S)}{C - \frac{1}{2}(\bar{x}_L + \bar{x}_S)} \right| \right] \right\}^2}{\sum D_c}$$

(Note: the parallel vertical lines, | |, around the second term denote absolute value.)

where: (1) the second term is "+" if $\bar{x}_i$ is closer to $\bar{x}_{trem.}$ than to $\bar{x}_{grand.}$ and "-" if closer to $\bar{x}_{grand.}$

(2) $C = \bar{x}_S + 2\, s_{\bar{x}_S}$ if $\bar{x}_i < \frac{1}{2}(\bar{x}_L + \bar{x}_S)$
and $C = \bar{x}_L - 2\, s_{\bar{x}_L}$ if $\bar{x}_i > \frac{1}{2}(\bar{x}_L + \bar{x}_S)$

(3) the maximum limit of the absolute value of the second term is 1.0

Example: Contribution of leaf dentation to the Affinity Index of tree T6.

Reference populations' values:

Since $\bar{x}_{trem.} = 29.90$, $\bar{x}_{grand.} = 8.89$, $s_{\bar{x}_{tre.}} = 1.38$, and $s_{\bar{x}_{grand.}} = 0.43$,
$\therefore \bar{x}_L = 29.90$, $\bar{x}_S = 8.89$, $-2s_{\bar{x}_L} = 2.76$ and $+2s_{\bar{x}_S} = 0.86$.
And since $\bar{x}_i = 32.75$ and is greater than $\frac{1}{2}(\bar{x}_L + \bar{x}_S)$, $C = \bar{x}_{trem.} - 2s_{\bar{x}_{trem.}}$

and a "+" is assigned to the second term for trembling affinity.

P. tremuloides Otisco Rd. P. grandidentata Otisco Rd.

$$\text{Contribution to I} = 1.62\left(+\left|\frac{32.75 - 19.40}{27.14 - 19.40}\right|\right)$$

$$= 1.62\ (+1.73)$$

but since the second term value > 1.0, 1.0 is substituted for 1.73 so that the contribution to I by dentation equals 1.62.

In the example at the bottom of table 4, the contribution of leaf dentation to the Affinity Index of tree T6 is + 1.62, a strong trembling affinity. This is expected since the mean dentation for T6 (xi = 32.75) is much larger than the mean for the Otisco Road reference population (xL, = 29.90) as shown in the drawing. The calculation of the contribution to the index is given below the drawing. The quotient of xi minus the midpoint value (19.40) divided by xL - 2s (27.14) minus the midpoint value is 1.73, but since this is larger than the limit (Condition 3), 1.0 is substituted in the calculations. It is given a "+" sign, so that the value contributes a trembling affinity, and then multiplied by D_c (1.62) to give a weighted contribution of + 1.62 to the Affinity Index for tree T6. The weighted contribution for all traits are summed, and the total value is multiplied by the correction term to give a standardized value that can range from + 2.0 to - 2.0. If the Index for tree T6 were based only on leaf dentation, it would have a value of + 2.0 (i.e. + 1.62 x 2/1.62 indicating that the tree was within the expected range of trembling aspen tree mean variation in the Otisco Road reference population.

The Affinity Indices of trees from unrelated populations and introgressants would likely fall between the limits of the index values for two reasons: (1) some traits for a given tree would show trembling affinity and others bigtooth affinity with signed contributions of "+" and "-", respectively; and (2) the mean values of some traits would not exceed the value two standard errors closest to the midpoint value from the mean of the population the trait more closely resembles so that the absolute value of the quotient would be less than 1.0. Either situation would cause the sum of the weighted contributions to be less than ED_e (the denominator of the correction term) so that the Affinity Index would fall between + 2.0 and - 2.0. Any positive index value, however, shows a greater resemblance to trembling aspen, and a negative value, to bigtooth aspen. An index value of "0" would be expected if all traits analyzed for the tree were exactly intermediate (i.e. equal to the midpoint value) or the sum of the weighted contributions for trembling affinity equaled those for bigtooth. An interspecific hybrid would not be expected to have an index of "0" as any degree of dominance or epistasis of genes contributed by one species would result in the trait showing an affinity to that species.

RESULTS

The results of the statistical analysis for the 45 traits in the P. tremuloides and P. grandidentata sample populations at Otisco Road are given in table 5. In addition to the population mean and standard deviation for each species, the results of a "t" test for a difference in the species' means and the value of the Character State Distance (D_o) for each trait are given. It should be noted that the difference in means is statistically significant (P s .05) for 35 of the 44 traits tested, indicating that each of these traits would be useful in distinguishing between these two populations. The last trait, frequency of branching, was not tested as the estimates of its means and standard deviations are based on regression analyses. The relatively small difference between the adjusted species means and the relatively large standard deviation of Y for fixed X, $S_{Y.X}$ for trembling aspen, however, indicate that this trait would not be a reliable index of difference between these populations.

Table 5.--Character means and standard deviations of trembling and bigtooth aspen populations at Otisco Road.

Results of "t" tests for difference between the species' means and the Character State Distance (D_c) for each trait are given. Values for measured traits are in cm, degrees, or exposure meter units. The remaining traits are coded units or ratios.

Character	D_c	Trembling		Bigtooth		"t" test
		$\bar{x}$	s	$\bar{x}$	s	
Mature Leaf						
Dentation, No. Teeth	1.62	29.90	6.16	8.89	1.92	**
Blade Length	.84	4.89	1.03	7.32	1.78	**
Blade Width	.37	4.76	.86	6.34	1.71	**
Blade L/W Ratio	.50	1.03	.09	1.17	.12	**
Apex Angle	- 1.89	106.5	15.87	99.2	11.36	**
Base Angle	- .42	159.6	20.44	145.2	14.44	**
Widest Point	- 2.63	2.3	.71	2.0	.68	**
Petiole Length	.61	4.11	1.11	6.08	1.63	**
Fall Color						
Earliness	.25	2.60	1.82	4.33	1.21	NS
Rapidity	.96	2.33	.58	3.67	.82	*
Young Leaf						
Thickness	- .24	18.00	1.55	17.26	.11	**
Amount Pubescence On						
Margin	1.70	3.88	.33	5.0	0	**
Petiole	1.74	2.36	.68	5.0	0	**
Upper Surface	1.68	1.43	.70	4.82	.39	**
Lower Surface	1.81	2.16	.55	5.0	0	**
Type Pubescence On						
Margin	- 4.19	3.33	2.06	3.0	0	NS
Petiole	- .68	2.22	2.07	3.0	0	**
Upper Surface	- 2.90	2.80	.98	3.0	0	NS
Lower Surface	1.18	5.01	1.66	3.0	0	**
Branch Terminal Buds						
Length	- 15.49	.56	.16	.58	.09	NS
Width	.85	.28	.06	.38	.06	**
L/W Ratio	.70	2.04	.41	1.51	.28	**
Widest Point	- 15.61	2.86	.35	2.90	.35	NS
Pubescence	1.87	1.00	0	3.85	.36	**

- Continued -

Table 5 (Concluded)

Character	D_c	Trembling		Bigtooth		"t" test
		$\bar{x}$	s	$\bar{x}$	s	
Branch Lateral Buds						
Length	- .37	.49	.21	.67	.22	**
Width	.40	.20	.10	.32	.09	**
L/W Ratio	- 4.77	2.49	.87	2.28	.58	NS
Widest Point	- .57	2.86	.35	2.62	.27	**
Pubescence	1.71	1.00	0	3.30	.67	**
Divergence	- 2.89	.31	.18	.38	.16	**
Floral						
Catkin Length	-117.47	5.97	1.02	5.82	.91	NS
Catkin, No. Flr./Side	- 1.91	20.72	4.14	22.45	2.63	**
Bract Length	- 1.42	.36	.08	.40	.06	**
Bract Width	.51	.17	.05	.23	.04	**
Bract No. Clefts	.93	4.62	1.35	7.35	1.59	**
Bract Cleft Depth	- .93	.16	.04	.14	.03	**
Flowers; No. Anthers	.74	9.19	1.16	11.40	1.62	**
Flowers; Anther/Stigma Color	- 1.00	25.4	2.04	26.65	1.72	*
Twigs						
Length Growth '60, '64 & '68	- .23	16.41	18.30	6.22	4.38	**
Middle Diam. '60, '64 & '68	- 3.32	.89	.85	.66	.35	*
Middle Diam. '68 only	- 2.08	.30	.10	.34	.06	NS
L/D '68	.24	37.51	32.69	15.23	6.49	**
Internode Length	- .16	1.06	.72	.60	.27	**
Branching 1962-67 Growth						
Survival	.33	.08	.04	.03	.04	NS
Frequency[1]	- .20	1.76	1.51	1.01	.14	--

[1] Based upon $\hat{y}$ and $s_{y \cdot x}$, i.e. the estimated mean no. of branches (Y) adjusted for year of growth (X) in regression analysis.

** Statistically significant at the 1% level of probability.

* Statistically significant at the 5% level of probability.

NS Statistically non-significant

Character State Distance

The reliability of a trait in discriminating between two species should be not only a function of the difference between the means, but also the overlap in variation as this commonly occurs among closely related species and even genera (SokaJ. and Sneath, 1963). This is particularly important if one wishes to construct a quantitative scale for rating each trait for its discriminatory value. The Character State Distance (D_c) is such a measure, based upon the populations' means and their standard deviations as a measure of variation.

The Character State Distance values for the 45 traits vary from -117.47 for catkin length to +1.87 for pubescence on branch terminal buds. Twenty-two traits have a D_c value greater than 0 and are considered of value in discriminating between the Otisco Road populations. Of these, eight exhibit little or no overlap and have D_c values greater than +1.0. These eight traits, leaf dentation and seven pubescence traits of young leaf parts and buds, are rated "Prime Value." The remaining fourteen, with D_c greater than 0 but equal to or less than + 1.0, are rated as "Some Value." Each, however, exhibits considerable overlap in variation in the sample populations. Twenty-three of the 45 traits have D_e values equal to or less than 0, and are rated "No Value." Most are extremely variable in both species and exhibit a large overlap in variation. A few, however, have a small variation, but the species means are so similar that the overlap is substantial.

Two kinds of comparisons were made by Harrison (1971) and summarized here which show that the patterns of similarity or difference for the 45 traits in the sample populations of the two species at Otisco Road also occur in unrelated populations. Five trembling and six bigtooth aspen trees from three natural stands and two trembling from a fourth location (see Table 1) were used for these comparisons. The means of unrelated trees for a given trait are within one standard deviation of their species mean at Otisco Road in 76.3 percent of the comparisons and more extreme in the direction opposite to that of the other species mean, an additional 9.2 percent of cases. The second type of comparison is whether the unrelated tree mean is closer to its species mean than to the other species mean at Otisco Road. In 74.5 percent of the cases, this holds. Both criteria are common to 72.4 percent of the comparisons. If traits rated as "No Value" are eliminated from these comparisons, the percentages are 86.1, 81.1, and 75.3, respectively.

The Otisco Road populations, however, do not appear to be representative of the expression and/or variation of a small number of traits of these two species. The two fall color traits, rated as "Some Value" for distinguishing the species at Otisco Road, are unreliable at Marcellus and Camden. Almost all trees of each species at these locations are more similar to trees of the other species at Otisco Road than to the same species for one or both traits. Six traits rated as "No Value" at Otisco Road, in contrast, appear reliable elsewhere. These include mature leaf widest point, apex, and base angles; lateral bud divergence; depth of floral bract cleft; and young leaf thickness. In general, however, the patterns do not vary much between the different unrelated populations and are similar to those exhibited by the Otisco populations.

Twenty of the twenty-two traits with positive D values in the Otisco Road sample populations, therefore, appear to be representative of differences between these aspen species in central New York State. These traits have been deemed useful for classifying an unknown tree by numerical methods to determine its phenetic relationship to these two species. In addition, since the numerical value of the Character State Distance of a trait is a measure of its reliability in discriminating between the two species, it appears justifiable to use the D_c value to weight the contribution of each trait to a numerical index that shows the similarity of the unknown tree to the reference populations. The Character State Distance values calculated for the trembling and bigtooth populations at Otisco Road are used as the weighting factors in the Affinity Index.

Affinity Index

The Affinity Indices of the Otisco Road putative hybrids, all trees from the unrelated natural stands, and the known F_1 interspecific hybrids are given in table 6.

Table 6.--Affinity indices of Otisco hybrids and trees from other populations.

Tree origin	Trembling			Bigtooth			Hybrids		
	Tree	No. traits	I	Tree	No. traits	I	Tree	No. traits	I
Otisco							H1	19	1.32
							H2	19	.44
Marcellus	T6	15	1.44	GX	12	- .74	H3	18	.19
	T7	12	1.44	G7	12	-1.37	H4	10	.93
				G8	19	-1.04			
Camden	T8	12	1.60	G9	12	- .95	HQ	10	1.64
	T9	19	1.72	G10	19	-1.22			
Ontario	OT	5	1.44	OG	5	-1.83	OH	5	-.37
Heiberg F. & F_1 hybrids	CR	12	1.79				12-3	10	.38
	CS	12	1.85				12-10	10	1.43
							12-11s	10	1.08
							12-11l	10	.89
Mean I			1.61			-1.19	(Excluding HQ)		.70

The index values of the seven unrelated trembling aspen range from +1.44 to +1.85, with a mean of +1.61. The two clones at Heiberg Forest (CR and CS) show the closest likeness to the Otisco Road population, based upon 12 mature leaf, bud, twig, and branching traits. The range in values for the bigtooth aspen is from -.74 to -1.83, with a mean of -1.19. The Ontario tree (OG) index, which shows the greatest affinity to the P. grandidentata reference population and is well separated from the rest of the values, is based only on five leaf traits. The average index of the five trees from stands near Syracuse is -1.06. It is obvious that members of these two species from unrelated stands differ somewhat from their respective species at Otisco Road. This method, however, does separate members of the two species into two widely spaced groups. These results clearly support the conclusion that the Affinity Index herein proposed serves as a reliable tool in classifying an "unknown" tree by numerical methods based upon the phenetic affinity to the reference populations.

It should be noted that the Affinity Indices for the known F_1 hybrids and the putative natural hybrids, except for tree HQ, lie between the values obtained for the seven trembling and six bigtooth aspen. In addition, the ranges for the known hybrids and the putative hybrids from central New York (excluding HQ) are approximately the same, +.38 to + 1.43 and +.19 to +1.32, respectively. It would appear that the trees from natural stands are introgressants of these species. All hybrid trees except for the Ontario hybrid (OH) exhibit a strong tremuloides affinity, with the upper limit of their range of index values just below the smallest value (+1.44) obtained for the trembling aspen. This suggests that the trembling aspen parent from central New York populations is partially dominant or epistatic for many of these traits or makes a greater contribution to the additive component of the phenotypic variance of quantitative traits.

In the Ontario hybrid, however, the bigtooth contribution is greater for the five leaf traits studied. The exceptional putative hybrid, HQ, with an Index of +1.63, appears to be P. tremuloides, although it may have had a hybrid ancestor. In the field it was considered a questionable hybrid because of the predominance of trembling traits, with only a few bigtooth-like leaf traits.

DISCUSSION

This study was undertaken to provide information on taxonomic differences in trembling and bigtooth aspen and introgressants of these species, preliminary to more extensive field studies. Two indices of taxonomic relationships are proposed, the Character State Distance (D_0) and the Affinity Index (I). The results show that the Character State Distance provides a valid estimate of the reliability of traits for distinguishing between the two aspen species and the Affinity Index, a numerical measure of the overall phenetic or phenotypic similarity of an "unknown" tree to these species in central New York State. Since both indices are based upon measurements of natural variation within and among trees in reference populations of the two species, they are dependent upon the reliability of the estimates of the true tree and population parameters. Therefore, sample size and sampling procedures are of paramount importance. Limitations in sampling, however, are generally necessary in numerical taxonomy because of the inordinate amount of time required to measure and analyze large numbers of traits in populations. In this study, since the main objectives were to identify and rate large numbers of traits according to their usefulness in distinguishing the two species and to characterize the within-tree variation so that efficient sampling procedures could be planned, multiple samples were taken for each tree and a limitation was imposed on the number of trees comprising the reference populations of each species.

The size of each of the sample populations at Otisco Road serving as the reference populations is very small, five trembling and six bigtooth aspen, so that the estimates of the true population parameters for many traits may not be very reliable. Comparisons of species differences in unrelated populations, however, indicate that the patterns of variation are similar to those in the Otisco Road groups for about 75 to 85 percent of the traits, depending upon the method of comparison. This suggests that the estimates are reliable. If this is the case, the affinity Indices of unrelated trees should cluster by species into two distinct groups, well separated in numerical value and sign. This was obtained. The indices of the six trembling aspen from four central New York stands range from +1.44 to +1.85, and the five bigtooth aspen from three of the same stands, from -.74 to - 1.37. It would appear, therefore, that the estimates of the true population parameters are reasonably good for most traits and that the variation in the relatively small and isolated populations at Otisco Road is representative of the variability and the taxonomic differences in the central New York populations of the two species.

A statistical analysis of the phenotypic variation in each of the reference populations by Harrison (1971) showed trembling to be considerably more variable than bigtooth aspen. Most of the variation in trembling aspen was "Between trees," with 22 of the 41 traits tested, statistically significant ($P \leq .05$). This compares with only 14 that were significant of the 39 traits which could be tested for "Within-tree" variation (i.e. "Between exposures" or "Between years of growth"). Bigtooth aspen, in contrast, showed little "Between-tree" variation (10 of the 41 traits were significant) and about the same "Within-tree" variation as trembling (16 of the 39 traits were significant). These results indicate that reference populations should include a larger number of trees for reliable estimates of the parameters of the populations. This would be especially important for more extensive populations in which both environmental and genetic variation would be greater than in the small, relatively isolated stand at Otisco Road.

Sampling procedures which minimize within-tree variation would not only increase the efficiency of the experiment by reducing the number of samples required for reliable estimates of the tree mean and variance but also increase the Character State Distance values since the phenotypic variance of one or both of the reference populations would decrease. A decrease in variance equivalent to 25 percent for each reference population, for example, would cause an increase in D_c of 0.33 if the population means did not change and their difference wasn't so small as to be limiting. Measures of the source and amount of variability by the "Within-tree" source of variation in the Analysis of Variance and the Coefficient of Variability indicate that the D_c values for 15 of the 22 traits rated as "Some Value" or as "Prime Value" could be increased by stratification in sampling. The ratings of four traits considered of "No Value," with Dc values between -.37 and -.16, would probably be changed to "Some Value" ($E > 0$), and an additional three traits might be affected sufficiently to change their ratings if a marked reduction in variance were obtained. The close similarity of the means of nine traits rated as "No Value," however, would probably prevent even a substantial reduction in variance from changing their ratings.

The amount of overlap in variation for traits rated as being of value ($D_c > 0$) in discriminating between the two species is large for the smaller

D_c values. This was permitted in order to minimize the number of traits rejected as being of "No Value" on the basis of the estimates of the variation in the small populations at Otisco Road. It was felt that the variation might be less than that characteristic of central New York populations because the rather uniform site and exposure would result in a smaller environmental contribution to the phenotypic variances and the size and isolation could cause a smaller genetic contribution due to inbreeding and/or genetic drift. To partially compensate for this, the standard deviation based upon the total phenotypic variance is used as the measure of variation in the reference populations for the Character State Distance equation. The "Between-tree" component of the variation, however, is the meaningful portion because the tree is the taxonomic unit and populations of trees are being compared for differences.

The use of an experimental design and sampling procedures for increasing the reliability and the numerical value of the Character State Distance of each trait is of primary importance for future studies. The reliability of the mean and components of variance as estimates of the true parameters of the populations would be improved by larger sample size. The contribution to D_c by the difference between the means, therefore, would be more reliable. Stratification in sampling would decrease the total phenotypic variance by minimizing the "Within-tree" and the "Error" variances. The net effect would be to increase the D_c values. The reliability would also be improved because the "Between-tree" variation would constitute a greater part of the total variance so that the standard deviation would be a better estimate of the overlap in variation of the two populations. Perhaps, the most important effect would be the increased sensitivity of the Affinity Index as a measure of species affinity. A greater proportion of the genetic differences between the species would contribute to the index since a larger number of traits would be rated as valuable for distinguishing the species. The contribution of each trait to the index would also be increased since it is weighted by the Character State Distance.

A much greater improvement of the Character State Distance would be accomplished by substituting the standard error of the mean for the standard deviation in the equation. This would result in marked increases in the D_l values as the variation in tree means is much smaller than for the unit of measure, so that the overlap in variation would be considerably reduced. Many traits rated as "No Value" would become valuable for distinguishing between the two populations. The effect on the Affinity Index would also be substantial. The numerical values for trees of each of the two species would probably approach the limits of the index, i.e. +2.0 for trembling and -2.0 for bigtooth aspen. This modification of the equation was not done in this study because of the small number of trees in each of the reference populations.

The Affinity Index method successfully separates the known interspecific hybrids and the putative natural hybrids from the two parental species except for one questionable hybrid, HQ. The range in value of the indices is rather large, with the extremes represented by the Ontario hybrid (I_{OH} = -.37) and the questionable hybrid (I_{HQ} = +1.64). The range of the hybrids with central New York parentage (excluding HQ) is +.19 to +1.43, with largest value bordering the lower limit of the trembling range (+1.44). The greater affinity to the trembling than to the bigtooth parent is shown by the positive sign and is obvious in comparisons of the tree mean for each trait in each hybrid with the reference populations' means. Fifty to 90 percent of the traits exhibit a trembling affinity. The Ontario hybrid (OH), in contrast, shows bigtooth affinity for three of the five traits studied, one intermediate, and only one with trembling affinity and may be due to a backcross to the bigtooth parent. The questionable hybrid (HQ) is most likely trembling aspen. Nine of the ten traits studied show strong trembling affinity and only one, strong bigtooth affinity. This might represent a difference between the Otisco Road reference population and the unrelated population as dissimilarities were found in the comparisons of unrelated trees of each species to the Otisco Road populations. It could, however, be an introgressant with a bigtooth parent in its distant ancestry. Anderson (1962) points out that progeny from the second or third successive backcross to one of the parent species are extremely similar to the recurrent parent. Even after a single backcross, the plant might be considered a variant of the species if only casually observed.

The distribution of traits showing affinity to one or the other parent species does not appear random. All hybrid trees from central New York exhibit an affinity to the same species for nine traits. Trembling affinity is found for seven traits (four were measured in all eight trees and the other three in only two or three trees) and bigtooth affinity for two traits (based on only three trees). For the remaining nine traits studied in two or more trees, however, an affinity to each of the two species is shown by at least one tree, and for two of these traits, one or two trees show an intermediate expression. These results indicate that for seven traits, the trembling aspen parent and for two traits, the bigtooth parent contribute dominant or epistatic genes or a greater proportion of the additive genetic variance of quantitative traits to these hybrid progeny.

The study of larger numbers of introgressants, especially as groups comprising a hybrid swarm, will be required to resolve many of the questions raised by this study. Improvement of the sensitivity of the Affinity Index by methods previously discussed is also necessary for the separation of introgressants into groups representing different degrees of relationship to the parent species, i.e. F_1 hybrids, F_2 and backcross progeny.

SUMMARY

The phenotypic variation of forty-five traits was studied intensively in a small, relatively isolated stand containing both trembling and bigtooth aspen. An index of taxonomic difference called the Character State Distance was devised to provide a measure of the reliability of a trait in distinguishing between the two species. The numerical value of this statistic depends upon the difference in the means and the amount of overlap in the variation of the trees of each species serving as reference populations. Twenty-two traits are considered reliable for distinguishing between the two reference populations. Two, however, were found to be unreliable for unrelated populations in central New York State, hence were not used.

A numerical measure of the overall phenotypic or phenetic similarity of an unknown tree to the reference populations is termed the Affinity Index. The contribution of each trait to the index is weighted by the Character State Distance, i.e. by the reliability of the trait in distinguishing between

the species. The index values for six trembling and seven bigtooth aspen trees from unrelated populations cluster by species into two well defined groups, confirming the validity of this quantitative method for taxonomic classification. The Affinity Indices of six putative hybrids from natural stands and four interspecific hybrids produced by controlled crosses fall between the values obtained for the unrelated trees of the two species except for one questionable hybrid with an index in the range of trembling aspen. Eight of the remaining nine trees show marked affinity for trembling aspen, and the ninth, a putative hybrid from Ontario, shows a bigtooth affinity. Methods for improving the reliability of the Character State Distance and the sensitivity of the Affinity Index are discussed.

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