Anerkennung

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Discriminant Analysis of Interspecific Hybridization in Betula

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1. Abstract

Three species of birch, *Betula alleghaniensis* Britton, *Betula papyrifera* Marsh., and *Betula populifolia* Marsh., and their hybrids, were classified with the use of leaf, seed, and bract variables in a discriminant analysis. Intraspecific crosses were classified correctly using leaf variables (ratio of petiole length to blade length and ratio of blade length to blade width), and hybrids were placed close to the mean of the female parent. Seed and bract measures (seed width, seed length, length of strobilus, and ratio of midbract lobe length to side lobe length) correctly classified intraspecific crosses and placed hybrids between the means of the intraspecific crosses. The application of our equations to data from other studies indicated that intraspecific crosses and hybrids from those studies will be classified correctly with our discriminant equations.

Key words: Discriminant analysis, hybridization, *Betula*.

Zusammenfassung

Es erfolgte eine Klassifizierung der drei Birkenarten, *Betula alleghaniensis* Britton, *Betula papyrifera* Marsh., und *Betula populifolia* Marsh. und ihrer Hybriden an Hand von Blatt-, Samen- und Brakteen-Variablen mit Hilfe einer Diskriminanzanalyse. Intraspezifische Kreuzungen wurden mit Hilfe von Blattvariablen richtig klassifiziert (Verhältnis der Blattstiellänge zur Blattlänge und Verhältnis der Blattlänge zur Blattbreite). Die Hybriden lagen näher an den Mittelwerten der weiblichen Eltern. Die Messung von Samen und Deckblättern (Samenbreite, Samenlänge, Strobililänge und das Verhältnis der mittleren Länge der Deckblattlappen zur seitlichen Lappenlänge) ergab eine richtige Klassifizierung der Kreuzungen zwischen reinen Arten und stellte die Hybriden zwischen deren Mittelwerte. Die Anwendung unserer Diskriminanzgleichungen an Daten anderer Untersuchungen zeigten, daß intraspezifische Kreuzungen und ihre Hybriden richtig klassifiziert werden konnten.

2. Introduction

Interspecific hybridization in the genus *Betula* is common in nature and has received a great deal of attention from geneticists and taxonomists over the last few decades. Several questions have been of particular interest, including the degree of interchange of genetic material between parent species, how these interspecific crosses affect the variability in parent populations, the evolutionary changes brought about by hybridization, and the genetic integrity of the (F_1) hybrids themselves.

Examples of natural hybridization among the North American birches have been reported, including *Betula alleghaniensis* Britton × *Betula papyrifera* Marsh. (Barnes *et al.* 1974, Clausen 1966), *B. alleghaniensis* × *Betula lenta* L. (Sharik and Barnes 1971), *B. papyrifera* × *Betula pumila* L. (Clausen 1962), and *B. alleghaniensis* × *B. pumila* (Dancik and Barnes 1972). Because of their sympatry and great amount of polymorphism, frequent crossings between *B. papyrifera* and *B. populifolia* have long been suspected

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(ALAM and GRANT 1972, DUGLE 1966). Other successful artificial crosses have been *Betula pubescens* EHRH. × *B. papyrifera* and *Betula verrucosa* EHRH. × *B. papyrifera* (JOHNSSON 1949), and a wide variety of intra- and interspecific crosses between a number of North American and European birch (CLAUSEN 1966).

Techniques for evaluating introgression and hybridization have been based primarily on descriptive statistics of morphological traits and on various graphical displays. These include hybrid indices (ANDERSON 1949, CLAUSEN 1962, DUGLE 1966, SHARIK and BARNES 1971), biometrical studies (ALAM and GRANT 1972, DANCIK and BARNES 1974, JENTYS-SZAFEROWA 1937, SHARIK and BARNES 1971), pictorial scatter diagrams (ANDERSON 1949, CLAUSEN 1962, DANCIK and BARNES 1974, DUGLE 1966), and polygonal graphs (CLAUSEN 1962, GUERRIERO *et al.* 1970). Others have studied chromosome number and morphology (ALAM and GRANT 1972, BRITTAIN and GRANT 1967, DUGLE 1966, JOHNSSON 1949) and chromatography of plant extracts (CLAUSEN 1963, DUGLE 1966, GRANT 1971).

Recent studies have evaluated hybridization by canonical correlation analysis (BARNES *et al.* 1974, DANCIK and BARNES 1974, NAMKOONG 1966) and principal component analysis (DANCIK and BARNES 1974, RICHENS and JEFFERS 1975, SHARIK and BARNES 1971). Discriminant analysis has been used for studies of introgression since interpopulational differences are maximized (DANCIK and BARNES 1974, LEDIG *et al.* 1969, MERGEN *et al.* 1966, NAMKOONG 1963, SHARIK and BARNES 1971, SMOUSE 1972).

The purpose of this study was to develop discriminant functions based on tree, leaf, and seed variables to identify individuals and place them into categories of intraspecific crosses or hybrid offspring of *B. alleghaniensis* (yellow birch), *B. papyrifera* (paper birch), and *B. populifolia* (gray birch).

3. Methods

Trees meeting the taxonomic description of *B. alleghaniensis*, *B. papyrifera*, and *B. populifolia* were selected at different locations in Connecticut and Massachusetts by the Northeastern Forest Experiment Station. In 1946, progeny from controlled self-pollinated, intraspecific, and interspecific crosses were planted in row plots at the Standing Stone Experimental Forest, Stone Valley, Pennsylvania.

In the summer of 1969, three trees per cross were selected at random for measurement of leaf, catkin, and seed characteristics. If less than three trees per cross were available, all trees were sampled. Together, these made a total of 54 trees representing 25 crosses *(Table 1)*. Seventeen of these were controlled-pollination hybrids of yellow × paper birch and gray × paper birch. Hybrids between gray birch and yellow birch were not represented since the species are thought to be incompatible (CLAUSEN 1966).

A total of 26 measurements and combinations of measurements were made on branches, leaves, seeds, and bracts. These measurements were used as variables in a series of stepwise discriminant analyses (KENDALL and STUART 1966). The most important variables were used to separate known species *(Table 2)*.

Table 1. — Number of trees sampled for self-pollinated, intraspecific, and interspecific crosses.

	Yellow birch (2n=84)			Paper birch (2n=84)			Gray birch (2n=28)		
Birch species	Self-pollinated	Intra-specific	Inter-specific	Self-pollinated	Intra-specific	Inter-specific	Self-pollinated	Intra-specific	Inter-specific
Yellow									
Crosses	4	3	--	--	--	3	--	--	--
Trees	8	6	--	--	--	4	--	--	--
Paper									
Crosses	--	--	3	1	3	--	--	--	1
Trees	--	--	7	3	8	--	--	--	1
Gray									
Crosses	--	--	--	--	--	2	3	2	--
Trees	--	--	--	--	--	5	7	5	--

Table 2. — Mean values of shade leaf. seed, and bract variables for intraspecific and hybrid crosses.

Source	Variable	*Betula alleghaniensis*	*B. alleghaniensis* X *B. papyrifera*	*B. papyrifera* X *B. alleghaniensis*	*Betula papyrifera*	*B. papyrifera* X *B. populifolia*	*B. populifolia* X *B. papyrifera*	*Betula populifolia*
Tree	A - number of nodes	14.43	16.00	16.86	18.27	31.00	22.80	59.00
	B - petiole length (mm)	9.36	9.00	18.43	21.82	23.00	23.60	21.92
	C - blade length (mm)	83.71	74.00	77.86	76.36	58.00	71.20	57.83
	D - blade width (1/4) (mm)	38.57	34.50	44.71	44.18	36.00	40.40	38.75
Leaf	E - blade width (1/2) (mm)	45.21	37.00	46.29	47.18	30.00	35.00	26.42
	F - blade width (3/4) (mm)	28.36	24.00	24.57	28.18	13.00	12.60	7.75
	G - vein number	12.36	11.50	9.29	8.81	7.00	8.60	7.50
	H - serration number/25.4 (mm)	13.14	13.50	13.43	12.36	14.00	13.80	14.92
	I - petiole-leaf angle	99.50	88.50	92.29	82.54	84.00	66.00	77.00
	J - strobilus length (mm)	24.07	22.91	37.16	43.72	52.32	42.20	22.72
	K - strobilus width (mm)	12.07	10.57	8.08	7.95	7.01	5.93	5.79
	L - seed length (mm)	3.51	3.11	2.13	1.93	1.71	1.73	1.58
Seed and Bract	M - seed width (mm)	1.60	1.61	1.30	1.30	1.01	1.14	.74
	N - mean seedwings width (mm)	.94	.71	.96	1.18	1,11	1.00	.91
	O - samara width (mm)	3.48	3.03	3.22	3.66	3.23	3.14	2.56
	P - mean stigma width (mm)	.73	.63	.67	.63	.45	.67	.45
	Q - total bract length (mm)	8.67	9.31	6.05	6.02	5.08	4.26	3.37
	R - middle lobe length (mm)	3.36	2.89	2.07	1.84	1.76	1.73	1.07
	S - mean side lobe length (mm)	4.78	4.32	2.38	2.13	2,11	1.86	1.82
	T - mean total side lobe length (mm)	7.97	8.46	4.87	4.72	3.54	3.26	2.87
	U - mean lobe angle	38.93	34.50	72.29	68.18	87.00	80.20	80.75
	B/C	.11	.12	.25	.29	.39	.33	.39
	C/E	1.85	2.01	1.67	1.63	1.93	2.05	2.20
Ratio	D/F	1.36	1.43	1.82	1.57	2.77	3.21	5.00
	E/F	1.59	1,54	1.88	1.67	2.31	2.78	3.41
	Q/S	.55	.46	.40	.36	.42	.44	.54

Since there are morphological differences between leaves from various parts of a tree's crown (Clausen and Kozlowski 1965, Jentys-Szaferowa 1937), branches of approximately the same age were selected from the midcrown of each tree. The number of branch nodes joining the central branch were recorded for each branch (A). Twenty leaves were randomly selected from four locations on the branch. Five sun leaves were taken from older shoots (spring leaves) and five from elongating shoots (summer leaves). Ten shade leaves were also selected--five spring and five summer leaves. Measurements made on each leaf included: petiole length (B); blade length (C); blade width at 1/4 (D), at 1/2 (E), and at 3/4 (F) of the length; number of veins per side of midvein (G); number of serrations per unit (H); and, the average degree of base angle between the petiole and blade (I) *(Figure 1)*. Five female strobili were chosen at random from each tree. The length (J) and width (K) of the green strobili were measured, and five bracts and five seeds were randomly selected from the middle of the catkin. Seed and bract measurements included length (L) and width (M) of the seed, average width of the seedwings (N), total samara width (O), average length of stigma (P), length of total bract (Q), length of middle lobe (R), average length of side lobes taken from middle of bract (S), average length of side lobes taken from bract base (T), and average angle between bract lobes (U) *(Figure 1)*.

4. Results and Discussion

The general problem in discrimination is to fit a function of the form:

$$Z = B_0 + B_1 X_1 + B_2 X_2 + B_3 X_3 + \ldots B_K X_K$$

where X_1, X_2, X_3 . . . X_K are the measured variables, and B_1, B_2 . . . B_K are the corresponding weights. The coefficients of the discriminant function arise from maximizing the ratio of the square of the difference between the means of the groups to the sum of squares within groups. After testing, nonsignificant variables are eliminated from the equation and only significant variables used for discrimination. To determine the variables to be used in the best discriminant function, a least squares analysis was conducted for most of the dependent variables (Harvey 1968). Individual analyses of variance were used to determine any differences among the three species, and between two crosses (self-pollinated and intraspecific crosses), trees within crosses, two branch positions (sun, shade), and two leaf categories (spring, summer). These preliminary results indicated large differences between species, branches, and leaves, requiring separation of the data into appropriate groups to determine the best discriminator. Differences between trees and self-pollinated and intraspecific crosses and their interactions were nonsignificant, so the data were combined and separate discriminate functions developed for each of the leaf-branch combinations with no interaction effect. Classification of an individual was determined by the Mahalanobis D^2 and from the posterior probability for each group.

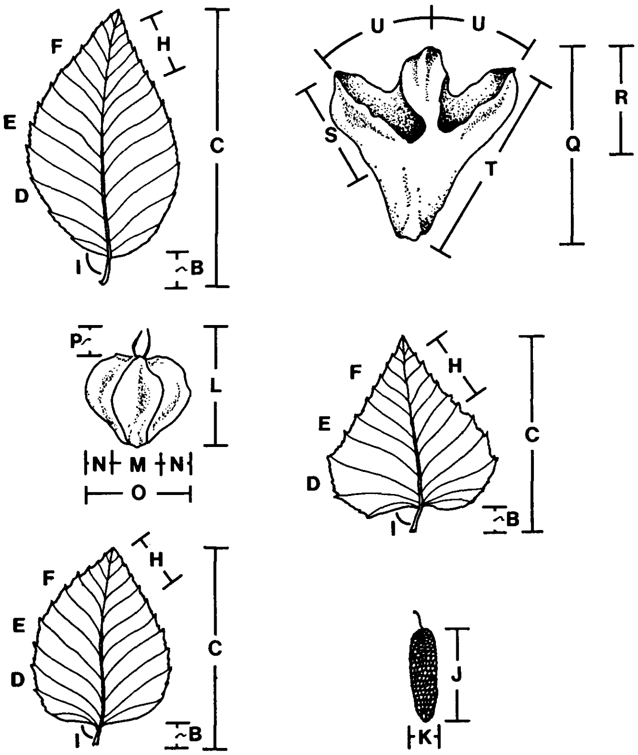

Figure 1. — Measurements made on leaves, seeds, and bracts.

4.1 — Leaf Variables

4.1.1 — Intraspecific crosses

To determine which group of leaves would provide the best basis for discriminating between species, a series of analyses were run on all variables for each of the leaf categories after separating the data *(Table 3)*. In general, spring leaves were better discriminators than summer leaves and shade leaves better than sun leaves. Summer leaves were the poorest discriminators because of the variability among leaves; the greater morphological consistency of spring leaves developing on short shoots is well known in birch (Clausen and Kozlowski 1965, Dancik and Barnes 1974, Jentys-Szaferowa 1937). Since there is little difference between spring/shade leaves and summer/shade leaves as a result of very small elongation of shade branches, all shade leaves were combined and used to develop discriminant functions *(Table 2)*. No significant improvement was obtained by further pooling the leaf data; subsequent analyses using leaf variables were restricted to the midcrown shade leaves.

The ratio of petiole length to blade length (B/C) was the most effective leaf variable for discriminating among all three intraspecific crosses, and particularly for separating yellow birch from the other two species *(Table 3)*[2]. Others have found that the ratio of petiole length to blade length is an important variable for discriminating between different species of birch (Barnes *et al.* 1974, Dancik and Barnes 1975, Jentys-Szaferowa 1937, Sharik and Barnes 1971). The addition of the ratio of blade length to blade width (C/E) to the model was significant, and led to correct classification of all three species. Variables that did not improve the leaf equation were blade width at 1/4 of the length divided by width at 3/4 of the length, blade width at 1/4 of the length, number of veins on one side of midvein, and number of serrations per unit.

Although all three species were classified correctly, some additional variables led to significant improvements in the discriminant functions *(Table 3)*. These provided an additional basis upon which to discriminate between paper birch and gray birch. For purposes of testing, each leaf sample was classified using a function built upon the other observations, but which excluded that sample from the function. This process is referred to as "jackknifing" (La-

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[2] Solomon, D. S.: Discrimination among three species of *Betula*. Unpubl. Rep. Northeast. For. Exp. Stn., Orono, Maine (1970).

Table 3. — Order of entry of significant variables and the percentage of trees classified by species for the different categories of leaves when samples are "jackknifed."

Variable	Yellow birch	Paper birch	Gray birch	Variable	Yellow birch	Paper birch	Gray birch	Variable	Yellow birch	Paper birch	Gray birch
	All Leaves				Sun Leaves				Shade Leaves		
B/C	100	90.9	91.7	B/C	100	72.7	91.7	B/C	100	90.9	83.3
D/F	100	100	91.7	D/E/F	100	100	91.7	C/E	100	100	100
G	100	100	91.7	D/F	100	100	91.7	D/F	100	100	100
B	100	100	91.7	*				I	100	100	100
C/E	100	100	91.7					B	100	100	100
H	100	100	91.7					D	100	100	100
D	100	100	91.7					G	100	100	100
I	100	100	100					H	100	100	100
E	100	100	100					*			
*											
	Spring Leaves				Spring-Sun Leaves				Spring-Shade Leaves		
B/C	100	90.9	100	B/C	100	81.8	91.7	B/C	100	90.9	100
D/E/F	100	100	91.7	D/F	100	90.9	100	D/E/F	100	100	100
I	100	100	100	C/E	100	90.9	91.7	B	100	100	100
D	100	100	100	I	100	90.9	100	C	100	100	91.7
C	100	100	100	G	100	100	100	E	100	100	91.7
B	100	100	100	*				*			
E	100	100	100								
*											
	Summer Leaves				Summer-Sun Leaves				Summer-Shade Leaves		
B/C	100	63.6	83.3	B/C	100	63.6	68.3	D/F	85.7	60.0	91.7
D/F	100	90.9	83.3	D/E/F	100	90.9	91.7	G	85.7	100	100
E/F	100	100	91.7	D/F	100	90.9	91.7	C/E	100	100	91.7
D/E/F	100	100	91.7	*				B	100	100	100
G	100	100	91.7					I	100	100	100
D	100	100	91.7					*			
*											

* Other variables were not significant at the 0.05 level.

Table 4. — Equations of leaf, seed, and bract variables used to discriminate among the three species of birch.

		Z_1			Z_1		
Equation	Variable	Coefficient	Intercept	Canonical correlation	Coefficient	Intercept	Canonical correlation
1	Petiole/blade length ratio (B/C)	-26.924	8.872	0.96	-5.149	-7.936	0.71
	Blade length/width (C/E)	-1.082			4.870		
2	Seed length (L) (mm)	3.646	-4.303	.97	1.403	-9.184	.89
	Strobilus length (J) (mm)	-0.153			0.197		
3	Seed length (L) (mm)	2.945	-10.097	.96	-3.250	-2.332	.83
	Seed width (M) (mm)	2.423			8.257		
4	Seed length (L) (mm)	4.627	-16.497	.98	-0.774	-0.877	.94
	Total bract length ratio (Q/S)	18.713			-13.498		
	Seed width (M) (mm)	-3.109			7.585		
5	Seed length (L) (mm)	3.234	-7.336	.99	-1.786	3.646	.96
	Seed width (M) (mm)	-0.097			6.155		
	Strobilus length (J) (mm)	-0.120			0.073		
	Petiole/leaf length ratio (B/C)	-16.680			-10.876		
	Total bract length ratio (Q/S)	15.124			-12.926		

chenbruch and Mickey 1968). Frequently, all species were classified correctly after the addition of the second variable. However, one gray birch was consistently classified as a paper birch when jackknifed, and additional variables provided a broader discriminating base.

Of the list of leaf variables (*Table 2*), only the two most significant variables were used due to the high correlation between measures of leaf width, and because all three species when self-pollinated were classified correctly. The best equations for discriminating among the three species are given in *Table 4*.

The discriminant scores (Z-values) for each observation were plotted in *Figure 2*. Intraspecific crosses of yellow birch individuals were tightly clustered around their group mean, indicating low variability within this species on the disriminating variables. Intraspecific crosses of paper birch trees were somewhat more variable with gray birches highly variable. Separation of gray and paper birch was a persistent problem, which may indicate either the existence of some introgressed genes in these two species or a high degree of polymorphism in leaf shape and size in each (Brittain and Grant 1965).

4.1.2 — *Hybrids*

Yellow birch-paper birch hybrids were classified as being most like the female parent in a majority of cases. All individuals with a yellow birch female parent were determined to be yellow birch and four of seven individuals with a paper birch female parent were grouped with paper birch (*Figure 2*). However, the mean of the hybrids was between the means of the intraspecific crosses and closer to the female parent.

Mean values of the basic leaf variables (not combined ratios) for which hybrid values were intermediate between the two parent species indicated that five of seven means were more like the female parent *(Table 2)*. However, four variables (B, D, E, H) of the yellow × paper and two variables (D, F) of the paper × yellow hybrids had mean values more extreme (higher or lower) than the mean of their female parent. Only three of the hybrid variables (C, F, H) had means higher or lower than the mean of the male parent.

The single paper × gray birch hybrid was classified as a gray birch *(Figure 2)*. Of the five gray × paper birch hybrids, all were classified as gray birch, but were located between the means of the intraspecific crosses. Mean values for leaf characteristics of the hybrids were more like gray than paper when gray was the female parent *(Table 2)*.

4.2 — Seed and bract variables

4.2.1 — Intraspecific crosses

The classification of trees to a species group using leaf variables worked well for intraspecific crosses of yellow birch and of paper birch. However, one gray birch was misclassified when jacknifed. Therefore, a similar stepwise discriminant analysis of the data for seeds and bracts *(Table 2)* was used to determine if other variables could improve the classification of this species.

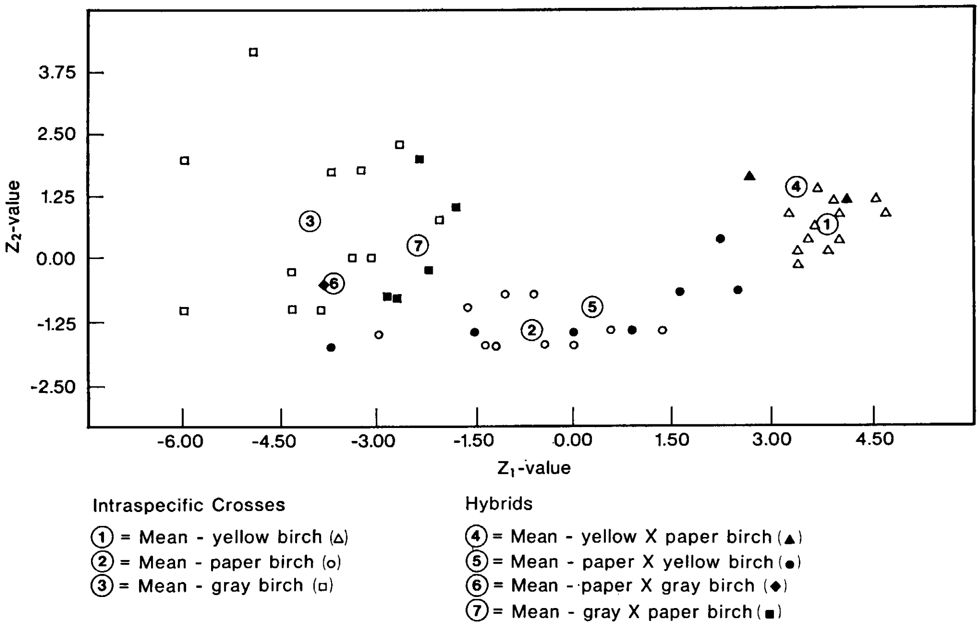

Figure 2. — Shade leaves of intraspecific crosses and hybrids of yellow, paper, and gray birch as classified by the discriminant function using variables (B/C) and (C/E) in equation 1.

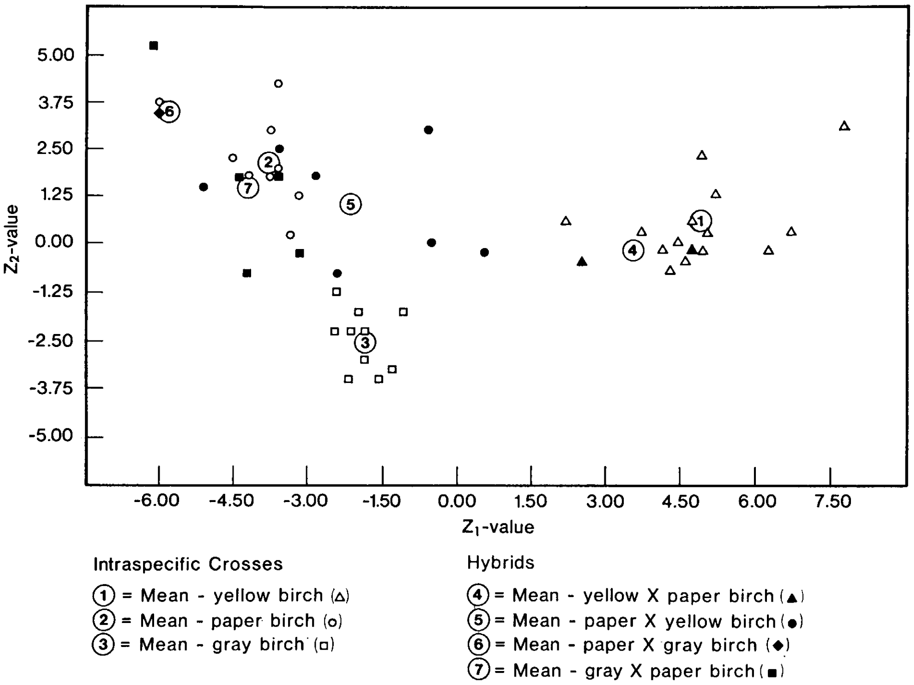

Figure 3. — Seeds and strobilus of intraspecific crosses and hybrids of yellow, paper, and gray birch as classified by the discriminant function using variables (L) and (J) in equation 2.

Table 5. — Significant seed and bract variables and percentage of trees correctly classified.

Variable	Yellow birch	Paper birch	Gray birch
All Variables Combined			
L - seed length (mm)	100	81.8	91.7
J - strobilus length (mm)	100	100	100
Q/S - bract length ratio	100	100	100
S - length of side lobe (mm)	100	100	100
Q - total bract length (mm)	100	100	100
N - wing width (mm)	100	100	100
K - strobilus width (mm)	100	100	100
M - seed width (mm)	100	100	100
Best Combinations of Variables			
L - seed length (mm)	100	81.8	91.7
J - strobilus length (mm)	100	100	100
L - seed length (mm)	100	81.8	91.7
M - seed width (mm)	100	100	100
L - seed length (mm)	100	81.8	91.7
Q/S - bract length ratio	100	90.9	100
M - seed width (mm)	100	100	100

Seed length (L) and length of the green strobilus (J) together were sufficient to classify all intraspecific crossed individuals correctly *(Table 5)*. Other significant measured variables were seed width (M), seedwing width (N), strobilus width (K), total bract length (Q), length of the bract side lobe (S), and bract lobe length ratio (Q/S). Dancik and Barnes (1975) also found many of these variables to be useful for discrimination between yellow birch and bog birch *(B. pumila* L.). Equations developed using the best discriminating seed and bract variables are given in *Table 4.*

In contrast to the results obtained with the leaf variables, gray birch showed the greatest within-group consistency when seed and bract variables were used in the function; yellow birch showed the least consistency *(Figure 3)*. However, all intraspecific crosses were classified correctly with this function.

4.2.2 — *Hybrids*

When the seed and bract variables (L, J) were used, the yellow birch × paper birch hybrids were classified as being more similar to yellow birch. Four of seven paper birch × yellow birch hybrids were classified as paper birch and the remaining three were classified as gray *(Figure 3)*. The maternal effect seems to be dominant for yellow birch-seed and bract. The classification of the hybrids becomes more distinct when different sets of variables are combined *(Table 4)*. When the discriminant function is based on seed width (M) and seed length (L), and equation 3 is applied, all hybrids are classified as paper birch *(Table 5)*. Removing strobilus length (J) causes the discriminant function to shift hybrid values from the overall mean closer to the mean value of paper birch.

The single paper birch × gray birch hybrid, and three of five gray × paper birch hybrids were classified as paper birch when seed length (L) and strobilus length (J) were used as discriminant variables. Other functions also classified most of these paper-gray hybrids as paper birch. This may be due to higher ploidy in paper birch (Alam and Grant 1972). Two gray birch × paper birch hybrids were classified as gray birch.

4.3 — *All variables combined*

Leaf, bract, and seed variables were combined to produce equation 5 *(Table 4)*. The best variables for discriminating between intraspecific crosses were seed length (L), strobilus length (J), ratio of petiole length to leaf length (B/C), ratio of side lobe length to total bract length (Q/S), and seed width (M). The correlation matrix for these variables is given in *Table 6*. The dimensions of the strobilus and the bract seem to be related, while the petiole-blade length ratio is highly correlated to the seed measurements.

The combination of leaf, seed, and bract variables to derive equation 5 leads to the results found previously when each set of variables was analyzed separately and all of the intraspecific crosses were classified correctly. Individuals of gray birch clustered very closely about their group mean, while paper and yellow birch had slightly more variation *(Figure 4)*.

All yellow × paper birch crosses were classified as yellow birch, while paper × yellow were classified as paper birch with one classified as gray birch. The gray × paper and paper × gray were classified as paper birch, apparently because strobilus length was included in the equation.

The length of strobilus seems to be controlled by the species with the higher ploidy number. The means for hybrids of paper and gray birch are close to the means for the intraspecific crosses of paper birch. The hybrid means of paper and yellow birch, which have the same chromosome number, are grouped closer to the mean of the female parent. Most hybrid means are located between the means of the intraspecific crosses and close to the mean of the female parent, though none fell close to gray birch.

4.4 — *Application*

As a measure of the reliability of our equations, existing studies and data (both published and unpublished) were used in conjunction with our functions to determine if the trees would be classified to the same species group as originally identified. Mean values of leaf petiole length (B), blade length (C), and blade width (E) were available for yellow birch (Dancik and Barnes 1972) and for yellow and paper birch and their hybrids (Barnes *et al. 1974)*.

Table 6. — Percentage of trees correctly classified and correlation coefficients between significant variables when leaf, bract, and seed measurements were combined.

Variable	Birch			Correlation coefficient			
	Yellow	Paper	Gray	Strobilus length (J)	Petiole-blade length ratio (B/C)	Lobe-total bract length ratio (Q/S)	Seed width (M)
Seed length (L) (mm)	100	81.8	91.7	-.593	-.951	.444	.886
Strobilus length (J) (mm)	100	100	100		.491	-.779	-.289
Petiole-blade length ratio (B/C)	100	100	100			-.194	-.974
Lobe-total bract length ratio (Q/S)	100	100	100				.000
Seed width (M) (mm)	100	100	100				

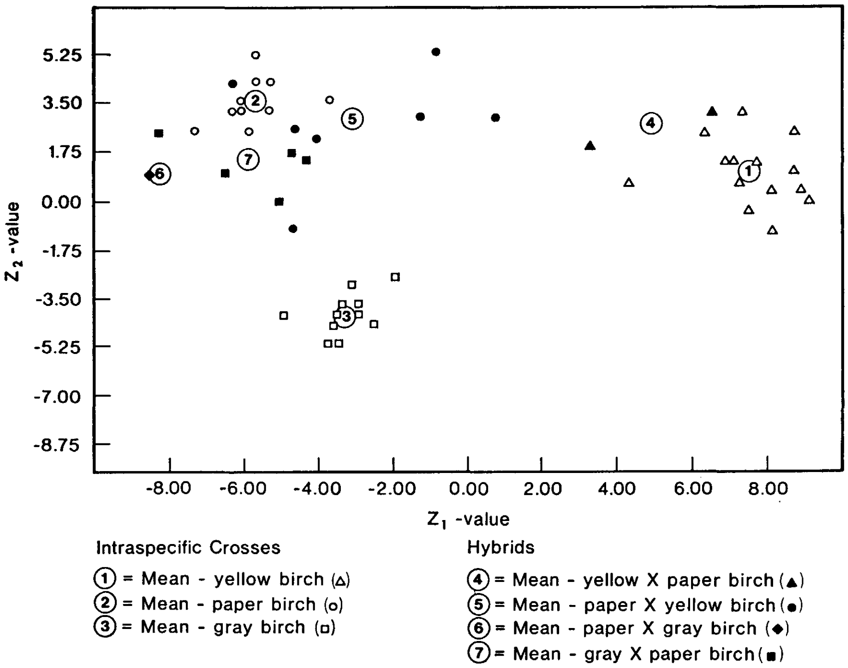

Figure 4. — Combinations of leaves, seeds, and bracts of intraspecific crosses and hybrids of yellow, paper, and gray birch as classified by the discriminant function using variables (J), (L), (M), (B/C), and (Q/S) in equation 5.

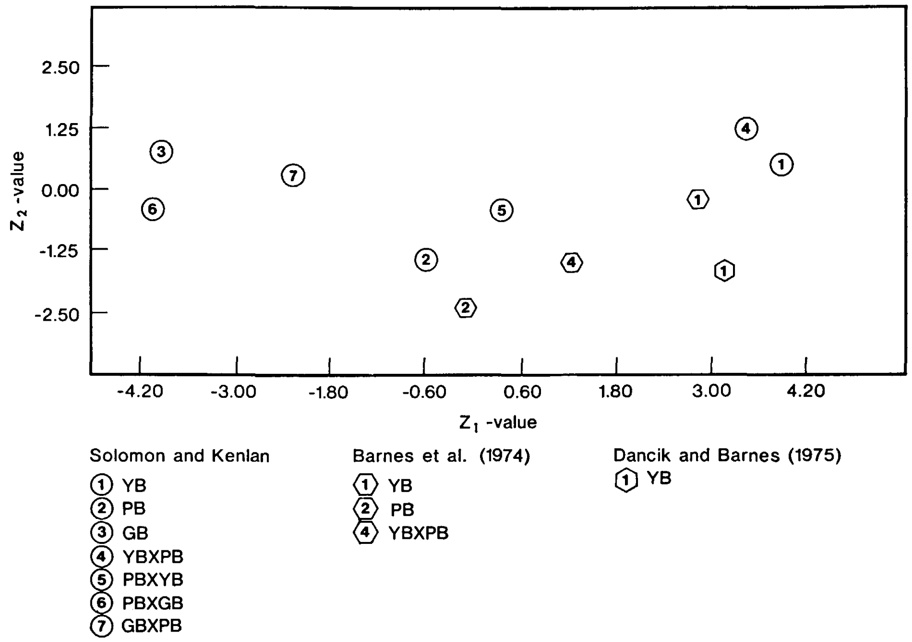

Figure 5. — Comparison of intraspecific crosses and hybrids of yellow, paper, and gray birch using leaf variables (B/C) and (C/E) in equation 1.

Most of these mean values were slightly higher than those found in our study *(Table 7)*. However, when classified using equation 1, the intraspecific crosses were assigned to their proper groups *(Figure 5)*. The yellow-paper birch hybrids from the Barnes *et al.* (1974) study are located between our paper × yellow birch and yellow × paper birch group means. This may be because the Barnes *et al.* (1974) collection represents a mixture of hybrid parentages with seed being collected from female parents of both species. The collective mean of our two yellow-paper birch hybrid types and the mean of the Barnes *et al.* trees *(Table 7)* support this conclusion.

Seed and bract equations (2 and 3) also were used to classify species based on data from other sources using our own data to generate the functions. Seed length (L) and strobilus length (J) values were available for paper birch from two studies (Barnes *et al.* 1974 and Guerriero *et al.* 1970), for yellow birch from two studies (Barnes *et al.* 1974 and Clausen 1968), for blue birch *(Betula caerulea* Blanch.) and mountain paper birch *(Betula cordifolia* Regel) from Guerriero *et al.* (1970), and for yellow-paper birch hybrids from Barnes *et al.* (1974). Equation 2 was highly successful in classifying the intraspecific species correctly, especially gray and yellow birch which were

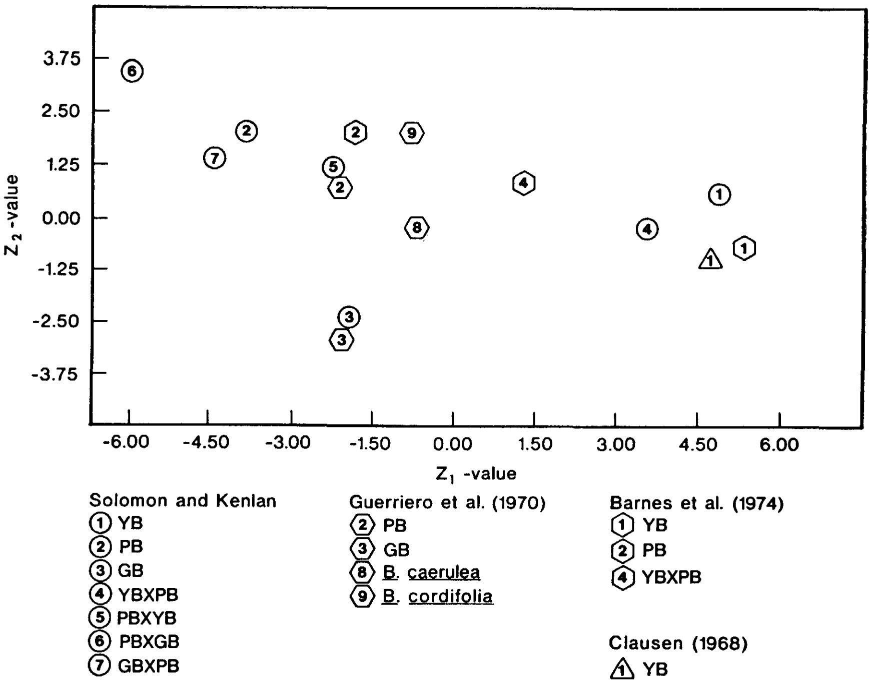

Figure 6. — Comparison of intraspecific crosses of yellow, paper, and gray birch with known and unknown intraspecific crosses and hybrids using variables (L) and (J) in equation 2.

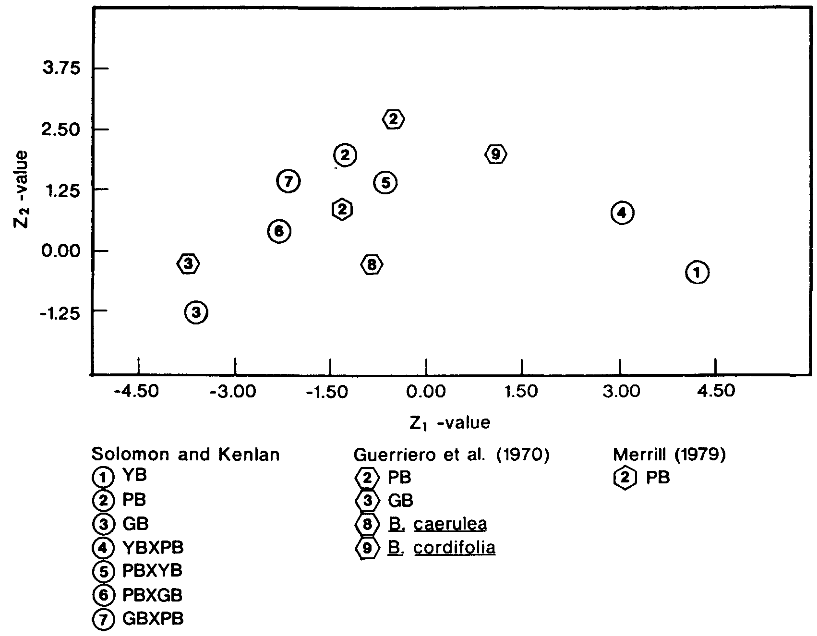

Figure 7. — Comparison of intraspecific crosses of yellow, paper, and gray birch with known and unknown intraspecific crosses and hybrids using variables (L) and (M) in equation 3.

tightly clustered around our group means *(Figure 6)*. The yellow-paper birch hybrid was intermediate between our two classes of these hybrids for reasons presented earlier. Mountain paper birch was classified as a paper birch but was more distant from our paper birch mean than the other test samples. Blue birch was classified as gray birch *(Figure 7)*.

Seed length (L) and seed width (M) variables were available for paper birch from Merrill[3] and Guerriero *et al.* (1970). Equation 3 correctly classified both intraspecific crosses from the Guerriero study *(Figure 7)*. In Merrill's study[3]) seed and bract samples of putative paper birch had been collected from throughout the species' range. Using his data from 129 stands, our function classified 91 percent as paper birch, 8 percent as gray birch, and 1 percent as yellow birch. The overall mean is close to our group mean for paper birch *(Figure 7)*.

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[3]) Merrill, R. E.: Phenotypic variation in white birch *(Betula papyrifera* Marsh.) bracts and fruits. Unpubl. Rep. Univ. Maine, Orono, Maine (1979).

Blue birch and mountain paper birch were classified as paper birch, though there was a 13 percent posterior probability that blue birch could have been classified as gray birch. This may reflect the uncertain geneology of the blue birches (GUERRIERO *et al.* 1970).

5. Conclusion

Intraspecific crosses of yellow, paper, and gray birch were classified successfully when leaf, seed, and bract variables were used. Shade leaves proved to be more reliable for discrimination than sun leaves. Although spring leaves were less variable and led to a better discrimination than summer leaves, midcrown shade leaves were sufficient to classify intraspecific crosses correctly.

Discrimination between intraspecific species was possible using either leaf variables (ratio of petiole length to blade length, and ratio of blade length to blade width) or seed and bract variables (seed length, seed width, strobilus length, and ratio of bract side lobe to midlobe length). Trees were classified correctly with two variables in all cases.

Seed and bract variables seem to be more useful for discrimination between species and classification of hybrids than are the leaf variables. Using leaf variables alone, *B. populifolia* × *B. papyrifera* and *B. papyrifera* × *B. populifolia* tend to be classified as *B. populifolia*. However, when seed and bract variables were used, the hybrids of *B. papyrifera* and *B. populifolia* were intermediate between the intraspecific crosses. Including strobilus length as a variable led to variation in classification of hybrids, because yellow and gray birch have similar strobilus lengths, and the strobilus of paper birch are, on average, longer. Classification using the best combination of leaf, seed, and bract variables named *B. papyrifera* and *B. populifolia* crosses as *B. papyrifera*.

Paper birch crosses with yellow birch females were classified as yellow birch. Most yellow birch crosses with paper birch females were classified as paper birch, though one was classified as a gray birch, possibly because of the similarities in strobilus length.

Comparison of data from this study with those of other studies indicates that the mean values of hybrids are located half way between the mean values of intraspecific

Table 7. — Mean values of data from other studies for variables used in leaf, seed, and bract and combined equations.

Variables	Merrill[a]	Dancik/ Barnes (1972)	Barnes *et al.* (1971)	Clausen (1968)	Guerriero *et al.* (1970)	Solomon/ Kenlan[b]
	- - - - - - -	- - - - - - -	- - - (mm) - - -	- - - - - - -	- - - - - - -	- - - - - - -
Petiole length						
papyrifera		--	25			21.8
alleghaniensis		13.7	17			9.4
alleghaniensis X *papyrifera*		--	22			9.0
Blade length						
papyrifera		--	90			76.4
alleghaniensis		87.7	109			83.7
alleghaniensis X *papyrifera*		--	99			74.0
Petiole/blade length ratio						
papyrifera		--	0.28			0.29
alleghaniensis		0.16[c]	.16			.11
alleghaniensis X *papyrifera*		--	.22			.12
Blade width						
papyrifera		--	63.0[d]			47.2
alleghaniensis		61.6	62.1			45.2
Blade width/length ratio						
papyrifera		--	0.70[e]			1.63
alleghaniensis		0.70[e]	0.57			1.85
alleghaniensis X *papyrifera*		--	0.64			2.01
Seed width						
papyrifera	1.2		2.9		1.41	1.30
alleghaniensis	--		3.8		--	1.60
populifolia	--		--		0.84	0.74
caerulea	--		--		1.13	--
cordifolia	--		--		1.52	--
alleghaniensis X *papyrifera*	--		2.8		--	1.61
Seed length						
papyrifera	2.0		2.3	--	2.05	1.93
alleghaniensis	--		3.4	3.2	--	3.51
populifolia	--		--	--	1.46	1.58
caerulea	--		--	--	2.20	--
cordifolia	--		--	--	2.54	--
alleghaniensis X *papyrifera*	--		2.8	--	--	3.10
Strobilus length						
papyrifera			39.9	--	35.8	43.7
alleghaniensis			19.0	18.1	--	24.1
populifolia			--	--	20.9	22.7
caerulea			--	--	29.5	--
cordifolia			--	--	38.2	--
alleghaniensis X *papyrifera*			31.0	--	--	22.9

[a] MERRILL, R. E.: Phenotypic variation in white birch (*Betula papyrifera* MARSH.) bracts and fruits. Unpubl. Rep. Univ. Maine, Orono, Maine (1979).

[b] Results of present study.

[c] Computed values for equation 1.

[d] Computed values using blade width/length ratio and blade length for equation 1.

[e] Reciprocal values were used for equation 1.

crosses. This is true when all natural crosses are combined. However, in cases where the female parents were known, the hybrids were classified closer to the mean of the female parent of intraspecific cross. The use of more than one equation with different leaf, seed, or bract variables may be necessary to classify some trees as particular hybrids and types of cross.

6. Literature Cited

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Corrections

RE: Erratum for the MS Khalil **30** (6): 179—181, Page, 179, Introduction:

From: "Consequently, the data of the four-year height growth of progenies of individual trees in the study *would also establish the groundwork from which* determine the correlation between caracters of cone morphology and seed weight with juvenile growth."

To: "Consequently, the data of the four-year height growth of progenies of individual trees in the study *reported earlier (Khalil 1974) were analyzed to* determine the correlation between caracters of cone morphology and seed weight with juvenile growth."

RE: Erratum for the MS El-Kassaby *et al.* **30** (6): 182—184, Page 182, Abstract:

From: "However, if such inbreeding levels *in progenies from* are common, some of the improvement in performance seen pollinated controls, may result from the break up of the family structure of the stands."

To: "However, if such inbreeding levels are common, some of the improvement in performance seen *in progenies from seed orchards over progenies from wind-pollinated* controls, may result from the break up of the family structure of the stands."

Buchbesprechungen

Experiments in Plant Tissue Culture. By John H. Dodds and Lorin W. Roberts. Cambridge University Press, Cambridge, 1982. pp. 178. £ 15.00 (Hardcover) / 5.95 (Paperback).

In recent years a number of books have appeared in the field of plant cell and tissue culture. Some of these books address to specialized problems of plant species, while others may be classified as general tissue culture information and technique books. Experiments in plant tissue culture belongs to the latter category. This book is divided into 14 chapters, and covers various aspects of plant tissue culture technology in a concise form. At the end of each chapter, there are three to five questions for discussion, an appendix (additional experiments), and a list of selected references (upto 1980). The first two chapters cover history of plant tissue culture and background information on sterilization and aseptic techniques. Chapter 3 provides information on the composition and preparation of different nutrient media. The remaining chapters in the book are devoted to laboratory experiments using particular in vitro procedures for such areas as: initiation and maintenance of callus, cell suspension, xylem differentiation, organogenesis, somatic embryogenesis, culture of shoot apex, isolation, culture, and fusion of plant protoplasts, anther culture for haploidy, production of secondary metabolites, and quantitation of tissue culture procedures. At the end of the book there is a list of "Commercial Sources of Supplies" (mostly British and American firms), for purchase of chemicals and equipment required in tissue culture work.

As pointed out by the authors, this book is mainly written for college undergraduates, although biology students in high school may also find many portions of the book not too difficult. I should add, however, that even those interested or working in the area of plant tissue culture may like to consult this book for basic *in vitro* techniques.

M. R. Ahuja