Identification of *Quercus* and *Celtis* Species Using Morphological and Electrophoretic Data

Gregory W. Buck and James E. Bidlack
Department of Biology, University of Central Oklahoma, Edmond, Oklahoma 73034-5209

Combining morphological and electrophoretic data may provide new information for identifying oaks (*Quercus* L. Fagaceae) and hackberries (*Celtis* L. Ulmaceae) in central Oklahoma. A total of 140 trees were sampled from the Arcadia Lake region in central Oklahoma, representing 20 each of four oak and three hackberry species. Approximately 58 morphological characters, within 15 categories, and 50 isozyme bands, representing phosphoglucomutase and malate dehydrogenase, were recorded in presence or absence format. Cluster analysis of the oak data revealed four, five, and five groups, whereas analysis of the hackberry data demonstrated four, four, and three groups for morphological, isozymic, and combined data sets, respectively. Oak clusters were grouped significantly on the basis of leaf characteristics, crown shape, and isozyme bands, whereas hackberry clusters were grouped significantly on the basis of leaf and twig characteristics, bark color, and isozyme bands. The best match between a priori field identification and a posteriori cluster identification was obtained from the morphological data set for both oaks and hackberries, although hackberry clusters were less representative of a priori identification than oak clusters. Clusters obtained from isozymic data showed poor representation of field identification for both genera. The combined data set for oaks produced four clusters of the a priori species and suggested a cluster containing *Quercus stellata* × *Q. macrocarpa* and *Q. stellata* × *Q. muehlenbergii* hybrids.

INTRODUCTION

Morphological similarities and differences cannot be directly translated into an analysis of the genotype of a species (*1*,*2*). In eastern Oklahoma, for instance, identification of oaks (*Quercus* L. Fagaceae) may be complicated by overlapping morphological traits that result from hybridization (*3*). It is plausible that similar hybridization events occur among oak species in the cross timbers of central Oklahoma, and the same may be true of hackberries (*Celtis* L. Ulmaceae).

Electrophoretic data can enhance species-level identification if it is incorporated with other types of data (*2*,*4*,*5*). Isozyme analysis has been used to study plant relationships (*6*,*7*,*8*) and to examine the possible hybridization and introgression of closely related species (*9*,*10*).

Previous studies of oaks have shown that isozymes are of little value for species-level identification, but are useful in demonstrating the relationships among subgenera (*10*, *11*, *12*). Evidence demonstrating the hybridizing nature of oaks (*3*,*13*,*14*) necessitates the incorporation of isozymic data with classical taxonomy to further explain the relationships among the genera. This may also be true in the case of hackberries that display hybridization characteristics more than those observed in oaks (*15*). Limited work has been done to show whether or not electrophoretic data can be used to identify hackberries. The use of zymograms or the presence of specific allozymes may enable scientists to distinguish between hackberries that have overlapping morphological traits (*16*) and may provide evidence of hackberry hybridization.

A small population of oaks and hackberries from central Oklahoma was studied to evaluate the use of morphological and isozymic data for species- level identification and to provide evidence, if any, of hybridization within genera. The objectives were: a) to determine whether or not isozymic data can be used independently or in combination with other types of data to improve the identification of oaks

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TABLE 1. *Quercus* and *Celtis* morphological categories and characteristics within categories used for classification and identification.

Category	Characteristics Within Category				
Crown shape	Round, Open	Round, Dense	Spreading, Open	Spreading, Dense	—
Bark texture	Thick, Ridges	Thick, Plates	Thin, Scaly	Thin, Warty	—
Bark color	Black	Dark brown	Light brown	Grey	Reddish-brown
Leaf shape	Ovate	Obovate	Lanceolate	Elliptical	—
Leaf base shape	Truncate	Obtuse	Acute	Asymmetrical	—
Abaxial trichome	Dendroid	Stellar	Fasciculate	Simple, Uniform	Simple, Scattered
Adaxial surface	Glabrous	Waxy	Rough	Pubescent	—
Twig color	Reddish-brown	Brown	Grey	Reddish-grey	Red
Twig shape	Stout	Moderately stout	Slender	—	—
Terminal bud	0–1	Clustered	—	—	—
Lobe No.	0	1–5	>5	—	—
Leaf length (cm)	0.0–3.0	3.1–7.5	7.6–15.0	>15.0	—
Leaf width (cm)	0.0–5.0	5.1–7.5	7.6–11.0	>11.0	—
Leaf sinus (cm)	0.0–2.5	2.6–4.0	>4.0	—	—
Petiole length (cm)	0.0–1.5	1.6–3.5	>3.5	—	—

and hackberries and b) to evaluate the use of these data sets in detecting oak and hackberry hybrids.

MATERIALS and METHODS

Leaves and twigs from a total of 140 individual trees were sampled from an area (T13N R02W) east of Interstate 35 and southwest of the Arcadia Lake Project in Edmond, Oklahoma. Twenty specimens of each species were collected from the first of May through the end of June 1992. The seven species sampled were post oak *(Quercus stellata* Wangenh.), blackjack oak *(Q. marilandica* Muench.), chinkapin oak *(Q. muehlenbergii* Engelm.), bur oak (*Q. macrocarpa* Michx.), sugarberry *(Celtis laevigata* Willd.), netleaf hackberry (*C. reticulata* Torr.), and northern hackberry *(C. occidentalis* L.). The samples were collected from all four sides of each tree. To allow the use of nominal, discrete, and continuous variables in the same study, the data were scaled into ranges or groups of values in a method of recording data similar to that used by Jensen (*17*) in an analysis of the scarlet oak complex *(Quercus* subg. *Erythrobalanus).* Data were recorded as the absence or presence of characteristics within categories (Table 1).

Collection of Morphological Data: The nominal variables were crown shape, bark texture, bark color, leaf shape, leaf base shape, abaxial trichome type, adaxial surface texture, twig color, and twig shape. Crown shape, bark texture, bark color, leaf shape, and leaf base shape were recorded as general descriptions based on those of Little (*16*). A hand-held lens was used to examine the appearance and type of abaxial oak trichomes (*18*). Those trichomes with arms radiating out from the epidermis and having no visible stalk or base were recorded as stellate. Those with radiating arms in all directions and a stalk were designated as dendroid. Leaf hairs in bundles were referred to as fasciculate. Leaf hairs that were raised off the surface of the leaf without branching were referred to as simple leaf hairs. Twig color and shape were based on the relative color and size of the twig for each individual (*16*).

The discrete variables were the number of lobes and terminal buds. The number of leaf lobes was recorded as none, one to five, or greater than five. A lobe was considered distinct if there was a vein running toward the tip of the lobe. A lobe was considered to be indistinct if no vein was observed. The terminal bud was recorded as a discrete value of none to one or as a cluster of buds.

To obtain values for continuous variables, five to 12 individual leaves from each specimen were measured, and an average was used to record the presence or absence of the characteristics within each category. Leaf blade length was measured from the bottom of the leaf blade, or the top of the petiole, to the furthest point of the leaf from the base of the petiole. Leaf width was measured from the furthest reaching point to the furthest point in a line perpendicular to the central axis of the leaf. The sinus depth was taken from the greatest depth of any one sinus measured in a perpendicular line from a line connecting the tips of two adjacent lobes. The petiole length was measured on the adaxial side from the point of connection with the stem to the base of the leaf blade.

Electrophoretic Procedures and Interpretation: All chemicals were purchased from SIGMA Chemical Company, St. Louis, Missouri. Within 2 to 3 h of collection, three to five leaves from each specimen were ground in 25 mL of buffer solution containing 0.4 M sucrose, 50 mM N-tris(hydroxymethyl)-methyl-2-aminoethanestulfonic acid (TES), 5 mM ethylenediaminetetraacetic acid (EDTA), 4 mM L-cysteine, and 2% polyvinylpyrrolidone (PVP). Buffered extracts were centrifuged at 1,250 *g* and 10 °C for 10 min to remove any solids and the supernatant was stored at –60 °C until it was analyzed. A similar extract was prepared from sycamore (*Platanus occidentalis* L.) and used as a standard for all electrophoretic analyses.

Electrophoresis was performed on Titan III cellulose acetate gels (Helena Laboratories, Beaumont, Texas), using procedures adapted from Soltis et al. (*19*) and Rennie et al. (*20*). Cellulose acetate gels were used because they are faster, easier, and usually provide a resolution of isozymes that is at least equal to that of starch gels (*21*). The gels were pre-

soaked with the appropriate electrode buffer at least 20 min prior to electrophoresis. Extracts of eight specimens were loaded at 2.5 cm from the base of each prepared gel using a Super Z applicator kit (Helena Laboratories). The gel was run at 200 V for 15 min at room temperature in a Zip Zone chamber (Helena Laboratories) with paper wicks to allow buffer flow to the gels.

The isozymes resolved were phosphoglucomutase (PGM) and malate dehydrogenase (MDH). The electrophoresis of PGM used an electrode buffer of 65 mM L-histidine and 20 mM citric acid with a pH of 8.5; MDH used the same buffer system with a pH of 9.1. The PGM staining solution contained 25 mL of electrode buffer, 1 mL of 1 M $MgCl_2$, 1 mL of 15 mM NAD, 1 mL of 2.5 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 0.3 mL of 3.3 mM phenazine methosulfate (PMS), 1 mL of 75 mM glucose-1-phosphate, and 1.5 mL of glucose-6-phosphate dehydrogenase (G-6-PDH) containing 50 units per mL. The MDH staining solution contained 25 mL of electrode buffer, 1 mL of 1 M $MgCl_2$, 1 mL of 15 mM NAD, 1 mL of 2.5 mM MTT, 0.3 mL of 3.3 mM PMS, and 1 mL of 0.75 M malic acid. A mixture containing 2 mL of the appropriate staining solution and 2 mL of 1.5% heated agar was prepared after the electrophoresis was complete. This mixture was poured, within 1 min of preparation, over the gel to develop zymograms.

After staining for 20 min at 30°C, the gels were rinsed in cold water to remove the agar and any remaining stain reagents. The bands were marked on the back of the gels with a permanent marker and then placed in a drier for 20 min. The marked bands were measured and recorded for analysis. The relative mobility (Rm) was calculated on each gel as the distance of sample band migration divided by the distance of the standard sycamore band migration. The standard error of the mean was calculated for Rm from multiple analyses of the sycamore standard and was used to determine whether or not the sample Rm values were significantly different from each other. Starting at zero mobility, if the Rm of a specific band exceeded the standard error of the mean, it was determined to be in the next band position.

Each allozyme was recorded as present or absent for each sample specimen according to the Rm value of all possible bands. Approximately 34 possible band positions, indicated by significantly different Rm values, for PGM (PGM1-PGM34) and 16 possible band positions for MDH (MDH1-MDH16) were evaluated for each sample. To create the data set for analysis, band positions were recorded as individual bands, but not as different loci with accompanying alleles.

Statistical Analyses: Separate data analyses were conducted for oaks and hackberries using Ward's agglomerative hierarchal cluster analysis in SAS PROC CLUSTER, and variables were evaluated using stepwise discriminant analysis in PROC SAS STEPDISC (*22*). All data within their specified data sets were used for analysis except for a priori field identification. To determine the number of clusters obtained from each data set, the variables used to obtain those clusters, and the agreement between a priori and a posteriori identification of individual species, the morphological, isozymic, and combined data sets were evaluated for each of the two genera.

RESULTS and DISCUSSION

Statistical analyses, performed separately for *Quercus* and *Celtis,* revealed unique clustering, different variables used for clustering, and distinct matching between a priori and a posteriori species identification. The results from the two genera are thus presented in separate sections.

***Quercus* Clustering, Discriminant Analysis, and Identification:** While there has never been a definitive method of determining the optimum number of clusters from any given data analysis, the SAS program does give an objective numerical estimation called the cubic clustering criterion (CCC), which evaluates the level of clustering (*22*). Morphological data provided a strong, reliable grouping at four different clusters (Fig. 1, top left panel). This detection of four clusters was

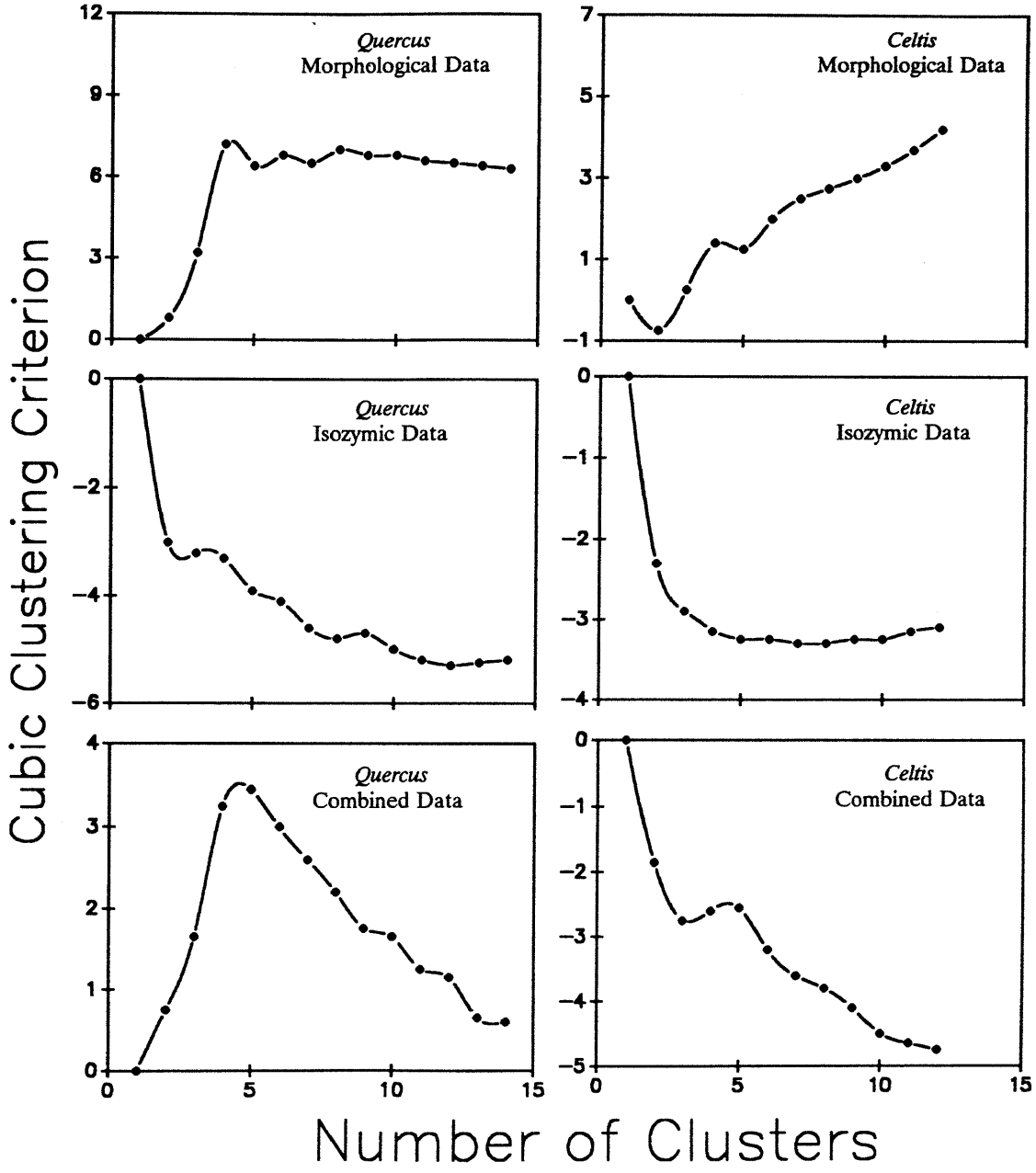

Figure 1. Cubic clustering criterion as determined by Ward's agglomerative hierarchal cluster analysis of data collected from species sampled at the Arcadia Lake region near Edmond, Oklahoma.

acceptable because the a priori identification within *Quercus* included 20 specimens for each of four species. The peak CCC value for the morphological data was about 7.5, which was well above the minimum value of 2-3 for accurate clusters (*22*). Isozymic data, on the other hand, had a slight inverse peak at five clusters, with a CCC value of *minus*4.0 (Fig. 1, middle left panel). When the CCC value is negative and decreasing, the distribution is probably unimodal (*22*). In the case of the isozymic data, Ward's cluster analysis made groups primarily on the basis of one or two band positions. Combining isozymic data with morphological data caused the CCC graph to demonstrate a sharp peak at five clusters, with a CCC value of 3.5 (Fig. 1, bottom left panel). The sharp peak demonstrated by the analysis of the combined data set indicated that including morphological data with the isozymic data was more reliable in forming the five clusters compared to the isozymic data alone. The five clusters revealed by the isozymic and combined data sets suggested an additional cluster of hybrids because only four a priori *Quercus* species were sampled.

TABLE 2. *Quercus* top 10 variables selected by stepwise discriminant analysis using morphological, isozymic, and combined data sets.

Step	Data Set Used		
	Morphological	Isozyme	Combined
	Variables Selected[a]		
1	Absence of leaf lobes	PGM8	Leaf length 7.6–15.0 cm
2	Round & dense crown	MDH14	MDH15
3	Round & open crown	PGM18	Absence of leaf lobes
4	Rough adaxial leaf surface	MDH13	Black bark
5	Leaf length 7.6–15.0 cm	MDH2	Round & dense crown
6	Pubescent[b]	PGM16	Round & open crown
7	Leaf width >11.0 cm	MDH6	Leaf width 5.1–7.5 cm
8	Trichomes[c]	MDH9	Leaf sinus 0–2.5 cm
9	Glabrous[d]	PGM13	Leaf width 0–5.0 cm
10	Waxy adaxial leaf surface	MDH3	Spreading & open crown

[a] All variables listed are significant at $p < 0.05$.
[b] Pubescent adaxial leaf surface.
[c] Simple & scattered abaxial trichomes.
[d] Glabrous adaxial leaf surface.

The top 10 significant variables used to form *Quercus* clusters are shown for both morphological and isozymic data sets in Table 2. The top 10 variables chosen from the morphological data set included leaf characteristics and crown shape. The absence of leaf lobes provided a good separation of *Q. muehlenbergii* from other oaks and was the first variable chosen to make clusters. Round and dense or round and open crown shapes, observed in *Q. marilandica* were contrasted with the spreading crown shapes of the other species. Other morphological characteristics provided the additional data needed to form the four unique clusters of *Quercus* species. Isozyme bands, on the other hand, were not necessarily representative of the a priori species identification. Bands designated as PGM8, MDH14, and PGM18, for instance, were found in three of four a priori species and caused mixing of these species in each cluster.

When isozymic data were combined with morphological data, only one allozyme, MDH15, was among the top 10 variables chosen by using stepwise discriminant analysis (Table 2). Most of the variables used to make clusters were morphological characteristics. Four of the 10 variables listed for the combined data set were the same as those listed for the morphological data set. The other six variables revealed by the combined data set were leaf, bark, and crown characteristics that, when combined with isozymic data, provided good separation of the species. Even though only one isozyme band was listed among the top 10 variables chosen by using stepwise discriminant analysis, there were other isozyme bands that were significant at $p<0.05$. These bands included MDH1, MDH4, MDH8, PGM16, PGM17, PGM24, PGM27, and PGM32, which may have provided additional information to separate species beyond that of the morphological data. The inclusion

of isozymic data may have enabled the detection of hybrids among *Quercus* species.

Morphological data revealed a close match between a priori field identification and a posteriori cluster identification of Quercus species (Table 3A). The clustering suggested four unique groups of approximately 20 specimens each. Clusters one and four represented complete groups containing 20 specimens of *Q. marilandica* and *Q. muehlenbergii*, respectively. As the only representative of *Erythrobalanus, Q. marilandica* formed a unique group of individuals with round crown shape. Among the white oaks (subgenus *Leucobalanus*), all individuals of *Q. muehlenbergii* were found in the same cluster because they were the only trees that lacked leaf lobes. Other members of *Leucobalanus* were distinguished from each other on the basis of leaf characteristics. However, one individual of *Q. macrocarpa* was grouped with the *Q. stellata* cluster. This particular *Q. macrocarpa* specimen was sampled from a stand containing several *Q. stellata* trees and may have resulted from *Q. stellata*×*Q. macrocarpa* hybridization. A *Q. stellata*×*Q. macrocarpa* hybrid would be difficult to detect because crosses between oaks may have characteristics intermediate of the two parents (*3,13,14*). Using isozymic data to match a priori field identification with a posteriori cluster identification of *Quercus* species was difficult (Table 3B). Cluster three, which contained 10 members of *Q. marilandica*, may have been the most uniquely formed group because this species was the only representative of the red oak subgenus. The only other cluster that contained at least half of the representative species was cluster one, which contained 11 of the 20 a priori *Q. macrocarpa* specimens. The remaining clusters contained a mixture of at least three of the four *Quercus* species studied.

TABLE 3. *Quercus* clusters — various data analyzed with number of individuals and a priori field identification of each cluster.

No. Clstr.[b]	No. Ind.[b]	ID[a] (freq.[b])
A. Morphological Data		
1	20	mar[c] (20)
2	21	stel[c] (20), mac (1)
3	19	mac[c] (19)
4	20	mueh[c] (20)
B. Isozymic Data		
1	23	mac[c] (11), stel (7), mueh (4), mar (1)
2	21	mueh[c] (7), stel[c] (7), mar (5), mac (2)
3	10	mar[c] (10)
4	19	mueh[c] (8), mar (4), stel (4), mac (3)
5	7	mac (4), stel (2), mueh (1)
C. Combined Morph. and Isozymic Data		
1	19	mueh[c] (19)
2	20	mar[c] (20)
3	17	mac[c] (17)
4	13	stel[c] (13)
5	11	stel (7), mac (3), mueh (1)

[a] *Quercus* species: mac = *macrocarpa*; mar = *marilandica*; mueh = *muehlenbergii*; stel = *stellata*.

[b] Clstr. = cluster; Ind. = individuals; freq. = frequency.

[c] Best match a posteriori identification.

The analysis of the combined data set revealed five clusters (Table 3C), four of which contained sole representatives of the four a priori species, and one which contained a mixture of seven *Q. stellata*, three *Q. macrocarpa*, and one *Q. muehlenbergii* specimens. *Quercus stellata* hybridizes more frequently than most oaks (*13*); studies have suggested that it will cross with both *Q. macrocarpa* and *Q. muehlenbergii* (*13,14*). Specimens of *Q. stellata* were usually in close proximity to, or in the same stand as, *Q. macrocarpa* and *Q. muehlenbergii*, which would provide the opportunity for hybridization. When oak species cross, they usually produce offspring that have characteristics intermediate of the two parent species (*14*). By using morphological data, and incorporating the isozymic data, two more possible *Q. stellata*×*Q. macrocarpa* hybrids were suggested. The combination of morphological and isozymic data may have also revealed a *Q. stellata*×*Q. muehlenbergii* hybrid found in cluster five. The seven *Q. stellata* individuals listed in cluster five were not grouped with *Q. stellata* of cluster four, which suggested that these individuals were also hybrids.

Celtis Clustering, Discriminant

Analysis, and Identification: Cluster analysis of *Celtis* data revealed four different clusters, as indicated by the CCC graph in the top right panel of Fig. 1. The disclosure of four clusters suggested that hybridization or overlapping traits caused an intermixing of species and a separation beyond the expected three a priori species. The peak at four clusters demonstrated by morphological data had a CCC value of 1.5, which is below the minimum value of 2-3 for accurate clusters (*22*). Moreover, the continual increase of the CCC value indicated that the data may be scattered or not complete enough to provide distinct clusters. Additional problems were encountered in the cluster analysis of *Celtis* isozymic data, for which CCC values beyond zero were negative and decreased continuously. An inverse peak demonstrated by isozymic data was barely discernable at four clusters, with a CCC value of *minus*3.2 (Fig. 1, middle right panel), but this peak was not as distinct as that revealed by the combined data set (Fig. 1, bottom right panel). The inverse peak at three clusters, demonstrated by the combined data set, was most analogous to the three anticipated groups of three *Celtis* species. However, the negative CCC values demonstrated by the combined and isozymic data sets indicated that the distribution of these data was unimodal (*22*). The unimodal nature of the isozymic and combined data sets indicated that *Celtis* species may hybridize or be so closely related that distinct clustering is not possible with the data used in this investigation.

The six significant variables for the morphological data used to form *Celtis* clusters are shown in Table 4. The significant variables included leaf, twig, and bark characteristics. Fewer than 10 morphological characteristics were significant because the remaining variables did not contribute significantly toward the separation of clusters. Simple and uniform abaxial trichomes were rarely seen in *C. laevigata* and enabled the separation of this species from *C. reticulata*, which often possessed these trichomes. Simple and uniform abaxial trichomes were also observed in *C. occidentalis*, but not as often as they were seen in *C. reticulata.* The inconsistency of trichome type demonstrated by *C. occidentalis* made it difficult to distinguish this species, on the basis of abaxial trichomes, from other *Celtis* species. While few *C. reticulata* specimens demonstrated a glabrous adaxial leaf surface, many *C. laevigata* specimens and an intermediate number of *C. occidentalis* specimens demonstrated a glabrous adaxial leaf surface. As was the case with trichomes, inconsistencies made it difficult to distinguish *C. occidentalis*, on the basis of adaxial leaf surface, from other *Celtis* species. Many representatives of *C. occidentalis* demonstrated leaves that were longer than other species (7.6-15.0 cm), but the leaves of some *C. laevigata* trees were just as long. As was the case for the most of the data, a characteristic thought to be represented by a single species was observed in at least two of the three *Celtis* species in this investigation.

The top 10 significant isozyme bands used to cluster *Celtis* data are shown in the middle column of Table 4. The bands designated as MDH12, MDH4, and MDH6, were found in all three of the a priori *Celtis* species, indicating that these bands provided poor separation into the expected a priori groups. Isozyme bands PGM10, PGM14, PGM18, MDH2, and MDH10 were found in at least two of the three a priori *Celtis* species. *Celtis reticulata* demonstrated exclusively PGM7 and PGM4 isozyme bands, but these bands were present in only two of the 20 *C. reticulata* specimens. These data indicated that similarities among MDH and PGM isozymes in *Celtis* did not allow the separation of clusters into acceptable a priori species.

Combining morphological and isozymic data for *Celtis* resulted in the selection of two of the 10 significant variables to be repeated from the other data sets (Table 4). These variables were simple and uniform abaxial trichomes and isozyme band MDH6. The trichome type helped to distinguish *C. laevigata* from the other species, but isozyme band MDH6 did little to separate clusters into a priori species. Perhaps the most useful characteristics revealed by the combined data set were those not listed in the other data sets.

For instance, lanceolate leaf shape was most common to *C. laevigata* and reddish-brown twig color was found mostly in *C. reticulata.* Although these data appeared to distinguish among the three a priori species, overlapping traits caused most of these characteristics to be represented by more than one species.

Clusters created by the morphological data of *Celtis* were not consistently representative of a priori field identification (Table 5A). Most clusters were a mix of the three species, with *C. occidentalis* being represented in all clusters. The cluster most representative of a priori field identification was cluster two, which contained 15 *C. laevigata* specimens, three *C. occidentalis* specimens, and one *C. reticulata* specimen. Cluster two, thus, represented *C. laevigata* and suggested a mix of three *C. occidentalis*×*C. laevigata* and one *C. reticulata*×*C. laevigata* hybrids. Cluster one was fairly representative of *C. reticulata* with 13 *C. reticulata* specimens and five possible *C. occidentalis*×*C. reticulata* hybrids. Clusters three and four contained mixes of the remaining specimens that may have resulted from hybridization or displayed overlapping characteristics that did not enable distinction among the three a priori *Celtis* species.

Isozymic data did little to provide a match between a priori field identification with a posteriori clusters (Table 5B). Clusters one, two, and three contained representatives of all of the species, and cluster four contained two of the three species. These results suggested that MDH and PGM isozymes were so closely related among *Celtis* species that it was not possible to identify *Celtis* species on the basis of MDH and PGM isozymes alone.

Including the isozymic data with the morphological data reduced the number of clusters obtained, but did not substantially improve the match between a priori field identification and a posteriori clusters (Table 5C). The three clusters of *Celtis* obtained from the combined data set included a mix of all species in cluster one, a group containing mostly *C. laevigata* in

TABLE 4. *Celtis* top 10 variables selected by stepwise discriminant analysis using morphological, isozymic, and combined data sets.

Step	Data Set Used		
	Morphological	Isozyme	Combined
	Variables Selected[a]		
1	Uniform trichomes[b]	MDH12	Uniform trichomes[b]
2	Glabrous[c]	MDH4	Lanceolate leaf
3	Leaf length 7.6–15.0 cm	MDH6	Rough adaxial leaf surface
4	Moderately stout twig	PGM10	Leaf length 3.1–7.5 cm
5	Dark brown bark color	PGM14	Moderately stout twig
6	Scattered trichomes[d]	PGM7	MDH6
7	—	PGM4	Leaf width 0–5.0 cm
8	—	PGM18	MDH1
9	—	MDH2	PGM15
10	—	MDH10	Reddish-brown twig color

[a] All variables listed are significant at $p < 0.05$.
[b] Simple and uniform abaxial trichomes.
[c] Glabrous adaxial leaf surface.
[d] Simple and scattered abaxial trichomes.

TABLE 5. *Celtis* clusters — various data analyzed with number of individuals and a priori field identification of each cluster.

No. Clstr.[b]	No. Ind.[b]	ID[a] (freq.[b])
A. Morphological Data		
1	18	ret[c] (13), occ (5)
2	19	laev[c] (15), occ (3), ret (1)
3	9	occ[c] (6), laev (3)
4	14	occ[c] (6), ret (6), laev (2)
B. Isozymic Data		
1	28	occ[c] (13), laev (10), ret (5)
2	12	laev[c] (5), ret (4), occ (3)
3	15	ret[c] (7), laev (4), occ (4)
4	5	ret[c] (4), laev (1)
C. Combined Morph. and Isozymic Data		
1	16	ret[c] (7), occ (5), laev (4)
2	27	laev[c] (16), occ (11)
3	17	ret[c] (13), occ (4)

[a] *Celtis* species: laev = *laevigata*; occ = *occidentalis*; ret = *reticulata.*
[b] Clstr. = cluster; Ind. = individuals; freq. = frequency.
[c] Best match a posteriori identification.

cluster two, and a group containing mostly *C. reticulata* in cluster three. The presence of *C. occidentalis* in all three clusters suggested the likelihood of *C. occidentalis* × *C. laevigata* and *C. occidentalis* × *C. reticulata* hybrids. The results from this combined data set, as well as results from the morphological and isozymic data sets, demonstrated that congruity among *Celtis* species may have resulted from hybridization. More research with actual crosses of pure *Celtis* species is needed to determine whether or not such hybrids exist.

CONCLUSIONS

When easily measurable morphological data were used for identification of *Quercus* species, the resulting clusters closely resembled traditional taxonomy. This was not the case for *Celtis* species, as the clusters did not consistently represent a priori field identification. Overlapping traits and a greater susceptibility of *Celtis* to hybridization may have caused a poor match between these clusters and traditional taxonomy.

Isozymic data alone did not contain sufficient information to provide a close match between a priori field identification and clusters for *Quercus* or *Celtis* species. However, the combination of isozymic data with morphological data provided a good match between a priori identification and clusters for *Quercus* and suggested the presence of *Q. stellata* × *Q. macrocarpa* and *Q. stellata* × *Q. muehlenbergii* hybrids. The combined data set for hackberries further demonstrated congruity among species which may have resulted from hybridization.

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