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Morphological Differentiation and Crossability among Populations of the *Turnera ulmifolia* L. Complex (Turneraceae)

JOEL S. SHORE and SPENCER C. H. BARRETT

Department of Botany, University of Toronto,
Toronto, Ontario M5S 1A1, Canada

ABSTRACT. The *Turnera ulmifolia* complex is a polymorphic assemblage of perennial weeds native to the Neotropics. Thirteen population samples from a wide geographical range, representing six taxonomic varieties, were selected for a comparison of morphological differentiation and crossability. The complex is polyploid with diploid and tetraploid populations exhibiting distyly and hexaploid populations homostyly. Thirty-eight morphological characters were measured on plants grown under uniform glasshouse conditions and seed set data collected from 2195 controlled crosses made in all combinations among the thirteen populations. Discriminant analysis and clustering techniques were employed to investigate phenetic relationships among populations and quantitative methods were used to compare the structure of the data from the morphological and crossability studies. Analysis of the phenetic and crossability data sets, using the Kc statistic, indicated that these data sets were significantly associated although discrepancies were apparent in the dendrograms produced. A dendrogram generated from the crossability data was composed solely of clusters of populations of the same ploidal levels, while the dendrogram based on phenetic data included one cluster containing populations at all ploidal levels. It is suggested that autopolyploidy or segmental allopolyploidy is responsible for the discordance between data sets. Morphological, cytological, and crossability data indicate that the complex might be better treated taxonomically as a number of separate species.

The use of controlled crosses among differentiated populations of plants is a basic experimental technique in systematic studies. Crossability data are often used to infer genetic or patristic relationships among groups and are useful in demonstrating hybridization potential or the existence of isolating mechanisms. In association with morphological, cytological, and chemical characters, data from artificial hybridization studies provide the most commonly used sources of information in the construction of classifications of flowering plants. Where both morphological and crossability data are available a comparison of the relationships implied by the alternative data sets may be helpful in clarifying taxonomic decision-making as well as providing insights into the processes of evolutionary differentiation among taxa.

The *Turnera ulmifolia* L. complex is variable in morphology, chromosome number, and breeding system. The plants are native throughout the Neotropics and occur as weeds of roadsides and open waste ground (Barrett 1978). Geographic races have been treated as varieties of *T. ulmifolia* or as separate species in different regions of the Neotropics (e.g., Woodson et al. 1967; Adams 1970; Bentley 1979; Arbo and Fernandez 1983) and the Old World (Lock 1904; Van Steenis 1931; Backer 1951), where adventive populations occur. Adams (1972) has suggested that the species is one of the most morphologically variable weeds indigenous to the American tropics.

Since no modern taxonomic revision of the group is available, in this study we follow Urban's (1883) treatment, which recognizes 12 intergrading varieties of *T. ulmifolia*. This approach is adopted for convenience; our data support dissolution of the complex into several distinct species, although their number and circumscription requires further work. This paper presents the results of a comparison of morphological variability and crossing relationships among 13 populations of *T. ulmifolia* comprising six varieties. The varieties investigated were: *T. ulmifolia* vars. *orientalis* Urb., *elegans* Urb., *intermedia* Urb., *angustifolia* Willd., *surinamensis* Urb., and *grandiflora* Urb. The populations were chosen to include a broad range of morphological variation and to represent samples from a wide geographical area. The specific objectives of the study were: 1) to as-

TABLE 1. Varietal status, source, and sample sizes for the thirteen populations of the *Turnera ulmifolia* complex used in glasshouse studies.

Population	Variety	Source	No. of plants		No. of crosses	
			Phenetic study	Crossability study	♀	♂
S	*surinamensis*	Santarém, Brazil	9	5	101	90
G	*grandiflora*	Corrientes, Argentina	20	16	173	174
I1	*intermedia*	Barreirinhas, Brazil	20	7	215	218
I2	*intermedia*	Calabozo, Venezuela	20	9	217	212
I3	*intermedia*	Caracas, Venezuela	20	6	178	189
I4	*intermedia*	Dagua, Colombia	20	7	182	196
E1	*elegans*	Selangor, Malaya	20	5	188	173
E2	*elegans*	Manaus, Brazil	20	8	240	216
E3	*elegans*	Belém, Brazil	20	7	186	174
A1	*angustifolia*	Panama City, Panama	20	3	103	83
A2	*angustifolia*	Selangor, Malaya	15	8	108	168
A3	*angustifolia*	Cañas, Costa Rica	20	6	130	180
O	*orientalis*	Corrientes, Argentina	20	25	174	122

sess phenetic similarity among populations based on comparisons of 38 morphological characters; 2) to assess crossability relationships among populations and provide methods for their presentation; and 3) to evaluate procedures for the comparison of similarity matrices obtained from crossability and phenetic data.

METHODS

All experimental work including morphological measurements and controlled crosses was undertaken on plants grown under glasshouse conditions. Plants are easily cultured and flower year round, provided glasshouse temperatures are maintained above 20°C. Seed germination levels vary depending on conditions; the best results were obtained by burying seeds 1 cm deep in a soil mixture consisting of 2 parts peat:1 part clay-loam:2 parts sand, by volume. The seeds were sown in 7.5 cm pots and allowed to imbibe water for 1 week by placing the pots in water-filled trays positioned 50 cm below a bank of fluorescent lights in a warm glasshouse (25–35°C). The seeds began to germinate within 2 weeks after the time of sowing. Seedlings were transplanted into 7.5 cm pots for morphological comparisons and 13 cm pots for crossability studies.

The locality and varietal status of the 13 population samples of *T. ulmifolia* used in the study are presented in table 1. The number of plants used for morphological comparisons and controlled crosses is also presented. Seeds used in the study were obtained from bulk collections of natural populations. Chromosome numbers of plants from the 13 populations were determined from squashes of anthers after fixing flower buds in ethanol and acetic acid (3:1). The buds were stained according to the procedure of Snow (1963). Vouchers are deposited at TRT.

Morphological differentiation. To assess the phenetic relationships among populations a total of 38 vegetative and reproductive characters was measured for each individual. Measurements were made on all plants during the summer of 1979, except those from the *T. ulmifolia* var. *angustifolia* populations (A1, A2, A3), which were grown the following summer. All measurements were made on plants of approximately the same age grown on a glasshouse bench in a randomized design. This procedure was adopted to reduce phenotypic variation attributable to environmental and age effects.

The characters measured on each plant are presented in table 2. Twenty fresh pollen grains were measured for each individual. Pubescence characteristics were scored according to hair density. Petal color, anther dehiscence, and breeding system were almost invariant within each population. Petal spot color was polymorphic in populations I1 and I4. The single flower

TABLE 2. Morphological characters used in phenetic comparison of populations of the *Turnera ulmifolia* complex.

Petiole length (mm), leaf blade length (mm), maximum leaf width (mm), length from leaf base to maximum leaf width (mm), leaf tooth shape (0 = acute, 1 = mixed, 2 = rounded), leaf tooth number, maximum leaf tooth depth (0.1 mm), leaf vein number, basal angle of leaf, apical angle of leaf, abaxial leaf pubescence (0 = glabrous, 1 = intermediate, 2 = pubescent), adaxial leaf pubescence (0 = glabrous, 1 = intermediate, 2 = pubescent), bracteole length (mm), bracteole width (0.1 mm), bracteole shape (0 = linear, 1 = oblong, 2 = ovate), bracteole tooth number, number of bracteolar appendages, hypanthium length (mm), flower diameter (mm), sepal length (mm), sepal width (mm), petal length (mm), maximum petal width (mm), length from petal base to maximum petal width (mm), petal tip shape (0 = truncated, 1 = rounded, 2 = acute), degree of petal overlap (0 = no overlap, 1 = moderate, 2 = considerable), petal shape (0 = oblanceolate, 1 = obovate, 2 = broadly obovate), petal color (0 = white, 1 = cream, 2 = pale yellow, 3 = dark yellow), petal spot color (1 = not visible, 2 = purple, 3 = violet, 4 = yellow), anther dehiscence (0 = extrorse, 1 = introrse), breeding system (0 = distylous, 1 = homostylous), reproductive organ length (style length + stamen length, mm), stigma-anther separation (mm), pollen size (μm), fruit length (mm), maximum fruit diameter (mm), seeds per capsule, fruit pubescence (0 = glabrous, 1 = intermediate, 2 = pubescence).

measured on each plant was borne upon the leaf measured. The number of seeds per capsule was obtained by crossing the next flower on the shoot apex to another compatible individual from the same population.

The following numerical procedures were used to evaluate the phenetic relationships among populations. Means of all characters were calculated for each population. A similarity matrix was constructed by performing pairwise correlations among populations across all 38 characters. Populations were clustered using the UPGMA procedure (Sneath and Sokal 1973). Fifteen continuous characters were used in a discriminant functions analysis (Nie et al. 1975) to ordinate population centroids in a character space of reduced dimensionality. One-way ANOVAs were performed for each of these characters.

Crossability. To assess the crossability relationships among populations a controlled crossing program was initiated in April 1979 in which an attempt was made to cross each individual in a population sample with all other compatible individuals from the remaining populations (see below). Plants were three years of age when crossing commenced and because of their larger stature many flowers were available per plant for the various pollinations. All crosses were performed by removing all anthers from the male parent with fine forceps and rubbing them on the stigmas of the emasculated female parent.

Since distyly occurs within the complex (Urban 1883) and individuals within distylous populations are self- and intra-morph incompatible (Barrett 1978), long-styled plants were crossed only to short-styled plants and vice versa. Individuals from homostylous populations (A1, A2, A3, O) were used as female parents only in crosses with short-styled plants and as male parents only with long-styled plants, as preliminary studies indicated that these were the only compatible pollinations between distylous and homostylous populations. Since plants from homostylous populations exhibit self-compatibility, their flowers were emasculated one day prior to anthesis to prevent autogamy.

The number of seeds produced per capsule was recorded for all pollinations. A detailed description of the sample sizes for each cross is provided (tables 1 and 5). Differences in the number of pollinations performed are a reflection of flowering propensity and the number of individuals in the population sample. Samples from populations O and G were not obtained until about half of the other crosses had been performed. Since these plants were smaller and flowered less profusely a larger sample of plants was used.

The crossability data collected here are presented as a rectangular matrix (table 5). In the absence of differences between reciprocal crosses, which can be tested by diallel analysis of variance (Yates 1947; Hayman 1954; Griffing 1956), the mean of reciprocals may be calculated, thus yielding a symmetrical similarity matrix. Numerical methods may then be employed to cluster populations in a hierarchical manner. If populations differ in seed set then the original matrix is likely to be asymmetrical. Differences in seed set may be recognized by

performing an analysis of variance among entries along the principal diagonal of the crossability matrix (a one-way ANOVA among within population crosses). Asymmetry may be removed by dividing the mean of each cross by the mean of its maternal parent's within population cross value. This standardization procedure is the crossability index suggested by McDade and Lundberg (1982). The occurrence of asymmetry may again be tested using a diallel analysis of variance.

In our study, the original crossability matrix was found to be asymmetrical (table 6), and populations were found to differ in seed set (table 7). The standardization procedure stated above was applied to the data but did not remove the asymmetry (table 8). When this situation occurs we suggest the following procedures. 1) Consider the standardized crossability matrix to be an operational taxonomic unit (OTU) by character matrix, where male parents are OTUs and female parents are characters. The reverse situation may also be considered, and the results compared. A similarity matrix is then constructed by computing pairwise correlations among OTUs. Clustering may then be employed to produce a dendrogram. 2) Alternatively, the raw crossability data may be standardized and clustered according to the method of Slater (1976) termed the iterative proportional fitting clustering procedure (IPFC). A scaling procedure is used to transform the original data and the resulting similarity matrix is clustered. For the present study both of the above procedures were employed and the results compared. The clustering method of Slater (1976) was performed using the IPFC procedure of SAS (Reinhardt 1980) with the non-null diagonal option. All other clustering procedures were performed using the unweighted pair-group (UPGMA) clustering procedure of NTSYS (Rohlf et al. 1974).

Comparison of phenetic and crossability data. The degree of similarity among the phenetic and crossability data sets was assessed by calculating the Kc statistic of association (Dietz 1983) for pairs of similarity matrices generated by the different procedures. For the IPFC procedure, the cophenetic matrix implied by the dendrogram was used rather than the similarity matrix due to asymmetry of the latter. The Kc statistic can be used both as a descriptive measure of association between two matrices and as a test statistic for the significance of association (Dietz 1983). Significance tests were performed between the phenetic similarity matrix and all three crossability similarity matrices. For each significance test the following procedure was performed: the pair of matrices to be compared were randomly permuted five hundred times, and the Kc statistic calculated for each permutation. This provides a probability distribution for Kc under the null hypothesis of no association between the matrices. Thus, the probability of obtaining a result as large as or larger than the observed Kc by chance could be calculated.

Results

The range of morphological variation exhibited by the 13 populations of *T. ulmifolia* grown under uniform glasshouse conditions was large. Each population possessed a unique combination of characters enabling the experienced observer to classify a single plant to its source of origin without difficulty. The major characters distinguishing varieties are presented in table 3. Two groups within *T. ulmifolia* were evident based on flower color, those varieties with cream-yellow flowers (vars. *elegans, intermedia, angustifolia,* and *orientalis*) and those with pale blue-purple flowers (vars. *surinamensis* and *grandiflora*). Characters of the bracteoles (e.g., width and tooth number) were particularly useful in discriminating varieties.

Most populations were easily identified to varietal status on the basis of Urban's (1883) treatment of the complex. However, the placement of populations I1 and I4 was problematic and these populations were placed in var. *intermedia* for convenience. Both populations exhibited morphological characters found in *T. ulmifolia* vars. *intermedia* and *elegans*. Additional sampling by us in South America has indicated the complex nature of the variation in these taxa, particularly in Brazil.

The *T. ulmifolia* complex is polyploid with a base number of $x = 5$. Diploid, tetraploid, and hexaploid populations occur. An association between ploidal level and breeding system was evident among the populations sampled. Populations of the diploid and tetraploid varieties are distylous and self-incompatible, whereas populations of the hexaploid varieties (*angustifolia* and *orientalis*) are homostylous and self-compatible. More extensive sampling has

TABLE 3. Major characters distinguishing varieties of the *Turnera ulmifolia* complex. Values given for quantitative characters are the mean (standard deviation) of glasshouse grown plants. Di = distylous; Ho = homostylous; NS. = no visible spot.

	Variety and population												
	surinamensis	*grandiflora*	*intermedia*				*elegans*			*angustifolia*			*orientalis*
Character	S	G	I1	I2	I3	I4	E1	E2	E3	A1	A2	A3	O
Chromosome number (*2n*)	10	10	10	10	10	20	20	20	20	30	30	30	30
Breeding system	Di	Di	Di	Di	Di	Di	Di	Di	Di	Ho	Ho	Ho	Ho
Flower color	Pale blue	Pale blue	Pale yellow	Yellow	Yellow	Yellow	Cream	Cream	Cream	Yellow	Yellow	Yellow	Pale yellow
Petal spot color	yellow	violet	NS. or purple	NS.	NS.	NS. or purple	Purple	Purple	Purple	NS.	NS.	NS.	NS.
Petal length (mm)	18 (2)	32 (5)	22 (3)	23 (3)	21 (3)	20 (2)	27 (3)	33 (4)	34 (5)	26 (3)	24 (3)	25 (2)	17 (2)
Hypanthium length (mm)	7.9 (0.9)	10.0 (1.3)	4.3 (0.7)	4.9 (0.5)	4.2 (0.7)	5.0 (0.7)	5.0 (0.7)	4.7 (0.6)	5.0 (0.9)	11.0 (0.9)	7.8 (0.9)	8.6 (1.0)	4.8 (0.7)
Bracteole length (mm)	6.1 (1.6)	4.8 (0.8)	9.1 (2.5)	11 (1.5)	9.0 (1.4)	8.6 (1.5)	12 (1.4)	12 (1.5)	15 (3.1)	16 (1.8)	9.9 (0.9)	13 (1.5)	9 (1.4)
Bracteole width (mm)	0.7 (0.2)	0.8 (0.1)	0.9 (0.2)	0.9 (0.1)	0.9 (0.1)	0.8 (0.1)	1.3 (0.2)	1.2 (0.2)	1.1 (0.3)	6.2 (0.1)	1.7 (0.3)	4.6 (0.6)	1.0 (0.1)
Maximum leaf width (mm)	10 (3)	17 (4)	25 (5)	21 (4)	29 (6)	24 (3)	29 (4)	30 (6)	34 (5)	23 (3)	18 (5)	25 (4)	27 (4)

confirmed this relationship (Barrett and Shore unpubl. data).

Phenetic relationships. One-way ANOVA revealed significant differences among populations for all morphological characters (table 4). Discriminant functions analysis was performed on the morphological data to ordinate populations in a character space. The absolute magnitude of the standardized discriminant function coefficients determine the relative importance of the characters in discriminating among populations. Bracteole width and reproductive organ length are important discriminators for function 1, which accounts for 67.2% of the variation, and hypanthium length and bracteole length are important discriminators for function 2, which accounts for 15.0% of the variation. Population centroids are plotted in a space defined by the first three functions from the discriminant analysis (fig. 1). The three functions cumulatively account for 88.4% of the variation in the data set. From the ordination it can be seen that both diploid and tetraploid populations of var. *intermedia* form a coherent group, with var. *elegans* (4*x*) and var. *orientalis* (6*x*) closely adjacent. Populations of var. *angustifolia* (6*x*) form a less discrete group indicating that there is greater interpopulational variation among the samples used in the study than is observed for the other varieties. Varieties *grandiflora* and *surinamensis* are more closely associated with one another than with any other population. The dendrogram generated from the phenetic data showed four major clusters (fig. 1). These clusters consist of: 1) all populations of var. *angustifolia,* 2) all populations of var. *elegans,* 3) all populations of var. *intermedia* and the single population of var. *orientalis,* and 4) the blue-flowered varieties, *grandiflora* and *surinamensis.*

TABLE 4. Characters, *F*-values (univariate), and standardized canonical discriminant function coefficients for the first three functions from a discriminant analysis of morphological variation.

Character	*F*-ratio	Func. 1	Func. 2	Func. 3
Petiole length	21.7	−0.08	0.30	−0.09
Leaf blade length	23.4	0.13	−0.11	0.07
Leaf blade width	34.0	−0.13	0.22	−0.12
Leaf length to max. width	16.2	−0.07	−0.29	0.13
Leaf tooth #	18.3	0.04	0.32	−0.33
Leaf max. tooth depth	58.1	−0.05	0.28	0.43
Leaf vein number	15.3	0.20	0.16	0.03
Bracteole length	62.0	−0.07	0.39	−0.04
Bracteole width	405.0	0.69	0.41	0.04
Hypanthium length	160.0	0.19	−0.58	0.05
Flower diameter	38.7	0.11	0.14	0.11
Sepal length	40.0	−0.16	0.03	0.45
Petal length	48.9	−0.26	−0.27	0.51
Petal length to max. width	33.3	−0.25	0.21	−0.14
Reproductive organ length	289.1	0.67	−0.20	0.07
% variance explained		67.2	15.0	6.2

Analysis of crossability data. The crossing program involved a total of 112 individuals from 13 populations. During the two-year period in which pollinations were conducted 2195 crosses were made. Of the 169 possible combinations 35 (20.7%) yielded no seed. Table 5 presents the mean number of seeds produced per pollination for each population combination and reciprocals. Crosses among populations within a variety were generally highly fertile, although the number of seeds produced varied greatly. Certain crossing combinations yielded higher seed set in one direction than the reciprocal. For example, hexaploid populations tended to set more seed when used as female parents in crosses than as males. This is apparent from inspection of the row means (♂) in comparison with the column means (♀) for hexaploid populations (table 5). Statistical confirmation of a general asymmetry in crossing relationships is evident in the analysis of variance of crossability data (table 6). The ANOVA indicates that there is a significant reciprocal source of variation ($P < 0.001$) in the data. This asymmetry is, in part, the result of significant differences in seed set of intrapopulation crosses (table 7). The standardization procedure described above failed to remove this asymmetry (table 8), although the *F*-value for this source of variation was reduced in comparison with the non-standardized analysis (table 6).

Clustering procedures were performed to visualize the crossability relationships among populations. The IPFC method of Slater (1976) deals explicitly with asymmetrical matrices. The dendrogram produced from this analysis was very similar to the other crossability dendrograms and hence is not presented. Clustering

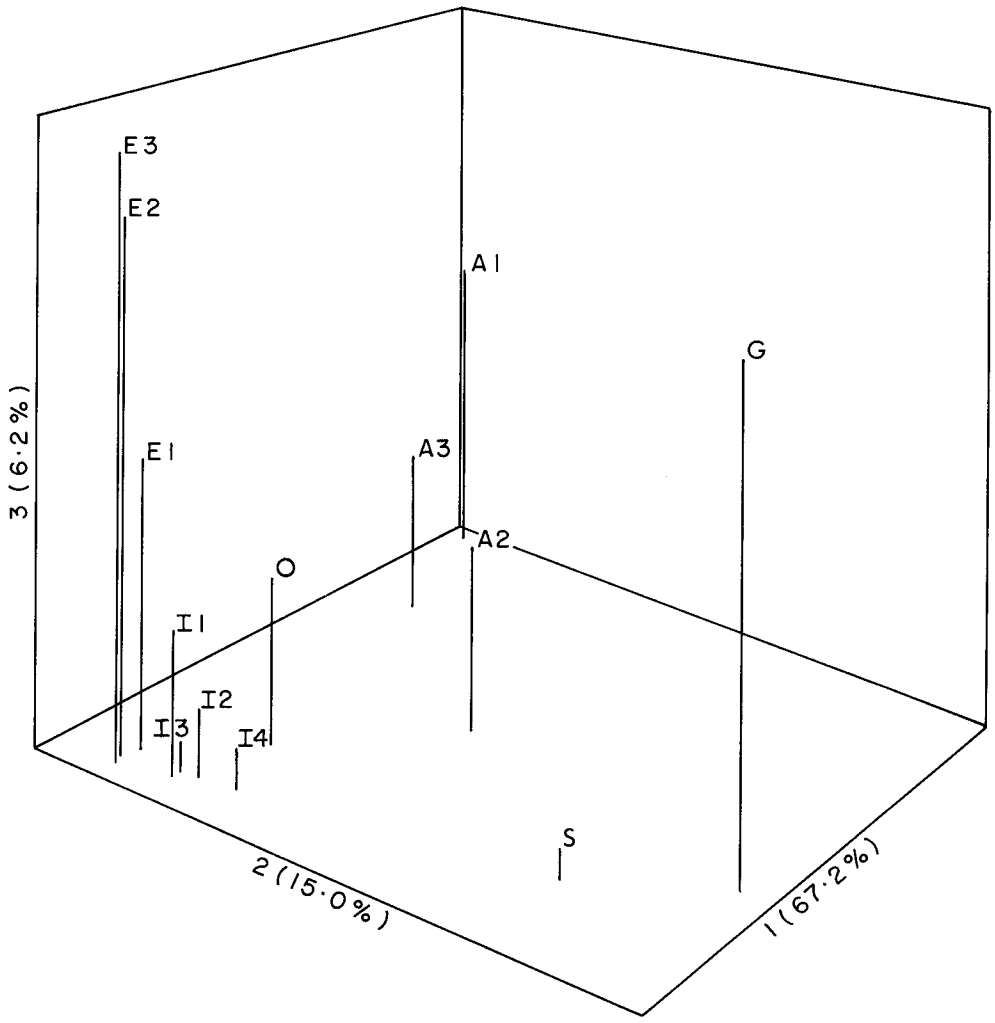

FIG. 1. Ordination of centroids of 13 populations of *Turnera ulmifolia* in a space defined by the first three functions of a discriminant functions analysis based upon 15 characters.

also was performed using data from the standardized crossability matrix considering male and female parents to be OTUs. The dendrogram produced for males is presented (fig. 3).

To compare clustering methods, the Kc statistic was computed among all possible pairs of similarity matrices (table 9). In addition both the cophenetic correlation coefficient (Sneath and Sokal 1973) and its Kc analog were computed to measure the amount of distortion caused by the clustering procedure. While it is not possible to assess the significance of the Kc statistic of association when the comparisons involve matrices generated from the same data set, it is possible to use the Kc statistic as a measure of the strength of association (Dietz 1983). All three clustering procedures employed on the crossability data were moderately associated (table 9). The congruence of these matrices was further substantiated by observation of the dendrograms produced by the three procedures. In all dendrograms clustering reflected differences in ploidal level among the populations. Hexaploids form one group, and the tetraploids another. At the diploid level, however, at least two clusters are apparent. Variety *intermedia* populations form one group, while vars. *grandiflora* and *surinamensis* form

TABLE 5. Mean crossability (standard deviation) and sample size for 13 populations of the *Turnera ulmifolia* complex based upon replicate measures of seeds produced per pollination. Diagonal values in boldface are within population crosses.

Female parent	Male parent													Mean
	S	G	I1	I2	I3	I4	E1	E2	E3	A1	A2	A3	O	
S	**13** (5.0) 7	8 (7.0) 11	0 (0) 11	0.6 (1.0) 7	0 (0) 13	0.3 (0.4) 4	0 (0) 9	3 (4.0) 6	0 (0) 9	0 (0) 2	0 (0) 7	0 (0) 6	0 (0) 9	1.9
G	16 (11.0) 12	**36** (17.0) 24	0.8 (1.0) 15	3 (5.0) 18	5 (6.0) 14	2 (4.0) 17	0.5 (1.0) 13	3 (4.0) 15	4 (4.0) 11	17 (9.0) 5	11 (10.0) 12	7 (15.0) 10	6 (4.0) 7	8.6
I1	9 (9.0) 9	7 (5.0) 15	**20** (12.0) 46	20 (12.0) 13	24 (8.0) 15	1 (2.0) 19	0.7 (1.0) 25	2 (2.0) 18	2 (2.0) 18	5 (4.0) 6	2 (3.0) 11	7 (9.0) 8	7 (10.0) 12	8.2
I2	3 (6.0) 7	1 (2.0) 12	11 (7.0) 13	**9** (9.0) 67	18 (8.0) 10	0.4 (0.7) 11	0.5 (2.0) 22	0.9 (2.0) 14	0.7 (2.0) 14	0.4 (0.5) 7	0.9 (2.0) 11	0 (0) 11	2 (4.0) 18	3.7
I3	5 (9.0) 12	2 (5.0) 16	21 (7.0) 14	17 (6.0) 11	**12** (8.0) 40	5 (5.0) 12	4 (7.0) 15	6 (5.0) 13	10 (7.0) 17	0 (0) 7	0 (0) 10	0 (0) 8	0 (0) 3	6.3
I4	0 (0) 2	2 (3.0) 14	2 (4.0) 12	0.2 (0.5) 12	1 (2.0) 14	**12** (8.0) 52	17 (7.0) 13	12 (8.0) 12	20 (12.0) 13	0 (0) 7	0 (0) 11	0 (0) 12	0 (0) 8	5.1
E1	0.7 (2.0) 7	1 (2.0) 10	0 (0) 28	0 (0) 20	0.1 (0.2) 16	22 (7.0) 12	**13** (8.0) 25	13 (7.0) 11	20 (10.0) 14	0.5 (1.0) 8	0 (0) 7	0 (0) 16	0 (0) 14	5.4
E2	0 (0) 2	3 (3.0) 12	0.2 (0.4) 20	0.1 (0.2) 17	0.1 (0.2) 16	25 (9.0) 22	28 (12.0) 11	**20** (10.0) 70	18 (9.0) 23	0 (0) 7	0.3 (0.6) 16	0 (0) 11	0 (0) 13	7.3
E3	1 (2.0) 6	12 (8.0) 12	2 (3.0) 24	2 (3.0) 16	2 (3.0) 16	30 (7.0) 18	24 (9.0) 17	17 (9.0) 23	**17** (13.0) 24	0.2 (0.4) 5	0 (0) 9	0 (0) 10	0 (0) 6	8.2
A1	0 (0) 4	5 (4.0) 10	0 (0) 10	0 (0) 7	0 (0) 5	2 (4.0) 5	0.3 (0.8) 6	2 (5.0) 7	0.2 (0.4) 5	**9** (10.0) 7	27 (10.0) 13	37 (7.0) 17	0.6 (1.0) 7	6.4
A2	0.7 (2.0) 7	9 (9.0) 7	29 (15.0) 8	18 (11.0) 7	15 (13.0) 7	43 (5.0) 5	33 (2.0) 3	25 (11.0) 6	40 (15.0) 7	73 (20.0) 2	**19** (20.0) 34	49 (24.0) 12	25 (8.0) 3	26.1
A3	10 (5.0) 3	22 (12.0) 10	32 (24.0) 5	16 (6.0) 6	11 (15.0) 9	0 (0) 5	2 (3.0) 9	6 (7.0) 6	5 (4.0) 8	16 (16.0) 11	48 (31.0) 9	**36** (23.0) 41	26 (13.0) 8	17.7
O	4 (6.0) 12	6 (6.0) 21	17 (12.0) 12	11 (11.0) 11	14 (12.0) 14	11 (10.0) 14	3 (3.0) 5	3 (6.0) 15	16 (10.0) 11	17 (8.0) 9	24 (13.0) 18	13 (14.0) 18	**36** (12.0) 14	13.5
Mean	4.8	8.8	10.0	7.5	7.9	11.8	9.7	8.7	11.8	10.6	10.2	11.5	7.9	

TABLE 6. Diallel analysis of variance of raw crossability data. Each main effect is tested against the error term immediately below it.

Source	d.f.	Sum of sq.	Mean sq.	F	P
Population	12	7.71	0.64	1.91	>0.05
Error	73	26.25	0.34		
Reciprocal	12	7.38	0.61	8.79	<0.001
Error	66	4.61	0.07		

TABLE 8. Diallel analysis of variance of the standardized crossability data. Each main effect is tested against the error term immediately below it.

Source	d.f.	Sum of sq.	Mean sq.	F	P
Population	12	11.6	0.965	1.48	>0.05
Error	78	50.9	0.653		
Reciprocal	12	8.5	0.709	4.78	<0.001
Error	66	9.8	0.148		

another. The tetraploid population of var. *intermedia* clustered with tetraploid var. *elegans* populations. As a result of the similarity among the different dendrograms generated from the crossability data only a single dendrogram is illustrated (fig. 3).

Comparisons of phenetic and crossability data. Quantitative comparisons among the phenetic and crossability data were made using the Kc statistic of association calculated for all possible pairs of similarity matrices (table 9). Values of Kc ranged from 176 to 214, indicating that there is a statistically significant association between the phenetic similarity matrix and each of the crossability similarity matrices. Despite these significant associations several disparities are evident from the two analyses and these can be seen by visual comparison of the phenetic and crossability dendrograms (figs. 2–3). In the crossability study, population I4 (var. *intermedia*, 4*x*) clustered with the var. *elegans* populations (4*x*), while for the phenetic study it was more closely associated with the other var. *intermedia* populations (2*x*). In addition, in the crossability study, the var. *orientalis* population (6*x*) clustered with var. *angustifolia* populations (6*x*), but was more closely allied with the var. *intermedia* populations (2*x* and 4*x*) in the phenetic study.

TABLE 7. One-way ANOVA among population crosses. A random sample of 7 replicate crosses from the raw crossability data was used for each population.

Source	d.f.	Sum of sq.	Mean sq.	F	P
Population	12	56.13	4.68	4.53	<0.01
Error	78	80.54	1.03		

DISCUSSION

Data were collected to aid in interpreting systematic relationships among selected taxa within the *Turnera ulmifolia* complex, and simultaneously, quantitative methods for the evaluation and comparison of phenetic and crossability data were developed. The populations chosen for study represent a diverse sampling collected from a broad geographical range. While the study is based on a limited sample of variability the results clearly indicate that the complex is composed of several differentiated assemblages reproductively isolated from one another by polyploidy and crossability barriers. This would suggest that the complex might be better treated taxonomically as a number of separate species. If this view is followed, the blue-flowered varieties *grandiflora* and *surinamensis* clearly require recognition as one or two separate species. All analyses performed resulted in the separation of these varieties from the remainder of the complex. Fur-

TABLE 9. The relationships among numerical studies as assessed by computing the Kc statistic for association among similarity matrices. Values on the diagonal represent the Kc analog to the cophenetic correlation coefficient, followed by the cophenetic correlation coefficient in parentheses. * Significance tests were performed for these comparisons; all were statistically significant at $P \leq 0.008$; NA = not applicable.

	Female OTU	Male OTU	Phenetic	IPFC
Female OTU	400 (0.90)			
Male OTU	302	432 (0.86)		
Phenetic	214*	176*	418 (0.82)	
IPFC	210	211	196*	NA (0.40)

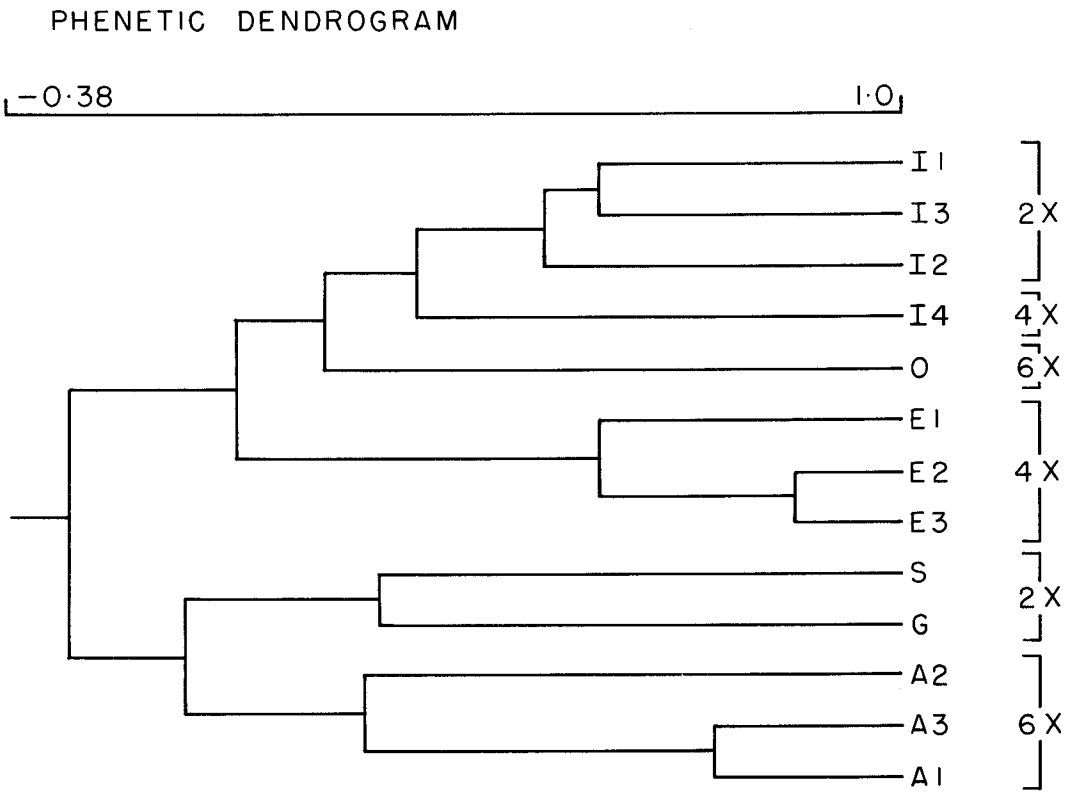

FIG. 2. Dendrogram generated from UPGMA clustering of a phenetic similarity matrix. The ploidal level of each population is indicated ($x = 5$).

ther, only small amounts of seed were obtained in crosses between the blue- and yellow-flowered diploid assemblages and we have not been able to raise viable hybrids from the seed. Crosses between the blue-flowered varieties yielded large amounts of seed but hybrid fertility was only 20%, as assessed by percent pollen stainability with cotton blue in lactophenol. The occurrence of an additional blue-flowered variety (*T. ulmifolia* var. *caerulea* Urb.) from Mexico and Bolivia (Urban 1883), which shares characteristics of vars. *grandiflora* and *surinamensis*, further complicates the taxonomic picture. Unfortunately, this variety was not available for study.

Morphological variation among the cream- and yellow-flowered populations is also complex and reproductive isolation results from the presence of three ploidal levels ($2x$, $4x$, $6x$). *Turnera ulmifolia* var. *elegans* has been frequently treated at the specific rank as *T. subulata* Smith (Backer 1951; Brizicky 1961; Backer and Bakhuizen 1963) or *T. trioniflora* Sims (Lock 1904; Ridley 1922; Bentley 1979). We noted above that there was some difficulty in assessing the varietal status of populations I1 and I4 from Brazil and Columbia, although the phenetic analyses indicate that these populations are most appropriately assigned to var. *intermedia* rather than to var. *elegans*. This study and subsequent work by us indicates that var. *elegans* ($4x$) is interfertile with some $4x$ populations of var. *intermedia*. Hybrids between different populations of the two varieties exhibit pollen fertilities ranging from 70–80%. The morphological intergradation of vars. *intermedia* and *elegans* points to the need for extensive sampling of both taxa throughout their geographical ranges before firm taxonomic conclusions can be reached.

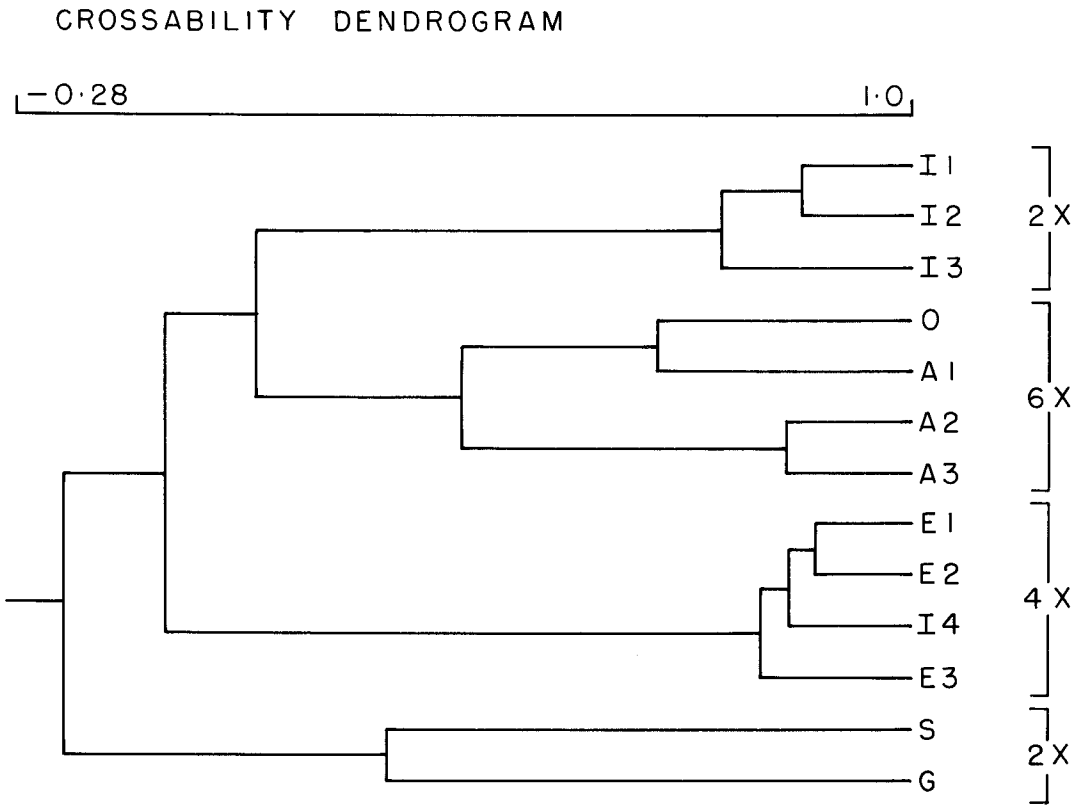

FIG. 3. Dendrogram generated from UPGMA clustering of the crossability similarity matrix using male parents as OTUs. The ploidal level of each population is indicated ($x = 5$).

Two different hexaploid varieties were examined and both exhibited different phenetic similarities with the diploid and tetraploid populations. The clustering and close association upon ordination of yellow-flowered diploid, tetraploid, and hexaploid populations (I1–I4 and O) of vars. *intermedia* and *orientalis* suggests they are related through hybridization and polyploidy (figs. 1-2). Populations of var. *angustifolia* were not closely associated with the cream- or yellow-flowered populations, suggesting that genetic divergence has occurred since polyploidization or that distinct diploid genomes are involved in their origin. Although crosses between the two hexaploid varieties (vars. *angustifolia* and *orientalis*) yielded abundant seed, the resultant hybrids are highly pollen sterile with pollen stainability of less than 4%.

A correlation exists between breeding system and ploidal level among the 13 populations examined. Diploids and tetraploids are distylous and self-incompatible while hexaploids are homostylous and self-compatible. We have recently extended our sampling to include an additional 27 populations of the three ploidal levels and the correlation has remained unchanged. Among the additional populations examined was var. *velutina* from the northern limit of the range of the complex in Mexico. This variety is homostylous, hexaploid, and intersterile with the other hexaploid varieties. Dowrick (1956) has suggested that there is a tendency for heterostyly to break down to homostyly in polyploids but this pattern is not evident in all heterostylous groups (Ockendon 1968).

The homostylous varieties in *Turnera ulmifolia* are morphologically distinct, allopatric, intersterile, and occur at different margins of the range of the species complex. This suggests that heterostyly has broken down to homostyly on

at least three occasions within the species complex, always in association with the hexaploid condition. The reason for the association between hexaploidy and homostyly is unclear. We have synthesized hexaploids, using colchicine, by doubling triploid seedlings from crosses between diploid and tetraploid populations of var. *intermedia*. The resulting hexaploids are distylous and exhibit self-incompatibility. Thus, at its inception, hexaploidy per se does not necessarily cause homostyly. However, it is possible that breakdown could occur in segregating generations.

Crossability data are most commonly presented in the form of crossing diagrams but may be presented as frequency distributions or crossability maps (McDade and Lundberg 1982). For ease of comparison with phenetic data, the crossability data are presented here in the form of a dendrogram. Since a crossability matrix is a similarity matrix, a clustering procedure such as UPGMA (Sneath and Sokal 1973) may be used to construct the dendrogram. If the crossability matrix is asymmetrical alternative procedures such as those described above may be employed. Two different methods for dealing with asymmetrical matrices were applied to the crossability data presented here. As all dendrograms generated from the crossability data were structurally similar and moderately associated, we recommend the use of the first method described (see Methods) since it is easier to implement than the IPFC procedure as it does not require the use of an iterative scaling procedure for standardization.

Statistical comparisons of dendrograms generated in this study were made using the Kc statistic of association. This statistic has certain desirable properties, including high power, and invariance under monotone transformations. In addition, it incorporates within matrix comparisons in assessing between matrix associations (Dietz 1983). Unfortunately, as the statistic is not commonly used in systematics and because it is not constrained always to lie within a particular range it is often difficult to have an intuitive grasp of the meaning of any particular value. For a matrix containing n objects the Kc statistic will have a maximum value of $n(n-1)(n-2)/2$. For the present study, $n = 13$, therefore the maximum value of Kc is 858 (the minimum possible value is -858). To provide the reader with some additional means of assessing the value of the Kc statistic we have provided both the cophenetic correlation coefficient and its Kc analog for three of the four dendrograms generated. The Kc values obtained are about half of the maximum possible value while the more familiar correlation coefficient is about 80% of its maximum value.

An additional analysis novel to systematic literature employed by us in this study was the use of the diallel analysis of variance to evaluate the presence of asymmetry in the crossability matrix. The original presentation of the method used percentage seed set data from controlled crosses of *Trifolium hybridum* (Yates 1947), yet the method seems not to have been employed in a systematic context. Quantitative geneticists and plant breeders have extended the experimental designs and analysis of diallels (Hayman 1954; Griffing 1956; Mather and Jinks 1971). While we have not employed these further analyses, the concepts of general and specific combining ability (Griffing 1956) may provide the most useful form of interpretation of diallel analysis for systematists. These analyses should allow workers to pinpoint the different sources of variation that contribute to the overall pattern of crossability or F_1 fertility. Specific combining ability estimates also may provide useful measures of similarity among the OTUs examined. In this study only the reciprocal source of variation was evaluated. After standardization of the crossability data the asymmetry remained and hence the asymmetry of the original data was not solely the result of differences in mean seed set among the populations studied. The presence of asymmetry is a common phenomenon in interploidal crosses. More seed is produced when a population at a higher ploidal level is used as the maternal parent than the reverse (Stebbins 1958; Woodell and Valentine 1961; Ockendon 1968; Levin 1971).

Where workers have compared morphological and crossability data as indices of relationship (e.g., Vickery 1964; Mulcahy 1965; Ornduff 1966; Rhodes et al. 1968; Waser et al. 1982) it has frequently been found that crossing relationships are not entirely in accord with the patterns of morphological differentiation among accessions. This may be the result of genetic differences at one or a few loci involved in controlling crossability, as well as the degree of chromosome pairing and fertility of

hybrids (Lewis and Crowe 1958; Stebbins 1958; Riley and Chapman 1958; Pandey 1969; Levin 1971). In addition polyploidy will have a major influence on the crossing relationships among related populations. The phenetic analyses conducted in this study revealed associations of individuals of different ploidal levels (figs. 1–2). In contrast, such associations were not apparent following analysis of the crossability data where groupings were influenced in large part by ploidal level.

We suggest that the discrepancy between the data sets is a result of the occurrence of autopolyploidy or segmental allopolyploidy within the *Turnera ulmifolia* complex. Cytological studies by Raman and Kesavan (1964) and Arbo and Fernandez (1983) provide evidence for the occurrence of autoploidy in var. *elegans*. Polyploidization will result in the production of phenetically similar populations that differ in ploidal level. Differences in ploidal level generally lead to a reduction in seed set upon interploidal crosses (Stebbins 1958; Woodell and Valentine 1961; Levin 1971). Thus, phenetically similar populations related via polyploidy will not cluster in an analysis of crossability data, leading to differences among phenetic and crossability dendrograms. This hypothesis can best be tested by meiotic analysis of chromosome pairing (Ownbey 1950; Stebbins 1971; Jackson 1984) and by electrophoretic comparison of polyploids and their putative diploid progenitors (Roose and Gottlieb 1976; Gottlieb 1977; Crawford 1983). We are currently undertaking such studies to aid in clarifying the phylogenetic relationships among members of the *Turnera ulmifolia* complex.

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