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J. Kapteyn · P. B. Goldsbrough · J. E. Simon

Genetic relationships and diversity of commercially relevant *Echinacea* species

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Abstract The genus *Echinacea* is comprised of nine species, which are perennial herbs indigenous to North America and which have been traditionally used as medicinal plants for centuries. Three *Echinacea* species, *E. angustifolia*, *E. purpurea*, and *E. pallida*, are currently being traded internationally in the natural products market. *Echinacea* products constitute a significant portion of this growing, multi-billion dollar industry. The increasing popularity of *Echinacea* products has led to the expansion of wildcrafting and commercial cultivation to meet the growing demand for plant material. *Echinacea* is considered of value as a nonspecific immune stimulant, and claims of its efficacy have been tentatively supported by both laboratory and clinical studies. This study used random amplified polymorphic DNA (RAPD) markers to determine the genetic relationships of the three *Echinacea* species of commercial interest, to evaluate the level of diversity present within germplasm of each of the three species, and to compare accessions of each species available from different sources. A total of 101 RAPD markers were generated for the 76 individuals of four species included in the analysis. NTSYS-pc was used to evaluate the genetic relationships of the three species and to determine the general level of overall diversity. Analysis of molecular variance (AMOVA) was performed using pruned marker sets corrected for the dominant nature of RAPD markers. AMOVA revealed that most of the variation occurred within accessions of the same species, though some accessions of both *E. pallida* and *E. angustifolia* were found to be significantly different from other accessions of the same species.

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P.B. Goldsbrough
Department of Horticulture and Landscape Architecture,
Purdue University, West Lafayette, IN 47907, USA

J. Kapteyn · J.E. Simon (✉)
Center for New Use Agriculture and Natural Plant Products,
Rutgers University, New Brunswick, NJ 08901, USA
e-mail: jesimon@aesop.rutgers.edu
Fax: +1-732-9329441

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Introduction

Echinacea Moench species are indigenous to North America and are distributed throughout the eastern and central U.S. and southern Canada. Three *Echinacea* species, *E. purpurea* (L.) Moench, *E. pallida* (Nutt.) Nutt., and *E. angustifolia* DC var. angustifolia, are commercially important sources of herbal phytopharmaceuticals and natural product preparations. *Echinacea* is a true Native American medicinal plant and was used for a variety of medicinal purposes by various American Indian tribes. *Echinacea* entered mainstream herbal medicine over a century ago, and *Echinacea* products currently constitute a significant portion of the rapidly growing, multi-billion dollar natural products industry. This popularity has led to an interest in the expansion of commercial cultivation of these species, which has previously existed primarily for the production of ornamental perennials, to meet the increasing demands of the phytopharmaceutical market.

The long history and current popularity of *Echinacea* has spurred scientific investigation into the validity of claims of efficacy of plant extracts and the active constituents of those extracts. Over 200 publications have resulted from the search for the active principles in *Echinacea* since 1940 (Tyler 1993). The findings of recent reviews of clinical trials conclude that *Echinacea* products appear to be beneficial and safe, with the majority reporting positive results in the effect of *Echinacea* in moderating the incidence, duration and severity of symptoms associated with the common cold and acute upper respiratory infections (Barret et al. 1999; Percival 2000). This increasing body of laboratory and clinical pharmacological studies should contribute to the continued popularity of *Echinacea* products and serve to confirm the efficacy of biologically active compounds. Increasing the level of these compounds has become a central goal for improvement of *Echinacea* through traditional breed-

ing efforts or via biotechnology. Bauer and others have profiled the accumulation of various classes of compounds suspected of being biologically active in several species of *Echinacea* (Bauer et al. 1988; Bauer and Foster 1991; Bauer and Wagner 1991; Cheminat et al. 1988; Glowniak et al. 1996; Pietta et al. 1998), primarily for chemotaxonomic purposes and for the evaluation of their potential therapeutic value. However, no large-scale screenings of available *Echinacea* germplasm have been reported for the identification of accessions accumulating elevated levels of compounds with demonstrated biological activity. Such research would facilitate the development of an *Echinacea* breeding program with the goal of establishing lines specific for commercial cultivation to meet the demands of the natural products market.

Breeding efforts would also be facilitated by information regarding the genetic diversity present in available germplasm resources, such as lines available from commercial seed sources and from institutions such as the USDA National Plant Germplasm System (NPGS). Commercial supplies of *E. purpurea* are obtained from cultivated sources; *E. angustifolia* and *E. pallida* have been supplied largely from indigenous habitats in the United States (Foster 1993). The threat to the genetic diversity present in wild populations due to indiscriminant overharvesting and the need for preservation of these genetic resources creates an additional incentive for the determination of the genetic variability present within these three species.

The advent of DNA fingerprinting techniques such as (Random Amplified Polymorphic DNA) RAPDs (Williams et al. 1990) has allowed for the identification of taxa and the determination of phylogenetic relationships and intraspecific diversity at a molecular genetic level. The use of such techniques for germplasm characterization facilitates the conservation and utilization of plant genetic resources, permitting the identification of unique accessions or sources of genetically diverse germplasm. This research has applied the RAPD technique for this purpose using germplasm of the three commercially important *Echinacea* species to characterize the interspecific relationships, assess intraspecific diversity, and identify unique accessions at the level of the genome itself.

Materials and methods

Plant material

A total of 19 accessions of *Echinacea* species were used in this study, including one outgroup *E. atrorubens* (Norton) Cronquist accession. Table 1 lists the accessions and their sources. Seed from each accession was germinated after stratification with 2-chloroethylphosphonic acid (Sari et al. 1999), and at least six individuals of each accession were grown in a greenhouse at Purdue University, West Lafayette, Indiana. DNA was isolated separately from four plants of each accession for RAPD analysis.

Table 1 *Echinacea* accession codes, species, and source for material used in RAPD analysis

Code	Species	Source
ang2	*E. angustifolia*	Richters, Goodwood, Ontario, Canada
ang5	*E. angustifolia*	Prairie Moon Nursery, Winona, Min.
ang12	*E. angustifolia*	Prairie Nursery Inc., Westfield, Wis.
ang14	*E. angustifolia*	USDA Ames 14446
ang15	*E. angustifolia*	USDA PI 312814
ang16	*E. angustifolia*	USDA PI 421331
ang18	*E. angustifolia*	USDA PI 421372
atr30	*E. atrorubens*	USDA PI 597602
pal3	*E. pallida*	Richters
pal6	*E. pallida*	Prairie Moon Nursery
pal13	*E. pallida*	HT/MO Wild Flowers
pal17	*E. pallida*	Johnny's, Albion, Me.
pal19	*E. pallida*	USDA PI597603
pal20	*E. pallida*	USDA PI597604
pal21	*E. pallida*	USDA Ames 23368
pur1	*E. purpurea*	Richters
pur4	*E. purpurea*	Prairie Moon Nursery
pur9	*E. purpurea*	Seeds of Change, Santa Fe, N.M.
pur10	*E. purpurea*	Johnny's

DNA isolation and RAPD analysis

DNA was isolated from young leaf tissue using a modified method of Doyle and Doyle (1987). One gram of leaf tissue was ground to a fine powder in liquid nitrogen and added to 20 ml of CTAB buffer (1.4 *M* NaCl, 100 m*M* Tris–HCl pH 8.0, 20 m*M* EDTA pH 8.0, 2% CTAB, 1% PVPP, 0.25% β-mercaptoethanol). Samples were incubated at 65 °C for 60 min, 10 ml of chloroform-isoamyl alcohol (24:1) was then added and the mixture was gently mixed for 15 min. After centrifugation at 15,000 rpm for 10 min, the aqueous layer was removed, added to 50 ml of CTAB precipitation buffer (100 m*M* Tris–HCl pH 8.0, 20 m*M* pH 8.0, 2% CTAB, 0.25% β-mercaptoethanol), and allowed to incubate for 30 min at room temperature. The precipitated DNA was pelleted by centrifugation at 15,000 rpm for 10 min. DNA was dissolved in 4 ml of 1.0 *M* NaCl and subsequently re-precipitated with 2.5 volumes of ice-cold ethanol. The DNA was spooled, air-dried briefly, and resuspended in 300 µl TE.

DNA was quantified using a TKO 100 Fluorometer (Hoefer Scientific Instruments, Calif.). DNA samples for RAPD analysis were prepared at a concentration of 12.5 ng/µl. To verify the concentration and quality of the DNA, we ran 50 ng of each DNA sample on a 1.0% agarose gel and compared each sample against a DNA standard of known concentration.

RAPD reactions were performed in 25-µl aliquots containing 25 ng of template DNA, PCR buffer (50 m*M* KCl; 10 m*M* Tris–HCl pH 8.8; 0.1% Triton X-100), 3.0 m*M* $MgCl_2$, 0.25 m*M* each dNTP, 0.2 m*M* primer, and 1.25 U *Taq* DNA polymerase. Amplification reactions were performed in a Perkin-Elmer 9600 Thermal Cycler programmed for 40 cycles of 94 °C for 30 s, 45 °C for 60 s, and 72 °C for 60 s, with a 72 °C hold for 10 min after the completion of 40 cycles.

PCR products were separated on 1.6% agarose/0.5X TBE gels. Gels were stained in a 1 µg/ml ethidium bromide solution for 60 min, destained in dH_2O for 30 min, and visualized and photographed on a UV transilluminator.

Data analysis

RAPD products were scored for presence (1) or absence (0) of each amplicon evaluated. Only those bands that could be unequivocally scored across all samples were included in the analysis. Variations in intensity between bands of the same molecular weight across samples were not considered to be polymorphisms.

Pairwise similarity matrices were generated using three different similarity coefficients, and four clustering methods were then used to produce dendrograms, in the same manner as described by Mace et al. (1999). Cophenetic matrices were derived from the dendrograms using the COPH (co-phenetic values) program, and the goodness-of-fit of the clustering to the data matrix was calculated by comparing the original similarity matrices with the co-phenetic value matrices using the Mantel matrix correspondence test (Mantel 1967) in the MXCOMP program. Principal coordinate analysis (Gower 1966) was also performed, using DCENTER to double-center the similarity matrix and EIGEN to extract eigenvectors and to display the relationships in three dimensions. All of the above statistical procedures were performed using NTSYS-pc (Rohlf 1998).

To determine whether accessions of the same species differed from one another, we used Arlequin (Schneider et al. 2000) to analyze the population genetic structure of the RAPD data within each of the three species. Prior to AMOVA analysis using Arlequin, the haplotypic data for each species was pruned, removing those bands whose observed frequency was greater than or equal to 1-(3/N) to ensure that unbiased estimates of population-genetic parameters could be achieved (Lynch and Milligan 1994). Subsequent AMOVA analysis proceeded with 67 markers for *E. purpurea*, 70 for *E. pallida*, and 72 for *E. angustifolia*. For this analysis we assumed that each accession represented a separate population in Hardy-Weinberg equilibrium, with the four individuals of each accession representing the population sample. A matrix of Euclidian square distances was computed using the pairwise difference method. This matrix was used for the analysis of genetic structure including partitioning of variation among and within populations and the calculation of pairwise population Fst values, which were subsequently tested for significance.

Results

Initially, 57 primers were tested for their ability to generate amplification products. Of the 43 primers that produced amplification products, 22 were chosen for their ability to generate RAPD bands that could be scored without ambiguity. A total of 101 bands were scored (including 7 bands monomorphic across all samples), with an average of 4.6 bands scored per primer. The number of scoreable bands generated by a single primer ranged from as few as one to as many as eight. Product sizes ranged from 400 bp to 2,100 bp. The concentrations of several of the reactants and the thermal cycling conditions used in this study differed from those of Wolf et al. (1999) due to the independent application and optimization of the RAPD technique to *Echinacea* species. Many other visibly polymorphic fragments were generated, however they were not considered in the analysis due to their weak or non-reproducible amplification, or due to difficulties in resolving closely migrating fragments.

Several primers generated products that were useful for distinguishing among taxa. Seventeen diagnostic markers suitable for the discrimination of these three species plus *E. atrorubens* were identified (Table 2). A marker was considered diagnostic if it was present at a frequency equal to or greater than 0.95 for all individuals of a given taxon and present at a frequency lower than or equal to 0.05 for all individuals of each taxon being discriminated against. The reciprocal case was also considered to be diagnostic.

Table 2 Diagnostic RAPD markers identified capable of distinguishing among *Echinacea* species and their frequencies. The markers are listed alphabetically

Marker	*Echinacea* spp.			
	purpurea	*angustifolia*	*pallida*	*atrorubens*
A-01_{1175}	0.00	1.00	0.00	0.00
C-09_{514}	1.00	0.00	0.00	0.00
C-11_{677}	1.00	0.00	1.00	0.00
G-02_{1353}	0.00	0.00	0.00	1.00
G-04_{1395}	0.00	0.00	0.00	1.00
G-09_{1497}	1.00	0.00	0.00	0.00
H-13_{1126}	1.00	0.96	0.04	1.00
H-19_{1764}	0.00	0.96	0.04	0.75
O-13_{800}	0.00	0.96	0.00	0.00
X-03_{1157}	1.00	0.00	0.00	0.00
X-03_{549}	0.00	0.00	0.00	1.00
X-03_{504}	0.00	0.00	1.00	1.00
X-03_{463}	0.00	0.00	0.00	1.00
X-06_{1175}	1.00	0.00	0.07	0.00
X-06_{1118}	1.00	0.00	0.00	0.00
X-06_{482}	0.00	1.00	0.00	0.75
X-07_{633}	0.94	0.04	1.00	0.00

Table 3 Comparison of co-phenetic correlation values obtained from combinations of three similarity coefficients and four clustering methods employed for analysis of RAPD data

Clustering method	Similarity coefficients		
	Jaccard's	SM	DICE
UPGMA	0.981	0.973	0.972
WPGMA	0.978	0.972	0.970
Complete linkage	0.968	0.966	0.963
Single linkage	0.974	0.966	0.959

Pairwise genetic similarities (data not shown) generated using Jaccard's coefficient of similarity (Jaccard 1908) ranged from as low as 0.185 between pur4-2 and ang16-2 (*E. purpurea* and *E. angustifolia*, respectively) to as high as 0.978 between pur1-1 and pur4-3 (both *E. purpurea*). Genetic similarity was highest among *E. atrorubens* at 0.873, followed by *E. pallida* at 0.816, *E. purpurea* at 0.802, and *E. angustifolia* at 0.790. Only the mean similarity of *E. atrorubens* was significantly different from the other taxa ($P = 0.05$).

Cluster analysis of the genetic similarity values was performed to generate dendrograms illustrating the overall genetic relationships between the species studied and the accessions and individuals within those species. The dendrograms, constructed using four different clustering methods (UPGMA, WPGMA, complete linkage, and single linkage) for each of the three different similarity matrices generated using the Jaccard (1908); Dice (1945), and SM coefficients of similarity, were used to produce co-phenetic matrices. The correlation of the co-phenetic matrices derived from the dendrograms and the original similarity matrices was then compared. The UPGMA clustering method consistently produced dendrograms with stronger correlation to the original similarity matri-

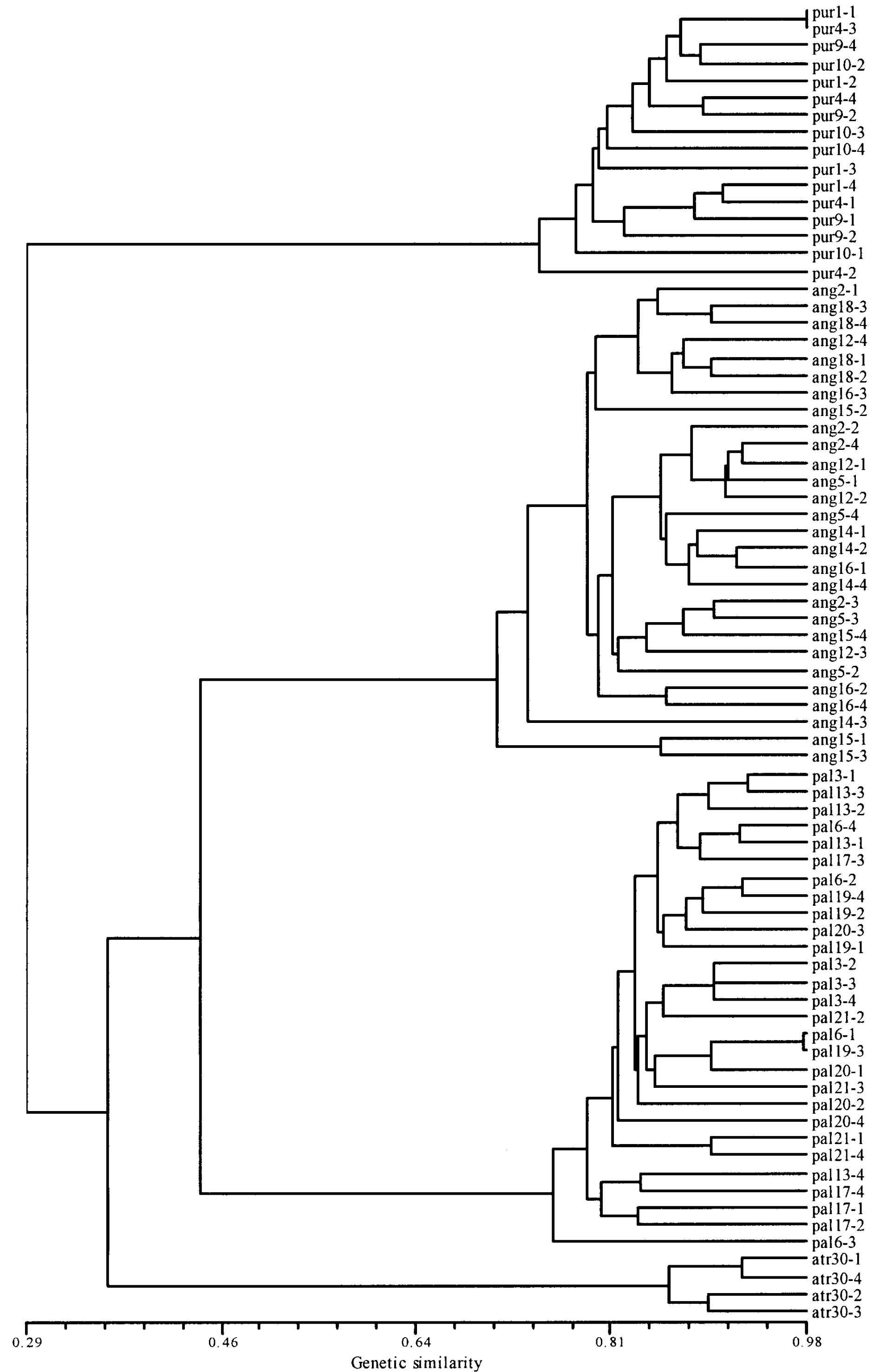

Fig. 1 Dendrogram showing the genetic relationships of 76 *Echinacea* individuals. The dendrogram was constructed using Jaccard's coefficient of similarity and UPGMA clustering. The individuals are labeled with the codes listed in Table 1, plus an individual designator

ces than the other clustering methods, and the matrix and dendrograms derived using Jaccard's coefficient of similarity in all cases gave the highest co-phenetic correlation values (Table 3). The dendrogram displayed in Fig. 1 was constructed using Jaccard's coefficient of similarity and UPGMA clustering, and all similarity values reported here were derived using Jaccard's coefficient of similarity. Four distinct clusters comprised of each of the four *Echinacea* species were formed. The *E. angustifolia* and *E. pallida* clusters were most closely linked, joining at a similarity of 0.45. The *E. atrorubens* cluster joins this cluster at a similarity of 0.37, and *E. purpurea* joins the cluster comprised of the previous three species at a similarity of 0.29.

Principle coordinates analysis was performed in addition to cluster analysis according to the recommendation

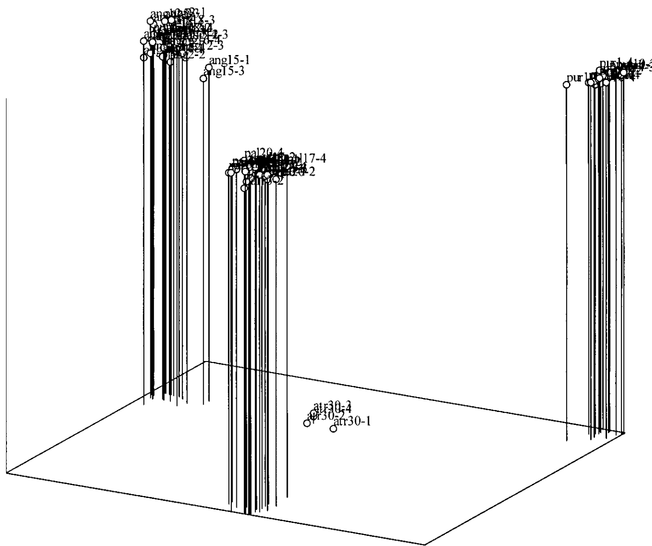

Fig. 2 Three-dimensional plot of the principle coordinates analysis of RAPD data. The samples are labeled with the codes listed in Table 1, plus an individual designator

of Sneath and Sokal (1973), and the ordinations displayed in three dimensions (Fig. 2). This method also clearly displays the intraspecific genetic similarity relative to the interspecific similarities, which are indicated by the distances of the four clusters relative to one another.

Application of the frequency parameters of Lynch and Milligan (1994) for obtaining unbiased estimates of population-genetic parameters to the data sets of each of the three *Echinacea* species separately resulted in the elimination of 34 markers for *E. purpurea*, 31 markers for *E. pallida*, and 29 markers for *E. angustifolia*. The remaining markers that satisfied the specified criteria were used in the subsequent AMOVA analysis of each species.

AMOVA analysis using the Arlequin program enabled a partitioning of the overall RAPD variation between the within accession and among accession covariance components (Table 4). AMOVA analysis did not reveal any significant differences between the *E. purpurea* accessions; all of the diversity (98.0%) was attributable to variation within the accessions. Variation was similar-

Table 4 Summary of AMOVA analysis. Statistics include: degrees of freedom (*df*), sum of squares (SSD), variance-component estimates (CV), and percentages of the total variance (% Total) contributed by each component

Analysis	Source of variation	*df*	SSD	CV	% Total
E. purpurea	Among populations	3	11.5	0.07	2.0 NS[a]
	Within populations	12	42.5	3.54	98
E. pallida	Among populations	6	32.4	0.62	17.5
	Within populations	21	31.5	2.93	82.6
E. angustifolia	Among populations	6	36.1	0.79	21.8
	Within populations	21	59.8	2.85	78.2

[a] NS, Not significant (significance tests after 1,023 permutations)

Table 5 Matrix of significant Fst *P* values for pairwise *E. pallida* accession comparisons. A “+” indicates a significant difference between accessions

Accession	pal3	pal6	pal13	pal17	pal19	pal20	pal21
pal3		–	+	+	+	+	–
pal6	–		+	+	–	–	–
pal13	+	+		–	+	+	–
pal17	+	+	–		+	+	+
pal19	+	–	+	+		–	–
pal20	+	–	+	+	–		–
pal21	–	–	–	+	–	–	

Table 6 Matrix of significant Fst *P* values for pairwise *E. angustifolia* accession comparisons. A "+" indicates a significant difference between accessions

Accession	ang2	ang5	ang12	ang14	ang15	ang16	ang18
ang2		–	–	+	–	–	+
ang5	–		–	–	–	–	+
ang12	–	–		+	–	–	+
ang14	+	–	+		+	–	+
ang15	–	–	–	+		+	+
ang16	–	–	–	–	+		+
ang18	+	+	+	+	+	+	

ly partitioned for *E. pallida* and *E. angustifolia*, with most variation again being found within the accessions (82.6% and 78.2%, respectively). Partitioning of variation between accessions was significant for both of these species, however.

Pairwise population comparisons were performed as the calculation of Fst values (data not shown) and a test of significance of these values using a non-parametric permutation approach (Excoffier et al. 1992). Tables 5 and 6 show matrices of the results of the tests of significance of Fst values for *E. pallida* and *E. angustifolia*, respectively. Of 21 pairwise population comparisons, 11 found accessions to be different for *E. pallida*, and 10 of 21 pairwise comparisons were found to be significant for *E. angustifolia*. Of particular note is accession ang18, which is significantly distinct from all other *E. angustifolia* accessions based on our analysis.

Discussion

RAPD markers were chosen to examine the inter- and intraspecific genetic diversity of the three commercially important *Echinacea* species because the RAPD technique can quickly and cost-effectively generate markers for species with no existing genomic sequence information, a condition that precludes the use other techniques, such as RFLPs and SSRs. The RAPD technique has been successfully used in a variety of taxonomic and genetic diversity studies (Jain et al. 1994; Li et al. 1999; McGrath et al. 1999; Nebauer et al. 1999; Rodriguez et al. 1999), and was found by Wolf et al. (1999) and by us to be suitable for use with *Echinacea* species in its ability to reproducibly generate polymorphic markers. As this study was being completed, Wolf et al. (1999) published a report demonstrating the utility of the RAPD technique for the discrimination of *E. purpurea*, *E. angustifolia*, and *E. pallida* using two different primers. Our study reports 17 RAPD markers capable of distinguishing among the commercially relevant *Echinacea* species and *E. atrorubens*, in addition to those reported by Wolf et al. (1999), and extends the application of these markers to the identification of the genetic relationships between the species and the diversity and structure present within the species. The large number of individuals from each of the three important taxa included in this study, obtained from commercial sources and germplasm repositories, along with the use of twice the number of loci recommended by Nei (1978), allows for a high degree of confidence in the conclusions presented. The complete concordance of the dendrograms produced using the different similarity coefficients and clustering methods (data not shown) also indicates that the data are robust in terms of the relative similarity between and within taxa.

The phylogenetic conclusions presented here are supported by other morphological and genetic studies. McGregor (1968), in his detailed monograph of the genus, provides morphological evidence for certain relationships within the genus. Our results are consistent with McGregor's conclusion as to the relative dissimilarity of *E. angustifolia* and *E. purpurea* based on morphological differences and geographic separation. *E. purpurea* is the most widespread species and is morphologically distinct from the other members of the genus (McGregor 1968). This distinctiveness is reflected at a genetic level by the relative distance of *E. purpurea* from the other three species in this study. *E. angustifolia* and *E. pallida* have been frequently confused due to their similar morpholgy, though they can be differentiated based on several macroscopic features, including plant height, pollen color, and ligule shape (McKeown 1999). Their overall morphological similarity is evidence of their close genetic relationship, a conclusion supported by the relative similarity between the two species shown here using RAPD markers.

Baskauf et al. (1994) assayed the genetic diversity present in natural populations of *E. angustifolia*, *E. purpurea*, and *E. tennesseensis* (Beadle) Small using isoenzyme analysis. The morphologically distinct *E. purpurea* provided a context in which to evaluate the genetic relationship of the other two species, which are considered to be closely related, and for which the separation into two taxa has been the subject of debate (McGregor 1968 vs. Cronquist 1980). The reported mean genetic identity values were 0.984 for *E. angustifolia* and 0.904 for *E. purpurea* (0.991 for *E. tennesseensis*). The interspecific identities were found to be significantly lower, with a pairwise identity of 0.826 for *E. angustifolia-E. tennesseensis* and 0.784 for *E. angustifolia–E. purpurea*. Their identification of a higher level of genetic similarity between the morphologically similar *E. angustifolia* and *E. tennesseensis* versus that for the distinct *E. angustifolia* and *E. purpurea* is congruent with our conclusion regarding *E. purpurea's* distance from the morphologically similar *E. angustifolia*, *E. pallida*, and *E. atrorubens*. The genetic variability for *E. angustifolia* and *E. purpurea* reported by Baskauf et al. (1994) is lower than that reported here, and the interspecific similarity higher for the pair, according to their study. This would be expected when assaying the variability of soluble enzymes versus the variability of the genome itself. RAPD analysis provides a broad survey of the genome, and RAPD polymorphisms may often represent variability in non-coding regions, which are more free to diverge.

Restriction site analysis of the chloroplast genomes of the tribe Heliantheae (Urbatsch and Jansen 1995), which included most species of *Echinacea* (but did not include *E. angustifolia*), revealed little diversity within the genus. The low levels of variation at the species level precluded firm phylogenetic conclusions, with the only strongly defined relationships consisting of *E. atrorubens* and *E. paradoxa* as sister taxa, which were collectively sister to *E. purpurea*. According to the consensus tree derived from their restriction site data, the relationship of *E. pallida* to the above taxa was more distant, though the significance with which this was shown was not strong. Subsequent integration of internal transcribed spacer region data (Urbatsch et al. 2000) with the above

chloroplast data contributed more resolution within *Echinacea*, resulting in a rearrangement of several branches, separation of species by a higher number of character differences, and more robust bootstrapping to support the branching. The second analysis, which again did not include *E. angustifolia*, resulted in *E. pallida* and *E. atrorubens* clustering together and joined by a much more distant branch to a cluster which included *E. purpurea*. Though the objective of their research was to determine the phylogeny of several genera of coneflowers and related genera, their conclusions regarding *Echinacea* are in agreement with the results of our RAPD marker analysis.

Partitioning of the RAPD variation between the within and among accessions levels revealed that most of the variation (78.2–98.0%) occurred within accessions, which would be expected for obligate outcrossing species such as *Echinacea* species. The observation that the highest within-accession variation occurred in *E. purpurea* and that there was no significant partitioning of variation among accessions was somewhat surprising given that each accession was obtained from a different source and that this species had been domesticated and bred for ornamental purposes for over a century. Though the accessions were not chosen for any visible phenotypic or known chemotypic differences, if these commercial offerings had been independently developed we would have expected some significant level of partitioning of variation between the accessions and a higher level of homogeneity within the accessions. Of greater interest is the lack of significant partitioning of variation between accessions of the other two species, especially between different accessions of the same species obtained from the USDA NPGS.

Evaluation of 16 pairwise population comparisons for the four *E. purpurea* accessions did not reveal any accessions as significantly different from the others. However, several accessions of both *E. pallida* and *E. angustifolia* were found to be distinct from other accessions and, conversely, some pairs of accessions of the same species obtained from the same source (USDA) were not found to be distinct based on our analysis. None of the three *E. pallida* accessions obtained from the USDA (PI597603, PI597604, Ames 23368) were found to be distinct from each other despite their documented origin from populations located in different states (Oklahoma, Kansas, and Nebraska, respectively). In contrast, all of the pairwise comparisons of *E. angustifolia* accessions from the USDA (Ames 14446, PI 421331, PI 421372, PI 597602) (Kansas, Nebraska, Oklahoma Nebraska, respectively) showed significant differences except for ang14–ang16 (Ames 14446–PI 421372). ang18 (USDA PI 597602) appeared to be unique from the other *E. angustifolia* accessions based on RAPD markers and could potentially serve as a source of unique genetic material for future breeding efforts. Information regarding the origins of seed from commercial sources could not be obtained. The lack of significant observable differences between the USDA *E. pallida* accessions may reflect continuous gene flow throughout the range of native populations, including those from which these accessions originated. The notable differences between several of the USDA *E. angustifolia* accessions may result from a more discontinuous distribution with obstructions to gene flow and some level of resulting differentiation between the original populations. The use of these RAPD markers, coupled with appropriate data handling and robust statistical analysis, can provide an efficient means of evaluating germplasm collections of *Echinacea* species, serving to identify unique genetic material as well as potential duplicate accessions and contributing to efficient conservation and management of *Echinacea* genetic resources. In conclusion, these results clearly demonstrate the utility of using RAPD markers to characterize interspecific relationships, evaluate germplasm diversity, and identify potential sources of unique genetic material in *Echinacea* species.

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