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A GENETIC ANALYSIS OF A RARE ARIZONA CACTUS: *ECHINOCEREUS TRIGLOCHIDIATUS* VAR. *ARIZONICUS*

by

Deborah Ann Alongi

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SIGNED: Deborah A. Alongi

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

David Mount — 5/6/96

David Mount
Professor of Molecular and Cellular Biology

Date

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ABSTRACT

Echinocereus triglochidiatus var. *arizonicus* is a federally endangered plant occurring in central Arizona. Since its 1979 listing, some botanists have questioned the definition of this taxon and its presumably limited range. Similar plants occurring in southeastern Arizona, classified by Benson (1982) as *E. t.* var. *neomexicanus*, are claimed to belong to the same taxon. RAPD analysis of plants from *E. t.* var. *arizonicus*, var. *neomexicanus* and the well-defined *E. t.* var. *triglochidiatus* were compared to determine genetic distance within and among groups. Genetic variability within groups was high and average genetic distance between groups nearly equal. Average heterozygosity levels within groups were not statistically different. RFLP analysis of noncoding chloroplast regions reveals a pattern of restriction sites and fragment lengths in three var. *neomexicanus* plants not present in plants of the other two taxa. The nature of the change was not determined but is consistent with a rearrangement of the region in question.

INTRODUCTION

Background

Echinocereus triglochidiatus var. *arizonicus* (Rose) L. Benson, commonly known as the Arizona hedgehog cactus, is a rare plant that occurs in central Arizona in the mountains and canyons between Globe and Superior. Historically, it has been thought to be endemic to this region (Benson 1982). It is a robust, dark green hedgehog cactus with a deep red "claret-cup" flower. In 1979, *E. t.* var. *arizonicus* was placed on the Federal endangered species list. The reasons for its endangerment were primarily illegal collecting, mining and mineral exploration, and degradation of its habitat due to livestock grazing (U.S. Fish and Wildlife Service 1979).

Since its listing, various botanists have questioned the definition of the taxon and its range, and confusion has surrounded its identification in the field. Each of the currently known varieties of *E. triglochidiatus* is highly variable with intergradation reported to occur between them (Benson 1982, Taylor 1985, Ferguson 1989). Fish and Wildlife personnel have expressed a need for improved understanding of *E. t.* var. *arizonicus* to help scientists and managers identify the plant in the field. Because of the plasticity and overlap of morphological characters in *E. t.* var. *arizonicus* and its close relatives, a genetic analysis may be of special benefit in clarifying the status of this taxon. Specifically, a comparison of *E. t.* var. *arizonicus* and other geographically nearby varieties of this species at the genetic level may shed light on the differences among these highly variable taxa.

Taxonomy

The taxonomy of the *E. triglochidiatus* species complex has been controversial for many years. Benson (1982) described eight varieties of *E. triglochidiatus* in The Cacti of the United States and Canada. In his introduction to the species he states:

> The status of *E. triglochidiatus* has been both misunderstood and hotly debated. The species is composed of a complex group of local populations, and the appearance of the extreme types differs remarkably. The most striking variable characters are stem size and the number, size and smoothness or angularity of the spines. Differing combinations of these readily visible characters produce extreme types that are seemingly distinct, and as a result many new "species" have been segregated. These determinations have resulted not from the study of natural populations in the field but from observation of a few plants in cultivation.

He concludes that "only extensive, intensive, and long-continued study of natural populations in the field can reveal the nature of the problems underlying the classification of the group." However, the documentation for Benson's classification of this species consists solely of a partial list of existing herbarium specimens. Taylor (1985) essentially followed Benson's (1982) classification of *E. triglochidiatus* in his book The Genus Echinocereus, although he departed from Benson's classification by recognizing *Echinocereus polyacanthus* as a separate species from *E. triglochidiatus*. Benson considered *E. polyacanthus* (now known to be tetraploid and reproductively isolated from the diploid *E. triglochidiatus*) to be a synonym for *E. t.* var. *neomexicanus*. Benson's (1982) *E. t.* var. *neomexicanus* included plants that are known to be *E. polyacanthus* and as such was polyphyletic.

Benson (1982) based his classification on: stem number and size; number of ribs; spine color, size and shape; flower measurements; elevation and floristic association. Based on variations in these characters he defined eight varieties of *E. triglochidiatus*, *E. t.* vars. *melanacanthus*, *mojavensis*, *neomexicanus*, *gurneyi*, *paucispinus*, *arizonicus*, *gonacanthus*, and *triglochidiatus*.

In recent years, chromosome counts of individuals of each variety have revealed that some of Benson's varieties are tetraploid and others are diploid (Pinkava et al. 1973, Pinkava et al. 1977; Weedin and Powell, 1978; Ross, 1981; Pinkava and Parfitt, 1982; Pinkava et al., 1985). *Echinocereus triglochidiatus* vars. *melanacanthus*, *paucispinous* and some members of var. *neomexicanus* are now known to be tetraploid. The criterion

of reproductive isolation requires the recognition of a new species for the tetraploid plants previously recognized as varieties of *E. triglochidiatus*. The tetraploid varieties now comprise the species *E. coccineus,* based on an early name for *E. t.* var. *melanocanthus* (Ferguson 1989, Powell et al. 1991, Hoffman 1992, Zimmerman 1992). This study concerns the diploid species, *E. triglochidiatus*, including *E. t.* vars. *arizonicus*, *mojavensis*, *triglochidiatus* and the diploid plants described by Benson as var. *neomexicanus*. Benson's (1982) classification of each of the diploid varieties of *E. triglochidiatus* is described below.

Variety *triglochidiatus*: Stems: Few, 15-30 cm long, ± 7.5 cm diameter. Stem ribs: 5-8, tuberculate. Spines (in general): Gray, to 1.9-2.5 cm long, nearly straight, 3-angled. Central spines: None (or rarely 1 and then like the radials; see below). Radial spines: 3-6, as long as central (when it is present), spreading or recurving, to 1.5 mm diameter. Flower: Broad, ±5 cm diam, 5-6.2 cm long. Style: No data. Elevation: 1,300-2070 m (4,350-6,900). Floristic association: Southern Juniper-Pinyon woodland.

Variety *mojavensis*: Stems: Many, up to 500, 3.8-7.5 (15) cm long, 2.5-5 (6.2) cm diameter. Stem ribs: Mostly 9 or 10, tuberculate. Spines (in general): Gray, pink, or at first straw-colored, to 4.4-7 cm long, curving and twisting, smooth or angled. Central spines: 1-2, light in color, usually twisting, often striate, to 0.7 mm basal diameter. Radial spines: 5-8, half to sometimes nearly as long as central(s), straight. Flower: Slender, ±3.8-5 cm diameter, 3-5(6.2) cm long. Style: 1 mm diameter; equal to or longer than perianth. Elevation: 1,050-2,400 (3,000) m (3,500-8,000 or 10,000 ft). Floristic association: Northern Juniper-Pinyon Woodland; desert-edge California Chaparral; upper Mojavean Desert; lower Rocky Mountain Montane Forest.

Variety *arizonicus*: Stems: Few, 22.5-40 cm long, 7.5-10 cm diameter. Stem ribs: ±10, tuberculate. Spines (in general): Dark gray (but radials pinkish-tan), to 2.5-

3.8 cm long, nearly straight, not angled. Central spines: 1-3, gray or pinkish, the largest deflexed, with minute striations, to 1.5 mm basal diameter. Radial spines: 5-11, shorter than central(s), often slightly curved. Flower: Broad, ±5 cm diameter, ±7 cm long. Style: 2 mm diameter; equal to perianth. Elevation: 1,050-1,410 m (3,500-4700 ft). Floristic association: Southwestern Oak Woodland; Southwestern Chaparral.

Variety *neomexicanus*: Stems: Mostly 5-45, 20-30 cm long, 7.5-10 cm diameter. Stem ribs: 8-12 (mostly 10), not markedly tuberculate. Spines (in general): Tan or pink, becoming light gray, to 3.8 cm long, nearly straight, not angled. Central spines: 2-4, gray, spreading, smooth, to 0.5-1+ mm basal diameter. Radial spines: 9-12, ± half as long as centrals, straight. Flower: Slender, ±3.8-5 cm diameter, 5-7 cm long. Style: 1 mm diameter; equal to or longer than perianth. Elevation: 1,350-2,100m (4,500-7,000 ft). Floristic association: Southwestern Oak Woodland and oak woodlands of Texas and northwest Mexico; Southern Juniper-Pinyon Woodland; Desert Grassland.

Since the publication of Benson's (1982) classification, some botanists working in the field have refuted his interpretations of the varieties of *E. triglochidiatus* (Ferguson 1989; Zimmerman, personal communication). The range of *E. t.* variety *arizonicus* was questioned by David Ferguson (1989) in a paper entitled "Revision of the U.S. members of the *Echinocereus triglochidiatus* group." Ferguson moved *E. t.* var. *arizonicus* into *E. coccineus*, assuming at the time that it was tetraploid. In his description of var. *arizonicus* he states, "This name was based upon plants of the robust, thick-spined type common in s.e. Arizona and s.w. New Mexico. The variety has traditionally been interpreted as including only those plants from the type locality, but this type of plant is in reality quite widespread." However, Ferguson presents no data to support this claim. Subsequent chromosome counts have repeatedly demonstrated that plants at the type locality of *E. t.* var. *arizonicus* are diploid and properly belong in *E. triglochidiatus*.

Figure 1a. *Echinocereus triglochidiatus* var. *arizonicus* at the type locality. A plant from site DA #6 (plant #6E).

Figure 1b. *E. t.* var. *arizonicus* near Devil's Canyon, approximately 4-5 miles from the type locality. A plant from site DA #4.

Figure 2a. Benson's *Echinocereus triglochidiatus* var. *neomexicanus* in the Gunnison Hills near Dragoon, Arizona. A plant from site AZ #2913.

Figure 2b. Benson's *E. t.* var. *neomexicanus* in the Chiricahua Mountains between Paradise and Portal, Arizona. A plant from site AZ #2915.

Figure 3a. *Echinocereus triglochidiatus* var. *triglochidiatus* near Tijeras Canyon, New Mexico. A plant from site DA #7.

Figure 3b. *E. t.* var. *triglochidiatus* near Alamogordo, New Mexico. A plant from site DA #10 (plant #10D).

The controversy surrounding the definition of *E. t.* var. *arizonicus* has caused confusion in the treatment and management of this endangered plant. Because Benson's *E. t.* var. *neomexicanus* included both diploid and tetraploid plants, the taxonomic standing of the diploid plants in southeastern Arizona is in question. Are these plants similar enough to *E. t.* var. *arizonicus* to belong in the same taxon? If so, the range of *E. t.* var. *arizonicus* is much larger than defined by Benson (1982). If not, the diploid plants in southeastern Arizona and southwestern New Mexico should be described and named as a separate variety of *E. triglochidiatus*.

To address this question, the degree of differentiation between accepted, presumably well-defined varieties of this species must be determined. How different are *E. t.* var. *arizonicus*, var. *triglochidiatus* and var. *mojavensis*? If the same degree of differentiation is present between *E. t.* var. *arizonicus* and the diploid plants in southeastern Arizona as is present between var. *arizonicus* and var. *triglochidiatus* or var. *mojavensis*, then it would seem reasonable to describe the SE Arizona plants as a separate variety. A genetic analysis to determine population genetic structure may help answer this question.

In addition, a genetic analysis can be used to make inferences about the genetic "health" of the rare and endangered *E. t.* var. *arizonicus*. Rare plants and animals whose populations are reduced often show decreased levels of genetic variation (Avise, 1994). Heterozygosity levels, most often detected with allozymes, have been used in many analyses of rare and endangered species to reveal genetic impoverishment. In non-selfing plant species, decreased genetic variation due to inbreeding depression appears to be potentially detrimental (Hunnekke, 1994). Lack of genetic variation within a population can influence the ability of individual members to exploit a patchy environment, to survive stochastic events, to maintain high levels of reproductive performance, or to adjust to novel or fluctuating environments (Hunnekke, 1994).

However, low heterozygosity levels should be interpreted with care. A causal link between reduced molecular variation and reduced population viability has not been established as a general rule. Different populations exhibit widely varying costs associated with reduced genetic variation due to inbreeding depression (Avise, 1994). Thus, each case should be evaluated individually.

Gene flow between populations is another central issue in this study. More than 1–2 effective migrants per generation can be enough to prevent significant differentiation between populations (Hartl and Clark, 1989). The pollination and seed dispersal biology of these plants is critical to understanding mechanisms of gene flow among these populations. Individuals of *E. triglochidiatus* are obligate out-crossers. They have red, cup- or funnel-shaped flowers with a long receptacle tube enclosing a large or very elongate nectar chamber that often holds a large quantity of nectar (Taylor, 1985). The flowers have been assumed to be hummingbird-pollinated because of their morphology (Taylor, 1985). However, a study of *E. coccineus*, the tetraploid counterpart to *E. triglochidiatus* which possesses similar flower morphology, revealed the most common pollinators visiting plants in a New Mexico population were two species of halictid bee: *Agapostemon* sp. and *Dialictus* sp. Pollen taken from the bodies and pollen sacs of these bees was from *E. coccineus*. No hummingbird visits were observed (Hoffman, 1992). The fruits of *E. triglochidiatus* are fleshy and juicy and often smell slightly of strawberries (Taylor, 1985). Seed dispersal is presumed to be by small mammals such as *Neotoma* species. It should be noted that no comprehensive study of the pollination or seed dispersal mechanisms in this species have been carried out.

Because individuals of *E. triglochidiatus* are long-lived perennials and obligate out-crossers, they are likely to exhibit relatively high levels of genetic variability within populations (Hamrick and Godt, 1989). If the major pollinators are bees, then long-

distance gene flow (50+ miles) between populations of different varieties would be limited. However, if migratory hummingbirds are pollinating these plants then gene flow across long distances seems plausible. The seed dispersal of *E. triglochidiatus* by small animals suggests that new plants become established close to their natal site. As a consequence, loci inherited in Mendelian fashion on the nuclear genome should reveal patterns consistent with levels of pollen flow between populations, and an analysis of the maternally inherited chloroplast genome should show results consistent with seed dispersal close the maternal parent.

The objectives of this study are (1) to test the hypothesis that diploid plants in southeastern Arizona are taxonomically the same as *E. triglochidiatus* var. *arizonicus* by determining the degree of genetic differentiation between *E. t.* var. *arizonicus* and *E. t.* var. *neomexicanus* as compared to the outgroup, *E. t.* var. *triglochidiatus*, and (2) to investigate the level of genetic variation within the rare and endangered var. *arizonicus* as compared to the more widespread taxa, var. *neomexicanus* and var. *triglochidiatus*.

Data from nuclear loci will provide estimates of genetic heterozygosity and allele frequencies and permit inferences about gene flow via pollen transfer. Data from chloroplast markers will provide evidence regarding gene flow via seed dispersal. If gene flow is occurring via pollen transfer, then nuclear loci should show similar heterozygosity levels and allele frequencies between populations. If gene flow is occurring via seed dispersal, then chloroplast markers should show a lack of differentiation between populations. Data from nuclear loci will reveal possible reduced genetic variation within *E. t.* var. *arizonicus* as compared to variation within var. *neomexicanus* and var. *triglochidiatus*.

Approach to the problem

Diploid plants from southeastern Arizona classified as *E. t.* var. *neomexicanus* by

Benson (1982) will be compared to plants from at or near the type locality of *E. t.* var. *arizonicus*. Both of these groups will be compared to plants of *E. t.* var. *triglochidiatus*. Variety *triglochidiatus* was chosen as an outgroup because its taxonomic status as a well-defined variety of this species is not in question, and because its range is close to that of both *E. t.* var. *arizonicus* and *E. t.* var. *neomexicanus*. These three groups are referred to throughout this study as var. *arizonicus*, var. *neomexicanus* and var. *triglochidiatus* to avoid confusion. Pictures of plants representative of each group are shown in Figures 1–3 on pp. 13–15.

To detect genetic differences at the varietal level, this study was directed at rapidly evolving DNA sequences. The random amplified polymorphic DNA (RAPD) technique (Williams et al., 1990) was chosen because it is relatively easy to apply, the genome is sampled randomly, and an essentially unlimited number of loci can be examined. Random amplified polymorphic DNAs are now commonly used to estimate genetic relationships among closely related populations or species of plants (Rieseberg, 1996). In particular, these markers have been used to determine the taxonomic identity of varieties, and in resolving the phylogenetic relationships of taxa (Arnold et al., 1991). RAPD loci are highly variable and are thought to most often come from noncoding regions (Lynch and Milligan 1994), making them useful for studies of populations at low taxonomic rank.

However, RAPD markers present some practical problems in population genetic analysis. Most RAPD markers are "dominant" in that the marker allele masks the presence of the null allele. As a consequence, only the null/null genotype can be observed. Both marker/null and marker/marker genotypes appear as a band and are indistinguishable. However, in spite of this difficulty, estimates of allele frequencies can be calculated using the statistical methods outlined by Lynch and Milligan (1994).

Another characteristic of RAPDs that must be considered when using this technique to estimate genetic relationships is the critical assumption that comigrating fragments in different individuals are homologous. A recent report of homology testing of RAPD fragments showed that 91% of 220 fragments tested were homologous, indicating that similarity of fragment size is a good indicator of homology, at least among closely related populations or species (Rieseberg, 1996). However, 13% (26) of the homologous loci were thought to be from paralogous regions (generated through gene duplication) rather than orthologous regions (derived via speciation). These findings indicate that RAPD data sets contain some noise. Steps to reduce noise in RAPD data sets recommended by Rieseberg include increased gel resolution of fragment size and bootstrapping of trees generated by genetic distance estimates to assess the internal consistency of the data and the strength of support for suggested relationships.

A second type of genetic analysis, restriction fragment length polymorphism analysis of noncoding regions of the chloroplast genome, was chosen to examine maternally inherited sequences. Species may exhibit qualitatively different patterns of geographic population structure at genes with biparental transmission (most nuclear loci) versus those transmitted through only one parent (Avise, 1994). Thus, noncoding regions of the maternally-inherited chloroplast genome may show different patterns of differentiation than the nuclear-encoded RAPD loci. Chloroplasts are assumed to be maternally inherited in *Echinocereus* as they are in the vast majority of flowering plants studied to date (Mogensen, 1996). Although the slow rate of evolution of chloroplast DNA at the structural and sequence levels has traditionally limited its use as a source of genetic variation below the species level (Banks and Birky, 1985; Palmer, 1987), intraspecific variation has recently proved more common than first believed (reviewed in Soltis et al., 1992). Using highly conserved primers flanking noncoding regions, amplification of

variable regions of the chloroplast genome are possible (Arnold et al., 1991; Demesure et al., 1995).

MATERIALS AND METHODS

Population Sampling

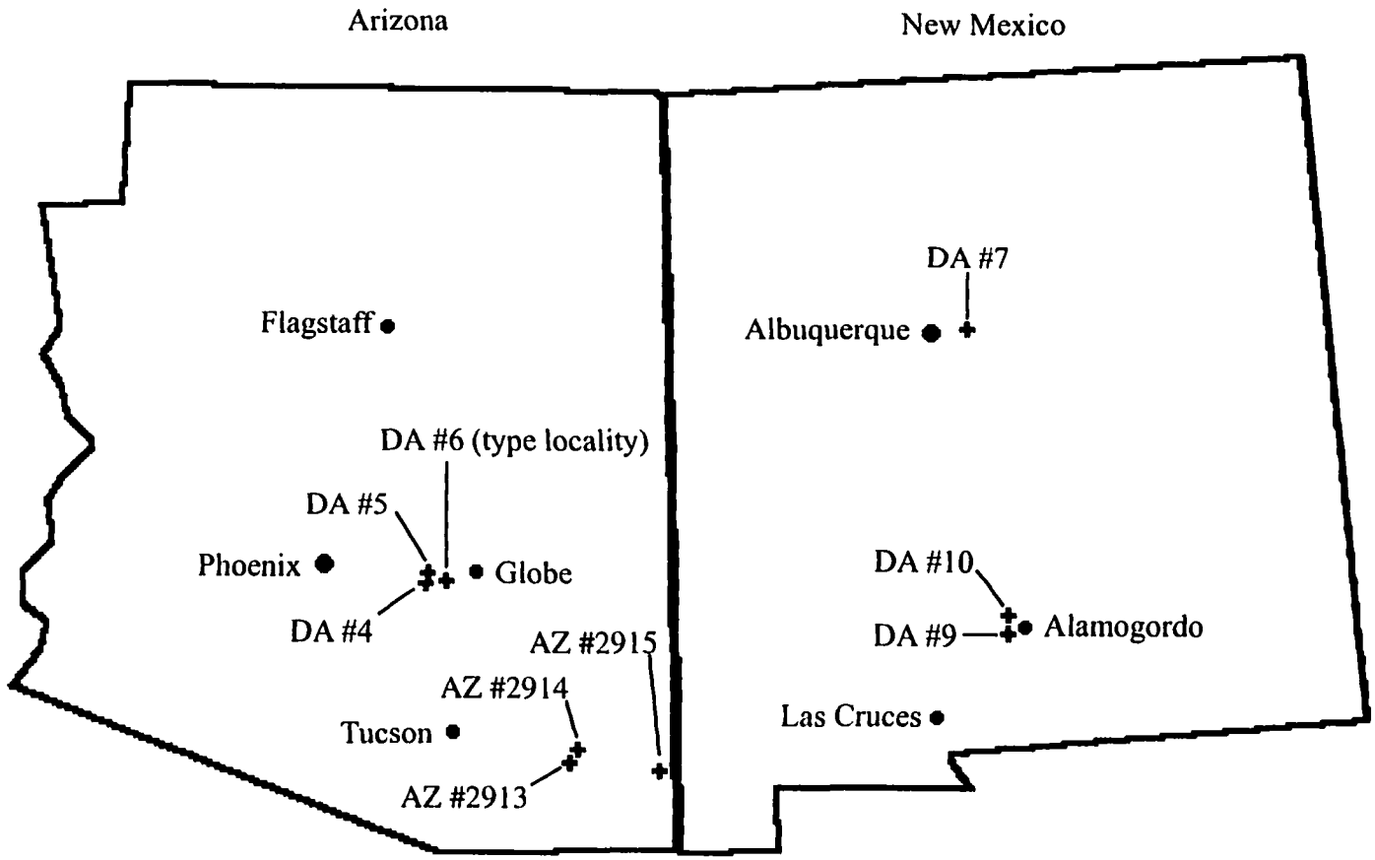
Within each of the three study taxa (*E. t.* var. *arizonicus*, *E. t.* var. *neomexicanus*, and *E. t.* var. *triglochidiatus*), 12 plants were sampled, for a total of 36 plants. Tissue was collected from each plant according to the method of Dr. Allan Zimmerman (personal communication): One small, easily accessible stem from a plant containing multiple stems was collected by cutting through the stem at the base of the plant. Several steps were taken to minimize potential damage to the plants as a result of collecting. Excess tissue was trimmed off of the remaining stump so that very little wet tissue was left exposed to the air, minimizing subsequent water loss. One or more rocks were then placed snugly over the cut surface to prevent herbivory by small animals. Each sampled plant was tagged and labeled with a collection number and recorded in my field notes. Photos were taken of many of the plants.

Within each taxon, four plants from each of three subpopulations were sampled. Plants were selected for sampling as they were discovered. If many plants were present in a small region, plants containing multiple stems were chosen over plants with one or a few stems. The locations of the subpopulations of each taxon are shown on the map in Figure 4 on page 22 and described in Appendix A.

DNA Isolation

Plant tissue was prepared for DNA isolation by first removing the spine clusters with a clean razor blade and then washing and drying the tissue. Stem tissue was trimmed to remove the interior storage and vascular tissue, saving the dark green chlorenchymatous tissue for DNA isolation. The tissue was cut into approximately 1.5 × 1 cm pieces and either extracted fresh or frozen at -70°C for later extraction. Half of the stem of one plant from each subpopulation was prepared for an herbarium voucher

Figure 4. Map of collection sites. *E. t.* var. *arizonicus* sites are DA #4, DA #5, and DA #6. *E. t.* var. *neomexicanus* sites are AZ #2913, AZ #2914, and AZ #2915. *E. t.* var. *triglochidiatus* sites are DA #7, DA #9, and DA #10.



specimen.

Attempts to isolate DNA from the tissue of *E. triglochidiatus* using standard CTAB DNA isolation methods (Doyle and Doyle, 1987) proved to be unsuccessful due to the presence of extremely large quantities of complex polysaccharides. During the phenol/chloroform extractions, the polysaccharides formed large coalesced precipitates extending through both aqueous and organic phases, making removal of the aqueous phase extremely difficult, and sometimes impossible. The polysaccharide molecules chelate to the DNA molecules, interfering both physically and chemically with the DNA isolation process (Robert Wallace, personal communication). A modification of a protocol developed by Robert Wallace at Iowa State University was used to eliminate most of the mucilage from the samples before beginning a "standard" CTAB extraction. This method is called the Organelle Pellet Method (Robert Wallace, personal communication).

With the Organelle Pellet Method, the nuclei and organelles remain intact during the grinding process. The plant tissue is ground in a blender in at least 50 volumes of an isotonic sorbitol buffer to dilute the mucilage. The grindate is then filtered and centrifuged, producing a pellet of nuclei, chloroplasts and other organelles. After the mucilaginous supernatant is poured off, the pellet is resuspended in hot CTAB extraction buffer and DNA isolation proceeds using a modification of the Doyle and Doyle method (1987).

Approximately 10 grams of fresh or frozen prepared tissue was placed in a chilled blender with just enough ice-cold (4°C) sorbitol grinding buffer to cover the tissue. If the tissue was frozen, it was allowed to thaw for a few minutes. The tissue was ground on high speed with short bursts until it was coarsely chopped. Then it was ground more thoroughly with 3-5 second bursts. More ice-cold sorbitol grinding buffer was added to bring the volume to at least 50 times the volume of the tissue (500 ml) and the mixture was again blended thoroughly on high speed. If the tissue appeared extremely mucilagi-

nous, the volume of grinding buffer was brought to 750 ml.

The grindate was filtered through four layers of cheesecloth with one layer of Miracloth™ on the bottom. It was allowed to drain mostly by gravity into a sterile, ice-cold flask. However, as filtering proceeded, gentle pressure was applied to force the fluid through the Miracloth more quickly (20-30 minutes) than gravity alone would allow.

The filtrate was poured into clean 250 ml centrifuge bottles and spun in a Sorvall GSA rotor at 3,000 rpm for 90 minutes. If the filtrate was extremely mucilaginous, centrifugation was continued for another 30 minutes. The supernatant was gently poured off while holding the pellet "up". If the pellets were "tight" enough, the bottles were inverted briefly to drain off as much of the mucilaginous supernatant as possible.

The pellets were resuspended in approximately 10-15 ml of hot (60°C) CTAB extraction buffer. The suspension was poured into 50 ml centrifuge tubes, 1 ml of 5% Sarkosyl was added to lyse the organellar membranes, and the solution was stirred well. The solution became viscous upon stirring. The samples were incubated for at least 1 hour at 60°C and then extracted with an equal volume of a 1:1 phenol-chloroform solution. The samples were spun at 6,000 rpm for ten minutes and the aqueous phase transferred to a new, sterile 50 ml tube where it was extracted again with 24:1 chloroform-isolamyl alcohol and spun. The clear, viscous aqueous phase was pipetted off and the DNA precipitated with ice-cold isopropanol. The precipitate was spooled on a glass hook and resuspended in about 1 ml of TE buffer, depending upon the amount of precipitate.

Quantification of the DNA samples was initially performed with spectrophotometry. The genomic DNA samples were then checked for quality/degradation by loading approximately 1 μg of sample per well of a 0.8% agarose gel. Later quantification of the amount of DNA in each sample was performed with fluorometry.

Recipes for buffer solutions are listed in Appendix B.

Random Amplified Polymorphic DNA Analysis (RAPDs)

Procedure

DNA from all 36 plants was amplified with decanucleotide primers of random sequence purchased from Operon Technologies. Seventeen primers were chosen after primer screens showed that each amplified several distinct loci. The sequences of these primers are listed in Appendix C. PCR reaction mixtures contained 25 ng DNA, 10 mM Tris-Cl (pH 8.8), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% w/v gelatin, 50 µM each dATP, dCTP, dGTP, dTTP, 1 unit Taq polymerase (Stratagene) and 0.5 mM primer, for a total reaction volume of 30 µl. Reactions were carried out in a Cetus/Perkin-Elmer thermocycler with the following reaction conditions: 45 cycles at 94° for 30 seconds, 40° for 30 seconds, and 72° for 2 minutes. The amplified products were then size-fractionated on a 1.4% agarose gel containing ethidium bromide and visualized under UV illumination.

Analysis of Data

RAPD loci were scored for presence or absence of the marker allele in each plant given the following assumptions and interpretations: (1) Within a population, each RAPD locus was assumed to be a two allele system: a "marker allele" represented by the presence of a band and a "null allele" represented by the absence of a band at that locus. (2) Amplification of a RAPD band of the same approximate size in two plants was interpreted to mean that the plants share a particular DNA sequence and are similar in the corresponding regions of the plant genome. (3) Absence of a particular RAPD marker allele in two plants in different populations was not used to indicate relatedness, because many genetic changes could cause such an absence. (4) It is not possible to differentiate between plants with a given RAPD marker allele on one chromosome (heterozygotes) and plants with the marker on both chromosomes (homozygotes). Because of these

properties of RAPD markers, only the frequency of bands *shared* between plants was calculated for each plant pair and was used as an indicator of genetic relatedness.

Genetic distances were calculated for each plant pair, both within population and between population, using the formula,

$$D_{xy} = 1 - \frac{n_{xy}}{n_x + n_x - n_{xy}},$$

where n_{XY} is the number of shared marker alleles between plants X and Y, n_X is the number of marker alleles present across all loci in plant X, and n_Y is the number of marker alleles present across all loci in plant Y (Marsolais et al 1993). This genetic distance formula does not count the *shared absence* of a band as an indicator of relatedness because it cannot be safely assumed that plants in different populations have lost or gained a RAPD primer site in the same manner. The mean genetic distance and standard deviation were calculated both within and between populations. The range of genetic distance, mean and standard deviation were graphed for each population to show overlap. Statistical tests to determine whether or not the genetic distance means are different, such as analysis of variance, student's t-test, or the nonparametric equivalents of these tests, were not useful because of the nonindependence of the data.

A triangular matrix of genetic distances between each pair of individual plants was used to construct a dendrogram with the neighbor-joining method (Saitou and Nei, 1987; PHYLIP version 3.5). The dendrogram was constructed using a plant (DA #7A) from *E. t.* var. *triglochidiatus* as the "outgroup."

As a measure of "gene diversity," average heterozygosities were calculated from RAPD data using the method of Lynch and Milligan (1994). Only those loci in which marker alleles could be unambiguously scored as present or absent were utilized. The gels were scored by at least two persons to verify the presence or absence of bands. The

loci utilized for the heterozygosity analysis were limited to those in which the marker allele was present in less than 1-(3/N) × N plants. In this case, only those loci in which the marker allele was present in 8 or fewer plants were used in the analysis (1 - 3/12 = 0.75 × 12 = 8). The loci used in the analysis were "pruned" in this way because the frequencies of the marker and null alleles are estimated from the frequency of the null/ null genotype. When the null/null geneotype is rare in a population, the estimates are less accurate (Lynch and Milligan, 1994). Expected heterozygosity (H) under Hardy-Weinberg equilibrium was estimated from allele frequencies for each locus. The assumption of Hardy-Weinberg equilibrium for the populations in this study appears to be appropriate with the possible exception of the rare variety *arizonicus*. The populations of var. *neomexicanus* and var. *triglochidiatus* are widespread and mating should be "random" as these plants are obligate out-crossers. Average heterozygosities were then calculated for each taxon along with an estimate of the standard error of the mean.

To test statistically whether or not the mean heterozygosities were homogeneous across taxa, a test of the difference between the means was undertaken. The mean standard error was calculated according to standard methods (Snedcor, 1956) and the difference between the means calculated with a t statistic for a 95% confidence interval. If the range of the difference between the means was found to contain 0, the means of the taxa were assumed to be the same.

To determine whether the three taxa are in genetic equilibrium, i.e. whether there is gene flow, with respect to RAPD loci, the upper and lower limits of the 95% confidence intervals for the frequency of the null allele at 15 loci were compared across all three taxa. Taxa in which the frequency of the null alleles overlapped were noted as well as those taxa in which the frequencies did not overlap. This test was done to determine whether or not the frequencies of the alleles at polymorphic loci were the same across

taxa. The assumption is that similar allele frequencies may indicate gene flow between populations, and differing allele frequencies may indicate selection or drift in isolated populations experiencing little gene flow between them.

Restriction fragment length polymorphisms in non-coding chloroplast regions

Procedure

The intergenic spacer region adjacent to the *rbc*L gene was amplified using the ORF106 and Z1264 primers (ORF106, 5'-ACTACAGATCTCATACTACCCC-3'; Z1264, 5'-GTAGCTTTAGAAGCCTGTGTACAAGCTCGTAA-3') (Arnold et al 1991). PCR amplification of this cpDNA region was accomplished with the following reaction mixtures (30-50 µl): 100 mM Tris•HCl (pH 8.8); 500 mM KCl; 15 mM MgCl2; 1 mM each dATP, dTTP, dCTP, and dGTP; 50 pmol of each primer; 25-50 ng of genomic DNA; 5.0% glycerol; and 0.5 units of *Taq* DNA polymerase (Stratagene). The amplifications were carried out in a Perkin-Elmer/Cetus DNA thermocycler using one cycle of two minutes at 95°C, 30 cycles at 95°C for 45 seconds, 52°C for 45 seconds, 72°C for 1 minute with a 4 second extension every 3rd cycle, and 1 cycle at 72°C for five minutes.

The non-coding region between the coding regions for two tRNA genes was also amplified using primer *trn*C [tRNA-Cys(GCA), 5'-CCAGTTCAAATCTGGGTGTC-3'] and primer *trn*D [tRNA-Asp (GUC), 5'-GGGATTGTAGTTCAATTGGT-5'] (Demusure et al 1995). PCR amplification of this cpDNA region was accomplished with the following reaction mixtures (50 µl): 100 mM Tris•HCl (pH 8.8); 500 mM KCl; 15 mM MgCl2; 1 mM each dATP, dTTP, dCTP, and dGTP; 0.2 µM of each primer; 25-50 ng of genomic DNA; 5.0% glycerol; 1 ng of BSA; and 0.5 units of *Taq* DNA polymerase (Stratagene). The amplifications were carried out in a Perkin-Elmer/Cetus DNA thermocycler using one cycle of 4 minutes at 94°C, 30 cycles of 45 seconds at 92°C, 45 seconds at 58°C, and 4 minutes at 72°C, and 1 cycle of 10 minutes at 72°C.

All plants were screened for amplification with each primer pair. Because amplification of these regions was successful in only some plants, one plant that amplified well from each subpopulation was chosen for RFLP analysis (3 plants per taxon, 9 plants total with each primer pair). This was done because there were some subpopulations in which only one plant amplified, and it was assumed that plants in each subpopulation would exhibit the same chloroplast profile. PCR product from each reaction was digested with 4 base-pair cutting enzymes. Restriction digest reactions contained 8–12 µl amplification product, 1–4 units of restriction endonuclease, 2.5 µl of enzyme buffer solution per vendor, and 1 ng of BSA as required in a 25 µl reaction. Reactions were incubated at 37°C overnight (14–16 hours). Restriction fragments were size-fractionated on 2% agarose gels containing ethidium bromide and visualized under UV illumination.

Analysis of Data

Restriction digests were analyzed for detectable polymorphisms by scoring the number of fragments produced by plants in each taxon. The number of fragments shared between each pair of taxa was then scored. The percent nucleotide substitution between two taxa was calculated from the proportion of shared fragments using the method of Upholt (1977). The proportion of shared fragments was calculated with the formula

$$F = \frac{2N_{xy}}{(N_x + N_y)}$$

where N_x is the number of bands in taxon X, N_y is the number of bands in taxon Y and N_{xy} is the number of bands shared between taxa X and Y. The percent nucleotide substitution is then calculated by

$$p = 1 - [0.5\{-F + (F^2 + 8F)^{0.5}\}]^{1/r}$$

where r is the number of base pairs in the enzyme recognition site. The percent nucleotide substitution was then compared between each pair of taxa.

RESULTS AND DISCUSSION

DNA Isolation

Genomic DNA isolation was difficult as described in Materials and Methods. The precipitates contained both clear and whitish strands, and were usually very copious. I believe the clear portion of the precipitates were polysaccharide molecules co-precipitating with the DNA (whitish strands); however, the precipitates were not assayed to confirm this. It was impossible to separate the clear from the white precipitates when spooling them. However, spooling was necessary because the clear gelatinous precipitate prevented a tight pellet from forming upon centrifugation. As a result, the samples of DNA resuspended in TE buffer still contained a lot of complex polysaccharides, some samples being very viscous.

Quantification of the DNA in each sample also proved difficult. Quantification using UV spectrophotometry yielded values between 65 and 790 ng/µl. Sample amounts varied between 200 and 1,300 µl, indicating recovery of very large amounts of DNA. However, a quality check of the DNA on a 0.8% agarose gel revealed the presence of much smaller quantities of DNA in each sample than suggested by spectrophotometry. The quality check did reveal that the DNA was not degraded.

Quantification using fluorometry revealed 10- to 100-fold lower yields compared to the concentrations determined by UV spectrophotometry. DNA concentrations with this method ranged from 2-73 ng/µl, most samples ranging between 5 and 20 ng/µl.

The difficulty in isolating clean DNA from the tissue of *E. triglochidiatus* did not prevent successful RAPD marker amplification. However, because of the difficulty in quantifying the concentration of DNA samples, the DNA concentration in PCR reactions for each plant are likely different. I diluted my DNA samples to what I estimated to be 0.25 ng/µl. However, in reality, the concentrations were probably variable. In a few

plants, the RAPD bands were noticeably fainter, probably due to low concentrations of DNA in the PCR reaction. However, the lower concentration of DNA in these plants did not seem to alter the RAPD markers amplified with each primer. Repeat amplification using the same RAPD primer and increased amounts of DNA produced the same banding patterns, with brighter bands.

Amplification of noncoding chloroplast regions with primer pairs ORF106/Z1264 (intergenic spacer adjacent to *rbc*L gene) and *trn*C/*trn*D (noncoding region between two tRNA genes) proved to be more difficult, especially for the *rbc*L spacer. In only 22 of the 36 plant DNA samples was I able to get amplification of the *rbc*L spacer. However, at least one plant from each subpopulation was amplifiable for this region. Amplification with the *trn*C/*trn*D primer pair was more successful, yielding amplification in 29 out of 36 plants, again for at least one plant from each subpopulation. Possible reasons for the difficulty in chloroplast amplification include varying concentrations of chloroplast DNA in each sample or varying degrees of polysaccharide contamination in each sample. Centrifugation of the DNA samples in a cesium chloride gradient would eliminate the polysaccharide contamination, and likely make amplification with specific primers more successful (Robert Wallace, personal communication).

Random Amplified Polymorphic DNA Analysis

Genetic analysis using the Random Amplified Polymphormic DNA (RAPD) method revealed a high degree of detectable polymorphism within populations. Seventeen primers were amplified yielding a total of 85 loci. The data for each of the three taxa are shown in Appendix D. Of those 85 loci, 51 were detectably polymorphic in at least one of the taxa. *E. t.* var. *arizonicus* had the most detectably polymorphic loci (39/85). *E. t.* var. *neomexicanus* had 32/85 detectably polymorphic loci, and 34/85 were detectably polymorphic in var.*triglochidiatus*. Table 1 shows the 51 polymorphic loci scored for all

Table 1. 51 polymorphic RAPD loci scored for presence (1) or absence (0) of marker allele across all three taxa. Plants 4A-6E are *E. t.* var. *arizonicus*, plants 13A-15D are *E. t.* var. *neomexicanus* and plants 7A-10D are *E. t.* var. *triglochidiatus*.

RAPD Locus	4A	4C	4D	4E	5A	5B	5C	5D	6A	6B	6D	6E	13A	13B	13C	13D	14A	14B	14C	14D	15A	15B	15C	15D	7A	7B	7C	7D	9A	9B	9C	9D	10A	10B	10C	10D
OPG4 0.30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPG8 1.18	1	0	0	0	1	0	1	1	0	0	0	1	0	1	1	1	0	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	0
OPG8 1.08	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OPG8 0.65	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OPG8 0.45	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
OPG11 1.56	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OPG11 1.74	0	0	0	1	0	1	1	0	0	1	0	0	0	0	1	1	1	1	0	1	1	0	1	0	1	0	0	0	1	0	1	0	0	0	0	0
OPG11 0.79	0	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	1	0	1	1	1	1	1
OPG11 0.59	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	0	1	0	0	1	1	1	0	1	1	0
OPG11 0.53	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
OPG11 0.51	0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPG9 1.18	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1
OPG12 1.75	1	0	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	0	1	0	0	1	1	0	1	0	1	1	1	1	1	1	1	1	1
OPG12 1.28	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1	0	0	1	0	0	0	0	1	1	1	1	1	0	0	1	1	1
OPG12 0.76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	0	0
OPG12 0.41	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OPG12 0.32	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1
OPG18 1.20	1	1	0	0	1	0	1	1	1	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	1
OPG18 1.15	1	0	0	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
OPG18 1.04	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
OPG18 0.46	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OPG18 0.40	1	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OPG18 0.31	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1
OPH7 1.25	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
OPH7 0.98	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	0	1	0	1	1	1
OPH7 0.80	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
OPH7 0.33	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	0	1	1	0	1
OPG19 1.20	1	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OPG19 1.14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
OPG19 1.02	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1
OPH12 1.25	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
OPH12 0.61	1	0	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1

Table 1 - continued. 51 polymorphic RAPD loci scored for presence (1) or absence (0) of marker allele across all three taxa.

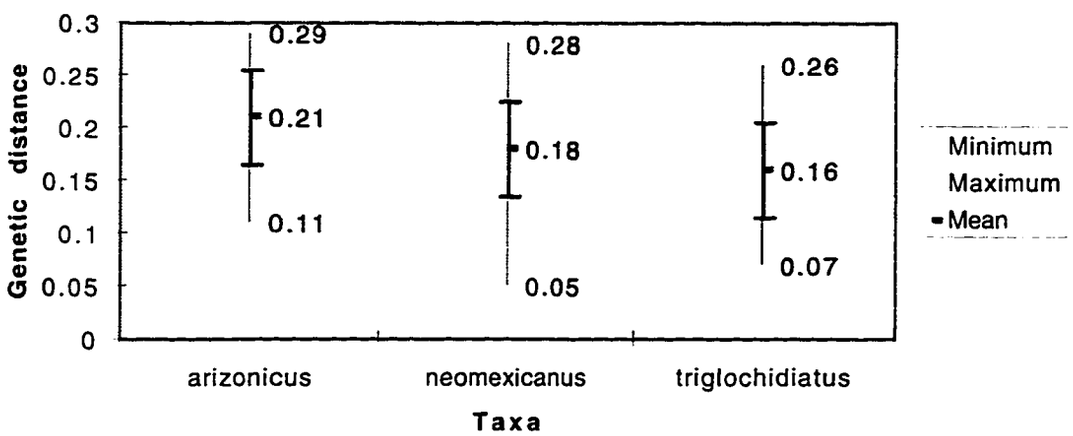
OPH12 0.42	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
OPH14 0.72	1	1	1	0	0	1	1	0	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	0	0	1	0	1	1
OPH14 0.43	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OPH14 0.39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1
OPH15 1.40	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0
OPH15 0.89	1	0	0	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0
OPH15 0.58	1	1	0	0	1	1	1	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1
OPH20 1.20	1	1	0	0	0	1	0	0	0	1	1	1	0	0	0	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.20	1	0	0	1	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
OPH2 1.05	0	1	0	0	0	1	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0
OPH3 1.15	0	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	0	0	1	1	0
OPH3 1.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OPH4 0.80	0	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
OPH17 0.70	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
OPH1 1.58	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0
OPH1 O.73	0	0	0	0	1	0	0	0	1	1	0	0	1	1	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0
OPH1 0.52	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	0	0	0
OPH1 0.89	0	0	0	1	0	1	1	1	1	1	0	0	0	0	1	1	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0
OPH1 0.65	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

plants across all three taxa.

Genetic distance within and between populations

Pairwise comparisons revealed genetic distances within populations to be highly variable. Genetic distances between plants within var. *arizonicus* ranged from 0.11 to 0.29 (mean 0.21). Within var. *neomexicanus* and var. *triglochidiatus* genetic distances ranged from 0.05 to 0.28 (mean 0.18) and 0.07 and 0.26 (mean 0.16), respectively. The genetic distances between all plants within populations are given in Appendix E. The standard deviation of the mean for each population is ±0.044. Figure 4 shows the genetic distance ranges, means and standard deviation of the means for each taxon. It was not possible to do an analysis of variance (or its non-parametric equivalent) to test whether the mean genetic distances were different because of the nonindependence of the data. The genetic distance data is nonindependent due to multiple comparisons of each plant to all other plants. The overlap between the ranges and standard deviations suggests that the means are not different. However, in the absence of a test it is not possible to form a conclusion. All three populations exhibit a high degree of variability within population.

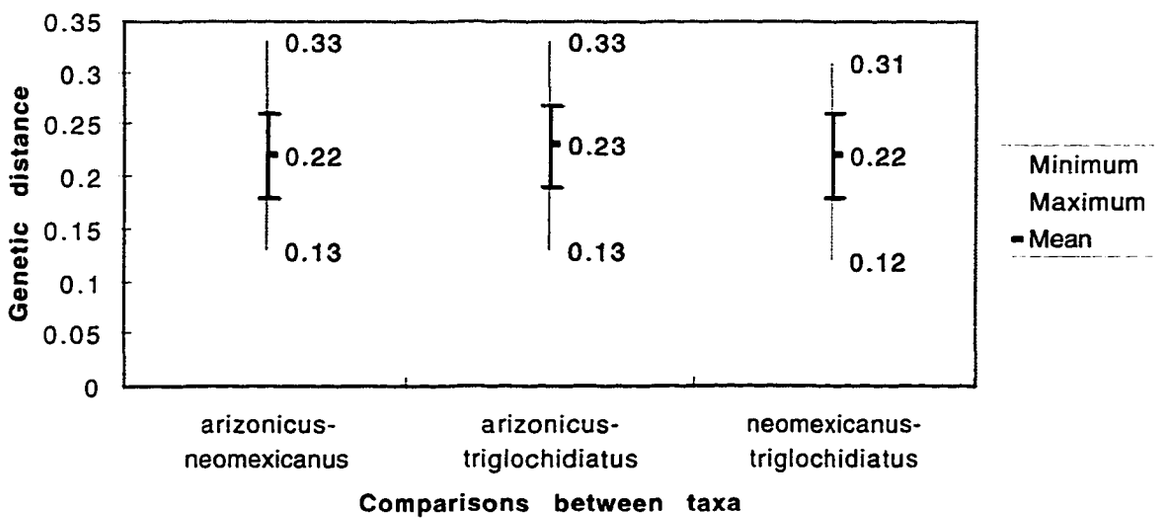
Figure 5. Within-taxon genetic distance ranges, means, and standard deviation of the means.



Pairwise comparisons *between* taxa revealed slightly higher genetic distances than within taxa. The pairwise genetic distances between individuals belonging to different

taxa are given in Appendix F. Genetic distance between plants in different taxa was highly variable, ranging from 0.12 to 0.33. Figure 5 shows the ranges, means and standard deviation of the means between each pair of taxa. The standard deviation of the mean genetic distance between var. *arizonicus* and var. *neomexicanus* was ±0.043, and the standard deviation between var. *arizonicus*/var. *triglochidiatus* and var. *neomexicanus*/var. *triglochidiatus* was ±0.040. Given these standard deviations for the means of the pairwise genetic distances between taxa, the mean genetic distances between taxa cannot be considered different. The high degree of overlap between the means in each comparison suggests there is no significant difference in genetic distance between any of the three varieties. Again, it was not possible to do an analysis of variance (or non-parametric equivalents) to test the hypothesis that the means are the same because of the nonindependence of the data.

Figure 6. Between-taxon genetic distance ranges, means, and standard deviation of the means.



Average heterozygosity within and between population

Average heterozygosity values and standard errors for each of the three varieties are as follows: *E. t.* var. *arizoncus* 0.27 (± 0.035), *neomexicanus* 0.24 (± 0.036) and

triglochidiatus 0.23 (± 0.041). The 95% confidence interval for each average heterozygosity value is: var.*arizonicus* 0.19–0.35, var. *neomexicanus* 0.16–0.32, and var. *triglochidiatus* 0.14–0.32. These ranges all overlap, indicating no significant differences in the amount of intrapopulation variation. A student's t-test of the difference between the means was performed (Snedecor, 1956), assuming normal frequency distributions. The tests revealed the upper and lower limits of the 97.5% confidence intervals for the difference between the means of each pair of taxa include 0 (Table 2). Thus, the null hypothesis is not rejected; the mean average heterozygosities within each taxon are not statistically different.

Table 2. Confidence intervals of differences between mean heterozygosity levels.

Paired taxa	Upper and lower limits of 97.5% confidence intervals for the difference between the means of each pair of taxa
var. *arizonicus*- var. *neomexicanus*	-0.13 to 0.19
var. *arizonicus*- var. *triglochidiatus*	-0.13 to 0.21
var. *neomexicanus*- var. *triglochidiatus*	-0.16 to 0.18

The results from these RAPD data are consistent with other studies of genetic variation within and between plant populations. However, relatively few studies have assessed RAPD variation among native plants. Most studies of population genetic structure to date have used allozyme markers (Hamrick and Godt, 1989). Allozymes have been shown to be useful in providing information on subspecific population genetics of plants and the detection of genetic variation within populations. In a detailed review of allozyme analyses of some 450 plant species, it has been shown that widespread species exhibit greater genetic diversity than do narrowly distributed species, that genetic diver-

sity is greater at both the species and population levels for outcrossers than for selfers, and that long-lived, wind-pollinated perennials exhibit greater within than among population variation (Hamrick and Godt, 1989).

In an attempt to correlate allozyme and RAPD diversity, a comparison of allozyme and RAPD markers was undertaken by Peakall, et al. (1995). They analyzed genetic variation within and between populations of buffalograss (*Buchloë dactyloides*) and found that overall patterns within and among populations were concordant between RAPD and allozyme markers, but that RAPD markers detect more genetic variation among individuals. They found considerable RAPD variation within populations, with each of 48 individuals being genetically unique. Variation was generally less among populations than within, and variation among primarily sexually reproducing populations was lower than among primarily vegetatively reproducing populations. An unrooted tree based on 98 RAPD loci produced good separation of populations but was not concordant with a tree based on allozyme markers.

While there are few studies using RAPD markers to investigate population genetic structure of native plants, there are even fewer genetic analyses of cactus species. Wallace and Fairbrothers (1986) analyzed allozymes in an asexually reproducing cactus species, *Opuntia humifusa*. They found within-population enzymatic variation to be absent at a minimum of 15 loci examined with the exception of one population. Between population proteins were found to be "generally similar with some variability." Parker and Hamrick (1992) used allozymes to investigate the population genetic structure of the clonal species *Lophocereus schottii* in southern Arizona. They found mean allozyme diversity at the species level to be 0.145 and within-population diversity to be 0.126. Diversity was calculated for each locus by $H_e = 1 - \sum p_i^2$, where p_i is the frequency of the ith allele, averaged over all loci in each population for the within-popuation value. I

found no published genetic analyses of cactus species using RAPD markers.

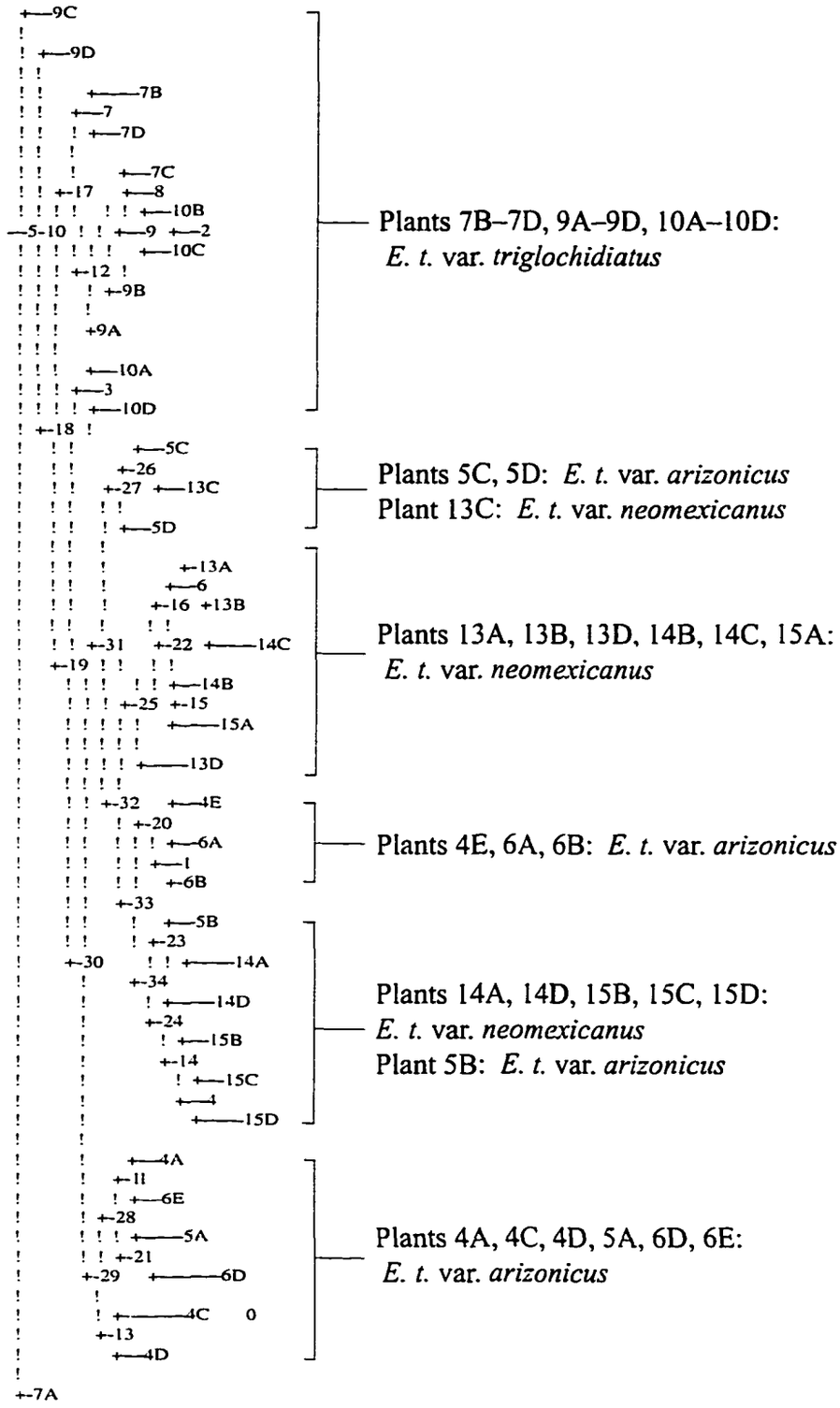
In this study, the variability of RAPD markers in the varieties of *E. triglochidiatus* is consistent with levels of allozyme variability found in other widespread, long-lived, outcrossing plants. There is a high level of genetic variability within-population and low levels of genetic subdivision among populations.

Phylogenetic tree using the neighbor-joining method

A dendrogram based on 85 RAPD loci, using a plant (DA #7A) from *E. t.* var. *triglochidiatus* as an outgroup, showed that all plants of *E. t.* var. *triglochidiatus* cluster together at the base of the dendrogram. Plants of var. *arizonicus* and var. *neomexicanus* form a polyphyletic group with several monophyletic sublineages within it. A clade of six var. *arizonicus* plants (4A, 6E, 5A, 6D, 4C, and 4D) grouped together while the other six var. *arizonicus* plants were scattered throughout the rest of the tree, sometimes grouped together (4E, 6A and 6B) and twice grouped with var. *neomexicanus* plants. Likewise, the var. *neomexicanus* plants grouped together in one clade of four plants (14D, 15B, 15C, 15D) and another of six (13A, 13B, 14C, 14B, 15A, 13D) within the larger complex group (Figure 7). Each of these clades was separated from the others by short branch lengths.

The tree seems somewhat counterintuitive to the genetic distance values from which it was generated (see Appendices D and E). The high variability of genetic distances within and between taxa resulted in some plants within one taxon being genetically more similar to plants in another taxon. This observation leads one to speculate that individuals of the three taxa would form a polyphyletic group. The formation of var. *triglochidiatus* as a monophyletic group using the neighbor-joining algorithm may be due to the overall lower mean genetic distance within taxon than within the other two taxa. However, in the absence of a statistical test of the differences between genetic distance

Figure 7. Dendrogram generated from 85 RAPD loci using the neighbor-joining method (PHYLIP 3.5). Plant DA #7A from *E. t.* var. *triglochidiatus* was used as an "outgroup."



means, the significance of the differences has not yet been determined.

The overall results are suggestive of incomplete lineage sorting, each taxon having been genetically isolated long enough to show differences, but not long enough to have achieved a level of genetic differentiation sufficient to delineate each taxon as a separate monophyletic group. In this case, it would appear that var. *triglochidiatus* has been isolated longer and more completely than var. *arizonicus* and var. *neomexicanus*. Another possible explanation is that there is enough gene flow between the populations of var. *arizonicus* and var. *neomexicanus* to prevent complete differentiation.

Frequencies of polymorphic alleles across taxa

To test a hypothesis of gene flow between taxa, a comparison of the frequencies of polymorphic alleles in each of the three populations was done. In the presence of sufficient gene flow across taxa, the frequencies of polymorphic alleles across taxa should be similar. Assuming Hardy-Weinberg equilibrium *within* each population, the frequency of the null allele was calculated for those loci in each taxa in which the marker allele was present in fewer than nine individuals, as recommended by Lynch and Milligan (1994). Fifteen polymorphic loci fit this criterion across all three taxa. The variance of the null allele frequency was then calculated for each locus and used to estimate the 95% confidence interval of the allele frequency at each locus for each population. Of these 15 loci, 2 show overlapping frequencies in all three taxa, 5 show no overlap at all and 8 show overlap between two taxa but not the third. Table 2 summarizes these results.

These variations in 13/15 allele frequencies between the three varieties of *E. triglochidiatus* suggest there may be limited gene flow between them, allowing drift in allele frequencies at many RAPD loci across these taxa. However, this assumes there is no sampling bias in these small sample sizes. Twelve plants were sampled within each variety, which is probably not a sufficient number to make confident estimates of allele

Table 3.

RAPD Locus	Confidence Interval for RAPD Locus Null Allele Frequency for Variety:					
	arizonicus	*arizonicus*	*neomexicanus*	*neomexicanus*	*triglochidiatus*	*triglochidiatus*
	lower	upper	lower	upper	lower	upper
OPG4 0.30	**1.000**	**1.000**	*0.875*	*0.951*	**1.000**	**1.000**
OPG11 1.74	**0.770**	**0.875**	*0.588*	*0.727*	**0.823**	**0.915**
OPG11 0.59	**0.875**	**0.951**	**0.770**	**0.875**	*0.588*	*0.727*
OPG11 0.53	*0.934*	*0.986*	**0.770**	**0.875**	**0.875**	**0.951**
OPG11 0.51	*0.650*	*0.779*	*0.934*	*0.986*	*1.000*	*1.000*
OPG12 0.76	*1.000*	*1.000*	*0.770*	*0.875*	*0.512*	*0.662*
OPG12 0.41	*0.875*	*0.951*	**1.000**	**1.000**	**1.000**	**1.000**
OPG18 0.40	*0.770*	*0.875*	*1.000*	*1.000*	*0.934*	*0.986*
OPG19 1.02	**1.000**	**1.000**	**1.000**	**1.000**	*0.650*	*0.779*
OPH15 0.58	*0.588*	*0.727*	**0.823**	**0.915**	**0.823**	**0.915**
OPH2 1.20	*0.650*	*0.779*	*1.000*	*1.000*	*0.934*	*0.986*
OPH2 1.05	**0.708**	**0.827**	**0.823**	**0.915**	**0.875**	**0.951**
OPH3 1.15	*0.770*	*0.875*	*1.000*	*1.000*	*0.512*	*0.662*
OPH1 0.73	**0.823**	**0.915**	**0.650**	**0.779**	**0.770**	**0.875**
OPH1 0.65	*0.934*	*0.986*	**1.000**	**1.000**	**1.000**	**1.000**
Confidence intervals that overlap are shown in bold type and those that do not overlap in italics.						
The frequency of the null allele is shown.						

frequencies (Lynch and Milligan, 1994).

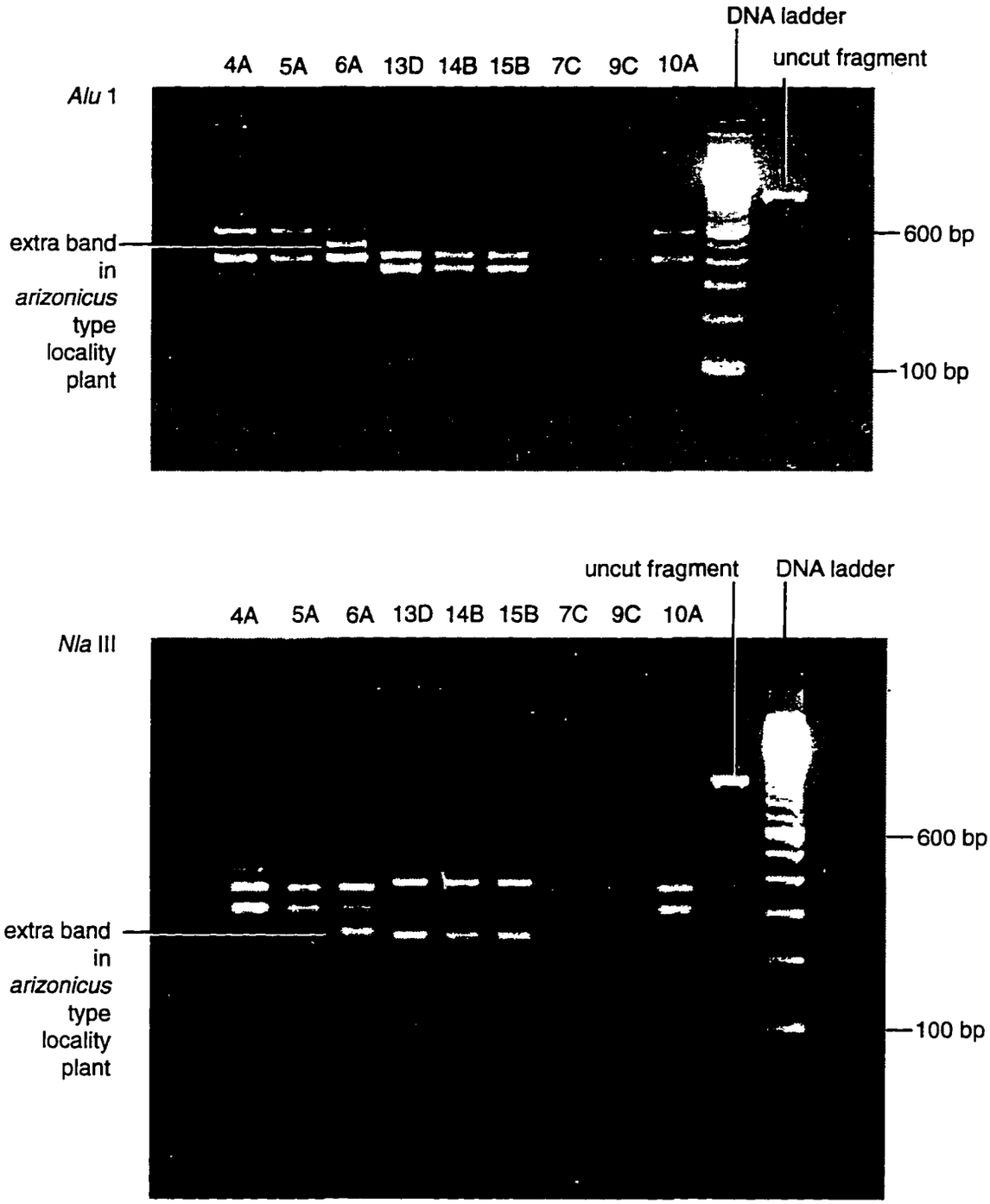
RFLPs in Non-coding Chloroplast Regions

The intergenic spacer adjacent to the *rbc*L gene generated complex restriction fragment length polymorphisms. The uncut spacer was determined to be approximately 1040 bp in length. This region appeared to be the same length in all plants, ruling out a length polymorphism as an explanation of the complex polymorphisms.

Enzymes *Alu* I and *Nla* III produced an extra band in the plant from the type locality (6A) of var. *arizonicus* (Figure 8). The extra bands were produced on repeated digests. To rule out DNA contamination or some other type of error, a second plant from the type locality (6B) was also analyzed and produced the same results. The total number of base pairs in the restriction fragments from these plants sum to more than the number of base pairs in the uncut fragment. Incomplete digestion does not seem to be an explanation as the other plants in the digest appear completely digested. Digests were performed with an excess of enzyme and were incubated for 16 hours. Possible explanations for this observation are that there are two distinct chloroplast types in the plants at the type locality (heteroplasmy) or, that there is a duplication of this spacer region on the same chloroplast. This phenomenon deserves further study; however, more extensive investigation was not feasible in the current study.

Excluding the extra bands present in the plants of var. *arizonicus* from the type locality, the digests of the *rbc*L spacer show that plants of var. *arizonicus* and var. *triglochidiatus* have the same profiles for five of the six enzymes used. The sixth, *Bst*U I, did not cut the amplified product of any plants. The var. *neomexicanus* plants in southeastern Arizona consistently produce different bands than the var. *arizonicus* and var. *triglochidiatus* plants. The patterns from the *Alu* I digestion of DNA from the var. *neomexicanus* plants (13D, 14B and 15B) cannot be explained by loss of a restriction site.

Figure 8. Restriction digests of *rbc*L spacer region with *Alu* I and Nla III. Plants 4A-6A are *var. arizonicus*, plants 13D-15B are var. *neomexicanus*, and plants 7C-10A are var. *triglochidiiatus*.



The source of the polymorphic variation between the plants of var. *arizonicus*/var. *triglochidiatus* and var. *neomexicanus* is difficult to determine (see Figure 8). The most probable explanation is that a sequence rearrangement has occurred in the var. *neomexicanus* plants subsequent to their isolation from an ancestral population of vars. *arizonicus* and *triglochidiatus*, or vice versa. Digestion with *Nla* II and *Bfa* I also reveals the presence of a complex change between the plants of var. *neomexicanus* and the other two taxa. Interestingly, digestion of these DNAs with *Aci* I and *Mse* II was not affected by this change but revealed loss of three enzyme recognition sites in var. *neomexicanus* of six that were present in var. *arizonicus* and var. *triglochidiatus*. These enzymes appear to cut outside of the presumed rearrangement in the var. *neomexicanus* plants.

Because it was not possible to identify loss or gain of restriction sites in the plants of each taxon, the restriction digests were analyzed for number of fragments produced and shared between pairs of taxa. These values are summarized in Table 4. The proportion of shared fragments (F) between var. *arizonicus* and var. *neomexicanus* is 0.33 and between var. *arizonicus* and var. *triglochidiatus* is 1.0. The percent nucleotide substitution (p) between var. *arizonicus* and var. *neomexicanus* was calculated to be 9.7% and between var. *arizonicus* and *var. triglochidiatus* was 0%. These calculations suggest an inordinately high number of nucleotide substitutions between the var. *neomexicanus* plants and the other two varieties. This is likely due to the assumption in this analysis that each RFLP change is an independent event. In this case, if the observed RFLPs are due to a rearrangement, this assumption is not valid. Nonetheless, these data suggest a closer relationship between var. *arizonicus* and var. *triglochidiatus* than between var. *arizonicus* and var. *neomexicanus*.

Amplification of the noncoding region between tRNA genes with primers *trn*C/ *trn*D produced a fragment approximately 3,000 base pairs in length. Digestion of this

Table 4. Analysis of RFLPs in the intergenic spacer adjacent to the *rbc*L gene.

Intergenic spacer adjacent to *rbc*L gene						
Enzyme	Number of fragments (N) in each variety			Number of shared fragments (N_{xy})		
	arizonicus	*neomexicanus*	*triglochidiatus*	*arizonicus-neomexicanus*	*arizonicus-triglochidiatus*	*neomexicanus-triglochidiatus*
Alu I	3	4	3	0	3	0
Aci I	3	1	3	0	3	0
Nla III	5	4	5	2	5	2
Bfa I	4	3	4	2	4	2
Mse II	5	4	5	2	5	2
Total	20	16	20	6	20	6

region with four-cutter endonucleases produced a total of 26 bands, corresponding to 20 four-base enzyme recognition sites. All plants produced the same sized fragments except for one polymorphism observed in the *Bfa* I digest of the var. *triglochidiatus* plants. This polymorphism consists of a fragment that is approximately 20 base-pairs shorter than the presumably corresponding fragments in the plants of the other two taxa. This noncoding region of the chloroplast genome is much less variable than the *rbc*L spacer, representing a sequence variation of 1/80 (1.25%), 10-fold lower than the *rbc*L spacer. These data do not permit inferences about degree of relatedness and indicate only that plants of var. *triglochidiatus* are differentiated from the other two.

The occurrance of a rearrangement, such as an inversion, in the *rbc*L spacer region in an isolated variety of *E. triglochidiatus* does not seem unusual. Comparative molecular analyses of the chloroplast genome have revealed complex patterns of mutational change across taxa. Non-coding regions of cpDNA have been shown to diverge through insertion/deletion changes that are sometimes site dependent (Clegg, et al., 1994). In the genus *Helianthus*, three insertion/deletions and seven site modifications were detected in a survey of 36 wild species (Serror, et al., 1990). Three inversions were found in grasses and other monocots relative to the arrangement typically found in most other flowering plants (Doyle, et al., 1992). The true nature of the change in the spacer region responsible for the observed polymorphism between var. *neomexicanus* and the other two taxa should be determined via sequencing.

SUMMARY AND CONCLUSIONS

Two types of genetic analysis, random amplified polymorphic DNA and restriction fragment length polymorphisms in noncoding chloroplast regions, reveal that the three varieties of *Echinocereus triglochidiatus* sampled in this study show evidence of incomplete divergence. The following conclusions can be drawn from the results of the analysis, and will be discussed below.

1. The degree of RAPD variation within each variety is consistent with allozyme variation in other widespread, long-lived, out-crossing plant populations. None of the varieties, including the endangered var. *arizonicus*, appear to be genetically depauperate.

2. The average genetic distances between varieties were nearly equal. The average genetic distances do not indicate that any one of these three varieties of *E. triglochidiatus* is any closer to another, including var. *arizonicus* to var. *neomexicanus*.

3. A dendrogram generated with the neighbor-joining method and using a var. *triglochidiatus* plant as an "outgroup" shows that plants of var. *triglochidiatus* cluster at the base of the tree and plants of var. *arizonicus* and var. *neomexicanus* form a polyphyletic group. Within the polyphyletic group, there are several monophyletic sublineages of var. *arizonicus* plants and var. *neomexicanus* plants. This tree suggests either incomplete lineage sorting of the var. *arizonicus* and var. *neomexicanus* plants or the presence of some gene flow between var. *arizonicus* and var. *neomexicanus*.

4. Differing allele frequencies in these three varieties of *E. triglochidiatus* suggests that gene flow between them is low or absent. For 5 of 15 RAPD loci analyzed across all three taxa, allele frequencies did not overlap between any of the three taxa, for 8 of the loci the frequencies overlapped between two taxa but not the third, and in the 2 remaining loci there was overlap among all three taxa.

5. Restriction fragment length polymorphisms of noncoding regions of the

chloroplast genome indicate a complex change present in the *rbc*L spacer region of the var. *neomexicanus* plants that is not present in either the var. *arizonicus* or var. *triglochidiatus* plants. This maternally-inherited polymorphism suggests a closer relationship between var. *arizonicus* and var. *triglochidiatus* than between var. *arizonicus* and var. *neomexicanus*.

The conclusions drawn above would benefit from a further analysis of the data. The RAPD data could be further analyzed by completing the Lynch and Milligan (1994) calculations to determine gene diversity within populations. Specifically, population subdivision (F_{ST}) could be estimated using the average heterozygosity values already calculated. However, it must be remembered that estimates of allele frequencies calculated with the methods of Lynch and Milligan are more accurate if the number of individuals sampled is large. In this study, for logistical and practical reasons, 12 plants were sampled in each taxon. This number is much lower than the number recommended by Lynch and Milligan (100). In addition, due to the dominance property of RAPDs, estimates of gene frequencies are less accurate than for other co-dominant markers. Any conclusions drawn from these estimates must take these properties of RAPD markers into consideration.

The RAPD data could also be further analyzed by an application of the new Analysis of Molecular Variance (AMOVA) technique (Huff, et al., 1993). A Euclidean distance matrix between all pairs of individual plants could be constructed and used with the AMOVA technique to apportion the variation among individuals within taxa and among taxa (Huff, et al., 1993). This may be done even if every individual is genetically distinct, as is the case here. The central idea of AMOVA is to convert the inter-individual distance matrix into an equivalent analysis of variance. This would overcome the problem of the nonindependence of the data in using a standard analysis of variance.

The RAPD data should also be re-sampled in a bootstrap analysis to test the robustness of the tree generated by the neighbor-joining algorithm. Given the probability of the presence of noise in a RAPD data set, a bootstrap analysis can help assess the internal consistency of the data and the strength of support for suggested relationships.

To further analyze the restriction fragment length polymorphisms found in the noncoding chloroplast regions between these taxa, all plants would need to be amplfied and analyzed. To accomplish this, the DNA samples would need to be further purified, probably by centrifugation in cesium chloride. In addition, to determine the nature of the change or changes in the *rbc*L spacer of the var. *neomexicanus* plants, this region should be sequenced.

The implications of this data for conservation of the federally endangered var. *arizonicus* are not yet determined. It does seem clear that the populations of each of these varieties, including var. *arizonicus*, are not genetically depauperate. The degree of RAPD variation found within and between populations is consistent with allozyme variation found in other widespread, long-lived, out-crossing native plant populations. RAPD variation patterns have been found to be consistent, at a general level, with allozyme variability in other plants. Some degree of divergence does appear to be present between all three of the groups tested.

A better understanding of the natural history of this species is also indicated in order to better understand any threats to its survival. Pollination and seed dispersal biology should be investigated. Data obtained from these studies would then be available for correlation with genetic data, providing a much more complete picture. The taxonomy of this species would also benefit from a reevaluation of diagnostic morphological characters and a thorough survey of the ranges of plant populations in the field. All of these studies would, of course, take a significant amount of time. However, in the ab-

sence of good populational, morphological, natural history and adequate genetic data, any decisions regarding listing or delisting of the currently endangered var. *arizonicus* are premature.

APPENDIX A:

DESCRIPTIONS OF SUBPOPULATION LOCATIONS

Echinocereus triglochidiatus* var. *arizonicus

Site DA #4A: ARIZONA: Pinal County: between Superior and Miami: northwest rim of Devil's Canyon where Highway 60 descends into the canyon at the east edge of Oak Flat, about 1 miles east of Oak Flat Campground. T1S, R13E, Sect. 27, center of west edge. This site is approximately 4.3 air-miles southwest of the type locality. Elevation is approximately 4150 feet.

Site DA #5: ARIZONA: Pinal County: between Superior and Miami: a few hundred meters northwest of the rim of Devil's Canyon. T1S, R13E, extreme northwest corner of Section 22. This site is approximately 1.5 air-miles north (upstream) of collection site #4 and about 3.5 air-miles west-southwest of the type locality. Elevation is approximately 4300 feet.

Site DA #6: ARIZONA: Gila County: at Pinal County line, half-way between Superior and Miami along Highway 60. T1S, R13E, Section 7, sw 1/4, 10-100 meters south of Highway 60. Elevation 4600 feet. This is the type locality.

Echinocereus triglochidiatus* var. *neomexicanus

Site AZ #2913: ARIZONA: Cochise County: Gunnison Hills, between Dragoon and Cochise: isolated southeasternmost hill of the range. T16S, R23E, slightly west of center of west half of section 14. Elevation approximately 4570 feet.

Site AZ #2914: ARIZONA: Cochise County: Gunnison Hills, between Dragoon and Cochise: eastern flank of the south end of the hills. T26S, R23E, center of north edge of section 15. Elevation approximately 4650 feet. About 3/4 air-mile northwest of collecting site AZ #2913.

Site AZ #2915: ARIZONA: Cochise County: Chiricahua Mountains: east edge of Coronado National Forest on road from Portal to Paradise, 1.5 miles northwest of Portal, 3 air-miles due north of Sunny Flat Campground. T17S, R31E, section 21, SE quarter of SE quarter. Elevation is approximately 5100 to 5200 feet.

Echinocereus triglochidiatus* var. *triglochidiatus

Site DA #7: NEW MEXICO: Bernalillo County: Tijeras Canyon: about 1 mile north of I40 at Tijeras exit on Canyon Estates Road to dead-end at trailhead. Approximately 200 meters west up SE-facing slope. Elevation approximately 6500 feet.

Site DA #9: NEW MEXICO: Otero County: Alamogordo: south side of Alamogordo near the junction of Highway 70 with Highway 54, on the NW side of Highway 70 about 100 meters west of the railroad tracks. Elevation approximately 4300 feet.

Site DA #10: NEW MEXICO: Otero County: Alamogordo: north of Alamogordo, just north of the junction of Highway 82 with Highway 70/54, about 100-200 meters west of the railroad tracks. Approximately 10-12 miles north of collecting site #9. Elevation approximately 4300 feet.

APPENDIX B:

RECIPES OF DNA ISOLATION BUFFERS

GRINDING BUFFER
From Organelle Pellet Method by Robert Wallace, Iowa State University

GRINDING BUFFER: Store at 4°C. **USE ICE COLD!**

0.35M Sorbitol (F.W. = 182.2)
50 mM Tris-HCl pH 8.0 (F.W. = 157.6)
5 mM EDTA (F.W. = 380.2)

Immediately before use add:
bovine serum albumin to 0.1%
2-mercaptoethanol to 5 mM
[i.e. 1.0 g BSA, 1 ml 2-ME per 1 liter buffer]

5X GRINDING BUFFER:

To make one liter of 5X isolation/grinding buffer:
Sorbitol 318.85 g
Tris-HCl 39.4 g
EDTA 9.505 g
in 1 L ddH20
pH to 8.0 with 10N NaOH

Dilute to 1X and add 1.0 g BSA and 1 ml 2-ME per liter of 1X buffer just before use.

CTAB ISOLATION BUFFER
Adapted from Focus 12(1): 13-15 (1990) by GH

	500 mL	100 mL	
CTAB (2% solution w/v)	10.00 g	2.00 g	
NaCl (1.4 M)	38.40 g	7.68 g	
ß-mercaptoethanol (0.2% v/v)	1000 µL	200 µL	
EDTA (20 mM)	20 mL	4 mL	of a 0.5 M
Tris-HCl, pH 8.0 (100 mM)	50 mL	10 mL	of a 1.0 M

Add ddH_20 for a final volume
Store at room temperature

APPENDIX C:

SEQUENCES OF OPERON RAPD PRIMERS

Code	5' to 3'
OPG-04	AGCGTGTCTG
OPG-08	TCACGTCCAC
OPG-09	CTGACGTCAC
OPG-11	TGCCCGTCGT
OPG-12	CAGCTCACGA
OPG-18	GGCTCATGTG
OPG-19	CTCAGGGCAA
OPH-01	GGTCGGAGAA
OPH-02	TCGGACGTGA
OPH-03	AGACGTCCAC
OPH-04	GGAAGTCGCC
OPH-07	CTGCATCGTG
OPH-12	ACGCGCATGT
OPH-14	ACCAGGTTGG
OPH-15	AATGGCGCAG
OPH-17	CACTCTCCTC
OPH-20	GGGAGACATC

APPENDIX D:

PRESENCE (1) OR ABSENCE (0) OF RAPD MARKER ALLELES ACROSS ALL LOCI IN EACH TAXON

E. t.* var. *arizonicus

RAPD Locus	Polymorphic?	4A	4C	4D	4E	5A	5B	5C	5D	6A	6B	6D	6E
OPG4 1.44		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 1.34		0	0	0	0	0	0	0	0	0	0	0	0
OPG4 1.15		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.92		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.72		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.34		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.30		0	0	0	0	0	0	0	0	0	0	0	0
OPG8 1.38		1	1	1	1	1	1	1	1	1	1	1	1
OPG8 1.18	Polymorphic	1	0	0	0	1	0	1	1	0	0	0	1
OPG8 1.08	Polymorphic	1	1	1	1	0	1	1	1	1	1	0	1
OPG8 0.65	Polymorphic	1	0	1	1	1	1	1	1	1	1	0	1
OPG8 0.45		1	1	1	1	1	1	1	1	1	1	1	1
OPG11 1.56		1	1	1	1	1	1	1	1	1	1	1	1
OPG11 1.74	Polymorphic	0	0	0	1	0	1	1	0	0	1	0	0
OPG11 1.21		1	1	1	1	1	1	1	1	1	1	1	1
OPG11 0.79	Polymorphic	0	0	1	1	0	1	0	1	1	1	1	1
OPG11 0.59	Polymorphic	0	0	0	0	0	0	1	1	0	0	0	0
OPG11 0.53	Polymorphic	0	0	0	0	0	0	1	0	0	0	0	0
OPG11 0.51	Polymorphic	0	1	1	1	1	0	0	0	1	1	0	0
OPG9 1.55		1	1	1	1	1	1	1	1	1	1	1	1
OPG9 1.18	Polymorphic	1	1	1	1	1	1	1	1	0	1	1	1
OPG9 0.80		1	1	1	1	1	1	1	1	1	1	1	1
OPG9 0.64		1	1	1	1	1	1	1	1	1	1	1	1
OPG12 1.75	Polymorphic	1	0	0	1	1	1	1	1	1	1	1	0
OPG12 1.28	Polymorphic	1	1	1	0	1	1	1	1	1	1	1	1
OPG12 0.76		0	0	0	0	0	0	0	0	0	0	0	0
OPG12 0.41	Polymorphic	0	0	0	0	0	0	0	0	1	1	0	0
OPG12 0.32	Polymorphic	1	1	1	1	1	0	1	1	1	1	1	1
OPG18 1.20	Polymorphic	1	1	0	0	1	0	1	1	1	0	1	1
OPG18 1.15	Polymorphic	1	0	0	0	1	0	1	0	0	0	0	0
OPG18 1.04		1	1	1	1	1	1	1	1	1	1	1	1
OPG18 0.46	Polymorphic	1	1	1	0	1	1	1	1	1	1	0	1
OPG18 0.40	Polymorphic	1	0	1	1	0	0	0	0	1	0	0	0
OPG18 0.31	Polymorphic	1	0	1	1	1	1	1	1	0	0	1	1
OPH7 1.25		1	1	1	1	1	1	1	1	1	1	1	1
OPH7 0.98	Polymorphic	1	1	1	1	0	1	1	1	1	1	0	1
OPH7 0.80	Polymorphic	1	1	1	1	1	1	1	1	1	1	0	1
OPH7 0.36		1	1	1	1	1	1	1	1	1	1	1	1
OPH7 0.33	Polymorphic	1	1	1	1	1	1	1	1	0	1	0	1
OPG19 1.20	Polymorphic	1	0	0	1	1	1	0	1	1	1	1	1
OPG19 1.14		1	1	1	1	1	1	1	1	1	1	1	1
OPG19 1.02		0	0	0	0	0	0	0	0	0	0	0	0
OPH12 1.25	Polymorphic	1	1	1	1	1	1	1	1	0	0	0	0
OPH12 0.61	Polymorphic	1	0	1	1	0	1	1	0	1	1	0	1

OPH12 0.49		1	1	1	1	1	1	1	1	1	1	1	1
OPH12 0.42		1	1	1	1	1	1	1	1	1	1	1	1
OPH14 1.50		1	1	1	1	1	1	1	1	1	1	1	1
OPH14 0.72	Polymorphic	1	1	1	0	0	1	1	0	1	0	1	1
OPH14 0.65		1	1	1	1	1	1	1	1	1	1	1	1
OPH14 0.43	Polymorphic	0	1	1	1	1	1	1	1	1	1	1	1
OPH14 0.39		1	1	1	1	1	1	1	1	1	1	1	1
OPH15 1.40	Polymorphic	1	0	1	1	1	1	1	1	1	1	1	1
OPH15 0.89	Polymorphic	1	0	0	1	1	1	1	1	1	1	1	1
OPH15 0.58	Polymorphic	1	1	0	0	1	1	1	1	0	0	0	1
OPH15 0.54		1	1	1	1	1	1	1	1	1	1	1	1
OPH15 0.34		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 1.73		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 1.20	Polymorphic	1	1	0	0	0	1	0	0	0	1	1	1
OPH20 0.68		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 0.60		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.65		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.30		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.23		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.20	Polymorphic	1	0	0	1	1	0	0	0	1	0	1	1
OPH2 1.05	Polymorphic	0	1	0	0	0	1	1	0	0	0	1	1
OPH2 0.95		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 0.68		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 0.54		1	1	1	1	1	1	1	1	1	1	1	1
OPH3 1.36		1	1	1	1	1	1	1	1	1	1	1	1
OPH3 1.15	Polymorphic	0	0	1	0	0	0	1	0	1	1	0	0
OPH3 1.11		0	0	0	0	0	0	0	0	0	0	0	0
OPH4 0.85		1	1	1	1	1	1	1	1	1	1	1	1
OPH4 0.80	Polymorphic	0	0	1	1	1	1	1	1	1	1	1	0
OPH17 1.30		1	1	1	1	1	1	1	1	1	1	1	1
OPH17 0.79		1	1	1	1	1	1	1	1	1	1	1	1
OPH17 0.70	Polymorphic	0	0	0	0	0	1	0	0	0	0	0	1
OPH17 0.51		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 1.58		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 1.45		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 0.81		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 O.73	Polymorphic	0	0	0	0	1	0	0	0	1	1	0	0
OPH1 0.57		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 0.52	Polymorphic	0	0	0	0	1	0	0	0	1	1	0	0
OPH1 0.89	Polymorphic	0	0	0	1	0	1	1	1	1	1	0	0
OPH1 0.65	Polymorphic	0	0	0	0	0	0	0	0	0	0	1	0
Total bands/plant		65	57	61	63	64	66	69	64	66	66	58	65

E. t. var. *neomexicanus*

RAPD locus	Polymorphic?	13A	13B	13C	13D	14A	14B	14C	14D	15A	15B	15C	15D
OPG4 1.44		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 1.34		0	0	0	0	0	0	0	0	0	0	0	0
OPG4 1.15		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.92		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.72		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.34		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.30	Polymorphic	0	0	0	0	1	0	0	1	0	0	0	0
OPG8 1.38		1	1	1	1	1	1	1	1	1	1	1	1
OPG8 1.18	Polymorphic	0	1	1	1	0	1	1	1	0	0	0	0
OPG8 1.08	Polymorphic	1	1	0	1	1	1	1	1	1	1	1	1
OPG8 0.65		1	1	1	1	1	1	1	1	1	1	1	1
OPG8 0.45		1	1	1	1	1	1	1	1	1	1	1	1
OPG11 1.56	Polymorphic	1	1	1	1	1	1	1	1	0	1	1	1
OPG11 1.74	Polymorphic	0	0	1	1	1	1	0	1	1	0	1	0
OPG11 1.21		1	1	1	1	1	1	1	1	1	1	1	1
OPG11 0.79	Polymorphic	1	1	1	1	1	1	1	1	0	0	1	0
OPG11 0.59	Polymorphic	0	0	0	0	0	0	0	1	0	1	1	1
OPG11 0.53	Polymorphic	1	0	0	1	0	0	1	0	1	0	0	0
OPG11 0.51	Polymorphic	0	0	0	0	0	0	0	0	1	0	0	0
OPG9 1.55		1	1	1	1	1	1	1	1	1	1	1	1
OPG9 1.18	Polymorphic	1	1	1	1	1	1	0	0	1	1	0	0
OPG9 0.80		1	1	1	1	1	1	1	1	1	1	1	1
OPG9 0.64		1	1	1	1	1	1	1	1	1	1	1	1
OPG12 1.75	Polymorphic	1	1	1	0	1	0	0	1	0	0	1	1
OPG12 1.28	Polymorphic	0	1	1	1	1	0	1	0	0	1	0	0
OPG12 0.76	Polymorphic	0	0	1	0	0	0	0	0	0	1	1	1
OPG12 0.41		0	0	0	0	0	0	0	0	0	0	0	0
OPG12 0.32	Polymorphic	1	1	1	0	1	1	0	1	1	1	1	1
OPG18 1.20	Polymorphic	0	0	1	1	0	0	0	0	0	0	0	0
OPG18 1.15	Polymorphic	1	1	1	0	1	0	1	1	0	1	1	1
OPG18 1.04		1	1	1	1	1	1	1	1	1	1	1	1
OPG18 0.46	Polymorphic	1	1	1	1	1	1	0	0	0	1	1	1
OPG18 0.40		0	0	0	0	0	0	0	0	0	0	0	0
OPG18 0.31	Polymorphic	1	1	1	1	0	1	1	1	1	1	1	0
OPH7 1.25		1	1	1	1	1	1	1	1	1	1	1	1
OPH7 0.98		1	1	1	1	1	1	1	1	1	1	1	1
OPH7 0.80		1	1	1	1	1	1	1	1	1	1	1	1
OPH7 0.36		1	1	1	1	1	1	1	1	1	1	1	1
OPH7 0.33	Polymorphic	1	1	1	0	1	1	1	0	1	1	1	1
OPG19 1.2		1	1	1	1	1	1	1	1	1	1	1	1
OPG19 1.14	Polymorphic	1	1	1	0	0	1	1	1	1	1	0	1
OPG19 1.02		0	0	0	0	0	0	0	0	0	0	0	0
OPH12 1.25	Polymorphic	1	1	1	1	1	1	0	1	1	1	1	0
OPH12 0.61	Polymorphic	1	1	1	1	1	1	1	1	1	1	0	0
OPH12 0.49		1	1	1	1	1	1	1	1	1	1	1	1
OPH12 0.42		1	1	1	1	1	1	1	1	1	1	1	1
OPH14 1.50		1	1	1	1	1	1	1	1	1	1	1	1
OPH14 0.72	Polymorphic	1	1	1	0	1	1	1	1	1	1	1	1
OPH14 0.65		1	1	1	1	1	1	1	1	1	1	1	1
OPH14 0.43		1	1	1	1	1	1	1	1	1	1	1	1

OPH14 0.39		1	1	1	1	1	1	1	1	1	1	1	1
OPH15 1.4		1	1	1	1	1	1	1	1	1	1	1	1
OPH15 0.89	Polymorphic	0	0	1	0	1	0	1	1	0	1	1	0
OPH15 0.58	Polymorphic	0	0	0	1	1	0	0	0	0	0	0	1
OPH15 0.54		1	1	1	1	1	1	1	1	1	1	1	1
OPH15 0.34		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 1.73		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 1.20	Polymorphic	0	0	0	0	1	0	0	1	0	1	0	1
OPH20 0.68		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 0.60		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.65		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.30		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.23		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.20		0	0	0	0	0	0	0	0	0	0	0	0
OPH2 1.05	Polymorphic	0	0	1	1	0	0	0	0	0	1	0	0
OPH2 0.95		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 0.68		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 0.54		1	1	1	1	1	1	1	1	1	1	1	1
OPH3 1.36		1	1	1	1	1	1	1	1	1	1	1	1
OPH3 1.15		0	0	0	0	0	0	0	0	0	0	0	0
OPH3 1.11	Polymorphic	0	0	0	1	0	0	0	0	1	1	1	1
OPH4 0.85		1	1	1	1	1	1	1	1	1	1	1	1
OPH4 0.80	Polymorphic	1	1	0	1	1	1	1	1	1	1	1	1
OPH17 1.30		1	1	1	1	1	1	1	1	1	1	1	1
OPH17 0.79		1	1	1	1	1	1	1	1	1	1	1	1
OPH17 0.70	Polymorphic	0	0	0	0	1	0	1	0	0	0	0	0
OPH17 0.51		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 1.58		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 1.45		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 0.81		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 0.73	Polymorphic	1	1	0	1	1	1	1	0	0	0	0	0
OPH1 0.57		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 0.52		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 0.89	Polymorphic	0	0	1	1	0	0	0	1	0	1	0	1
OPH1 0.64		0	0	0	0	0	0	0	0	0	0	0	0
Total bands/plant		63	64	67	65	67	62	62	65	60	67	63	62

E. t.* var. *triglochidiatus

RAPD Locus	Polymorphic?	7A	7B	7C	7D	9A	9B	9C	9D	10A	10B	10C	10D
OPG4 1.44		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 1.34		0	0	0	0	0	0	0	0	0	1	0	0
OPG4 1.15		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.92		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.72		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.34		1	1	1	1	1	1	1	1	1	1	1	1
OPG4 0.30		0	0	0	0	0	0	0	0	0	0	0	0
OPG8 1.38		1	1	1	1	1	1	1	1	1	1	1	1
OPG8 1.18	Polymorphic	1	1	1	1	1	1	1	1	1	1	1	0
OPG8 1.08		1	1	1	1	1	1	1	1	1	1	1	1
OPG8 0.65		1	1	1	1	1	1	1	1	1	1	1	1
OPG8 0.45	Polymorphic	1	0	1	1	1	1	1	1	1	1	1	1
OPG11 1.56		1	1	1	1	1	1	1	1	1	1	1	1
OPG11 1.74	Polymorphic	1	0	0	0	1	0	1	0	0	0	0	0
OPG11 1.21		1	1	1	1	1	1	1	1	1	1	1	1
OPG11 0.79	Polymorphic	1	0	1	0	1	1	0	1	1	1	1	1
OPG11 0.59	Polymorphic	1	0	1	0	0	1	1	1	0	1	1	0
OPG11 0.53	Polymorphic	0	1	0	1	0	0	0	0	0	0	0	0
OPG11 0.51		0	0	0	0	0	0	0	0	0	0	0	0
OPG9 1.55		1	1	1	1	1	1	1	1	1	1	1	1
OPG9 1.18		1	1	1	1	1	1	1	1	1	1	1	1
OPG9 0.80		1	1	1	1	1	1	1	1	1	1	1	1
OPG9 0.64		1	1	1	1	1	1	1	1	1	1	1	1
OPG12 1.75	Polymorphic	0	1	0	1	1	1	1	1	1	1	1	1
OPG12 1.28	Polymorphic	0	0	1	1	1	1	1	0	0	1	1	1
OPG12 0.76	Polymorphic	1	1	1	1	0	0	1	1	1	1	0	0
OPG12 0.41		0	0	0	0	0	0	0	0	0	0	0	0
OPG12 0.32	Polymorphic	1	0	1	1	1	1	1	1	1	1	1	1
OPG18 1.20	Polymorphic	1	1	1	0	1	1	1	1	1	1	1	1
OPG18 1.15		0	0	0	0	0	0	0	0	0	0	0	0
OPG18 1.04	Polymorphic	1	1	1	1	1	1	1	1	1	1	0	0
OPG18 0.46		1	1	1	1	1	1	1	1	1	1	1	1
OPG18 0.40	Polymorphic	0	1	0	0	0	0	0	0	0	0	0	0
OPG18 0.31	Polymorphic	1	1	0	1	1	1	1	1	1	1	1	1
OPH7 1.25	Polymorphic	1	1	1	1	1	1	1	1	0	1	1	0
OPH7 0.98	Polymorphic	1	0	1	0	1	1	0	1	0	1	1	1
OPH7 0.80	Polymorphic	1	1	1	1	1	1	1	1	1	0	0	1
OPH7 0.36		1	1	1	1	1	1	1	1	1	1	1	1
OPH7 0.33	Polymorphic	1	1	1	0	1	1	1	0	1	1	0	1
OPG19 1.2		1	1	1	1	1	1	1	1	1	1	1	1
OPG19 1.14		1	1	1	1	1	1	1	1	1	1	1	1
OPG19 1.02	Polymorphic	0	1	1	1	0	0	0	0	0	1	1	1
OPH12 1.25		1	1	1	1	1	1	1	1	1	1	1	1
OPH12 0.61		1	1	1	1	1	1	1	1	1	1	1	1

OPH12 0.49		1	1	1	1	1	1	1	1	1	1	1	1
OPH12 0.42	Polymorphic	1	1	1	1	1	0	1	1	1	1	1	1
OPH14 1.50		1	1	1	1	1	1	1	1	1	1	1	1
OPH14 0.72	Polymorphic	1	0	1	1	0	1	0	0	1	0	1	1
OPH14 0.65		1	1	1	1	1	1	1	1	1	1	1	1
OPH14 0.43		1	1	1	1	1	1	1	1	1	1	1	1
OPH14 0.39	Polymorphic	0	1	1	1	1	1	0	0	1	1	1	1
OPH15 1.40	Polymorphic	1	1	1	1	1	1	1	1	0	1	1	0
OPH15 0.89	Polymorphic	1	0	0	0	0	0	0	0	0	0	0	0
OPH15 0.58	Polymorphic	0	0	0	0	0	0	0	0	1	1	0	1
OPH15 0.54		1	1	1	1	1	1	1	1	1	1	1	1
OPH15 0.34		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 1.73		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 1.20		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 0.68		1	1	1	1	1	1	1	1	1	1	1	1
OPH20 0.60		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.65		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.30		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.23		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 1.20	Polymorphic	0	1	0	0	0	0	0	0	0	0	0	0
OPH2 1.05	Polymorphic	0	0	0	0	0	0	0	1	0	1	0	0
OPH2 0.95		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 0.68		1	1	1	1	1	1	1	1	1	1	1	1
OPH2 0.54		1	1	1	1	1	1	1	1	1	1	1	1
OPH3 1.36		1	1	1	1	1	1	1	1	1	1	1	1
OPH3 1.15	Polymorphic	1	0	1	1	1	1	1	0	0	1	1	0
OPH3 1.11		1	1	1	1	1	1	1	1	1	1	1	1
OPH4 0.85		1	1	1	1	1	1	1	1	1	1	1	1
OPH4 0.80	Polymorphic	1	1	1	1	1	1	0	1	1	1	1	1
OPH17 1.30		1	1	1	1	1	1	1	1	1	1	1	1
OPH17 0.79		1	1	1	1	1	1	1	1	1	1	1	1
OPH17 0.70	Polymorphic	0	0	0	0	0	0	0	0	0	0	1	1
OPH17 0.51		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 1.58	Polymorphic	1	1	1	1	1	1	1	1	0	0	0	0
OPH1 1.45		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 0.81		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 0.73	Polymorphic	0	1	0	0	0	1	0	0	0	1	1	0
OPH1 0.57		1	1	1	1	1	1	1	1	1	1	1	1
OPH1 0.52	Polymorphic	1	0	0	0	0	0	1	1	1	0	0	0
OPH1 0.89	Polymorphic	0	1	0	0	0	0	0	1	0	0	0	0
OPH1 0.64		0	0	0	0	0	0	0	0	0	0	0	0
Total bands/plant		67	65	66	64	65	66	64	65	62	69	65	62

APPENDIX E:

GENETIC DISTANCES BETWEEN PLANTS *WITHIN-TAXON*

Genetic distances were calculated with the formula

$$D_{xy} = 1 - \frac{N_{xy}}{N_x + N_y - N_{xy}},$$

where N_x is the number of bands in Plant X, N_y is the number of bands in Plant Y and N_{xy} is the number of bands shared between X and Y.

Var. *arizonicus*–var. *arizonicus*

Pair-wise comparisons	Nx	Ny	Nxy	Dxy
4A-4C	65	57	53	0.232
4A-4D	65	61	56	0.200
4A-4E	65	63	57	0.197
4A-5A	65	64	59	0.157
4A-5B	65	66	59	0.181
4A-5C	65	69	62	0.139
4A-5D	65	64	59	0.157
4A-6A	65	66	57	0.230
4A-6B	65	66	56	0.253
4A-6D	65	58	53	0.243
4A-6E	65	65	61	0.116
4C-4D	57	61	53	0.185
4C-4E	57	63	50	0.286
4C-5A	57	64	52	0.246
4C-5B	57	66	54	0.217
4C-5C	57	69	55	0.225
4C-5D	57	64	53	0.221
4C-6A	57	66	51	0.292
4C-6B	57	66	52	0.268
4C-6D	57	58	49	0.258
4C-6E	57	65	55	0.179
4D-4E	61	63	57	0.149
4D-5A	61	64	54	0.239
4D-5B	61	66	57	0.186
4D-5C	61	69	58	0.194
4D-5D	61	64	56	0.188
4D-6A	61	66	57	0.186
4D-6B	61	66	57	0.186
4D-6D	61	58	50	0.275
4D-6E	61	65	56	0.200
4E-5A	63	64	56	0.211

4E-5B	63	66	59	0.157
4E-5C	63	69	58	0.216
4E-5D	63	64	58	0.159
4E-6A	63	66	58	0.183
4E-6B	63	66	59	0.157
4E-6D	63	58	52	0.246
4E-6E	63	65	56	0.222
5A-5B	64	66	56	0.243
5A-5C	64	69	59	0.203
5A-5D	64	64	59	0.145
5A-6A	64	66	57	0.219
5A-6B	64	66	57	0.219
5A-6D	64	58	53	0.232
5A-6E	64	65	57	0.208
5B-5C	66	69	62	0.151
5B-5D	66	64	60	0.143
5B-6A	66	66	57	0.240
5B-6B	66	66	60	0.167
5B-6D	66	58	54	0.229
5B-6E	66	65	61	0.129
5C-5D	69	64	62	0.127
5C-6A	69	66	59	0.224
5C-6B	69	66	59	0.224
5C-6D	69	58	53	0.284
5C-6E	69	65	60	0.189
5D-6A	64	66	57	0.219
5D-6B	64	66	58	0.194
5D-6D	64	58	53	0.232
5D-6E	64	65	59	0.157
6A-6B	66	66	62	0.114
6A-6D	66	58	53	0.254
6A-6E	66	65	57	0.230
6B-6D	66	58	52	0.278
6B-6E	66	65	57	0.230
6D-6E	58	65	54	0.217
Average genetic distance				**0.21**
Minimum genetic distance				**0.11**
Maximum genetic distance				**0.29**
Standard deviation				**0.044**
Standard deviation of all 3 populations				**0.047**

Var. *neomexicanus*–var. *neomexicanus*

Pair-wise comparisons	Nx	Ny	Nxy	Dxy
13A-13B	63	64	62	0.046
13A-13C	63	67	59	0.169
13A-13D	63	65	57	0.197
13A-14A	63	67	60	0.143
13A-14B	63	62	60	0.077
13A-14C	63	62	58	0.134
13A-14D	63	65	58	0.171
13A-15A	63	60	56	0.164
13A-15B	63	67	59	0.169
13A-15C	63	63	58	0.147
13A-15D	63	62	56	0.188
13B-13C	64	67	61	0.129
13B-13D	64	65	58	0.183
13B-14A	64	67	61	0.129
13B-14B	64	62	61	0.062
13B-14C	64	62	59	0.119
13B-14D	64	65	59	0.157
13B-15A	64	60	56	0.176
13B-15B	64	67	60	0.155
13B-15C	64	63	58	0.159
13B-15D	64	62	56	0.200
13C-13D	67	65	59	0.192
13C-14A	67	67	60	0.189
13C-14B	67	62	59	0.157
13C-14C	67	62	57	0.208
13C-14D	67	65	60	0.167
13C-15A	67	60	55	0.236
13C-15B	67	67	62	0.139
13C-15C	67	63	59	0.169
13C-15D	67	62	56	0.233
13D-14A	65	67	58	0.216
13D-14B	65	62	58	0.159
13D-14C	65	62	56	0.211
13D-14D	65	65	56	0.243
13D-15A	65	60	55	0.214
13D-15B	65	67	58	0.216
13D-15C	65	63	55	0.247
13D-15D	65	62	53	0.284
14A-14B	67	62	59	0.157
14A-14C	67	62	58	0.183
14A-14D	67	65	60	0.167
14A-15A	67	60	55	0.236
14A-15B	67	67	60	0.189
14A-15C	67	63	58	0.194

14A-15D	67	62	57	0.208
14B-14C	62	62	57	0.149
14B-14D	62	65	58	0.159
14B-15A	62	60	57	0.123
14B-15B	62	67	58	0.183
14B-15C	62	63	57	0.162
14B-15D	62	62	54	0.229
14C-14D	62	65	57	0.186
14C-15A	62	60	54	0.206
14C-15B	62	67	57	0.208
14C-15C	62	63	55	0.214
14C-15D	62	62	53	0.254
14D-15A	65	60	55	0.214
14D-15B	65	67	59	0.192
14D-15C	65	63	59	0.145
14D-15D	65	62	57	0.186
15A-15B	60	67	57	0.186
15A-15C	60	63	55	0.191
15A-15D	60	62	53	0.232
15B-15C	67	63	60	0.143
15B-15D	67	62	60	0.130
15C-15D	63	62	58	0.134
Average genetic distance				**0.178**
Minimum genetic distance				**0.046**
Maximum genetic distance				**0.284**
Standard deviation				**0.044**

Var. *triglochidiatus*–var. *triglochidiatus*

Pair-wise comparisons	Nx	Ny	Nxy	Dxy
7A-7B	67	65	57	0.240
7A-7C	67	66	63	0.100
7A-7D	67	64	59	0.181
7A-9A	67	65	62	0.114
7A-9B	67	66	62	0.127
7A-9C	67	64	62	0.101
7A-9D	67	65	62	0.114
7A-10A	67	62	59	0.157
7A-10B	67	69	61	0.187
7A-10C	67	65	59	0.192
7A-10D	67	62	56	0.233
7B-7C	65	66	58	0.205
7B-7D	65	64	59	0.157
7B-9A	65	65	59	0.169
7B-9B	65	66	58	0.205
7B-9C	65	64	57	0.208
7B-9D	65	65	58	0.194
7B-10A	65	62	56	0.211
7B-10B	65	69	58	0.237
7B-10C	65	65	56	0.243
7B-10D	65	62	54	0.260
7C-7D	66	64	61	0.116
7C-9A	66	65	62	0.101
7C-9B	66	66	63	0.087
7C-9C	66	64	60	0.143
7C-9D	66	65	60	0.155
7C-10A	66	62	57	0.197
7C-10B	66	69	63	0.125
7C-10C	66	65	61	0.129
7C-10D	66	62	58	0.171
7D-9A	64	65	60	0.130
7D-9B	64	66	61	0.116
7D-9C	64	64	59	0.145
7D-9D	64	65	58	0.183
7D-10A	64	62	57	0.174
7D-10B	64	69	60	0.178
7D-10C	64	65	59	0.157
7D-10D	64	62	56	0.200
9A-9B	65	66	63	0.074
9A-9C	65	64	61	0.103
9A-9D	65	65	60	0.143
9A-10A	65	62	58	0.159
9A-10B	65	69	62	0.139
9A-10C	65	65	60	0.143

9A-10D	65	62	58	0.159
9B-9C	66	64	60	0.143
9B-9D	66	65	60	0.155
9B-10A	66	62	58	0.171
9B-10B	66	69	63	0.125
9B-10C	66	65	62	0.101
9B-10D	66	62	58	0.171
9C-9D	64	65	60	0.130
9C-10A	64	62	57	0.174
9C-10B	64	69	60	0.178
9C-10C	64	65	57	0.208
9C-10D	64	62	54	0.250
9D-10A	65	62	58	0.159
9D-10B	65	69	61	0.164
9D-10C	65	65	58	0.194
9D-10D	65	62	55	0.236
10A-10B	62	69	59	0.181
10A-10C	62	65	56	0.211
10A-10D	62	62	58	0.121
10B-10C	69	65	63	0.113
10B-10D	69	62	59	0.181
10C-10D	65	62	59	0.132
Average genetic distance				**0.163**
Minimum genetic distance				**0.074**
Maximum genetic distance				**0.260**
Standard deviation				**0.044**

APPENDIX F:

GENETIC DISTANCES BETWEEN PLANTS *BETWEEN-TAXON*

Var. *arizonicus*–var. *neomexicanus*

Pair-wise comparisons	Nx	Ny	Nxy	Dxy
4A-13A	65	63	57	0.197
4A-13B	65	64	59	0.157
4A-13C	65	67	60	0.167
4A-13D	65	65	55	0.267
4A-14A	65	67	59	0.192
4A-14B	65	62	56	0.211
4A-14C	65	62	55	0.236
4A-14D	65	65	57	0.219
4A-15A	65	60	53	0.264
4A-15B	65	67	59	0.192
4A-15C	65	63	55	0.247
4A-15D	65	62	55	0.236
4C-13A	57	63	51	0.261
4C-13B	57	64	52	0.246
4C-13C	57	67	53	0.254
4C-13D	57	65	51	0.282
4C-14A	57	67	53	0.254
4C-14B	57	62	51	0.250
4C-14C	57	62	48	0.324
4C-14D	57	65	49	0.329
4C-15A	57	60	50	0.254
4C-15B	57	67	54	0.229
4C-15C	57	63	49	0.310
4C-15D	57	62	51	0.250
4D-13A	61	63	57	0.149
4D-13B	61	64	58	0.134
4D-13C	61	67	56	0.222
4D-13D	61	65	54	0.250
4D-14A	61	67	56	0.222
4D-14B	61	62	57	0.136
4D-14C	61	62	54	0.217
4D-14D	61	65	54	0.250
4D-15A	61	60	55	0.167
4D-15B	61	67	57	0.197
4D-15C	61	63	54	0.229
4D-15D	61	62	52	0.268
4E-13A	63	63	57	0.174
4E-13B	63	64	57	0.186
4E-13C	63	67	58	0.194

4E-13D	63	65	55	0.247
4E-14A	63	67	57	0.219
4E-14B	63	62	57	0.162
4E-14C	63	62	54	0.239
4E-14D	63	65	58	0.171
4E-15A	63	60	56	0.164
4E-15B	63	67	57	0.219
4E-15C	63	63	56	0.200
4E-15D	63	62	53	0.264
5A-13A	64	63	57	0.186
5A-13B	64	64	59	0.145
5A-13C	64	67	59	0.181
5A-13D	64	65	56	0.233
5A-14A	64	67	58	0.205
5A-14B	64	62	56	0.200
5A-14C	64	62	55	0.225
5A-14D	64	65	55	0.257
5A-15A	64	60	53	0.254
5A-15B	64	67	57	0.230
5A-15C	64	63	55	0.236
5A-15D	64	62	54	0.250
5B-13A	66	63	58	0.183
5B-13B	66	64	59	0.169
5B-13C	66	67	61	0.153
5B-13D	66	65	59	0.181
5B-14A	66	67	62	0.127
5B-14B	66	62	58	0.171
5B-14C	66	62	57	0.197
5B-14D	66	65	59	0.181
5B-15A	66	60	55	0.225
5B-15B	66	67	61	0.153
5B-15C	66	63	57	0.208
5B-15D	66	62	56	0.222
5C-13A	69	63	59	0.192
5C-13B	69	64	60	0.178
5C-13C	69	67	63	0.137
5C-13D	69	65	60	0.189
5C-14A	69	67	60	0.211
5C-14B	69	62	58	0.205
5C-14C	69	62	57	0.230
5C-14D	69	65	60	0.189
5C-15A	69	60	56	0.233
5C-15B	69	67	62	0.162
5C-15C	69	63	58	0.216
5C-15D	69	62	57	0.230
5D-13A	64	63	57	0.186
5D-13B	64	64	59	0.145

5D-13C	64	67	61	0.129
5D-13D	64	65	58	0.183
5D-14A	64	67	58	0.205
5D-14B	64	62	58	0.147
5D-14C	64	62	55	0.225
5D-14D	64	65	58	0.183
5D-15A	64	60	53	0.254
5D-15B	64	67	59	0.181
5D-15C	64	63	57	0.186
5D-15D	64	62	56	0.200
6A-13A	66	63	57	0.208
6A-13B	66	64	58	0.194
6A-13C	66	67	58	0.227
6A-13D	66	65	56	0.253
6A-14A	66	67	58	0.227
6A-14B	66	62	56	0.222
6A-14C	66	62	56	0.222
6A-14D	66	65	57	0.230
6A-15A	66	60	53	0.274
6A-15B	66	67	57	0.250
6A-15C	66	63	55	0.257
6A-15D	66	62	55	0.247
6B-13A	66	63	58	0.183
6B-13B	66	64	59	0.169
6B-13C	66	67	59	0.203
6B-13D	66	65	57	0.230
6B-14A	66	67	61	0.153
6B-14B	66	62	58	0.171
6B-14C	66	62	56	0.222
6B-14D	66	65	58	0.205
6B-15A	66	60	55	0.225
6B-15B	66	67	59	0.203
6B-15C	66	63	56	0.233
6B-15D	66	62	56	0.222
6D-13A	58	63	51	0.271
6D-13B	58	64	52	0.257
6D-13C	58	67	54	0.239
6D-13D	58	65	50	0.315
6D-14A	58	67	52	0.288
6D-14B	58	62	50	0.286
6D-14C	58	62	50	0.286
6D-14D	58	65	52	0.268
6D-15A	58	60	48	0.314
6D-15B	58	67	53	0.264
6D-15C	58	63	50	0.296
6D-15D	58	62	49	0.310
6E-13A	65	63	56	0.222

6E-13B	65	64	58	0.183
6E-13C	65	67	60	0.167
6E-13D	65	65	57	0.219
6E-14A	65	67	59	0.192
6E-14B	65	62	57	0.186
6E-14C	65	62	57	0.186
6E-14D	65	65	56	0.243
6E-15A	65	60	53	0.264
6E-15B	65	67	59	0.192
6E-15C	65	63	54	0.270
6E-15D	65	62	54	0.260
Average genetic distance				**0.217**
Maximum genetic distance				**0.329**
Minimum genetic distance				**0.127**
Standard deviation				**0.043**

Var. *arizonicus*–var. *triglochidiatus*

Pair-wise comparisons	Nx	Ny	Nxy	Dxy
4A-7A	65	67	58	0.216
4A-7B	65	65	57	0.219
4A-7C	65	66	58	0.205
4A-7D	65	64	57	0.208
4A-9A	65	65	59	0.169
4A-9B	65	66	59	0.181
4A-9C	65	64	57	0.208
4A-9D	65	65	56	0.243
4A-10A	65	62	56	0.211
4A-10B	65	69	58	0.237
4A-10C	65	65	56	0.243
4A-10D	65	62	56	0.211
4C-7A	57	67	52	0.278
4C-7B	57	65	49	0.329
4C-7C	57	66	54	0.217
4C-7D	57	64	51	0.271
4C-9A	57	65	53	0.232
4C-9B	57	66	53	0.243
4C-9C	57	64	51	0.271
4C-9D	57	65	51	0.282
4C-10A	57	62	51	0.250
4C-10B	57	69	52	0.297
4C-10C	57	65	50	0.306
4D-10D	57	62	52	0.224
4D-7A	61	67	57	0.197
4D-7B	61	65	53	0.274
4D-7C	61	66	58	0.159
4D-7D	61	64	56	0.188
4D-9A	61	65	58	0.147
4D-9B	61	66	58	0.159
4D-9C	61	64	54	0.239
4D-9D	61	65	54	0.250
4D-10A	61	62	53	0.243
4D-10B	61	69	56	0.243
4D-10C	61	65	55	0.225
4D-10D	61	62	54	0.217
4E-7A	63	67	57	0.219
4E-7B	63	65	56	0.222
4E-7C	63	66	55	0.257
4E-7D	63	64	54	0.260
4E-9A	63	65	58	0.171
4E-9B	63	66	56	0.233
4E-9C	63	64	54	0.260
4E-9D	63	65	56	0.222

4E-10A	63	62	53	0.264
4E-10B	63	69	55	0.286
4E-10C	63	65	53	0.293
4E-10D	63	62	53	0.264
5A-7A	64	67	56	0.253
5A-7B	64	65	56	0.233
5A-7C	64	66	55	0.267
5A-7D	64	64	55	0.247
5A-9A	64	65	57	0.208
5A-9B	64	66	57	0.219
5A-9C	64	64	56	0.222
5A-9D	64	65	55	0.257
5A-10A	64	62	55	0.225
5A-10B	64	69	57	0.250
5A-10C	64	65	54	0.280
5A-10D	64	62	53	0.274
5B-7A	66	67	59	0.203
5B-7B	66	65	56	0.253
5B-7C	66	66	58	0.216
5B-7D	66	64	57	0.219
5B-9A	66	65	60	0.155
5B-9B	66	66	59	0.192
5B-9C	66	64	56	0.243
5B-9D	66	65	58	0.205
5B-10A	66	62	56	0.222
5B-10B	66	69	59	0.224
5B-10C	66	65	57	0.230
5B-10D	66	62	58	0.171
5C-7A	69	67	61	0.187
5C-7B	69	65	57	0.260
5C-7C	69	66	60	0.200
5C-7D	69	64	59	0.203
5C-9A	69	65	61	0.164
5C-9B	69	66	61	0.176
5C-9C	69	64	59	0.203
5C-9D	69	65	59	0.213
5C-10A	69	62	56	0.253
5C-10B	69	69	61	0.208
5C-10C	69	65	58	0.237
5C-10D	69	62	56	0.253
5D-7A	64	67	59	0.181
5D-7B	64	65	56	0.233
5D-7C	64	66	59	0.169
5D-7D	64	64	56	0.222
5D-9A	64	65	60	0.130
5D-9B	64	66	60	0.143
5D-9C	64	64	57	0.197

5D-9D	64	65	59	0.157
5D-10A	64	62	56	0.200
5D-10B	64	69	60	0.178
5D-10C	64	65	57	0.208
5D-10D	64	62	56	0.200
6A-7A	66	67	57	0.250
6A-7B	66	65	55	0.276
6A-7C	66	66	57	0.240
6A-7D	66	64	55	0.267
6A-9A	66	65	57	0.230
6A-9B	66	66	58	0.216
6A-9C	66	64	54	0.289
6A-9D	66	65	56	0.253
6A-10A	66	62	53	0.293
6A-10B	66	69	56	0.291
6A-10C	66	65	56	0.253
6A-10D	66	62	53	0.293
6B-7A	66	67	59	0.203
6B-7B	66	65	55	0.276
6B-7C	66	66	58	0.216
6B-7D	66	64	56	0.243
6B-9A	66	65	60	0.155
6B-9B	66	66	59	0.192
6B-9C	66	64	57	0.219
6B-9D	66	65	57	0.230
6B-10A	66	62	54	0.270
6B-10B	66	69	58	0.247
6B-10C	66	65	56	0.253
6B-10D	66	62	54	0.270
6D-7A	58	67	52	0.288
6D-7B	58	65	50	0.315
6D-7C	58	66	52	0.278
6D-7D	58	64	52	0.257
6D-9A	58	65	53	0.243
6D-9B	58	66	53	0.254
6D-9C	58	64	50	0.306
6D-9D	58	65	52	0.268
6D-10A	58	62	50	0.286
6D-10B	58	69	53	0.284
6D-10C	58	65	52	0.268
6D-10D	58	62	50	0.286
6E-7A	65	67	59	0.192
6E-7B	65	65	55	0.267
6E-7C	65	66	59	0.181
6E-7D	65	64	56	0.233
6E-9A	65	65	59	0.169
6E-9B	65	66	59	0.181

6E-9C	65	64	56	0.233
6E-9D	65	65	57	0.219
6E-10A	65	62	56	0.211
6E-10B	65	69	59	0.213
6E-10C	65	65	57	0.219
6E-10D	65	62	57	0.186
Average genetic distance				**0.231**
Maximum genetic distance				**0.329**
Minimum genetic distance				**0.130**
Standard deviation				**0.040**

Var. *neomexicanus*–var. *triglochidiatus*

Pair-wise comparisons	Nx	Ny	Nxy	Dxy
13A-7A	63	67	58	0.194
13A-7B	63	65	56	0.222
13A-7C	63	66	57	0.208
13A-7D	63	64	57	0.186
13A-9A	63	65	58	0.171
13A-9B	63	66	59	0.157
13A-9C	63	64	55	0.236
13A-9D	63	65	57	0.197
13A-10A	63	62	56	0.188
13A-10B	63	69	57	0.240
13A-10C	63	65	56	0.222
13A-10D	63	62	55	0.214
13B-7A	64	67	59	0.181
13B-7B	64	65	56	0.233
13B-7C	64	66	59	0.169
13B-7D	64	64	58	0.171
13B-9A	64	65	60	0.130
13B-9B	64	66	61	0.116
13B-9C	64	64	57	0.197
13B-9D	64	65	58	0.183
13B-10A	64	62	57	0.174
13B-10B	64	69	59	0.203
13B-10C	64	65	58	0.183
13B-10D	64	62	56	0.200
13C-7A	67	67	61	0.164
13C-7B	67	65	56	0.263
13C-7C	67	66	59	0.203
13C-7D	67	64	57	0.230
13C-9A	67	65	60	0.167
13C-9B	67	66	59	0.203
13C-9C	67	64	59	0.181
13C-9D	67	65	60	0.167
13C-10A	67	62	57	0.208
13C-10B	67	69	59	0.234
13C-10C	67	65	56	0.263
13C-10D	67	62	55	0.257
13D-7A	65	67	58	0.216
13D-7B	65	65	57	0.219
13D-7C	65	66	57	0.230
13D-7D	65	64	56	0.233
13D-9A	65	65	59	0.169
13D-9B	65	66	58	0.205
13D-9C	65	64	56	0.233
13D-9D	65	65	59	0.169

13D-10A	65	62	55	0.236
13D-10B	65	69	59	0.213
13D-10C	65	65	56	0.243
13D-10D	65	62	54	0.260
14A-7A	67	67	59	0.213
14A-7B	67	65	54	0.308
14A-7C	67	66	58	0.227
14A-7D	67	64	56	0.253
14A-9A	67	65	59	0.192
14A-9B	67	66	59	0.203
14A-9C	67	64	56	0.253
14A-9D	67	65	56	0.263
14A-10A	67	62	56	0.233
14A-10B	67	69	58	0.256
14A-10C	67	65	57	0.240
14A-10D	67	62	57	0.208
14B-7A	62	67	60	0.130
14B-7B	62	65	55	0.236
14B-7C	62	66	58	0.171
14B-7D	62	64	56	0.200
14B-9A	62	65	59	0.132
14B-9B	62	66	59	0.145
14B-9C	62	64	56	0.200
14B-9D	62	65	57	0.186
14B-10A	62	62	56	0.176
14B-10B	62	69	57	0.230
14B-10C	62	65	56	0.211
14B-10D	62	62	54	0.229
14C-7A	62	67	56	0.233
14C-7B	62	65	53	0.284
14C-7C	62	66	54	0.270
14C-7D	62	64	54	0.250
14C-9A	62	65	55	0.236
14C-9B	62	66	56	0.222
14C-9C	62	64	52	0.297
14C-9D	62	65	53	0.284
14C-10A	62	62	52	0.278
14C-10B	62	69	54	0.299
14C-10C	62	65	54	0.260
14C-10D	62	62	52	0.278
14D-7A	65	67	60	0.167
14D-7B	65	65	54	0.289
14D-7C	65	66	57	0.230
14D-7D	65	64	56	0.233
14D-9A	65	65	58	0.194
14D-9B	65	66	58	0.205
14D-9C	65	64	56	0.233

14D-9D	65	65	59	0.169
14D-10A	65	62	55	0.236
14D-10B	65	69	56	0.282
14D-10C	65	65	56	0.243
14D-10D	65	62	53	0.284
15A-7A	60	67	57	0.186
15A-7B	60	65	53	0.264
15A-7C	60	66	55	0.225
15A-7D	60	64	55	0.203
15A-9A	60	65	56	0.188
15A-9B	60	66	55	0.225
15A-9C	60	64	54	0.229
15A-9D	60	65	54	0.239
15A-10A	60	62	53	0.232
15A-10B	60	69	53	0.303
15A-10C	60	65	52	0.288
15A-10D	60	62	52	0.257
15B-7A	67	67	62	0.139
15B-7B	67	65	57	0.240
15B-7C	67	66	61	0.153
15B-7D	67	64	59	0.181
15B-9A	67	65	59	0.192
15B-9B	67	66	60	0.178
15B-9C	67	64	59	0.181
15B-9D	67	65	61	0.141
15B-10A	67	62	57	0.208
15B-10B	67	69	60	0.211
15B-10C	67	65	57	0.240
15B-10D	67	62	56	0.233
15C-7A	63	67	60	0.143
15C-7B	63	65	53	0.293
15C-7C	63	66	57	0.208
15C-7D	63	64	55	0.236
15C-9A	63	65	57	0.197
15C-9B	63	66	57	0.208
15C-9C	63	64	56	0.211
15C-9D	63	65	57	0.197
15C-10A	63	62	55	0.214
15C-10B	63	69	56	0.263
15C-10C	63	65	54	0.270
15C-10D	63	62	53	0.264
15D-7A	62	67	57	0.208
15D-7B	62	65	54	0.260
15D-7C	62	66	57	0.197
15D-7D	62	64	55	0.225
15D-9A	62	65	55	0.236
15D-9B	62	66	56	0.222

15D-9C	62	64	55	0.225
15D-9D	62	65	57	0.186
15D-10A	62	62	55	0.203
15D-10B	62	69	56	0.253
15D-10C	62	65	53	0.284
15D-10D	62	62	53	0.254
Average genetic distance				**0.218**
Minimum genetic distance				**0.116**
Maximum genetic distance				**0.308**
Standard deviation				**0.040**

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