

**GEOGRAPHIC VARIATION IN *HESPEROCYPARIS*
(*CUPRESSUS*) *ARIZONICA* AND *H. GLABRA*:
RAPDS ANALYSIS**

Robert P. Adams

Biology Department, Baylor University, Box 727, Gruver, TX, 79040
Robert_Adams@baylor.edu

Jim A. Bartel

U.S. Fish and Wildlife Service, Carlsbad Fish and Wildlife Office
6010 Hidden Valley Road, Suite 101
Carlsbad, CA 92011-4213

ABSTRACT

RAPDs were analyzed from five *Hesperocyparis* (= *Cupressus*) *arizonica* and three *H. glabra* populations. This analysis supports the continued recognition of these taxa at the specific level. *Phytologia* 91(2): 244-250 (August, 2009).

KEY WORDS: *Hesperocyparis* (= *Cupressus*) *arizonica*, *H. glabra*, RAPDs, geographic variation, taxonomy.

Hesperocyparis (= *Cupressus*) *arizonica* (Greene) Bartel and *H. glabra* (Sudw.) Bartel are two closely related taxa that have a variable taxonomic history. Table 1 summarizes the taxonomic treatments. Wolf (1948) recognized both taxa at the specific level (Table 1), while Little (1970) reduced *C. glabra* to a variety (*C. arizonica* var. *glabra*). Though Bartel (1993) and Eckenwalder (1993) included *C. glabra* within *C. arizonica*, Farjon (1998) followed Little (1970) in recognizing *C. glabra* as a variety of *C. arizonica* (Table 1). All these classifications were based strictly on morphology.

Askew and Schoenike (1982) concluded that bark texture (fibrous and not peeling = *H. arizonica* versus smooth and peeling in thin plates or strips = *H. glabra*) correctly identified the taxa 89% of the time, while resin gland occurrence worked 85% of the time. However, Little

(2006) separated these taxa using only resin glands (on < 5% of leaves =*H. arizonica* versus on >5% of leaves =*H. glabra*) in his key..

Table 1. Taxonomic treatments of *H. arizonica* and *H. glabra*.

Treatment	<i>arizonica</i>	<i>glabra</i>
Wolf (1948)	<i>C. arizonica</i>	<i>C. glabra</i> .
Little (1970)	<i>C. arizonica</i> var. <i>arizonica</i>	<i>C. arizonica</i> var. <i>glabra</i> (Sudw.) Little (= <i>C. arizonica</i>)
Bartel (1993)	<i>C. arizonica</i>	(= <i>C. arizonica</i>)
Eckenwalder (1993)	<i>C. arizonica</i>	(= <i>C. arizonica</i>)
Farjon (1998)	<i>C. arizonica</i> var. <i>arizonica</i>	<i>C. arizonica</i> var. <i>glabra</i> (Sudw.)
Bartel et al. (2003)	<i>C. arizonica</i>	<i>C. glabra</i> Sudw.
D. P. Little (2006)	<i>Callitropsis arizonica</i> (Greene) D. P. Little	<i>Callitropsis. glabra</i> (Sudw.) D. P. Little
Adams et al.(2009)	<i>Hesperocyparis arizonica</i> (Greene) Bartel	<i>Hesperocyparis glabra</i> (Sudw.) Bartel

A recent analyses using RAPDs fingerprinting (Bartel et al., 2003) found *H. glabra* to be distinct from *H. arizonica* (Fig. 1).

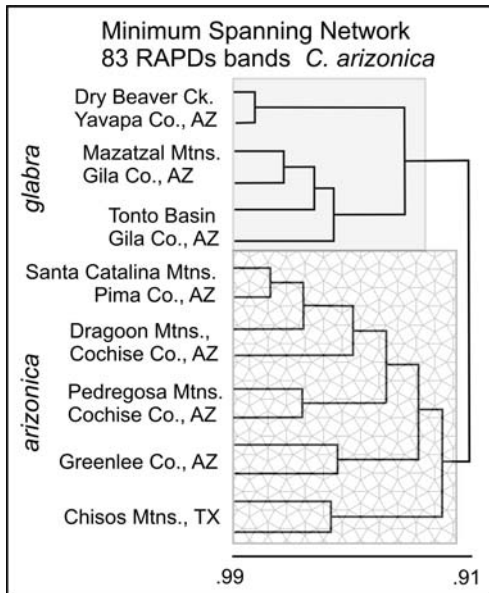


Figure 1. Minimum spanning network (from Bartel et al., 2003).

Recent DNA sequencing of *Cupressus sensu lato* (Little et al., 2004, Little, 2006) demonstrated that the Western Hemisphere species form a well-supported clade quite separated from the Eastern Hemisphere cypresses. As a result, Little (2006) not only confined the genus *Cupressus* to the Eastern Hemisphere, he used *Callitropsis nootkatensis* and its generic epithet for the Western Hemisphere cypresses and *Xanthocyparis vietnamensis*.

Little (2006) found very limited nucleotide differences among any of the Western Hemisphere *Hesperocyparis* species. However, he did find differences that supported the recognition of *H. arizonica* and *glabra* (Table 2) and he maintained these two taxa as distinct species.

Table 2. Summary of DNA sequencing support for the recognition of *H. arizonica* and *H. glabra* (from Little, 2006).

<u>Chloroplast genes</u>	<u>Nuclear genes</u>	
<i>matK+rbcL+trnL</i>	nrDNA(ITS)	<i>NEEDLY</i>
60% support	56% support	no differences

Debreczy et al. (2009) argued on morphological grounds that *Ca. nootkatensis* is a monotypic genus. Sequencing by Adams et al. (2009) of two additional nuclear genes and petN-psbM further supported the recognition of *Ca. nootkatensis* as a monotypic genus. Because *Callitropsis* should not be applied to the Western Hemisphere cypresses, Bartel and Price in Adams et al. (2009) described a new genus, *Hesperocyparis*, for the Western Hemisphere cypresses (exclusive of *Xanthocyparis vietnamensis* and *Callitropsis nootkatensis*). In Adams et al. (2009), Bartel made the new combinations of *Hesperocyparis arizonica* (Greene) Bartel and *H. glabra* (Sudw.) Bartel. The present paper will analyze geographical variation within and between *H. arizonica* and *H. glabra*.

MATERIALS AND METHODS

Collection information for specimens utilized:

Hesperocyparis arizonica: Adams 9268-9269, Boot Spring, Chisos Mtns., Brewster Co., TX, USA; Lab # 9378-9379,

Bartel,1580A,B, upper Bear Canyon, 11.8 mi n of Houghton Rd along Catalina Hwy, N 32 21' 47.9", W 110 42' 50.3", 1695m, Santa Catalina Mtns., Pima Co., AZ; Lab # 9380-9381, Bartel, 1581A,B, Stronghold Canyon East, 7.3 mi from US 191, along Ironwood Rd., N 31 56 26.9", W 109 57' 27.8"1457m, Dragoon Mtns., Cochise Co., AZ; Lab # 9382-9383, Bartel,1582A, B, Rucker Canyon, 6.1 mi from Leslie Canyon Rd along Rucker Canyon Rd., N 31 45' 18.3", W 109 22' 39.5", 1676m, Pedregosa Mtns., Cochise Co., AZ, 9384-9385, Bartel, 1583A,B, Metcalf, w of Chase Creek, 9.6 mi from lower Eagle Creek Rd, along US191, N 33 08' 01.5", W 109 22' 38.7", 1683m, Greenlee Co., AZ,

Hesperocyparis glabra, 9386-9387, Bartel,1584A,B, upper Slate Creek, 7.1 mi sw if SR 188, along SR87, N 33 57' 28.5", W 111 24' 21.8", 1009m, Mazatzal Mtns., Gila Co., AZ, 9388-9389, Bartel, 1585A,B, se of Tonto Natural Bridge St. Park, jct along SR87, nw of East Verde River, N 34 18' 58.6", W 111 23' 12.6", 1483m, Gila Co., AZ, 9390-9391, Bartel, 1586A,B, upper Dry Beaver Creek, 0.1 mi. e of SR 179 along Wild Horse Mesa Rd., N 34 46' 07.7", W 111 45' 46.4", 1225m, Yavapai Co., AZ.

Adams' specimens are deposited at BAYL herbarium, Waco, Texas. Bartel's specimens currently are held in his personal herbarium, Carlsbad, California.

One gram (fresh weight) of foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20 C until the DNA was extracted. DNA was extracted from the leaves by use of the Qiagen DNeasy Mini-plant extractors. Ten-mer primers were purchased from the University of British Columbia (5'-3'): 131, GAA ACA GCG T; 153, GAG TCA CGA G; 204, TTC GGG CCG T; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 239, CTG AAG CGG A; 244, CAG CGA ACC G; 250, CGA CAG TCC C; 327, ATA CGG CGT C; 338 CTC TGG CGG T; 346, TAG GCG AAC G; 347, TTG CTT GGC G; 389 CGC CCG CAG T; 413, GAG GCG GCG A;

PCR was performed in a volume of 15 ml containing 50 mM Tris-HCl (pH 9), 2.0 mM MgCl₂, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 mM primers, 0.3 ng genomic DNA, 15 ng BSA and 0.6 unit of Taq DNA polymerase (Promega). A control

PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94 C (1.5 min) for initial strand separation, then 40 cycles of 38 C (2 min), 72 C (2 min), 91 C (1 min). Two additional steps were used: 38 C (2 min) and 72 C (5 min) for final extension. Bands that occurred once or did not show fidelity within the two replicated samples of each taxon were eliminated. It should be noted that these bands contain very useful information for the study of genetic variance and individual variation, but are merely "noise" in the present taxonomic study. Bands were scored in 4 classes: very bright (=6); medium bright (=5), faint (=4) and absent (=0). See Adams and Demeke (1993) for details on electrophoresis and RAPD band scoring.

Similarity measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric, Gower 1971; Adams 1975). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966).

RESULTS AND DISCUSSION

Contoured clustering based on 83 RAPD bands (Figure 2) shows that the populations cluster by geographical proximity. The most similar *H. arizonica* populations are Santa Catalina Mtns. - Dragoon Mtns. (0.969), followed by Dragoon Mtns. - Pedregosa Mtns. (0.943), then Pedregosa Mtns. - Greenlee Co. (0.932). The Chisos Mtns., TX population is clearly quite differentiated (linkage of 0.926 to the Dragoon Mtns. population).

The most similar *H. glabra* populations are Mazatzal Mtns. - Tonto Basin (0.959), then Tonto Basin - Dry Beaver Creek (0.937). The *H. arizonica* - *H. glabra* populations are finally linked by Greenlee Co. - Mazatzal Mtns. (0.916).

The linkage of populations of both taxa by geographically near neighbors suggests that differentiation is due to restricted gene exchange perhaps leading to genetic drift. Due to the likely short distances of cone/ seed dispersal, it seems probable that pollen dispersal

over long distances may be the principal agent of gene flow among these populations.

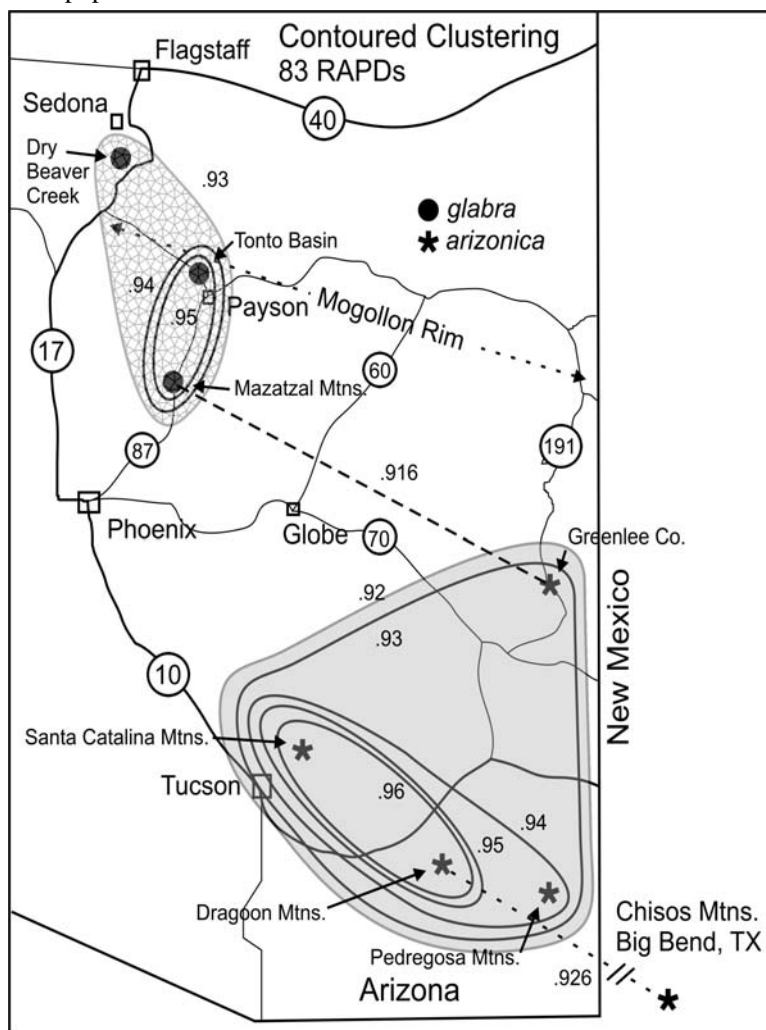


Figure 2. Contoured clustering of populations of *H. arizonica* and *H. glabra* based on 83 RAPDs bands.

ACKNOWLEDGEMENTS

This research supported in part with funds from U. S. Fish and Wildlife Service, Grant 814307J011. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

LITERATURE CITED

- Adams, R. P., 1975. Statistical character weighting and similarity stability. *Brittonia* 27: 305-316.
- Adams, R. P., J. A. Bartel and R. A. Price. 2009. A new genus, *Hesperocyparis*, for the cypresses of the western hemisphere. *Phytologia* 91(1): 160-185.
- Askew, G. R. and R. E. Schoenike. 1982. Identification of characteristic traits of two varieties of Arizona cypress. *Silvae Genetica* 31: 158-160.
- Bartel, J. A. 1993. Cypress family-Cupressaceae. *J. Ariz. Nev. Acad. Sci.* 27: 195-200.
- Bartel, J. A., R. P. Adams, S. A. James, L. E. Mumba and R. N. Pandey. 2003. Variation among *Cupressus* species from the western hemisphere based on Random Amplified Polymorphic DNAs (RAPDs). *Biochem. Syst. Ecol.* 31: 693-702.
- Eckenwalder, J. E., 1993. *Cupressus* In: Morin, N.R. (Ed.), *Flora of North America. Pteridophytes and Gymnosperms*, vol. 2. Oxford University Press, New York, pp. 405-408.
- Farjon, A., 1998. *World checklist and bibliography of conifers*. Royal Bot. Gard., Kew, London.
- Gower, J. C., 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* 53: 326-338.
- Gower, J. C., 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857-874.
- Little, E. L., 1970. Names of New World cypresses (*Cupressus*) *Phytologia* 20: 429-445.
- Little, D. P. 2006. Evolution and circumscription of the true Cypresses (Cupressaceae: *Cupressus*) *Syst. Bot.* 31: 461-480.
- Little, D. P., A. E. Schwarzbach, R. P. Adams and C-F. Hsieh. 2004. The circumscription and phylogenetic relationships of *Callitropsis* and the newly described genus *Xanthocyparis* (Cupressaceae). *Am. J. Bot.* 91: 1872-1881.
- Wolf, C. B., 1948. The New World cypresses. *Aliso* 1: 1-250.