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# GENETIC POLYMORPHISM IN THE MORPHOLOGICALLY REDUCED DWARF MISTLETOES (ARCEUTHOBIMUM, VISCACEAE): AN ELECTROPHORETIC STUDY<sup>1</sup>

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## ABSTRACT

*Arceuthobium* is a well defined genus of obligate parasites of conifers. Assessment of taxonomic and phylogenetic relationships in this group is difficult owing to morphological reductions and complex host relationships. In the present study, genetic relationships within and among 19 taxa were examined using starch gel electrophoresis of triploid seed endosperm tissue. Allelic frequency data for eleven polymorphic loci were derived from analysis of 40 natural populations collected from the U.S. and Mexico. The genus showed remarkably high levels of genetic diversity: averaged across the 19 taxa, 66.7% of the loci were polymorphic with an average of 2.23 alleles per locus. This level of polymorphism is approximately double the average value reported for many dicotyledons and stands in contrast to the overall uniform morphology of these parasites.

Unweighted pair group cluster analysis (UPGMA) of genetic similarity measures was conducted for all sampled populations and the results compared to a phenetic system of classification for the genus. Similarities between the two studies include the recognition of two subgenera based upon the segregation of the verticillately branched *Arceuthobium americanum* from the remainder of the taxa examined. Analysis of isozyme data supported a grouping of six taxa: *A. vaginatum* ssp. *cryptopodium*, *A. vaginatum* ssp. *durangense*, *A. gillii*, *A. rubrum*, *A. divaricatum*, and *A. douglasii*. The placement of the latter three taxa in other groups by phenetic criteria provides evidence for molecular divergence not seen using morphological features. The *Campylopodium* group of taxa comprised eleven members that were linked at similarity values of 80% or greater. Populations were not unambiguously grouped according to species as defined by the phenetic study or at similarity levels comparable to other well defined species in this study. This group is either not reproductively isolated or molecular differentiation is cryptic due to rapid adaptive radiation onto numerous host tree species.

*ARCEUTHOBIMUM* M. VON BIEBERSTEIN (dwarf mistletoes) is a clearly defined genus of obligate parasites of the Pinaceae in the New World and the Pinaceae and Cupressaceae in the Old World. These plants are currently treated as members of the family Viscaceae, considered distinct from the Loranthaceae *sensu stricto* (Kuijt, 1969; Wiens and Barlow, 1971). The dwarf mistletoes are characterized by extreme morphological reductions and lack leaves, trichomes, true roots, and non-essential floral parts. As the number of useful characters for taxonomic analysis is limited, determination of intrageneric phylogenetic relationships has proven difficult.

The first major classification of *Arceuthobium* (Gill, 1935) strongly weighted host colonization, in some cases irrespective of observed morphological differences or similarities in the parasites themselves (see Hawksworth and Wiens, 1972, p. 41). Kuijt (1955) proposed a subgeneric classification not based upon this host form concept where, for example, the two parasites of ponderosa pine, *Arceuthobium vaginatum* and *A. campylopodium*, were considered separate yet morphologically variable species. Kuijt (1955) maintained, however, that the parasites of hemlock, larch, digger pine, and many other host trees were all part of one species, *A. campylopodium*.

The first comprehensive monograph of the genus, worldwide in scope, documented the geographic and host ranges of 32 taxa in 28 species (Hawksworth and Wiens, 1972). A major tenet of this system was that morphological integrity of the mistletoe is maintained on separate host species. The taxonomic placement of species was influenced by the results of a numerical phenetic analysis (Hawksworth, Estabrook and Rogers, 1968) which used 60 char-

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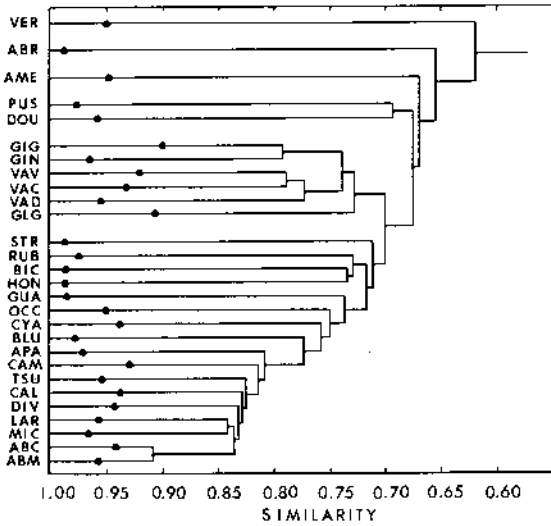


Fig. 1. Dendrogram showing the results of a phenetic analysis of most New World dwarf mistletoes (reproduced from Hawksworth and Wiens, 1972). The full scientific names can be found in Table 1. The scale shows overall phenetic similarity based upon a combined analysis of 60 morphological, physiological, and host characters. The dot shows the level of intrataxon similarity, i.e., the further the dot from 1.0, the more variable the taxon.

acters involved with host choice, parasite morphology, physiology, phenology, and secondary chemistry (Fig. 1). Further analysis of flavonoid chemistry of *Arceuthobium* (Crawford and Hawksworth, 1979) utilized 36 of the 38 known North American taxa but showed few differences between established subgenera, sections or species. Nearly all taxa have been examined cytologically (Wiens, 1968) and these show gametic chromosomes of similar morphology and base number ( $X = 14$ ). In addition, no hybridization or polyploidy has been reported for the genus. The classification system of Hawksworth and Wiens (1972) mainly differed from that of Kuijt (1955) by treating the forms of *A. campylopodum* as distinct species. An updated classification for New World dwarf mistletoes (Hawksworth and Wiens, 1984) is shown in Table 1 and the abbreviations assigned to each taxon will be used throughout this paper.

Despite these taxonomic studies, species relationships, species boundaries, and phylogenetic patterns within these parasites remain unclear. Electrophoresis has proven extremely useful for quantitatively estimating the amount of genetic variation present within populations (Gottlieb, 1981; Crawford, 1983) and for generating systematic information in plants (Avisé, 1974; Gottlieb, 1977). Accurate genotype determinations can be inferred from gel banding patterns given the Mendelian mode of in-

TABLE 1. Subgeneric classification of New World dwarf mistletoes (Hawksworth and Wiens, 1984)

Name	Abbreviation
<b>I. Subgenus <i>Arceuthobium</i> (no sections)</b>	
<i>A. abietis-religiosae</i>	ABR
<i>A. americanum</i> <sup>a</sup>	AME
<i>A. verticilliflorum</i>	VER
<b>II. Subgenus <i>Vaginata</i></b>	
<b>A. Section <i>Vaginata</i></b>	
<i>A. aureum</i> ssp. <i>aureum</i>	AUR
<i>A. aureum</i> ssp. <i>petersonii</i>	AUP
<i>A. gillii</i> ssp. <i>gillii</i> <sup>a</sup>	GIG
<i>A. gillii</i> ssp. <i>nigrum</i>	GIN
<i>A. globosum</i> ssp. <i>globosum</i>	GLO
<i>A. globosum</i> ssp. <i>grandicaule</i>	GLG
<i>A. vaginatum</i> ssp. <i>vaginatum</i>	VAV
<i>A. vaginatum</i> ssp. <i>cryptopodum</i> <sup>a</sup>	VAC
<i>A. vaginatum</i> ssp. <i>durangense</i> <sup>a</sup>	VAD
<b>B. Section <i>Campylopoda</i></b>	
<b>1. Series <i>Campylopoda</i></b>	
<i>A. abietinum</i> f. sp. <i>concoloris</i> <sup>a</sup>	ABC
<i>A. abietinum</i> f. sp. <i>magnificae</i> <sup>a</sup>	ABM
<i>A. apachecum</i> <sup>a</sup>	APA
<i>A. blumeri</i> <sup>a</sup>	BLU
<i>A. californicum</i> <sup>a</sup>	CAL
<i>A. campylopodum</i> <sup>a</sup>	CAM
<i>A. cyanocarpum</i> <sup>a</sup>	CYA
<i>A. divaricatum</i> <sup>a</sup>	DIV
<i>A. guatemalense</i>	GUA
<i>A. laricis</i> <sup>a</sup>	LAR
<i>A. microcarpum</i> <sup>a</sup>	MIC
<i>A. occidentale</i> <sup>a</sup>	OCC
<i>A. pendens</i>	PEN
<i>A. tsugense</i> <sup>a</sup>	TSU
<b>2. Series <i>Rubra</i></b>	
<i>A. bicarinatum</i>	BIC
<i>A. hondurensis</i>	HON
<i>A. rubrum</i> <sup>a</sup>	RUB
<b>3. Series <i>Stricta</i></b>	
<i>A. strictum</i>	STR
<b>C. Section <i>Minuta</i></b>	
<i>A. douglasii</i> <sup>a</sup>	DOU
<i>A. pusillum</i> <sup>a</sup>	PUS

<sup>a</sup> Dwarf mistletoe species used in this study.

heritance, the highly conserved number and subcellular compartmentalization of plant isozymes (Gottlieb, 1982), and the conservative nature of quaternary structure of many isozymes of higher plants (Gottlieb, 1981).

Most isozyme analyses dealing with polyploid tissue have involved even numbers (tetraploids, hexaploids, etc.). The relationship between band number, band intensity and position, and tissue ploidy level has been documented for ADH in hexaploid wheat-rye addition lines (Tang and Hart, 1975) and PGI in tetraploid rye (Nielsen, 1980). Those investigations using tissues with uneven ploidy levels (such as triploid) have been with crop plants such as wheat and maize (Scandalios, 1969)

and few have utilized such tissue in systematic electrophoretic studies.

For the present study, electrophoresis was chosen as a method for obtaining new evidence relating to microevolutionary processes in *Arceuthobium*. To date, electrophoresis has not been utilized for measuring genetic variation in any loranthaceous mistletoe and the only analyses of viscaceous mistletoes have been by Nickrent (1984) and Nickrent, Guttman, and Eshbaugh, (1984) who used triploid seed endosperm and Linhart (1984) who used diploid stem tissue. The objectives of this study, using 19 taxa of New World dwarf mistletoes, were to assess the amount of genetic variation present within populations, to compare the species delimitations based upon isozyme evidence with those based upon the phenetic study of Hawksworth and Wiens (1972), and to contribute an additional, independent source of information which might be useful in determining phylogenies within the genus.

**MATERIALS AND METHODS**—Seed material and herbarium voucher specimens of 19 taxa of dwarf mistletoes were obtained from 40 populations in the U.S. and Mexico (locality information in Nickrent, 1984 and Nickrent et al., 1984). Voucher specimens are deposited at the Willard S. Turrell Herbarium, Miami Univ., OH (MU) and the Univ. of Illinois Herbarium (IL). Samples from 20 randomly selected trees per population were obtained when feasible. In both the field and laboratory, low temperatures (2–4 C) and high humidity proved optimal for seed storage.

Seeds were placed in distilled water overnight and then transferred to 2% H<sub>2</sub>O<sub>2</sub> (for sterilization and to initiate germination) at 14 C with 12 hr of fluorescent light. Germination was used as an indicator of viability and could be observed as early as four days after these treatments. Enzyme extraction was facilitated by first removing the viscin coat and (if necessary) the diploid radicle tissue from each seedling. Endosperm tissue was frozen (–70 C) and stored until required and enzyme activity could be detected after more than two years.

Early in the study problems were encountered with protein extraction from the small (2.0 mg or less wet wt) seeds of some dwarf mistletoe taxa. This was alleviated by using a plexiglass plate with 20 small, cylindrical wells and a stainless steel pestle connected to a motor driven homogenizer. The plate was frozen at –70 C prior to use and was kept on ice during the grinding procedure. Single endosperms were ground in a HEPES buffer (35–50  $\mu$ l, 0.01 M)

with 2-mercaptoethanol (0.025 M) and polyvinylpyrrolidone (PVP-40, 10% w/v). The crude extract was absorbed directly onto two filter paper wicks (Whatman #3, 4.0  $\times$  9.0 mm) and the plate was immediately refrozen.

Enzyme separation was performed with 15% horizontal starch gels as described by Shaw and Prasad (1970). The following enzyme systems were used in this study: alcohol dehydrogenase (ADH, E.C. 1.1.1.1), glucose 6-phosphate dehydrogenase (G-6-PDH, 1.1.1.49), glucose-phosphate isomerase (GPI, 5.3.1.9), glutamate dehydrogenase (GDH, 1.4.1.2), isocitrate dehydrogenase (IDH, 1.1.1.42), malate dehydrogenase (MDH, 1.1.1.37), 6-phosphogluconate dehydrogenase (6-PGD, 1.1.1.44), and phosphoglucomutase (PGM, 2.7.5.1). ADH and GPI were resolved on a phosphate buffer (pH 6.7) as reported in Selander et al. (1971); MDH, IDH, 6-PGD, and G-6-PDH on a modification of the citrate-morpholine buffer (pH 6.5 gel, pH 6.1 electrode) as reported in Clayton and Tretiak (1972); and PGM and GDH were resolved on a Tris-EDTA-Borate gel (pH 9.1) as reported in Ayala et al. (1972). Enzyme staining protocols were derived from Shaw and Prasad (1970), Selander et al. (1971), Siciliano and Shaw (1976), O'Malley, Wheeler and Guries (1980), and Soltis et al. (1983).

Bands for each enzyme locus were assigned relative mobility designations as compared to *Arceuthobium vaginatum* ssp. *cryptopodum* (VAC) seeds run as standards on each gel (assigned R.M. values of "100"). For enzyme systems with two or more loci present on the gel, the most anodal locus was designated number one. Allele frequencies were tabulated for each locus and population and these frequencies subjected to analysis with BIOSYS-1, a FORTRAN IV computer program designed to analyze genetic variation (Swofford and Selander, 1981). The data matrix of allele frequencies for 11 polymorphic loci for each of the 40 dwarf mistletoe populations is not reproduced here because of its size. This matrix can be seen in Nickrent (1984) or can be obtained from the author upon request.

**RESULTS AND DISCUSSION**—Eight enzyme systems representing eleven presumed loci were used for statistical analysis: 6-PGD, IDH, MDH-2, MDH-3, G-6-PDH, GPI, ADH-1, ADH-2, PGM-1, PGM-2, and GDH. One seed did not generate enough protein sample to assay across the three different gel buffers, hence different individuals from the same population or seeds from the same female parent plant were used for different gels. As one individual did not provide genotypes across all eleven

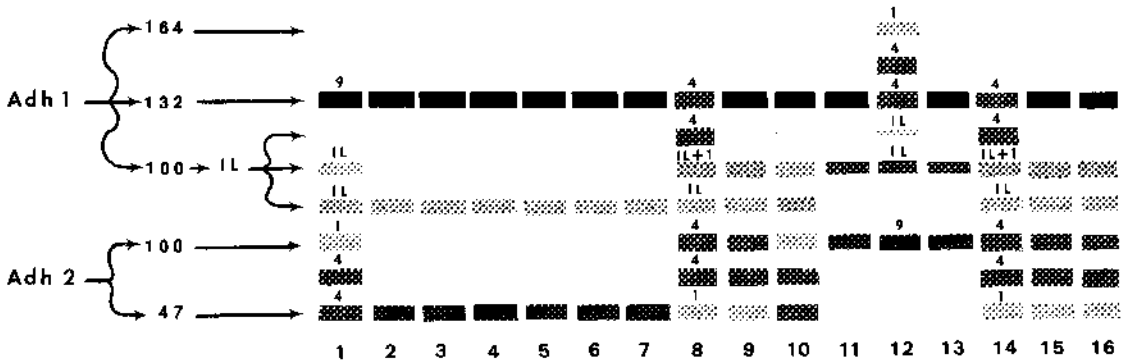


Fig. 2. Schematic diagram of an ADH zymogram of 16 seed endosperms from *Arceuthobium divaricatum*. The numbers 100, 132, and 164 refer to relative mobility phenotypes for enzymes coded by the ADH-1 locus, 100 and 47 for relative mobilities for enzymes coded by the ADH-2 locus. The small numbers above the bands refer to enzyme "dosage," i.e., the predicted proportion of enzyme molecules for that position given homozygote or heterozygote individuals (see text). IL is a band representing the position of the interlocus heterodimeric enzyme.

loci, frequencies of alleles at each locus for each of the forty populations were used. The number of individuals sampled per locus and per population varied; however, all sample sizes were ten or greater.

*Interpretation of isozymes in triploid endosperm*—Triploid endosperm has seldom been used to generate isozyme data for systematic purposes, despite the fact that more than 80% of all angiosperms have monosporic and bisporic embryo sacs which produce this tissue type following fertilization (Palser, 1975). In *A. minutissimum*, female gametophyte development conforms to the *Allium* (bisporic) type (Bhandari and Nanda, 1968). This type of embryo sac development would result in triploid endosperm following fertilization. The ploidy level of *Arceuthobium* endosperm was confirmed by banding patterns observed for the 19 dwarf mistletoe taxa examined in this study. Although seldom used for population genetic analyses, triploid endosperm can be advantageous in showing linkage relationships (Schoen, 1979, 1980; Nickrent, 1984) and in conducting outcrossing analyses (Schoen, 1982).

Results of the analysis of endosperm from 40 populations of *Arceuthobium* showed that banding patterns for monomeric and dimeric enzymes followed theoretical predictions with respect to band number, position, and intensity (Nickrent, 1984). Alcohol dehydrogenase (ADH) obtained from dwarf mistletoe seed endosperm will be used to illustrate banding patterns typical of most dimeric enzymes used in this study. ADH has been intensively studied in cultivated higher plants such as maize (Freeling, 1974) and sunflower (Torres, 1976)

as well as wild species such as *Tragopogon* (Roose and Gottlieb, 1980). Usually three zones of activity are reported which represent enzymes coded by two gene loci and an interlocus heterodimer region.

In a schematic representation of a zymogram resulting from electrophoresis of 16 seed endosperms from pinyon dwarf mistletoes (DIV) stained for ADH (Fig. 2), three zones of activity are indicated: ADH-1, ADH-2, and an interlocus (IL) zone. The numbers 164 and 132 represent relative mobility designations for allozymes (isozymes coded by chromosomal alleles). For this enzyme system interlocus heterodimers are coincident with one of the three allozymes of ADH-1 (ADH-1<sup>100</sup>) but do not overlap ADH-2. Despite the overlap with ADH-1, the staining intensity at specific positions and a knowledge of expected proportions of allozymes for heterozygotes allow accurate genotype assessment of all individuals.

One dark band at ADH-2<sup>47</sup> (slots 2-7) indicates that the sampled individuals are homozygous for that locus and that particular allele (i.e., all three chromosome sets have loci coding for allozymes with the same electrophoretic mobility). Heterozygotes, such as individual 1, displayed three bands with intensities of 4:4:1. Staining intensity is a direct reflection of the number of enzyme molecules present at the band position. The expected proportion of dimeric isozymes derived from a heterozygous (AAB) triploid nucleus may be obtained by the expression  $(\frac{2}{3} A + \frac{1}{3} B)^2$  which represents a maternal chromosome contribution of two and a paternal contribution of one. The coefficients of the resulting expression  $(\frac{4}{9} AA + \frac{4}{9} AB + \frac{1}{9} BB)$  predict the enzyme dosage (and staining intensity) for each of the three

TABLE 2. Genetic variability for 19 taxa of dwarf mistletoes

Taxon abbrev.	Number of pops. (N)	% Polymorphic loci (P)	Mean number of alleles per locus ( $\bar{k}$ )
ABC	2	50.0	1.90
ABM	4	79.5	2.45
AME	4	79.5	2.63
APA	4	59.0	2.18
BLU	1	45.5	2.00
CAL	1	90.0	2.70
CAM	3	78.8	2.37
CYA	2	63.6	1.80
DIV	1	81.8	2.60
DOU	3	72.7	2.70
GIG	1	54.5	2.50
LAR	1	63.6	1.70
MIC	2	59.1	2.10
OCC	1	81.8	2.20
PUS	5	50.9	1.78
RUB	1	45.5	1.50
TSU	1	72.7	1.90
VAC	2	63.6	2.60
VAD	1	72.7	2.80
Mean =		66.6	2.23

electrophoretic phenotypes. This 4:4:1 pattern differs from that obtained from a diploid heterozygote in that the latter shows a 1:2:1 intensity difference for the three bands; i.e.,  $(1/2 A + 1/2 B)^2 = 1/4 AA + 1/2 AB + 1/4 BB$ . Staining intensity (reflecting enzyme dosage) can be quantified densitometrically, however this method was unnecessary here as particular bands are derived from one individual and are only compared with other bands within the same slot.

**Electrophoretic results**—Considering the 19 taxa collectively, ten or more alleles per locus were observed for all loci except G-6-PDH, ADH-2, and GDH and greater than 20 alleles were detected for 6-PGD and GPI. For the 19 taxa, all sampled loci are polymorphic; however, GDH and G-6-PDH are often monomorphic within individual species. Though little GDH variability exists within a taxon, fixation for different alleles between taxa makes this system useful in showing general taxonomic affinities.

An estimation of genetic variability per taxon as measured by the mean percent of loci polymorphic per taxon (P) and the mean number of alleles per locus per taxon ( $\bar{k}$ ) is given in Table 2. A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99. The mean percentage of loci polymorphic for the 19 taxa examined is 66.6, a value placing dwarf mistletoes among

the most genetically diverse dicotyledonous plants examined electrophoretically. *Arceuthobium* may be nearly twice as genetically variable as the average dicotyledon (31.28%, in Hamrick, Mitton and Linhart, 1981), and the level of polymorphism approaches that of the gymnosperms (67.01%). This high level of polymorphism probably results from the extensive geographic range of many dwarf mistletoe species, a perennial growth habit, and an obligately outcrossing breeding system (dioecy) involving both wind and insect pollination (Penfield, Stevens and Hawksworth, 1976; Gilbert and Punter, 1984). The average number of polymorphic loci is 51% and 18% for species with outcrossing versus selfing breeding systems (Gottlieb, 1981).

Given two alleles at a locus, heterozygous triploid tissue will have two homozygote (AAA, BBB) and two heterozygote (AAB, ABB) genotypes. For heterozygous diploid tissue, only one genotype (AB) can occur. The presence of two heterozygote classes in triploids precludes the use of standard measures such as mean levels of heterozygosity (Nei, 1978) and conformation to Hardy-Weinberg equilibrium. An allozyme analysis of diploid shoot tissue of *Arceuthobium americanum* and *A. vaginatum* ssp. *cryptopodum* with three loci (ADH, GPI, and PER) has been reported by Linhart (1984). The observed genotype frequencies for these populations did not differ significantly from those expected given Hardy-Weinberg equilibrium conditions.

Phenograms were generated by the unweighted pair group method with arithmetic averaging (UPGMA) as described in Sneath and Sokal (1973). The genetic similarity measure reported here is that of Rogers (1972) and the resulting phenogram is shown in Fig. 3. Other measures, such as Nei's (1978) coefficient of genetic similarity, resulted in comparable phenograms but had higher (56.7 vs. 22.4) percent standard deviation (Fitch and Margoliash, 1967). Branching arrays generated by the two measures differed only for taxa within the *Campylopodum* complex.

A matrix of genetic distance values (Nei, 1978) averaged by species is shown in Table 3 (mean distance values only). The 19 taxa considered in this study were arranged according to the sectional hierarchy of Hawksworth and Wiens (1972, 1984) in Table 1. The SIMAVE option in BIOSYS was used to average the distance coefficients between the groups at various levels of this hierarchy. Ranges of the distance coefficients were then used to determine whether the group contained closely or distantly related members (Table 4).

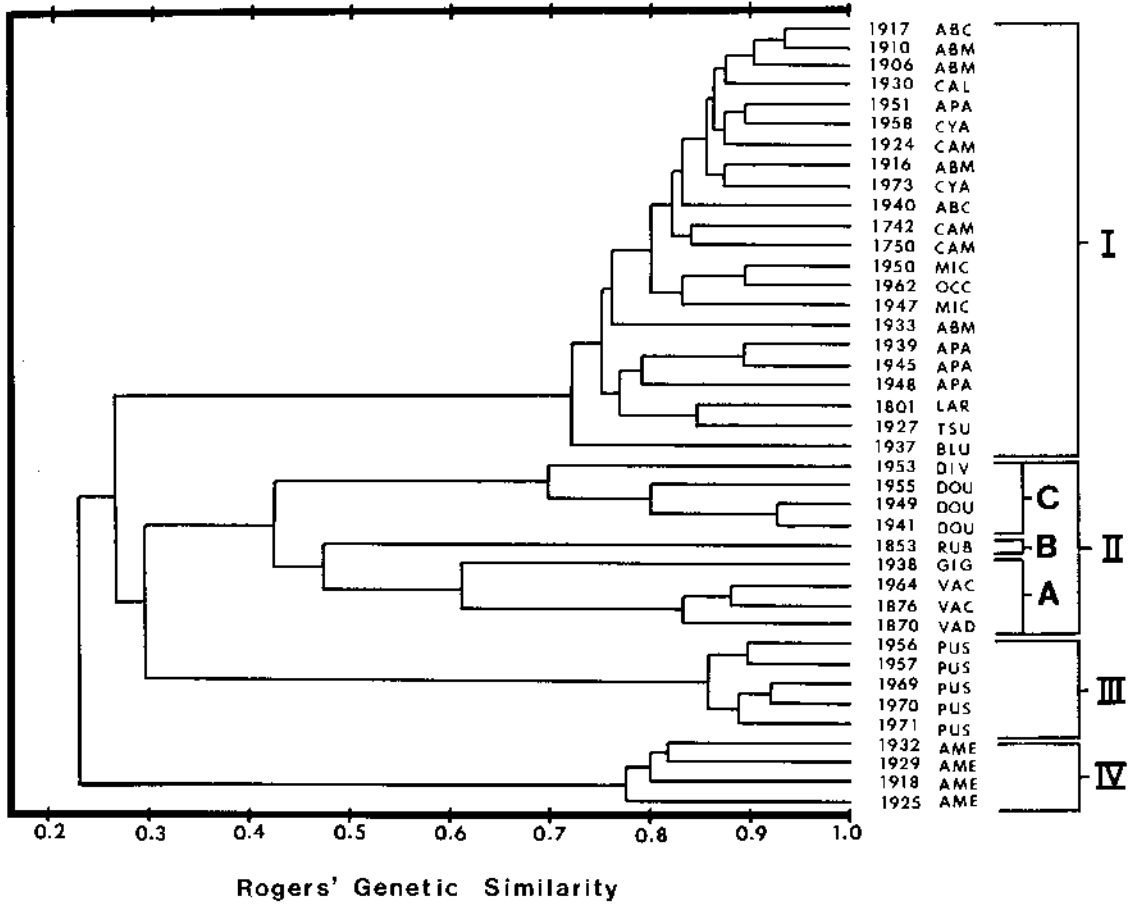


Fig. 3. UPGMA phenogram for 40 dwarf mistletoe populations utilizing Rogers' (1972) coefficient of genetic similarity. Full scientific names are given in Table 1 and locality information for population numbers can be seen in Nickrent (1984). See Table 5 for the groupings identified by roman numerals.

An alternate hierarchal arrangement (Table 5), was based upon a 50% "phenon" line (Sneath and Sokal, 1973) of the isozyme phenogram (Fig. 3). The means and ranges of the distance values, given this arrangement, are shown in Table 6. The names for the groupings shown in Tables 5 and 6 are in some cases similar to names used by Hawksworth and Wiens (1972) but are only used here to facilitate identification of groupings based on an analysis of isozyme data.

The ranges of the distance coefficients (Table 4) indicate that the most heterogeneous group in the genus is section *Minuta* (mean distance of 0.674, range from 0.010–1.327). This contrasts with the mean intrasectional distance value for section *Americana* (0.101) and *Vaginata* (0.248). Table 6 shows the distance values given the hierarchal arrangement based on the isozyme phenogram and six levels: I, IIA, IIB, IIC, III, and IV. This arrangement reduces

the variation in the *Campylopodium* group from a mean of 0.360 to 0.122. High distance values for section *Campylopoda* apparently results from the inclusion of two taxa (DIV and RUB) which show greater affinity to other sections according to isozyme evidence. Distance coefficient values are identical for Section *Vaginata* and subgroup IIA (*Vaginatium*) because both hierarchal arrangements contain identical taxa (GIG, VAC, VAD). The mean distance value (0.248) is high compared to other groups (Table 6) which indicates greater intragroup heterogeneity. The mean distance coefficient for *Divaricatum* (0.18) is low which suggests this taxon should be segregated as a subgroup of Group II.

A number of similarities and differences appear when the phenetic analysis of Hawksworth and Wiens (1972) is compared with the present study based on isozyme data (cf. Fig. 1, 3). Both methods segregate AME from the

TABLE 3. Interspecific genetic distance values (Nei, 1978)

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.
1. AME	0.101 <sup>a</sup>																		
2. GIG	1.463	*****b																	
3. VAD	1.683	0.336	*****																
4. VAC	1.751	0.489	0.069	0.037															
5. OCC	2.670	1.449	1.114	1.201	*****														
6. CYA	2.636	1.512	1.105	1.184	0.063	0.034													
7. BLU	2.944	1.641	0.974	1.008	0.196	0.173	*****												
8. APA	2.526	1.356	1.039	1.127	0.181	0.110	0.281	0.130											
9. CAM	2.529	1.430	1.107	1.200	0.086	0.062	0.188	0.148	0.063										
10. TSU	3.063	1.588	1.150	1.215	0.203	0.136	0.285	0.119	0.192	*****									
11. CAL	2.565	1.397	1.038	1.119	0.030	0.044	0.121	0.135	0.059	0.150	*****								
12. DIV	1.463	1.007	0.580	0.597	1.995	2.019	1.535	1.947	1.898	2.372	1.886	*****							
13. LAR	2.756	1.474	1.081	1.161	0.130	0.142	0.259	0.174	0.166	0.052	0.112	2.122	*****						
14. MIC	2.525	1.395	1.071	1.148	0.039	0.089	0.252	0.201	0.140	0.202	0.075	2.030	0.147	0.079					
15. ABC	2.637	1.491	1.077	1.158	0.080	0.044	0.118	0.133	0.067	0.184	0.030	1.917	0.177	0.124	0.037				
16. ABM	2.557	1.437	1.055	1.150	0.094	0.006	0.186	0.131	0.083	0.135	0.054	1.967	0.122	0.140	0.074	0.077			
17. RUB	2.562	0.784	0.733	0.777	1.229	1.248	1.043	1.257	1.255	1.231	1.199	1.423	1.160	1.090	1.230	1.209	*****		
18. DOU	1.465	1.166	0.816	0.826	2.375	2.164	1.505	2.229	2.096	2.331	2.146	0.279	2.409	2.546	2.111	2.171	1.408	0.080	
19. PUS	1.920	1.552	1.354	1.423	1.912	1.952	2.105	1.766	1.825	2.240	1.808	1.156	2.027	1.912	1.710	1.901	2.194	1.204	0.057

<sup>a</sup> Ranges for means in Nickrent (1984) or Nickrent et al. (1984).

<sup>b</sup> Only one population included.



TABLE 4. Distance coefficients (Nei, 1978) averaged by sections<sup>a</sup>

Section	# Populations	I.	II.	III.	IV.
I. <i>Campylopoda</i>	24	0.360 (0.005–2.372)			
II. <i>Vaginata</i>	4	1.167 (0.580–1.641)	0.248 (0.037–0.529)		
III. <i>Minuta</i>	8	1.946 (0.218–2.850)	1.239 (0.668–1.666)	0.674 (0.010–1.327)	
IV. "Americana"	4	2.564 (1.344–3.611)	1.662 (1.236–2.124)	1.750 (1.292–2.255)	0.101 (0.070–0.127)

<sup>a</sup> Sectional arrangement according to Hawksworth and Wiens (1972, 1984).

remainder of the genus and both also suggest that the *Vaginatum* group exhibits more variation, both morphological and genetic, than the *Campylopodum* group. The results of isozyme analysis do not, however, support the placement of both DOU and PUS into section *Minuta*. In addition, the pinyon dwarf mistletoe, DIV, is allied most closely with DOU and not the *Campylopodum* complex as suggested by the morphometric analysis. *Arceuthobium rubrum* (RUB), which was placed within its own series of section *Campylopoda* by Hawksworth and Wiens (1972), clusters with three taxa of the *Vaginatum* group. The morphometric analysis suggests that this taxon, along with *A. strictum* (not included in this study), has greater affinity with section *Vaginata* than any other members of Section *Campylopoda*. It appears that RUB may occupy a position within the *Vaginatum* group but with some affinity with the *Campylopodum* complex. A similar situation may also exist with BLU which is placed in the *Campylopodum* group in this study. Both of these taxa are distributed mainly in Mexico and the isozyme evidence collected to date indicates that the *Campylopodum* group, exclusive of BLU, does not occur in Mexico.

For section *Campylopodum*, the isozyme analysis does not support clustering of taxa as discrete species as defined by Hawksworth and Wiens (1972) or at similarity levels consistent with values grouping other members of the genus (lower half of Fig. 3). *Arceuthobium vaginatum s. lat.*, AME, DIV, DOU, GIG, PUS, and RUB are separated before the 70% similarity level. The two subspecies of *A. vaginatum*, VAC and VAD, separate at ca. 82% similarity, yet ten taxa considered separate species within section *Campylopodum* (Hawksworth and Wiens, 1972) show equally high (80% or greater) levels of identity (ABC, ABM, APA, CAL, CAM, CYA, LAR, MIC, OCC, and TSU). Six of these ten taxa (ABC, ABM, APA, CAM, CYA, and MIC) are represented in this study

by more than one population, but cluster analysis does not unambiguously group these populations as discrete species. Populations of *Arceuthobium abietinum s. lat.*, CAM, and APA show some tendency to cluster according to such species limits.

*A comparison of classifications*—The above evidence indicates that the two classifications, one based on morphology, physiology, and host information (Hawksworth and Wiens, 1972), and the second based on isozyme analysis, lead to different interpretations of relationships in the genus. This is not unusual as taxonomic data derived from disparate sources can often

TABLE 5. Classification of 19 dwarf mistletoes based on isozyme evidence

I. <i>Campylopodum</i> group	
	<i>A. abietinum</i> f. sp. <i>concoloris</i>
	<i>A. abietinum</i> f. sp. <i>magnificae</i>
	<i>A. apachecum</i>
	<i>A. blumeri</i>
	<i>A. californicum</i>
	<i>A. campylopodum</i>
	<i>A. cyanocarpum</i>
	<i>A. laricis</i>
	<i>A. microcarpum</i>
	<i>A. occidentale</i>
	<i>A. tsugense</i>
II. <i>Vaginatum</i> group	
A. <i>Vaginatum</i> subgroup	
	<i>A. gillii</i> ssp. <i>gillii</i>
	<i>A. vaginatum</i> ssp. <i>cryptopodum</i>
	<i>A. vaginatum</i> ssp. <i>durangense</i>
B. <i>Rubrum</i> subgroup	
	<i>A. rubrum</i>
C. <i>Divaricatum</i> subgroup	
	<i>A. divaricatum</i>
	<i>A. douglasii</i>
III. <i>Pusillum</i> group	
	<i>A. pusillum</i>
IV. <i>Arceuthobium</i> group	
	<i>A. americanum</i>

TABLE 6. Distance coefficients (Nei, 1978) averaged by isozyme groups

Group	# Populations	I.	IIA.	IIB.	IIC.	III.	IV.
I. <i>Campylopodium</i>	22	0.122 (0.005-0.418)					
IIA. <i>Vaginatum</i>	4	1.206 (0.832-1.641)	0.248 (0.037-0.529)				
IIB. <i>Rubrum</i>	1	1.210 (1.008-1.433)	0.768 (0.733-0.800)	***** (*****-*****)			
IIC. <i>Divaricatum</i>	4	2.137 (1.412-2.850)	0.855 (0.580-1.194)	1.412 (1.392-1.436)	0.180 (0.010-0.324)		
III. <i>Pusillum</i>	5	1.881 (1.351-2.459)	1.438 (1.258-1.666)	2.194 (1.892-2.463)	1.192 (1.053-1.327)	0.057 (0.014-0.121)	
IV. <i>Americanum</i>	4	2.614 (1.814-3.611)	1.662 (1.236-2.124)	2.562 (2.330-2.968)	1.465 (1.292-1.732)	1.920 (1.528-2.255)	0.101 (0.070-0.127)

\* Only one population included.

lead to different classifications (Mayr, 1981; Patton and Avise, 1983) and morphological studies alone may not be sufficient to adequately determine evolutionary relationships in dynamic species complexes (Sytsma and Schaal, 1985). In parasitic plants such as *Arceuthobium*, reductions tend to obscure phylogenetic relationships by limiting the number of characters, both vegetative and reproductive, available for study. The chances for "errors in assignment" especially at higher taxonomic rank, become quite real (Kuijt, 1969).

The placement of DIV nearer to DOU than to any taxon in the *Campylopodium* complex is a major departure from the treatment in Hawksworth and Wiens (1972). One possible explanation for this surprising relationship involves biochemical (but not morphological) divergence from the *Campylopodium* group resulting in coincidental similarity to DOU. Supposedly similar (homologous) isozymes between different taxa have been shown to be different by altering electrophoretic conditions (Coyne et al., 1979). For DIV and DOU, a more likely explanation is that of similarity by descent since both show fixation for the GDH<sup>66</sup> allele which is absent in other taxa and they share 6-PGD<sup>65</sup>, 6-PGD<sup>100</sup>, PGI<sup>154</sup>, PGI<sup>178</sup>, ADH-1<sup>100</sup>, ADH-1<sup>132</sup>, ADH-1<sup>164</sup>, ADH-2<sup>100</sup>, PGM-1<sup>76</sup>, PGM-1<sup>97</sup>, PGM-2<sup>97</sup>, PGM-2<sup>100</sup>, G-6PDH<sup>100</sup>, MDH-2<sup>76</sup>, MDH-2<sup>100</sup>, and MDH-3<sup>205</sup>. Independent evolution of this complement of alleles in two unrelated taxa (biochemical convergence) is considered a less parsimonious explanation of the results than patristic relationship.

The complement of electromorphs shared by DIV and DOU could be viewed as shared primitive states (symplesiomorphies) and the different suite of electromorphs in the *Campylopodium* group as shared derived states (synapomorphies). Assuming that AME represents the most primitive taxon utilized in this study, the polarity of this evolutionary trend is supported by lower genetic distance values between *Divaricatum* and *Americanum* than between *Divaricatum* and *Campylopodium* (Table 6). Apparently DIV and DOU have retained biochemical similarity despite developing quite different host preferences (pinyons and douglas fir respectively). As reported by Wiens (1968), DIV is included in the direct summer flowering group (summer meiosis and summer flowering), as are all members of the *Campylopodium* complex, whereas DOU is in the indirect spring flowering group (spring meiosis for the carpellate plants, summer meiosis for the staminate plants, and spring flowering). From the phenological and the above electrophoretic

evidence, a probable phylogenetic scenario is that DOU has diverged from ancestral stock with flowering characteristics retained by DIV and the bulk of the *Campylopodum* complex.

Species delimitation in the *Campylopodum* complex is difficult because of the overall high degree of genetic similarity. A possible explanation is that the complex is composed of closely related sibling species with relatively uniform morphological features. Because the group is undergoing more rapid (and more recent) radiation than other members of the genus, significant biochemical differentiation between members has not yet occurred. The data from this study are also consistent with the relationships within the *Campylopodum* complex as proposed by Kuijt (1955) where all members are forms of one variable species, *A. campylopodum*. How species boundaries have been shaped by past evolutionary history and by adaptation to specific hosts remains unanswered. Evolutionary divergence may be independent of reproductive isolation and species can be envisioned as the "total assemblage of adaptive peaks" (Levin, 1979). For a member of the *Campylopodum* complex, one such adaptive peak certainly corresponds to the major host species. A rational approach to dwarf mistletoe classification is to attempt to use information from many areas to synthesize a phylogeny.

**CONCLUSION AND SUMMARY**—*Arceuthobium* shows levels of genetic variability greater than most dicot species which have been studied electrophoretically. The initial segregation of *A. americanum* from the remainder of the genus supports the concept of two subgenera, *Arceuthobium* and *Vaginatum* (Hawksworth and Wiens, 1972). Further work with other verticillately branched New World taxa (such as *A. abietis-religiosae* and *A. verticilliflorum*) and Old World members of this subgenus is in order. *A. douglasii* and *A. pusillum* are not recent derivatives from *Campylopodum* stock and the phylogenetic affinities of the former species is with the *Vaginatum* group. In addition, both taxa, as shown by isozyme evidence, are not closely related to each another. The pinyon dwarf mistletoe, *A. divaricatum*, does not appear to have affinity with the *Campylopodum* group but with *A. douglasii* and the *Vaginatum* group of species.

The affinities of *Arceuthobium vaginatum* ssp. *cryptopodum*, *A. vaginatum* ssp. *durangense*, and *A. gillii* as proposed by Hawksworth and Wiens (1972) are supported by the present isozyme study. This group is more variable both morphologically and genetically than oth-

er members of the genus. In contrast, designation of species within the *Campylopodum* complex is difficult because of high levels of genetic similarity (ca. 80%) among the eleven taxa examined. For the purposes of classification it is important to interpret levels of similarity or distance consistently for all subgeneric groups. To synthesize the phylogeny of *Arceuthobium* it is essential to utilize all available information derived from a variety of systematic approaches.

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