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Electrophoretic Evidence for Genetic Differentiation in Two Host Races of Hemlock Dwarf Mistletoe (*Arceuthobium tsugense*)*

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Key Word Index—*Arceuthobium tsugense*; Viscaceae; dwarf mistletoe; electrophoresis; allozymes; host race.

Abstract—Three host races of hemlock dwarf mistletoe have been described: the western hemlock race (mainly parasitic on *Tsuga heterophylla*), the shore pine race (mainly on *Pinus contorta* ssp. *contorta*) and the mountain hemlock race (mainly on *Tsuga mertensiana* and *P. monticola*). Mistletoe shoots from 21 populations representing the three host races and eight host species were obtained and analysed using starch gel electrophoresis. Over 900 individuals were examined and 13 electrophoretic loci were used. On average, 84% of the loci were polymorphic and mean heterozygosity was 0.260. The mistletoes from the Pacific Northwest (Washington, Vancouver Island, B.C. and Orcas Island) were less diverse genetically than mainland populations. Mistletoe populations parasitic on two more hosts per population had higher numbers of alleles per locus and a higher percentage of polymorphic loci than populations colonizing only one host.

The UPGMA phenogram showed a strong correspondence between geographic location and genetic distance indicating a clinal pattern from north to south. The most distinct cluster is composed of four populations from the Sierra Nevada in California. The population from Juneau, Alaska, is also distinct but shows greater affinity to the more northern populations using UPGMA analysis. The Oregon Cascade populations referred to the mountain hemlock race were allied with the western hemlock populations with UPGMA analysis but showed affinity to the Sierra Nevada populations when analysed with the distance Wagner procedure. Cluster and cladistic analyses did not result in discrete clusters of populations composed of either the western hemlock or shore pine races. Thus, the mountain hemlock dwarf mistletoe appears to be a separate taxon deserving of taxonomic recognition, possibly at the subspecies level. Isozyme data do not support the recognition of the shore pine race as distinct from the dwarf mistletoes on western hemlock.

Introduction

The hemlock dwarf mistletoe, *Arceuthobium tsugense* (Rosendahl) G. N. Jones is an important parasite of a number of economically important conifers in the western U.S. and Canada. This mistletoe will infect 12 host species under natural conditions and at least 20 species using artificial inoculations [1–3]. Among members of section *Campylopoda*, this species exhibits the greatest host breadth.

The existence of host races in *A. tsugense* (also called ecological races or pathotypes) has been suggested following field observation and inoculation trials [3–7]. Hawksworth [8] summarized the evidence that host races exist within this species. Table 1 lists the primary and secondary hosts of these races. The distributions of the three purported host races are shown in Fig. 1. The western hemlock race has a wider distribution than either the mountain hemlock or shore pine races. This form occurs from sea level to 1200 m in elevation and is found from a disjunct population along the coast of California in Mendocino Co. north to Juneau, Alaska. The principal hosts are western hemlock, pacific fir and noble fir. In mixed stands where both

TABLE 1. NATURAL HOSTS FOR THREE HOST RACES OF *A. TSUGENSE*

Race	Primary hosts	Secondary and rare hosts
I. Western hemlock	*Tsuga heterophylla*	*Tsuga mertensiana*
	Abies amabilis	*Pinus monticola*
	Abies procera	*Picea sitchensis*
II. Mountain hemlock	*Tusga mertensiana*	*Pinus albicaulis*
	Pinus monticola	*Abies lasiocarpa*
	Abies grandis	
	Picea engelmannii	
	Picea breweriana	
III. Shore pine	*Pinus contorta* ssp. *contorta*	*Tsuga heterophylla*
		Pinus monticola

*Part II in the series "Biochemical Systematics of the *Arceuthobium campylopodum* Complex (Dwarf Mistletoes, Viscaceae)". For Part I see Nickrent, D. L. and Butler, T. L. (1990) *Biochem. Syst. Ecol.* **18**, 253.

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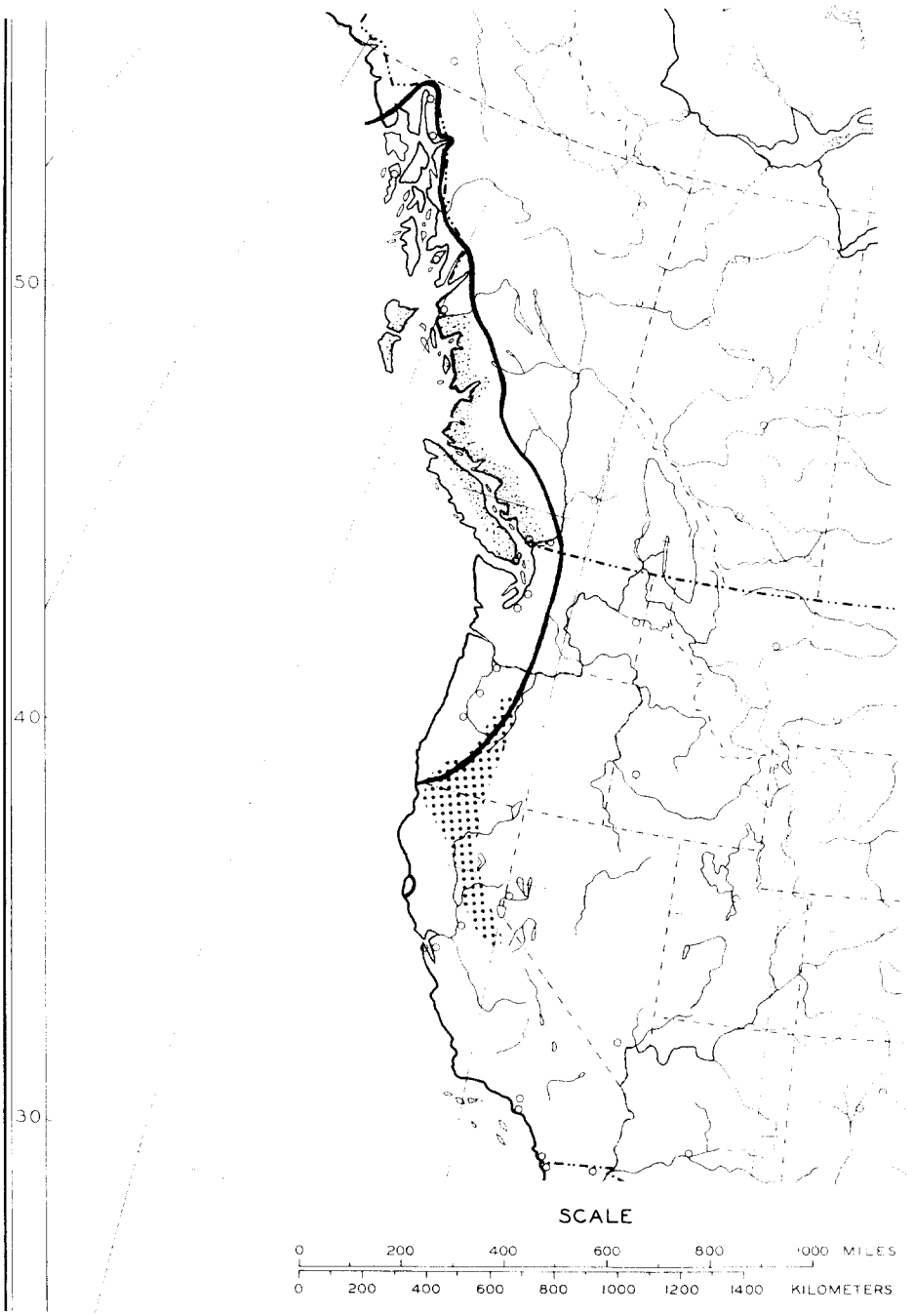

FIG. 1. DISTRIBUTION OF *A. TSUGENSE*. The solid outline encloses the range of the western hemlock race, the dots the mountain hemlock race, and the small stipple the shore pine race.

western and mountain hemlock occur, mountain hemlock is only rarely parasitized [6, 9]. The western hemlock race also rarely parasitizes *Pinus monticola* when these hosts occur sympatrically [10]. Smith [11] and Hunt and Smith [5] attempted inoculations of western hemlock dwarf mistletoe onto western white pine in British Columbia and northern Oregon; only rare infections or no infections were seen from these inoculations.

Several studies have focused upon the differences between the shore pine and western hemlock races [3–5, 12–15]. The shore pine race occurs from sea level to 780 m from the San Juan Islands off coastal Washington to the east coast of Vancouver Island and north to the Queen Charlotte Islands. This form also occurs along coastal British Columbia from Vancouver to Terrace. The principal host is *Pinus contorta* spp. *contorta* (shore pine). Secondary and rare hosts include *Pinus monticola* and *Tsuga heterophylla.*

Smith and Wass [3] conducted inter-host inoculations using the seeds from both the shore pine and western hemlock races of *A. tsugense.* The western hemlock pathotype showed low infection (0–2%) and moderate shoot production on shore pine whereas the shore pine pathotype showed moderate infection (3–12%) but low shoot production on western hemlock. The conclusion was that these data support the existence of host races in *A. tsugense.* Hawksworth and Wiens [7] suggested that perhaps these races should be treated as *"forma speciales"* (special forms) as was done for *A. abietinum* f. sp. *concoloris* and *A. abietinum* f. sp. *magnificae.* To date, no morphological differences have been reported that allow the two races to be distinguished.

The mountain hemlock race occurs at high elevation (1200 to 2500 m) from the Central Cascades in Oregon to the Sierra Nevada in Central California. The principal hosts are *Tsuga mertensiana* (mountain hemlock) and *P. monticola* (western white pine). Secondary and rare hosts include *Pinus albicaulis* (whitebark pine), *Abies lasiocarpa* (subalpine fir), *Picea engelmannii* (Engelmann spruce) and *P. breweriana* (brewer's spruce).

In the interest of clarifying the taxonomic status of the host races of the hemlock dwarf mistletoe, the goals of this project were to (1) obtain population samples of the three purported host races and determine genetic distance values based upon an isozyme data set, (2) test the genetic data set to determine whether trends exist in the partitioning of variation, for example with respect to geographic location, host species and host races, and (3) determine whether genetic variation of mistletoe populations colonizing multiple hosts differs in any way from that seen when they exist only on one host.

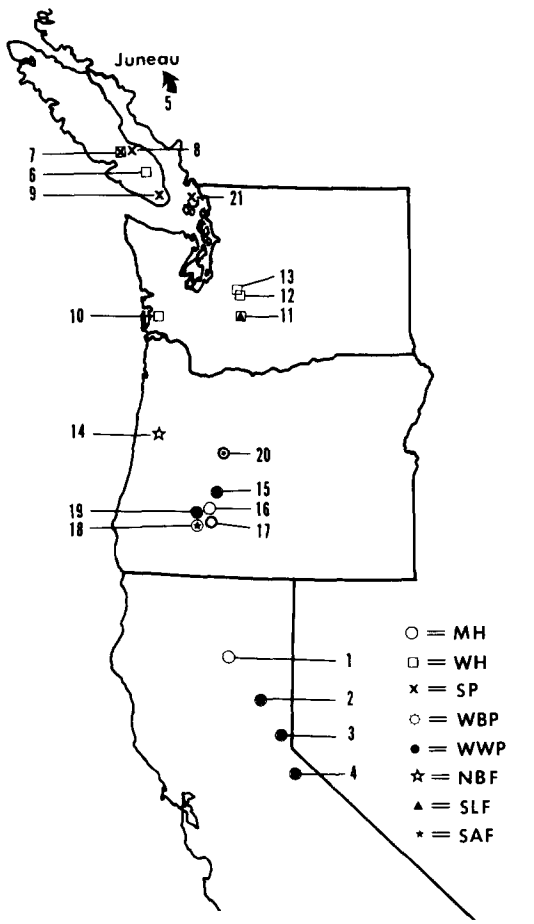

FIG. 2. LOCATIONS OF THE HEMLOCK MISTLETOE POPULATIONS COLLECTED FOR ISOZYME ANALYSIS. Symbols indicate host or hosts parasitized at each site. For multiple host colonizations, the symbols are used in combination. MH = mountain hemlock (*Tsuga mertensiana*), WH = western hemlock (*Tsuga heterophylla*), SP = shore pine (*Pinus contorta* ssp. *contorta*), WBP = white bark pine (*Pinus albicaulis*), WWP = western white pine (*Pinus monticola*), NBF = noble fir (*Abies procera*), SLF = silver fir (*Abies amabilis*) and SAF = subalpine fir (*Abies lasiocarpa*).

Results

Allelic diversity

In this study, all loci examined were polymorphic in at least one population. Several loci showed large numbers of alleles such as *ADH-1, IDH, MDH-3, PGM, 6-PGDH* and *PER-2*. In many instances, though, the bulk of the allelic variation was apportioned between only two alleles, such as with *MDH-3*^{100} and *MDH-3*^{80}. None of the 21 populations examined showed fixation for unique alleles. Populational genetic differentiation was generally marked by difference in allele frequencies. In general, populations from Washington, British Columbia and Alaska showed reduced genetic diversity (number of alleles) as compared with populations from the Oregon Cascades and the Sierra Nevada. This is apparent for *ADH-1*^{100} which is either fixed or present at a frequency of 0.9 or greater. Conversely, the populations from the Sierra Nevada have the *ADH-2*^{66} allele present at relatively high frequency as do those populations from the Cascades of Oregon. This contrasts with the populations from Washington and British Columbia which are fixed or nearly fixed for *ADH-2*^{100}.*

The frequencies of particular alleles at the *G-6-PDH* and *6-PGDH* loci appear different between major geographic areas. The *G-6-PDH*^{93} allele shows clinal variation with higher frequencies in the more northern populations (Washington, British Columbia) and lower frequencies in the southern populations (Oregon Cascades, California Sierra Nevada). A similar situation exists with the *6-PGDH*^{89} allele. As with the *ADH-2*^{66} allele, *IDH*^{121} is present only in the California and Oregon populations. The *PGM* locus showed two predominant alleles: *PGM*^{100} and *PGM*^{70}. For most populations, these alleles were roughly in equal proportions; however, the Mt Findlayson population (no. 9) was nearly fixed for *PGM*^{70} with a frequency of 0.917.

Host race genetic differentiation

Three populations of hemlock dwarf mistletoe that exhibit multiple host colonizations were chosen to test whether significant differences in allele frequencies exist between mistletoes on different host species. The Horne Lake "subpopulations" (2663 on *Tsuga heterophylla* and 2664 on *Pinus contorta*) represent an area where the two purported host races coexist. The other populations, as currently recognized [8], do not represent different sympatrically-occurring host races. These are: White Pass (2668 on *Tsuga heterophylla*, 2669 on *Abies amabilis*) and Alpine Meadow (2212 on *T. mertensiana* and 2213 on *Pinus monticola*). The *G*-test [15] with the Williams' correction [17] was used to calculate expected frequencies. Autapomorphic alleles were excluded from the analysis as were monomorphic loci and *PER* owing to its large number of different alleles. The diversity of alleles at *PER* resulted in certain expected allelic combinations that were not observed, an error associated partly with sample size. The sum of the values from these cells tended to give an erroneously high degree of significance. For the Horne Lake subpopulations, two of the 11 polymorphic loci showed significant differences in allele frequencies: *G-6-PDH* ($\chi^2 = 7.87$, 1 *df*, $P = 0.005$) and *PGM* ($\chi^2 = 11.9$, 1 *df*, $P < 0.001$). Of the nine polymorphic loci for the Alpine Meadow population, two showed significant allelic frequency differences: *MDH-4* ($\chi^2 = 20.4$, 1 *df*, $P < 0.001$) and *PGM* ($\chi^2 = 18.72$, 3 *df*, $P < 001$). The White Pass subpopulations showed no significant allelic differences across 11 polymorphic loci.

Genetic variability among populations

Genetic variability for the 21 populations across 13 loci is shown in Table 2. The mean number of alleles per locus (A) was 2.6, the percentage of loci polymorphic (P) was 84.2%, and the direct count of heterozygosity (H_o) was 0.26. Most populations deviate little from these mean values, with the exception of Mt Findlayson and Orcas Island which were both genetically depauperate. To determine whether a correlation exists between the number of hosts colonized and genetic diversity, populations of dwarf mistletoes present on one vs several hosts were pooled and genetic variability measures averaged. It can be seen (Table 3) that the number of alleles per locus and the percentage of polymorphic loci are higher in populations parasitizing more than one conifer host. Multiple host colonization has a geographic component, however, since the majority of populations occurring

*The table of allele frequencies can be obtained upon request from the senior author.

TABLE 2. GENETIC VARIABILITY AT THIRTEEN LOCI IN TWENTY-ONE POPULATIONS OF *A. TSUGENSE*

Population name and no.	Mean sample size per locus	Mean no. of alleles per locus (*A*)	Percentage of loci polymorphic* (*P*)	Mean heterozygosity Direct-count (H_o)	H.–W. expected† (H_e)
1. Hemlock Lake	16.2	3.1	100.0	0.267	0.367
2. Mt Elwell	37.2	2.3	76.9	0.258	0.352
3. Alpine Meadow	66.5	3.1	76.9	0.253	0.293
4. Mosquito Lake	36.8	3.2	84.6	0.264	0.302
5. Juneau	21.5	1.8	69.2	0.204	0.234
6. Cowichan	38.2	2.9	76.9	0.270	0.315
7. Horne Lake	90.5	2.8	92.3	0.230	0.253
8. Spider Lake	49.9	2.1	61.5	0.236	0.238
9. Mt Findlayson	23.9	1.6	61.5	0.094	0.125
10. Nemah	36.8	2.9	92.3	0.292	0.317
11. White Pass	87.1	3.2	92.3	0.285	0.313
12. Silver Creek	45.4	2.4	76.9	0.274	0.265
13. Huckleberry	62.6	2.8	84.6	0.250	0.292
14. Mary's Peak	31.5	2.4	84.6	0.296	0.280
15. Windigo Pass	29.5	2.8	100.0	0.246	0.326
16. Diamond Lake	27.1	2.5	92.3	0.339	0.346
17. Crater Lake	31.5	2.6	100.0	0.327	0.394
18. 1000 Springs	26.0	2.4	92.3	0.249	0.280
19. Beaver Meadows	46.6	3.2	92.3	0.298	0.343
20. McKenzie Pass	50.1	3.3	100.0	0.362	0.394
21. Orcas Island	31.5	2.2	61.5	0.185	0.211
Means	42.2	2.6	84.2	0.260	0.297

*A locus is considered polymorphic if more than one allele was detected.
†Unbiased estimate [27].

TABLE 3. GENETIC DIVERSITY FOR *A. TSUGENSE* POPULATIONS COLONIZING DIFFERENT NUMBERS OF HOST SPECIES

	No. of populations	Mean sample size per locus	Mean no. of alleles per locus (*A*)	Percentage of loci polymorphic* (*P*)	Mean heterozygosity Direct-count (H_o)	H.–W. expected† (H_e)
Populations on two or more hosts	10	50.1	2.89	90.7	0.277	0.325
Populations on one host	11	34.9	2.42	78.3	0.246	0.271
All populations	21	42.2	2.60	84.2	0.260	0.297

*A locus is considered polymorphic if more than one allele was detected.
†Unbiased estimate [27].

on more than one host occur in the southern portion of the range of this mistletoe species.

Partitioning of genetic diversity

The partitioning of genetic diversity within and between populations was examined using the Fixation Index [18]. The F_{IT} value is the fixation index of individuals relative to all populations, F_{IS} is the fixation index of individuals relative to their specific population, and F_{ST} measures the differentiation between populations relative to the limiting amount under complete fixation. An F_{ST} value of 0 indicates that all variance resides within populations. A value of 1.0 means that all of the variance is between populations, i.e. they are completely differentiated and have no alleles in common. For *A. tsugense*, the F_{ST} value averaged across the 13 loci is 0.216 (Table 4), which indicates a moderate amount of differentiation between populations.

To analyse population differentiation hierarchically [18], three arrangements of the populations were used. The first grouped the 21 populations shown in Table 2 according to host race, thus resulting in 22 populations (Table 5). The second method also grouped the populations according

TABLE 4. SUMMARY OF *F*-STATISTICS AT THIRTEEN LOCI FOR *A. TSUGENSE*

Locus	F_{IS}	F_{1}	F_{ST}
*ACO**	0.206	0.366	0.202
ADH-1	0.082	0.154	0.079
ADH-2	−0.012	0.318	0.326
ADK	0.217	0.260	0.054
GSR	−0.039	0.217	0.246
G-6-PDH	0.196	0.447	0.312
IDH	0.018	0.259	0.245
MDH-3	−0.133	0.033	0.088
MDH-4	0.221	0.405	0.236
PGI	0.081	0.181	0.108
PGM	0.034	0.169	0.141
6-PGDH	0.080	0.385	0.331
PER-2	0.228	0.383	0.201
Mean	0.086	0.283	0.216

*For locus abbreviations, see Experimental.

TABLE 5. HIERARCHICAL LEVELS FOR TWENTY-TWO POPULATIONS GROUPED BY HOST RACE

1. Western Hemlock	
Juneau	WH*
Cowichan	WH
Horne Lake	WH†
Nemah	WH
White Pass	WH–SLF
Huckleberry	WH
Mary's Peak	NBF
2. Mt Hemlock	
Hemlock Lake	MH
Mt Elwell	MH–WWP
Alpine Meadow	MH–WWP
Mosquito Lake	MH–WWP
Windigo Pass	MH–WWP
Diamond Lake	MH
Crater Lake	MH–WBP
1000 Springs	MH
Beaver Meadows	MH–WWP
McKenzie Pass	MH–WBP–SAF
3. Shore Pine	
Horne Lake	SP†
Spider Lake	SP
Mt Findlayson	SP
Orcas Island	SP

*For host race abbreviations, see Fig. 2.

†Horne Lake is geographically one population, split here into two subpopulations based upon host.

TABLE 6. VARIANCE COMPONENTS AND *F*-STATISTICS COMBINED ACROSS LOCI

Comparison		22 Populations; defined by host race*		28 Populations; defined by host race	
X	Y	Variance component	$F_{x,}$	Variance component	$F_{x,}$
Population	Total	0.87888	0.187	0.94739	0.197
Population	Host race	0.64659	0.145	0.72703	0.159
Host race	Total	0.23229	0.049	0.22036	0.046

*Sympatric dwarf mistletoes occurring on different hosts defined as same population (giving 22 total) or different subpopulations (28 total).

to host race but continued by dividing populations showing multiple host colonization into two or more subpopulations; e.g. White Pass on western hemlock and White Pass on silver fir were treated as separate subpopulations. This results in a total of 28 populations. The output from the hierarchical analysis provides a table giving variance components for each level of the hierarchy relative to another (Table 6). For the first two analyses, the levels are mistletoe population to total populations, mistletoe population to host race, and host race to total populations. The effect of dividing single populations into two or more populations based upon host species results in little change in the variance values and no change in the overall trend. In both cases, the greater amount of the total variance is explained by the interaction between mistletoe population and host race.

The third hierarchy grouped each population (here 28 in total) according to host genus and species (Table 7). As shown in Table 8, a large proportion of the variance is explained by the interaction between mistletoe population and host genus. Although less than the interaction between population and total, the comparison of population to host species also results in a high variance component.

Cluster analysis

The UPGMA phenogram shown in Fig. 3 used the chord distances between the 21 populations presented in Table 9. When populations showing multiple host colonization were divided based upon host (resulting in 28 populations as shown in Table 7), the derived sympatric populations clustered closer to each other than to any other population in five of the seven possible cases. This strongly suggests that in areas of sympatry, gene flow is occurring between dwarf mistletoes on different hosts.

The phenogram in Fig. 3 also illustrates a striking correspondence between geographic

TABLE 7. HIERARCHICAL LEVELS FOR TWENTY-EIGHT POPULATIONS GROUPED BY HOST GENUS AND SPECIES

1. *Tsuga*
    1. *T. heterophylla*
        Juneau
        Cowichan
        Horne Lake
        Nemah
        White Pass
        Silver Creek
        Huckleberry
    2. *T. mertensiana*
        Hemlock Lake
        Mt Elwell
        Alpine Meadow
        Mosquito Lake
        Windigo Pass
        Diamond Lake
        1000 Springs
        Beaver Meadows
        McKenzie Pass
2. *Pinus*
    1. *P. albicaulis*
        Crater Lake
        McKenzie Pass
    2. *P. contorta*
        Horne Lake
        Spider Lake
        Mt Findlayson
        Orcas Island
    3. *P. monticola*
        Mt Elwell
        Alpine Meadow
        Beaver Meadow
3. *Abies*
    1. *A. amabilis*
        White Pass
    2. *A. lasiocarpa*
        McKenzie Pass
    3. *A. procera*
        Mary's Peak

TABLE 8. VARIANCE COMPONENTS AND *F*-STATISTICS COMBINED ACROSS LOCI FOR TWENTY-EIGHT POPULATIONS DEFINED BY HOST SPECIES AND LOCALITY

Comparison X	Y	Variance component	F_{XY}
Population	Host genus	1.11131	0.224
Population	Total	0.94744	0.197
Population	Host species	0.71308	0.156
Host species	Host genus	0.39823	0.080
Host species	Total	0.23436	0.049
Host genus	Total	−0.16387	−0.034

location and genetic distance. The most distinct element appears as a cluster composed of populations 1–4 from the Sierra Nevada in California. These California populations, referred to the mountain hemlock race, join the remaining populations at the 0.35 level. The population from Juneau, Alaska, is also distinct but shows greater affinity to the remaining cluster of more northern populations. The six dwarf mistletoe populations from the Oregon Cascades, referred to the mountain hemlock race, form a discrete cluster at the 0.28 level. This cluster joins next with the Washington and British Columbia populations, and not the Californian Sierra Nevada populations as might be expected.

Populations included within the shore pine race do not form a discrete cluster in the phenetic analysis but appear intermixed within groupings composed of the western hemlock race. The Horne Lake population is composed of individuals parasitizing both *Tsuga heterophylla* and *Pinus contorta.* This population has greatest affinity with Spider Lake which is geographically most proximal. The Mary's Peak and Mt Findlayson populations emerge as the most dissimilar elements in the above cluster. This result stems partly from the finding that the Mt Findlayson population shows allele frequencies for several loci that deviate from other populations.

Distance Wagner analysis

The phylogenetic tree produced by the distance Wagner procedure is shown in Fig. 4. This tree had a total length of 2.547 and a cophenetic correlation of 0.906. As on the UPGMA phenogram, the populations assigned to the western hemlock and shore pine races are both contained within a clade distinct from the mountain hemlock race populations. The one exception involves the population from Juneau, Alaska, which does not cluster with the western hemlock race populations. The distant relationship of this population to the western hemlock race is also apparent on the UPGMA phenogram. Unlike the phenetic analysis of the isozyme data, the Wagner cladogram shows a clear relationship between the mountain hemlock populations from the Cascades and those from the Sierra Nevada. The latter populations show greater affinity with two populations from Oregon: Diamond Lake (no. 16) and Crater Lake (no. 17).

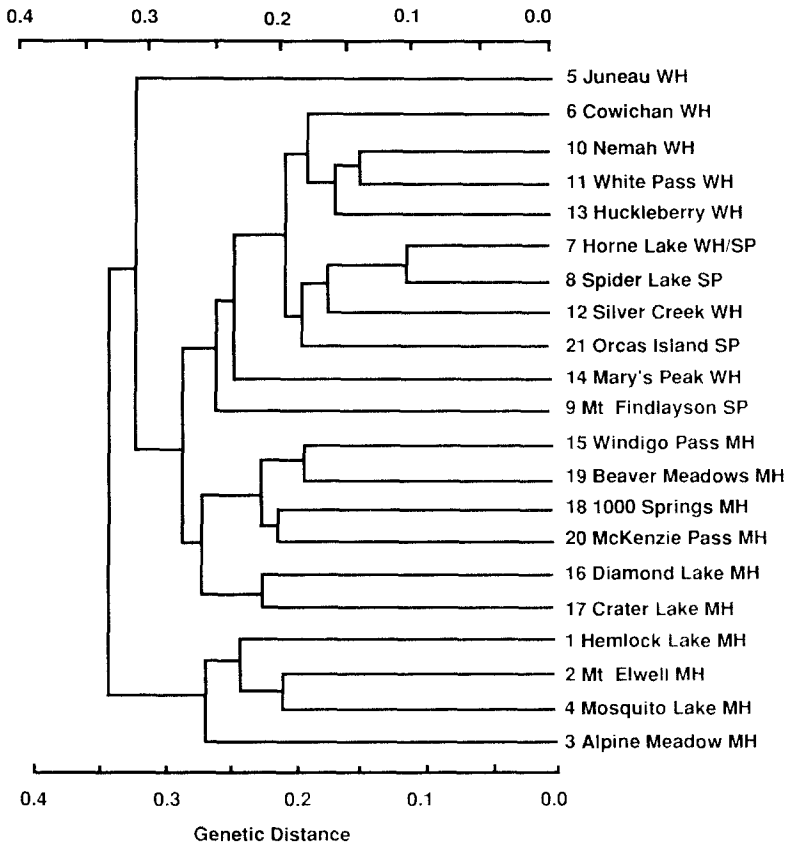

FIG. 3. UPGMA PHENOGRAM USING THE CHORD DISTANCES [28] FOR THE TWENTY-ONE NUMBERED POPULATIONS OF HEMLOCK DWARF MISTLETOE LISTED IN TABLE 2. WH = western hemlock race, SP = shore pine race and MH = mountain hemlock race.

Discussion

In a previous study of two closely related California dwarf mistletoe taxa, *Arceuthobium campylopodum* and *A. occidentale*, isozyme data were used to examine the amount and distribution of genetic variation [19]. For these taxa, as much variance exists between the two dwarf mistletoe species categories as between any of the populations sampled, hence the recognition of one rather than two species was appropriate. In contrast, this study of the hemlock dwarf mistletoe indicates that a moderate amount of genetic differentiation has occurred between populations as shown by an F_{ST} value of 0.216 and the UPGMA phenogram of genetic distances.

As shown in Table 2, the Mt Findlayson and Orcas Island populations are genetically depauperate compared with the mean values for all populations. These populations are geographically isolated from other populations, which may result in restricted gene flow. The low genetic diversity seen for these populations may be the result of a founder event (a small number of genetically "atypical" seeds colonizing the site) or the extreme truncation of a formerly more widespread population (a population crash). In either case, random genetic drift in the small population can result in fixation for alternate alleles and reduction of the population's overall genetic diversity as was observed for these two populations.

The pattern of relationships shown in the UPGMA phenogram (Fig. 3) indicates a strong clinal trend from north to south. A similar clinal pattern resulted from isozyme analysis of three species of closed-cone pines in California [20].

TABLE 9. MATRIX OF GENETIC DISTANCE COEFFICIENTS FOR *A. TSUGENSE**

Population no. and name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1. Hemlock Lake	***	0.271	0.276	0.228	0.359	0.306	0.370	0.381	0.411	0.322	0.327	0.358	0.331	0.378	0.311	0.261	0.347	0.325	0.302	0.319	0.368
2. Mt Elwell	0.182	***	0.270	0.215	0.378	0.313	0.385	0.393	0.387	0.327	0.355	0.379	0.346	0.367	0.313	0.283	0.327	0.302	0.302	0.299	0.376
3. Alpine Meadow	0.189	0.209	***	0.269	0.373	0.347	0.383	0.384	0.407	0.324	0.345	0.389	0.378	0.373	0.328	0.332	0.367	0.300	0.320	0.333	0.396
4. Mosquito Lake	0.156	0.147	0.195	***	0.344	0.286	0.343	0.350	0.384	0.288	0.291	0.343	0.307	0.317	0.294	0.272	0.299	0.283	0.277	0.280	0.359
5. Juneau	0.282	0.257	0.267	0.235	***	0.261	0.332	0.326	0.337	0.291	0.322	0.374	0.337	0.358	0.285	0.287	0.312	0.311	0.306	0.371	0.346
6. Cowichan	0.234	0.237	0.273	0.211	0.155	***	0.206	0.216	0.244	0.177	0.191	0.219	0.183	0.268	0.228	0.246	0.293	0.291	0.260	0.266	0.198
7. Horne Lake	0.272	0.301	0.308	0.261	0.216	0.143	***	0.111	0.280	0.218	0.192	0.171	0.202	0.251	0.243	0.314	0.294	0.332	0.289	0.283	0.206
8. Spider Lake	0.282	0.314	0.304	0.271	0.209	0.142	0.061	***	0.249	0.228	0.193	0.170	0.207	0.247	0.252	0.324	0.312	0.341	0.302	0.305	0.194
9. Mt Findlayson	0.320	0.301	0.344	0.285	0.234	0.162	0.197	0.182	***	0.259	0.268	0.270	0.272	0.309	0.308	0.337	0.394	0.347	0.321	0.355	0.206
10. Nemah	0.224	0.251	0.245	0.218	0.212	0.097	0.135	0.151	0.195	***	0.149	0.232	0.178	0.202	0.228	0.260	0.278	0.239	0.242	0.200	0.244
11. White Pass	0.236	0.285	0.266	0.233	0.215	0.116	0.110	0.115	0.200	0.102	***	0.185	0.160	0.208	0.227	0.294	0.303	0.280	0.250	0.225	0.217
12. Silver Creek	0.236	0.289	0.306	0.255	0.238	0.137	0.091	0.099	0.191	0.137	0.107	***	0.182	0.262	0.255	0.333	0.356	0.332	0.278	0.277	0.174
13. Huckleberry	0.244	0.267	0.312	0.235	0.199	0.100	0.126	0.132	0.172	0.098	0.100	0.100	***	0.232	0.249	0.295	0.326	0.299	0.255	0.252	0.200
14. Mary's Peak	0.271	0.270	0.293	0.243	0.275	0.187	0.157	0.158	0.232	0.153	0.145	0.172	0.184	***	0.294	0.326	0.285	0.282	0.288	0.224	0.288
15. Windigo Pass	0.221	0.227	0.268	0.233	0.209	0.146	0.179	0.204	0.247	0.138	0.159	0.162	0.173	0.230	***	0.244	0.256	0.231	0.196	0.209	0.266
16. Diamond Lake	0.189	0.172	0.255	0.174	0.190	0.173	0.239	0.254	0.243	0.176	0.230	0.245	0.206	0.230	0.195	***	0.227	0.285	0.274	0.287	0.315
17. Crater Lake	0.269	0.207	0.252	0.214	0.240	0.227	0.249	0.257	0.330	0.204	0.235	0.261	0.261	0.197	0.195	0.183	***	0.292	0.302	0.267	0.357
18. 1000 Springs	0.221	0.207	0.227	0.181	0.192	0.190	0.230	0.257	0.263	0.166	0.212	0.218	0.206	0.225	0.152	0.164	0.204	***	0.236	0.215	0.351
19. Beaver Meadows	0.207	0.196	0.237	0.195	0.192	0.183	0.207	0.239	0.257	0.173	0.203	0.181	0.190	0.250	0.121	0.180	0.200	0.130	***	0.225	0.316
20. McKenzie Pass	0.248	0.223	0.254	0.224	0.295	0.200	0.214	0.238	0.314	0.138	0.153	0.209	0.195	0.169	0.140	0.215	0.178	0.162	0.187	***	0.317
21. Orcas Island	0.283	0.281	0.315	0.247	0.206	0.097	0.142	0.130	0.117	0.152	0.130	0.122	0.124	0.206	0.189	0.233	0.279	0.239	0.247	0.242	***

*Above diagonal: chord distance [28]; below diagonal: genetic distance [29].

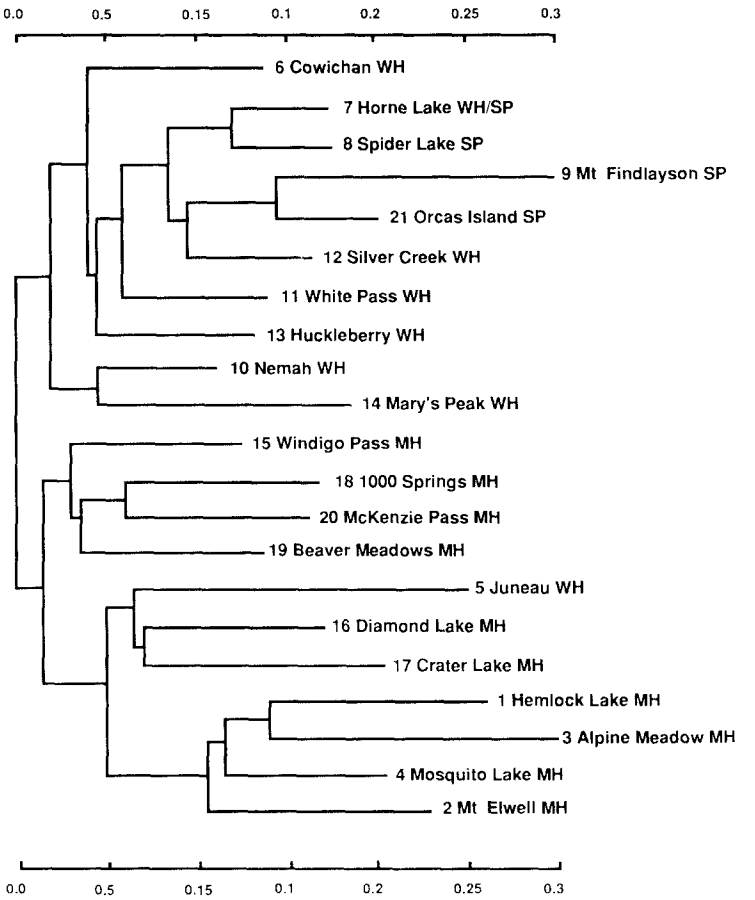

FIG. 4. DISTANCE WAGNER CLADOGRAM USING THE CHORD DISTANCES GIVEN IN TABLE 2. WH = western hemlock race, SP = shore pine race and MH = mountain hemlock race.

The relationship between the California Sierra Nevada populations of hemlock dwarf mistletoe and the Cascade populations is only clearly seen when distance Wagner trees are generated. This indicates that abrupt genetic differences between populations do not occur but instead interpopulational affinities gradually increase with decreased geographic distance.

The UPGMA phenogram and the Wagner cladogram both indicate the existence of three groups; the western hemlock–shore pine populations, the Cascade Range populations from Oregon, and the Sierra Nevada populations. The distinctiveness of the western hemlock–shore pine group is in agreement with field observations and inoculation trials [4–6, 8, 9]. Stands where western hemlock and western white pine occur together are relatively rare and unfortunately none of the sites sampled for this isozyme study included both these hosts. Mathiasen and Hawksworth [10] reported infection percentages for these two hosts at five sites near Union Creek, Douglas Co., Oregon; however, specific locality information was lacking. In that study, infection of *P. monticola* in *T. heterophylla* stands was rare. Unfortunately, no population of dwarf mistletoe on western hemlock was obtained from this area for isozyme analysis. One population, located 15 miles N.E. of Union Creek (Beaver Meadow, no. 19), was used for this isozyme study and showed heavy infestation of both mountain hemlock and western white pine. This indicates that within very short distances, infection characteristics vary widely

among host species. If the western and mountain hemlock races are indeed sympatric near Union Creek, populations from this area should be subjected to further sampling for isozyme analyses.

Host race differentiation

The electrophoretic evidence does not at present indicate a comparable level of genetic differentiation between the western hemlock and purported shore pine races as was seen with the mountain hemlock race. Two of the four populations referred to the shore pine race (Spider Lake and Horne Lake) cluster together in the UPGMA phenogram, whereas the other two (Mt Findlayson and Orcas Island) do not. With distance Wagner analysis, these populations do show some tendency to cluster together. The shore pine race is not monophyletic, however, since the clade that includes these four populations also contains a member of the western hemlock race (Silver Creek). Given that both shore pine and western hemlock were both heavily infected at the Horne Lake population, and the high level of genetic similarity among mistletoes from the two hosts, it appears that these plants are not reproductively isolated. Since genetic differentiation was not detected using isozymes, the recognition of two host races at this location would be artificial.

Two of the 11 polymorphic loci between the Horne Lake subpopulations showed statistically significant differences in allele frequencies. Likewise, two of the nine polymorphic loci at the Alpine Meadow site also showed significant differences. This indicates that as much differentiation has occurred between the mistletoes on different hosts considered one host race (MH) as between mistletoes on different hosts considered two host races (SP and WH). If one accepts that these allozymic differences reflect genetic and reproductive isolation, then one must conclude that host race differentiation is taking place in the Sierra Nevada of California as well as in the Pacific Northwest. A more acceptable alternative is to recognize no distinction between the WH and SP host races, at least where they occur sympatrically.

Artificial inoculations and genetic distinctiveness

Since the western hemlock and shore pine pathotypes were the subject of the artificial inoculation trials [3], a review of these findings is in order. Table 10 summarizes the results of that study and indicates the percentage germination, percentage infection, swelling dimensions, shoot number and shoot heights. Seeds from each dwarf mistletoe pathotype result in more infections on the primary host than on the alternate host. Seeds derived from the shore pine race, however, resulted in 12 and 3% infection on western hemlock for 1970 and 1971, respectively. Shoot production following infection was low, however, for this host–parasite combination. These results indicate that the shore pine race is more "aggressive", even on an alternate host, than is the western hemlock race. This is also shown by the significantly higher infection percentages obtained on its primary host (39 and 58%). The 12% infection rate for shore pine pathotype seeds on western hemlock in 1970 is nearly as high as the percentage infection of western hemlock pathotype seeds placed on western hemlock in 1971 (13%). The seed source for the shore pine pathotype of *A. tsugense* was collected at Horne Lake, Vancouver Island. This is the same locality as population no. 7 in this study. As already noted, both shore pine and western hemlock are naturally parasitized at this site. This fact was not mentioned by Smith and Wass [3] but is relevant given the relatively high rate of infection on western hemlock in 1970.

Since no morphological differences are apparent between the shore pine and western hemlock pathotypes of *A. tsugense*, inoculation studies [3–5] remain the primary evidence supporting their recognition. Although these studies provide valuable information relative to the existence of local pathotypes, it is not clear how these features reflect upon the population biology of these parasites. Studies employing artificial inoculations with dwarf mistletoe seeds are often difficult owing to wide variation in infection success between years, sites and hosts [21, 22]. Smith and Wass [3] did not mention how many dwarf mistletoe individuals were used as seed sources. Given the high level of genetic variability seen in these plants, it is not clear how infection percentages would change given a broader sampling of plants as seed sources. In the above study, only one population each of the shore pine pathotype (Horne Lake) and western

TABLE 10. SUMMARY OF RESULTS OF THE *A. TSUGENSE* PATHOTYPE CROSS INOCULATION STUDY BY SMITH AND WASS [3]*

	Hosts									
	Western hemlock (WH) Victoria, B.C.					Shore pine (SP) Victoria, B.C.				
Dwarf mistletoe and source host	Germ. (%)	Infect. (%)	Swell. length (mm)	No.	Shoot height (mm)	Germ. (%)	Infect (%)	Swell. length (mm)	No.	Shoot height (mm)
TSU–WH	47†	20				52	0			
Cowichan			75‡	18	37			35	5	43
B.C.	39	13				79	2			
TSU–SP	74	12				82	39			
Horne Lk.			19	0.2	2			45	16	37
B.C.	61	3				85	58			

*Percentages based upon 300 seeds in each mistletoe–host–location combination.

†Top figure for 1970, bottom for 1971.

‡Averaged for both years.

hemlock pathotype (Cowichan Lake) was used. Projects involving additional accessions of dwarf mistletoe seeds collected throughout the range of the taxon and then inoculated onto hosts at several sites would make such studies more meaningful.

Information on the genetic make-up of the host and parasite populations as well as individual genotypes of plants being used for experimentation would be valuable. Sampling from the entire range of the taxon has already been mentioned. This will present a more representative picture of the range of genetic variability of the mistletoe. Since electrophoretic studies have shown that members of the *Campylopodum* complex, including *A. tsugense*, are variable at most isozyme loci examined, it is reasonable to assume that the genes responsible for host pathogenicity also exist with multiple alleles. This assumes that the variation measured by electrophoretic means provides some indication of the total genetic variation, including those genes associated with host parasitism. Evidence that electrophoretically characterized genetic variation and host latitude are at least loosely correlated is shown in this study where *A. tsugense* populations colonizing more than one host are genetically more variable than populations colonizing a single host. This correlation could be further tested by choosing electrophoretically variable and monomorphic populations and using these seeds for inoculation trials on several different host species.

Infraspecific classification

The results of this study indicate a higher level of genetic differentiation between *A. tsugense* populations than was seen for *A. campylopodum* and *A. occidentale* [19]. For the western hemlock and the mountain hemlock races, documented host preference differences, and now genetic differences visualized from isozymes, raise a question concerning the correct placement of these taxa within the taxonomic hierarchy. To test the validity of these host races using the biological species concept requires information on reproductive isolation. Direct (experimental) evidence involving crossing experiments is unfortunately lacking for these host races. The isozyme data reported here do not indicate strong isolating barriers but show a continuum of genetic distance values, albeit with some indication of a break between the mountain hemlock race and the remaining populations. The intermediate status of the Cascade populations does not indicate genetic isolation but that gene flow is occurring. The western hemlock and mountain hemlock dwarf mistletoes are, for the most part, allopatric based upon host preference and the ranges of their principal host species. For populations that are morphologically identical, not reproductively isolated, and allopatric, the taxonomic rank of subspecies was suggested by Mayr [23, 24]. This level seems appropriate for the "races" of the mountain and western hemlock dwarf mistletoe.

Experimental

Collection methods. The sampling strategy and method of mistletoe shoot collection was essentially as described previously [19]. Sample sizes varied but were usually greater than

20 plants per population (see Table 2). Generally no more than one pistillate and one staminate individual was taken from the same tree. Attempts were made to sample from at least 15 trees within each population.

Population nos. 1 to 5 were obtained during the fall of 1986. The remaining collections were made in September of 1987. These populations span the entire geographic range of the hemlock dwarf mistletoe and nearly the entire host range (eight of the 12 hosts infected in nature). The sampled populations include seven from the western hemlock race (414 individuals), 10 from the mountain hemlock race (385 individuals), and four from the shore pine race (129 individuals). Population no. 7 from Horne Lake, Vancouver Island, contained dwarf mistletoes parasitic on both shore pine and western hemlock. For the purpose of the above tabulation, the mistletoes from each host species were treated as different host races. This is the only location sampled during this study where the primary host for two host races were parasitized with equal frequency.

Other populations with multiple host colonizations (nos. 2–4, 7, 11, 15, 17–20) are so indicated in Fig. 2 by symbol combinations. These represent secondary or rare host species for the particular host race as indicated in Table 1. In one population (no. 20), three host species were parasitized. In all populations with multiple host colonizations, collections of dwarf mistletoes from different hosts were given different accession numbers to allow various statistical tests of the isozyme data. When this is done, 28 populations and subpopulations are obtained instead of 21.

Collection localities. Complete collection information for the 21 populations sampled for isozyme analysis (Fig. 2) is detailed below with the population names and numbers given in parentheses.

Mountain hemlock race

Alpine Co., CA: on *Tsuga mertensiana* (Nickrent 2216) and *Pinus monticola* (Nickrent 2217), Toiyabe National Forest, along the south side of Mosquito Lake and along trail to Heiser Lake, *ca* 6.0 miles northeast of Alpine along S.H. 4, (Mosquito Lake, 4). Placer Co., CA: on *Tsuga mertensiana* (Nickrent 2212) and *Pinus monticola* (Nickrent 2213), Tahoe National Forest, along the Roundhouse ski lift above the Alpine Meadow ski resort, *ca* 5.0 miles west of Tahoe City, (Alpine Meadow, 3). Plumas Co., CA: on *Tsuga mertensiana* (Nickrent 2194) and *Pinus monticola* (Nickrent 2195), Plumas National Forest *ca* 6.0 air miles S.W. of Graegle along trail from Lakes Basin Group campground to Mt Elwell, near Silver Lake, (Mt Elwell, 2). Shasta Co., CA: on *Tsuga mertensiana* (Nickrent 2187), Lassen National Park, near Hemlock Lake, along Rt 89, (Hemlock Lake, 1). Benton Co., OR: on *Abies procera* (Nickrent 2673), Siuslaw National Forest, on Mary's Peak near the microwave relay station, 0.7 miles W. of the campground turnoff, (Mary's Peak, 14). Douglas Co., OR: on *Tsuga mertensiana* (Nickrent 2676), Umpqua National Forest, along Rt. 138, 1.8 miles N. of jnc. with 230, just E. of Diamond Lake, (Diamond Lake, 16); on *Pinus monticola* (Nickrent 2681) and *Tsuga mertensiana* (Nickrent 2682), Rogue River National Forest along Rt. 230, 15.5 miles N.E. of Union Creek, (Beaver Meadows, 19). Jackson Co., OR: on *Tsuga mertensiana* (Nickrent 2679), Rogue River National Forest at Thousand Springs area, 3.0 miles S.E. of Rt. 62 along F.H. 800, 1.0 mile west of Crater Lake National Park boundary, (1000 Springs, 18). Klamath Co., OR: on *Tsuga mertensiana* (Nickrent 2674) and *Pinus monticola* (Nickrent 2675), Deschutes National Forest, 1.5 miles N.E. of Windigo Pass along F.H. 60, (Windigo Pass, 15); on *Pinus albicaulis* (Nickrent 2677) and *Tsuga mertensiana* (Nickrent 2678), Palisade Point, along rim drive, Crater Lake National Park, (Crater Lake, 17). Lane Co., OR: parasitic on *Tsuga mertensiana* (Nickrent 2684), *Abies lasiocarpa* (Nickrent 2685) and *Pinus albicaulis* (Nickrent 2686), Willamette National Forest, Washington Wilderness area, in volcanic rock zone 1.5 miles W. of McKenzie Pass along Rt. 242, (McKenzie Pass, 20).

Western hemlock race

Vancouver Island, British Columbia, Canada: on *Tsuga heterophylla* (Nickrent 2661), just outside the Cowichan Forestry Research Station gate, Lake Cowichan, (Cowichan, 6); on *Tsuga heterophylla* (Nickrent 2663) and *Pinus contorta* (Nickrent 2664), Horne Lake Rd, along north side of Horne Lake and along the north beach of Horne Lake, *ca* 8 km S.W. of Qualicum, (Horne Lake, 7). Juneau, Alaska: on *Tsuga heterophylla*, along the Juneau road system (Paul Hennon and Elaine Loopstra, S.N.). Lewis Co., WA: on *Tsuga heterophylla* (Nickrent 2668) and *Abies amabilis* (Nickrent 2669), Gifford-Pinchot National Forest, just north of Goat Rocks Wilderness area, 4.0 miles N.E. of White Pass along Rt. 12, (White Pass, 11). Pacific Co., WA: parasitic on *Tsuga heterophylla* (Nickrent 2667), along North Nemah Rd that parallels U.S. 101 to Nemah, (Nemah, 10). Pierce Co., WA: on *Tsuga heterophylla* (Nickrent 2670), Mt Baker-Snoqualmie National Forest at Silver Creek, around parking lot at information booth, just outside Mt Rainier National Park, (Silver Creek, 12); on *Tsuga heterophylla* (Nickrent 2671), Mt Baker-Snoqualmie National Forest at Huckleberry Camp (U.S. Army) road along F.H. 73, just off Rt. 410, (Huckleberry, 13).

Shore pine race

Vancouver Island, British Columbia: on *Pinus contorta* (Nickrent 2665), along the beach at Spider Lake, *ca* 6 km S. of Qualicum, (Spider Lake, 8); on *Pinus contorta* (Nickrent 2666), summit of Mt Findlayson, just E. of Rt. 1 and *ca* 14 air km west of Victoria, (Mt Findlayson, 9). San Juan Co., WA: on *Pinus contorta*, Mt Constitution, Orcas Island (F. G. Hawksworth, SN), (Orcas Island, 21).

Isozyme methods. The extraction method and buffer, and gel–electrode buffers for hemlock dwarf mistletoe are already reported [19]. The following 11 enzyme systems coding for 13 putative loci were used (with loci abbreviations, enzyme commission numbers, and buffer system in parentheses): aconitase (*ACO*, E.C. 4.2.1.3, B); adenylate kinase (*ADK-1*, E.C. 2.7.4.3, A); alcohol dehydrogenase (*ADH-1, ADH-2*, E.C. 1.1.1.1, B); glucose-6-phosphate dehydrogenase (*G-6-PDH*, E.C. 1.1.1.49, A); glutathione reductase [25] (*GSR-1*, 1.6.4.2, A); isocitrate dehydrogenase (*IDH*, E.C. 1.1.1.42, A); malate dehydrogenase (*MDH-3, MDH-4*, E.C. 1.1.1.37, B); peroxidase (*PER-2*, E.C. 1.11.1.7, B); phosphoglucose isomerase (*PGI*, E.C. 5.3.1.9, A or C); phosphoglucomutase (*PGM*, E.C. 5.4.2.1, C); and 6-phosphogluconate dehydrogenase (*6-PGDH*, E.C. 1.1.1.44, B).

The genotypic data were analysed for genetic variability using the computer program BIOSYS-1 [26]. Hardy–Weinberg expected heterozygosity levels were determined by the formula of Nei [27]. The chord distance of Cavalli-Sforza and Edwards [28] and the genetic distance of Rogers [29] were calculated using this program. *F*-statistics were after Nei [30] and hierarchical *F*-statistics followed the formulae of Wright [18]. UPGMA phenograms [31] were generated from the chord distances shown in Table 9. Distance Wagner [32] cladograms were generated from the chord distances shown in Table 9.

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*Part I in this series.