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Comparative population structure and genetic diversity of *Arceuthobium americanum* (Viscaceae) and its *Pinus* host species: insight into host–parasite evolution in parasitic angiosperms

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Abstract

In a recent study we revealed that the parasitic angiosperm *Arceuthobium americanum* is comprised of three distinct genetic races, each associated with a different host in regions of allopatry. In order to assess the role of host identity and geographical isolation on race formation in *A. americanum*, we compared the genetic population structure of this parasite with that of its three principal hosts, *Pinus banksiana*, *Pinus contorta* var. *latifolia* and *Pinus contorta* var. *murrayana*. Despite the fact that *A. americanum* was divided into three genetic races, hosts were divided into only two genetic groups: (i) *Pinus banksiana* and hybrids, and (ii) *P. contorta* var. *latifolia* and var. *murrayana*. These findings suggest that factors such as geographical isolation and adaptation to different environmental conditions are important for race formation in the absence of host-driven selection pressures. To assess factors impacting population structure at the fine-scale, genetic and geographical distance matrices of host and parasite were compared within *A. americanum* races. The lack of a relationship between genetic and geographical distance matrices suggests that isolation-by-distance plays a negligible role at this level. The effect of geographical isolation may have been diminished because of the influence of factors such as random seed dispersal by animal vectors or adaptation to nongeographically patterned environmental conditions. Host–parasite interactions might also have impacted the fine-scale structure of *A. americanum* because the parasite and host were found to have similar patterns of gene flow.

Keywords: AFLP, *Arceuthobium americanum*, genetic structure, parasitic angiosperm, *Pinus banksiana*, *Pinus contorta*

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Introduction

Knowledge of population structure is important to understanding evolution as it reflects the extent of gene flow between populations, and hence, the evolutionary potential of an organism (Price 1980; Thompson 1994; Nadler 1995). Genetic population structure is affected by life history characteristics such as mating patterns and dispersal systems, as well as by numerous other influences such as selection pressures, geographical range, stochastic forces and historical events. Parasites are unique, however, in that their population structure is also influenced by their intimate relationship with their hosts (Mulvey *et al*. 1991; Nadler 1995; Nadler *et al*. 1995).

In host–parasite interactions, the evolutionary outcome is dependent upon the amount of gene flow among populations of both the host and the parasite (Dybdahl & Lively 1996). For example, high gene flow by host and parasite could lead to decreased local adaptation (Gandon *et al*. 1996; Morand *et al*. 1996). In contrast, low gene flow by both host and parasite could lead to increased adaptation, particularly if the parasite and host have similar patterns of gene flow (Price 1980; Kirkpatrick & Barton 1997; Gandon & Van Zandt 1998). In this latter case, however, the individual (host or parasite) with the higher gene flow is most

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likely to have a selective advantage due to the introduction of novel alleles into the population (Gandon *et al*. 1996). This would occur as a result of gene-for-gene interactions between parasites and their hosts. Adaptation to hosts is important to the evolution of parasites as populations may eventually become differentiated into races as they adapt to the divergent selection pressures driven by different host taxa or genotypes (Thompson 1994). Parasite populations may additionally differentiate into races independent of the role played by interaction with its host. This may occur as a result of decreased gene flow because of limited migration (i.e. isolation-by-distance) (Price 1980; Thompson 1994) or as a result of divergent selection pressures imposed by different environmental conditions (Orr 1995; Orr & Orr 1996; Via *et al*. 2000).

Given the importance of relative gene flow and adaptation, researchers (Thompson 1994; Dybdahl & Lively 1996) have suggested that knowledge of the population structure of both the parasite and its host is necessary to fully understand evolution of these pathosystems. Adaptation of a parasite to a given host species may be reflected by higher genetic similarity between populations of parasites found within, relative to between, different host species (Price 1980). The effect of isolation-by-distance would be reflected by congruence between genetic and geographical distances (Dybdahl & Lively 1996). If environmental parameters are patterned geographically, such an influence could be detected in the same manner as is isolation-by-distance.

In recent years, empirical studies have employed comparisons of host and parasite population structures in order to further understand the evolution of parasites (Nadler *et al*. 1990; Mulvey *et al*. 1991; Dybdahl & Lively 1996; Davies *et al*. 1999; Delmotte *et al*. 1999; Martinez *et al*. 1999; Jobet *et al*. 2000). Unfortunately, this approach has not been applied to parasitic angiosperms. Edmunds & Alstad (1978) predicted, however, that parasitic plants such as dwarf mistletoes would show strong adaptation to local host populations, and even to host individuals, because of their dependence on toxin-defended, long-lived host trees. This hypothesis of local adaptation has never been examined thoroughly within parasitic angiosperms to test for genetic races that have resulted from isolation-by-distance or adaptation to their hosts.

This is the second of two studies that examine the evolutionary biology of *Arceuthobium americanum* Nutt. ex Engelm., a parasitic plant that infects three principal hosts [*Pinus banksiana* Lamb., *Pinus contorta* Douglas ex Loudon var. *latifolia* Englem. and *Pinus contorta* var. *murrayana* (Greville and Balfour) Engelm.]. In the first study (Jerome & Ford 2002a), the population structure of *A. americanum* was examined using amplified fragment length polymorphism (AFLP) analysis. It was found that *A. americanum* is divided into three genetic races, each associated with a different principal host in regions of allopatry. In this second study, the population genetic structures of the three principal *Pinus* spp. hosts (*P. banksiana*, *P. contorta* var. *latifolia* and *P. contorta* var. *murrayana*) were also determined using AFLP. The genetic distance matrices of both host and parasite were then compared with each other and with geographical distance. The objectives of this study were to: (i) determine the population genetic structures of *P. banksiana*, *P. contorta* var. *latifolia* and *P. contorta* var. *murrayana*; (ii) evaluate the influence of host identity in shaping the overall genetic structure of *A. americanum*; and (iii) examine the role played by isolation-by-distance and host–parasite interactions in shaping the fine-scale genetic structure of *A. americanum*.

Methods

Collections

Parasite and host plant tissue were collected from 29 populations: (i) 11 from *Pinus banksiana* — Belair (MB-2), Candle Lake (SK-3), Cowan (MB-4), Ft. McMurray (AB-6), Grand Rapids I (MB-7), La Loche (SK-9), La Ronge (SK-10), The Pas (MB-11), Prince Albert I (SK-12), Smeaton (SK-14) and Smoky Lake (AB-15); (ii) five from *P. banksiana* × *P. contorta* var. *latifolia* hybrids, High Level (AB-18), Slave Lake (AB-19), Whitecourt (AB-20), Whitemud/Peace River (AB-21) and Wood Buffalo National Park (AB-22); (iii) 10 from *P. contorta* var. *latifolia* hosts, Banff (AB-24), Castlegar (BC-25), Cypress Hills (AB-26), DTR (AB-27), Jasper (AB-29), John Day (OR-38), Ketchum (ID-40), Manila I (UT-41), Red Feather Lakes I (CO-43) and Yellowstone (WY-44); and (iv) three from *P. contorta* var. *murrayana* hosts, Ft. Klamath (OR-47), Lee Vining I (CA-48) and Mt. Shasta (CA-49). These populations represent a subset of the 51 *Arceuthobium americanum* populations sampled in the first study (see Jerome & Ford 2002; for locality information). For each population, host and parasite individuals were sampled from a single witches' broom from 10 different trees. Prior to DNA extraction, plant tissue was lyophilized as described previously (Jerome & Ford 2002).

DNA extractions and AFLP analysis

Parasite DNA extractions using a modified protocol for the DNeasy Plant Mini Kit (Qiagen 69106) were described previously (Jerome & Ford 2002). Host DNA was extracted in the same manner except that 15–20 mg of lyophilized plant tissue was used.

The AFLP procedure for *A. americanum* using *Mse*I and *Eco*RI restriction enzymes was carried out as per Vos *et al*. (1995) with modifications as described in Jerome & Ford (2002). The AFLP procedure for *Pinus* spp. was similar to that used for *A. americanum*. However, for *Pinus* spp., a +1/

+2 primer combination [E-A (GACTGCGTACCAATTCA) and M-CC (GATGAGTCCTGAGTAACC)] was used for pre-amplification instead of a +1/+1 combination. Reaction conditions and cycle parameters for *A. americanum* have been described in Jerome & Ford (2002). For *Pinus* spp., reaction mixes were the same as those for *A. americanum*. Cycle parameters were also similar except that the polymerase chain reaction (PCR) cycle was repeated 28 times (rather than 20 times) and the pre-amplification product was diluted 1:6 (rather than 1:4) with sterile dH_2O.

Selective amplification PCR for *A. americanum* using two +3/+3 primer combinations was described previously (Jerome & Ford 2002). Selective amplification PCR for *Pinus* spp. was similar to that for *A. americanum*. However, because of the large size of the *Pinus* genome, +3/+4 primer combinations were used for selective amplification in order to reduce the number of fragments observed on a gel (D. Remington, Forest Biotech. Group, North Carolina State University, Raleigh, personal commun.). A preliminary study was performed using five +3/+4 selective PCR primer combinations, yielding 122 loci across 20 *Pinus* spp. populations. The unweighted pair-group means of analysis (UPGMA) dendrogram based on Nei's and F_{ST} genetic distances from these five primers had the same overall topology as that obtained with only two primer combinations. Thus, further analyses were restricted to loci obtained from these two +3/+4 primer combinations: (i) M-CCAG (GATGAGTCCTGAGTAACCAG), E-AGG (GACTGCGTACCAATTCAGG); and (ii) M-CCGC (GATGAGTCCTGAGTAACCGC), E-ACG (GACTGCGTACCAATTCACG). Reaction conditions and cycles were similar to that for *Arceuthobium*. However, for *Pinus*, the selective amplification was carried out in a 25-μL reaction volume and an initial denaturation step at 94.0 °C for 3 min was added to the cycle parameters. In addition, once the annealing temperature had ramped to 56.0 °C, 23 cycles were carried out holding this annealing temperature constant.

AFLP products were run on 5% polyacrylamide gels, fixed in 10% glacial acetic acid, silverstained using the Silver Sequence(tm) DNA Sequencing System kit (Promega), and scored as described by Jerome & Ford (2002).

Data analysis of Arceuthobium *and* Pinus *AFLP*

Genetic distance and diversity measures for *Pinus* spp. and *A. americanum* were calculated using the two approaches described in Jerome & Ford (2002). The first approach was based on Nei's distance and diversity measures unbiased for sample size (Nei 1978). The frequency of presence/absence bands of both *Arceuthobium* and *Pinus* spp. were adjusted using the correction factor of Lynch & Milligan (1994) prior to calculation of distance and diversity measures. Because of the outcrossing nature of *Arceuthobium*, Jerome & Ford (2002) found this approach to be appropriate for *A. americanum*. This approach is also suitable for *Pinus* as members of the genus are known to transport their pollen over extremely long distances (Nichols *et al*. 1987; Ledig 1998; Campbell *et al*. 1999). In addition, molecular studies have shown high similarity between observed and expected heterozygosities for *P. banksiana* and *P. contorta* var. *latifolia* (Yeh & Layton 1979; Dancik & Yeh 1983; Yeh *et al*. 1985), thereby supporting the outcrossing nature of these taxa. For both *Pinus* spp. and *A. americanum*, UPGMA cluster analysis was used to create a dendrogram based on these modified Nei's genetic distances (matrices available upon request from CAJ).

Within-population variability measures for *A. americanum* [including the proportion of polymorphic loci (% poly.), number of alleles per locus, and expected heterozygosities (H_E)] were determined previously (Jerome & Ford 2002) using BIOSYS-2 (Swofford & Selander 1992). This program was recompiled by Kermit Ritland (Department of Forest Sciences, University of British Columbia, Vancouver) to handle 200 loci and 100 populations. Within-population variability measures were estimated for *Pinus* spp. in the same manner.

Group-level genetic diversity measures including genetic identity, total genetic diversity (H_T), average within-population diversity (H_S), among-population diversity (D_{ST}) and the coefficient of genetic differentiation (G_{ST}) were calculated using Nei & Chesser's (1983) procedure (unbiased for sample size) using the output from BIOSYS-2. These measures were determined for *A. americanum* as a whole, for the four groups of *A. americanum* defined on the basis of host identity, and for each of the four *Pinus* spp. host groups (*P. banksiana*, *P. contorta* var. *latifolia*, *P. banksiana* × *P. contorta* var. *latifolia* hybrids and *P. contorta* var. *murrayana*). Analysis of variance and two-sample *t*-tests were performed using DATADESK® VERSION© 4.1 (Vellman 1993) to determine if these groups possessed significantly different levels of genetic variation (as reflected by percentage of polymorphic loci or H_E).

Genetic data were also examined using an analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992). A population-level AMOVA was used to examine diversity within and between populations for each of *A. americanum*, *P. banksiana*, *P. contorta* var. *latifolia* and *P. contorta* var. *murrayana*. In addition, a nested AMOVA was performed on the four groups of *A. americanum* to assess the amount of diversity within and between host groups of this parasite. Euclidean distances and AMOVA (Excoffier *et al*. 1992; Huff *et al*. 1993) were calculated using the software program ARLEQUIN (Schneider *et al*. 2000). An UPGMA dendrogram for both *A. americanum* and *Pinus* spp. was constructed using pairwise population F_{ST} genetic distance values (matrices available upon request from CAJ) with the program SYN-TAX 5.1 (Podani 1998).

Partial correlations between distance matrices were calculated using three-way Mantel tests to examine the relationship of parasite genetic distance (represented as $F_{ST}/(1 - F_{ST})$] vs. either geography [calculated as log (distance in km)] or host genetic distance (represented as $F_{ST}/(1 - F_{ST})$], independent of the other variable (Smouse *et al*. 1986; Rousset 1997, 1999). Geographic distances (km) were calculated by assuming that dispersal between populations occurred via a straight-line geographical distance ('as the crow flies') between populations. If parasites were impacted by isolation-by-distance, a correlation between genetic and geographical distances would be expected. However, if the genetic distance between populations of parasites and hosts was correlated, this would indicate that these taxa have similar patterns of gene flow, and hence, the potential to become locally adapted (Dybdahl & Lively 1996).

Prior to Mantel analysis, parasite and host populations were divided into groups based on identity of the host such that the effect of local host genotype could be assessed independent of the role played by host identity. Only two (*P. contorta* var. *latifolia* and *P. banksiana*) of the potential four host groups were examined. The small number of populations examined from *P. contorta* var. *murrayana* ($n = 3$) precluded the analysis of this group because of bias during matrix randomization. The hybrid host group was not examined since results would be complicated by the nature of these individuals that represent a variety of introgressed forms between *P. banksiana* and *P. contorta* var. *latifolia*. The significance of the standardized correlation (r) for the Mantel tests was determined using matrix randomization. Correlations were considered significant if the probability of obtaining the observed value of 'r' by chance alone among 1000 reshuffled matrices was small ($P < 0.05$). Mantel tests were performed using the Mantel option in the computer program ARLEQUIN (Schneider *et al*. 2000).

Scatterplots of pairwise distances for pairs of populations were used to portray parasite genetic distance vs. host genetic distance, as well as parasite genetic distance vs. geographical distance. Scatterplots were constructed using the computer program DATA DESK® VERSION 4.1 © (Vellman 1993).

Results

The two primers used in the analysis of *Pinus* spp. yielded 79 scorable loci ranging in size from 400 to 1100 bp. Of these loci, 61 were polymorphic and 18 were monomorphic. Marker information for *Arceuthobium americanum* was presented previously (Jerome & Ford 2002).

Overall genetic structure of parasite and host populations

The dendrogram (Fig. 1) based on F_{ST} genetic distances shows the division of the 29 *A. americanum* populations into three distinct genetic races each associated with a different host in regions of allopatry. This pattern was shown previously when all 51 *A. americanum* populations were included in the analysis (Jerome & Ford 2002). In this second study, however, the pattern observed for *A. americanum* can be interpreted in light of knowledge about the genetic structure of hosts. Surprisingly, the UPGMA dendrogram (Fig. 2) based on F_{ST} genetic distances among host populations suggested that *Pinus* spp. were divided into only two distinctive genetic groups: (i) *P. banksiana* and hybrids, and (ii) *P. contorta* var. *latifolia* and var. *murrayana*. UPGMA dendrograms based on Nei's unbiased genetic distances (available upon request from CAJ) yielded the same overall topology as those from F_{ST} genetic distances. The high genetic identity found within either *P. banksiana* (0.976) or *P. contorta* (0.969–0.973) relative to the low identity between these species (0.837–0.839) suggests that they are well-differentiated taxa (Table 1). However, the two varieties of *P. contorta* had similar between (0.970) and within (0.969–0.973) group genetic identities.

Comparison of parasite and host genetic/geographic distances

Partial correlations (Table 2) were performed using Mantel tests to examine the relationship between parasite genetic structure compared with either: (i) host genotype (without the confounding influence of geographical isolation); or (ii)

Table 1 Matrix of Nei's unbiased genetic identity coefficients (range) (Nei 1978) for pairwise comparisons among four *Pinus* spp.

Species	*Pinus banksiana* ($n = 11$)	*Pinus contorta* var. *latifolia* ($n = 10$)	Hybrids ($n = 5$)	*Pinus contorta* var. *murrayana* ($n = 3$)
Pinus banksiana	0.976 (0.955–0.995)			
Pinus contorta var. *latifolia*	0.837 (0.748–0.915)	0.969 (0.931–0.997)		
Hybrids	0.955 (0.918–0.985)	0.897 (0.834–0.967)	0.967 (0.940–0.986)	
Pinus contorta var. *murrayana*	0.839 (0.797–0.903)	0.970 (0.937–0.993)	0.896 (0.862–0.947)	0.973 (0.966–0.986)

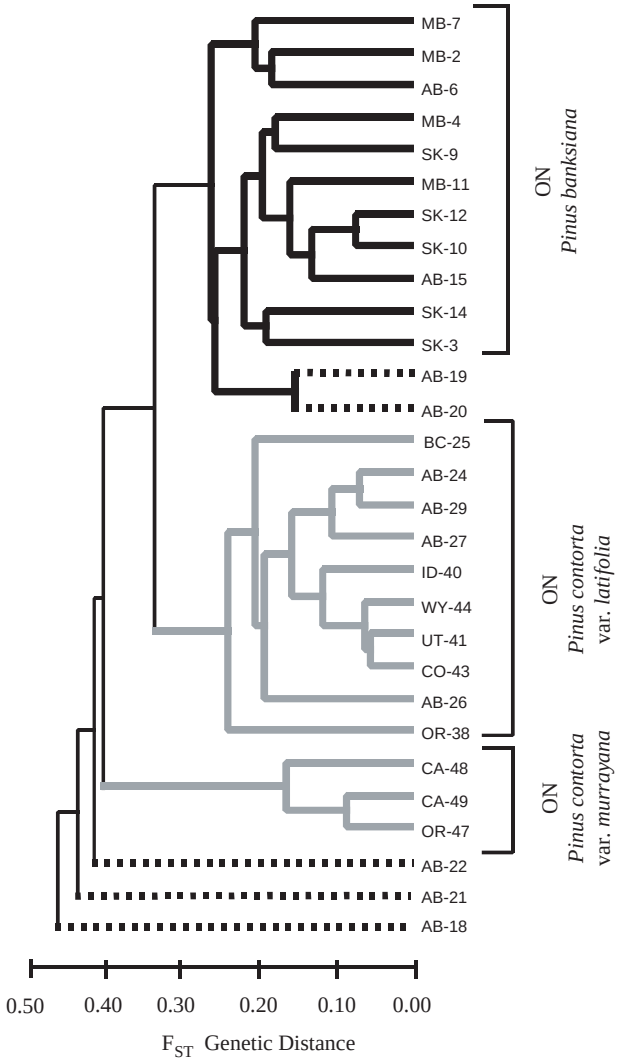


Fig. 1 UPGMA dendrogram of 29 *Arceuthobium americanum* populations based on F_{ST} genetic distances from AMOVA (Excoffier *et al*. 1992). Line shading indicates host taxon from which the parasite was isolated: black lines indicate *Pinus banksiana* hosts, grey lines indicate *Pinus contorta* var. *latifolia* and var. *murrayana* hosts, dashed lines indicate *Pinus banksiana* × *P. contorta* var. *latifolia* hybrid hosts.

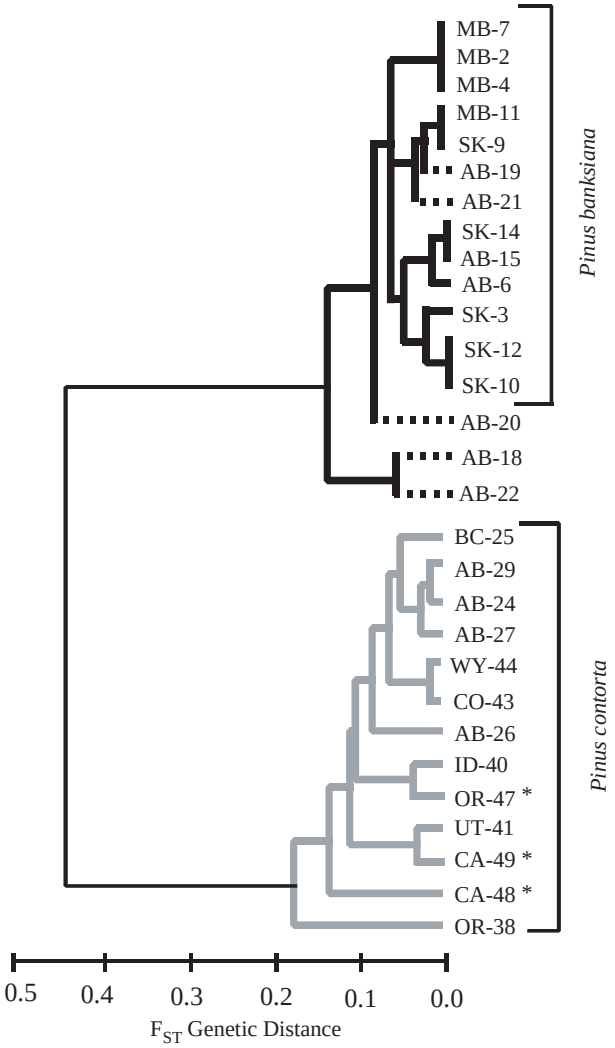


Fig. 2 UPGMA dendrogram of 29 *Pinus* spp. populations based on F_{ST} genetic distances from AMOVA (Excoffier *et al*. 1992). Black lines indicate *P. banksiana*, grey lines indicate *P. contorta* var. *latifolia* and var. *murrayana* (* indicates the latter variety), dashed lines indicate *P. banksiana* × *P. contorta* var. *latifolia* hybrids.

geographical distance (without the confounding influence of host). When partial correlations were examined for only those populations from *P. banksiana* hosts, 13.6% of the variance in the parasite genetic distance matrix was related with the host ($r = 0.349$, $P = 0.030$, Fig. 3a), whereas 0.0% was related to geography ($r = -0.031$, $P = 0.538$, Fig. 3b) (Table 2). When only those populations isolated from *P. contorta* var. *latifolia* hosts were examined, 24.5% of the variance in the parasite genetic distance matrix was related to host ($r = 0.482$, $P = 0.034$, Fig. 4a) and 2.5% was related to geography ($r = 0.129$, $P = 0.203$, Fig. 4b) (Table 2). Thus, geography has a surprisingly small impact on population structure within races of *A. americanum*. However, there is a similarity in patterns of gene flow in the parasite and host.

Parasite genetic variation and diversity measures

Overall variation. Population level variability measures for the 29 populations of *A. americanum* examined in this study were reported previously and discussed in Jerome & Ford (2002). Nei & Chesser's (1983) among-group diversity measures for *A. americanum* were calculated independently in this second study (Table 3) in order to allow for a more direct comparison with the 29 host populations examined. When all populations were considered as one group, *A. americanum* was found to be genetically diverse (H_T

Host	*Pinus banksiana* (n = 11)	*Pinus contorta* var. *latifolia* (n = 10)
Parasite vs. host	r = 0.349 P = 0.030	r = 0.482 P = 0.034
Parasite vs. geography	r = −0.031 P = 0.538	r = 0.129 P = 0.203
Proportion of genetic variation in parasite attributed to host	13.6%	24.5%
Proportion of genetic variation in parasite attributed to geography	0.0%	2.5%
Proportion of genetic variation in parasite accounted for by both host and geography combined	13.6%	27.0%

Table 2 Three-way Mantel test results showing partial correlations (r) between parasite (*Arceuthobium americanum*) and host (*Pinus* spp.) genetic distances, and between parasite genetic and geographical distances to determine the effect of one variable when the other variable is controlled. Number of populations sampled (n), correlation (r) and probability (P)

Host identity	H_T	H_S	D_{ST}	G_{ST}
All populations (n = 29)	0.234	0.163	0.071	0.303
Pinus banksiana (n = 11)	0.198	0.154	0.044	0.222
Pinus contorta var. *latifolia* (n = 10)	0.225	0.188	0.037	0.164
Canadian *Pinus contorta* var. *latifolia* (n = 5)	0.225	0.193	0.032	0.142
U.S.A. *Pinus contorta* var. *latifolia* (n = 5)	0.210	0.184	0.026	0.124
Pinus contorta var. *murrayana* (n = 3)	0.192	0.175	0.017	0.089
Hybrids (n = 5)	0.201	0.123	0.078	0.388

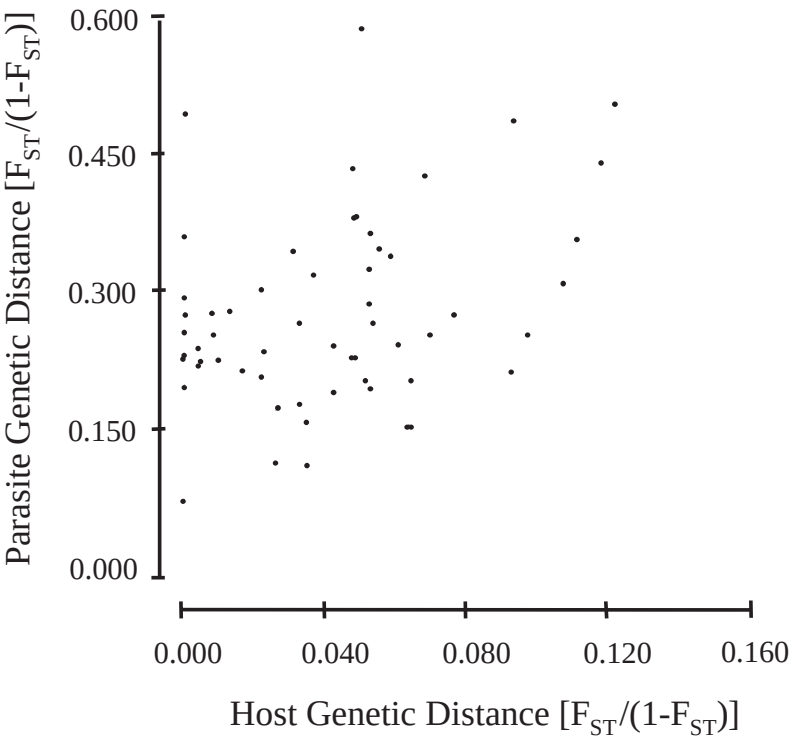
Table 3 Genetic diversity statistics (Nei & Chesser 1983) for 29 *Arceuthobium americanum* populations on its principal host taxa. Number of populations sampled (n), total genetic diversity (H_T), within-population genetic diversity (H_S), among-population genetic diversity (D_{ST}) and coefficient of genetic differentiation (G_{ST})

	d.f.	SSD	Variance components	% Total	P-value
Population Level					
Among Populations	28	1367.317	4.17771	32.93	<0.001
Within Populations	251	2135.833	8.50930	67.07	<0.001
Total	279	3503.150	12.68701	100.00	
Nested Level (Four groups)					
Among Groups	3	560.093	2.37773	17.80	<0.001
Among Populations within Groups	25	807.223	2.46593	18.47	<0.001
Within Populations	251	2135.833	8.50930	63.73	<0.001
Total	279	3503.150	13.35296	100.00	

Table 4 Analysis of molecular variance (AMOVA) for 29 populations of *Arceuthobium americanum*. Populations were analysed at both the population-level by considering all populations as a single group, and at the nested level using four groups based on host identity (*Pinus banksiana*, *P. contorta* var. *latifolia*, *P. banksiana* × *P. contorta* var. *latifolia* hybrids and *P. contorta* var. *murrayana*). Degrees of freedom (d.f), sums of square deviations (SSD), variance component estimates, the percentages of the total variance (% Total) contributed by each component, and the probability (P-value)

0.234, H_S 0.163) with fairly strong differentiation among populations (G_{ST} 0.303). The population-level AMOVA confirmed this observation as most of the variance was found within (67.07%) rather than between populations (32.93%) (P < 0.001) (Table 4). These results are similar to those seen when all 51 populations of *A. americanum* were included in the analysis (Jerome & Ford 2002).

Within-group variation. Diversity measures were also assessed for groups of *A. americanum* defined by host identity. This analysis indicated that total genetic diversity (H_T) measures were similar for the different host groups of *A. americanum* (0.192–0.201) with the exception of the higher diversity seen for the *P. contorta* var. *latifolia* group (0.225). Within-group genetic diversity values (H_S) showed a wider range of values (0.123–0.188). Likewise, values for population differentiation as measured by the coefficient of genetic differentiation (G_{ST} 0.089–0.388) ranged widely across groups (Table 3). When the four groups of *A. americanum* were examined using a nested AMOVA, 63.73% was found within the populations, 17.80% was found among the four groups, and 18.47% was found among the populations within these groups (P < 0.001) (Table 4).



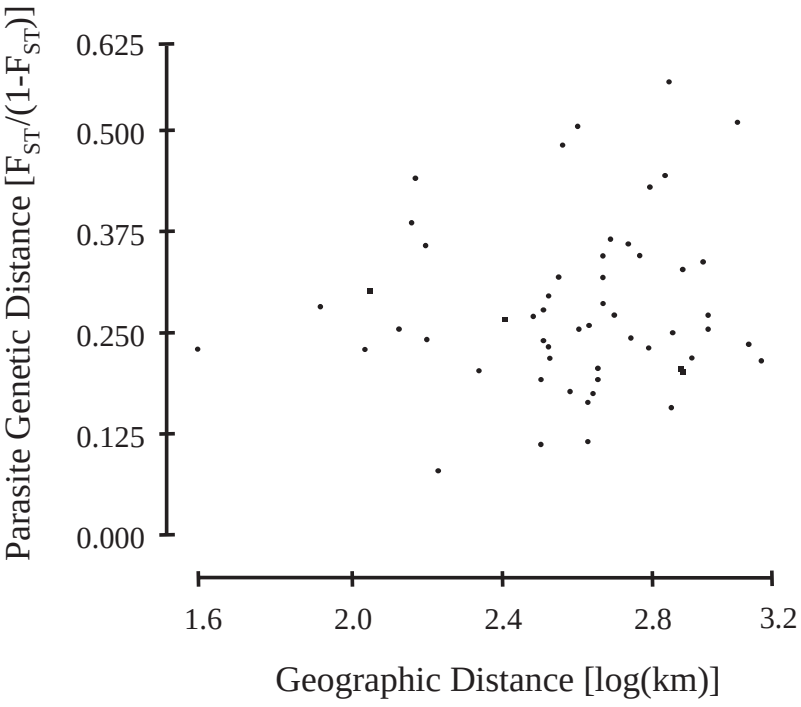
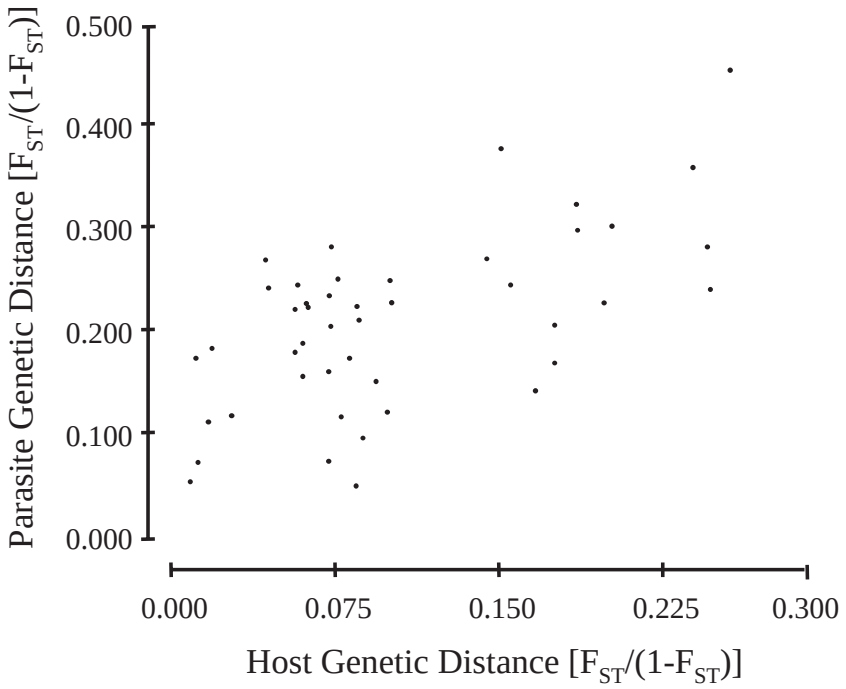


Fig. 3 Scatterplots of pairwise distances for 11 populations in the *Arceuthobium americanum*/*Pinus banksiana* pathosystem: (a) *A. americanum* genetic distance $[F_{ST}/(1 - F_{ST})]$ vs. *P. banksiana* genetic distance $[F_{ST}/(1 - F_{ST})]$; (b) *A. americanum* genetic distance $[F_{ST}/(1 - F_{ST})]$ vs. geographical distance [log (km)].

Host genetic variation and diversity measures

Population-level variability measures ranged considerably (37.7–62.3% poly.; H_E 0.130–0.219) across the 29 *Pinus* spp. host populations examined (Table 5). In contrast with that seen for *A. americanum* populations, the three hybrid pine populations from northern Alberta (AB-18, AB-21 and AB-22) had the highest proportion of polymorphic loci (54.1–55.7%, $P < 0.0001$) of all populations studied (37.7–62.3% poly.). As with the parasite, *P. contorta* var. *latifolia* spans regions with vastly different glacial histories in the Wisconsin period. When *P. contorta* var. *latifolia* populations



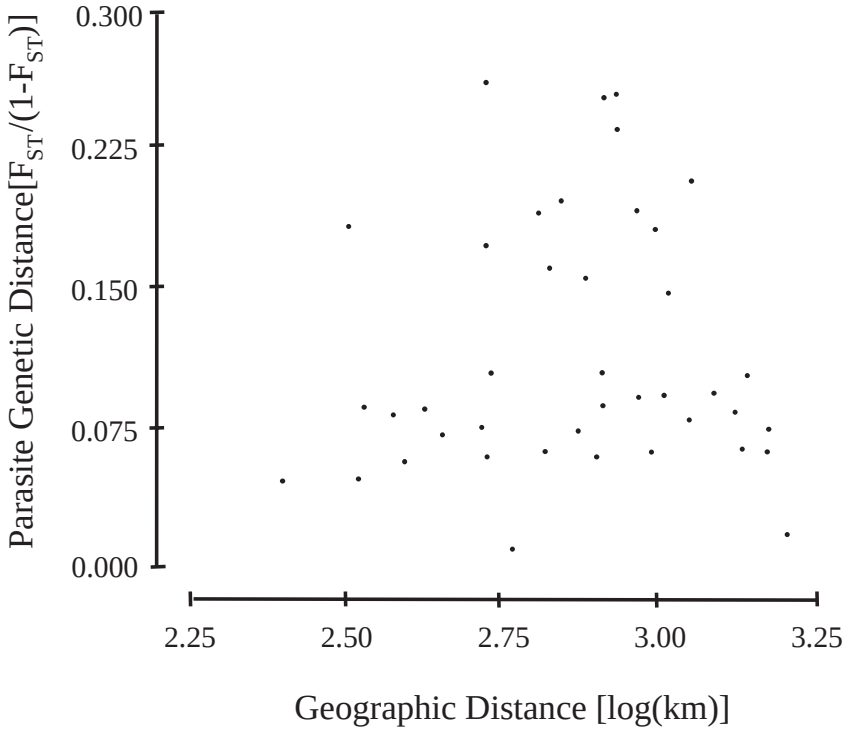


Fig. 4 Scatterplots of pairwise distances for 14 populations in the *Arceuthobium americanum*/*Pinus contorta* var. *latifolia* pathosystem: (a) *A. americanum* genetic distance [$F_{ST}/(1 - F_{ST})$] vs. *P. contorta* var. *latifolia* genetic distance [$F_{ST}/(1 - F_{ST})$]; (b) *A. americanum* genetic distance [$F_{ST}/(1 - F_{ST})$] vs. geographical distance [log (km)].

were split into two subgroups, those from previously glaciated regions had similar levels of polymorphic loci (45.9–54.1% poly., $P = 0.474$) and H_E (0.149–0.194, $P = 0.716$) as those from unglaciated regions (37.7–52.5% poly., H_E 0.131–0.185).

Nei & Chesser's (1983) among-group diversity measures for individual host taxa indicated that total genetic diversity (H_T) and within-group diversity (H_S) were similar across all pine species (H_T 0.174–0.192, H_S 0.166–0.172) with the exception of *P. banksiana* × *P. contorta* var. *latifolia* hybrids which had higher levels for both of these measures (H_T 0.217, H_S 0.193) (Table 6). As was observed for genetic variability measures, *P. contorta* var. *latifolia* populations

Table 5 Genetic variability measures for 29 populations of *Pinus* spp. Average number of individuals (*N*), mean number of alleles per locus ± 1 SE (*k*), percentage of polymorphic loci at 95% criterion (*P*) and expected heterozygosity ± 1 SE (H_E) (unbiased estimate Nei 1978)

Population	*N*	*k*	*P*	H_E
Pinus banksiana				
Belair (MB-2)	9.0 ± 0.0	1.5 ± 0.1	45.9	0.130 ± 0.027
Candle Lake (SK-3)	10.0 ± 0.0	1.5 ± 0.1	52.5	0.201 ± 0.028
Cowan (MB-4)	10.0 ± 0.0	1.6 ± 0.1	57.4	0.219 ± 0.029
Ft. McMurray (AB-6)	10.0 ± 0.0	1.4 ± 0.0	44.3	0.152 ± 0.026
Grand Rapids I (MB-7)	9.9 ± 0.0	1.4 ± 0.1	44.3	0.154 ± 0.027
La Loche (SK-9)	10.0 ± 0.0	1.5 ± 0.1	50.8	0.162 ± 0.025
La Ronge (SK-10)	10.0 ± 0.0	1.5 ± 0.1	54.1	0.188 ± 0.027
Prince Albert I (SK-11)	9.0 ± 0.0	1.5 ± 0.1	52.5	0.186 ± 0.028
Smeaton (SK-14)	10.0 ± 0.0	1.4 ± 0.1	44.3	0.164 ± 0.027
Smoky Lake (AB-15)	10.0 ± 0.0	1.5 ± 0.1	49.2	0.166 ± 0.026
The Pas (MB-11)	8.4 ± 0.1	1.5 ± 0.1	49.2	0.166 ± 0.026
Average	9.7	1.5	49.5	0.172
P. banksiana × *P. contorta* var. *latifolia* hybrids				
High Level (AB-18)	9.0 ± 0.0	1.6 ± 0.1	55.7	0.189 ± 0.027
Slave Lake (AB-19)	10.0 ± 0.0	1.5 ± 0.1	52.5	0.189 ± 0.027
Whitecourt (AB-20)	10.0 ± 0.0	1.6 ± 0.1	62.3	0.203 ± 0.027
Wood Buffalo National Park (AB-21)	9.5 ± 0.1	1.5 ± 0.1	54.1	0.190 ± 0.027
Whitemud/PR (AB-22)	9.5 ± 0.1	1.6 ± 0.1	55.7	0.196 ± 0.027
Average	9.6	1.6	56.1	0.193
Canadian *P. contorta* var. *latifolia*				
Banff (AB-24)	8.0 ± 0.0	1.5 ± 0.1	52.5	0.184 ± 0.27
Castlegar (BC-25)	9.0 ± 0.0	1.5 ± 0.1	49.2	0.168 ± 0.026
Cypress Hills (AB-26)	10.0 ± 0.0	1.5 ± 0.1	45.9	0.149 ± 0.025
David Thompson Resort (AB-27)	10.0 ± 0.0	1.5 ± 0.1	47.5	0.151 ± 0.025
Jasper (AB-29)	10.0 ± 0.0	1.5 ± 0.1	54.1	0.194 ± 0.027
Cdn. Average	9.4	1.5	49.8	0.169
U.S.A. *P. contorta* var. *latifolia*				
John Day (OR-38)	9.5 ± 0.1	1.5 ± 0.1	50.8	0.173 ± 0.027
Ketchum (ID-40)	10.0 ± 0.0	1.4 ± 0.1	37.7	0.131 ± 0.025
Manila I (UT-41)	10.0 ± 0.0	1.5 ± 0.1	52.5	0.169 ± 0.025
Red Feathers Lake I (CO-43)	9.5 ± 0.1	1.5 ± 0.1	49.2	0.185 ± 0.027
Yellowstone (WY-44)	10.0 ± 0.0	1.5 ± 0.1	47.5	0.164 ± 0.025
U.S.A. Average	9.8	1.5	47.5	0.164
Overall *P. contorta* var. *latifolia* Average	9.6	1.5	48.7	0.167
P. contorta var. *murrayana*				
Fort Klamath (OR-47)	10.0 ± 0.0	1.5 ± 0.1	49.2	0.187 ± 0.028
Lee Vining I (CA-48)	9.0 ± 0.0	1.4 ± 0.1	41.0	0.150 ± 0.027
Mount Shasta (CA-49)	9.0 ± 0.0	1.4 ± 0.1	44.3	0.162 ± 0.027
Average	9.3	1.4	44.8	0.166

from previously glaciated regions had similar levels of total genetic diversity (H_T 0.183) and within-group genetic diversity (H_S 0.169) as those from unglaciated regions (H_T 0.196; H_S 0.164) (Table 6). Interestingly, the parasite races were characterized by higher total genetic diversity (H_T 0.192–0.225) (Table 3) than the host taxa (H_T 0.174–0.217) (Table 6).

In the pines, the small divergence between H_T and H_S values resulted in low values of population differentiation as estimated by G_{ST} (0.046–0.130) (Table 6). In comparison, *A. americanum* had a G_{ST} of 0.303 (Table 3), indicating that populations of the parasite were 3–6 times more strongly structured than its hosts. As with Nei and Chesser's diversity measures, the AMOVA revealed that populations of *Pinus* spp. were much less differentiated (3.77–10.55%) (Table 7) than were populations of its parasite (32.93%) (Table 4).

Discussion

Factors influencing overall structure of Arceuthobium americanum

As observed previously with 51 populations (Jerome & Ford 2002), the 29 *A. americanum* populations examined

Species	H_T	H_S	D_{ST}	G_{ST}
Pinus banksiana (n = 11)	0.192	0.172	0.020	0.102
Pinus contorta var. *latifolia* (n = 10)	0.192	0.167	0.025	0.130
Canadian *Pinus contorta* var. *latifolia* (n = 5)	0.183	0.169	0.014	0.077
U.S.A. *Pinus contorta* var. *latifolia* (n = 5)	0.196	0.164	0.032	0.163
Pinus contorta var. *murrayana* (n = 4)	0.174	0.166	0.008	0.046
Hybrids (n = 5)	0.217	0.193	0.024	0.110

Table 6 Genetic diversity statistics (Nei & Chesser 1983) for *Pinus* spp. hosts. Number of populations sampled (n), total genetic diversity (H_T), within-population genetic diversity (H_S), among-population genetic diversity (D_{ST}) and coefficient of genetic differentiation (G_{ST})

	d.f.	SSD	Variance Components	% Total	P-value
Pinus banksiana					
Among Populations	10	73.610	0.20888	3.77	<0.001
Within Populations	96	511.633	5.32951	96.23	<0.001
Total	106	585.243	5.53840	100.00	
Pinus contorta var. *latifolia*					
Among Populations	9	102.055	0.58621	9.22	<0.001
Within Populations	85	490.861	5.77484	90.78	<0.001
Total	94	592.916	6.36105	100.00	
Pinus contorta var. *murrayana*					
Among Populations	2	19.033	0.51500	10.55	<0.001
Within Populations	27	117.900	4.36667	89.45	<0.001
Total	29	136.933	4.90311	100.00	

Table 7 Analysis of molecular variance (AMOVA) for 29 populations of *Pinus* spp. Populations were divided into three groups based on host identity (*P. banksiana*, *P. contorta* var. *latifolia* and *P. contorta* var. *murrayana*). Degrees of freedom (d.f.), sums of square deviations (SSD), variance component estimates, the percentages of the total variance (% Total) contributed by each component, and the probability (P-value)

in this study were divided into three genetic races, each infecting a different host taxon in regions of allopatry. This pattern suggested that identity of the host, isolation-by-distance and environmental parameters have facilitated the formation of three genetic races in *A. americanum.* In this second study, the impact of these factors was explored further by assessing the overall structure of *A. americanum* in light of information about genetic structure of the hosts.

Examination of hosts revealed that only two of the three taxa were genetically distinct. Because *Pinus banksiana* and *P. contorta* hosts were genetically divergent, they likely impose different selection pressures on their respective parasite populations. This role for host identity does not preclude, however, a role for isolation-by-distance and adaptation to environmental conditions in shaping populations of *A. americanum*. Indeed, these latter two factors likely play a significant role in the diversification of the *A. americanum* races associated with the two *P. contorta* varieties as these hosts were not distinct genetically. Geographic isolation appears to limit gene flow between populations on *P. contorta* var. *murrayana* in the Sierra Nevada and Cascade Mountain ranges and populations on *P. contorta* var. *latifolia* in the Blue, Salmon River, Uinta and Rocky Mountains. Geographical isolation is more likely to affect the parasite than the host because of intrinsic differences in their mating systems. For example, pollen of *Pinus* is carried over much longer distances (Ledig 1998; Campbell *et al*. 1999) than is *Arceuthobium* pollen (Penfield *et al*. 1976; Gilbert & Punter 1984).

Adaptive races?

The molecular data provided in this study show the presence of three genetically distinct parasite groups and two genetically distinct host groups. It is important to emphasize, however, that these groups do not necessarily represent 'adaptive races'. In order to ascertain the number of 'adaptive' races, it would be necessary to examine the genes involved in infectivity and resistance. Because AFLP analysis is designed to examine random regions across the entire genome of an organism, the majority of loci examined in this study are probably not directly involved in resistance or infectivity. Infectivity data pertaining to the ability for different parasite races to infect each host would provide important ancillary information that might help in the interpretation of the AFLP analysis. Unfortunately, such information is not available at this time.

Factors influencing patterns within A. americanum *races*

Geography and host. For *A. americanum* races found on both *P. banksiana* and *P. contorta* var. *latifolia*, there was virtually

no relationship between geography and parasite genetic distance. This observation suggests that isolation-by-distance plays a negligible role in shaping the population structure within *A. americanum* races.

The relationship between host and parasite genetic distance matrices, and the information that can be elicited from such knowledge, is somewhat less clear. For both races, there appears to be a relationship between the genetic structures of the host and parasite. This suggests that patterns of gene flow are similar for host and parasite. As a result, it is possible that host–parasite interactions have impacted population structure within *A. americanum* races. As mentioned previously, however, AFLP markers do not specifically target genes involved in local adaptation and resistance. For this reason, it is not possible to directly infer the role of host genotype in shaping the genetic structure within *A. americanum* races.

Local environmental conditions. In a previous study (Jerome & Ford 2002a), broad-scale environmental patterns associated with different ecoclimatic regions were implicated as facilitating race formation in *A. americanum*. Patterning within *A. americanum* races may also be affected by environmental patterns, but in this case, on a much finer scale. For example, the lack of structuring within *A. americanum* races could be a result of adaptation to local environmental conditions that are not geographically patterned. In a study by Cooper (2000), such factors were implicated as playing an important role in shaping the genetic structure in the southern brown bandicoot (a small marsupial). Cooper (2000) found no correlation between genetic and geographical distance, but found a strong correlation between genetic distance and both habitat type (swamp or forest) and annual rainfall. This researcher concluded that gene flow among populations was being limited because of selection against new migrants imposed by local habitat type and levels of rainfall. In *A. americanum*, local environmental conditions such as rainfall, light intensity and average temperature probably differ between populations. However, measures of these parameters were not available for the sites examined in this study. Nonetheless, these factors could account for some degree of the fine-scale structuring within *A. americanum* races.

Comparison with other parasites. Currently, there are no other published studies comparing the genetic structure of parasitic angiosperms and their hosts. However, a review of previous studies suggests that various factors likely explain fine-scale patterning in parasites. For example, Mulvey *et al*. (1991) found a lack of congruence between genetic and geographical distance matrices in a liver fluke parasite and its deer host. These researchers suggested that long-distance dispersal of parasites may have counteracted factors that would have otherwise led to spatial patterning in these populations. This finding is similar to that from this study, whereby rare instances of long-distance transport of *A. americanum* seeds have likely led to decreased structuring of populations. In contrast, some researchers have found the opposite pattern. For example, isolation-by-distance was found to play a more important role than local host genotype in shaping fine-scale structure in trematode parasites of snails (Dybdahl & Lively 1996) and cuckoo parasites of magpies (Martinez *et al*. 1999).

Comparison of host and parasite population differentiation

Price (1980) predicted that adaptation and strict dependence of a parasite on a host would lead to strong structuring of parasite populations (but see Nadler 1995). In our study, *A. americanum* populations were found to be 3–6 times more structured than any of the principal hosts. The strong differentiation in parasite populations may partially be attributed to selection pressures imposed by different host taxa and/or local host genotypes. Differential selection pressures imposed by environmental conditions throughout the range of *A. americanum* may also contribute to the strong partitioning of diversity in this parasite. Such selection pressures could favour specific genotypes of *A. americanum* in certain regions while selecting against others.

It is also likely that the strong structuring in *A. americanum* relative to its *Pinus* spp. hosts may be attributed to differences in breeding and dispersal mechanisms rather than the parasitic nature of *A. americanum*. Because of the prevalence of wind-pollination in gymnosperms, these taxa have the highest levels of gene flow recorded for any plant group (Hamrick & Godt 1990). This has been attributed to pollen morphology that allows *Pinus* pollen to be transported by wind over extremely long distances (hundreds to thousands of kilometres) (Ledig 1998; Campbell *et al*. 1999). Such high rates of gene flow have a homogenizing influence on the population structure of pines. In the genus *Arceuthobium*, however, there is no evidence to suggest that pollen can be carried by the wind over distances comparable with that seen for the *Pinus* hosts (Penfield *et al*. 1976; Gilbert & Punter 1984). For this reason, *A. americanum* populations are more likely to be differentiated.

Comparison with the literature. Some studies that have examined the degree of genetic structuring in parasites relative to their hosts have shown results similar to that seen for the *A. americanum*/*Pinus* spp. pathosystem. For example, Delmotte *et al*. (1999) showed that a fungal parasite was much more differentiated than its *Silene* plant host. These researchers attributed this finding to different mating systems because the fungal parasite undergoes routine selfing, whereas the host plant outcrosses. Martinez *et al*. (1999) also showed a similar pattern because

they found that great spotted cuckoo parasites were more strongly structured than their magpie hosts. It was suggested that the cuckoo parasites impose a selection pressure for their magpie hosts to disperse relatively long distances from their natal sites. Some researchers (Dybdahl & Lively 1996; Davies *et al*. 1999) have also observed the opposite pattern to that seen for the *A. americanum*/*Pinus* spp. pathosystem. In these studies, the primary hosts (snails) were found to be more strongly structured than their parasites (trematodes). The difference in structuring was attributed to dispersal mechanisms. For example, the primary hosts (snails) disperse themselves over short distances. However, the parasites are dispersed over longer distances by their subsequent and final hosts, birds (Dybdahl & Lively 1996) or humans (Davies *et al*. 1999).

As can be seen from our study and from the literature, the complex nature of forces acting on hosts and parasites will lead to varying degrees of differentiation in hosts and parasites, depending on the pathosystem in question (contra Price 1980). This is important as the degree to which a parasite and host are differentiated relative to each other may have an impact on the evolutionary outcome of host–parasite interactions (Dybdahl & Lively 1996).

Comparison of variability/diversity in A. americanum *and hosts*

A comparison of levels of genetic diversity within and between a parasite and its hosts is important for understanding their interaction as levels of genetic diversity can impact the outcome of an evolutionary arms race. In our study, the parasite (*A. americanum*) had a higher level of total genetic diversity (H_T) than its *Pinus* spp. hosts. This observation may be related to several factors. First, the geographical range of the parasite as a whole is larger than that of its individual host taxa. Empirical studies across a large number of plant species indicate that taxa with wide geographical ranges commonly possess higher levels of genetic diversity than do more narrowly distributed taxa (Hamrick & Godt 1990). In addition, *A. americanum* may maintain higher diversity to enable it to infect different host taxa with varying genetic characteristics over this range.

In only one situation were host populations more diverse than parasite populations. Both within-population variability values and within-group diversity measures indicated that the least diverse parasite populations (i.e. those from hybrid hosts in northern Alberta) were isolated from the most diverse host populations. For the parasite, it was previously speculated (Jerome & Ford 2002) that founder events and geographical isolation contributed to the low level of genetic diversity in these northern populations. For the hosts, the hybrid nature of organisms in these populations likely results in their higher genetic diversity because they represent a mixture of the genomes of two different species.

Effect of glaciation on genetic diversity. In this and a previous study (Jerome & Ford 2002), genetic diversity in *A. americanum* populations on *P. contorta* var. *latifolia* hosts was found to be greater in glaciated (on-ice) than unglaciated (off-ice) regions. This is in contrast to the pattern observed in most other taxa in which conspecifics and congeners from glaciated regions tend to be less genetically diverse than those from unglaciated regions (Copes 1981; Lewis & Crawford 1995; Broyles 1998). It was suggested that the aberrant pattern in *A. americanum* might be explained if this taxon survived the Wisconsin glaciation in a genetically diverse refugium in the Canadian Rocky Mountains. Interestingly, results from our study revealed that *P. contorta* var. *latifolia* from off-ice regions had a similar level of genetic diversity to those from on-ice regions. Thus, past glaciations have not greatly impacted genetic diversity in the host. Because the host and parasite would have both existed in this northern refugium, they must have been impacted differently. Expansion of the host and its parasite into newly deglaciated territory in the north may have impacted the host more strongly than it did the parasite. This could occur if the hosts moved into deglaciated regions at a much quicker rate than did the parasite. This is possible as the obligate parasite would have had to migrate behind the movement of the host.

AFLP as a marker for population genetic studies on pines

There is general congruence between the genetic diversity measures and differentiation values obtained in this study using dominant AFLP markers and that previously reported for these taxa based on codominant isoenzyme markers. In this study, values for population differentiation in *P. banksiana*, *P. contorta* var. *latifolia* and var. *murrayana* (G_{ST} 0.046–0.130) using AFLP data were similar to those determined for these taxa in several other studies using isoenzymes (G_{ST} 0.010–0.070, reviewed in Ledig 1998). In addition, the average within population genetic diversity (H_S 0.166–0.172) based on AFLP data from this study falls within the range of values reported for these taxa by several other authors using isoenzymes (H_S 0.143–0.185; reviewed in Ledig 1998). Thus, dominant markers such as AFLPs seem reliable for studying population structure in outcrossing plants once the L-M correction factor has been applied.

Conclusions

Observations from this and a previous study (Jerome & Ford 2002) support the concept that host identity plays an important role in shaping the overall structure of

Arceuthobium americanum into three genetic races. The findings from this study imply, however, that other factors contribute to race formation in the absence of divergent host selection pressures. These factors include geographical isolation and adaptation to different environmental conditions. Such factors likely play an important role in race formation in other parasitic plants as well. For example, genetic evidence and infectivity studies support the existence of geographical rather than host-specific races in *Striga hermonthica* Benth. (Bharathalakshimi *et al*. 1990; but see Olivier *et al*. 1998), *Striga gesneroides* Vierh. (Lane *et al*. 1996) and *Orobanche cumana* Wallr. (Gagne *et al*. 1998).

The idea that parasitic plants may also be impacted at the local level was addressed by comparing host and parasite genetic and geographical distance matrices within *A. americanum* races. At this level, isolation-by-distance appears to play a negligible role in shaping population structure, whereas host–parasite interactions may have some impact. Given the wide array of reproductive strategies and dispersal mechanisms found in parasitic angiosperms, it is likely that levels of gene flow and subsequent local adaptation will be quite variable across taxa. Pollination strategies in parasitic plants range from those that self (some *Orobanchaceae*) to those that outcross through wind and animal pollination (such as the *Loranthaceae*, *Viscaceae* and *Scrophulariaceae*). Seed dispersal mechanisms vary from those that disperse seeds by explosive discharge and animals (many *Loranthaceae* and *Viscaceae*) to those that disperse seeds by wind (such as the *Scrophulariaceae* and *Orobanchaceae*). As such, it is unlikely that a general rule can be applied with respect to the impact that isolation-by-distance and host–parasite interactions will have on parasitic angiosperms and their hosts.

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This research comprises part of Dr Cheryl Jerome's PhD thesis, which was concerned with examining evolutionary forces affecting the genetic structure of *Arceuthobium americanum*. Her long-term research interests are to gain a broad understanding of the evolutionary pressures acting on parasitic plants by examining taxa with different reproductive strategies, dispersal mechanisms, host specificities and degrees of parasitism. Dr Bruce Ford's primary research interest is the taxonomy and evolution of the genus *Carex*. His sojourn away from sedge systematics has been a welcome diversion. Through collaborative studies, he hopes to continue his research on the ecology and evolution of *Arceuthobium*.
