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Insular biogeography and population genetics of dwarf mistletoe (*Arceuthobium americanum*) in the Central Rocky Mountains

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Citation: Hill, R., J. L. Loxterman, and K. Aho. 2017. Insular biogeography and population genetics of dwarf mistletoe (*Arceuthobium americanum*) in the Central Rocky Mountains. Ecosphere 8(5):e01810. 10.1002/ecs2.1810

Abstract. Lodgepole pine dwarf mistletoe (*Arceuthobium americanum*), an aggressive parasite of lodgepole pine (*Pinus contorta* subsp. *latifolia*), is limited, in the Central Rocky Mountains, to montane host "islands." We sought to quantify the effect of the insularity and life history of *A. americanum* on its population genetic structure by considering three questions: (1) Do apparent limits to *A. americanum* pollen and seed dispersal affect gene flow at small spatial scales? (2) Are patterns of genetic diversity on montane "islands" and "mainlands" consistent with the predictions of island biogeographic theory? (3) What is the origin and genetic relationship of "island" and "mainland" populations of *A. americanum* within the Central Rocky Mountain study area? We used seven *A. americanum* microsatellite markers developed specifically for the project to genotype 241 individuals from six populations (four "island" and two "mainland" locations) at the western edge of the Central Rocky Mountains, Idaho, USA. All loci were unlinked and polymorphic with a range of 4–24 alleles per locus. To consider gene flow, we performed spatial analyses and compared *F*-statistics from our data to those from a wide range of species and functional groups. We tested the predictions of biogeographic theory by quantifying associations of genetic diversity with "island" area and isolation from "mainland" populations. To consider the genetic relationships of "island" and "mainland" regions, we applied agglomerative and Bayesian hierarchical classifications to datasets, and objectively identified the optimal numbers of clusters in these analyses. We found that F_{IS} values in *A. americanum* were similar to those reported for dispersal-limited species with similar life-history characteristics, and F_{ST} values were similar to those reported for plants with animal-facilitated seed dispersal. As predicted by biogeographic theory, island allelic richness was positively correlated with geographic size of the population and negatively correlated with distance from mainland populations. Average linkage (UPGMA) classifications and Bayesian cluster analyses both identified two groupings of island populations radiating from the mainland populations. Our results are likely to be of interest to foresters managing dwarf mistletoe infections, particularly in the Central Rocky Mountains, and to ecologists and geneticists working with insular population distributions.

Key words: dispersal; dwarf mistletoe; Greater Yellowstone Ecosystem; Island biogeography; population genetics; Rocky Mountains.

Received 9 January 2017; revised 27 March 2017; accepted 3 April 2017. Corresponding Editor: Jonathan R. Walter.
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INTRODUCTION

Forest ecologists and land managers require detailed species information, including genetic data, to effectively assess and maintain the natural diversity, stability, and resilience of forest ecosystems (Loo et al. 2014, UN-FAO 2014, Wickneswari et al. 2014). Particularly relevant are considerations of the geographic genetic structure of forest species in montane environments, including tree symbionts. The gene pools of those species are expected to become

increasingly fragmented as a result of global climate change (Romme and Turner 1991, Webber et al. 2005, Creeden et al. 2014) and other, often concomitant, anthropogenic impacts (Smith et al. 2008). Such pressures are likely to increase vulnerability of montane forest populations to extinction by altering natural patterns of genetic differentiation and gene flow (Piotti 2009, Piovania et al. 2010, Leonardi et al. 2012).

Arceuthobium americanum

Lodgepole pine dwarf mistletoe (*A. americanum* Nutt ex Engelm.) is an obligate parasite of lodgepole pine (*Pinus contorta* subsp. *latifolia* Dougl.), a species limited to montane ecosystems in western North America. All dwarf mistletoes (genus *Arcethobium* [Santalaceae]) are obligate parasites of conifers and require host trees to obtain water and nutrients (Hawksworth and Wiens 1996). Dwarf mistletoes often damage host conifers by depleting nutrients, decreasing respiration (Wanner and Tinnin 1986), and causing a tumor-like growth called "witch's broom" in most host species (Nicholls et al. 1987, Hawksworth and Wiens 1996). Witches' brooms tend to be more flammable than normal growth, increasing fire severity and coincident mortality of conifers in forest fires (Harrington and Hawksworth 1990). On the other hand, dwarf mistletoes may *positively* affect forest ecosystem diversity by providing food and shelter for animal species, and by increasing understory light, leading to higher understory plant productivity (Nicholls et al. 1984*a*, Hawksworth and Wiens 1996, Nicholls 2014).

Pollen dispersal.—Pollination mechanisms in *A. americanum* are dispersal-limited. Individual plants are dioecious with the potential for hundreds of flowers per inflorescence, and the production of approximately 11,000 pollen grains per ovule (Gregor et al. 1974, Penfield et al. 1976, Hawksworth and Wiens 1996). *Arceuthobium americanum*, however, flowers very early in the spring when few insect pollinators are active (Munro et al. 2014). As a result, when temperatures are low during anthesis, anemophily is likely to be the main pollen dispersal mechanism, whereas later in the growing season entomophily may be favored over anemophily (Munro et al. 2014).

Further, *A. americanum* pollen grains are in bundles and nectaries are open and flat, resulting in a morphology poorly suited to wind dispersal (Gregor et al. 1974, Penfield et al. 1976). On average, wind dispersion of pollen, some of which may be explosively released from anthers, occurs over short distances, ≈1.6 m (Penfield et al. 1976), with a maximum reported range of 512 m (Coppola 1989, Munro et al. 2014).

The major animal pollinators of *A. americanum* are generalist insects, including the hymenopteran *Formica fusca* Linnaeus (Formicidae), and dipterans, particularly *Philygria debilis* Loew (Ephydridae). Only unwinged workers of the primary pollinator, *F. fusca*, a common circumboreal forest ant (MacKay and Mackay 2002), visit inflorescences for nectar (Penfield et al. 1976). Other, smaller insects (e.g., *Protophormia terraenovae* Robineau-Desvoidy, *Hylemya ceralis* Gillette, *H. cinerella* Fallen, *and H. platura* Meigen [Anthomyiidae]) provide pollination services for *A. americanum* to a lesser degree (Penfield et al. 1976).

Seed dispersal.—Seed dispersal of *A. americanum* (and other dwarf mistletoes) is also constrained. Under the primary method of seed dispersal, hydrostatic pressure generated in the seed pod and thermogenesis—via the alternative oxidase pathway—is used to trigger ejection of the seed from the parent plant at velocities up to 27 m/s (Hinds et al. 1963 *qtd*. in Hawksworth and Wiens 1996, deBruyn et al. 2015). This mechanism propels seeds up to 16 m (Hawksworth and Wiens 1996); seeds, however, usually only travel 2–4 m before encountering and affixing to a host as the result of a sticky mucilaginous seed coating (Hawksworth and Wiens 1996). Approximately 40% of propelled seeds encounter viable hosts (Hawksworth and Wiens 1996). Animals serve as secondary seed dispersal agents of *A. americanum*. While ingested seeds are nonviable after passing through animal digestive tracts, external animal dispersal of adhesive *A. americanum* seeds may occur via small mammals and birds (Hudler et al. 1979, Nicholls and Hawksworth 1983, Nicholls et al. 1984*a*, *b*, Hawksworth et al. 1987, Nicholls 2014).

Dispersal and genetic structure.—The physical limitations to pollen and seed dispersal should constrain gene flow among and within populations of *A. americanum* (Hawksworth and Wiens 1996). Jerome and Ford (2002), however, argued that most populations of *A. americanum* are unlikely to be negatively affected by genetic drift because of large population sizes and dioecious

gender expression. This apparent conflict prompts further consideration of the dispersal characteristics of *A. americanum*, and their genetic consequences.

Insular biogeography and population genetics

The ecology of *A. americanum* requires its distribution to be highly insular. Specifically, *A. americanum* is a dispersal-limited species, obligate to host "islands" of lodgepole pine (cf. Wheeler and Guries 1982), whose Central Rocky Mountain distribution is in turn limited to montane sites with elevations ranging between 1670 and 2380 m (Barrett and Arno 1991). The situation strongly suggests application of island biogeographic perspectives.

Several important theoretical models have defined constraints to gene flow among island populations. As a seminal framework, the infinite island model (Wright 1931) posited that (1) populations can be subdivided into subpopulations or islands and (2) gene flow between islands increases multiplicatively with population size and dispersal rate. The model of Wright (1931) was later refined in the model of isolation by distance (Wright 1943, Malécot 1955), which stipulated that the amount of gene flow between subpopulations is a function of the distance between those subpopulations or islands, and more recently with metapopulation models (e.g., Levins 1970, Gilpin 1991), that account for divergence among demes as a function of extinction and recolonization rates.

The theory of island biogeography (MacArthur and Wilson 1963, 1967) famously proposed that, given sufficient time, species diversity on islands will come to an equilibrium resulting from continual processes of immigration and extinction. Equilibria will occur at higher levels of species diversity when islands are large (due to lower extinction rates) and distances from the mainland are small (due to higher immigration rates). Although originally intended for oceanic islands, the theory of island biogeography has widely been applied to terrestrial montane "islands" (Brown 1971, Lomolino et al. 1989, Lomolino and Perault 2001). Genetic corollaries of the theory have also been tested in the context of terrestrial islands (Wheeler and Guries 1982, Floyd et al. 2005, Marlowe and Hufford 2008).

Jaenike (1973) suggested that the isolation-by-distance model and theory of island biogeography implied similar mechanisms for gene flow, and could be conflated to explain and/or predict genetic diversity of species' populations. This view has been substantiated by Frankham (1996, 1997) and Vellend (2003) whose meta-analyses found that small isolated islands had the lowest levels of genetic diversity, with underlying causes ascribed to increased inbreeding and genetic drift.

In our study, we consider the applicability of predictions from island biogeographic theory to insular populations of *A. americanum* from the western edge of the Central Rocky Mountains in the Greater Yellowstone Ecosystem, near the northern edge of the Great Basin (Fig. 1). In this region, *P. contorta* var. *latifolia* host distributions are limited to a series of small, relatively isolated montane landscapes with potential metapopulation connections to each other and to extensive "mainland" populations to the north and northeast.

Hypotheses

We have three hypotheses:

1. Given limited capacities for pollen and seed dispersal, we predict that high levels of inbreeding will occur within *A. americanum* populations at small spatial scales and that patterns of genetic differentiation for *A. americanum* will be comparable to documented dispersal-limited species.
2. Based on the general predictions of island biogeographic theory and genetic extensions, we predict that large contiguous populations of *A. americanum* closest to a figurative "mainland" will have the highest levels of genetic diversity.
3. We consider two sub-hypotheses concerned with the geographical source of island populations of *A. americanum* at the northern edge of the Great Basin. We predict (1) that eastern island populations are the result of colonization from the Island Park (IP)/Yellowstone "mainland" area to the northeast and (2) that western island populations are the result of colonization from the Central Idaho "mainland" area to the north.

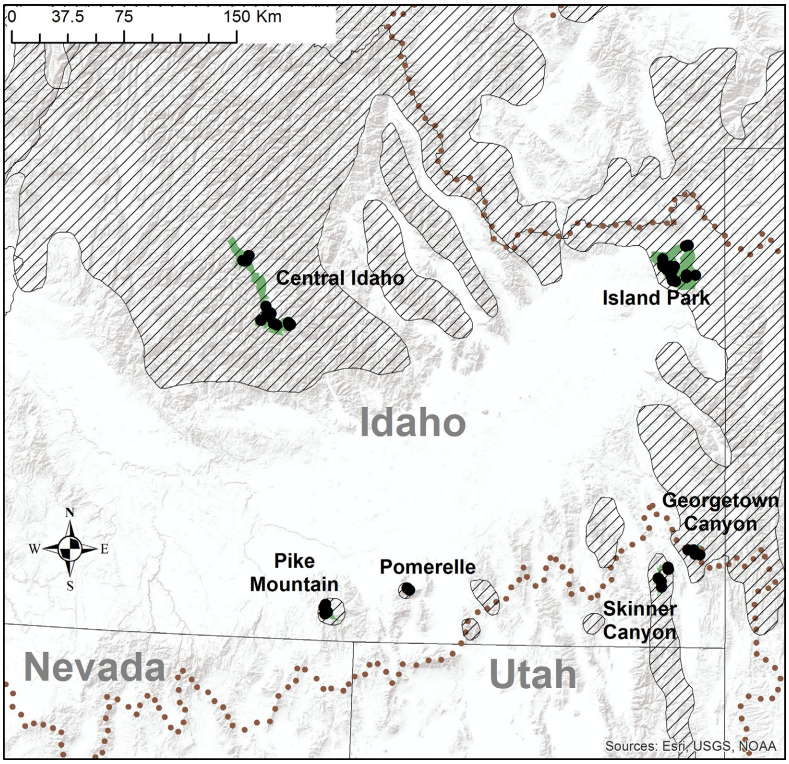


Fig. 1. Map of southeast and Central Idaho. Hatched areas denote coarse hulls (boundaries connecting outermost points) of contiguous *Pinus contorta* var. *latifolia* cover (U.S. Geological Survey 1999), green areas define bounds for inferential sampling, and black dots indicate individual sample locations. Dotted lines represent continental divides for the Great Basin (southeastern corner of map) and the Atlantic/Pacific oceans (northeastern corner of map).

Materials and Methods

Study area

Stems from 241 *Arceuthobium americanum* individuals were collected from Rocky Mountain lodgepole pines (*Pinus contorta* Dougl. var. *latifolia* Engelm.) during the summer of 2013 in the Targhee, Caribou, and Sawtooth National Forests within montane regions constituting the western edge of the Central Rocky Mountains. The locations of sampling (Fig. 1) corresponded to two large "mainland" areas: Island Park (IP), Central Idaho (CI), and four outlying "island" areas of varying size and degree of isolation. These were designated Skinner Canyon (SC), Georgetown Canyon (GTC), Pomerelle (PO), and Pike Mountain (PM). Sample numbers per site as well as physical characteristics are given in Table 1. Forest stands at the CI, IP, and PM sites are predominately *P. contorta* var. *latifolia*, whereas PO, SC, and GTC stands are largely mixtures of *P. contorta* var. *latifolia*, Rocky Mountain Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *glauca* (Beissn.) Franco), and subalpine fir (*Abies lasiocarpa* (Hooker) Nuttall). Winds are primarily westerly for all sites.

The sites have undergone varying degrees of natural and anthropogenic disturbance, with potential consequences to both gene flow and genetic structure. The CI mainland is positioned between the Sawtooth Mountain range and the White Clouds Wilderness. While permanent human settlements in the area are sparse, CI contains many recreational areas and campgrounds which have been sources for large forest fires. The shape of the CI mainland is highly complex, resulting in a much longer perimeter-to-area ratio than the IP mainland, which has a larger

Table 1. Names of collection sites for *Arceuthobium americanum* with population designation and other pertinent information.

Sites	Designation	Sample no.	Mean Lat.	Mean Long.	Mean elevation (m)	Mean annual precip. (mm)	Mean min. January temp (°C)	Mean max August temp (°C)	*Pinus contorta* areal cover (km^2)
CI	Mainland	75	44.00	−114.86	2165	29.4	−18.3	25.8	1009
PM	Island	25	42.19	−114.27	2102	23.4	−7.1	29.4	77
PO	Island	15	42.32	−113.61	2407	23.4	−7.1	29.4	8
IP	Mainland	83	44.28	−111.48	1958	60.2	−16.1	25.8	1170
GTC	Island	18	42.57	−111.30	2221	36.9	−13.3	28.6	151
SC	Island	25	42.42	−111.55	2275	36.9	−13.3	28.6	112

Notes: CI, Central Idaho; PM, Pike Mountain; PO, Pomerelle; IP, Island Park; GTC, Georgetown Canyon; SC, Skinner Canyon. Climate data were obtained from the National Climatic Data Center, which is a Department of the National Oceanic and Atmospheric Administration (NOAA, Asheville, North Carolina, USA). Gross estimates of *P. contorta* var. *latifolia* cover were based on topographical and satellite maps for the study area.

spatial area (Table 1). The IP mainland constitutes the western border of Yellowstone National Park and thus has high levels of associated tourism and recreational use. Mountain pine beetles (*Dendroctonus ponderosae*), which are associated with increased prevalence of *A. americanum* in *P. contorta var. latifolia* populations (Hawksworth and Wiens 1996), heavily infected the IP site between 1937 and 1947 and again in 1967 (Roe and Amman 1970). No appreciable pine beetle infestations have occurred at other sites. The southwestern PM and PO islands both contain ski resorts and have undergone prescribed *P. contorta* var. *latifolia* tree removal and control of *A. americanum* infection (K. Fuelling USFS, *personal communication*, 2016). The PM site has had a number of recent fires which may have affected both *P. contorta* var. *latifolia* population size and habitat quality (N. Poppino, *unpublished data*, K. Klass, *unpublished data*). The southeastern islands SC and GTC both harbor campgrounds, but experience anthropogenic disturbance to a lesser degree than other sites.

Field methods

Arceuthobium americanum tissue samples were acquired from sites using a tessellated stratified random process (Stevens and Olsen 2004). Specifically, for each island and mainland population, expanses of *P. contorta* var. *latifolia* forest were designated and grids with one square mile area cells were overlaid on digital maps using ArcGIS software (ESRI 2011). Cells were randomly selected from overlays, with the number of selected cells being proportional to estimated island/mainland area. Cell numbers were: IP = 17, CI = 15, SC = 5, GTC = 4, PO = 3, and PM = 5. From within each selected cell, five *P. contorta* hosts were then randomly chosen using mapping software for the extraction of *A. americanum* tissue. From each host, one male and one female individual were randomly selected, and one stem from those individuals was collected as a tissue sample. Male or female mistletoe representation of each host was subsequently determined using a randomization routine that maintained a one-to-one ratio of males to females at each island/mainland (see Appendix S1).

Molecular methods

We examined genetic diversity in *A. americanum* using seven microsatellite loci explicitly developed by the project, for the study organism (GenBank accession numbers: Pr032816302, Pr032816303, Pr032816304, Pr032816305, Pr032816306, Pr032816307, and Pr032816308; see Appendix S1). DNA extraction from *A. americanum* tissue was performed using the PowerPlant Pro DNA Isolation Kit (Mo Bio, Carlsbad, California, USA). Primers labeled with the DS-33 dye set were obtained from Applied Biosystems (Foster City, California, USA). Polymerase Chain Reaction (PCR) amplification was performed using HotStarTaq DNA Polymerase (Qiagen, Valencia, California, USA). Ten microliter reaction volumes were used with the following final concentrations: 1× of 10× PCR buffer, 200 μmol/L dNTP mix (Phenix, Candler, North Carolina, USA), 0.16 μmol/L forward primer, 0.16 μmol/L reverse primer, 0.5 units of HotStarTaq, and 2–10 ng genomic DNA. The thermal cycling protocol was as follows: one cycle at 95°C for 15 min, 30 cycles at 94°C for 30 s, 56°C for

45 s, 72°C for 45 s, another eight cycles at 95°C for 30 s, 53°C for 45 s, 72°C for 45 s, with a final extension of one cycle at 72°C for 30 min. Fragment analysis was performed in Molecular Research Core Facility at Idaho State University (Pocatello, Idaho, USA) using a 3130XL Genetic Analyzer (Applied Biosystems, Carlsbad, California, USA). GeneMapper software 4.0 (Applied Biosystems) was used to score the peaks, and Flexibin v2 (Amos et al. 2007) was used to bin the data. Primer sequences, GenBank accession numbers, primer characteristics, and Hardy-Weinberg equilibrium tests are detailed in Appendix S1.

Preliminary analyses suggested that three of our markers contained null alleles (Appendix S1). To address this issue, we used two different datasets for our analyses: (1) the full dataset with null alleles included (denoted NAI) and (2) a trimmed dataset with four loci (denoted 4L) resulting from elimination of the three markers with evidence of null alleles. *F*-statistics corrected for null alleles using the NAI dataset are given the abbreviation CNA (cf. Roques et al. 1999, Chapuis and Estoup 2007).

Statistical analysis

Euclidean spatial distance of sample locations was calculated, and spatial autocorrelation analyses were performed using the software package GenAlEx 6.502 (Peakall and Smouse 2006, 2012, Smouse et al. 2008). Functions in diveRsity 1.9.89 (Keenan et al. 2013), a package in the R 3.1.3 statistical environment (R Core Team 2015), were used to calculate Weir and Cockerham's (1984) F_{ST}, and Wright's (1951) F_{IS} for both the NAI and 4L datasets, and to calculate Wright's (1951) subpopulation F_{IS}, and average allelic richness (AR) per subpopulation for the 4L dataset. The package was also used to obtain bias-corrected and accelerated (Efron 1987) bootstrap (10,000 iterations) confidence intervals for all calculated fixation indices. FREENA software (Chapuis and Estoup 2007) was used to mathematically correct for the presence of null alleles in the NAI dataset, to provide corresponding CNA estimates for Weir's (1996) F_{ST} and pairwise F_{ST} statistics, and to calculate CNA bootstrap (10,000 iterations) confidence intervals for these indices using the excluding null alleles method (Chapuis and Estoup 2007). INEST software (Chybicki and Burczyk 2009) was used to provide CNA estimates and significance tests for Wright's (1951) F_{IS}. To facilitate inferences concerning F_{IS}, two distinct Bayesian frameworks were defined for the CNA data using default INEST beta distribution priors for inbreeding coefficients. In the first, F_{IS} was designated to be >0, while in the second F_{IS} was fixed at 0. The parsimony of these frameworks was considered using the Deviance Information Criterion (DIC, Spiegelhalter et al. 2002, Aho et al. 2017).

To test our predictions involving island biogeographic theory, we relied on both conventional ordinary least-squares (OLS) and ranged major axis (RMA; Snedecor and Cochran 1967, Legendre and Legendre 1998) regression models. Ranged major axis regressions addressed the lack of researcher control over predictor variables (island size and mainland distance), and potential random errors in measurements. Ranged major axis models were obtained using the R package lmodel2 (Legendre 2014). Ranged major axis *P* values were the result of 10,000 data permutations.

To identify genetic relationships among islands and mainlands, hierarchical agglomerative cluster analyses of CNA pairwise F_{ST} values were performed using unweighted pair group method with arithmetic mean linkage (UPGMA; Sokal and Michener 1958). Dendrograms were generated using the R package cluster (Maechler et al. 2015). Pruning solutions of dendrogram hierarchies were assessed using average silhouette width (Kaufman and Rousseeuw 1990, Aho et al. 2008). To complement UPGMA classifications, Bayesian cluster analyses were performed with 50,000 iterations, following a 5000 iteration burn-in, using TESS 2.3 (Chen et al. 2007). The maximum number of Bayesian clusters (K_{MAX}) was verified using GENELAND 4.0.5 (Guillot et al. 2005).

BOTTLENECK 1.2.02 software (Cornuet and Luikart 1996) was used with the NAI and 4L datasets to identify the presence of potential bottlenecks in island populations. The bottleneck analysis was performed with 1000 iterations, using the two-phase model (TPM), recommended for microsatellite data (Cornuet and Luikart 1996, Peery et al. 2012) with associated sign tests and mode-shift measures. The variance for TPM was set at 0.36 and proportion of the stepwise mutation model (SMM) in TPM was set at 0% as recommended by Cornuet and Luikart (1996) and then varied, as recommended by Peery et al. (2012), around those settings (variance for

TPM ± 0.15, proportion of SMM in TPM up to 40%) to examine stability of results.

Results

Genetic diversity and gene flow

Spatial autocorrelation.—The genetic makeup of *Arceuthobium americanum* individuals was strongly spatially dependent for distances up to 50 m. The spatial autocorrelation testing procedure used (see *Materials and methods*) compares bootstrapped confidence intervals for the true allelic correlation, within particular distance classes, under an assumption of no spatial structure (dashed lines in Fig. 2) to bootstrapped confidence intervals for true allelic correlations based on data (error bars in Fig. 2). At distances greater than or equal to 50 m, all bootstrapped (9999 iterations) 95% confidence intervals for true genetic correlation overlapped with the 95% confidence envelope under H_0. Conversely, at distances less than or equal to 25 m, the lower confidence limit for the true genetic correlation was greater than the envelope under H_0.

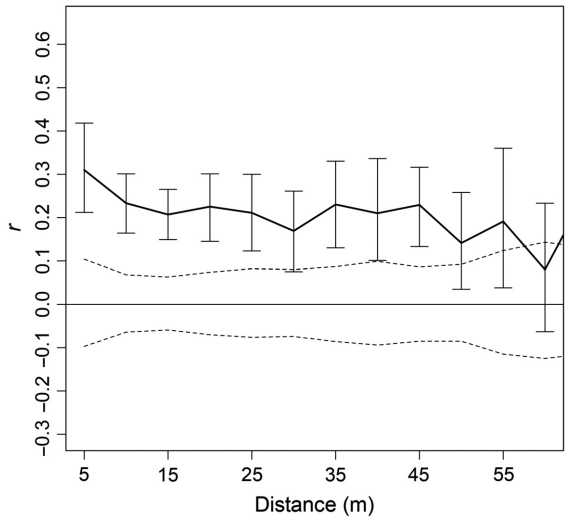


Fig. 2. Autocorrelation diagram for *Arceuthobium americanum* given 5-m distance classes. The thick solid line is the observed average element by element correlation of the allelic distance matrices of mistletoe individuals. The error bars are bootstrapped 95% confidence intervals for the true correlation. The dashed lines are the upper and lower 95% confidence envelopes under a null hypothesis of no correlation (true $r = 0$).

Intrapopulation gene flow.—Dataset-level F_{IS} scores were relatively high, indicating either pronounced levels of inbreeding or the occurrence of the Wahlund effect (Wahlund 1928) within our populations. Values for the entire NAI and 4L were as follows: F_{IS} estimate (95% bootstrap confidence interval): 0.516 (0.459, 0.558) and 0.396 (0.349, 0.442), respectively (Table 2). When using the CNA data, Bayesian comparison of $F_{IS} > 0$ and $F_{IS} = 0$ frameworks using the DIC demonstrated much greater parsimony for the $F_{IS} > 0$ perspective (ΔDIC = 51). In related analyses, the Bayesian posterior median F_{IS} was 0.104 (average $F_{IS} = 0.107$) with the 95% credible interval (0.057, 0.167; Table 2).

Bootstrapped (across all loci) confidence intervals were also calculated for the F_{IS} values of each island and mainland location using 4L dataset (Table 3). F_{IS} values for island and mainland locations could not be calculated using the CNA dataset due to programming limitations in INEST, the software package used to correct for null alleles (see *Materials and methods*). All sites except PO had F_{IS} values significantly greater than zero (Table 3). The highest result was detected on the CI mainland; F_{IS} estimate (95% bootstrap confidence interval) = 0.481 (0.416, 0.537), followed by IP = 0.450 (0.346, 0.536), PM = 0.399 (0.129, 0.562), SC = 0.335 (0.156, 0.467), GTC = 0.305 (0.148, 0.384), and PO = 0.122 (−0.096, 0.239).

Interpopulation gene flow.—Dataset-level F_{ST} (fixation index) scores, while significantly different from zero, suggested a relatively low probability of identity by descent and corresponding high levels of gene flow among populations. Values for the entire NAI, 4L, and CNA datasets were as follows: F_{ST} estimate (95% bootstrap confidence interval): 0.035 (0.020, 0.054), 0.045 (0.029, 0.063), and 0.044 (0.010, 0.112). Twelve of 15 CNA pairwise F_{ST} values were significantly different from zero (Table 4). Nonsignificant pairwise F_{ST} values were those for CI and PO, CI and GTC, and PO and SC (Table 4).

Biogeography

Allelic richness.—Classic biogeographic theory uses richness of regional species pools (gamma-diversity) to quantify the effect of island size and

Table 2. Overall F_{ST} or G_{ST} and F_{IS} values for *Arceuthobium americanum* in this study (denoted "This study") and other studies, and comparisons with other plant species and groups in the literature.

Species	F_{IS}	F_{ST} or G_{ST}	Reference
A. americanum—NAI	0.516	0.035	This study
A. americanum—CNA	0.107	0.044	This study
A. americanum—4L	0.396	0.045	This study
A. americanum	N/A	0.286†	Jerome and Ford (2002)
A. americanum—USA, *on Pinus contorta* v. *latifolia*	N/A	0.142†	Jerome and Ford (2002)
P. contorta	0.060	0.007	Yeh et al. (1985)
Reforested P. contorta	0.360	0.028	Thomas et al. (1999)
Mimulus platycalyx	0.543	N/A	Dole and Ritland (1993)
Mimulus guttatus	0.168	N/A	Dole and Ritland (1993)
Phlox drummondii	0.352	N/A	Watkins and Levin (1990)
Antirrhinum cirrhigerum	0.068	0.132	Mateau-Andre and De Paco (2005)
Antirrhinum latifolium	0.034	0.093	Mateau-Andre and De Paco (2005)
Antirrhinum linkianum	0.017	0.176	Mateau-Andre and De Paco (2005)
Antirrhinum litigiosum	0.250	0.100	Mateau-Andre and De Paco (2005)
Antirrhinum maius	0.030	0.148	Mateau-Andre and De Paco (2005)
Antirrhinum silicum	0.628	0.270	Mateau-Andre and De Paco (2005)
Antirrhinum tortuosum	0.235	0.061	Mateau-Andre and De Paco (2005)
Non-colonizing conifers	N/A	0.002	Govindaraju (1989*a*)
Colonizing conifers	N/A	0.002	Govindaraju (1989*a*)
Ingested dispersed seed plants	N/A	0.051†	Hamrick et al. (1992)
Sexual and asexual reproductive plants	N/A	0.051†	Hamrick et al. (1992)
Animal-attached dispersed seed plants	N/A	0.065†	Hamrick et al. (1992)
Wind-dispersed seed plants	N/A	0.076†	Hamrick et al. (1992)
Wind-pollinated plants	N/A	0.084†	Govindaraju (1988*b*)
Sexual reproductive plants	N/A	0.086†	Hamrick et al. (1992)
Outcrossing plants	N/A	0.091†	Govindaraju (1988*a*)
Explosive-dispersed seed plants	N/A	0.092†	Hamrick et al. (1992)
Gravity-dispersed seed plants	N/A	0.131†	Hamrick et al. (1992)
Animal-pollinated plants	N/A	0.178†	Govindaraju (1988*b*)
Mixed mating plants	N/A	0.216†	Govindaraju (1988*a*)
Primarily outcrossing plants	N/A	0.277†	Govindaraju (1989*b*)
Self-pollinating plants	N/A	0.446†	Govindaraju (1988*a*, *b*, 1989*b*)

† G_{ST} value.

Table 3. F_{IS}, allelic richness, and evidence for the presence (+) or absence (−) of strong recent bottlenecks of *Arceuthobium americanum* populations using the 4L dataset.

Sites	F_{IS}	Allelic richness, mean (95% CI)	Evidence of Bottleneck
CI	0.481†	6.86 (5.50, 8.25)	−
PM	0.399†	5.05 (4.00, 5.75)	+
PO	0.122	5.10 (4.25, 5.75)	±
IP	0.450†	5.86 (4.50, 7.25)	−
GTC	0.305†	6.33 (5.00, 7.50)	−
SC	0.335†	5.37 (4.25, 6.50)	−

Notes: See Table 1 for explanations of abbreviations of sites. ± Indicates a positive bottleneck result for NAI dataset, but a negative result for 4L dataset.

† Significantly ($\alpha = 0.05$) different from 0 at $\alpha = 0.05$.

isolation on *species* diversity (MacArthur and Wilson 1967). Similarly, insular genetic models, which are motivated by the need to quantify the effect of population size and insularity on gene flow and genetic drift, often use AR to measure *genetic* diversity (Cornuet and Luikart 1996, Petit et al. 1998, Castric and Bernatchez 2003, Greenbaum et al. 2014).

To address the confounding effect of null alleles on AR, we used the 4L dataset to estimate the AR of island and mainland areas (Table 3). Under this approach, CI had the highest AR per *A. americanum* individual: estimate (95% bootstrap CI) = 6.86 (5.50, 8.25), followed by GTC = 6.33 (5.00, 7.50), IP = 5.86 (4.50, 7.25),

Table 4. Spatial distance (in km) (gray lower triangle) and corrected pairwise F_{ST} (upper triangle) excluding null alleles method (Chapuis and Estoup 2007) Weir (1996) matrix for the sampled populations.

Sites	CI	PM	PO	IP	GTC	SC
CI		0.0603†	0.0587	0.0204†	0.0050	0.0506†
PM	207.6		0.1968†	0.1220†	0.0752†	0.2015†
PO	212.9	56.8		0.0227†	0.0494†	0.0201
IP	271.5	324.7	277.7		0.0155†	0.0344†
GTC	329.2	248.2	191.6	190.3		0.0489†
SC	320.7	225.9	169.8	206.2	26.1	

Note: See Table 1 for explanations of abbreviations of sites.
† F_{ST} significantly ($\alpha = 0.05$) different from 0 based on bootstrapped 95% confidence intervals (10,000 bootstraps).

SC = 5.37 (4.25, 6.50), PO = 5.10 (4.25, 5.75), and PM = 5.05 (4.00, 5.75).

Pinus contorta var. *latifolia* contiguous cover (i.e., island size) and AR were significantly positively associated at $\alpha = 0.05$ from the perspective of both RMA regression (Fig. 3, $r^2 = 0.54$, $n = 6$, permutational upper-tailed P value = 0.050) and OLS regression ($r^2 = 0.54$, $t_4 = 2.15$, upper-tailed $P = 0.049$). Additionally, island AR was significantly negatively associated with distance from nearest mainland $\alpha = 0.05$ when applying RMA regression (Fig. 4, $r^2 = 0.70$, $n = 4$, permutational lower-tailed P value = 0.043), although this signal was weaker when using OLS regression ($r^2 = 0.70$, $t_2 = -2.77$, lower-tailed P value = 0.080). In accordance with previous work (MacArthur and Wilson 1963, Brown 1971, Kodric-Brown and Brown 1993), our regression models acknowledged a log-linear relationship between island insularity and AR and a power association (linear after log transformation of x and y) between island area and AR (Figs. 4, 5).

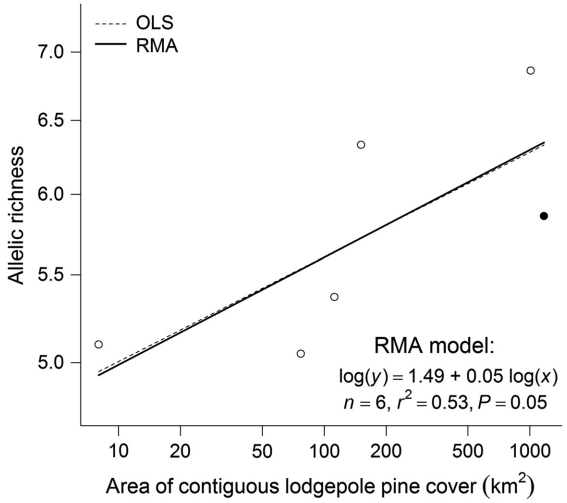


Fig. 3. Bootstrapped allelic richness estimates obtained from the 4L dataset (see *Materials and Methods*) as a function of gross estimates of contiguous lodgepole pine cover (km^2). The solid line depicts the reduced major axis (RMA) regression ($r^2 = 0.535$, upper-tailed permutational P value = 0.050), whereas the dashed line depicts the ordinary least-squares (OLS) regression fit ($r^2 = 0.535$, upper-tailed $P = 0.049$). The point representing the Island Park mainland is colored black.

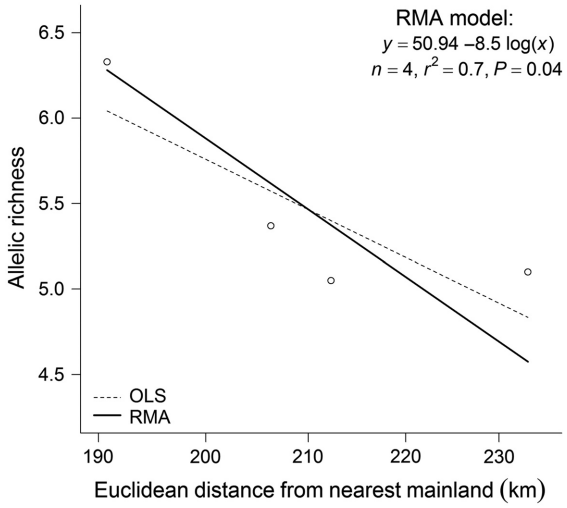


Fig. 4. Bootstrapped allelic richness estimates obtained from the 4L dataset (see *Materials and Methods*) as a function of Euclidean distance from nearest mainland (km). The solid line depicts the reduced major axis (RMA) regression ($r^2 = 0.70$, permutational lower-tailed P value = 0.043), whereas the dashed line depicts the ordinary least-squares (OLS) regression fit ($r^2 = 0.70$, lower-tailed $P = 0.080$).

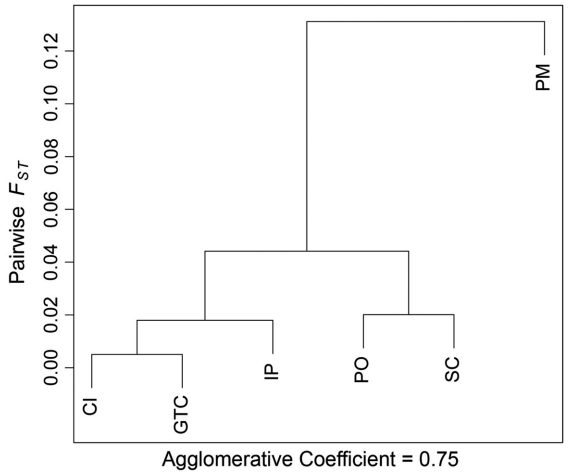


Fig. 5. UPGMA cluster diagram for *Arceuthobium americanum*. Optimal pruning resulted in two clusters. Pairwise F_{ST}'s were calculated using Weir (1996) pairwise F_{ST} with the excluding null alleles correction procedure (Chapuis and Estoup 2007). CI, Central Idaho; PM, Pike Mountain; PO, Pomerelle; IP, Island Park; GTC, Georgetown Canyon; SC, Skinner Canyon.

Cluster analyses

An UPGMA dendrogram (agglomerative coefficient = 0.75; Fig. 5) was constructed to depict the hierarchical genetic relationships of island and mainland locations based on CNA pairwise F_{ST} values. Subsequent average silhouette width analysis suggested that two-cluster solution (average silhouette width = 0.579) provided a reasonable representation of genetic structure (Kaufman and Rousseeuw 1990), and optimally represented our populations. The first included CI, GTC, IP, PO, and SC, whereas the second cluster contained only PM.

In accordance with UPGMA results, evidence of only two clusters was found (K_{MAX} = 2) in the Bayesian island classification. All *A. americanum* individuals at PO were assigned membership to cluster one, the larger of the two clusters. Other important contributors to cluster one were SC (98% of individuals) and IP (89% of individuals). Lesser contributors were GTC (62%) and CI (59%) (Table 5). Cluster two had strong contribution from PM (84%) with very little input (<50%) from all other sites (Table 5). Notably, there was no difference in optimal cluster number and contributions of island to the clusters when using the CNA and 4L datasets.

Table 5. Bayesian probabilistic assignment of *Arceuthobium americanum* island and mainland sites into two clusters.

	Clusters	
Sites	1	2
CI	0.5914	0.4086
PM	0.1566	0.8434
PO	1.0000	0.0000
IP	0.8941	0.1059
GTC	0.6192	0.3808
SC	0.9820	0.0180

Note: See Table 1 for explanations of abbreviations of sites.

Bottlenecks

Patterns of allelic diversity and heterozygosity suggested bottlenecks (i.e., a pronounced reduction in individuals, or a recent colonization) in some populations (Table 3). In particular, when using the NAI dataset, PM and PO displayed mode shifts (cf. Cornuet and Luikart 1996), indicating a possible recent bottleneck. From the perspective of the 4L dataset, however, only PM had a shifted mode. Corresponding sign-rank tests were used to test the null hypothesis of no bottlenecks. These analyses were not statistically significant for any population, prompting caution in the interpretation of mode-shift results.

Discussion

This study quantified diversity at microsatellite loci to examine the population genetic structure of lodgepole pine dwarf mistletoe on two montane "mainlands" and four montane "islands" across a large region (139,648 km^2) in the West-Central Rocky Mountain. These data were used to assess the possible limits to pollen and seed dispersal in *A. americanum*, test the applicability of classic biogeographic theories, and estimate the potential origins of island populations within the study area.

Gene flow and F*-statistics*

Intrapopulation gene flow.—Our study supports previous work (Gregor et al. 1974, Penfield et al. 1976, Coppola 1989, Hawksworth and Wiens 1996) suggesting that *A. americanum* is relatively dispersal-limited with high levels of inbreeding (F_{IS}) within subpopulations (Wright 1965) and

low levels of allelic fixation (high pairwise F_{ST}) between some subpopulations. A spatial correlogram (Fig. 2) demonstrates the presence of gene flow between individuals in subpopulations for distances of only 30 m or less, with highest levels at distances around 5 m. Notably, these constraints coincide with *A. americanum* average reported distances for explosive seed dispersal (2–4 m; Hawksworth and Wiens 1996). This information is likely to help forest managers assess both the vulnerability of host trees to dwarf mistletoe infection, and the genetic isolation of *A. americanum* individuals and populations.

For two of our three general analytical perspectives, the overall F_{IS} was high for a mandatory outcrossing species (CNA F_{IS} = 0.107, 4L F_{IS} = 0.396, NAI F_{IS} = 0.516, Table 3). In comparison, lodgepole pine, the *A. americanum* host, is typically outcrossing with much lower values of F_{IS} in most populations (F_{IS} = 0.06, Table 2; Yeh et al. 1985). As a contrasting reference, *Antirrhinum siculum* Miller (Plantaginaceae) and *Mimulus platycalyx* Pennell (Scrophulariaceae) are self-compatible species with concomitant high inbreeding coefficient values, F_{IS} = 0.628 and 0.543, respectively (Table 2; Dole and Ritland 1993, Mateau-Andre and De Paco 2005). The inbreeding coefficient of *A. americanum* appears similar to that of *Phlox drummondii* Hook (Polemoniaceae; F_{IS} = 0.352, Table 2), an outcrossing species with infrequent selfing, but elevated levels of inbreeding in high-density wild populations (Watkins and Levin 1990). Small insects, which are less vagile, increase inbreeding in insect-pollinated plants (Loveless and Hamrick 1984), thus increasing F_{IS} values. Given that entomophily is favored over anemophily in *A. americanum* during most of the growing season (Munro et al. 2014), our data support a hypothesis of pollination reliance on movement-limited pollinators proposed by Penfield et al. (1976).

Interpopulation gene flow.—Our measure of overall genetic differentiation among *A. americanum* subpopulations (F_{ST}) is much lower (CNA F_{ST} = 0.044, 4L F_{ST} = 0.045, NAI F_{ST} = 0.035, Table 2) than the value reported by Jerome and Ford (2002; G_{ST} = 0.286, Table 2). This may be due in part to our smaller sampled geographical area (220 km latitudinal by 280 km longitudinal area) compared to Jerome and Ford (2002; 2800 km latitudinal by 2400 km longitudinal area). Our pairwise F_{ST} values were mostly less than 0.2, in accordance with Jerome and Ford's (2002) F_{ST} values for data subsets with similar geographic areas and distances. Further, while Jerome and Ford's (2002) total population G_{ST} value is likely reliant on a spatial area too large for comparison, the authors provide a G_{ST} value for *A. americanum* limited in distribution to the United States on *P. contorta* var. *latifolia* hosts. This USA G_{ST} value (G_{ST} = 0.142) lines up between the outcrossing plants (G_{ST} = 0.091) with explosive seed dispersal (G_{ST} = 0.092) and animal-pollinated plants (G_{ST} = 0.178), as might be expected.

We assumed that fixation index values for *A. americanum* would be similar to those reported for other plant species, measured at similar spatial scales, with similar life-history traits—those with mandatory outcrossing, explosively dispersed seeds, and animal pollination (Table 2). Our measured F_{ST} value (CNA F_{ST} = 0.044), however, is much smaller than all of these. This outcome may be due in part to differences in spatial scale compared to other studies, but also to previously observed long-distance transportation of viscous *A. americanum* seeds by mammals and birds (Hudler et al. 1979, Nicholls and Hawksworth 1983, Nicholls et al. 1984*a*, *b*, Nicholls 2014). Notably, Hawksworth et al. (1987) found that during one seed dispersal peak in North-Central Colorado, 22% of individuals among 10 bird species and 20% of individuals within four trapped mammal species carried *A. americanum* seeds. Dispersal via migratory birds—whose fall migratory times coincide with *A. americanum* seed release—may allow the establishment of remote new populations, and/or facilitate gene flow over long distances. Plants with animal-attached seed dispersal (F_{ST} = 0.065) and particularly plants with animal-internal seed dispersal (F_{ST} = 0.051) have similar mean fixation index values to those measured for our populations of *A. americanum* (Table 2).

We acknowledge that the effects of pollen and seed dispersal cannot be distinguished in our work. Previous studies, however, have shown that seed dispersal generally affects genetic differentiation and gene flow more than pollen dispersal for the obvious reason that seeds carry twice as much genetic information as pollen

(Loveless and Hamrick 1984, Williams and Guries 1994). Given this factor, the frequent occurrence of animal–seed attachment, and the F_{ST} properties noted above, we suggest that long-distance animal dispersal of seeds has strongly affected genetic properties of *A. americanum* populations in the study region.

Insular biogeography

In agreement with our second hypothesis, and previous considerations of genetic diversity and island size (e.g., Frankham 1996), we found a positive correlation between island size and AR (Fig. 3) and a negative correlation between distance from mainland and AR (Fig. 4). Thus, our results support the genetic predictions of the isolation-by-distance model (Wright 1943) and the more general predictions of the theory of island biogeography (MacArthur and Wilson 1963, 1967). While third highest among the populations under consideration, the AR (5.86) for IP was lower than might be expected for a mainland area (Fig. 3). The IP region underwent a massive U.S. Forest Service-directed salvage of lodgepole pine killed by mountain pine beetles (*Dendroctonus ponderosae*) in the 1970s. This operation, effects of which are visually striking more than 40 years later, likely decreased both host and parasite genetic diversity.

Our work can be criticized on the grounds that only a small number of major lodgepole/mistletoe islands exist for biogeographic inference in the West-Central Rockies, at the northern edge of the Great Basin (Fig. 1). We respond by noting that (1) biogeographic relationships were statistically significant in our work (the power in our study was adequate at $\alpha = 0.05$), (2) sample sizes are generally constrained for large spatial-scale ecological comparisons (Ellison and Gotelli 2004:150–151), and (3) important findings in biogeography have typically been (and often must be) made given small sample sizes (Simberloff 1976, Hortal 2012, Triantis et al. 2012).

While similar to OLS, our RMA regression models demonstrated greater power (smaller *P* values), for detecting biogeographic trends (Figs. 4, 5), and we emphasize the latter over the former here. Although applied only sporadically (e.g., Price and Phillimore 2007), model II regression methods, including RMA, are clearly a more appropriate choice in most biogeographic studies because predictors cannot generally be controlled by researchers (but see Simberloff 1976) and are thus subject to sampling error (Legendre and Legendre 2012, Aho 2014).

Relationship of Arceuthobium americanum *islands*

Our UPGMA and Bayesian cluster analyses were in agreement regarding genetic relationships and the ontogeny of *A. americanum* islands. Both analyses found strongest evidence for two clusters: one including CI, GTC, IP, PO, and SC, and the other consisting of PM.

These results do not align perfectly with our predictions. In particular, our Bayesian analyses indicated that the SC population is more genetically similar to the IP mainland than CI and that PM is more genetically similar to CI than IP. Georgetown Canyon, however, appears to be a genetic mixture of both mainland populations. Though, *A. americanum* distributional maps (Hawksworth and Wiens 1996) indicate that GTC has the potential for additional island and mainland populations (e.g., Colorado populations) in closer proximity to GTC than other islands in this study. Skinner Canyon is spatially proximal to GTC (32 km, Fig. 1), but it may have greater genetic connectivity with the Utah populations of *A. americanum*. This could explain the observed genetic differentiation between SC and GTC. Also contrary to our predictions, the PO population was more genetically similar to the IP mainland than CI. The PO population, however, showed evidence of possible bottlenecking in the NAI dataset and had low AR (Table 3). Luikart et al. (1998) demonstrated that population bottlenecks may cause a reduction or loss of rare alleles, decreasing genetic diversity and confounding phylogenetic inferences.

Pike Mountain showed evidence of a bottleneck in both NAI and 4L datasets and had low AR (Table 3). This suggests that PM and perhaps PO (spatially adjacent sites) were recently colonized and/or have had recent dramatic reductions in population size. We note that these locations have a history of recent human-driven population reductions. Both PM and PO are heavily used for recreational activities (e.g., ski resorts, camping, off road vehicles) and have

undergone prescribed *P. contorta* var. *latifolia* tree removal and active control of *A. americanum* infection to maintain forest health (K. Fuelling USFS, *personal communication*, 2016). Pike Mountain has also had a history of recent fires which likely reduced both *A. americanum* population size and the extent of host habitat (L. Poppert, *unpublished data*, K. Klass, *unpublished data*).

Conclusions

We found genetic evidence that *Arceuthobium americanum* is a dispersal-limited species with intrapopulation gene flow essentially ending at 10–30 m, and inbreeding coefficient values similar to high-density/dispersal-limited species. Relatively low fixation index values, however, indicate appreciable gene flow among subpopulations in our study, likely facilitated by dispersal of animal-attached seeds. Our work indicates that *A. americanum* on contiguous *P. contorta* var. *latifolia* islands are affected by their insular biogeography, leading to predictably higher levels of genetic diversity on larger islands with close proximity to mainland source populations. Finally, our work provides a phylogenetic framework for *A. americanum* populations at the northern edge of the Great Basin. We hope our work will be helpful to foresters managing areas infected with dwarf mistletoe and to researchers considering dwarf mistletoes (particularly those in the Central Rockies) or other species with insular distributional characteristics.

Acknowledgments

We thank Dr. Rick Williams for his comments on early drafts of this manuscript and helpful comments on our submission from two anonymous Ecosphere reviewers. We also thank staff at the Molecular Core Research Facility at ISU for assistance including financial support, the College of Science and Engineering at ISU for partial support of microsatellite development costs, and supervisory personnel at the Caribou-Targhee and Sawtooth National Forests (DOI-USFS) for guidance and information.

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Supporting Information

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