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Population structure of a vector-borne plant parasite

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Abstract

Parasites are among the most diverse groups of life on Earth, yet complex natural histories often preclude studies of their speciation processes. The biology of parasitic plants facilitates in situ collection of data on both genetic structure and the mechanisms responsible for that structure. Here, we studied the role of mating, dispersal and establishment in host race formation of a parasitic plant. We investigated the population genetics of a vector-borne desert mistletoe (*Phoradendron californicum*) across two legume host tree species (*Senegalia greggii* and *Prosopis velutina*) in the Sonoran desert using microsatellites. Consistent with host race formation, we found strong host-associated genetic structure in sympatry, little genetic variation due to geographic site and weak isolation by distance. We hypothesize that genetic differentiation results from differences in the timing of mistletoe flowering by host species, as we found initial flowering date of individual mistletoes correlated with genetic ancestry. Hybrids with intermediate ancestry were detected genetically. Individuals likely resulting from recent, successful establishment events following dispersal between the host species were detected at frequencies similar to hybrids between host races. Therefore, barriers to gene flow between the host races may have been stronger at mating than at dispersal. We also found higher inbreeding and within-host individual relatedness values for mistletoes on the more rare and isolated host species (*S. greggii*). Our study spanned spatial scales to address how interactions with both vectors and hosts influence parasitic plant structure with implications for parasite virulence evolution and speciation.

Keywords: host race formation, host switching, host-parasite interactions, *Phoradendron*, population genetics, speciation

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Introduction

Parasitism is among the most successful life-history strategies (Poulin & Morand 2000). Evolutionary transitions to parasitism lead to increased diversification rates (Mitter *et al.* 1988; Morand *et al.* 2015; Wiens *et al.* 2015), but the processes by which parasites diversify remain under debate (Poulin & Morand 2000; Hoberg & Brooks 2015). Host-switching events are implicated in the diversification of a variety of parasites (Desdevises *et al.* 2002; Ricklefs *et al.* 2004; Harbison & Clayton 2011; De Vienne *et al.* 2013; Santiago-Alarcon *et al.* 2014; Choi & Thines 2015). Studies of parasite population genetic structure can inform how speciation via host-switching unfolds. Two main factors determining parasite population genetic structure within and among hosts include the following: (i) where potential mates are located (mating) and (ii) where parasite propagules are dispersed (dispersal) and successfully establish. Host race formation, the accumulation of genetic differences between parasite populations on different host species, may be an antecedent to speciation following

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colonization of new host species (Drès & Mallet 2002). When parasites infect different host species in allopatry, evolutionary divergence can lead to speciation. However, when gene flow between host races occurs in sympatry or during secondary contact, natural selection for host specialization and the evolution of barriers to gene flow are necessary for speciation (Le Gac & Giraud 2004; Giraud *et al.* 2008). At one extreme, mating, dispersal and establishment of the parasites can be random with respect to host species, leading to a panmictic population of a generalist parasite. At the other extreme, mating, dispersal and establishment can occur only within a host race, leading to speciation of each parasite lineage. Mating/ecology pleiotropy, when adaptation to a host is accompanied by mating within the host, may lead to the evolution of sympatric host races (Bolnick & Fitzpatrick 2007). As host races evolve, reproductive isolation through differences in the timing of mating can reduce hybridization (the mating of individuals from genetically divergent host races). Similarly, immigration (the dispersal of propagules of parasites from one host race to the other host species) can also be affected by phenology (the timing of life-history events). For example, host race formation in apple maggot flies (*Rhagoletis pomonella* species complex) is the result of temporal isolation that reduces between-host species mating and dispersal (Dambroski & Feder 2007). Finally, if fitness tradeoffs are strong, immigrants between host species should have lower fitness values than hybrids between host races. Thus, divergence will ultimately be determined by the frequency of hybridization and reproduction following immigration between host races.

Parasitic plants provide a convenient system for exploring the genetic structure of parasites within and between host species. Evidence both for and against the presence of host races has been demonstrated in a variety of parasitic plant systems (e.g. Mutikainen & Koskela 2002; Lara *et al.* 2009; Le Corre *et al.* 2014). Mistletoes comprise over 1300 aerial stem parasite species in the sandalwood order (Santalales) and vary widely in their host ranges (Norton & Carpenter 1998). Mistletoes include species that are forestry pests, critical animal food resources and important contributors to nutrient cycling (Watson 2001; Aukema 2003; March & Watson 2010). As sessile ectoparasites of plants, mistletoes allow for the collection of in situ phenotypic and genetic data across many hosts. Desert mistletoe (*Phoradendron californicum*) is a dioecious hemiparasite of leguminous tree species throughout the Sonoran and Mojave deserts. In southeastern Arizona, it primarily infects *Prosopis* spp., *Senegalia greggii*, *Parkinsonia* spp. and *Olneya tesota*. While desert mistletoe can infect all of these species in sympatry, relative abundances and infection prevalence are spatially variable (Aukema & Martinez del Rio 2002). Desert mistletoe is an excellent system for studying how interactions with vectors and hosts influence parasite population genetic structure (Aukema 2003). Extensive research has been performed on the foraging behaviour of and seed deposition by the phainopepla (*Phainopepla nitens*), the specialized, primary avian seed vector of desert mistletoe (Walsberg 1975, 1978; Aukema 2001; Martínez del Rio & Aukema 2002). While generalist bird species do consume desert mistletoe berries, the phainopepla is the primary disperser of desert mistletoe, effectively dispersing an order of magnitude more seeds per female plant than the next most common disperser (Larson 1991, 1996). Desert mistletoe seed deposition is spatially correlated at 70 m, about the scale of individual phainopepla territories (~4000 m^2; Walsberg 1978; Aukema 2001, 2004). Preferential perching and foraging behaviour of phainopeplas on tall, heavily infected trees causes aggregated seed rain (Aukema 2001, 2004). Phainopeplas are attracted to and defend trees rich in mistletoe berries, such that when mistletoes are experimentally removed from a tree, seed deposition is drastically reduced (Martínez del Rio & Aukema 2002). These behaviours lead us to hypothesize that dispersal of most mistletoe seeds will be limited to a subset of hosts within a given phainopepla's territory.

The existence of host races has been previously studied in desert mistletoe. In the Mojave and Colorado deserts, divergence was found in four of four allozymes and three of four morphological characters over two hosts that only occasionally grow in sympatry, catclaw acacia (*Senegalia greggii*) and honey mesquite (*Prosopis glandulosa*; Glazner *et al.* 1988). In Baja California Norte, reduced establishment rates were found for desert mistletoe seeds experimentally transplanted to a different host species (Overton 1997). Overton hypothesized that differences in flowering phenology of mistletoes across hosts allows for partial reproductive isolation of host races. While host species were drivers of population structure in these previously studied populations, geography may be a more important factor than host species for desert mistletoe across the species' entire range (Lira-Noriega *et al.* 2014). Therefore, host races are likely common, but may not be universal across the range in this species. Here, we revisit the question of host race formation in desert mistletoe across two of the most common hosts infected in sympatry in the Sonoran desert: velvet mesquite (*Prosopis velutina*), the predominant host at our sites, and catclaw acacia, a less prevalent host at our sites. We use microsatellite markers to characterize the population genetic structure across a variety of scales: within-host individuals, between-host individuals across geographic sites and distance and between-host species. Our study provides insight into the prevalence of desert mistletoe host races

across the range and the extent of hybridization and immigration between host races.

For desert mistletoe, a vector-transmitted parasite, interactions with vectors and hosts determine whether host races initially form and are then reinforced or eroded. Gene flow at mating is mediated by pollen dispersal by small, flying insects, primarily generalist Halictidae (Hymenoptera) and Syrphidae (Diptera) (K. M. Yule, unpublished data). Due to the short flight distances of these groups of insects, pollen dispersal most likely occurs between individual mistletoes growing in close proximity with overlapping flowering phenologies (Waddington 1979; Herrera 1987). Mistletoe fruits are then dispersed by phainopepla primarily within their territories and between hosts on which mistletoes overlap in fruiting phenology. Due to the local scale of these vector interactions, mistletoes on a rare host species may have few potential mates or appropriate establishment sites (local adaptation) outside of their own host individual. Thus, these mistletoes are predicted to be more inbred than those on common hosts. However, long-distance dispersal events may be common enough due to generalist dispersers or during phainopepla migration to obscure spatial structuring across different host individuals. In this study, we tested whether host races of desert mistletoe are present, whether reproductive phenology differs between host species and whether predictions generated from phenology are supported by population genetic data. If host races are present, we predict the following: (i) between-host race hybridization rates (pollen flow) will reflect the degree of overlap in flowering phenology across hosts, (ii) between-host immigration rates (seed dispersal) will reflect the degree of overlap in fruiting phenology across hosts, and (iii) mistletoes on the rare host will show greater within-host individual structure than those on the common host.

Materials and methods

Plant material

Living stem material was collected from desert mistletoe (*Phoradendron californicum*) individuals parasitizing living host trees across three sites near Tucson, AZ [Santa Rita Experimental Range (SRER), Tumamoc Hill (TH) and Catalina Regional Park (CRP)] and two host species [velvet mesquite (*Prosopis velutina*) and catclaw acacia (*Senegalia greggii*)]. Sites were chosen that contained locally interspersed, sympatric infected populations of both host species (see Figs S1–S4, Supporting information for maps). TH is 65 km north of SRER and 40 km south of CRP. Within SRER, no two samples were taken from hosts more than 10 km apart, while no two samples were taken from hosts more than 1 km apart within CRP and TH. At all sites, velvet mesquites have higher population density than catclaw acacia. Tissue was sampled from one mistletoe individual/host individual for 10–26 hosts/species/site (CRP: 22 mesquites, 24 acacias; TH: 15 mesquites, 10 acacias; SRER: 26 mesquites, 24 acacias). At SRER, an additional 40 mistletoe individuals over 40 mesquites were collected to increase our power to test for isolation by distance within a site on a single host species (for a total of 66 mistletoes on mesquite hosts at SRER and 161 mistletoes across species and sites). For the study of intrahost population genetic structure, 20 randomly chosen mistletoes were sampled from intrahost populations on two hosts of each of the two host species at SRER (a total of 80 additional mistletoes). Each host individual was distant (~5.3 km) from the other sampled conspecific host and near (66 or 77 m) to one sampled host of the other species. A distance of ~70 m between nearby sampled host trees was chosen as this corresponds to the scale of individual phainopepla territories. Mistletoe seed deposition is spatially autocorrelated at these short distances and at distances of >4000 m, represented by the distant (~5 km) intrahost populations in our study (Aukema 2004).

Reproductive phenology

The flowering and fruiting phenology curves of mistletoes on sympatric mesquite and acacia hosts were recorded at SRER. Censuses of tagged mistletoes (n = 76 on mesquite; 60 on acacia) were conducted at approximately biweekly intervals from September 2013 to May 2014. The presence or absence of open flowers/ripe fruits was recorded for each individual at each census. The degree of overlap between flowering/fruiting phenologies for each pair of individuals, c, was calculated as $c = a/b$. Here, a is the number of days between the first and last census in which both individuals were recorded as flowering/fruiting, and b is the number of days between the first and last census in which the individual with the shorter flowering/fruiting interval was recorded as flowering/fruiting (Primack 1980). This index, c, gives 1.0 for complete flowering/fruiting overlap and 0 for no flowering/fruiting overlap in our censuses. Differences in the distribution of overlap values for male and female mistletoes flowering on each host species were evaluated by t-test. To investigate the relationship between phenology and genetic ancestry, the first flowering date in 2015 was recorded for a subset of mistletoes (n = 57 individuals) sampled for genetic analysis. The relationship between ancestry proportions from genetic clusters determined by STRUCTURE (see Between-host species genetic analyses section below)

and the flowering phenology of these individuals was determined using a general linear model (logit-link function). All statistical tests were conducted using R v. 3.0.3 (R Core Team 2014).

Microsatellite development

From the primer development procedure outlined in the Appendix S1 (Supporting information), we found seven new and three previously published (Arroyo *et al.* 2013) polymorphic loci that amplified consistently (Table S1, Supporting information). Variation across all microsatellite loci was tested for linkage disequilibrium, departure from Hardy–Weinberg equilibrium (HWE), and the presence of null alleles using GENEPOP v. 4.2 (Raymond & Rousset 1995). None of the loci used in this study showed evidence of significant genetic linkage disequilibrium using the log-likelihood ratio statistic (*G*-test). F_{IS} was highly variable and several locus by population combinations showed significant departures from HWE (Table S2, Supporting information). Departures were all due to heterozygote deficiencies, an unsurprising result in parasite population genetic studies (Dharmarajan *et al.* 2011). Heterozygote deficiencies could have arisen due technical issues (null alleles, genotyping errors), population subdivision (Wahlund effect) or inbreeding. Macroparasites that show hierarchically nested population structure, like desert mistletoe (Aukema 2004), are particularly prone to deviating from HWE due to Wahlund effects and kin structure (Dharmarajan *et al.* 2011). We used the EM method (Dempster *et al.* 1977) for estimating null allele frequencies, which assumes that deviations from HWE are caused by incomplete data (i.e. null alleles). Estimated null allele frequencies per locus in mistletoe host species × site populations were generally <0.10 and never >0.30, which are likely to be overestimates due to the HWE assumption. While null alleles can produce bias in population measures, the presence of null alleles at this frequency is unlikely to alter the conclusions of population genetic analyses, with the exception of parentage analysis, which we did not perform (Dakin & Avise 2004; Carlsson 2008; Kelly *et al.* 2011). Additionally, F_{IS} values were not significantly correlated with the amount of missing data for each locus by population combination (slope = 0.0017 ± 0.0026, $P = 0.52$). A positive relationship is expected when deviations from HWE are caused by null alleles, which are recorded as missing data when homozygous (Dharmarajan *et al.* 2011). Genotyping errors could have also caused deviations from HWE, but jackknife analysis did not indicate any 'influential individuals' causing an overestimation of disequilibrium (Morin *et al.* 2009). Results were qualitatively similar when the locus most deficient in heterozygotes (1HP) was excluded. For these reasons and due to the difficulty of developing new microsatellites capable of amplifying consistently across populations from different host species, none of the ten loci described here were discarded from the analysis.

Intrahost population genetic analyses

The estimated number of migrants (N_m), *F*-statistics and Slatkin's allele size-based *R*-statistics were calculated at different levels of population structure using GENEPOP v. 4.2 (Raymond & Rousset 1995; Slatkin 1995). Slatkin's R_{ST} is calculated from the sum of squared differences in the size of alleles, making it more appropriate than F_{ST} for microsatellite markers evolving following a stepwise mutation model. Differentiation between pairs of mistletoe populations based on pairwise R_{ST} was tested using 110 permutations of haplotypes across individuals. Relationship coefficients (*R*) were calculated for pairs of individuals using the algorithm of Queller & Goodnight (1989) in SPAGEDI v. 1-4 (Hardy & Vekemans 2002). Four predefined contrast tests of difference in *R* were conducted by permuting parasite genotypes 1000 times across host individuals. Those contrast tests were as follows: (i) mistletoes on the same vs. different acacia host individual, (ii) mistletoes on the same vs. different mesquite host individual, (iii) all mistletoes on the same vs. different host species and (iv) all mistletoes on a host individual of a different species located near (~70 m) vs. distant (~5 km).

Among host individuals genetic analyses

A hierarchical analysis of molecular variance (AMOVA) was performed using ARLEQUIN 3.5.2 with individuals nested within host species grouped within sites (Excoffier & Lischer 2010). All of the mistletoe samples except the intrahost populations were used in this analysis ($n = 161$). Tests of isolation by distance were performed on host species by site populations at SRER and CRP, where more than 20 individuals from each host were sampled, by permuting the geographic coordinates of the individuals across their genotypes 10 000 times using SPAGEDI v. 1-4 (Hardy & Vekemans 2002). The distribution of expected slopes between genetic relatedness and geographic distance given no isolation by distance was compared to the observed slope of the relationship.

Between-host species genetic analyses

The estimated proportion of ancestry originating from different genetic clusters was determined using STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000). This analysis included all sampled individuals ($n = 161$), except the

intrahost populations, which could bias results due to within-host structure. The optimal number of genetic clusters was found via the delta K method using results from 20 independent runs with 100 000 Markov Chain Monte Carlo reps (burnin length of 10 000) for each possible *K* from 1 to 10 (Evanno *et al.* 2005). For calculations of hybrid index, pure parental acacia or mesquite-associated mistletoe populations were defined as all individuals with >99% of ancestry from the cluster associated with the host species on which they were sampled. Using these parental populations, hybrid index and between-population heterozygosity were estimated using the introgress R package (Gompert & Buerkle 2010). The number and frequency of private alleles for each host-associated population was calculated after removing putative immigrant individuals (those with >90% of ancestry associated with the species host on which they do not live) from the sample.

Results

Reproductive phenology

Desert mistletoes (*Phoradendron californicum*, hereafter referred to as 'mistletoe') parasitizing velvet mesquites (*Prosopis velutina*, hereafter, 'mesquite') showed a differentiated, but overlapping flowering phenology relative to mistletoes parasitizing catclaw acacia (*Senegalia greggii*, hereafter, 'acacia') (Fig. 1B). Mistletoes on acacia reached peak flowering in early February, while mistletoes on mesquite peaked in mid-March. Male mistletoes peaked in flowering about 2 weeks earlier than females on the same host species. Female mistletoes overlapped more in flowering phenology with male mistletoes on the same host species than those on different host species. Females on mesquite showed a greater overlap with males on mesquite ($c = 0.812$) than with males on acacia ($c = 0.108$, $t = 91.6$, $P < 0.001$). The difference was less striking, but still significant between the flowering overlap of female mistletoes on acacia with males on acacia ($c = 0.791$) vs. with males on mesquite ($c = 0.566$, $t = 18.2$, $P < 0.001$). Overlap with males on the opposite host species was significantly lower for females on mesquite than for females on acacia ($t = 41.9$, $P < 0.001$). The overlap in flowering of male and female mistletoes on different host species and, thus, the predicted potential for hybridization between mistletoes on different host species was greatest from mid-February to early March. In contrast to the divergence in flowering, mistletoe fruiting phenology differed little by host species (Fig. 1B). Females infecting both host species produced ripe fruits from early September to early May. The overlap in fruiting between mistletoes on mesquite and acacia is large

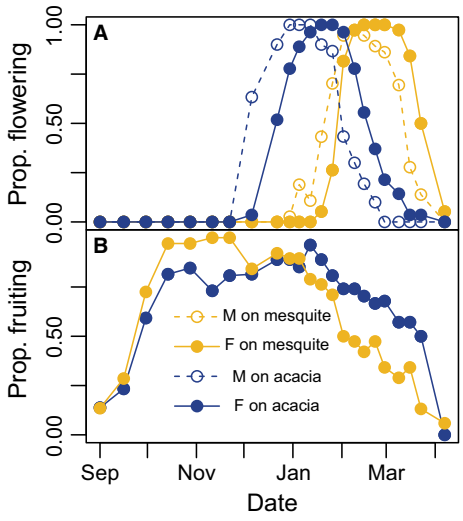

Fig. 1 Reproductive phenology of desert mistletoes (*Phoradendron californicum*) parasitizing sympatric acacia (*Senegalia greggii*) and mesquite (*Prosopis velutina*) hosts at the Santa Rita Experimental Range. Phenology was recorded as the proportion of plants with (A) open flowers and (B) ripe fruit per census from September 2013 to May 2014.

($c = 0.932$) and similar to the degree of overlap in fruiting between individuals within a host (acacia, $c = 0.930$; mesquite, $c = 0.962$).

Intrahost genetic structure

Differentiation (R_{ST}) between intrahost populations of mistletoes was greater when host individuals were of different species rather than the same species. That is, intrahost populations on acacia and nearby (~70 m) intrahost populations on mesquite were significantly differentiated (pairwise $R_{ST} > 0$: $R_{ST} = 0.276$, $P < 0.001$; $R_{ST} = 0.261$, $P < 0.001$), Similarly, intrahost populations on acacia were significantly differentiated from distant (~5 km) intrahost populations on mesquite (pairwise $R_{ST} > 0$: $R_{ST} = 0.223$, $P < 0.001$; $R_{ST} = 0.313$, $P < 0.001$). The intrahost populations of mistletoes on different acacia individuals (~5 km) were genetically differentiated from one another (pairwise $R_{ST} > 0$: $R_{ST} = 0.061$, $P = 0.018$), but the intrahost populations on different mesquite individuals were not (pairwise $R_{ST} > 0$: $R_{ST} = -0.016$, $P > 0.05$). As predicted, intrahost populations on acacia were more inbred ($R_{IS} = 0.38$, $R_{IS} = 0.29$) than intrahost populations on mesquite ($R_{IS} = -0.01$, $R_{IS} = 0.14$). The relationship coefficient (R) between pairs of mistletoes on the same acacia individual was greater than the relationship coefficient between pairs of mistletoes on different acacia individuals (~5 km) (Fig. 2A). In contrast, pairs of mistletoes infecting mesquite showed similar levels of relatedness, regardless of

whether they shared a host individual. The average R of pairs of mistletoes on different host species was negative and lower than between pairs of mistletoes on the same host species, consistent with host species-associated genetic differentiation. Whether the host individual was nearby (~70 m) or distant (~5 km) did not affect the relatedness of mistletoe pairs on different host species. The estimated number of migrants (N_m) between the intrahost populations was 0.8 on acacia and 2.07 on mesquite, corrected for mean sample sizes per population per locus of 12.5 and 15.8, respectively.

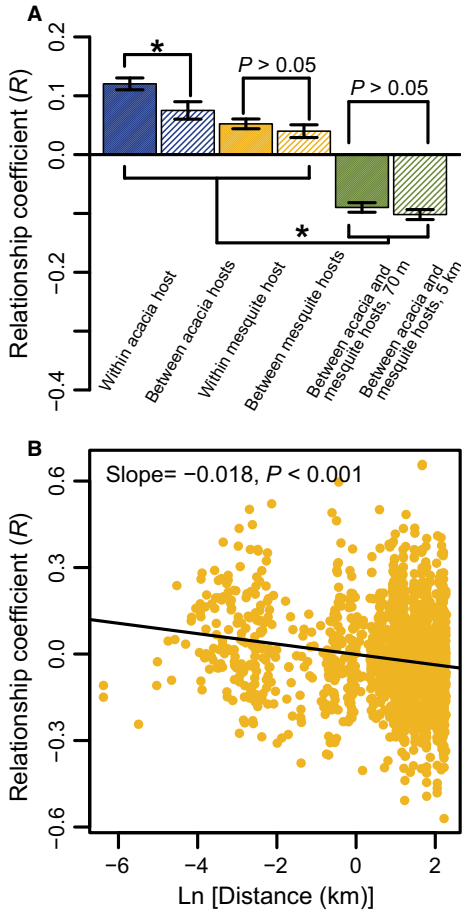

Fig. 2 Hierarchical population genetic structure of desert mistletoe (*Phoradendron californicum*) at the Santa Rita Experimental Range. (A) Within- and between-host individual populations: Relationship coefficients ($R \pm$ SEM) of mistletoe intrahost populations within host individuals, between host individuals within a species, between close (70 m) host individuals of different species and between distant (5 km) host individuals of different species ($n = 20$ per host individual, two host individuals/species). Contrast tests are represented by brackets, $^*P < 0.05$. (B) By geographic distance: pairwise relationship coefficient (R) on mesquite ($n = 65$ mistletoes) relative to geographic distance. The linear relationship shown is stronger than expected by permutation test (expected slope: mean $\pm$ SD = 0.000007 $\pm$ 0.0026).

Among host individual genetic structure

Within a site and host species, the relationship coefficient (R) between pairs of mistletoe individuals decreased with increasing geographic distance (Fig. 2B). However, geographic distance explained only a small proportion of the variation in R for pairs of mistletoes on mesquites (mean randomized slope jackknifed over loci $\pm$ SEM = -0.016 ± 0.009, $r^2 = 0.030$). For other geographic site by host species populations, results were qualitatively similar with either weak or no significant signal of isolation by distance (Fig. S5, Supporting information). Furthermore, while geographic site itself did not contribute significantly to the overall molecular variance of mistletoes across all hosts, host species within a site did contribute significantly (hierarchical AMOVA, Table 1). When mistletoe populations on different host species at the same site were compared, eight of nine of the R_{ST} values between pairs of populations were indicative of significant differentiation (Table 2). In contrast, when populations from the same host species but at different sites were compared, three of six of the pairs did not show significant differentiation. The estimated number of migrants (N_m) between the three sites on mesquite, corrected for mean sample size of 30.23 individuals per population per locus, was 4.20. The estimated number of migrants (N_m) between the three sites on acacia, corrected for mean sample size of 16.10 individuals per population per locus, was 2.11.

Between-host species population genetic structure

The number of genetic clusters that best explained the genetic variation of the mistletoes was $K = 2$. Estimated proportions of ancestry associated with each cluster strongly corresponded to host species (Fig. 3A).

Table 1 Analysis of molecular variance (AMOVA) across ten microsatellite loci for populations of desert mistletoe (*Phoradendron californicum*) on velvet mesquite (*Prosopis velutina*) and catclaw acacia (*Senegalia greggii*) hosts at Catalina Regional Park, Tumamoc Hill and the Santa Rita Experimental Range

Source of variation	d.f.	Sum of squares	Variance component	Per cent of variation
Among sites	2	12 382	9.6	2.14
Between-host species	3	10 130	77.0	17.21***
Within-host species within site	316	113 949	360.6	80.65***
Total	321	136 461	447.2	100

$^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 2 Genetic differentiation as measured by sum of squared allele size differences (R_{ST}) for pairs of desert mistletoe (*Phoradendron californicum*) populations on velvet mesquite (*Prosopis velutina*) and catclaw acacia (*Senegalia greggii*) at three sites: Catalina Regional Park (CRP), Tumamoc Hill (TH) and the Santa Rita Experimental Range (SRER)

	Mistletoe populations on acacia			Mistletoe populations on mesquite		
	CRP	TH	SRER	CRP	TH	SRER
Mistletoe populations on acacia						
CRP	0					
TH	−0.005	0				
SRER	0.088*	0.070*	0			
Mistletoe populations on mesquite						
CRP	0.157***	0.169***	0.026	0		
TH	0.333***	0.340***	0.222***	0.044**	0	
SRER	0.128***	0.082*	0.156*	−0.106	0.012	0

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Mistletoes on acacia had an average of 93.2% (±2.7% SEM) ancestry from the first genetic cluster, while mistletoes on mesquite had an average of 89.3% (±2.4% SEM) ancestry from the second cluster. Few individuals had intermediate ancestry from each of the two clusters (15 of 161 had <90% estimated ancestry from one of the two clusters). While 39 individuals had intermediate hybrid indices (between 0.25 and 0.75), those individuals did not show high between-host-associated population heterozygosity (mean ± SEM = 0.32 ± 0.034, only four individuals of intermediate hybrid index had a between-host-associated population heterozygosity >0.5), indicating a lack of F1 hybrids between host-associated populations but frequent backcrosses in our sample. Putative immigrants between the host species (defined as mistletoe having >90% of their ancestry from the genetic cluster associated with the other host species) were present and identifiable, but did not differ in frequency between the host species (Fisher's Exact test: three of 58 on acacia, six of 103 on mesquite, $P > 0.99$). Immigrants and hybrids (defined as mistletoes having <90% ancestry from either cluster) between the host races did not differ in frequency across both hosts (Fisher's exact test: $P > 0.99$). Excluding immigrants, mistletoes on mesquite showed a greater proportion of ancestry on average from the acacia-associated cluster than mistletoes on acacias did from the mesquite-associated cluster (one-sided *t*-test: $t = 1.92$, d.f. = 122, $P = 0.029$). This result was inconsistent with the hypothesis that the genetic background of

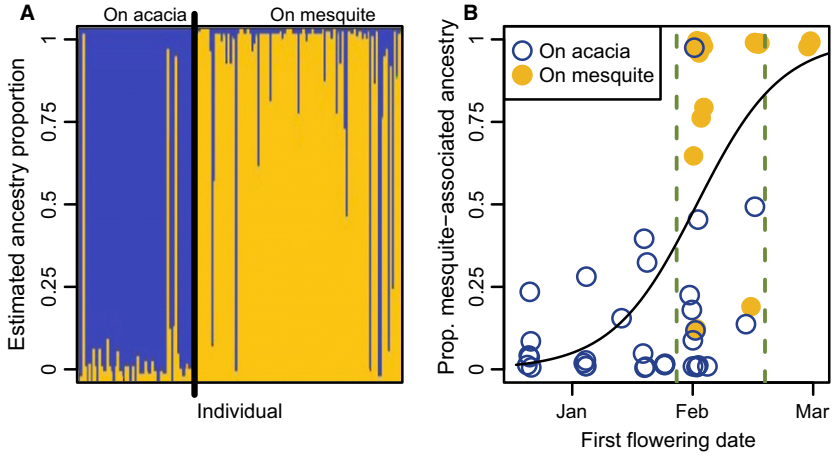

Fig. 3 Genetic differentiation between desert mistletoe (*Phoradendron californicum*) parasitizing sympatric catclaw acacia (*Senegalia greggii*) and velvet mesquite (*Prosopis velutina*) and the relationship to reproductive phenology. (A) STRUCTURE results estimating proportion ancestry of each individual (vertical lines) from the most probable genetic cluster ($K = 2$). The black line delineates mistletoes sampled on acacia ($n = 58$) and mesquite ($n = 103$). (B) Proportion ancestry from the mesquite-associated cluster increases with first flowering date (binomial glm, slope = 0.095, $z = 3.133$, $P = 0.002$, $n = 23$ on mesquite, 34 on acacia). Phenological overlap between mistletoe on acacia and mesquite is shown between the dashed lines.

the adult mistletoe population reflects overlap in flowering phenology between male and female plants, regardless of host species. The estimated number of migrants (N_m) between the host species, corrected for a mean sample size of 68.7 individuals per locus per host species, was 2.89. Mistletoes associated with each host species, excluding immigrants, exhibited a private allele proportion of 0.17 per locus on average (Table S3, Supporting information). R_{ST} between mistletoes on different hosts was 0.29. These patterns were indicative of host-associated races with low, but detectable levels of hybridization and immigration of parasites between the two host species.

Mistletoes that initiated flowering later in the season had a higher proportion of ancestry associated with the mesquite-associated genetic cluster (binomial glm, slope = 0.095, $z = 3.133$, $P = 0.002$), as expected based on the differences in flowering phenology between mistletoes on the different host species (Fig. 3B). Of the mistletoe individuals for which we measured flowering time ($n = 57$), three had an ancestry mismatched to their host-associated cluster. These individuals began flowering during the period from mid-February to early March, when coflowering of mistletoes on the two host species was most common.

Discussion

We found that desert mistletoes were primarily structured by host species. This may be due to the divergence in flowering time we observed on mistletoes infecting different host species. Mistletoes on the more rare host species showed greater structuring among host individuals than those on the more common host, potentially due to greater isolation. Across host individuals, mistletoes were more closely related to nearby individuals, but geography did not play a strong role in genetic structure at the scale of our study. Altogether, these patterns are indicative of two host-associated races of mistletoe in our populations. Individual mistletoes resulting from pollen (hybrids) or seed (immigrants) dispersal between the two host races were rare, but present.

Studies of multiple parasite species infecting the same host individual have increased our understanding of how parasite life-history strategies impact the potential for horizontal transmission and subsequent population genetic structuring (Whiteman *et al.* 2007). For example, parasite species with a greater likelihood of being dispersed between bat colonies show less population differentiation than those transmitted within colonies (Van Schaik *et al.* 2015). Studies, like ours, of a single parasite species infecting multiple sympatric hosts can similarly elucidate how differences between host species contribute to differences in parasite population genetic structure. We hypothesized that a greater proportion of parasite mating and dispersal occurs within individual hosts when the host species is rare compared to within common hosts. When territorial vectors forage on rare host species, most successful mistletoe offspring are likely to be those that are dispersed within the same host individual. When reproductive phenology differs between host races, parasites on rare hosts will have few nearby potential mates other than those that share a host individual. Together, these factors may lead to the increased isolation and inbreeding of parasites on rare host species, as observed in our study. Although the small number of intrahost populations sampled precludes inference at the scale of the host populations, this pattern is consistent with the hypothesis that the different host races experience different levels of relatedness with neighbouring mistletoes. Parasites sharing a host compete for host resources, and the outcome of competition can increase host resource depletion. Relatedness of parasites on the same host individual can influence optimal virulence levels (Frank 1992, 1994). The ability of mistletoes to recognize and respond to kin or the degree to which they compete with other mistletoes is unknown, but if competition does not increase with relatedness, we predict that mistletoes on acacia would be less virulent than those on mesquite. Future work should investigate the possibility for mistletoe signalling through host or parasite vascular tissue or through volatiles, as has been found for several plant species (Bhatt *et al.* 2011; Karban *et al.* 2013).

Above the level of individual hosts, we found that mistletoes are strongly genetically structured by host species, but divergence is not complete. Gene flow between parasite populations on different host species is likely substantial when parasites are not vertically transmitted, vectors are not host species-constant and hosts occur in sympatry. The reduced viability of experimentally transplanted immigrant mistletoes between the host species has been documented (Glazner *et al.* 1988; Overton 1997; Clay *et al.* 2015), but whether immigrants between the host species exist in natural populations was not known prior to our study. The existence of these potentially less fit individuals can cause selection for reinforcement of prezygotic reproductive isolation, such as the divergent parasite flowering phenology we observed. However, flowering phenology alone was not sufficient to predict the population genetic structure of the adult population. While females and males on the same host species overlapped significantly in flowering time, female mistletoes on acacia overlapped more in flowering with males on mesquite than females on mesquite overlapped with males on acacia. Therefore, we expected that asymmetry in pollen

flow would lead to more hybrids between the host races on acacia than on mesquite. However, the distribution of individuals with intermediate ancestry between the host races did not support this prediction. Other factors, such as relative densities of each host race, short pollen dispersal distances, pollen discrimination mechanisms or low hybrid seed establishment rates, could influence gene flow between the host races at mating (Hopkins 2013). Interestingly, immigrants were found at frequencies similar to hybrids between host races, despite the prediction that immigrants should have lower establishment rates than hybrids. Dispersal between the two host species is likely more common than the observed frequency of immigrants due to relatively low establishment success (Overton 1997). This fact, coupled with the synchronicity of fruiting on the two host species, is consistent with greater barriers between the host races at pollination than at dispersal. Successful reproduction of hybrid and immigrant adults should erode differentiation between the host races in the absence of reinforcement. Therefore, future experiments investigating reinforcement should be undertaken (Hopkins 2013).

Our findings highlight the critical role vectors can play in determining parasite population genetic structure and speciation. If vectors have foraging preferences, constancy in host usage can promote divergence of existing host races. If, however, vectors show little constancy in host usage, they provide frequent opportunities for parasites to colonize different host species and may disperse the antecedents of host-switching speciation events (Brooks 1988, 1993; Ròzsa 1993; Sorenson *et al.* 2003; Whiteman *et al.* 2004; Hoberg & Brooks 2008; Harbison & Clayton 2011). The presence of immigrants between host species suggests plasticity or standing variation in host range, a factor also hypothesized to correlate with host-switching ability (Hoberg & Brooks 2015). Indeed, preliminary analyses of cophylogenetic patterns of mistletoes in the Phoradendreae tribe and their hosts are consistent with the broad importance of diversification through host-switching events (Appendix S2, Fig. S5, Supporting information).

We cannot determine whether observed divergence in flowering phenology was the result of reinforcement or, more generally, adaptation. Asynchrony in host race flowering phenologies could be the result of differences in host physiologies that have subsequently promoted host race formation by limiting gene flow. Differences between mesquite and acacia in defence against mistletoe, the presence of a taproot to mitigate drought and relationship with nitrogen-fixing bacteria may impact their interactions with mistletoes (Schulze & Ehleringer 1984; Overton 1997). Alternatively, differences in flowering phenology could have arisen due to pleiotropic effects of genes involved in local adaptation to the host environment. Finally, differences in phenology could be due to selection minimizing the frequency of hybrids between the host races via reduced fitness. Differences in desert mistletoe flowering phenology between the host races are consistent across sites, but not consistent with flowering time differences of their host species. Catclaw acacia flowers later in the year than velvet mesquite (K. M. Yule, unpublished data). Similar to model plant systems (Grillo *et al.* 2013), variation in desert mistletoe flowering time is likely determined in part by heritable, quantitative genetic variation, as immigrants between the host races have intermediate flowering times. Host-associated reproductive phenology is a clear case of mating/ecology pleiotropy, a powerful isolating mechanism that can facilitate sympatric speciation (Drès & Mallet 2002; Bolnick & Fitzpatrick 2007).

Our study and previous studies from other areas of the range have provided strong evidence for locally adapted host races (Glazner *et al.* 1988; Overton 1997). Conversely, these studies have found little evidence that geography structures mistletoe populations independent of host species. In contrast, the single range-wide study of desert mistletoe genetic structure found geography to be more important than host species in explaining chloroplast haplotype variation (Lira-Noriega *et al.* 2014). Therefore, mistletoes may have repeatedly diverged with respect to host species across the range. Our results cannot distinguish between allopatric or sympatric origins for the host races or determine which host race is derived from the other, warranting future work on the phylogeography of desert mistletoe. However, the evolutionary history of desert mistletoe has likely been marked by repeated host-switching events followed by local adaptation and partial reproductive isolation, resulting in host races that commonly occur in sympatry.

Conclusion

Parasites comprise a large proportion of the species of life on Earth and can have profound effects on their hosts (Dobson & Hudson 1986; Poulin & Morand 2000; Dobson *et al.* 2008). Studies on the evolution of parasite species are critical to understanding mechanisms driving diversification and to identifying the processes leading to emerging infectious disease agents. Parasitic plants are excellent models for dissecting fundamental questions in parasite biology: they are sessile, they often require animal vectors, complete infection inventories of individual hosts can be compiled, and they share many epidemiological patterns with parasitic animals, such as helminths (Aukema 2003). Our study outlines how differences in reproductive phenology, dispersal mediated

by vectors and local adaptation to hosts can interact to influence the population genetic structure of mistletoes from within-host individuals to between-host species. Our results may also have implications for the evolution of virulence and the mechanisms of diversification in the mistletoes.

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References

Arroyo JM, Munguia-Vega A, Rodríguez-Estrella R, Bascompte J (2013) Isolation of 18 microsatellite loci in the desert mistletoe *Phoradendron californicum* (Santalaceae) via 454 pyrosequencing. *Applications in Plant Sciences*, **1**, 1300048.

Aukema JE (2001) *Dispersal and spatial distribution of the desert mistletoe,* Phoradendron californicum*, at multiple scales: patterns, processes and mechanisms*. PhD Thesis, University of Arizona, USA.

Aukema JE (2003) Vectors, viscin, and Viscaceae: mistletoes as parasites, mutualists, and resources. *Frontiers in Ecology and the Environment*, **1**, 212–219.

Aukema JE (2004) Distribution and dispersal of desert mistletoe is scale-dependent, hierarchically nested. *Ecography*, **27**, 137–144.

Aukema JE, Martinez del Rio C (2002) Variation in mistletoe seed deposition: effects of intra- and interspecific host characteristics. *Ecography*, **25**, 139–144.

Bhatt MV, Khandelwal A, Dudley SA (2011) Kin recognition, not competitive interactions, predicts root allocation in young *Cakile edentula* seedling pairs. *New Phytologist*, **189**, 1135–1142.

Bolnick DI, Fitzpatrick BM (2007) Speciation: sympatric models and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 459–487.

Brooks DR (1988) Macroevolutionary comparisons of host and parasite phylogenies. *Annual Review of Ecology and Systematics*, **19**, 235–259.

Brooks WR (1993) Protection of symbiotic cnidarians by their hermit-crab hosts: evidence for mutualism. *Symbiosis*, **15**, 1–13.

Carlsson J (2008) Effects of microsatellite null alleles on assignment testing. *Journal of Heredity*, **99**, 616–623.

Choi Y-J, Thines M (2015) Host jumps and radiation, not co-divergence drives diversification of obligate pathogens. A case study in downy mildews and Asteraceae. *PLoS ONE*, **10**, e0133655.

Clay K, Dements D, Rejmanek M (2015) Experimental evidence for host races in mistletoe (*Phoradendron tomentosum*). *American Journal of Botany*, **72**, 1225–1231.

Dakin EE, Avise JC (2004) Microsatellite null alleles in parentage analysis. *Heredity*, **93**, 504–509.

Dambroski HR, Feder JL (2007) Host plant and latitude-related diapause variation in *Rhagoletis pomonella*: a test for multifaceted life history adaptation on different stages of diapause development. *Journal of Evolutionary Biology*, **20**, 2101–2112.

De Vienne DM, Refrégier G, López-Villavicencio M *et al*. (2013) Cospeciation vs host-shift speciation: methods for testing, evidence from natural associations and relation to coevolution. *New Phytologist*, **198**, 347–385.

Dempster AP, Laird NM, Rubin DB (1977) Maximum likelihood from incomplete data via the EM algorithm. *Journal of the Royal Statistical Society, Series B (Methodological)*, **39**, 1–38.

Desdevises Y, Morand S, Jousson O, Legendre P (2002) Coevolution between *Lamellodiscus* (Monogenea: Diplectanidae) and Sparidae (Teleostei): the study of a complex host-parasite system. *Evolution*, **56**, 2459–2471.

Dharmarajan G, Beasley JC, Rhodes OE (2011) Heterozygote deficiencies in parasite populations: an evaluation of interrelated hypotheses in the raccoon tick, *Ixodes texanus*. *Heredity*, **106**, 253–256.

Dobson AP, Hudson PJ (1986) Parasites, disease and the structure of ecological communities. *Trends in Ecology & Evolution*, **1**, 11–15.

Dobson AP, Lafferty KD, Kuris AM, Hechinger RF, Walter J (2008) Homage to Linnaeus: how many parasites? How many hosts? *Proceedings of the National Academy of Sciences of the USA*, **108**, 11482–11489.

Drès M, Mallet J (2002) Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **357**, 471–492.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Frank SA (1992) A kin selection model for the evolution of virulence. *Proceedings of the Royal Society of London B: Biological Sciences*, **250**, 195–197.

Frank SA (1994) Kin selection and virulence in the evolution of protocells and parasites. *Proceedings of the Royal Society of London B: Biological Sciences*, **258**, 153–161.

Giraud T, Refrégier G, Le Gac M, de Vienne DM, Hood ME (2008) Speciation in fungi. *Fungal Genetics and Biology*, **45**, 791–802.

Glazner JT, Devhn B, Ellstrand NC (1988) Biochemical and morphological evidence for host race evolution in desert mistletoe, *Phoradendron californicum* (Viscaceae). *Plant Systematics and Evolution*, **161**, 13–21.

Gompert Z, Buerkle CA (2010) *introgress*: a software package for mapping components of isolation in hybrids. *Molecular Ecology Resources*, **10**, 378–384.

Grillo MA, Li C, Hammond M, Wang L, Schemske DW (2013) Genetic architecture of flowering time differentiation between locally adapted populations of *Arabidopsis thaliana*. *New Phytologist*, **197**, 1321–1331.

Harbison CW, Clayton DH (2011) Community interactions govern host-switching with implications for host-parasite coevolutionary history. *Proceedings of the National Academy of Sciences of the USA*, **108**, 9525–9529.

Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Herrera CM (1987) Components of pollinator "quality": comparative analysis of a diverse insect assemblage. *Oikos*, **50**, 79–90.

Hoberg EP, Brooks DR (2008) A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host-parasite systems. *Journal of Biogeography*, **35**, 1533–1550.

Hoberg EP, Brooks DR (2015) Evolution in action: climate change, biodiversity dynamics, and emerging infectious disease. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **370**, 20130553.

Hopkins R (2013) Reinforcement in plants. *New Phytologist*, **197**, 1095–1103.

Karban R, Shiojiri K, Ishizaki S, Wetzel WC, Evans RY (2013) Kin recognition affects plant communication and defense. *Proceedings of the Royal Society of London B: Biological Sciences*, **280**, 20123062.

Kelly AC, Mateus-Pinilla NE, Douglas M *et al.* (2011) Microsatellites behaving badly: empirical evaluation of genotyping errors and subsequent impacts on population studies. *Genetics and Molecular Research*, **10**, 2534–2553.

Lara C, Perez G, Ornelas JF (2009) Provenance, guts, and fate: field and experimental evidence in a host-mistletoe-bird system. *Ecoscience*, **16**, 399–407.

Larson DL (1991) *Ecology of desert mistletoe seed dispersal*. PhD Thesis, University of Illinois at Chicago, USA.

Larson DL (1996) Seed dispersal by specialist versus generalist foragers: the plant's perspective. *Trends in Ecology & Evolution*, **76**, 113–120.

Le Corre V, Reibel C, Gibot-Leclerc S (2014) Development of microsatellite markers in the branched broomrape *Phelipanche ramosa* L. (Pomel) and evidence for host-associated genetic divergence. *International Journal of Molecular Sciences*, **15**, 994–1002.

Le Gac M, Giraud T (2004) What is sympatric speciation in parasites? *Trends in Parasitology*, **20**, 207–208.

Lira-Noriega A, Toro-Nunez O, Oaks JR, Mort ME (2014) The roles of history and ecology in chloroplast phylogeographic patterns of the bird-dispersed plant parasite *Phoradendron* californicum (Viscaceae) in the Sonoran Desert. *American Journal of Botany*, **102**, 149–164.

March WA, Watson DM (2010) The contribution of mistletoes to nutrient returns: evidence for a critical role in nutrient cycling. *Austral Ecology*, **35**, 713–721.

Martínez del Rio C, Aukema JE (2002) Where does a fruit-eating bird deposit mistletoe seeds? Seed deposition patterns and an experiment. *Ecology*, **83**, 3489–3496.

Mitter C, Farrell B, Wiegmann B (1988) The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? *The American Naturalist*, **132**, 107.

Morand S, Krasnov BR, Littlewood DJ (2015) *Parasite Diversity and Diversification: Evolutionary Ecology Meets Phylogenetics*. Cambridge University Press, Cambridge.

Morin PA, Leduc RG, Archer FI *et al.* (2009) Significant deviations from Hardy–Weinberg equilibrium caused by low levels of microsatellite genotyping errors. *Molecular Ecology Resources*, **9**, 498–504.

Mutikainen P, Koskela T (2002) Population structure of a parasitic plant and its perennial host. *Heredity*, **89**, 318–324.

Norton DA, Carpenter MA (1998) Mistletoes as parasites: host specificity and speciation. *Trends in Ecology & Evolution*, **5347**, 101–105.

Overton JM (1997) Host specialization and partial reproductive isolation in desert mistletoe (*Phoradendron californicum*). *The Southwestern Naturalist*, **42**, 201–209.

Poulin R, Morand S (2000) The diversity of parasites. *The Quarterly Review of Biology*, **75**, 277–293.

Primack RB (1980) Variation in the phenology of natural populations of montane shrubs in New Zealand. *Journal of Ecology*, **68**, 849–862.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

R Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org/.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ricklefs RE, Fallon SM, Bermingham E (2004) Evolutionary relationships, cospeciation, and host switching in avian malaria parasites. *Systematic Biology*, **53**, 111–119.

Ròzsa L (1993) Speciation patterns of ectoparasites and "straggling" lice. *International Journal for Parasitology*, **23**, 859–864.

Santiago-Alarcon D, Rodríguez-Ferraro A, Parker PG, Ricklefs RE (2014) Different meal, same flavor: cospeciation and host switching of haemosporidian parasites in some non-passerine birds. *Parasites & Vectors*, **7**, 286.

Schulze ED, Ehleringer JR (1984) The effect of nitrogen supply on growth and water-use efficiency of xylem-tapping mistletoes. *Planta*, **162**, 268–275.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.

Sorenson MD, Sefc KM, Payne RB (2003) Speciation by host switch in brood parasitic indigobirds. *Nature*, **424**, 928–931.

Van Schaik J, Dekeukeleire D, Kerth G (2015) Host and parasite life history interplay to yield divergent population genetic structures in two ectoparasites living on the same bat species. *Molecular Ecology*, **24**, 2324–2335.

Waddington KD (1979) Flight patterns of three species of sweat bees (Halictidae) foraging at *Convolvulus arvensis*. *Journal of the Kansas Entomological Society*, **524**, 751–758.

Walsberg GE (1975) Digestive adaptations of *Phainopepla nitens* associated with the eating of mistletoe berries. *The Condor*, **77**, 169–174.

Walsberg GE (1978) Brood size and the use of time and energy by the phainopepla. *Ecology*, **59**, 147–153.

Watson DM (2001) Mistletoe: a keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics*, **32**, 219–249.

Whiteman NK, Santiago-Alarcon D, Johnson KP, Parker PG (2004) Differences in straggling rates between two genera of dove lice (Insecta: Phthiraptera) reinforce population genetic and cophylogenetic patterns. *International Journal for Parasitology*, **34**, 1113–1119.

Whiteman NK, Kimball RT, Parker PG (2007) Co-phylogeography and comparative population genetics of the threatened Galapagos hawk and three ectoparasite species: ecology shapes population histories within parasite communities. *Molecular Ecology*, **16**, 4759–4773.

Wiens JJ, Lapoint RT, Whiteman NK (2015) Herbivory increases diversification across insect clades. *Nature Communications*, **6**, 8370.

K.M.Y., J.A.H.K. and N.K.W. designed the research; J.A.H.K. and L.R.J. designed the microsatellite markers; K.M.Y., J.A.H.K., N.M.A. and L.R.J. carried out field collections and laboratory work; K.M.Y. and J.A.H.K. analysed the microsatellite genotypes; K.M.Y. performed the phenological surveys, statistical analysis and cophylogenetic analysis; K.M.Y. and N.K.W. wrote the manuscript.

Data accessibility

Sampling locations, phenology data, microsatellite genotypes, Structure input file and parameters: Dryad doi: 10.5061/dryad.vt45p.

Supporting information

Additional supporting information may be found in the online version of this article.

Figs. S1–S4 Maps of study site locations and distribution of velvet mesquite (*Prosopis velutina*) and catclaw acacia (*Senegalia greggii*) hosts of surveyed desert mistletoe (*Phoradendron californicum*).

Fig. S5 Pairwise relationship coefficient (*R*) between pairs of mistletoes by geographic distance.

Fig. S6 Cophylogeny of Phoradendreae and representative host plant species. Phorandendreae phylogeny reproduced from consensus tree of Ashworth (2000).

Table S1 Characteristics of seven new microsatellite loci isolated from *Phoradendron californicum*.

Table S2 Desert mistletoe (*Phoradendron californicum*) population genetic statistics by locus and host species x site population.

Table S3 Host race-associated private alleles in desert mistletoe (*Phoradendron californicum*).

Appendix S1 Methods.

Appendix S2 Co-phylogenetic analyses.