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ORIGINAL ARTICLE

Genetic diversity and population structure of the mistletoe *Tristerix corymbosus* (Loranthaceae)

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Abstract The genetic structure of a plant species is influenced by life-history traits, geographical range and ecological interactions that shape gene flow. We examined the genetic structure of the South American mistletoe *Tristerix corymbosus* using random amplification of polymorphic DNA. This species is found mainly in Chile and inhabits two biomes, the Chilean matorral and the temperate forest. The main pollinator agent, a hummingbird, is the same across the whole range, but the disperser assemblage varies between biomes. We selected 22 populations, eight of which were located in the Chilean matorral, where fruits are yellow and birds act as seed dispersers, and 14 populations in the temperate forest, where fruits are green and a marsupial disperses the mistletoe seeds. A total of ten primers were used to generate amplification products for 121 individuals (ca. six individuals per population) and 91 bands were scored. Results show that this mistletoe species is highly variable with 81 % of the bands polymorphic and a Shannon's diversity index among populations of 0.634. The temperate forest shows slightly higher diversity indices than the Chilean matorral. The central region of the mistletoe geographic range was more variable than the north and the south regions, suggesting that it is a genetically mixed zone. It is likely that gene flow occurs mainly via hummingbirds moving pollen between biomes and birds moving seeds from north to south during spring migrations.

Keywords Seed disperser · Chilean matorral · Genetic variation · Parasitic plant · Population genetics · RAPD · South America · Temperate forest

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Introduction

Genetic structure is influenced by life-history traits, species distribution and ecological processes responsible for gene dispersal. For seed plants, mating system has been reported to be one of the main predictors of population differentiation (Hamrick et al. 1992; Hamrick and Godt 1997; Nybom and Bartish 2000; Nybom 2004). However, different genetic markers can reflect different factors. In particular, it has been reported that the genetic structure revealed by chloroplast markers better reflects the influence of factors that vary with the geographic range, whereas nuclear markers better reflect the influence of plant mating system (Duminil et al. 2007).

Mistletoes (stem-parasitic plants) have life histories with a number of constraints because of their intimate association with other organisms (Kuijt 1969; Norton and Carpenter 1998; Mathiasen et al. 2008). Specifically, many loranthaceous mistletoes are highly dependent not only on a host plant, but also on animals for pollination and seed dispersal. These close, and many times obligate, relationships with animals have resulted in numerous morphological modifications in the mistletoes (Kuijt 1969; Kirkup 1998; Watson 2001). Thus, it can be expected that such interactions with other organisms (hosts, pollinators and seed dispersers) might also influence mistletoe's genetic structure.

The genus *Tristerix* (Loranthaceae) is composed of 11 species distributed along the Andes and nearby areas (Kuijt 1988; Amico et al. 2007). *Tristerix corymbosus* L. is endemic to southern South America and is the most austral species in the genus. This species is present in two different biomes, the matorral of central Chile (hereafter the Chilean matorral) with a Mediterranean climate and the temperate forest of South America (hereafter the temperate forest). Across its entire geographic range, this mistletoe is pollinated mainly by the hummingbird *Sephanoides sephaniodes* (Kuijt 1988; Aizen 2003). The flowering season of the mistletoe in both biomes occurs during fall and winter, providing the principal food for the hummingbird when alternative flowering resources are scarce or nonexistent. Instead, during summer, this hummingbird can use the flowers of many different species, pollinating nearly 20 % of the woody genera endemic to the Patagonian temperate forests (Riveros and Smith-Ramirez 1996; Aizen and Ezcurra 1998). In contrast, the seed disperser assemblage varies geographically and this variation correlates with fruit color. In the Chilean matorral, fruits are yellow and their seeds exclusively dispersed by three bird species, whereas in the temperate forest, fruits are green and their seeds efficiently dispersed by a marsupial, *Dromiciops gliroides* (Amico and Aizen 2000; Amico et al. 2011). The genetic structure of *T. corymbosus* was previously explored by Amico and Nickrent (2009), who used two chloroplast markers to document the presence of three clades. None of these clades were directly associated with biome or ecological factors such as host and seed dispersers. One of the three clades is found in the northern and central regions, and the other two occur in the southern and the central regions. However, as pointed out above, the use of chloroplast markers, and more generally of maternally inherited markers, can provide only a partial view of the factors affecting a species genetic structure over its geographic range.

The objective of this study was to use random amplification of polymorphic DNA (RAPD), to evaluate the genetic structure along the entire geographical range of *T. corymbosus*, and gain an alternative perspective on the geographic-varying factors affecting this structure by the use of genetic nuclear-based markers. Below the species rank, RAPDs are a widely used DNA-based approach that allows relatively rapid acquisition of genetic data from a large number of loci within and across populations (Hadrys et al. 1992; Wang et al. 2011). One potential problem with this approach is reproducibility (Harris 1999); however, RAPDs can provide valuable reproducible data on patterns of genetic variation, provided that the polymerase chain reaction (PCR) amplifications are conducted with a stable polymerase and with standardized genomic DNA concentrations (Lynch and Milligan 1994;

Table 1 Collection information for 22 populations of *Tristerix corymbosus*

Population	Code	Lat. (S)	Long. (W)	Altitude	Biome	Region	*N*	Chloroplast clade
Ovalle	Ov	30°39′30″	71°40′53″	450	CM	N	6	III
Fray Jorge	FJ	30°38′27″	71°22′57″	550	CM	N	6	III
Chinchillas	Cc	31°30′15″	71°07′37″	365	CM	N	6	III
Illapel	Il	31°46′13″	71°19′07″	460	CM	N	4	III
San Felipe	SF	32°47′05″	70°51′35″	460	CM	N	6	III
Yerba Loca	YL	33°20′22″	70°19′56″	1,800	CM	N	6	III
Talca	Ta	35°24′32″	71°37′33″	160	CM	C	4	III
Los Tilos	LT	36°46′35″	72°18′48″	100	CM	C	6	II
Queules	Qu	35°58′58″	71°41′42″	250	TF	C	5	I
Chillán	Cl	36°49′44″	71°43′01″	985	TF	C	5	II
Nahuelbuta	Nb	37°49′26″	71°57′54″	1,205	TF	C	6	II
San Ramón	SR	37°51′01″	72°57′50″	1,023	TF	C	6	II
Ñielol	Ni	38°44′40″	72°35′17″	105	TF	C	5	III
San Martín	SM	39°38′57″	73°11′23″	40	TF	S	5	I
Pucará	Pu	40°09′55″	71°37′35″	640	TF	S	5	II
Rió Bueno	RB	40°20′58″	72°55′31″	450	TF	S	6	I
Puyehue	Pu	40°39′51″	71°12′58″	809	TF	S	5	II
Quetrihue	Qt	40°47′59″	71°32′40″	785	TF	S	6	I
Llao Llao	LL	41°03′00″	71°32′40″	785	TF	S	6	I
Tacul	Tc	41°04′06″	71°32′67″	780	TF	S	5	I
Linao	Li	41°59′01″	73°30′38″	15	TF	S	6	I
Huillinco	Hu	42°40′45″	73°54′21″	25	TF	S	6	II

Chloroplast clades from Amico and Nickrent (2009). Only the most common clade per locality is shown

Biomes: *CM* Chilean matorral, *TF* temperate forest

Geographical region: *N* northern, *C* central, *S* southern

Wang et al. 2011). This technique has been used successfully in a number of plant groups, but has never been used in mistletoes. We used RAPDs to evaluate genetic structure associated with biome as well as geographic region. Given our previous results from the chloroplast genome (Amico and Nickrent 2009), we might expect to find genetic structure associated with contrasting biomes and their seed disperser using RAPDs. However, RAPDs reflect recent gene flow, whereas the chloroplast genome shows more ancient structure; thus, it is not unlikely that the genetic structure inferred from these two different markers will be different, perhaps nuclear-based markers stressing the role of paternal gene flow movement via the hummingbird pollinator.

Materials and methods

Population sampling

Samples from 22 populations of *T. corymbosus* were collected across its entire geographic range. Eight populations were located in the Chilean matorral and 14 in the temperate forest (Table 1; Fig. 1). Up to six plants, at least 20 m apart, were randomly sampled from each population. Fresh leaves from all sampled individuals were dried in silica gel and later used for DNA isolation. Host species was recorded for each individual collected. Because sample sizes were low, we do not know whether overall genetic variability in the population was represented, although this was partially compensated by including many populations. For analytical purposes, the populations were grouped by biome (Chilean matorral and temperate forest) and geographical region (north, central and south) (Table 1; Fig. 1).

DNA methodologies

DNA was extracted from dried leaf tissue using a modified CTAB protocol for high carbohydrate plants (Tel-Zur et al. 1999). After extraction, we estimated DNA concentration using a fluorometer (Hoefer DQ200) and diluted the samples to a final concentration of 5 ng/µL. Thirty 10-base oligonucleotide primers (Operon Technologies) were tested and the ten that showed intense and reproducible bands (Table 2) were selected. RAPD amplifications for six individuals of two populations (Yerba Loca and Llao Llao) were repeated at least twice to check for band reproducibility. All PCR experiments from these individuals repeatedly showed the same banding pattern. Each amplification (25 µL total volume) contained 1× reaction buffer, 1.5 mM $MgCl_2$, 50 µM of each dNTP, 20 ng of primer, 1.0 unit Taq DNA polymerase, and 5 ng template DNA. The PCR was conducted in a GeneAmp 9700 thermal cycler (Applied Biosystem) using both positive and negative

Fig. 1 Geographical location of *Tristerix corymbosus* populations in the Chilean matorral (*squares*) and temperate forest (*circles*)

controls to detect the efficiency of the enzyme and the absence of contamination. The amplification program consisted of one DNA denaturation cycle for 3 min at 94 °C, followed by 45 cycles of 94 °C for 1 min, 36 °C for 1 min, and 72 °C for 1.5 min, and a final elongation step of 72 °C for 10 min. The amplified products were resolved in 1 % TBE (Tris–borate EDTA) agarose gels stained with ethidium bromide. RAPD bands were visualized with UV transmitted light and photographed.

Data analysis

RAPD bands of equal size were scored by their presence/absence under the assumption that they are homologous

Table 2 Primers employed, the number of RAPD markers obtained, their sequence, the size of the fragments, number of polymorphic bands, the percentage of polymorphic bands, and genetic diversity for each primer

Primer	Number of Bands	Primer Seq (5′–3′)	Size (bp) min–max	Polymorphic bands	Total % Polymorphic	H'_{pop}	H'_{sp}	H'_{pop}/H'_{sp}	$1 - H'_{pop}/H'_{sp}$
OPA-17	9	GACCGCTTGT	300–1800	7	77.78	1.138	3.402	0.335	0.665
OPB-04	9	GGACTGGAGT	300–2,000	6	66.67	1.350	2.628	0.514	0.486
OPB-07	10	GGTGACGCAG	500–1,500	8	80.00	1.393	3.703	0.376	0.624
OPB-12	12	CCTTGACGCA	500–2,000	11	91.67	1.616	4.487	0.360	0.640
OPB-15	10	GGAGGGTGTT	450–2,100	9	90.00	1.436	4.009	0.358	0.642
OPA-04	7	AATCGGGCTG	350–900	5	71.43	0.597	2.659	0.225	0.775
OPB-18	5	CCACAGCAGT	400–1,200	3	60.00	0.682	1.274	0.535	0.465
OPB-20	10	GGACCCTTAC	450–1,800	9	90.00	1.128	3.340	0.338	0.662
OPF-11	8	TTGGTACCCC	300–2,000	7	87.50	1.136	2.963	0.383	0.617
OPL-12	11	GGGCGGTACT	500–2,400	10	90.91	1.407	4.075	0.345	0.655
	91		300–2,400	75	80.60	11.884	32.54	0.365	0.634

H'_{pop} = Shannon's diversity index over loci; H'_{sp} = Shannon's diversity index over primer, H'_{pop}/H'_{sp} = population genetic diversity, $1 - H'_{pop}/H'_{sp}$ = genetic differentiation among populations

and represent independent loci. The final data structure consisted of a binary (0/1) matrix of 121 rows (the individuals) by 91 columns (the scored RAPD markers). Since RAPD markers are dominant, it was assumed that each band represented the phenotype at a single biallelic locus (Williams et al. 1990).

Genetic diversity was measured using Shannon's diversity index, $S = -\sum p_i \log_2 p_i$, where p_i is the frequency of the presence or absence of a given RAPD fragment in the population for each RAPD locus. Genetic diversity was calculated over all populations, for the whole sample (species) and for each primer. The percentage of polymorphic loci was also calculated for each population. The genetic diversity measures from each biome and region were compared with analysis of variance (ANOVA). A Mantel test (significance given by 9,999 permutations) was applied to analyze the association between genetic and geographic distances for each sampled population using the R statistical software package (R Development Core Team 2013).

The data were first analyzed using non-metric multidimensional scaling (NMDS) based on Jaccard (1908) distances for binary data. NMDS calculated with three dimensions provided an adequate description of the data, with a remaining stress value below 20 %. Only the first two dimensions are shown in the ordination graph, as the third axis did not add much information to the spatial genetic pattern. The R statistical software (R Development Core Team 2013) and, specifically, the 'Vegan' package (Oksanen et al. 2007) were used for the analyses.

Genetic structure was explored using a Discriminant Analysis of Principal Components (DAPC). DAPC summarizes the genetic variability using linear combinations of the alleles as variables. It maximizes the variability between groups and minimizes the within-group variance (Jombart et al. 2010). DAPC provides group membership probabilities that can be seen as proximities of individuals to different clusters. This method first performs a principal component analysis in which a number of components are retained. We performed the *find.cluster* command in Adegenet v. 1.3-6 (Jombart 2008; Jombart et al. 2010) with 10^7 iterations and starting 1,000 times. At this stage, we retained 80 principal components and selected 9 clusters based on the Bayesian Information Criteria (adding more clusters did not improve the BIC score). For the DAPC, we retained 40 components and 8 discriminant functions.

To further explore the population genetic structure of *T. corymbosus*, we performed analyses of molecular variance (AMOVA) using the ARLEQUIN program (Excoffier et al. 2005). AMOVA analysis using pairwise differences was performed at different hierarchical levels associated with biomes and geographical regions (Table 1). Further grouping was done using the clades of localities obtained from the previous study using chloroplast markers (Amico and Nickrent 2009). In addition, AMOVA was performed comparing only the northern and southern localities, omitting the potential diluting effect of the hybrid zone represented by populations from the central localities (Table 1). Significance levels of the variance components estimated for each case were obtained by non-parametric permutation using 1,000 replicates.

Population genetic structure was also examined with a Bayesian hierarchical model developed for dominant data using the computer program Hickory v. 1.1 (Holsinger and Lewis 2003). This method estimates values for F_{st} without assumptions about within-population inbreeding or whether those genotypes within populations are in Hardy–Weinberg equilibrium. The program Hickory was run in

Table 3 Shannon's diversity (*S*), percent polymorphic bands (PPB) and DAPC groupings for all populations

Population	*S*	PPB	DAPC cluster analysis								
			1	2	3	4	5	6	7	8	9
Ovalle	1.22	35.16				6					
Fray Jorge	0.88	27.47						6			
Chinchillas	1.24	32.97			2				2		
Illapel	1.36	38.46			5			1			
San Felipe	0.64	17.58								6	
Yerba Loca	1.40	39.56							6		
Talca	1.33	37.36			3			1			
Los Tilos	0.77	20.88			1			5			
Queules	1.37	39.56			5						
Chillán	0.93	24.18									5
Nahuelbuta	0.96	25.27									6
San Ramón	1.94	50.55					6				
Ñielol	1.37	34.07									5
San Martín	1.62	42.86					5				
Pucará	1.20	31.87				5					
Rió Bueno	0.92	27.47		6							
Puyehue	1.36	38.46									5
Quetrihue	0.89	21.98					6				
Llao Llao	1.29	34.07		6							
Tacul	0.84	25.27									5
Linao	1.22	32.97		6							
Huillinco	1.39	37.36	6								

full model mode with non-informative priors for *f* (estimate of F_{is}) and θ_B (estimate of F_{st}) with the default settings (burn-in 50,000, sample 250,000 and thin 50). Several runs were performed to ensure consistent results. These results were compared with the AMOVA and the Shannon's index estimations.

Table 4 Shannon's diversity (*S*) and percent polymorphic bands (PPB) for RAPD loci for biome and geographical region

Biome	*S*	PPB
Chilean matorral	1.10	31.18
Temperate forest	1.23	33.28
Geographical region		
Northern	1.12	31.86
Central	1.24	33.12
Southern	1.19	32.48

Results

Genetic diversity

The 10 chosen primers consistently amplified a total of 91 scorable markers that ranged in size from 350 to 2,400 bp. The number of bands per primer ranged from 5 to 12 with an average of 9.1 bands/primer. Seventy-five of the 91 markers (81 %) were polymorphic (Table 2).

Shannon's diversity index (*S*) ranged from 0.64 (San Felipe) to 1.94 (San Ramon) (Table 3). The genetic diversity was higher in the temperate forest ($S = 1.23$) than in the Chilean matorral ($S = 1.10$) (Table 3). Despite these differences, the diversity measures were not significantly different between regions ($F = 0.09$, d.f $= 1$, $p = 0.34$). Populations from the central region had the highest genetic diversity ($S = 1.24$), followed by the ones in the southern region ($S = 1.19$) and the ones in the northern region had the least diversity ($S = 1.12$) ($F = 0.02$, d.f $= 2$, $p = 0.81$) (Table 4). Genetic distances among populations were not significantly correlated with geographic distances (Mantel test, $r = -0.13$, $p = 0.968$).

The percentage of polymorphic bands (PPB) within populations ranged from 17.6 to 50.5 % (Table 3). The

PPB was higher in the temperate forest (33.28 %) than in the Chilean matorral (31.18 %), but not significantly different ($F = 0.33$, d.f $= 1$, $p = 0.56$) (Table 4). Populations from the central region had the highest PPB (PPB = 33.12 %), followed by the ones in the southern region (PPB = 32.48 %); the ones in the northern region had the lowest genetic polymorphism (PPB = 31.86 %) (Table 4); however, they are not significantly different ($F = 0.03$, d.f $= 2$, $p = 0.96$).

The NMDS plot in general showed that most individuals within one population grouped together (Fig. 2). However, certain individuals from different populations were mixed, showing an area of overlap between the two biomes. This pattern shows that the separation by biomes was more evident than by geographic regions. A stress value of 31.01 % indicated that the observed distances among samples were well represented by the ordination on the NMDS plot.

The DAPC analysis conserved 91 % of the variance in nine clusters. As in the NMDS analysis, most populations retained their identity and most individuals from one population grouped in one cluster (Table 3). No cluster included individuals from the three geographical regions (Table 5). Three clusters (7, 8, 9) separated from the rest in the scatter plot (Fig. 3). One was represented by individuals from the central and southern regions (but all from the temperate forest) and the other two with individuals from the northern region. No cluster was composed exclusively of populations from the central region; however, two clusters included populations that were exclusive from the southern region (1, 2) and two from the northern region (7, 8). In the scatter plot, besides the three clusters already described, there were two distinct groups that involve three clusters each. The first group includes clusters (1, 2, 5) with individuals from the temperate forest (from the southern and central region) and the second group (clusters 3, 6, 4) includes individuals from both biomes and three regions.

Population genetic structure

The genetic differentiation found among the 22 populations of the mistletoe is 0.634 with the Shannon's index ($1 - H'_{pop}/H'_{sp}$ in Table 2) and 0.398 with the Hickory method. The within-population genetic diversity is 0.365 with the Shannon's index (Table 2), 0.221 with Hickory and 43 % with AMOVA (Table 6).

The 22 populations were arranged in different groups according to their biomes, geographical regions and chloroplast clades (Table 1). All variations were found to be highly significant (AMOVA $p < 0.001$). In the grouping by biome type, the AMOVA showed that 51 % of the molecular variance was found within each biome followed by 43 % within population, and only 6 % between biomes (Table 6). When grouping by geographical region, the major genetic

Table 5 DAPC cluster analysis by biome and geographical region

Cluster	1	2	3	4	5	6	7	8	9
Biome									
Chilean matorral	0	0	9	8	0	15	6	6	0
Temperate forest	6	18	5	5	17	0	0	0	26
Geographical region									
Northern	0	0	5	8	0	9	6	6	0
Central	0	0	9	0	6	6	0	0	16
Southern	6	18	0	5	11	0	0	0	10

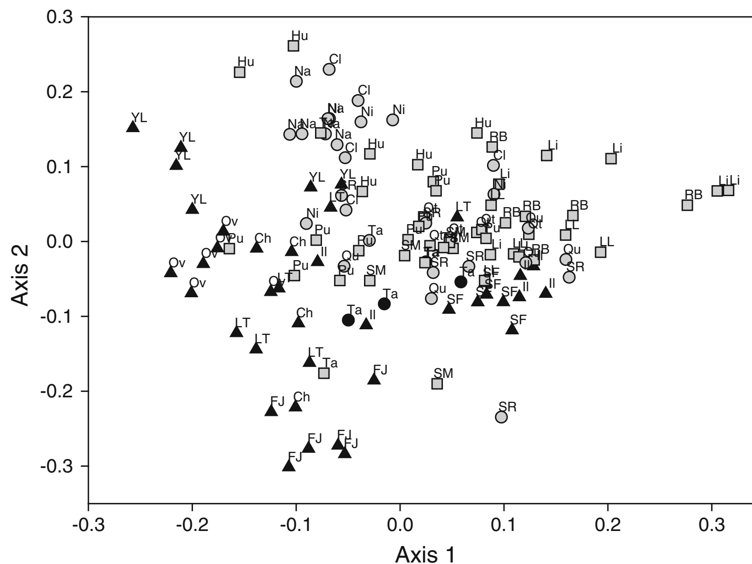

Fig. 2 Ordination using non-metric multidimensional scaling (NMDS) of Jaccard (1908) distances for 121 individuals of *Tristerix corymbosus* sampled from the 22 populations. The *shading* represents biomes: Chilean matorral (*black*) and Temperate forest (*gray*). The *symbols* represent the geographical regions: northern (*triangles*), central (*circles*) and southern (*squares*). Population abbreviations are the same as in Table 1

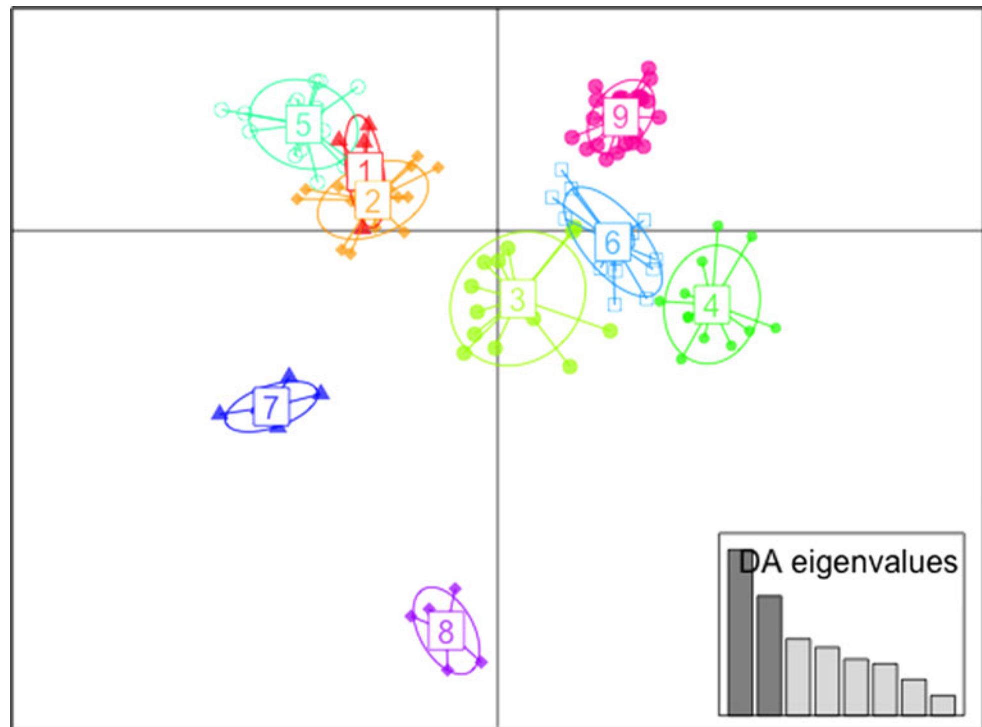

Fig. 3 Scatterplot of DAPC analysis showing the first two principal component of the analysis. Clusters and inertia ellipses are shown in *different colors*, and *dots* represent individuals. *Inset* shows the histogram of discriminant analysis eigenvalues

Table 6 Analyses of molecular variance (AMOVA) of RAPD given different groupings

Grouping	Level of variation	*df*	Sum of squares	Variance components	%	*p*
Biome	Among groups	1	110.0	1.00	6.25	<0.001
	Within groups	20	1046.2	8.26	50.88	<0.001
	Within locality	99	689.0	6.90	42.87	<0.001
	Total	120	1845.4	16.20		
Region	Among groups	2	182.3	0.98	6.15	<0.001
	Within groups	19	974.0	8.07	50.40	<0.001
	Within locality	99	689.1	6.96	43.45	<0.001
	Total	120	1845.4	16.01		
Clados	Among groups	2	171.0	0.82	5.12	<0.001
	Within groups	19	985.3	8.18	51.27	<0.001
	Within locality	99	689.1	6.96	43.61	<0.001
	Total	120	1845.4	15.96		
North–South	Among groups	1	116.6	1.54	9.22	<0.001
	Within groups	13	693.6	8.32	49.84	<0.001
	Within locality	69	471.9	6.84	40.94	<0.001
	Total	83	12.82.1	16.70		

variation was found within each region (50 %), with lesser amounts (40 %) within population. In the grouping of *T. corymbosus* by chloroplast clade only, 5 % of the genetic variation was found between clades (Table 6). All combinations of clustering showed that the greatest percentage of variation is within biomes, geographic regions and clades, and not at a larger scale (among biomes, or geographic regions). When grouping by geographic regions (comparing only the northern and southern), the AMOVA showed that 49 % of the molecular variance was found within each region followed by 40 % within population, and 9 % between regions (Table 6). This value doubles the genetic variation found when partitioning by the three regions; hence, excluding the localities from the central part of the distribution increases the percentage of variance explained between northern and southern regions.

Discussion

The genetic structure of *T. corymbosus* is not clearly associated with biome, with geographical region or chloroplast clades. This mistletoe has a wide geographic distribution (1,200 km) and is located in contrasting biomes (Chilean matorral and temperate forest). Given our previous work using chloroplast markers (Amico and Nickrent 2009), we expected to find genetic structure associated with these habitats; however, little (<10 %) of this species' genetic variability is associated with region, biome, or chloroplast clade. The DAPC analysis also showed that most variation is found between populations and that some genetic structure is associated with biome. There are some genotypes associated with the temperate forest (4 clusters exclusive of this biome) that mix elements from the southern and central regions, while there are three exclusive clusters from the Chilean matorral. Three clusters group together representing individuals from the three geographical regions (northern, central and southern), probably indicating that the central region is acting as a hybrid zone, mixing the genetic components of the southern and northern regions. In addition, Shannon indices and polymorphic bands showed that the central region is more variable that the northern or southern regions. We propose that the hummingbird pollinator must be moving genes and genotypes from one region to the other, thereby homogenizing the genetic pools from both biomes. This would explain why most variation is found within (not between) each biome and within each population in almost the same amounts.

Studies conducted for other mistletoe species in Viscaceae and Misodendraceae showed lower genetic variability than *T. corymbosus*. However, none of these studies used RAPDs as genetic marker. For Viscaceae, *Arcethobium americanum* (Jerome and Ford 2002b) and *Viscum album* (Mejnartowicz 2006) showed indices of gene diversity of 0.238 (AFLPs) and 0.468 (isozymes), respectively, compared to 1.14 for *T. corymbosus* (mean H'_{pop} in Table 2). The F_{st} of *V. album* was 0.277 (Mejnartowicz 2006), 0.286 for *A. americanum* (Jerome and Ford 2002a) and 0.20 (isozymes) for *Misodendrum punctulatum* (Vidal-Russell 2000), all lower than the value of *T. corymbosus* (0.634). It is possible that RAPD markers inflate genetic diversity as compared with other methods such as AFLP or isozymes. Unfortunately, there are no other mistletoe studies that use RAPDs as a genetic marker to compare with the present study.

Integrating RAPDs and chloroplast markers

The genetic structure of *T. corymbosus* using chloroplast markers was previously explored (Amico and Nickrent 2009). The chloroplast and the nuclear genome agree that the central region is the most variable; however, they differ as to where most genetic diversity is found. For the RAPD marker, most genetic diversity resides within each locality and within biome and not between biomes, thus showing that gene flow is homogenizing the two areas. In contrast to the chloroplast markers (*atpB–rbcL* spacer and *trnL-F* region), about half of the genetic diversity resides between biomes (Amico and Nickrent 2009). Although the other half of the genetic diversity is within each biome, this result indicates some structuring by biome. Specifically, when we omitted the populations of the central region from the RAPD analysis, the amount of genetic variation between northern and southern regions (Chilean matorral vs. temperate forest) almost doubles. Previously, Amico and Nickrent (2009) reported that about 40 % of the genetic diversity is among geographic regions and 50 % within each region, also suggesting geographic structuring, and only 10 % within each locality. The distribution across the geographic range shows a similar pattern as the RAPD loci. There is one chloroplast clade (5 haplotypes) restricted to the temperate forest and another clade with two haplotypes of which one is found in populations within the temperate forest or the Chilean matorral (central region). There is another clade with two haplotypes of which one is restricted to the Chilean matorral, and the other one is found within the temperate forest in the central region. Our results are in agreement with Duminil et al. (2007) who stated that the genetic structure indicated by chloroplast markers is more related to the seed dispersers (here biomes).

Biotic factor and gene flow

It has been suggested that interactions between mistletoes and their hosts can result in the formation of host races. In *Arceuthobium americanum* (dwarf mistletoe, Viscaceae), genetic structure (measured with AFLPs) was shaped mainly by the host, resulting in three distinct host races (Jerome and Ford 2002a, b). Host races have also been documented for *Phoradendron tomentosum*, Viscaceae (Clay et al. 1985) and various loranthaceous mistletoes (Norton and Carpenter 1998; Norton and de Lange 1999). *Tristerix corymbosus* is a generalist species, parasitizing at least 27 different hosts in 13 families, and these typically do not occur in large monospecific stands (Amico et al. 2007). For the 121 individuals of *T. corymbosus* sampled in this study, 16 species of small trees, shrubs or vines from 12 families were recorded as hosts. The structuring found with RAPDs is not associated with hosts of *T. corymbosus* (data not shown). This result has already been suggested using chloroplast markers (Amico and Nickrent 2009).

Currently, gene flow via the pollinator and seed disperser results in mixing the genotypes derived from different regions. The major pollinator of *T. corymbosus*, the hummingbird *S. sephaniodes*, migrates from south to north during the flowering season (March–September). Some individuals of this hummingbird species migrate from the cold southern regions (Lat. 54°S) to central Chile in areas where the mistletoe is present. The winter distribution of the hummingbird coincides with the whole range of the mistletoe. This hummingbird could be the agent responsible for moving mistletoe genotypes from southern to central and northern regions. The DAPC analysis supports this by showing that populations from the central region grouped with temperate forest or matorral populations, while there are clusters exclusive for each biome and none from the central region, thus indicating a hybrid zone in the center of the mistletoe distribution.

In contrast to the effects of the hummingbird pollinator, the migrations of the major seed dispersing birds in central Chile are from north to south. The migration route of the tyrant flycatcher *Elaenia albiceps* overlaps the distribution of *T. corymbosus*, and the timing of its arrival coincides with the mistletoe fruiting season. Bird migration could produce a seed rain in a north to south direction, thus generating the dispersal of chloroplast genomes from the central to the southern regions. This is evidenced by the genetic structure associated with biomes in the chloroplast marker (Amico and Nickrent 2009). This opposite and strong directional gene flow generated by the biotic agents could have the consequence of erasing past genetic patterns and generating the complex genetic structure found in *T. corymbosus* today.

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