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Nothofagus trees show genotype difference that influence infection by mistletoes, Misodendraceae

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Abstract. *Nothofagus* trees host *Misodendrum*, an endemic mistletoe of the subantarctic forests of Chile and Argentina. Differences in the infection intensity on a given host and patches of infected trees are observed within the forest. We used allozymes to test for genetic differences between uninfected and infected *Nothofagus* trees (*Nothofagus antarctica* (G. Forst.) Oerst.) by two species of *Misodendrum* (*Misodendrum linearifolium* DC. and *Misodendrum punctulatum* DC.) at three sites. Non-metric multidimensional scaling ordination was performed using the presence of each of 26 total alleles in 166 trees of *N. antarctica* (89 uninfected and 77 infected). Sites with higher degrees of infection by *M. punctulatum* can be distinguished in the ordination. The number of infections per tree has a significant correlation with the ordination axis. ANOSIM analysis showed significant differences between infected and uninfected trees when they were infected by *M. punctulatum* but not by *M. linearifolium.* Differences between sites were also found, but the two sites with higher degrees of infection by *M. punctulatum* did not differ from each other. The intrapopulation genetic structure of *N. antarctica* could be maintained by the mistletoe *Misodendrum* through host selection.

Additional keywords: isozyme, *Misodendrum linearifolium*, *Misodendrum punctulatum*, *Nothofagus antarctica*, parasitic plants, Patagonia, resistance.

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Introduction

Mistletoes are aerial parasitic plants in the order Santalales that infect several of vascular plant species. Parasitic plants are those that have a physiological bridge (haustorium) through which nutrients and water are transported from one organism to the other (Kuijt 1969). In general, parasitism can be defined as a prolonged interaction in time between host and parasite (Combes 2001). The host–parasite system may show simultaneous genetic variation of the pair across the environment. Host genetics may not be the primary reason that determines the infection variation among hosts. For example *Tristerix aphyllus* (Miers ex DC.) Barlow & Wiens (Loranthaceae), an aerial parasitic mistletoe, host infection is affected by the behaviour of the seed disperser, and this is believed to have mediated its speciation from a population of *Tristerix corymbosus* (L.) Kuijt (Amico *et al*. 2007). Some hosts establish mechanical barriers to haustorium formation, as is the case for some sorghum varieties resistant to the root parasite *Striga* (Williams 1959; Arnaud *et al*. 1999). Also genetic-based mechanisms, like the salicylic acid pathway, have been suggested for host resistance in some root parasites (Yoder and Scholes 2010).

Parasitic plants vary greatly in their pathological effects upon hosts. An aerial parasite can generate changes in growth habit, for example the formation of witches' brooms by some mistletoes (Hawksworth and Wiens 1996). Severely infected hosts may have also decreased reproduction or produce sterile seeds (Hawksworth and Wiens 1996; Kuijt 1969; Press and Phoenix 2005). For some mistletoes, (e.g. *Arceuthobium vaginatum* J.Presl on *Pinus ponderosa* Douglas ex Lawson & C. Lawson) the host branch beyond the point of infection commonly dies and in extreme cases complete host death may occur (Parker and Riches 1993; Hawksworth and Wiens 1996; Aukema 2003).

The sessile nature of plants has resulted in the evolution of different strategies to resist pathogen attack. The two main (and usually mixed) defensive tactics exhibited by plants are (i) tolerance and (ii) resistance (Núñez-Farfán *et al*. 2007). These strategies may be adaptive, thus, plant species potentially differ in their ability to survive and reproduce after damage (Strauss and Agrawal 1999). Many studies in plants have focussed on tolerance and resistance to herbivory, and in parasitic plants many studies have focussed on host reaction to infection by *Striga* and *Orobanche* (Orobanchaceae) – two economically important root-parasitic weeds (Yoder and Scholes 2010). However, comparatively few studies have focussed on the reaction of plants to infection and its potential genetic component by aerial parasitic plants. For example, Koskela *et al*. (2002) found genetic variation in tolerance and resistance of *Urtica dioica* L. to the aerial holoparasite *Cuscuta*

europaea L. (European dodder). These authors concluded that the cost of host resistance and tolerance to dodder might be maintaining the genetic variation in these traits. Other studies found that non-parasitised and parasitised populations of the host plant (*U. dioica* parasitised by *C. europaea*) did not differ in gene diversity measures or inbreeding coefficients (Mutikainen and Koskela 2002).

Parasites play an important role in natural systems influencing host genetic diversity (Altizer and Pedersen 2008). In particular, parasites represent powerful selective agents, given that they rapidly spread and may negatively impact host fitness (Hawksworth and Wiens 1996; Geils *et al*. 2002). Particularly, under high parasite loads, traits conferring resistance are expected to increase in frequency. The general pattern that hosts vary in their defence against parasites is rooted in life-history theory that assumes fitness costs of immune defence and trade-offs in the face of limited resources (Schmid-Hempel and Ebert 2003). Nevertheless, this may not be the case for slow-spreading parasitic plants. Therefore, it is important to understand intra- and inter-population variation patterns in host resistance traits. Polymorphism in host resistance within natural populations may be the result of different key mechanisms. Under frequency-dependent selection, the fitness of a locally adapted parasite to the most common genotype creates rare resistant hosts (Kaltz and Shykoff 1998), whereas under balancing selection the persistence of multiple alleles, or genetic polymorphisms in a population, is favoured (Altizer and Pedersen 2008). In addition, host resistance may vary in relation to environmental heterogeneity and/or be influenced by pleiotropic effects between resistance and other fitness-conferring traits (Schmid-Hempel and Ebert 2003).

In this study, we focussed on how inherent genetic variability of the host may affect and correlate the occurrence of an aerial hemiparasite. We investigated genetic variability of *Nothofagus*, which are dominant trees in the subantarctic forest of southern South America. They host an endemic mistletoe, *Misodendrum* (Misodendraceae). As has been repeatedly reported for other mistletoe species in Viscaceae (Smith and Wass 1976; Thomson and Mahall 1983; Clay *et al*. 1985; Glazner *et al*. 1988; Linhart 1989; Linhart *et al*. 1994; Overton 1994; Snyder *et al*. 1996) and Loranthaceae (Overton 1994), mistletoe infections are not evenly distributed, i.e. some trees are free of the mistletoe whereas others are heavily infected or have only one or two infections. Even trees in close proximity may differ in infection load, which suggests genetically-determined susceptibility (Linhart and Grant 1996). In mistletoes, these patterns were attributed to either bird behaviour affecting dispersal (Martinez del Rio *et al*. 1995; Norton and Smith 1999; Aukema and Martínez del Rio 2002; Bach *et al*. 2005; Rawsthorne *et al*. 2011) or an edge effect (Bach *et al*. 2005; Norton and Smith 1999). The former does not apply to the *Misodendrum–Nothofagus* system because the fruits of this mistletoe are wind dispersed. This dispersal mechanism does not discard mistletoe aggregation due to differential dispersal as it was described for mistletoes dispersed by animals (Aukema 2003; García *et al*. 2009). *Misodendrum punctulatum* DC. seeds do not disperse far from the parent plant and the dispersal distance and within tree autoinfection depends on the height of the infection of the seed generating mistletoe (Tercero-Bucardo and Rovere 2010).

We hypothesised that host trees would show resistance or tolerance in their defence mechanism, which could be structured in their genetics. We investigated whether the genetic structure of *Nothofagus antarctica* (G. Forst.) Oerst is associated with infection patterns by *Misodendrum punctulatum* DC. and *Misodendrum linearifolium* DC. at three different populations in temperate South America.

Materials and methods

Study species

Nothofagus and *Misodendrum* are widely distributed in temperate South America, and their distributions overlap from Neuquén to Tierra del Fuego along the Andes in Argentina and from Region VIII to the south in Chile (36°30′S to 55°S). Species of *Misodendrum* parasitise *Nothofagus* trees, thereby producing tumours on the host branches (Orfila 1978) and causing metabolic changes that have a deleterious effect on wood quality (Reyes *et al*. 1986). The wide ecological range (McQueen 1976) of *Nothofagus antarctica* (G. Forst.) Oerst. in temperate forests of southern South America is attained by a combination of phenotypic plasticity and high genetic diversity (Steinke *et al*. 2008). It reproduces both by seeds and by vegetative propagation (coppices and root suckers).

Infection level and site characteristics

Patterns of genetic variation of *N. antarctica* were examined at three sites – Espejo, Guillelmo, and Steffen – located within Nahuel Huapi National Park at ~41° latitude South in north-western Patagonia, Argentina. This corresponds to the northern end of the host distribution range. Espejo is located at the northern end of the Park, and is ~150 km from the other two sites. These two sites are located at the southern end, ~20 km from each other. Selected sites corresponded to similar *N. antarctica* morphotypes that mainly propagate sexually by seed, which, in turn, are ecologically and genetically similar (Steinke *et al*. 2008).

The infection level (prevalence) was evaluated in a 300 m transect in each site. This transect was extended 1.5 m on each side. For each *N. antarctica* tree that intercept the transect the mistletoe presence/absence was recorded. Two parasite species, *Misodendrum punctulatum* DC. and *Misodendrum linearifolium* DC., were present in Steffen and Guillelmo, but only *M. punctulatum* was present in Espejo.

Within each site, 10 stations were set every 50 m along another transect. At each station two trees were chosen in each cardinal direction, one infected and one uninfected. Thus, a total of 40 infected and 40 uninfected trees per site were studied (wherever possible). A small branch from each tree with ~10 fresh leaves was collected and kept refrigerated in a portable cooler until isozyme extraction could take place in the laboratory. The number of *Misodendrum* infections (*M. punctulatum* and *M. linearifolium*) was recorded for each sampled individual. Analysis of variance was performed to detect differences in the number of infections between sites. Normality assumption was not met for the number of infections (Kolmogorov–Smirnov test $D = 0.1421$ $P < 0.01$). Thus, a non-parametric Kruskal–Wallis test was conducted with *post-hoc* comparisons using the Mann-Witney *U*-test with significance assessed by a Studentised range ($P < 0.05$). This

analysis was performed using the software 'R' Ver. 3.1.2 (R Foundation for Statistical Computing: Vienna, Austria).

Isozyme extraction, allele score and character matrix assemble

Isozyme analysis was used to genetically characterise infected and uninfected trees at each population/site. Tissues extracts were prepared by grinding two to three leaves in 1 mL of grinding buffer (Mitton *et al*. 1979). Enzyme screening was performed in three different electrophoresis buffers that resolve 14 different enzyme stains. We chose the buffers that worked repeatedly and had clear bands. A total of eight enzymes were analysed coding 12 putative loci (Adh-1,-2; Idh-1,-2; Mdh; Me; Per; 6Pgd-1,-2; Pgi-1,-2; Skdh). Electrophoresis was performed in 12% w/v starch gels using a pH 7.5 morpholine and citric acid gel buffer (MC). Following electrophoresis, enzymes were stained in 2 mm gel slices using the protocol by Mitton *et al*. (1979). Bands were scored as distance from the origin, the allele closer to the origin received lower number. The genetic control of the inheritance for these enzymes was not performed, therefore the loci analysed are considered putative. However, the enzymes and band patterns found in this study were similar to the ones found in an isozyme study of another *Nothofagus* species (Premoli 1996).

To test for genetic differences between infected and uninfected *N. antarctica* trees at the three sites, a matrix was constructed with the allozyme genotypes. Columns in the matrix corresponded to the different alleles and the rows to the individuals. Values within the matrix varied from 0 to 2 where 0 indicated the absence of an allele, 1 the allele present in a heterozygote, and 2 the allele present as a homozygote. For example, in a locus with two alleles, a homozygous individual will have a value of 2 for allele 'a' and 0 for allele 'b'. For a heterozygous individual, alleles 'a' and 'b' will both have a value of 1.

Statistical analysis

Genetic variation parameters were calculated by the total number of alleles, allelic frequencies, number of rare alleles (i.e. those with frequencies <0.1), number of exclusive alleles (i.e. those present only in one population and/or group of infected or uninfected trees), mean number of alleles/locus (A), percent of polymorphic loci, expected and observed heterozygosity, and *F*-statistics using the BIOSYS computer program (Swofford *et al*. 1997). Estimates of F_{IS}, F_{ST}, and F_{IT} following work by Weir and Cockerham (1984) were calculated in FSTAT, which also calculates the 95% confidence intervals (95% CI) for these parameters (Goudet 2001). Statistical differences in by-locus gene diversity measures (H_E) and inbreeding coefficient (F_{IS}) between infected and uninfected host trees were assessed with a *t*-test.

A non-metric multidimensional scaling ordination (Kruskal 1964*a*, 1964*b*) using Bray-Curtis dissimilarity index was performed using the allele matrix. Ten random start and 200 iterations were run. Analysis of similarity (ANOSIM, (Clarke 1993) was performed to relate possible groups in the ordination with *a priori* known groups; significance was based on a 10 000 permutation test. The groupings were: (1) infected and uninfected trees; (2) infected and uninfected trees by *M. punctulatum*; (3) infected and uninfected trees by *M. linearifolium*; and (4) site.

The number of infections by the two mistletoe species was correlated with the ordination axis; significance values were obtained by 10 000 permutations. These vectors of maximum correlation helped to visualise the change of these variables in ordination space. This analysis were performed in DECODA Ver. 3.0 (Minchin 2004).

A total of 89 uninfected and 77 infected trees were analysed. The reduction of sample size to 166 from the maximum possible sample size was due to the exclusion of missing values for some loci and some sites had smaller sample sizes owing to differences in infection level.

To determine the influence of frequency-dependent selection (i.e. whether common host genotypes were more infected by *Misodendrum* than rare ones), a matrix of isozyme genotype by individual was generated. This matrix consisted of 0 and 1 if an allele was absent or present, respectively. Alleles with missing data were excluded from the matrix. A total of 18 alleles were used to build the matrix. The frequency of these genotypes was calculated for each site together with the number of infections by each *Misodendrum* species. Linear regression between genotype frequency and number of infections was calculated for each site and for each *Misodendrum* species. Boxplots for each of the genotype frequency classes were made. These analyses were performed in the software 'R' Ver. 3.1.2 (R Foundation for Statistical Computing).

Results

Degree of infection at each site

The infection level measured in the 300 m transect was equivalent at the three sites: 71% Espejo and Steffen, and 86% Guillelmo. However, Guillelmo and Espejo were the two sites with the highest infection level for *M. punctulatum*, at 71 and 33%, respectively, compared with Steffen at only 2%. The average number of mistletoes per tree differs among sites (Kruskal–Wallis $\chi^2 = 14.81$, d.f. = 2, $P < 0.01$), with Steffen having the smallest number of mistletoes per tree (Table 1).

Isozyme data

Seven of the 12 loci were polymorphic and all three sites shared most alleles (69%). Nonetheless unique alleles were present in Espejo (3), Guillelmo (2), and one in Steffen. In Steffen site unique alleles were shared between individuals infected and not infected by *Misodendrum*. In contrast, in Espejo and Guillelmo unique alleles were either from infected or uninfected trees (Table 2). Pooled populations in relation to the degree of infection yielded one and five unique alleles in resistant and susceptible trees respectively (Table S1, available

Table 1. Mean number (s.e.) of mistletoes (*Misodendrum*) per tree (*Nothofagus antarctica*)

Differences on degree of infection between sites were tested with Kruskal–Wallis. Values followed by different letters are significantly different ($P < 0.05$)

Site	*Misodendrum punctulatum*	*Misodendrum linearifolium*
Espejo	7.95a (0.92)	0.00a (0.00)
Guillelmo	3.84b (0.74)	6.11b (0.90)
Steffen	0.91c (0.28)	3.43b (0.60)

Table 2. Genetic variability measurements (s.e.) for *Nothofagus antarctica* trees uninfected (UI) and infected (I) by *Misodendrum punctulatum* at the three sites

	Espejo UI	I	Guillelmo UI	I	Steffen UI	I
Mean number of individuals/locus	20.1 (1.3)	47.5 (4.2)	21.5 (2.1)	36.1 (3.7)	35.6 (2.9)	15 (1.1)
Total number of alleles	19	21	16	21	18	16
Number of rare alleles	5	7	1	6	4	1
Exclusive alleles	1	2	0	2	0	0
Mean number of alleles/locus (A)	1.6 (0.3)	1.8 (0.3)	1.3 (0.2)	1.8 (0.3)	1.5 (0.2)	1.3 (0.2)
% Polymorphism (*sensu stricto*)	41.7	50.0	16.7	50.0	25.0	41.7
Expected Heterozygosity (H_E)	0.080 (0.047)	0.090 (0.040)	0.079 (0.047)	0.110 (0.054)	0.088 (0.051)	0.082 (0.055)
Observed Heterozygosity (H_O)	0.047 (0.020)	0.023 (0.013)	0.024 (0.014)	0.036 (0.018)	0.043 (0.032)	0.029 (0.024)

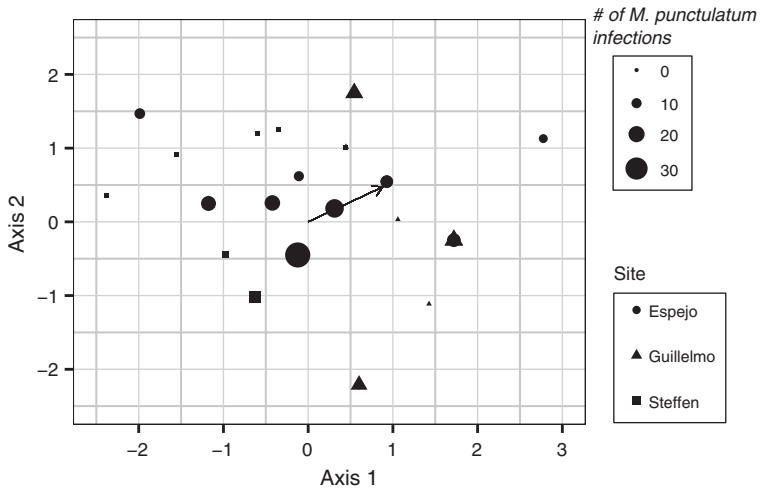

Fig. 1. Direction of the maximum correlation vector corresponding to the infection of *Misodendrum punctulatum* by means of a non-metric multidimensional scaling ordination analysis (NMDS). I and UI stand for infected and uninfected hosts respectively at each site.

as Supplementary Material to this paper). The mean number of alleles per locus was similar between Espejo (1.9) and Guillemo (1.8) and less in Steffen (1.5). The percentage of polymorphic loci (*sensu stricto*) was highest in Espejo (66.7%) followed by Guillelmo (50%) and less in Steffen (41.7%). All sites showed similar values for observed heterozygosity (Table 2) and these were approximately one third of the expected heterozygosity. These results suggest inbreeding in all sites. The F_{ST} over all loci was 0.059 (95% CI = 0.011–0.077) indicating most variability resides within each site. The overall inbreeding coefficient (F_{IS}) was significantly greater than zero (0.617, 95% CI = 0.530–0.851) indicating a significant deficiency of heterozygotes on average.

Genetic characterisation of infected and uninfected hosts

The uninfected host trees showed lower mean number of alleles per locus (1.8 vs 2.1), and had fewer total alleles (21 vs 25), low frequency (i.e. rare) alleles (6 vs 10), and unique alleles (1 vs 5) than infected ones. These results indicate that infected trees were more genetically diverse than uninfected ones. This is still evident when each site is examined independently, with the exception of Steffen, which, in turn, had the least number of individual infections (Table 2). The expected heterozygosity in uninfected trees was about twice the observed heterozygosity, whereas in infected trees it was three times higher. This indicates a lower number of heterozygotes than expected under Hardy–Weinberg equilibrium; which could be attributed to a higher inbreeding coefficient. However, the F_{IS} between uninfected (mean $F_{IS} = 0.524$, s.e. = 0.170) and infected (mean $F_{IS} = 0.611$, s.e. = 0.134) did not differ significantly ($t = –0.382$, d.f. = 14, $P > 0.05$), possibly owing to great variance of such values among loci. In terms of average gene diversity, the infected (H_E infected = 0.103, s.e. = 0.049) and uninfected hosts (H_E uninfected = 0.081, s.e. = 0.043) did not differ significantly ($t = –0.342$, d.f. = 22, $P > 0.05$).

The study sites differed significantly in their allele composition (ANOSIM test $r = 0.068$, $P < 0.01$) with Steffen being different from the other two (Steffen-Espejo: $r = 0.078$, $P < 0.01$; Steffen-Guillelmo: $r = 0.130$, $P < 0.01$). Also *N. antarctica* individuals infected by *M. punctulatum* had different allele makeup than the ones not infected by this species ($r = 0.2061$, $P < 0.01$). As can be seen in the ordination, individuals with more infections of *M. punctulatum* corresponded also to individuals from Espejo and Guillelmo (Fig. 1). The variable 'number of infections by *M. punctulatum*' was the only one significantly correlated with the ordination axis (Table 3).

A total of 33 genotypes were found in the 181 individuals from the three sites. These genotypes were assembled by pooling 18 alleles from the total 26 alleles derived from 14 isozymes (eight were excluded because of missing values). Sixteen of these 33

genotypes were unique to one individual (nine from Espejo, four from Guillelmo, and three from Steffen), and did not show any correspondence with infection status. Pooling the three sites together, the other genotypes vary in frequency from 0.01 to 0.32. At each individual site, the genotype frequencies (not counting individual unique genotypes) vary from 0.03 to 0.41 in Espejo, from 0.03 to 0.25 in Guillelmo and from 0.03 to 0.29 in Steffen. No genotype had a frequency higher than 50% at any site and the most common genotype in all three sites was the same. The second most common genotype varied between sites: in Espejo it was genotype 3 with a frequency of 0.21, which was shared with the other two sites; in Guillelmo it was genotype 16 with a frequency of 0.23 which was found only there; and in Steffen it was genotype 7 with a frequency of 0.12 which was shared also with the other two sites. Guillelmo was the site with a higher number of unique genotypes (five not counting the ones present in only one individual). These genotypes had relatively high frequencies (one with 0.23, two with 0.12, and two with 0.03). Steffen also had 4 unique genotypes but with lower frequencies (0.05 and 0.03). Espejo had two unique genotypes with low frequency (0.03).

No linear relationship was found between the number of infections by any of the mistletoe species (or both together) and the genotype frequency in any site ($F_{\text{Espejo-}M.\ punctulatum} = 0.0003$, $P > 0.05$; $F_{\text{Guillelmo-}M.\ punctulatum} = 0.027$, $P > 0.05$; $F_{\text{Steffen-}M.\ punctulatum} = 1.83$, $P > 0.05$; $F_{\text{Guillelmo-}M.\ linearifolium} = 0.188$, $P > 0.05$; $F_{\text{Steffen-}M.\ linearifolium} = 0.25$, $P > 0.05$). More frequent genotypes do not show higher number of mistletoe infections in any of the sites (Fig. 2). Most genotypes (60–70%) had between zero and five *Misodendrum* infections.

Table 3. Correlation of variables with NMDS ordinations axis, *P*-values correspond to a 10 000 permutations test

Significant differences are indicated: *, $P < 0.0.5$

Variable	N	MAX R	*P*-value
Infections of *Misodendrum linearifolium*	166	0.0669	0.688
Infections of *Misodendrum punctulatum*	166	0.2061	0.029*
Infected by *Misodendrum* (yes/no)	166	0.1606	0.123
Infected by *M. linearifolium* (yes/no)	166	0.1614	0.113
Infected by *M. punctulatum* (yes/no)	166	0.1693	0.089
More than five infections of *M. linearifolium*	166	0.1248	0.29
More than five infections of *M. punctulatum*	166	0.222	0.018*

Discussion

The three study sites differed in the *Misodendrum* infection level and they were also genetically distinct. Trees in Espejo had higher numbers of infections of *M. punctulatum* than those in the other two sites. We noted that at Steffen and Guillelmo, *N. antarctica* trees were infected by two species of *Misodendrum*, whereas in Espejo they were only infected by *M. punctulatum*. This fact could explain the differences found in the load of *M. punctulatum* per host tree among sites (Table 1). As the other species of mistletoe *M. linearifolium* was found in Steffen and Guillelmo, it is possible that the two species compete for space on a particular tree, thus setting a maximum load that a host can carry. This could be an explanation for the lower number of *M. punctulatum* infections in Steffen and Guillemo, but no difference in the total number of *Misodendrum* spp. infections between the three locations. One important fact is that *M. linearifolium* sprouts immediately after infection occurs, whereas *M. punctulatum* sprouts two years afterwards (Tercero-Bucardo and Kitzberger 2004). So at sites where both species are present, *M. linearifolium* can gain an advantage in acquiring 'host space'.

Allozyme differences were found between uninfected and infected trees by *M. punctulatum*; however, allozyme differences were also found between sites. Deficiency of heterozygotes was found in the three studied populations of *N. antarctica*, which agrees with the previous study by Steinke *et al*. (2008) and with the biology of the species. *Nothofagus antarctica* is a frequent resprouter and with occasional establishment by sexual means. Moreover, it has a low germination rate (Premoli 1991) that results in the spatial aggregation of similar genotypes (Premoli and Steinke 2008). These characteristics together with the genetic

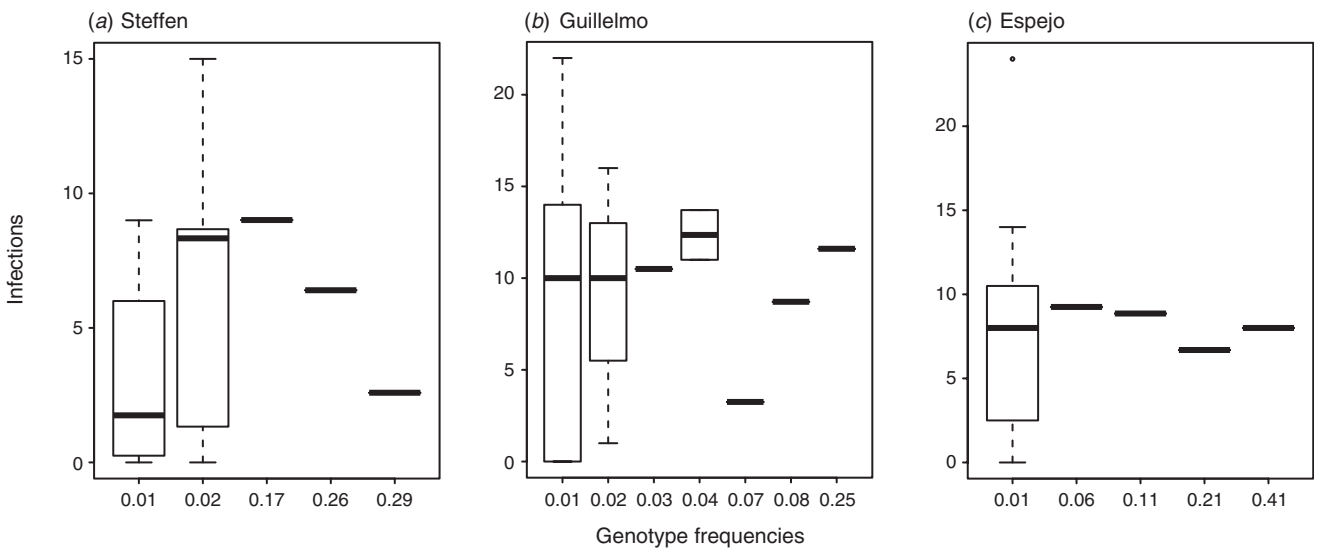

Fig. 2. Boxplot of mistletoe infections for each genotype frequency in each site: (*a*) Steffen, (*b*) Guillelmo and (*c*) Espejo.

difference between uninfected and infected trees could explain the patchy distribution of mistletoes in one stand.

The two sites with more infections by *M. punctulatum* did not differ from each other genetically but both sites differed from Steffen with only 2% of infection by this species of mistletoe. Numerous study cases exist in the literature of plant-herbivore and plant-pathogen interactions that have shown genetic variation in resistance, tolerance or both (Koskela *et al*. 2002). For example, genetic-based susceptibility occurs when some hosts lack parasite resistance and they tend to acquire the infection first, with resistant individuals acquiring the infection later or at a slower rate (Medel *et al*. 2004). This, in turn, may be genetic or environmentally influenced. The evidence presented here suggests that some allozyme differences exist between infected and uninfected trees, which maybe also related to the percentage of trees infected at the site. Although Steffen had the lowest genetic diversity, it also had one unique allele shared between resistant and susceptible trees. In contrast, unique alleles were private for susceptible or resistant trees in Espejo and Guillelmo. These two populations resulted in being the most genetically diverse. Particularly Guillelmo show the highest gene diversity and number of individual infections in addition to the presence of both species of mistletoe.

Genetically homogenous host populations are expected to have the highest prevalence and intensity of disease outbreaks (e.g. Ganz and Ebert 2010). In contrast, host heterogeneity may slow the spread of the infection, although may also increase the susceptibility to a wider range of parasites (van Baalen and Beekman 2006). It has been suggested that root and shoot parasites often perform better on hosts with a high nitrogen content (Press and Phoenix 2005) or those with accessible vascular systems (Kelly *et al*. 1988) and/or lower defence capacity (Cameron *et al*. 2005, 2006). Polymorphism in nitrogen content and in ramification rate exist among *N. antarctica* populations (Steinke *et al*. 2008). Although such differences in *Nothofagus* were measured at the population level, our study shows that the degree of infection may be acting at the individual level as a selective force maintaining genetically diverse populations. In particular, balancing selection may favour the long-lasting persistence of genetic polymorphisms for parasite resistance within host populations (Altizer and Pedersen 2008). This may be reinforced by spatial and temporal heterogeneity in host defences partially driven by environmental factors (Rolff and Siva-Jothy 2003).

Many studies have shown local adaptation of the parasite (Kaltz and Shykoff 1998; Lively and Dybdahl 2000; Gandon and Michalakis 2002; Laine 2005); however, most studies focus on animal parasites with little attention for parasitic plants. Two contrasting studies on different species of root parasitic plants, reported either local adaptation of the parasitic plant *Cuscuta* (Koskela *et al*. 2002) or absence of it in the hemiparasitic plant *Rhinanthus* (Mutikainen *et al*. 2000). Local adaptation may occur when a parasite increases its fitness by specialising on the most common host genotype (Kaltz and Shykoff 1998). We did not find evidence of local adaptation for the mistletoe *Misodendrum* since most common genotypes did not support more parasite infections.

The intrapopulation genetic structure of *N. antarctica* could be maintained by the mistletoe *Misodendrum* through host selection by the hemiparasite, or the other way round. This is to say that the host tree intrapopulation genetic structure could be maintained by a resistance mechanism of the host. In another species of *Nothofagus*, *Nothofagus pumilio*, tissue necrosis has been observed in certain trees around an infection of *Misodendrum*, thereby preventing mistletoe development (N Tercero Bucardo pers. comm). Genetic differences could be correlated with variation in another trait related to a defence mechanism of the host. However, this was not evaluated in the present study.

Several other studies on root parasites have focussed on parasite selective pressure, looking at host growth, reproduction, and local adaptation (Joshi *et al*. 2000; Koskela *et al*. 2000, 2002; Mutikainen *et al*. 2000). Results presented in this study indicate a genetic structure associated with the mistletoe, especially to *M. punctulatum*. Therefore, future studies should focus on measuring the fitness of the mistletoe grown on infected and uninfected host trees and investigate the defence strategy (tolerance/resistance) that has evolved in *Nothofagus* trees in response to *Misodendrum* infections.

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