Running head: Habitat transformation alters ecoevolution

**The hidden costs of living in a transformed habitat: ecological and
evolutionary consequences on a tripartite mutualistic system with a
keystone mistletoe**

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ABSTRACT

Land use change is one of the most important anthropogenic drivers of biodiversity loss. Nevertheless, the ecological and evolutionary consequences of habitat transformation remain less understood than those from habitat fragmentation. Transformed habitats are structurally simpler, altering species composition and their ecological interactions, potentially compromising gene flow and genetic diversity. We focused on a tripartite mutualistic system composed of a mistletoe (*Tristerix corymbosus*), its pollinator (*Sephanoides sephanioides*) and its seed disperser (*Dromiciops gliroides*) to assess changes on their ecological and evolutionary dynamics as a result of habitat transformation. We used eight microsatellite markers to compare genetic diversity, relatedness and gene flow among five mistletoe groups inhabiting native and transformed habitats (abandoned *Eucalyptus globulus* plantations). We found that these groups were genetically structured, with greater allelic richness and genetic diversity in their native habitat. Also, we found higher relatedness among mistletoe individuals in transformed habitats, which varied as a function of the geographic distance among plants, probably as a result of larger resource availability, which influenced mutualist visitation rates. We did not find differences in the current migration patterns, which suggests that *Tristerix corymbosus* may be resilient to habitat transformation, yet its highly specialized interactions along with changes in its spatial configuration depict a more complex scenario, which probably impose a cost in terms of lower genetic diversity and increased relatedness that might compromise its long-term viability.

Keywords: *Eucalyptus* plantation, microsatellite markers, relatedness, *Tristerix corymbosus*, spatial structure, South American temperate rainforest.

INTRODUCTION

Humans have altered almost every ecosystem on Earth, leading to habitat loss, fragmentation, degradation and profound transformation (Chapin III *et al.*, 2000; Corlett, 2015; Didham *et al.*, 2012; Fahrig, 2003; Ghazoul *et al.*, 2015; Perez-Mendez *et al.*, 2016; Sala *et al.*, 2000). Our understanding of the consequences of land use change as a driver of biodiversity loss has dramatically changed in the past two decades thanks to the development of new techniques including molecular approaches (Epps and Keyghobadi, 2015; Sunnucks, 2000). Such advancements have enabled the study of whole communities and their interactions rather than individual species, and the study of processes instead of patterns (Escudero *et al.*, 2003; Valiente-Banuet *et al.*, 2015). Therefore, assessing the effects of anthropogenic disturbance on natural ecosystems, using field methods and molecular markers, will also provide insight into their long-term consequences (Carpenter, 2002; Melo *et al.*, 2013).

One type of habitat transformation considers the total or partial replacement of the native vegetation by a single or multiple exotic species (Fontúrbel *et al.*, 2015), usually for commercial purposes (e.g., the establishment of *Pinus* spp. or *Eucalyptus* spp. plantations). According to FAO, 264 million ha were covered by exotic forest plantations around the world by 2015 (<http://www.fao.org/forestry/fra>). Unlike fragmented habitats, transformed habitats usually lack spatial discontinuities, resulting in habitat mosaics of native and transformed stands at the landscape level (Salazar and Fontúrbel, 2016). Although many native animal and plant species are capable of persisting in these mixed landscapes (García *et al.*, 2013; Lancaster *et al.*, 2011), transformed habitats can impose restrictions on ecological connectivity that can compromise gene flow at the landscape scale (Albert *et al.*, 2013; Lancaster *et al.*, 2011). Such restricted gene flow may result from the combination of

many possible changes, for example in pollinator and/or seed disperser behaviour (Lavabre *et al.*, 2014; Sasal and Morales, 2013), plant recruitment (Bravo *et al.*, 2015), habitat structure (Castaño-Villa *et al.*, 2014), microclimatic conditions (Fontúrbel and Medel, 2017), plant-animal interactions (Neuschulz *et al.*, 2016), plant-plant interactions (Candia *et al.*, 2014), spatial structure (Fontúrbel *et al.*, 2015) and neighbourhood effects (Lázaro *et al.*, 2014). There are two ecological interactions that determine most of plant gene flow across the landscape: pollination and seed dispersal. Animal pollinators and seed dispersers act as mobile links across the landscape (González-Varo *et al.*, 2017), but habitat transformation can affect both quantitative and qualitative components of pollination and seed dispersal interactions (Fontúrbel *et al.*, 2017a). These changes may alter landscape-level gene flow, changing genetic diversity and relatedness among individuals, potentially damaging their evolutionary potential and raising extinction probabilities (Carvalho *et al.*, 2016; Lancaster *et al.*, 2011). However, despite being a major driver of biodiversity change (Albert *et al.*, 2013), habitat transformation remains little known, particularly regarding its long-term consequences.

South American temperate rainforests (SATF hereafter) are facing increased habitat loss and degradation due to activities such including the establishment of exotic forestry plantations. The Valdivian Coastal Reserve (VCR), which protects 46 900 ha of native forest remnants, results from a failed private endeavour to establish 3,100 ha *Eucalyptus globulus* plantations (established by clear-cutting native forest 12-20 years ago, but never harvested or managed) on its 50,000-ha property. In this area, native forest stands and transformed habitats (abandoned *Eucalyptus* plantations with regeneration of native understory) form a complex and intertwined landscape mosaic.

A key species inhabiting the VCR is the hemiparasitic mistletoe *Tristerix corymbosus* (Loranthaceae), a winter-flowering plant that parasitises a range of host plants, and it is considered a keystone species of the SATF. In winter, *T. corymbosus* represents almost the only food source for its sole pollinator, and during summer represents a major food source for the seed disperser species (Aizen, 2003, 2005). This species features highly specialized interactions with its pollinator and seed disperser mutualists, with pollination being almost exclusively provided by one hummingbird species (*Sephanoides sephaniodes*), while seeds are dispersed by an arboreal marsupial (*Dromiciops gliroides*) (Aizen, 2005; Amico *et al.*, 2011). Thus, *T. corymbosus*, with its highly specialized interactions, offers a model to study the ecological and evolutionary effects of habitat transformation, since it is able to persist in both native and transformed habitats (Fontúrbel *et al.*, 2015).

We used the above species to ask whether anthropogenic habitat transformation affect the ecological and evolutionary dynamics of a mutualistic system. To address this aim, we determined the population structure, genetic diversity, relatedness and migration patterns of mistletoes inhabiting native and transformed habitats and changes in visitation rates of its pollinator and seed disperser mutualists due to habitat transformation. We hypothesized that changes in habitat structure and vegetation composition will impair mistletoe gene flow across the landscape and consequently increase its relatedness and reduce its genetic diversity, due to changes in pollination and seed dispersal. We expected mistletoes in transformed habitat to be more related and have less genetic diversity due to lower gene flow among populations.

MATERIALS AND METHODS

Study site and sampling protocol

We conducted this study in the Valdivian Coastal Reserve (VCR; 39°57'S, 73°34'W), a 50,000-ha private protected area, owned and managed by The Nature Conservancy (Delgado, 2010). This reserve protects an important fraction of the Valdivian rainforest ecosystem, considered a biodiversity hotspot due to its high proportion of endemic species (Mittermier *et al.*, 2005). The VCR features a complex mosaic composed of native and transformed habitats. The native forest habitat comprises of both old- and secondary-growth stands. In old-growth stands the dominant species are *Nothofagus dombeyi*, *N. pumilio*, *Fitzroya cupressoides*, *Laurelia philippiana*, *Lomatia ferruginea*, and *Mitraria coccinea*; whereas in the secondary forest the dominant species are *N. pumilio*, *N. dombeyi*, *Eucryphia cordifolia*, *Drimys winteri*, *Tepualia stipularis*, *M. coccinea* and *Chusquea quila*. The transformed habitat is dominated by the exotic tree *Eucalyptus globulus*, coexisting with many shade-intolerant understory native plants, such as *Rhaphithamnus spinosus*, *Aristotelia chilensis*, *Lapageria rosea* and *C. quila* (Fontúrbel *et al.*, 2015).

Between 2013 and 2015, we obtained samples from 123 adult *T. corymbosus* plants in the study area, representing a large fraction of remaining individuals in the VCR. We collected three to four young leaves from each mistletoe (one individual per host, to prevent sampling clones and resprouts), which were immediately stored in Ziploc bags, dried using silica gel, and kept dry until DNA extraction. We georeferenced sampled mistletoes, which came from five sampling locations harbouring the large majority of VCR plants, two in what could be classified as native habitats: N1 (N = 21; 39°59'25.71"S, 73°40'33.33"W; evidence for some disturbance by illegal logging), N2 (N = 20; 39°58'16.35"S, 73°36'13.28"W; pristine); and three in what could be classified as transformed habitats: T1

(N = 20; 39°57'0.46"S, 73°38'58.28"W), T2 (N = 22; 39°57'26.45"S, 73°39'13.79"W), T3 (N = 21; 39°57'57.42"S, 73°39'8.88"W) (Fig. 1). Sampling sites were ascribed *a priori*, based on the spatial clusters, obtained by georeferencing each plant. Sampling sites were classified either as native (N) or transformed (T) according to the dominant vegetation type, following Fontúrbel *et al.* (2015), on aerial photographs and on field surveys. Sampling sites were separated by between 1 and 6 km, as the largest movement distance of the seed disperser (*D. gliroides*) is recorded as 500 m (Fontúrbel *et al.*, 2012).

<Figure 1 about here>

Microsatellite amplification

Genomic DNA was isolated from the 123 samples collected in the field (51 and 72 from native and transformed habitats, respectively) using a DNEasy plant mini kit (QIAGEN, Valencia CA) using ~10-20 mg of dry plant material, ground using a Mini-Beadbeater-96 device (BioSpec Products, Bartlesville OK; Fontúrbel *et al.* (2016). We used the 10 species-specific microsatellites and conditions described by Fontúrbel *et al.* (2016). PCR products were genotyped in the sequencing core at Pontificia Universidad Católica de Chile (PUC), using the internal size standard LIZ 500 (Applied Biosystems, Foster City CA).

Descriptive statistics and population structure

We used GENEMARKER 1.85 software to build an allelic matrix and MICRO-CHECKER 2.2.3 software (Van Oosterhout *et al.*, 2004) to identify possible genotyping error and null alleles in the data. We estimated linkage disequilibrium for all pairs of loci and deviations from the Hardy-Weinberg Equilibrium (HWE) using GENETIX 4.5.2

(Belkhir *et al.*, 2004). To assess the representativeness of our sampling, we calculated rarefied allelic richness using the package hierfstat (Goudet, 2014) in R 3.4.2 (R Development Core Team, 2017) and estimated allele sampling effectiveness as the ratio between observed and expected allelic richness.

We used three approaches to determine population structure. First, we used GENETIX to estimate genetic differences between pairs of sites using F_{ST} (Weir and Cockerham, 1984) with 10,000 permutations to estimate statistical significance. To reduce Type I error, we applied a Bonferroni correction with $\alpha = 0.005$. To test for a geographical association with population differentiation, we conducted a Mantel test with 1,000 permutations using the GENETIX. Geographic distances between sites were estimated using ArcGIS 10.2 (ESRI, Redlands CA). Second, we used STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) to estimate the most likely number of Bayesian clusters present in the sample using the admixture and LOCPRIOR models, 400,000 burnin simulations, 1M analysed simulations and tested K=1 to K=8. Finally, we used GENELAND 4.0 (Guillot *et al.*, 2005) to determine the number of spatially explicit population clusters, run 10 times using correlated allele frequency model without spatial uncertainty in the locations (as recommended for plants, given that they are sessile organisms), with 500 000 iterations and thinning set at 500.

We estimated the number of alleles (N_A), allelic richness (AR) and genetic diversity (GD) for each population using GENETIX and FSTAT 2.9.32 (Goudet, 1995), respectively. We tested for differences in these parameters among populations from native and transformed habitats using a Wilcoxon signed rank test in R. We used the random mating model option and a critical allele frequency value of 0.05 (alleles with frequency

0.05 were excluded). Then we used the BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996) to determine if the assessed populations had undergone a recent bottleneck episode. A two-phase mutation model was applied with a 70 % stepwise mutation, and we assessed the significance using its prescribed Wilcoxon test.

Relatedness and its relationship with distance

To ensure that the sampled individuals represented a random subset of the population, we estimated relatedness according to Queller and Goodnight (1989), using the r_{xy} estimator calculated with IDENTIX 1.1 (Belkhir *et al.*, 2002). Within each population, the null hypothesis for the random distribution of related individuals was tested by using 1,000 permutations of the alleles present. We also estimated pairwise relatedness coefficients among the 104 mistletoes genotyped, and then constructed a relatedness matrix and performed a permutation ANOVA to compare relatedness values between native and transformed habitats. GPS coordinates for each genotyped sample were used to construct a distance matrix using PASSaGE 2 (Rosenberg and Anderson, 2011) and pairwise distances between habitats were compared using a permutation ANOVA.

We used the ade4 (Dray and Dufour, 2007), adegenet (Jombart, 2008) and PopGenReport (Adamack and Gruber, 2014) R packages to obtain genetic and geographic Euclidean distance matrices. Then, we estimated the correlation between relatedness and geographic distance matrices using a Mantel test (9,999 permutations). To estimate a threshold geographic distance of relatedness between plants, we conducted spatial autocorrelation (Vekemans and Hardy, 2004), using GenAlEx 6.5 (Peakall and Smouse, 2012) and obtained separate correlograms for each habitat type, using 10 distance classes in each case (distance class size was 1 000 m for the native habitat and 250 m for the

transformed habitat). The significance of each distance class and the correlation was estimated after 9,999 bootstraps.

Gene flow

To estimate contemporary migration (m), we used BAYESASS 3.0 (Wilson and Rannala, 2003) with a burn-in of 3,000,000 iterations, 30,000,000 iterations with sampling at 100 iterations and mixing parameters for allele frequencies, migration rates and inbreeding coefficients were defined as 0.5, 0.3, and 0.5, respectively. We performed five independent runs starting with different random seeds; results are expressed in terms of the average value of these five independent runs. To determine differences between both types of habitat we compared the current immigration rates of the native and transformed populations using a permutation ANOVA with the ImPerm package in R (Wheeler and Torchiano, 2016).

Resource availability, pollination and seed dispersal

To gain insight on the potential effects of the habitat transformation on ecological interactions and relate this to the genetics effects detected, we studied the relationship between resource availability (i.e., flowers and fleshy fruits) and the visitation rates of *S. sephaniodes* and *D. gliroides*. We monitored 24 plants (12 from each habitat type) using infrared camera traps (Bushnell Trophy Cam model 2011) following (Fontúrbel *et al.*, 2015; Fontúrbel *et al.*, 2017b). Plants were monitored at the beginning (March 2015) and peak (August 2015) of the flowering season, and at the beginning (November 2015) and peak (January 2016) of the fruiting season. Camera-traps (one per plant) were simultaneously operated for 72 continuous hours. We counted the number of flowers and

fruits on the monitored plants and the number of flowers and fruits on other mistletoes in a 250-m radius from the focal plant, as well as the number of flowers and fruits of other co-flowering / co-fruiting species within this area.

We quantified our visitation results as the number of effective visits (i.e., actual contact with the flower or fruit consumption). As resources could influence pollinators and seed dispersers in many ways, we expressed flower and fruit availability in three dimensions: (1) *T. corymbosus* flowers / fruits alone, (2) other co-flowering / co-fruiting plants with flowers / fruits, and (3) the total number of flowers / fruits (i.e., resources from both the mistletoe and the accompanying plants), enabling potential interaction triggers to be considered (i.e., the mistletoe resource itself, the resources of the neighbouring plants, or the combination). This kind of count data is better suited for examining concordances than cause-effect relationships in a more traditional way (Li *et al.*, 2012). Therefore, we examined the spatial concordance between the number of visits made by the pollinator / seed disperser animals with the resource availability in each of the three dimensions described above using the SADIE (Spatial Analysis by Distance Indices; (Perry *et al.*, 2002; Perry *et al.*, 1999). This approach uses two count variables (in this case, pollinator or disperser visits and number of flowers or fruits of each resource dimension) sharing the same geographic coordinates (i.e., the location of each mistletoe). SADIE works in two phases: first the degree of the clustering of each variable is estimated separately, followed by an association index (X_p) that ranges between 1 (complete association; e.g., where there are more flowers we register more hummingbird visits) and -1 (complete dissociation; e.g., where there are more fruits we register less marsupial visits), values not significantly different from zero indicate spatial independence. SADIE analysis used SADIEShell 2.0 (Conrad, 2001). We conducted separate analyses for the March, August, November and

January datasets. As we performed multiple comparisons, P-values were internally adjusted using a sequential Bonferroni adjustment.

RESULTS

Descriptive statistics and population structure

We were only able to retain 104 of 123 samples for the analysis, due to inconsistent genotype quality for 19 individuals. We used eight out of the ten microsatellite markers available, as the locus TRIS_80 showed evidence of null alleles in the five groups, while the locus TRIS_84 showed consistent deviations from the HWE at all sites. A summary of the characteristics of these loci is shown in Table S1 (available online as Supplementary Information). Overall allele sampling effectiveness was 86.6%, ranging between 81.4 and 92.1% among sampling sites.

The global F_{ST} in the VCR was 0.108 ($P < 0.001$), while pairwise analysis suggested that each sampling site comprised a separate genetic group, as F_{ST} values showed significant differences among all pairwise comparisons ($P < 0.005$) with values ranging from 0.071 to 0.132 (Table 1). STRUCTURE also showed a maximum likelihood value at $K = 5$ (Fig. S1), assigning 99% of individuals to their correct sampling site (Table S2). Similarly, GENELAND showed evidence of five populations in 100% of the 10 runs (Fig. 2a – 2f), assigning 97.1% of the individuals to their sampling site (Table S3). However, the Mantel test showed no evidence of a relationship between F_{ST} and geographic distance among populations ($r = -0.051$, $P = 0.649$). Thus, the three different methods used consistently inferred five mistletoe populations in the VCR. Thus, the genetic structure retained for subsequent analyses considered the presence of five populations: T1, T2, T3, N1, N2, which matched our sampling sites.

<Table 1 and Figure 2 about here>

The difference in N_A was marginally significant between native and transformed habitats (Wilcoxon signed rank test, $V = 14$, $P = 0.053$). Also, we found significant differences between native and transformed habitats for AR ($V = 19$, $P = 0.047$) and GD ($V = 35$, $P = 0.008$), in both cases transformed habitats showed lower values than native habitats (Table S4, Fig. S2; Table S5). For the bottleneck analysis, only one population show evidence for a recent bottleneck event (N_1 ; $P = 0.004$).

Relatedness and its relationship with distance

Individuals at all sampling sites were on average unrelated (global $r_{xy} = -0.014$, $P = 0.190$; Table S6). However, we found a significant difference between habitats (permutation ANOVA $F = 37.69$, $P < 0.001$), where mean values of relatedness were higher in transformed habitat (0.18 ± 0.04 (mean $\pm$ 1SE), $N = 63$) than in native habitat (0.07 ± 0.04 , $N = 41$). When examining geographic distance among individuals, we found the minimum pairwise distance to be 3.61 m in the native habitat and 1 m in the transformed habitat. Their mean pairwise distance among plants in the different habitats was significantly different (permutation ANOVA $F = 273.60$, $P < 0.001$), and was larger in native (1168.00 ± 247.75 m, $N = 41$) than transformed habitat (118.82 ± 15.46 m, $N = 63$), showing that as distances among plants decrease, their relatedness values increase. This relationship was confirmed by correlating genetic and geographic distances, which showed significant RV correlation coefficients at both habitats (native: $RV_{coef} = 0.357$, $P < 0.001$; transformed: $RV_{coef} = 0.225$, $P < 0.011$). Further, we found positive and significant spatial genetic autocorrelation at 1,000, 3,000 and 4,000 m for the native habitat, whereas at the transformed habitat we only found positive significant autocorrelation at 250 m, which

suggests that gene flow at the transformed habitat is more spatially limited than at the native habitat (Fig. 3).

<Figure 3 about here>

Gene flow

While gene flow estimates were higher for transformed habitat (Fig. 4), we found that the total immigration rate for native and transformed populations was not significantly different ($F = 0.17$, $P = 0.800$). Gene flow among populations ranged between 1.5 and 18.5%, being most of the recruitment (73 to 92%) originated in the same population. Populations from the transformed habitat had the greatest contribution to other populations, whereas the native habitat populations had the least contribution (Table S8).

<Figure 4 about here>

Resource offer, pollination and seed dispersal

At the beginning of the flowering season (March), flowers were more abundant in the transformed than the native habitat (616 ± 184 vs. 291 ± 26 flowers per plant), but no significant associations between *S. sephaniodes* pollination visits and flower availability were detected at any level or habitat. At the peak of the flowering season (August), flower abundance was similar between native and transformed habitats (112 ± 11 vs. 149 ± 23 flowers per plant, respectively), and both *T. corymbosus* flower abundance and the total number of flowers were spatially associated with the number of *S. sephaniodes* visits ($X_p = 0.537$, $P = 0.039$ and $X_p = 0.526$, $P = 0.042$, respectively) in native but not transformed habitat during this period.

For seed dispersal at the beginning of the fruiting season (November), fruit abundance was similar between native and transformed habitats (241 ± 36 vs. 202 ± 28

fruits per plant, respectively) and we found a significant association between *D. gliroides* visits and the number of *T. corymbosus* fruits in native habitat ($X_p = 0.714$, $P = 0.007$). At the peak of the fruiting season (January), fruits were similar between native and transformed habitats (139 ± 29 vs. 121 ± 25 fruits per plant, respectively) and here we found a significant association between *D. gliroides* visits and the number of *T. corymbosus* fruits in transformed habitat ($X_p = 0.611$, $P = 0.011$; Table S7).

DISCUSSION

We found striking population structure for *Tristerix corymbosus* inhabiting the VCR and mistletoe populations in transformed habitats showed lower genetic diversity and higher relatedness than the mistletoe populations in native habitats. However, we did not find differences in the immigration rates of transformed and native habitat populations. It is possible that recent changes in gene flow can result in an overestimation of migration rates (Samarasin *et al.*, 2017), and here this implies that current gene flow among mistletoe populations could be even lower than our estimates infer, which were below 5% in most cases.

Furthermore, habitat transformation was also found to alter resource availability and diversity, influencing pollinator and seed disperser visitation rates and at critical points of *T. corymbosus* reproductive cycle (Fontúrbel *et al.*, 2017b). This may contribute to reduced gene flow across the landscape by concentrating *S. sephaniodes* and *D. gliroides* activity in areas with large flower / fruit aggregations, a result partially confirmed by our association tests, where we found a positive association between the number of *T. corymbosus* fruits and *D. gliroides* visits at the transformed habitat during January, when most of the ripe fruit offer is concentrated (Fontúrbel *et al.*, 2017b).

Tristerix corymbosus and its mutualists have proven to be highly resilient to habitat transformation (Fontúrbel *et al.*, 2015; Fontúrbel *et al.*, 2017b), contrary to habitat fragmentation (Magrach *et al.*, 2013; Rodríguez-Cabal *et al.*, 2007). Increased resource availability seems to be the main factor influencing the persistence of *Tristerix corymbosus* and its pollination and seed dispersal interactions in disturbed habitats (Fontúrbel *et al.*, 2017b). Although pollination and seed dispersal interactions are able to persist in spite of the structural and microclimate changes, habitat transformation modifies the effectiveness of both interactions (Fontúrbel *et al.*, 2017a) as well as the spatial arrangement of mistletoe, resulting in the occurrence of dense plant aggregations in transformed habitats (Fontúrbel *et al.*, 2017c). Such evidence suggests that habitat transformation has a neutral -if not positive- effect on *T. corymbosus*. However, the molecular evidence presented here depicts a more complex scenario behind this apparent resilience. Gene flow at the landscape level is mostly below 5% (with the exception of T3 to T2 with a rate of 18.5% and T1 to N2 with a rate of 8.8%), which is likely to be the main cause of the high level of population structure found along the study area. We consistently found five highly differentiated groups, matching our sampling sites – with very few individuals left unsampled in the VCR (not shown).

Resource availability is known to affect interaction rates with pollinators and seed dispersers (Fontúrbel *et al.*, 2017b; Lázaro *et al.*, 2014; Morales *et al.*, 2012). Here we found that the availability of *T. corymbosus* flowers and fruits were spatially associated with *S. sephaniodes* and *D. gliroides* visits in some cases, particularly in native habitat, but highly variable between the beginning and the peak of the flowering and fruiting seasons. The observed genetic structure among populations could be influenced by the behaviour of the pollinator and the seed disperser vectors. *S. sephaniodes* is able to move several

kilometres across the landscape, but consistently returns to the same foraging locations (González-Gómez and Vásquez, 2006), which may drastically reduce pollen movement distances. *Dromiciops gliroides* has a limited movement range of < 500 m (Fontúrbel *et al.*, 2012) and is limited to forested areas (Fontúrbel *et al.*, 2010) constraining seed dispersal. Perhaps more important than distance travelled by the pollinator or the seed disperser, is the amount of time spent feeding at the same location, which has been shown to increase up to three times as resource offer increase for the seed disperser (15 vs 45 min at native and transformed habitats, respectively; Fontúrbel *et al.*, 2017c). *D. gliroides* was detected more frequently in transformed habitats at the beginning of the austral summer (January-February), but was more frequently detected in native habitats during late summer (March), probably as a response to fruit availability (Fontúrbel *et al.*, 2014; Fontúrbel *et al.*, 2017b; Salazar and Fontúrbel, 2016). Consequently, the reproductive biology of *T. corymbosus* (depending on one pollinator and one seed disperser species) may also contribute to the high genetic structure found, especially considering that the disturbance event is relatively recent (~20 years), over approximately 10 mistletoe generations.

We also found that mistletoes in the transformed habitat were more clustered than in native habitat (Fontúrbel *et al.*, 2017c) and more related to each other. As relatedness and geographic distance were negatively correlated, we expect that densely aggregated mistletoes are more closely related than sparse plants, as spatial aggregation can alter demographic patterns (Fedriani and Wiegand, 2014). Given that mistletoes thriving in transformed habitats tend to have large crop sizes (Fontúrbel *et al.*, 2015), relatedness among plants may increase as a result of increased *D. gliroides* feeding time at the same plant, consuming more fruits of the same plant or neighbouring plants and then defecating them together nearby the feeding site (di Virgilio *et al.*, 2014; Fontúrbel *et al.*, 2017c).

Also, the presence of an abundant and diverse flowering and fruiting neighbourhood in transformed habitats (Fontúrbel *et al.*, 2017b) could influence *S. sephaniodes* and *D. gliroides* foraging decisions (Morales *et al.*, 2012; Sasal and Morales, 2013). Therefore, the relatedness-distance relationship suggests a positive feedback between the ecological constraints imposed by the transformed habitat and its evolutionary consequences: as mistletoes become more aggregated, they also become more related to each other, resulting in less genetic diversity and more relatedness among plants (Fig. 5). Furthermore, since both habitats currently have immigration rates below 5%, it is possible that this scenario will worsen over time for populations in transformed habitats.

<Figure 5 about here>

Despite most of the original mistletoe population being lost at the time of the establishment of the *E. globulus* plantation the current migration rates are similar between the transformed and the native habitat, which suggests that this is a highly resilient system with stabilized gene flow. Yet, transformed populations may be facing important costs in terms of diminished genetic diversity and increased relatedness. However, we observed that native populations have low gene-flow to other populations, despite having been an important gene source in past colonization. This suggests that habitat transformation has modified *T. corymbosus* gene flow in time and space, leading to more isolated populations. The migration analyses showed that between 73-92% of the individuals result from self-recruitment within the populations. Gene flow among populations is variable and asymmetric.

These results could be explained by one or more of the following three scenarios (which are non-mutually exclusive): (1) A founder effect resulting from mistletoe recovery after clearing the native forest and the subsequent establishment of the *E. globulus*

plantation. However, we detected alleles exclusive to transformed habitats, which could come from small native remnants left among the planted areas (mostly corresponding to areas difficult to clear-cut and riparian vegetation) acting as propagule sources. (2) Despite no evidence for a bottleneck in transformed habitat populations, the native remnants (which survived clear-cutting) could still have experienced bottlenecks but recruitment from distant native populations may be masking them. We only found evidence for a bottleneck in population N1, which could be the result of selective logging in the past decade due to wood theft (which also may be the cause to be more aggregated than population N2). (3) Habitat transformation is known to alter pollination and seed dispersal (Fontúrbel *et al.*, 2017a), and host quality may play an important selective force on native habitat mistletoes, influencing the survival probabilities of individuals hosted by particular species (Fontúrbel *et al.*, 2017c).

Tristerix corymbosus, as with many other mistletoes around the world, seems to benefit from habitat modification (Bowen *et al.*, 2009). However, in the absence of a forest/non-forest habitat dichotomy, the effects of habitat modification are more difficult to assess along a gradient of heterogeneous landscapes (Herrera *et al.*, 2011). *Sephanoides sephanioides* and *D. gliroides* apparently benefit from the increased landscape heterogeneity (Tscharntke *et al.*, 2012), taking advantage of native remnants to nest and transformed stands to feed (Salazar and Fontúrbel, 2016). However, when we examine the demographic and genetic processes behind these patterns this apparent resilience to habitat transformation is may be costly in terms of relatedness and genetic diversity, therefore in their evolutionary potential and extinction probabilities. The observed gene flow patterns among mistletoe populations might result from a combination of changes in plant spatial aggregation (plants densely clumped at the transformed habitat), plant-animal interactions

(pollinators / seed dispersers spending more time on areas with large resource offer) and a neighbourhood effect (offering alternative flower / fruit resources). These three biotic factors together and microclimate changes can create a novel ecological scenario that is apparently beneficial for pollination and seed dispersal interactions in the short term, but it also has less obvious effects, such as the change in the selective forces influencing plant recruitment (Fontúrbel *et al.*, 2015; Fontúrbel and Medel, 2017), as well as the reduction of the genetic diversity and the increase of inter-individual relatedness.

DATA ARCHIVING

Data associated to this article is available from the figshare digital repository
<https://doi.org/10.6084/m9.figshare.4728721>

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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649

650 TABLES

651

652 Table 1. Pairwise F_{ST} and associated P-values obtained after 10000 permutations. * denote
 653 significant P-values ($P < 0.001$).

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Site	T1	T2	T3	N1	N2
T1	—	0.132*	0.124*	0.131*	0.077*
T2		—	0.071*	0.121*	0.104*
T3			—	0.111*	0.108*
N1				—	0.099*
N2					—

655

FIGURE CAPTIONS

Figure 1. Map of the study area: (a) location in South America, (b) detailed location in southern Chile, (c) detailed map of the sampling area showing native and transformed habitats. Mistletoe locations within each sampling site are shown in different colours (N = native forest habitat and T = transformed habitat).

Figure 2. (a) Plot of the number of populations simulated from the posterior distribution obtained with GENELAND; (b) to (f) GENELAND maps of individual assignments to clusters ($K = 5$). Cluster correspondence: (b) T1; (c) T2; (d) T3; (e) N1 and (f) N2. The probability of belonging to a given cluster ranges from 0.0 (red areas) to 1.0 (white areas); these probability values are also indicated by isolines. Black dots represent sampled mistletoes. The plot is based on the highest-probability run at that value of K .

Figure 3. Spatial genetic autocorrelograms for: (a) native habitat and (b) transformed habitats. Solid lines represented the observed autocorrelation, dashed lines represent the 95% confidence intervals. Black circles represent significant autocorrelations whereas white circles represent non-significant autocorrelations.

Figure 4. Migration rates (proportion of migrants from population x in population y , per generation) among *Tristerix corymbosus* populations at native and transformed habitats. Red arrows represent the largest inter-population migration.

679 Figure 5. Determinants of ecological and evolutionary changes of *T. corymbosus* at
680 transformed habitats.

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Figure 1

Figure 2

Figure 3

Figure 4

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Figure 5