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1 Running head: Habitat transformation alters ecoevolution

2

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

6

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18 ABSTRACT

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

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

41 INTRODUCTION

42 Humans have altered almost every ecosystem on Earth, leading to habitat loss,
43 fragmentation, degradation and profound transformation (Chapin III *et al.*, 2000; Corlett,
44 2015; Didham *et al.*, 2012; Fahrig, 2003; Ghazoul *et al.*, 2015; Perez-Mendez *et al.*, 2016;
45 Sala *et al.*, 2000). Our understanding of the consequences of land use change as a driver of
46 biodiversity loss has dramatically changed in the past two decades thanks to the
47 development of new techniques including molecular approaches (Epps and Keyghobadi,
48 2015; Sunnucks, 2000). Such advancements have enabled the study of whole communities
49 and their interactions rather than individual species, and the study of processes instead of
50 patterns (Escudero *et al.*, 2003; Valiente-Banuet *et al.*, 2015). Therefore, assessing the
51 effects of anthropogenic disturbance on natural ecosystems, using field methods and
52 molecular markers, will also provide insight into their long-term consequences (Carpenter,
53 2002; Melo *et al.*, 2013).

54 One type of habitat transformation considers the total or partial replacement of the
55 native vegetation by a single or multiple exotic species (Fontúrbel *et al.*, 2015), usually for
56 commercial purposes (e.g., the establishment of *Pinus* spp. or *Eucalyptus* spp. plantations).
57 According to FAO, 264 million ha were covered by exotic forest plantations around the
58 world by 2015 (<http://www.fao.org/forestry/fra>). Unlike fragmented habitats, transformed
59 habitats usually lack spatial discontinuities, resulting in habitat mosaics of native and
60 transformed stands at the landscape level (Salazar and Fontúrbel, 2016). Although many
61 native animal and plant species are capable of persisting in these mixed landscapes (García
62 *et al.*, 2013; Lancaster *et al.*, 2011), transformed habitats can impose restrictions on
63 ecological connectivity that can compromise gene flow at the landscape scale (Albert *et al.*,
64 2013; Lancaster *et al.*, 2011). Such restricted gene flow may result from the combination of

65 many possible changes, for example in pollinator and/or seed disperser behaviour (Lavabre
66 *et al.*, 2014; Sasal and Morales, 2013), plant recruitment (Bravo *et al.*, 2015), habitat
67 structure (Castaño-Villa *et al.*, 2014), microclimatic conditions (Fontúrbel and Medel,
68 2017), plant-animal interactions (Neuschulz *et al.*, 2016), plant-plant interactions (Candia *et*
69 *al.*, 2014), spatial structure (Fontúrbel *et al.*, 2015) and neighbourhood effects (Lázaro *et*
70 *al.*, 2014). There are two ecological interactions that determine most of plant gene flow
71 across the landscape: pollination and seed dispersal. Animal pollinators and seed dispersers
72 act as mobile links across the landscape (González-Varo *et al.*, 2017), but habitat
73 transformation can affect both quantitative and qualitative components of pollination and
74 seed dispersal interactions (Fontúrbel *et al.*, 2017a). These changes may alter landscape-
75 level gene flow, changing genetic diversity and relatedness among individuals, potentially
76 damaging their evolutionary potential and raising extinction probabilities (Carvalho *et al.*,
77 2016; Lancaster *et al.*, 2011). However, despite being a major driver of biodiversity change
78 (Albert *et al.*, 2013), habitat transformation remains little known, particularly regarding its
79 long-term consequences.

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

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

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

110

111 MATERIALS AND METHODS

112 *Study site and sampling protocol*

113 We conducted this study in the Valdivian Coastal Reserve (VCR; 39°57'S,
114 73°34'W), a 50,000-ha private protected area, owned and managed by The Nature
115 Conservancy (Delgado, 2010). This reserve protects an important fraction of the Valdivian
116 rainforest ecosystem, considered a biodiversity hotspot due to its high proportion of
117 endemic species (Mittermier *et al.*, 2005). The VCR features a complex mosaic composed
118 of native and transformed habitats. The native forest habitat comprises of both old- and
119 secondary-growth stands. In old-growth stands the dominant species are *Nothofagus*
120 *dombeyi*, *N. pumilio*, *Fitzroya cupressoides*, *Laurelia philippiana*, *Lomatia ferruginea*, and
121 *Mitraria coccinea*; whereas in the secondary forest the dominant species are *N. pumilio*, *N.*
122 *dombeyi*, *Eucryphia cordifolia*, *Drimys winteri*, *Tepualia stipularis*, *M. coccinea* and
123 *Chusquea quila*. The transformed habitat is dominated by the exotic tree *Eucalyptus*
124 *globulus*, coexisting with many shade-intolerant understory native plants, such as
125 *Rhaphithamnus spinosus*, *Aristotelia chilensis*, *Lapageria rosea* and *C. quila* (Fontúrbel *et*
126 *al.*, 2015).

127 Between 2013 and 2015, we obtained samples from 123 adult *T. corymbosus* plants
128 in the study area, representing a large fraction of remaining individuals in the VCR. We
129 collected three to four young leaves from each mistletoe (one individual per host, to prevent
130 sampling clones and resprouts), which were immediately stored in Ziploc bags, dried using
131 silica gel, and kept dry until DNA extraction. We georeferenced sampled mistletoes, which
132 came from five sampling locations harbouring the large majority of VCR plants, two in
133 what could be classified as native habitats: N1 (N = 21; 39°59'25.71"S, 73°40'33.33"W;
134 evidence for some disturbance by illegal logging), N2 (N = 20; 39°58'16.35"S,
135 73°36'13.28"W; pristine); and three in what could be classified as transformed habitats: T1

136 (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
137 (N = 21; 39°57'57.42"S, 73°39'8.88"W) (Fig. 1). Sampling sites were ascribed *a priori*,
138 based on the spatial clusters, obtained by georeferencing each plant. Sampling sites were
139 classified either as native (N) or transformed (T) according to the dominant vegetation type,
140 following Fontúrbel *et al.* (2015), on aerial photographs and on field surveys. Sampling
141 sites were separated by between 1 and 6 km, as the largest movement distance of the seed
142 disperser (*D. gliroides*) is recorded as 500 m (Fontúrbel *et al.*, 2012).

143 <Figure 1 about here>

144

145 *Microsatellite amplification*

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

154

155 *Descriptive statistics and population structure*

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

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

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

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

183 0.05 were excluded). Then we used the BOTTLENECK 1.2.02 (Cornuet and Luikart,
184 1996) to determine if the assessed populations had undergone a recent bottleneck episode.
185 A two-phase mutation model was applied with a 70 % stepwise mutation, and we assessed
186 the significance using its prescribed Wilcoxon test.

187

188 *Relatedness and its relationship with distance*

189 To ensure that the sampled individuals represented a random subset of the
190 population, we estimated relatedness according to Queller and Goodnight (1989), using the
191 r_{xy} estimator calculated with IDENTIX 1.1 (Belkhir *et al.*, 2002). Within each population,
192 the null hypothesis for the random distribution of related individuals was tested by using
193 1,000 permutations of the alleles present. We also estimated pairwise relatedness
194 coefficients among the 104 mistletoes genotyped, and then constructed a relatedness matrix
195 and performed a permutation ANOVA to compare relatedness values between native and
196 transformed habitats. GPS coordinates for each genotyped sample were used to construct a
197 distance matrix using PASSaGE 2 (Rosenberg and Anderson, 2011) and pairwise distances
198 between habitats were compared using a permutation ANOVA.

199 We used the ade4 (Dray and Dufour, 2007), adegenet (Jombart, 2008) and
200 PopGenReport (Adamack and Gruber, 2014) R packages to obtain genetic and geographic
201 Euclidean distance matrices. Then, we estimated the correlation between relatedness and
202 geographic distance matrices using a Mantel test (9,999 permutations). To estimate a
203 threshold geographic distance of relatedness between plants, we conducted spatial
204 autocorrelation (Vekemans and Hardy, 2004), using GenAlEx 6.5 (Peakall and Smouse,
205 2012) and obtained separate correlograms for each habitat type, using 10 distance classes in
206 each case (distance class size was 1 000 m for the native habitat and 250 m for the

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

209

210 *Gene flow*

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

220

221 *Resource availability, pollination and seed dispersal*

222 To gain insight on the potential effects of the habitat transformation on ecological
223 interactions and relate this to the genetics effects detected, we studied the relationship
224 between resource availability (i.e., flowers and fleshy fruits) and the visitation rates of *S.*
225 *sephaniodes* and *D. gliroides*. We monitored 24 plants (12 from each habitat type) using
226 infrared camera traps (Bushnell Trophy Cam model 2011) following (Fontúrbel *et al.*,
227 2015; Fontúrbel *et al.*, 2017b). Plants were monitored at the beginning (March 2015) and
228 peak (August 2015) of the flowering season, and at the beginning (November 2015) and
229 peak (January 2016) of the fruiting season. Camera-traps (one per plant) were
230 simultaneously operated for 72 continuous hours. We counted the number of flowers and

231 fruits on the monitored plants and the number of flowers and fruits on other mistletoes in a
232 250-m radius from the focal plant, as well as the number of flowers and fruits of other co-
233 flowering / co-fruiting species within this area.

234 We quantified our visitation results as the number of effective visits (i.e., actual
235 contact with the flower or fruit consumption). As resources could influence pollinators and
236 seed dispersers in many ways, we expressed flower and fruit availability in three
237 dimensions: (1) *T. corymbosus* flowers / fruits alone, (2) other co-flowering / co-fruiting
238 plants with flowers / fruits, and (3) the total number of flowers / fruits (i.e., resources from
239 both the mistletoe and the accompanying plants), enabling potential interaction triggers to
240 be considered (i.e., the mistletoe resource itself, the resources of the neighbouring plants, or
241 the combination). This kind of count data is better suited for examining concordances than
242 cause-effect relationships in a more traditional way (Li *et al.*, 2012). Therefore, we
243 examined the spatial concordance between the number of visits made by the pollinator /
244 seed disperser animals with the resource availability in each of the three dimensions
245 described above using the SADIE (Spatial Analysis by Distance Indices; (Perry *et al.*,
246 2002; Perry *et al.*, 1999). This approach uses two count variables (in this case, pollinator or
247 disperser visits and number of flowers or fruits of each resource dimension) sharing the
248 same geographic coordinates (i.e., the location of each mistletoe). SADIE works in two
249 phases: first the degree of the clustering of each variable is estimated separately, followed
250 by an association index (X_p) that ranges between 1 (complete association; e.g., where there
251 are more flowers we register more hummingbird visits) and -1 (complete dissociation; e.g.,
252 where there are more fruits we register less marsupial visits), values not significantly
253 different from zero indicate spatial independence. SADIE analysis used SADIEShell 2.0
254 (Conrad, 2001). We conducted separate analyses for the March, August, November and

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

257

258 RESULTS

259 *Descriptive statistics and population structure*

260 We were only able to retain 104 of 123 samples for the analysis, due to inconsistent
261 genotype quality for 19 individuals. We used eight out of the ten microsatellite markers
262 available, as the locus TRIS_80 showed evidence of null alleles in the five groups, while
263 the locus TRIS_84 showed consistent deviations from the HWE at all sites. A summary of
264 the characteristics of these loci is shown in Table S1 (available online as Supplementary
265 Information). Overall allele sampling effectiveness was 86.6%, ranging between 81.4 and
266 92.1% among sampling sites.

267 The global F_{ST} in the VCR was 0.108 ($P < 0.001$), while pairwise analysis suggested
268 that each sampling site comprised a separate genetic group, as F_{ST} values showed
269 significant differences among all pairwise comparisons ($P < 0.005$) with values ranging
270 from 0.071 to 0.132 (Table 1). STRUCTURE also showed a maximum likelihood value at
271 $K = 5$ (Fig. S1), assigning 99% of individuals to their correct sampling site (Table S2).
272 Similarly, GENELAND showed evidence of five populations in 100% of the 10 runs (Fig.
273 2a – 2f), assigning 97.1% of the individuals to their sampling site (Table S3). However, the
274 Mantel test showed no evidence of a relationship between F_{ST} and geographic distance
275 among populations ($r = -0.051$, $P = 0.649$). Thus, the three different methods used
276 consistently inferred five mistletoe populations in the VCR. Thus, the genetic structure
277 retained for subsequent analyses considered the presence of five populations: T1, T2, T3,
278 N1, N2, which matched our sampling sites.

279 <Table 1 and Figure 2 about here>

280 The difference in N_A was marginally significant between native and transformed
281 habitats (Wilcoxon signed rank test, $V = 14$, $P = 0.053$). Also, we found significant
282 differences between native and transformed habitats for AR ($V = 19$, $P = 0.047$) and GD (V
283 $= 35$, $P = 0.008$), in both cases transformed habitats showed lower values than native
284 habitats (Table S4, Fig. S2; Table S5). For the bottleneck analysis, only one population
285 show evidence for a recent bottleneck event ($N1$; $P = 0.004$).

286

287 *Relatedness and its relationship with distance*

288 Individuals at all sampling sites were on average unrelated (global $r_{xy} = -0.014$, $P =$
289 0.190 ; Table S6). However, we found a significant difference between habitats
290 (permutation ANOVA $F = 37.69$, $P < 0.001$), where mean values of relatedness were higher
291 in transformed habitat (0.18 ± 0.04 (mean \pm 1SE), $N = 63$) than in native habitat ($0.07 \pm$
292 0.04 , $N = 41$). When examining geographic distance among individuals, we found the
293 minimum pairwise distance to be 3.61 m in the native habitat and 1 m in the transformed
294 habitat. There mean pairwise distance among plants in the different habitats was
295 significantly different (permutation ANOVA $F = 273.60$, $P < 0.001$), and was larger in
296 native (1168.00 ± 247.75 m, $N = 41$) than transformed habitat (118.82 ± 15.46 m, $N = 63$),
297 showing that as distances among plants decrease, their relatedness values increase. This
298 relationship was confirmed by correlating genetic and geographic distances, which showed
299 significant RV correlation coefficients at both habitats (native: $RV_{coef} = 0.357$, $P < 0.001$;
300 transformed: $RV_{coef} = 0.225$, $P < 0.011$). Further, we found positive and significant spatial
301 genetic autocorrelation at 1,000, 3,000 and 4,000 m for the native habitat, whereas at the
302 transformed habitat we only found positive significant autocorrelation at 250 m, which

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

305 <Figure 3 about here>

306 *Gene flow*

307 While gene flow estimates were higher for transformed habitat (Fig. 4), we found
308 that the total immigration rate for native and transformed populations was not significantly
309 different ($F = 0.17$, $P = 0.800$). Gene flow among populations ranged between 1.5 and
310 18.5%, being most of the recruitment (73 to 92%) originated in the same population.
311 Populations from the transformed habitat had the greatest contribution to other populations,
312 whereas the native habitat populations had the least contribution (Table S8).

313 <Figure 4 about here>

314

315 *Resource offer, pollination and seed dispersal*

316 At the beginning of the flowering season (March), flowers were more abundant in
317 the transformed than the native habitat (616 ± 184 vs. 291 ± 26 flowers per plant), but no
318 significant associations between *S. sephaniodes* pollination visits and flower availability
319 were detected at any level or habitat. At the peak of the flowering season (August), flower
320 abundance was similar between native and transformed habitats (112 ± 11 vs. 149 ± 23
321 flowers per plant, respectively), and both *T. corymbosus* flower abundance and the total
322 number of flowers were spatially associated with the number of *S. sephaniodes* visits ($X_p =$
323 0.537 , $P = 0.039$ and $X_p = 0.526$, $P = 0.042$, respectively) in native but not transformed
324 habitat during this period.

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

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

333

334 DISCUSSION

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

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

351 *Tristerix corymbosus* and its mutualists have proven to be highly resilient to habitat
352 transformation (Fontúrbel *et al.*, 2015; Fontúrbel *et al.*, 2017b), contrary to habitat
353 fragmentation (Magrach *et al.*, 2013; Rodríguez-Cabal *et al.*, 2007). Increased resource
354 availability seems to be the main factor influencing the persistence of *Tristerix corymbosus*
355 and its pollination and seed dispersal interactions in disturbed habitats (Fontúrbel *et al.*,
356 2017b). Although pollination and seed dispersal interactions are able to persist in spite of
357 the structural and microclimate changes, habitat transformation modifies the effectiveness
358 of both interactions (Fontúrbel *et al.*, 2017a) as well as the spatial arrangement of mistletoe,
359 resulting in the occurrence of dense plant aggregations in transformed habitats (Fontúrbel *et*
360 *al.*, 2017c). Such evidence suggests that habitat transformation has a neutral -if not
361 positive- effect on *T. corymbosus*. However, the molecular evidence presented here depicts
362 a more complex scenario behind this apparent resilience. Gene flow at the landscape level
363 is mostly below 5% (with the exception of T3 to T2 with a rate of 18.5% and T1 to N2 with
364 a rate of 8.8%), which is likely to be the main cause of the high level of population
365 structure found along the study area. We consistently found five highly differentiated
366 groups, matching our sampling sites – with very few individuals left unsampled in the VCR
367 (not shown).

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

375 kilometres across the landscape, but consistently returns to the same foraging locations
376 (González-Gómez and Vásquez, 2006), which may drastically reduce pollen movement
377 distances. *Dromiciops gliroides* has a limited movement range of < 500 m (Fontúrbel *et al.*,
378 2012) and is limited to forested areas (Fontúrbel *et al.*, 2010) constraining seed dispersal.
379 Perhaps more important than distance travelled by the pollinator or the seed disperser, is the
380 amount of time spent feeding at the same location, which has been shown to increase up to
381 three times as resource offer increase for the seed disperser (15 vs 45 min at native and
382 transformed habitats, respectively; Fontúrbel *et al.*, 2017c). *D. gliroides* was detected more
383 frequently in transformed habitats at the beginning of the austral summer (January-
384 February), but was more frequently detected in native habitats during late summer (March),
385 probably as a response to fruit availability (Fontúrbel *et al.*, 2014; Fontúrbel *et al.*, 2017b;
386 Salazar and Fontúrbel, 2016). Consequently, the reproductive biology of *T. corymbosus*
387 (depending on one pollinator and one seed disperser species) may also contribute to the
388 high genetic structure found, especially considering that the disturbance event is relatively
389 recent (~20 years), over approximately 10 mistletoe generations.

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

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

408 <Figure 5 about here>

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

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

423 plantation. However, we detected alleles exclusive to transformed habitats, which could
424 come from small native remnants left among the planted areas (mostly corresponding to
425 areas difficult to clear-cut and riparian vegetation) acting as propagule sources. (2) Despite
426 no evidence for a bottleneck in transformed habitat populations, the native remnants (which
427 survived clear-cutting) could still have experienced bottlenecks but recruitment from distant
428 native populations may be masking them. We only found evidence for a bottleneck in
429 population N1, which could be the result of selective logging in the past decade due to
430 wood theft (which also may be the cause to be more aggregated than population N2). (3)
431 Habitat transformation is known to alter pollination and seed dispersal (Fontúrbel *et al.*,
432 2017a), and host quality may play an important selective force on native habitat mistletoes,
433 influencing the survival probabilities of individuals hosted by particular species (Fontúrbel
434 *et al.*, 2017c).

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

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

454

455 DATA ARCHIVING

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

458

459 CONFLICT OF INTEREST

460 The authors declare no conflict of interest.

461

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469

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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 ($P < 0.001$).

654

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

656 FIGURE CAPTIONS

657

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

662

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

669

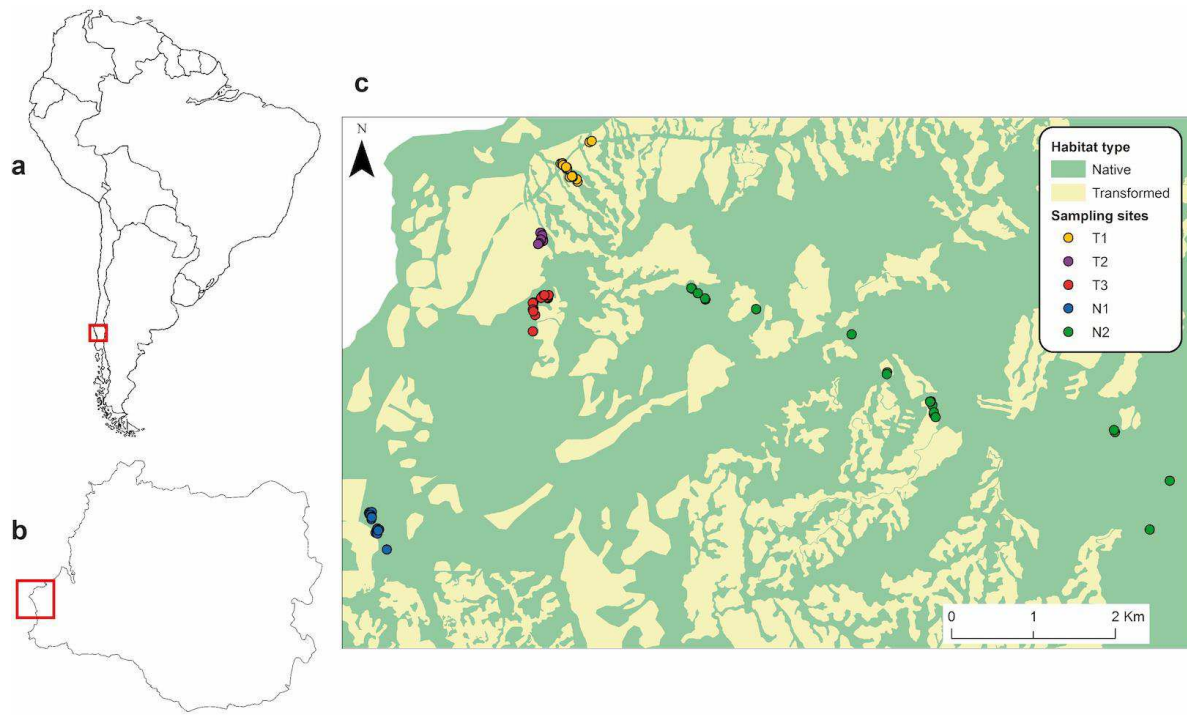
670 Figure 3. Spatial genetic autocorrelograms for: (a) native habitat and (b) transformed
671 habitats. Solid lines represented the observed autocorrelation, dashed lines represent the
672 95% confidence intervals. Black circles represent significant autocorrelations whereas
673 white circles represent non-significant autocorrelations.

674

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

678

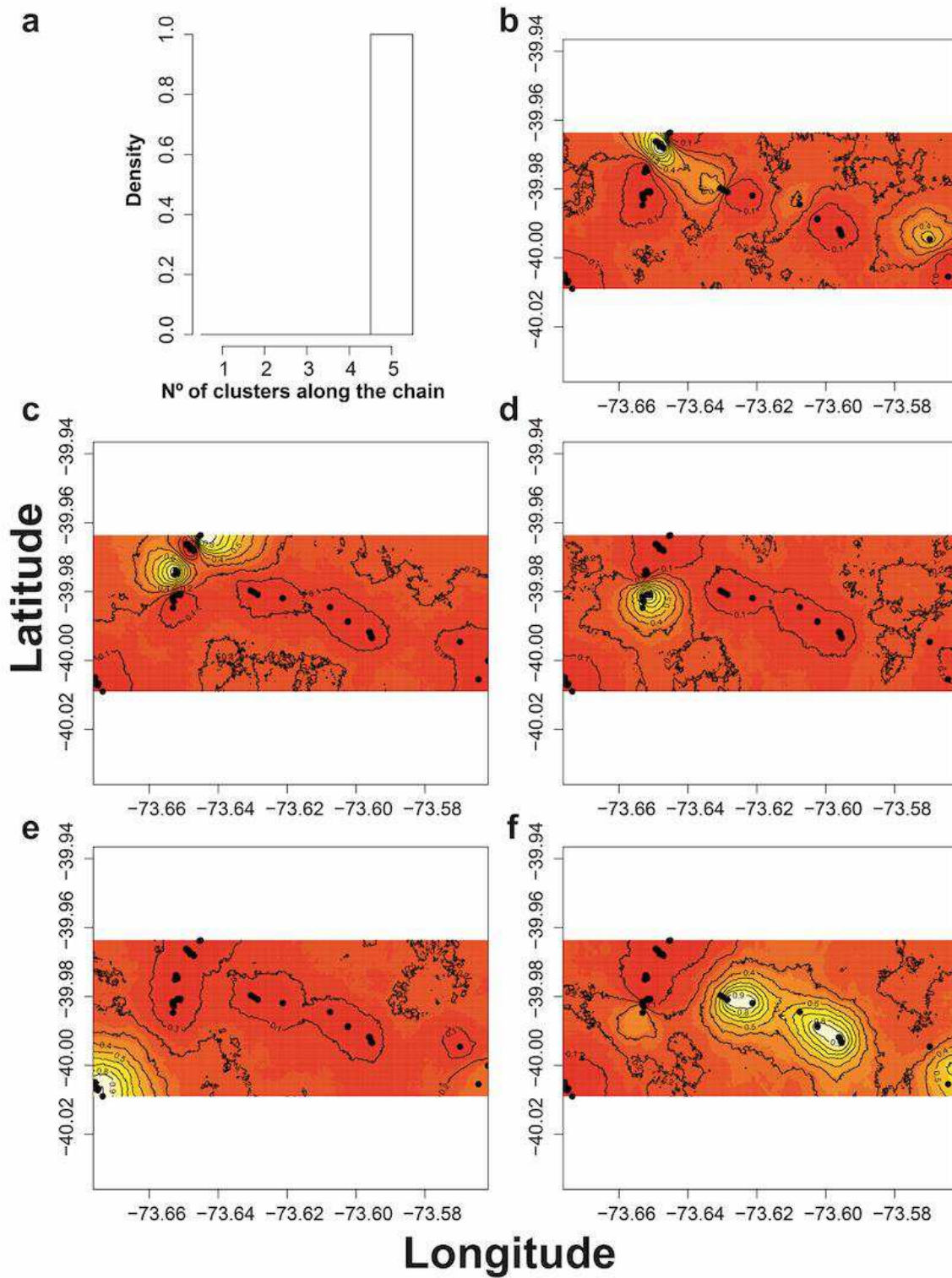
679 Figure 5. Determinants of ecological and evolutionary changes of *T. corymbosus* at
680 transformed habitats.



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Figure 1

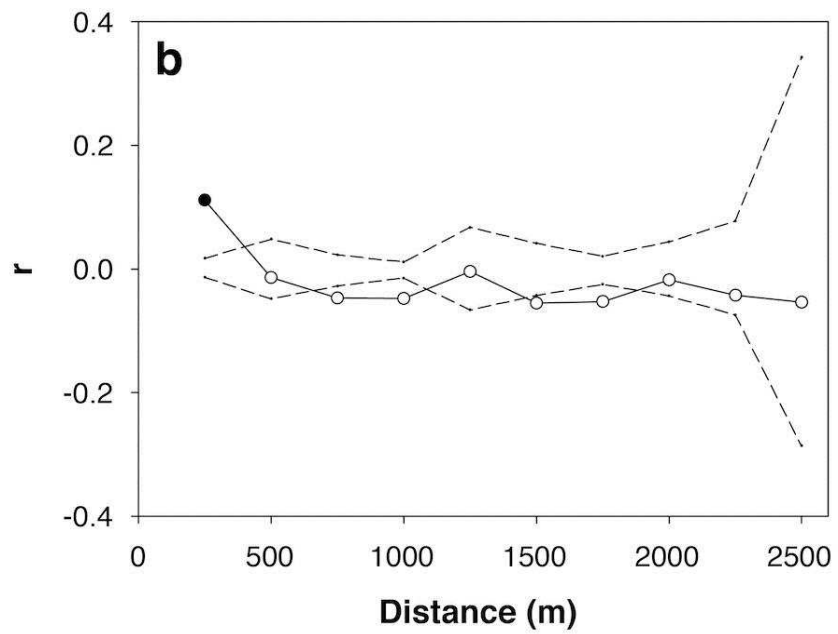
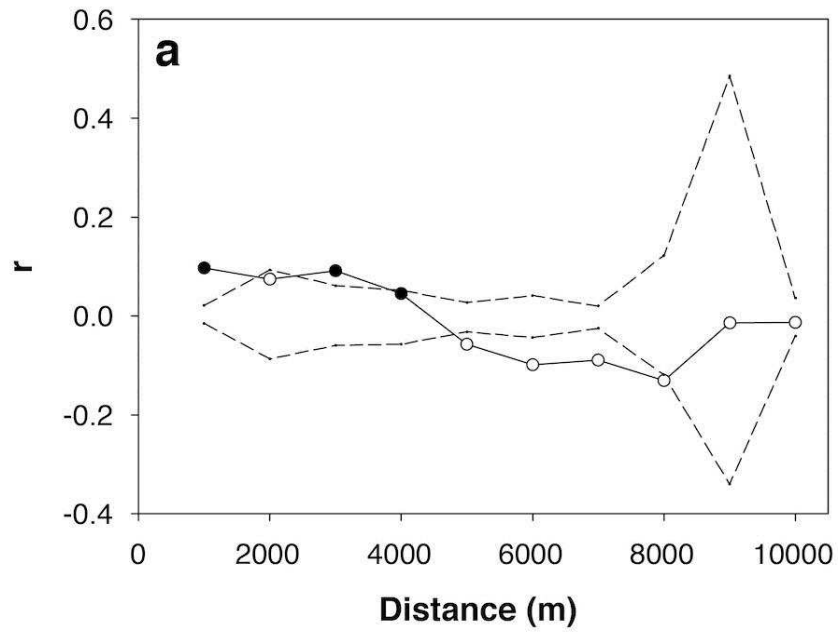


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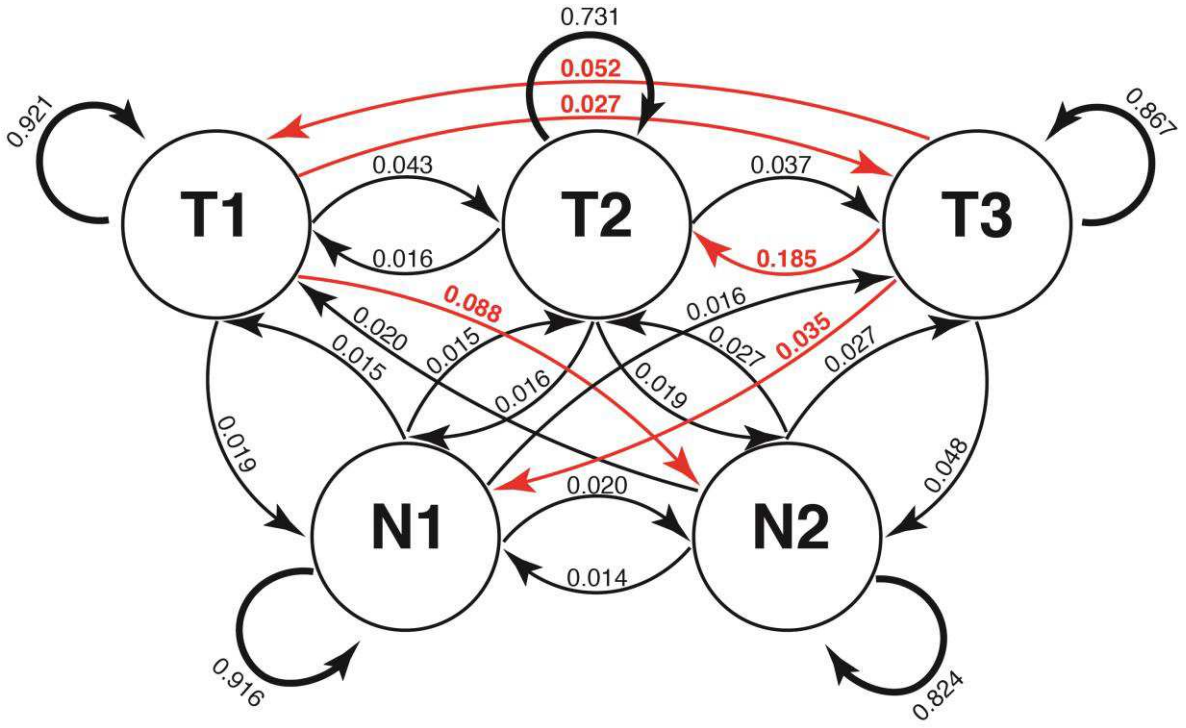
Figure 2



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Figure 3



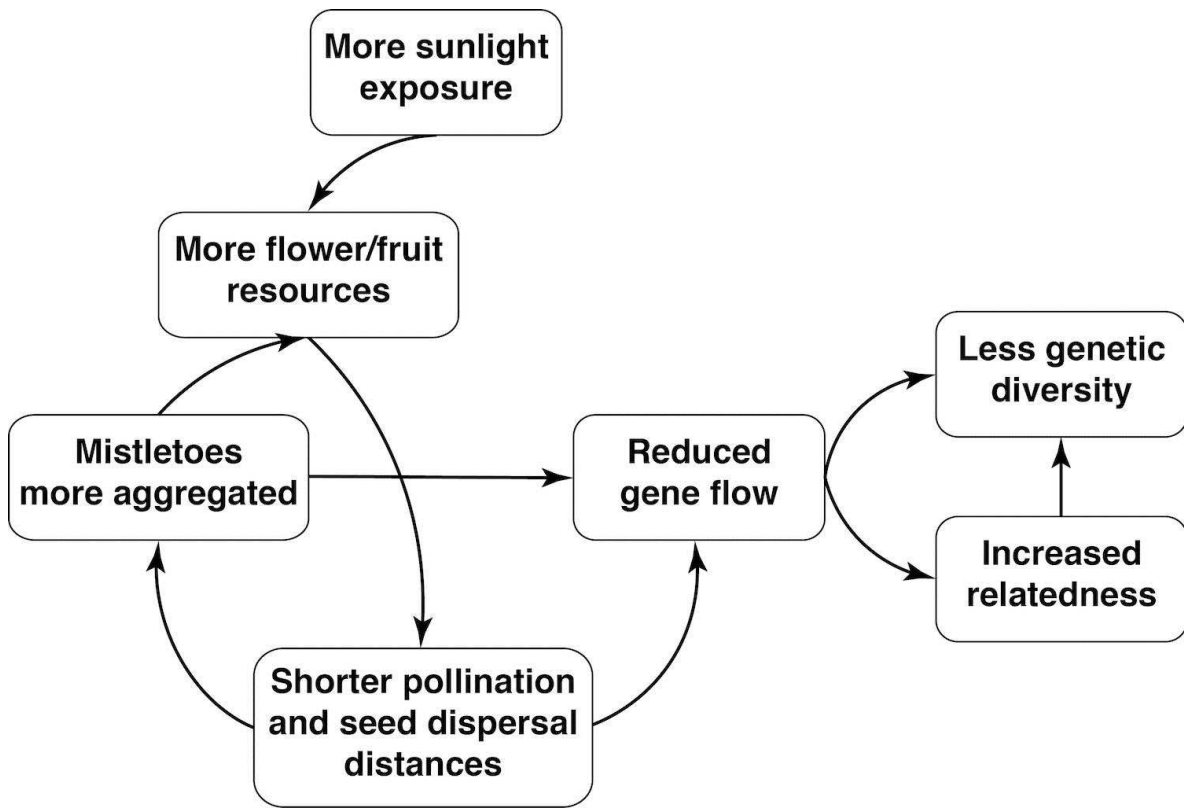
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Figure 4

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Figure 5