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 sephaniodes*) 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.

INTRODUCTION

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

Study site and sampling protocol

113   We conducted this study in the Valdivian Coastal Reserve (VCR; 39º57'S,

114   73º34'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; 39º59'25.71"S, 73º40'33.33"W;

134   evidence for some disturbance by illegal logging), N2 (N = 20; 39º58'16.35"S,

135   73º36'13.28"W; pristine); and three in what could be classified as transformed habitats: T1

136   (N = 20; 39º57'0.46"S, 73º38'58.28"W), T2 (N = 22; 39º57'26.45"S, 73º39'13.79"W), T3

137   (N = 21; 39º57'57.42"S, 73º39'8.88"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) of 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

8

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   autocorration (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 lmPerm 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.

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 ± 1SE), N = 63) than in native habitat (0.07 ±

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: RVcoef = 0.357, P < 0.001;

300   transformed: RVcoef = 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

13

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

14

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).

17

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*

18

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   *sephaniodes* and *D. gliroides* apparently benefit from the increased landscape heterogeneity

440   (Tscharntke *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

19

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

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.
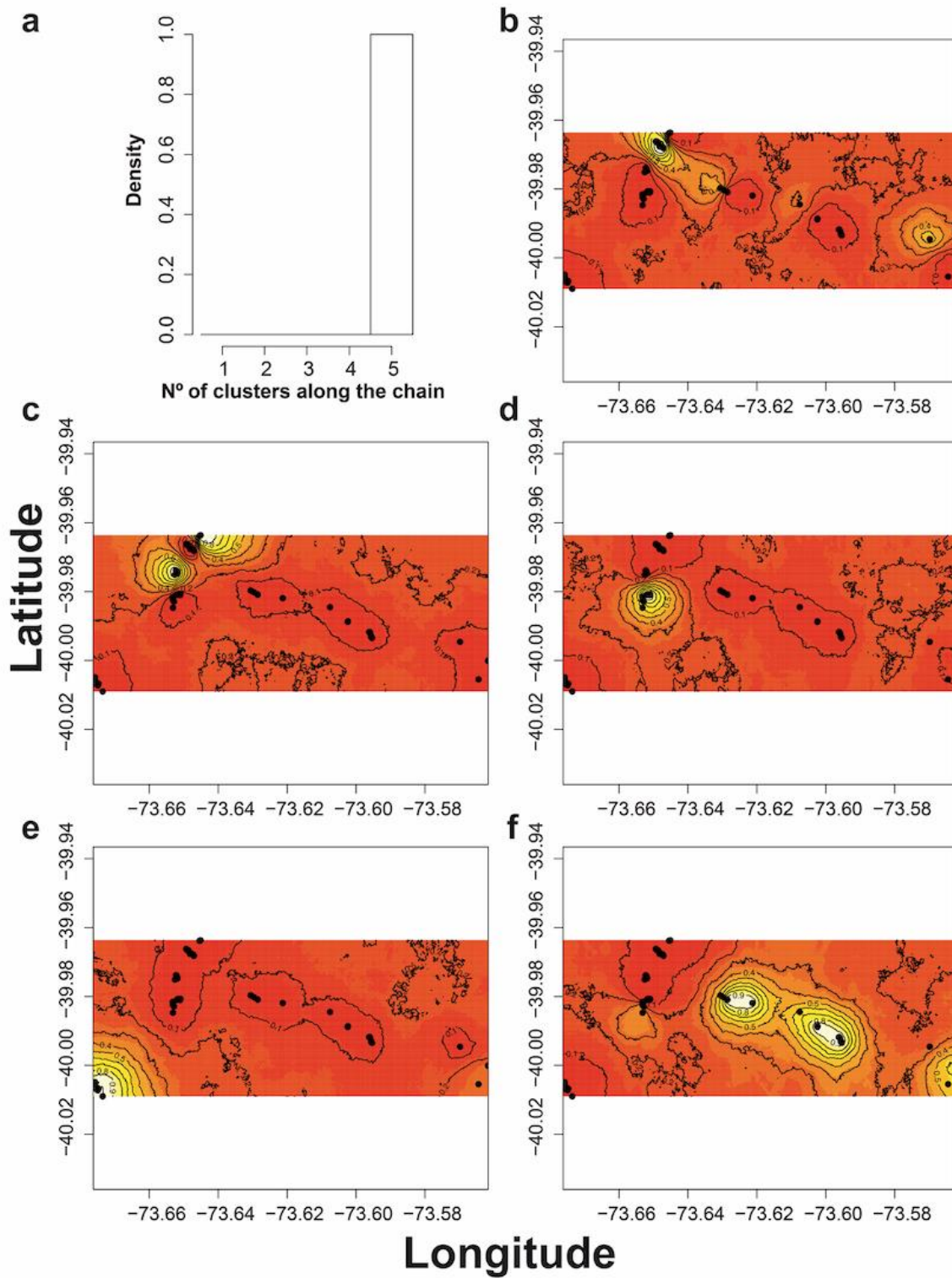
678

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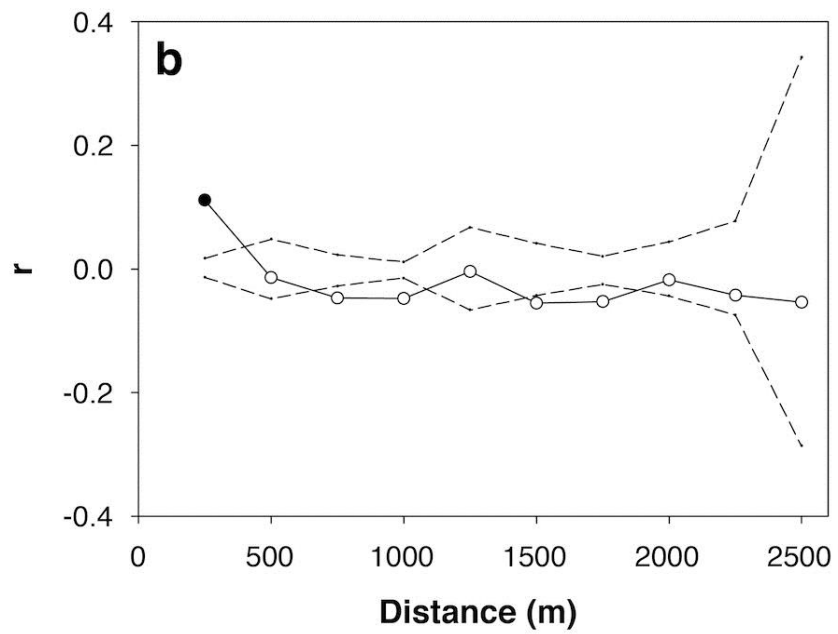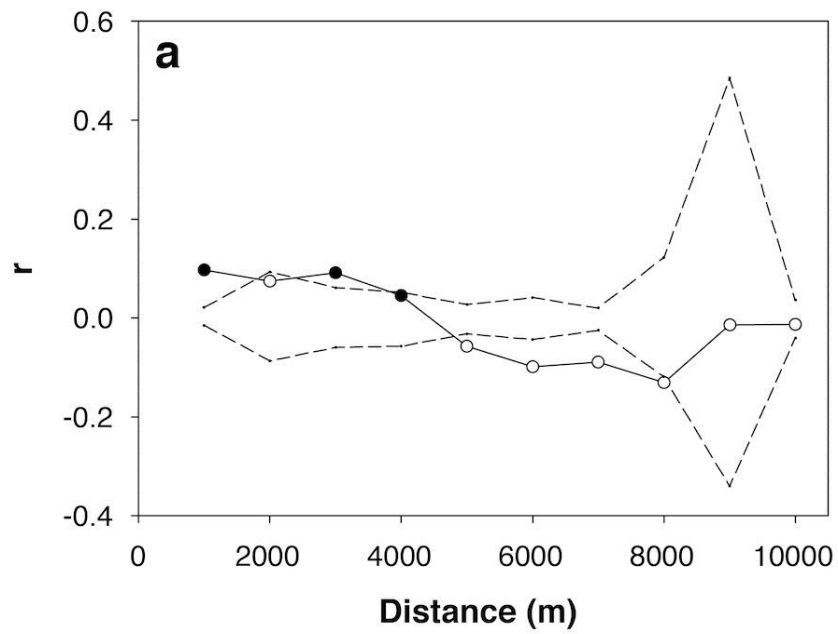
680   transformed habitats.

681 FIGURES
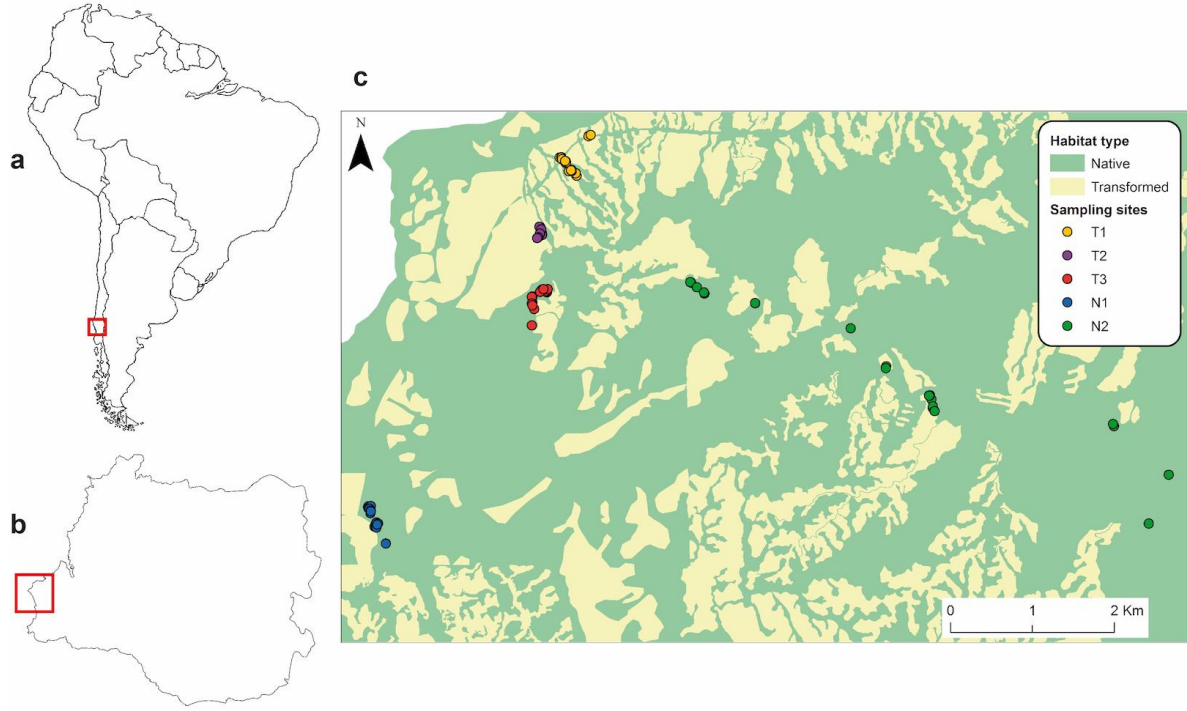
682

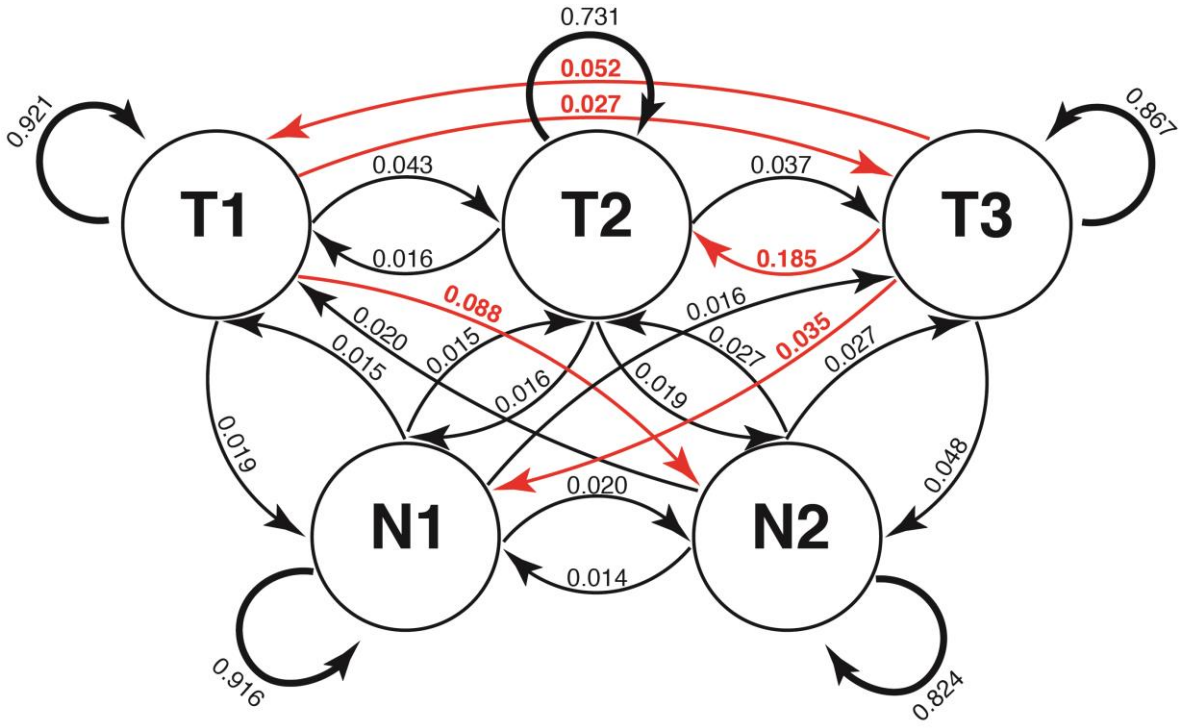683   Figure 1

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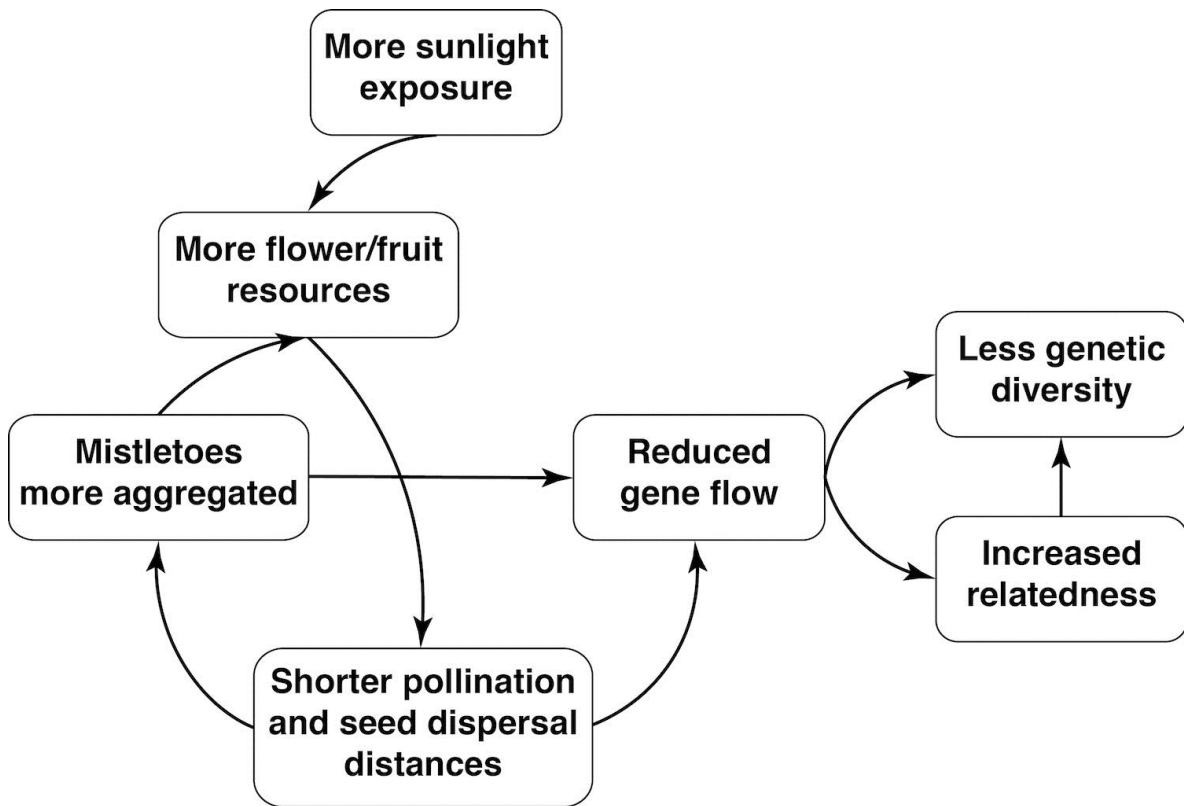690   Figure 4

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695
<div align="center">Figure 5</div>