

# Scale-dependent responses of pollination and seed dispersal mutualisms in a habitat transformation scenario

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## Summary

**1.** Transformed habitats are the result of deliberate replacement of native species by an exotic monoculture, involving changes in biotic and abiotic conditions. Despite the fact that transformed habitats are becoming more common and constitute a major biodiversity change driver, little is known about the scale-dependent responses of plant–animal mutualisms.

**2.** Aiming to test the multiscale responses of pollination and seed dispersal in a habitat transformation scenario, we examined a gradient of native and transformed habitats at three spatial scales (0–50, 50–100 and 100–250 m) and focused on a highly specialized mutualistic system composed of a hemiparasitic mistletoe (*Tristerix corymbosus*) that is almost exclusively pollinated by a hummingbird (*Sephanoides sephaniodes*) and dispersed by an arboreal marsupial (*Dromiciops gliroides*).

**3.** Even though mistletoes were found along the gradient, they were more abundant and more densely aggregated when the transformed habitat was dominant. Disperser and pollinator activity also increased as the transformed habitat becomes dominant, at the scale of 0–50 and 50–100 m, respectively. Furthermore, crop size and disperser activity covaried at broad and intermediate scales, whereas recruitment covaried at intermediate and fine scales. Moreover, disperser activity and the number of seedlings were spatially associated, stressing *D. gliroides*' role in the recruitment of the mistletoe.

**4. Synthesis.** This highly specialized mutualistic system seems to be responding positively to the habitat structure modifications associated with *Eucalyptus* plantations. However, the actual costs (e.g. reduced gene flow, increased herbivory) in these transformed habitats are yet to be assessed.

**Key-words:** Chile, *Dromiciops gliroides*, Moran's eigenvector maps, plant population and community dynamics, SADIE, *Sephanoides sephaniodes*, *Tristerix corymbosus*

## Introduction

A major goal in ecology is to recognize ecological patterns arising at different spatial scales and to relate them to particular ecological processes (Wiens 1989; Kotliar & Wiens 1990; Levin 1992). Several ecological patterns result from multiscale ecological processes, which are difficult to interpret adequately from a single-scale perspective. For example, patterns in plant demography and regeneration are scale-dependent phenomena usually affected by resource availability and habitat structure (García & Chacoff 2007; García, Zamora & Amico 2011), in which multiscale patterns may emerge from the fact that each interacting animal has a different response

scale of a plant's resources (e.g. a pollinator bird perceives a wider scale than a seed-predator rodent). Furthermore, in response to resource availability, animal activity might match the plant's spatial distribution (García, Rodríguez-Cabal & Amico 2009), creating a cyclic process in which plant resources influence the animal's behaviour which in turn shapes the population structure and spatial distribution of the plant (Sasal & Morales 2013).

The study of mutualistic interactions may shed light on the spatial scales at which key ecological processes are affected by human activities such as habitat loss, fragmentation and degradation, which are known to have scale-dependent effects on plant–animal mutualisms (e.g. García & Chacoff 2007; Rodríguez-Cabal, Aizen & Novaro 2007; González-Varo 2010). Additionally, invasive species are known to alter plant–animal interactions (Morales & Aizen 2006; Wandrag

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*et al.* 2013), being potentially disruptive in the case of mutualisms (Davis *et al.* 2010; Rodríguez-Cabal *et al.* 2013). However, how plant–animal interactions are affected when native species are replaced by an exotic monoculture (e.g. Murúa *et al.* 2010) is little explored yet, despite being relevant for the natural regeneration in degraded or abandoned productive lands.

Among a complex mosaic of scattered historical habitat remnants, second-growth stands and productive lands, transformed habitats are becoming more common as the result of human actions, involving not only the replacement of native species by an exotic monoculture, but also changes in environmental conditions (Hobbs *et al.* 2006, 2014; Melo *et al.* 2013), but we do not know how interspecific interactions are responding to these changes in habitat complexity at the landscape level.

Aiming to test multiscale habitat responses of ecological interactions in a habitat transformation scenario, we focused on a highly specialized mutualist system composed of a hemiparasitic mistletoe (*Tristerix corymbosus*) that is almost exclusively pollinated by one hummingbird species (*Sephanioides sephanioides*) and dispersed by one marsupial (*Dromiciops gliroides*) (Aizen 2003). We used this study system to answer the following questions: (i) Are pollination and seed dispersal interactions functional in transformed habitats? (ii) If so, how they affect plant recruitment? and (iii) Are those responses consistent through different spatial scales and which are the demographic consequences of potential scale discordances?

## Materials and methods

### STUDY SITE AND SPECIES

This study was conducted in the Valdivian Coastal Reserve (39°57' S 73°34' W), a 50 530-ha private protected area owned and managed by the NGO The Nature Conservancy (Delgado 2010). The Valdivian Coastal Reserve is the largest remnant of native temperate rain forest of southern South America, an ecosystem rich in endemic species but threatened by human activities (Myers *et al.* 2000; Mittermeier *et al.* 2005). This area represents a large forest continuum with a habitat mosaic composed by the following: (i) old-growth native stands (with large *Nothofagus dombeyi*, *N. pumilio*, and *Fitzroya cupressoides* canopy trees, and sparse understorey vegetation dominated by *Laurelia philippiana*, *Mitraria coccinea* and *Lomatia ferruginea*); (ii) secondary growth native stands (regenerated after been clear-cut once; presenting a canopy with sparse *N. pumilio*, *N. dombeyi* and *Eucryphia cordifolia* individuals, and an understorey dominated by *Drimys winteri*, *M. coccinea*, *Tepualia stipularis*, *Chusquea quila* and *Blechnum* spp. ferns); and (iii) exotic *Eucalyptus globulus* abandoned plantations (12–20 years old, never harvested or managed after their establishment) containing abundant understorey native vegetation (dominated by *Aristotelia chilensis*, *Rhaphithamnus spinosus*, *Ugni molinae*, *Luma apiculata*, *C. quila*, and *Lapageria rosea* vines climbing on the *Eucalyptus* stems).

We focused on the system composed of the hemiparasitic mistletoe *Tristerix corymbosus* (L.) Kuijt (Loranthaceae), which is a winter-flowering plant found on at least 30 different host trees and considered a keystone resource for forest-dwelling animals (Aizen 2003, 2005). This mistletoe presents two highly specialized mutualistic

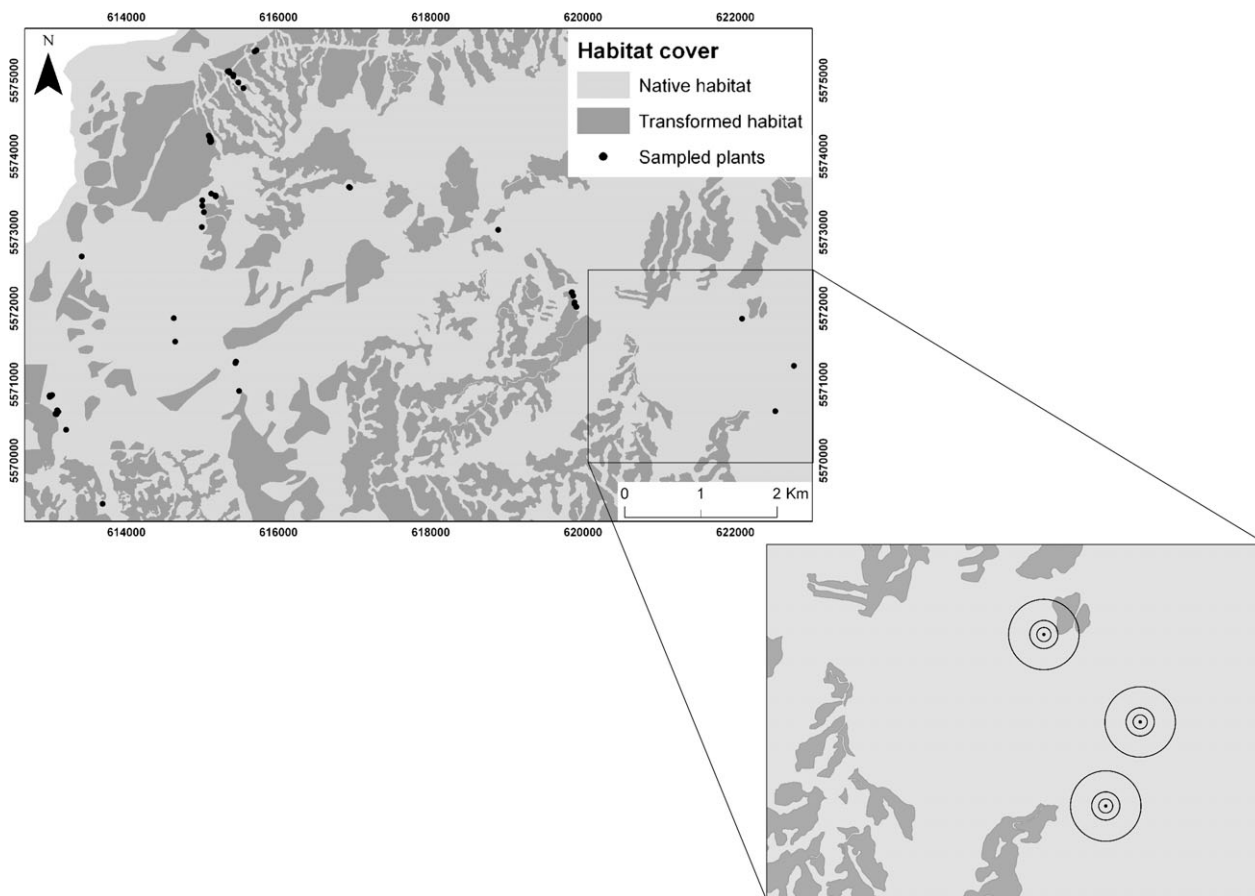
interactions for reproduction. On the one hand, *T. corymbosus* depends on the Green-backed Firecrown *Sephanioides sephanioides*, a small hummingbird that provides most of the pollination service (Aizen 2005). On the other hand, this mistletoe depends almost exclusively on the arboreal marsupial *Dromiciops gliroides* to disperse its seeds (Amico & Aizen 2000). The marsupial is the only legitimate disperser known in the southern (>37°S) part of its distribution range (Amico, Rodríguez-Cabal & Aizen 2011). This unique study system allows assessing the effects of habitat alteration on two highly specialized and sequential mutualisms that ultimately determine the plant's reproductive success.

### DATA COLLECTION

From July 2011 to November 2012, we searched the study area for mistletoes, using all roads and paths available (by car or walking), covering most of the northern sector of the Valdivian Coastal Reserve (where *Eucalyptus* were planted). From that search, we found 278 mistletoes in 197 different host plants, which were tagged and georeferenced. From December 2012 to March 2013 (austral summer season), we sampled 70 *T. corymbosus* plants (Fig. 1), which corresponded to all plants that had both flowers and fruits during the fieldwork and were accessible enough to take samples and monitor visits (see specific methods below). Fourteen mistletoes (20% of the sample) presented more than one mistletoe at the host plant (8 hosts presented two mistletoes, 2 hosts presented three and 4 hosts presented four); we worked only with the largest one (i.e. we sampled only one mistletoe per host). Mistletoes were found parasitizing 13 host species (detailed information available online in Table S1 in Supporting Information) being *Aristotelia chilensis* and *Rhaphithamnus spinosus* the most common hosts at the transformed habitat, and *Pluchea absinthioides* at the native forest. No mistletoes were found parasitizing *Eucalyptus* trees. Each plant was tagged and georeferenced using a Garmin Vista Cx GPS. For each sampled plant, we recorded the following information: (i) number of flowers; (ii) crop size; (iii) number of plants per host tree (as in many mistletoe species, it is common to find intense reinfection on the same host plant); and (iv) number of *T. corymbosus* seedlings present on the host tree (as a proxy of recruitment). We visually counted flowers, fruits, plants per host tree, and seedlings.

To quantify pollination and seed dispersal mutualisms, we used visitation rate as an interaction proxy, since this measure is known to be a good surrogate (Vásquez, Morris & Jordano 2005). We used infrared camera traps (Bushnell Trophy Cam 2011) set in video mode (resolution of 640 × 480, length 15 s, sensor at normal level). Cameras were placed in front of each sampled plant for 48 continuous hours. Visitation rate monitoring was conducted in a 6-day period (divided in three sets of 48 h). We expressed *S. sephanioides* and *D. gliroides* visitation rates as the number of recorded visits (in which we saw actual pollination or fruit consumption) per 48 h. Aiming to quantify the success of each phase of plant recruitment, we estimated fruit set as the ratio between the number of fruits produced and the number of flowers, also we estimated fruit removal by marking ten random fruits per plant with a non-toxic paint and counting the number of removed fruits after 7 days (we set seed traps to account for fallen fruits), and finally, we estimated seed germination by setting five seeds per plant in petri dishes with wet filter paper for 5 days. These three measures were expressed as a proportion.

As habitat modification involves changes in the abiotic conditions, we measured microclimate conditions that might affect both plants and animals (Cleary *et al.* 2007): air temperature, relative humidity



**Fig. 1.** Sampled plants and habitat cover configuration. Light grey areas correspond to native forest, whereas dark grey areas correspond to the transformed habitat (*Eucalyptus* plantation with native understorey). In the box, habitat rings at the three spatial scales defined are depicted.

(using a hand-held digital thermohygrometer) and luminosity (using a hand-held digital luxometer) below each sampled plant. Then, we used those measures as explanatory variables to contrast the measured response variables described above. Since these variables are measured in different units, values were standardized (mean = 0, variance = 1) before including those variables in the statistical models.

#### SPATIAL SCALES AND NATIVE HABITAT QUANTIFICATION

To assess the scale-dependent responses of mutualistic interactions, we defined three spatial scales by three non-overlapping concentric rings: the first from 0 to 50 m around each sampled plant, the second from 50 to 100 m and the third from 100 to 250 m. We chose the non-overlapping ring approach to avoid multicollinearity among scales (García & Chacoff 2007). The 0–50 m scale depicts the immediate vicinity of the plant, the 50–100 m scale depicts the plant neighbourhood and the 100–250 m scale involves the approximate foraging area of *D. gliroides*, since this species is known to have a home range of ca. 1.6 ha and a maximum displacement distance of 500 m (Fontúrbel *et al.* 2012).

Since the study area presents a complex habitat mosaic with a heterogeneous mixture of native and transformed (i.e. *Eucalyptus* plantations with abundant native understorey vegetation) forest stands, we employed an environmental gradient approach using aerial imagery and digital cartography of the study area to quantify the

proportion of native habitat surrounding each sampled plant at each spatial scale. The proportion of native habitat within a given radius from each sampled plant was considered as proxy of the strength of habitat alteration. All GIS procedures were conducted using ArcGIS 10.1 (ESRI, Redlands, CA, USA).

For comparative purposes, we plotted a set of 70 random points over the study area and repeated the same procedures described above in an attempt to obtain a random distribution of native habitat proportion for each spatial scale. Actual and random distributions at each spatial scale were compared using a bootstrap Kolmogorov–Smirnov test with 10 000 iterations.

#### DATA ANALYSIS

We used three analytical approaches: (i) pattern causality, (ii) pattern covariation and (iii) pattern concordance. We decided to analyse patterns in this way to first describe the responses of each measured variable to the spatial scales defined above; then, we aimed to assess whether pollination and seed dispersal processes were covarying at the same scale and then to assess whether pollination and seed dispersal patterns were concordant in a spatially explicit scenario. We used the proportion of native habitat at the three defined spatial scales as explanatory variables. Response variables included number of flowers, *S. sephaniodes* visitation rate, crop size, *D. gliroides* visitation rate, number of seedlings, and number of plants per host tree. As plants were non-randomly distributed in space and, hence, some

observations were not spatially independent from each other, we developed spatially explicit models to fit our data. Prior to model building, we made a preliminary spatial assessment of each response variable through inspection of potential spatial autocorrelation in the raw data. We found positive and significant spatial autocorrelation for number of flowers, crop size and *D. gliroides* visitation rate, but no autocorrelation was detected for the number of plants per host tree, seedlings per tree or *S. sephaniodes* visitation rate (Table S2).

We tested three analytical approaches (following Dormann *et al.* 2007): a regular generalized linear model (GLM) with a Poisson error distribution (log link function), a spatially explicit GLM with a Poisson error distribution incorporating a spatial covariate, and a spatially explicit generalized additive model (GAM) with a Poisson error distribution incorporating a spline term with the UTM coordinates of each plant. After comparing the performance of each approach (using AIC scores, residual fit and Moran's partial correlograms), GAMs were chosen (see Table S3 for model performance comparison). To assess causality, we fitted GAMs (Poisson error distribution, log link function), operating explanatory variables (i.e. the proportion of native habitat at the three scales defined) as linear terms and operating a spline term (based on the X,Y coordinates of each mistletoe) accounting for the spatial structure of the data for two purposes: (i) to assess the effect of the proportion of native habitat at the three spatial scales defined, and (ii) to assess the effects of the measured microclimate variables (i.e. temperature, humidity and luminosity) on the response variables quantified. We tested all GAMs for overdispersion (following Zuur *et al.* 2009), finding true overdispersion for the number of flowers and crop size. Therefore, we accounted for overdispersion on those models by using a quasi-Poisson error distribution (log link function) instead. Furthermore, to make a connection between the proportion of native habitat and microclimatic conditions, we calculated Spearman partial correlations for each microclimate variable, controlling by the remaining two variables.

To assess spatial covariation patterns, we used the Moran's eigenvector map approach [MEM hereafter, previously known as principal coordinates of neighbour matrices (PCNM)] that decomposes spatial variability into broad, intermediate and fine scales by conducting multiple regression analyses using the resulting positive MEM eigenvectors (Borcard & Legendre 2002; Borcard *et al.* 2004; Dray, Legendre & Peres-Neto 2006). We used an irregular bidimensional design, with which 23 out of 29 eigenvectors were positive and kept for further analyses. We split eigenvectors into three groups: eigenvectors V1 to V8 correspond to broad-scale variation, eigenvectors V9 to V16 to the intermediate-scale variation, and eigenvectors V17 to V23 to fine-scale variation. Eigenvectors were also used as explanatory variables in forward multiple regression models against our response variables (number of flowers, *S. sephaniodes* visitation rate crop size, *D. gliroides* visitation rate, number of seedlings, and number of plants per host tree). For each case, we estimated  $R^2$  and the overall significance of the multiple regression models and selected those eigenvectors with significant contributions at a given covarying scale.

Finally, to assess the spatial concordance of response variables, we employed the SADIE technique (Perry *et al.* 1999, 2002). SADIE is the acronym of spatial analysis by distance indices, involving the analysis of spatial coordinates and a count variable (e.g. number of flowers), which are used to determine the degree of spatial aggregation, as well as spatial correspondence when two data sets are compared. We used the software SADIESHELL v1.22 (Conrad 2001) to calculate (i) the extent of aggregation of each variable (Table S4; necessary for creating the cluster files needed for the next step) and (ii) the association index between variables ( $X_p$ ), which ranges between  $-1$

(complete spatial disassociation) and  $1$  (complete association), with  $0$  values indicating spatial independence. As multiple pairwise tests were performed,  $P$ -values were adjusted using a sequential Bonferroni procedure. All statistical analyses were conducted in R 2.15 (R Development Core Team 2012) and the packages vegan (Oksanen *et al.* 2013), mgcv (Wood 2001), spdep (Bivand 2014), spatstat (Baddeley & Turner 2005), Matching (Sekhon 2011) and mpmcorrelogram (Matesanz *et al.* 2011).

## Results

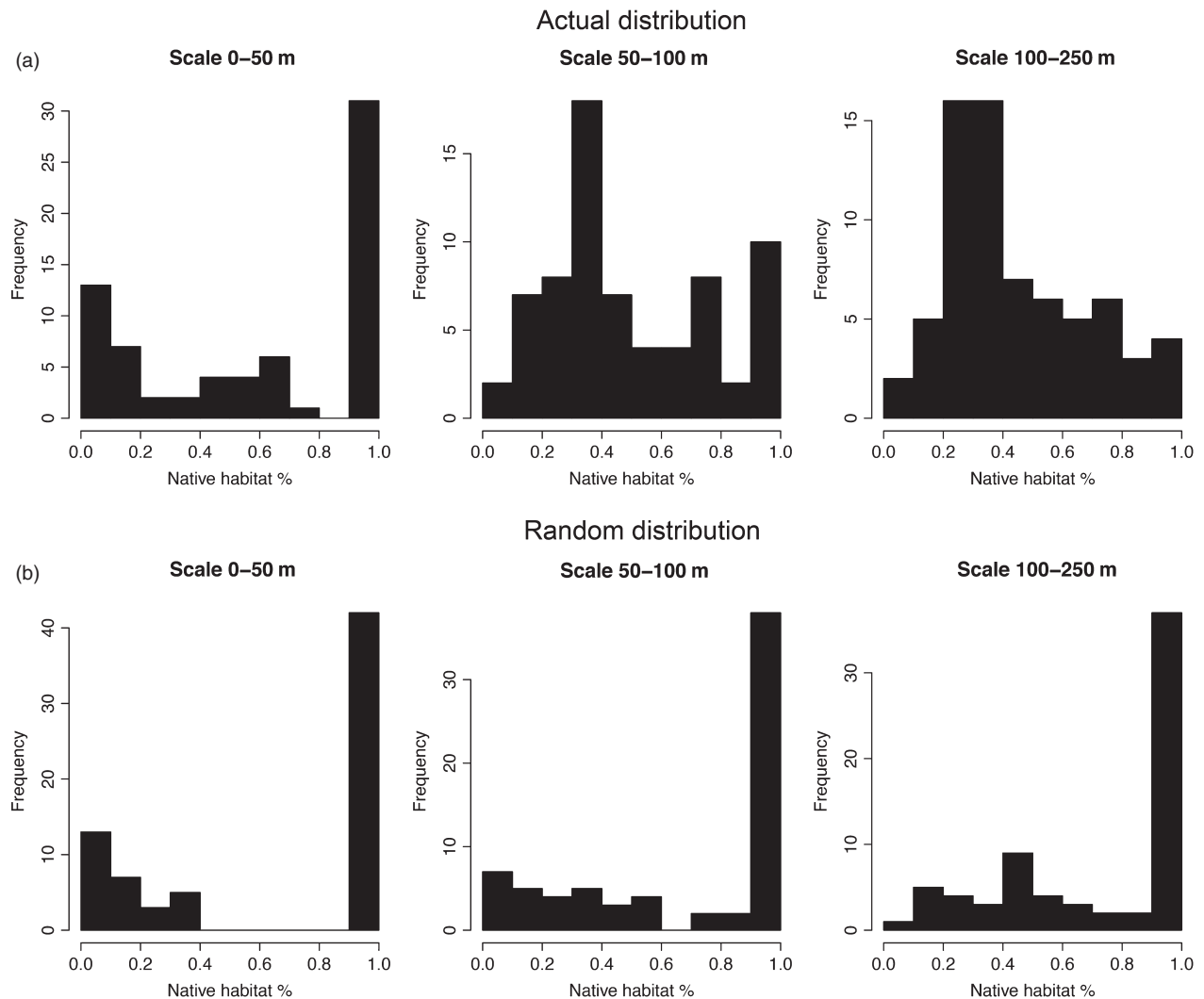
Sampled plants were distributed along native and transformed habitats at the study area, with spots of dense plant aggregation and some isolated individuals (Fig. 1). Highly aggregated plants had larger flower displays, crop sizes, more *D. gliroides* and *S. sephaniodes* visitation rates, and a larger number of seedlings and plants per host tree, after a visual inspection of the raw data (Fig. S1). Comparing the observed and random distributions, *T. corymbosus* was found in dominant native forest in a lower proportion than expected by its availability (Fig. 2), considering that 86.53% of the study area is native habitat. Comparisons of actual and random distributions differed at each of the three spatial scales: 0–50 m (Kolmogorov–Smirnov test,  $D = 0.27$ ,  $P = 0.012$ ), 50–100 m ( $D = 0.46$ ,  $P < 0.001$ ) and 100–250 m ( $D = 0.50$ ,  $P < 0.001$ ).

### PATTERN CAUSALITY

We first examined the causal relationships between the gradient of native habitat at the three defined spatial scales and a set of response variables relevant to the reproductive success and recruitment of *T. corymbosus* (Table 1). Regarding pollination, the number of flowers was not affected by native habitat proportion at any scale, whereas the visitation rate of the hummingbird *S. sephaniodes* decreased with an increased proportion of native habitat at the 50–100 m scale but increased with native habitat at 100–250 m. Both the number of flowers and visitation rates of *S. sephaniodes* responded to the spatial structure. Regarding fruit availability, crop size showed significant variation as a function of the spatial structure but was not affected by the amount of native habitat at any spatial scale, as happened with the flowers. Conversely, the visitation rate of the disperser *D. gliroides* was not affected by the spatial structure but was negatively affected by native habitat cover at two scales: 0–50 and 100–250 m. Regarding recruitment of *T. corymbosus*, the number of seedlings responded only to the spatial structure, whereas the number of mistletoes per tree (a proxy of reinfection) was not affected by the spatial structure or by the proportion of native habitat at any scale (Table 1).

We examined the correlation between the amount of native habitat with temperature, relative humidity and luminosity at the three defined scales through partial correlations. The proportion of native habitat was correlated with relative humidity in the three scales measured ( $P < 0.001$  in all cases), but temperature and luminosity did not correlate significantly. Fitting GAMs





**Fig. 2.** Histogram plots of the proportion of native habitat at three spatial scales: 0–50, 50–100 and 100–250 m. (a) Observed distributions measured from aerial imagery and GIS files; (b) random distribution generated from 70 random points in the study area.

**Table 1.** Summary of results of generalized additive model for each response variable measured, contrasted against the proportion of native habitat at three spatial scales, incorporating a spatially explicit nonlinear term (X,Y spline). Adjusted  $R^2$ -values, estimates, their standard error (in parentheses) and  $P$ -values are presented. Ss, *Sephaniodes sephaniodes*; Dg, *Dromiciops gliroides*. Significance levels: † $P < 0.1$ , \* $P < 0.05$ , \*\* $P < 0.01$

	$R^2$	Native habitat proportion			X,Y spline, $P$
		Scale 0–50 m	Scale 50–100 m	Scale 100–250 m	
No. of flowers	0.15	0.57 (0.46)	−0.02 (0.84)	−0.63 (0.96)	<0.01
Ss visit rate	0.69	1.71 (1.90)	−5.86 (2.80)*	6.71 (3.85)†	<0.01
Crop size	0.19	0.70 (0.42)	−0.13 (0.78)	−0.44 (0.89)	<0.01
Dg visit rate	0.84	−2.87 (1.05)**	−5.59 (6.77)	−53.96 (18.72)**	0.40
Seedlings	0.99	−1.59 (5.58)	4.50 (46.61)	11.44 (56.67)	0.02
No. of plants	0.05	0.16 (0.42)	−0.33 (0.85)	0.40 (1.02)	0.69

using microclimate features as explanatory variables revealed that the number of flowers, crop size and the visitation rates of *S. sephaniodes* and *D. gliroides* were influenced by microclimate conditions, whereas only the visitation rates of

*S. sephaniodes* and *D. gliroides* were influenced by the spatial structure. The number of seedlings was affected by relative humidity, and the number of plants per host tree was not affected by any of the microclimate features measured (Table 2).

**Table 2.** Summary of results of generalized additive model for each response variable analysed, contrasted against microclimate features (temperature, relative humidity and luminosity; based on standardized values), incorporating a spatially explicit nonlinear term (X,Y spline). Estimates, their standard error (in parentheses) and *P*-values are presented. Ss, *Sephaniodes sephaniodes*; Dg, *Dromiciops gliroides*. Significance levels: †*P* < 0.1, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001

	<i>R</i> <sup>2</sup>	Microclimatic variables			
		Temperature	Relative humidity	Luminosity	X,Y spline, <i>P</i>
No. of flowers	0.30	−0.48 (0.10)**	−0.55 (0.17)**	0.09 (0.09)	0.26
Ss visit rate	0.03	0.69 (0.42)	−0.17 (0.78)	−0.91 (0.24)***	<0.01
Crop size	0.31	−0.41 (0.17)*	−0.50 (0.16)**	0.11 (0.08)	0.21
Dg visit rate	0.36	−0.17 (0.28)	−0.52 (0.34)	0.48 (0.26)†	<0.01
Seedlings	0.65	−19.73 (17.89)	141.61 (68.65)*	2.42 (3.33)	0.72
No. of plants	0.25	−0.13 (0.18)	−0.18 (0.18)	0.01 (0.12)	0.93

#### PATTERN COVARIATION

Two out of the 23 positive eigenvectors resulting from the MEM analysis significantly accounted for the number of flowers and crop size variation; four eigenvectors were significant for *D. gliroides* visitation rates and one eigenvector was significant for the number of seedlings and for the number of plants per host tree. No eigenvector showed significant covariation with *S. sephaniodes* visitation rates. The number of flowers, crop size and visitation rate of *D. gliroides* varied at broad and intermediate scales, but not at the fine scale. The number of seedlings showed variation only at the fine scale, and the number of plants per host tree varied only at the intermediate scale (Table 3). The pattern of covariation shown by MEM analysis indicates that crop size and the activity of the disperser covaried at the same spatial scales, whereas the number of seedlings and reinfection on the same host (variables describing plant recruitment output) covaried at finer scales and *S. sephaniodes* activity seems to be

**Table 3.** Summary of multiple regression models fitting the number of flowers, crop size, *Dromiciops gliroides* (=Dg) visitation rates, the number of seedlings and the number of plants per host tree. Significant MEM vectors are shown with their respective *R*<sup>2</sup> and *P*-values in three progressively finer scales. The overall determination coefficient (*R*<sup>2</sup>) is shown for each variable

Scale	No. of flowers	Crop size	Dg visit rate	Seedlings	No. of plants
Broad					
MEM vectors	V1	V1	V1, V7		
<i>R</i> <sup>2</sup>	0.16	0.18	0.19		
<i>P</i> -value	0.025	0.024	0.018		
Intermediate					
MEM vectors	V13	V13	V11, V12		V11
<i>R</i> <sup>2</sup>	0.14	0.18	0.19		0.13
<i>P</i> -value	0.035	0.010	0.008		0.028
Fine					
MEM vectors				V18	
<i>R</i> <sup>2</sup>				0.40	
<i>P</i> -value				<0.001	
<i>R</i> <sup>2</sup> total	0.39	0.41	0.38	0.49	0.24

scale-independent although its resource covaries at broad and intermediate scales.

#### PATTERN CONCORDANCE

Examining pattern concordance through pairwise spatial association of the variables examined above, we found significant associations between the number of flowers and crop size (SADIE  $X_p = 0.82$ ,  $P < 0.01$ ), the number of flowers and *S. sephaniodes* visitation rates ( $X_p = 0.25$ ,  $P = 0.03$ ), crop size and the number of mistletoes ( $X_p = 0.27$ ,  $P = 0.01$ ), and *D. gliroides* visitation rates and the number of seedlings ( $X_p = 0.39$ ,  $P < 0.01$ ). However, the associations between the number of flowers and *S. sephaniodes* visitation rates, as well as crop size and the number of plants, lost its significance after sequential Bonferroni adjustment ( $P_{adj} = 0.28$  and  $P_{adj} = 0.11$ , respectively), but the associations between the number of flowers and crop size and between *D. gliroides* visitation rate and the number of seedlings retained its significance after adjustment ( $P_{adj} < 0.01$  in both cases).

#### MISTLETOE RECRUITMENT

The numbers of flowers and fruits were larger at the native habitats at the three spatial scales, but fruit set values were similar across scales (ranging from 83 to 86%). Pollinator and seed disperser visitation rates were variable across spatial scales, being larger at the transformed habitat at the 0–50 and 100–250 m scales, but larger at the native habitat at the 50–100 m scale. Fruit removal rates were larger at the transformed habitat regardless of the spatial scale. Germination rates were similar between habitats and among scales (ranging from 73 to 82%), but the number of seedlings and the number of plants per host (as a proxy of reinfection) were larger at the native habitat in all cases (Fig. 3).

#### Discussion

Mistletoes in the transformed habitat were more abundant than expected by chance, according to its availability in the landscape, as reported for other mistletoe species in Australia (Bowen *et al.* 2009). Such patterns may emerge from the

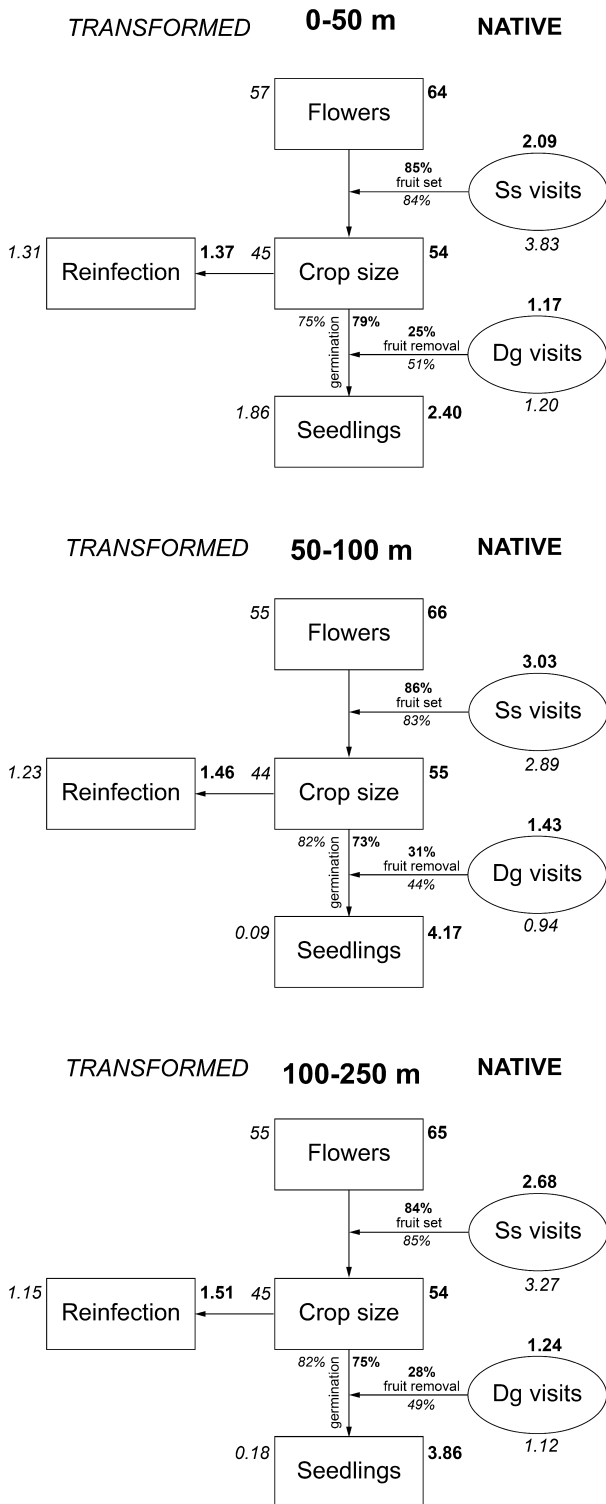
following: (i) the resource concentration of neighbouring mistletoes with larger crop sizes (see Fig. S1a) within the disperser's home range (ranging from 1.0 to 2.2 ha; Fontúrbel

*et al.* 2010), and (ii) the greater availability of alternative fleshy fruits from other shade-intolerant species characteristic of early successional stages (e.g. *Aristotelia chilensis*, *Rhaphithamnus spinosus*, *Ugni molinae*; see Table S5 for details), which usually have large fruit displays. A study conducted in Argentina showed that *D. gliroides* responded to greater resource availability by increasing frugivory activity and reducing dispersal distances, causing aggregation patterns at locations with dense fruit neighbourhoods (Morales *et al.* 2012). The same pattern has been described for frugivorous birds (Carlo & Morales 2008; Uriarte *et al.* 2011).

The relationship between the response variables and the proportion of native habitat cover shows that *S. sephanioides* was significantly affected by native habitat cover at the 50–100 m scale. Interactions were more frequent at those locations where transformed habitat was dominant; also there was an opposite but marginally significant effect at 100–250 m. Such opposite responses at different scales may result from a greater landscape complexity where neighbouring native and transformed habitats are complementary (see hypothesis 7 in Tschardtke *et al.* 2012), resulting in an increased resource offer as the analysis scale is enlarged. The activity of *S. sephanioides* showed a weak response to resource availability (i.e. number of mistletoe flowers). However, pollinator activity could be also influenced by the floral neighbourhood present in the transformed habitat, where *Eucalyptus* flowers and those from the woody vine *Lapageria rosea* are abundant; *L. rosea* has conspicuous and nectar-rich flowers and climbs the trunks of *Eucalyptus* trees (FE Fontúrbel, personal observation).

The visitation rates of *D. gliroides* were significantly affected at the 0–50 m scale (depicting the situation in the immediate vicinity of the plant). This result was not surprising as this arboreal marsupial depends on the fine habitat structure to move and reach the plant by climbing through bamboo stems and thin branches. However, contrary to our expectations, *D. gliroides* visited mistletoes more frequently in places dominated by transformed habitat rather than native forest. This finding is contrary to descriptions of this species as an old-growth native forest specialist (Hershkovitz 1999). Currently, there is a growing body of literature that suggests *D. gliroides* is a forest generalist, able to thrive on second-growth forests as long as they retain their three-dimensional structure and some key elements such as fallen logs, thin branches and bamboo (Rodríguez-Cabal & Branch 2011). Native habitat cover at the broadest scale (100–250 m) also influenced *D. gliroides* visitation rates, which might be related to the selection of foraging areas, which is coincident with the average extent of many of the mistletoe clusters found at the study area. Furthermore, *D. gliroides* activity varied at broad and intermediate spatial scales according to the MEM analyses performed, matching the scales at which resources (i.e. crop size) varied. This association was previously reported in Argentina (García, Rodríguez-Cabal & Amico 2009).

Changes in microclimate conditions have been recognized to affect the probability of fruit consumption in fragmented



**Fig. 3.** Mistletoe recruitment path at the three spatial scales defined. Values in bold (located at the right or the top) correspond to the native habitat, whereas values in italic (located at the left of the bottom) correspond to the transformed habitat. Correspondence to transformed or native habitat was determined by the median value of native habitat cover at each scale. Ss, *Sephanioides sephanioides*; Dg, *Dromiciops gliroides*.

habitats (Galetti, Alves-Costa & Cazetta 2003). In our study system, the only microclimatic factor that was correlated with the gradient of habitat alteration was relative humidity, because of the structural simplification. Temperature and luminosity also are affected by structural simplification, but their influence was weak. There was a strong positive effect of relative humidity on the number of seedlings that could be related to seed survival. As *T. corymbosus* seeds germinate glued on a host's branches they probably require humidity to maintain embryo moisture and ensure haustorium development until contacting the host xylem vessels.

*Tristerix corymbosus* recruitment benefited at the transformed habitat level because of large clusters of flowers and fruits, as well as an increased germination, compared to the native forest. Plant and animal responses seem to match at different spatial scales, making it possible for *T. corymbosus* to recruit in both habitat types. However, the low number of adult plants found at the native habitat may be related to safe sites (Reid 1989, 1991; Okubamichael *et al.* 2011), which in this case are related to the host quality as the most common host in the native habitat (*Pluchea absinthioides*) seems to be a low-quality host because of its seasonality and the high mistletoe mortality detected in field (FE Fontúrbel, personal observation). We found spatial concordance between the number of plants per host tree and crop size (although it was not significant after Bonferroni correction), suggesting that hosts with large mistletoe display are more likely to be reinfected (Medel *et al.* 2004; Cazetta & Galetti 2007). The clearest evidence linking *D. gliroides* with *T. corymbosus* recruitment is the strong spatial association between seedlings and *D. gliroides* visits. This fact confirms the patterns observed in Argentina (Rodríguez-Cabal, Aizen & Novaro 2007; Rodríguez-Cabal & Branch 2011), stressing the role of *D. gliroides* as the sole legitimate disperser of *T. corymbosus* in the southern portion of its distribution range (Amico, Rodríguez-Cabal & Aizen 2011).

*Tristerix corymbosus* was more abundant in transformed habitats (69% of the sampled mistletoes were found in transformed habitats; see Table S1), and was also found in denser aggregates than plants located in native habitat stands. Mistletoe spatial distributions are characteristically aggregated due to host and disperser effects (Medel *et al.* 2004; Rawsthorne, Watson & Roshier 2011). In this study, transformed habitats offer favourable conditions for *T. corymbosus* and other fleshy-fruited plants that provide a rich mixed neighbourhood attractive to frugivores (Carlo & Morales 2008), which may result from a greater landscape complexity (Tscharntke *et al.* 2012). It is likely that this spatial pattern reinforces the cyclic process of reduced dispersal distances that cause even more aggregation in the next generation (Morales & Carlo 2006).

Degradation of natural habitats or the abandonment of productive agroforestry systems may result on novel ecosystems, when biotic and abiotic conditions change simultaneously (Hobbs *et al.* 2006; Hobbs, Higgs & Harris 2009). To be considered as a novel ecosystem, a transformed habitat needs to meet four criteria: (i) result from an intentional human

alteration, (ii) present thresholds that differentiate them from natural, degraded or invaded habitats, (iii) comprise novel species compositions that are impossible to occur naturally, and (iv) to be self-sustaining without management or any human intervention (Morse *et al.* 2014). Our study site here may be also considered as a novel ecosystem, as it met the first three criteria, and our findings on ecological interactions strongly suggest that the fourth condition may also be met, opening a new research venue on this topic.

Here, we present a particular scenario of anthropogenic disturbance in which both habitat extent and geometry remained constant (contrary to what happens in a habitat fragmentation scenario), but habitat structure has been modified due to a 20-year-old replacement of native forest by a *Eucalyptus* plantation. Fragmented landscapes usually present a numerical response in which populations decrease as a result of habitat loss, but on degraded and transformed landscapes, species composition changes are more important (Caley, Buckley & Jones 2001; Melo *et al.* 2013). It is noteworthy that such specialized plant–animal interactions still remain functional in a habitat transformation scenario, in which mistletoes are abundant in transformed stands, and both mutualistic interactions seem to be reinforced in this new scenario. Highly specialized mutualistic systems are typical of insular ecosystems (e.g. Olesen & Valido 2003), but they can also be found at biogeographically isolated continental systems such as Chilean temperate rain forests, which are expected to be more sensitive to habitat disturbance since a depauperate fauna results in low (or none) functional redundancy among species. Therefore, it is expected for generalist interactions to persist in an anthropogenic scenario, but in this case we have a highly specialized mutualistic system thriving in a transformed habitat, dominated by exotic tree species. Nevertheless, the costs of thriving in such transformed habitats are virtually unknown yet. This habitat transformation scenario could be costly for plants (e.g. gene flow reduction, increased foliar and floral herbivory), and we need further research to understand the real impact of thriving in a transformed habitat on plant life cycle and its ecological interactions.

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## Data accessibility

Data available from the Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.11385> (Fontúrbel, Jordano & Medel 2015).



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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Host plant species parasitized by the sampled mistletoes.

**Table S2.** Multivariate partial Mantel correlograms results for raw response variables measured.

**Table S3.** Performance of standard GLMs, spatially covaried GLMs and GAMs with a spatial spline, for each response variable measured.

**Table S4.** Aggregation index results for the response variables assessed using SADIE.

**Table S5.** Number of ripe fleshy fruits counted in a 2.5 m radius from each focal mistletoe plant.

**Figure S1.** Spatially explicit bubble plots depicting the raw data of the measured response variables.