EFECTOS DE LA TRANSFORMACIÓN DEL HÁBITAT SOBRE LA DINÁMICA ECOEVOLUTIVA DE LOS MUTUALISMOS PLANTA–ANIMAL EN UN MUÉRDAGO HEMIPARÁSITO

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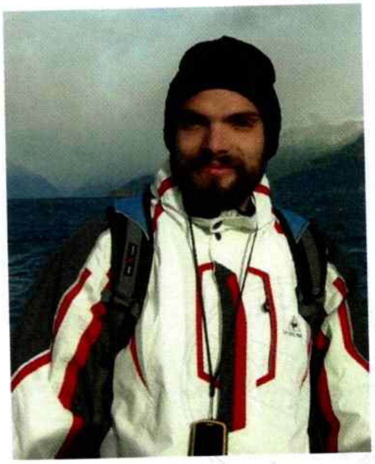

Nací el 5 de octubre de 1979 en Quito, Ecuador. En 2002 me titulé de licenciado en Ciencias Biológicas por la Universidad Mayor de San Andrés (La Paz, Bolivia) y posteriormente en 2008 recibí el grado de Magíster en Ciencias mención Producción, Manejo y Conservación de Recursos Naturales por la Universidad de Los Lagos (Osorno, Chile). Trabajé en consultorías, proyectos de investigación y docencia, en temas de medio ambiente, ecología y evolución. En 2009 me trasladé a Santiago e ingresé al Doctorado en Ciencias con Mención Ecología y Biología Evolutiva de la Universidad de Chile, donde expandí mis intereses académicos y consolidé mis líneas de investigación actuales. Los bosques templados del sur de Sudamérica han capturado mi interés desde hace ya varios años y además de constituir un sistema de estudio muy interesante, para mi son motivo de fascinación por lo compleja y diversa que es la naturaleza.

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RESUMEN

Examiné los efectos multi-escala de la transformación del hábitat sobre la dinámica eco-evolutiva de los mutualismos planta-animal, usando un sistema altamente especializado compuesto por el muérdago *Tristerix corymbosus* (Loranthaceae) que es polinizado por un picaflor (*Sephanoides sephaniodes*) y dispersado por un marsupial (*Dromiciops gliroides*). Respecto a los efectos escala-dependientes de la transformación del hábitat sobre la polinización y la dispersión, *S. sephaniodes* fue poco afectado por la transformación y respondió a una escala espacial más amplia que *D. gliroides*, cuya actividad aumentó según el hábitat transformado se hacía dominante, siendo ésta espacialmente concordante con el reclutamiento de *T. corymbosus*. El efecto acoplado de la polinización y la dispersión sobre la adecuación biológica de *T. corymbosus* a lo largo del gradiente de transformación mostró que el componente cuantitativo (tasa de visita) fue mayor en el hábitat nativo, mientras que el componente cualitativo (remoción de frutos), la germinación y la adecuación biológica fueron mayores en los bosques transformados. Examinando el escenario desde la perspectiva animal, es llamativo el haber encontrado a *D. gliroides* en un bosque de especies exóticas, siendo que se pensaba que esta especie estaba restringida al bosque nativo maduro. Ambas especies estuvieron presentes, fueron abundantes e interactuaron con *T. corymbosus* en ambos tipos de hábitat, estando la actividad de *D. gliroides* relacionada a un efecto de vecindario, que podría dar cuenta del patrón de agregación de *T. corymbosus* observado. Finalmente, los patrones de selección mediada por frugívoros mostraron que *D. gliroides* selecciona el tamaño de la semilla y el contenido de azúcares, siendo el primero más relevante en bosques nativos y el segundo en los transformados.

ABSTRACT

I have assessed the multi-scale effects of habitat transformation on the eco-evolutionary dynamics of plant-animal mutualisms, using a highly specialized system composed by the mistletoe *Tristerix corymbosus* (Loranthaceae), which is pollinated by one hummingbird (*Sephanoides sephaniodes*) and dispersed by one marsupial (*Dromiciops gliroides*). Regarding scale-dependent effects of habitat transformation on pollination and seed dispersal, *S. sephaniodes* has been little affected by habitat transformation and responded at a broad spatial scale than *D. gliroides*, whose activity increased as transformed habitat becomes dominant, and was spatially concordant with *T. corymbosus* recruitment. Then, the coupled effect of pollination and seed dispersal on *T. corymbosus*' fitness along a habitat transformation resulted in larger quantitative component (visit rates) for pollination and seed dispersal at native habitat, but dispersers' quality (fruit removal rate), germination, and plant fitness were favored at transformed stands. Further, assessing structural modification effects from the animal perspective, pinpointed the relevance of having found *D. gliroides* at an exotic-tree dominated forest since this species was thought to be restricted to old-growth forests. *Sephanoides sephaniodes* and *D. gliroides* were present, abundant, and interacting with *T. corymbosus* at both native and transformed forests, being *D. gliroides* activity also related to a mixed neighborhood, which might explain mistletoe's aggregation pattern. Finally, frugivore-mediated selection patterns on *T. corymbosus* along a transformation gradient showed that seed size and sugar content were significantly selected by *D. gliroides*, being the former more relevant at native habitats and the former at transformed habitat.

CAPÍTULO 1

INTRODUCCIÓN

Las interacciones planta-animal tienen un rol central en la generación y mantenimiento de la biodiversidad (Thompson 1994). Dentro de estas interacciones, los mutualismos planta-animal (i.e., polinización y dispersión de semillas) han sido ampliamente estudiados ya que de ellos depende el éxito reproductivo y la dinámica poblacional de muchas especies de plantas (Godínez-Alvarez & Jordano 2007, Ollerton *et al.* 2011, Sasal & Morales 2013), lo cual incide en los procesos de regeneración vegetacional en diversos tipos de ecosistemas (e.g., Gomes *et al.* 2008). La mayor parte de los mutualismos planta-animal existentes son el resultado de procesos coevolutivos difusos (Levey 1987), en los que una especie interactúa con más de una contraparte. Sin embargo, existen distintos grados de especialización al interior de las interacciones difusas, dando lugar a síndromes de polinización y dispersión de semillas (Lomáscolo *et al.* 2008, Lomáscolo *et al.* 2010, Reynolds *et al.* 2009), producto de la selección direccional y correlacional sobre un conjunto de rasgos fenotípicos (e.g., tamaño, color, contenido de azúcares, tamaño del fruto) ejercida por los animales (Pizo 2002).

Dada la importancia de los mutualismos planta-animal, éstos se han estudiado bajo distintos escenarios de perturbación antrópica, como son la pérdida y fragmentación del hábitat (e.g., Breed *et al.* 2012, Rodríguez-Cabal *et al.* 2007), la tala selectiva (e.g., Schleuning *et al.* 2011, Velho *et al.* 2012), y las invasiones biológicas (e.g., Gibson *et al.* 2012). Sin embargo, el estudio de estos mutualismos en un contexto de transformación del hábitat (i.e., la introducción deliberada de especies exóticas y/o la eliminación selectiva de especies nativas) es un tema reciente y relativamente poco explorado (e.g., Matías *et al.* 2010, Murúa *et al.* 2010), al igual que las consecuencias microevolutivas de la modificación de las interacciones ecológicas en ambientes bajo perturbación antrópica (Kinnison *et al.* 2007, Stockwell *et al.* 2003). A pesar que las perturbaciones antrópicas constituyen importantes fuerzas de evolución contemporánea, su incorporación formal en estudios de ecología es muy reciente (Kinnison & Hairston 2007).

Al modificarse la estructura del hábitat, se altera la distribución y abundancia de refugios y alimento (Kelm *et al.* 2008, Peh *et al.* 2006, Thiollay 1999), lo que a su vez puede alterar las interacciones ecológicas en que están comprometidas las especies afectadas. Los cambios en la abundancia de los mutualistas podrían ocasionar variaciones en el ambiente selectivo sobre ciertos rasgos fenotípicos de las plantas. Por lo tanto, tales cambios pueden influir indirectamente en la dinámica microevolutiva de las plantas. En teoría la transformación ambiental modifica los óptimos de adecuación biológica en los ambientes perturbados, generando nuevos paisajes adaptativos visualizables a través de coeficientes de selección hacia los nuevos óptimos. Si bien existen estudios al respecto enfocados en polinización (e.g., Murúa *et al.* 2010), es poco lo que se sabe del efecto de la transformación del hábitat en la evolución de la dispersión de semillas.

El reclutamiento de las plantas que interactúan con mutualistas animales ocurre mediante una serie de fases secuenciales (Godínez-Alvarez & Jordano 2007, Jordano 1987): (1) la polinización de las flores, (2) la remoción de los frutos, (3) la germinación de las semillas, (4) la deposición de las semillas en un lugar apropiado, y (5) el establecimiento de las plántulas, que posteriormente lleva a la producción de nuevas flores, reiniciando el ciclo. Dentro de este ciclo de mutualismos acoplados, la dispersión zoócora de las semillas constituye una fase crítica para el reclutamiento de las plantas, y más aun para aquellas plantas parásitas que dependen del dispersor para llegar a un lugar adecuado para establecerse (Reid 1989). El reclutamiento está influenciado por la efectividad de la dispersión de semillas, la cual depende del producto de dos componentes: uno cuantitativo y otro cualitativo (Schupp *et al.* 2010). El componente cuantitativo de la efectividad de dispersión de semillas está definido por la tasa de visitas y el número de semillas dispersadas por visita, mientras que el componente cualitativo está definido por la calidad germinativa de las semillas, la calidad del tratamiento en el tracto digestivo, y la conducta del dispersor de depositar la semilla en un lugar apropiado (Schupp *et al.* 2010).

En este sentido, dado que la estructura del hábitat se altera en los ambientes transformados, es esperable que se modifique la conducta de los dispersores (Sasal & Morales 2013), alterando así las decisiones de movimiento y forrajeo. El componente cualitativo de la dispersión, por otro lado, presenta una gran variabilidad de calidad germinativa en ambientes perturbados (e.g., Matías *et al.* 2010). Por lo tanto, la combinación de ambos componentes en ambientes transformados puede dar lugar a muchos posibles resultados dependiendo de la textura fina del hábitat, el microclima, y la oferta de recursos alternativos, entre otros factores.

Los mutualismos planta-animal son importantes agentes de evolución fenotípica (Rodríguez-Pérez & Traveset 2011, Thorpe *et al.* 2011), siendo de particular relevancia en los bosques templados de Sudamérica austral puesto que gran parte de las plantas dependen de interacciones mutualistas para asegurar el éxito reproductivo (Aizen *et al.* 2002). En este contexto biogeográfico, la interacción del quintral (*Tristerix corymbosus* (L.) Kuijt) con sus mutualistas, el picaflor chico (*Sephanoides sephaniodes*) que es el principal polinizador, y el monito del monte (*Dromiciops gliroides*) que es el único dispersor, conforman un sistema de estudio muy particular, donde el éxito reproductivo de la planta hemiparásita depende totalmente de dos interactuantes mutualistas (Aizen 2003, Aizen 2005, Amico & Aizen 2000). Se sabe que la fragmentación del hábitat ocasiona una reducción en el reclutamiento del quintral, como consecuencia de la disrupción del mutualismo al reducirse drásticamente la abundancia del dispersor (Rodríguez-Cabal *et al.* 2007). Por lo tanto, es esperable que los mutualismos planta-animal se modifiquen en un escenario de transformación del hábitat, debido a posibles modificaciones en la abundancia y/o comportamiento de los dispersores, relacionados con sus rasgos de historia de vida y requerimientos de hábitat (Breitbach *et al.* 2012, Cleary *et al.* 2007).

El mantenimiento a largo plazo de la biodiversidad no sólo depende de la persistencia de las especies sino también del mantenimiento de las interacciones ecológicas y la dinámica eco-evolutiva subyacente a ellas (Kinnison & Hairston 2007). En el escenario de los hábitats transformados, la dinámica eco-evolutiva de los mutualismos puede verse alterada por efectos de corto plazo (cambios en la efectividad) y largo plazo (cambios en los síndromes).

En base a lo anterior, se hipotetiza que la transformación del hábitat, al implicar una simplificación estructural y modificaciones en el microclima, tendrá un efecto negativo en la abundancia de los quintrales y sus mutualistas, y por ende en la frecuencia y efectividad de las interacciones en que éstas especies participan. Para verificar esta hipótesis, se propone como objetivo general el evaluar el efecto de la transformación del hábitat sobre los procesos ecológicos y de evolución fenotípica de mutualismos planta-animal, asociados al éxito reproductivo del quintral *T. corymbosus*.

Para cumplir con el objetivo propuesto, esta tesis se compone de cuatro capítulos secuenciales. El primer capítulo examina el efecto de la transformación del hábitat sobre los mutualismos de polinización y dispersión asociados a *T. corymbosus* a diferentes escalas mediante la causalidad, covariación y concordancia de los patrones espaciales. En el segundo capítulo se estudian las consecuencias de la transformación del hábitat sobre el paisaje de efectividad de los mutualismos en el quintral. Complementariamente, el tercer capítulo aborda el tema desde la perspectiva de los animales interactuantes (*S. sephaniodes* y *D. gliroides*). Finalmente, el cuarto capítulo examina las fuerzas de selección fenotípica que actúan sobre rasgos asociados a la dispersión de semillas de *T. corymbosus* en un gradiente de transformación del hábitat.

Adicionalmente, se incluyen tres apéndices en los que se resumen actividades relacionadas con el desarrollo de esta investigación. El primer apéndice resume los resultados de un seminario de título de pregrado (Biología Ambiental) derivado de esta investigación, en el que participé como co-tutor. El segundo apéndice resume las actividades de extensión y difusión que se llevaron a cabo para compartir la información con los actores sociales pertinentes. El tercer apéndice incluye imágenes de las especies de estudio en terreno.

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CAPÍTULO 2

SCALE-DEPENDENT EFFECTS OF HABITAT TRANSFORMATION ON PLANT-ANIMAL MUTUALISMS

(To be submitted to Ecography)

ABSTRACT

Habitat transformation is recognized as a major biodiversity loss driver, but its effects on ecological interactions have been poorly studied. Structural changes involved in habitat transformation processes are likely to alter ecological interactions dynamics, which operate at different spatial scales. Aiming to test the multi-scale effects of habitat transformation on ecological interactions, we examined a gradient of native forest replacement by *Eucalyptus* plantations at three spatial scales (0-50, 50-100, and 100-250 m), focused on a highly-specialized mutualistic system, composed by a hemiparasitic mistletoe (*Tristerix corymbosus*) that is solely pollinated by a hummingbird (*Sephanoides sephaniodes*) and dispersed by an arboreal marsupial (*Dromiciops gliroides*). Even though mistletoes were found along the gradient, they were more abundant and densely aggregated when the transformed habitat was dominant. Additionally, disperser and pollinator activity increased as transformed habitat become dominant, at the scale of 0-50 m and 50-100 m, respectively. Further, resource availability and disperser activity covaried at broad and intermediate scales, whereas recruitment covaried at intermediate and fine scales. Moreover, disperser's activity and the number of seedlings were spatially associated, stressing *D. gliroides*' role as mistletoe's regeneration agent. This highly specialized mutualistic system seems to be positively responding to a less complex habitat structure, but the enhanced fleshy fruit availability (a mixed-species neighborhood) appears to drive more clumped patterns than in the native forest. However, the actual costs (e.g., reduced gene flow, increased herbivory) at these novel habitats are yet to be assessed.

Key words: Chile, *Dromiciops gliroides*, Moran's eigenvector maps, SADIE, *Sephanoides sephaniodes*, *Tristerix corymbosus*.

INTRODUCTION

A major goal in ecology is to recognize ecological patterns arising at different spatial scales and relating them to particular ecological processes (Levin 1992). Several ecological patterns result from multi-scale ecological processes, which are not possible to be adequately interpreted from a single-scale perspective. Plant-animal interactions are scale-dependent phenomena, which are usually affected by resource availability and habitat structure (García & Chacoff 2007, García *et al.* 2011), in which multi-scale patterns may emerge from the fact that each interacting animal has a different perception scale of plant's resources (e.g., a pollinator bird perceives a wider scale than a seed-predator rodent). Further, in response to resource availability, the animal activity might match plant's spatial distribution (García *et al.* 2009), creating a cyclic process in which plant resources influence animal's behavior which in turn shapes plant population structure and spatial distribution (Sasal & Morales 2013). The study of plant-animal mutualisms (i.e., pollination and seed dispersal) is crucial to understand the multi-scale nature of plant's demography and regeneration.

The study of mutualistic interactions may shed light on the way key ecological processes are affected by human actions such as habitat loss, fragmentation, habitat degradation and transformation. While the effects of habitat loss and fragmentation on plant-animal mutualisms have been widely studied (e.g., García & Chacoff 2007, Gonzalez-Varo 2010, Rodríguez-Cabal *et al.* 2007), the effects of habitat transformation (understood as the total or partial replacement of native vegetation by exotic species) is a less developed topic, despite being a major biodiversity loss driver (Albert *et al.* 2013). Unlike habitat loss, habitat transformation deals with structural and microclimate change produced by the replacement of dominant species in an ecosystem. Such replacement usually conveys less complex habitats characterized by limitation of structural resources (e.g., perching branches and natural cavities) and microclimate change (e.g., a less complex habitat might be more exposed to sunlight). Two studies dealing with habitat transformation showed how changes in pollinator's assemblage affected the reproductive outcome related to pollinator-mediated selection (Murúa *et al.* 2010) and flower damage (Espinoza *et al.* 2012) in native forests versus pine plantations. However, to our best knowledge no study has addressed this issue from a multi-scale and multi-interaction approach.

Aiming to test multi-scale habitat transformation effects on ecological interactions, we focused on highly specialized mutualist system composed by a hemiparasitic mistletoe (*Tristerix corymbosus*) that is solely pollinated by one hummingbird (*Sephanoides sephaniodes*) and dispersed by one marsupial (*Dromiciops gliroides*). As habitat transformation implies structural and microclimate changes, we hypothesized that: (1) mistletoes would be more abundant in less transformed habitats, (2) the interaction intensity of pollination and seed dispersal tend to decrease as the transformed habitat becomes dominant, (3) the effect of habitat transformation on pollination and seed dispersal would operate at different spatial scales, beings more conspicuous on *D. gliroides* due to movement restrictions, and (4) plant recruitment and *D. gliroides* activity would be spatially associated.

METHODS

Study site and species

This work was conducted at 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 one of the extant large remnants of native temperate rainforest of southern South America, an ecosystem rich in endemic species but threatened by human activities (Mittermeier *et al.* 2005, Myers *et al.* 2000). This area represents a large forest continuum with a complex habitat mosaic comprising old-growth native stands, secondary growth native stands (regenerated after clear-cutting), and exotic *Eucalyptus* plantations (12-20 years old) containing abundant native regeneration.

We focused on the system composed by the hemiparasitic mistletoe *Tristerix corymbosus* (L.) Kuijt (Loranthaceae), which is a winter-flowering plant found on at least 30 different host trees and it is considered a keystone resource for forest-dwelling animals (Aizen 2003, Aizen 2005). This mistletoe presents two highly-specialized mutualistic interactions for reproductive success. On the one hand, *T. corymbosus* depends on the Green-backed Firecrown *Sephanoides sephaniodes*, a hummingbird that provides most of the pollination service, and also depends mostly on *T. corymbosus'* flowers for feeding during the winter season (Aizen 2003, Aizen 2005). On the other hand, this mistletoe depends almost exclusively on the arboreal marsupial *Dromiciops gliroides*, to disperse its seeds. The marsupial is the sole legitimate disperser known at the southern (below 37°S) part of its distribution range (Amico & Aizen 2000, Amico *et al.* 2011). This unique study system allows assessing the effects of habitat transformation on two highly specialized and sequential mutualisms that ultimately determine plant's reproductive success.

Data collection

During the 2012 and 2013 austral summer seasons, we sampled 70 *T. corymbosus* plants in the study site (Figure 1). Each plant was tagged and georeferenced using a Garmin

Vista Cx GPS. For each sampled plant, we recorded the following information: (1) crop size; (2) number of plants per tree (as in many mistletoe species, it is common to find intense reinfection on the same host plant); and (3) number of *T. corymbosus* seedlings present at the host tree (as a proxy of recruitment). To quantify the interaction strength in both mutualisms, we used visit rate as an interaction proxy since this measure is known to be a good surrogate (Vásquez *et al.* 2005). In doing so, we used infrared camera-traps (Bushnell Trophy Cam 2011) set in video mode (resolution of 640x480, length 15 s, sensor at normal level). Cameras were placed in front of each sampled plant for 48 continuous hours. We expressed *S. sephaniodes* and *D. gliroides* visit rates as the number of recorded visits (in which we saw actual pollination or fruit consumption) per 48h.

<Figure 1 about here>

As habitat transformation is usually related to microclimate conditions, which might affect both plants and animals, we measured air temperature, relative humidity (using a handheld digital thermohygrometer), and luminosity (using a handheld digital luxometer) below each sampled plant. Then, we used those measures as explanatory variables to contrast the measured response variables described above.

Spatial scales and native habitat quantification

To test the effect of habitat transformation on mistletoe 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. Further, the 50-100 m scale depicts the plant neighborhood, and the 100-250 m 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 forest stands (i.e., eucalyptus plantations with native regeneration in between), 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 transformation. All GIS procedures were conducted using ArcGIS 10.1 (ESRI Redlands CA).

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 Kolmogorov-Smirnov test.

Data analysis

We tested habitat transformation effects by the means of three analytic approaches: (1) pattern causality, (2) pattern covariation, and (3) pattern concordance. We used the proportion of native habitat at the three defined spatial scales as explanatory variables. Response variables included: crop size, number of plants per tree, number of seedling, *S. sephaniodes* visit rate, and *D. gliroides* visit rate. As plants were non-randomly distributed in space and hence many observations were not spatially independent from each other, we developed spatially explicit models to fit our data. Prior model building, we made a preliminary spatial assessment of each response variable through inspection of potential spatial correlation on raw data. We found positive and significant spatial correlations at crop size and *D. gliroides* visit rates, but no correlation was detected for the number of plants per tree, seedlings, and *S. sephaniodes* visit rates (see Table S1 in Supplementary Material for detailed information).

We tested three analytic approaches (following Dormann *et al.* 2007): a regular Generalized Lineal Models (GLM) with Poisson distribution, a spatially explicit Poisson GLM incorporating a spatial covariate, and a spatially explicit Generalized Additive Model (GAM) 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), GAM models were chosen (data not shown, available upon request). We fitted Poisson GAM models with a spline term accounting for the spatial structure of the data for two purposes: (1) assessing the effect of the proportion of native habitat at the three spatial scales defined, and (2) assessing the effects of the measured microclimate variables (i.e., temperature, humidity, and luminosity) on the dependent variables quantified. Further, to make a connection between the native habitat proportion and microclimate conditions, we conducted Spearman partial correlation tests for each microclimate variable, controlling by the remaining two variables.

To assess spatial covariation patterns we used a Moran's eigenvector maps approach (MEM hereafter, previously known as Principal Coordinates of Neighbor Matrices [PCNM]), that decomposes spatial variability in broad, intermediate, and fine scales by conducting multiple regression analyses using the resulting positive MEM eigenvectors (Borcard & Legendre 2002, Borcard *et al.* 2004, Dray *et al.* 2006). We used an irregular bi-dimensional design, from which 23 out of 29 eigenvectors were positive and kept for further analyses. We split eigenvectors in three groups: eigenvectors V1 to V8 corresponding to broad scale variation, eigenvectors V9 to V16 to intermediate scale, and eigenvectors V17 to V23 to fine scale variation. Further, eigenvectors were used as explanatory variables in forward multiple regression models against our response variables (i.e., crop size, *D. gliroides* visit rates, *S. sephaniodes* visit rates, number of seedlings, and number of plants per 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 spatial pattern concordance, we employed the SADIE technique (Perry *et al.* 1999, Perry *et al.* 2002) to assess the spatial association between pairs of the response variables measured. We used the software SADIEShell v1.22 (Conard 2001) to calculate (1) the extent of aggregation of each variable (data not shown but available upon request; necessary for creating the cluster files needed for the next step); and (2) the association index (X_p), which ranges between -1 (complete spatial disassociation) and 1 (complete association), with 0 values standing for spatial independence. As multiple pairwise tests were performed, P values were corrected using a sequential Bonferroni procedure. All statistical analyses were conducted using R 2.15 (R Development Core Team 2012) and external packages (vegan, mgcv, spdep, spatstat, mpmcorrelogram, ggplot2), unless otherwise indicated.

RESULTS

Sampled plants were distributed along a gradient of habitat transformation at the study area (Figure 2a), with spots of dense plant aggregation and some isolated individuals. Those highly aggregated plants had larger crop sizes, more *D. gliroides* and *S. sephaniodes* visit rates, as well as larger number of seedlings, and plants per tree, after a visual inspection of the raw data (Figure S1 available in Supplementary Material). At the 0-50 m spatial scale, 5.7% (4 out of 70) of the plants were surrounded by transformed habitat only, whereas 32.9% (23 out of 70) were surrounded by native habitat only. Examining the next spatial scale (50-100 m), no plant was completely surrounded by transformed habitat and four plants were surrounded by native habitat. Only two plants were surrounded by transformed habitat at the broader scale (100-250 m). As expected, plants located in extreme situations (i.e., only transformed or native habitat) at broader scales were nested subsets of the preceding scale. Comparing actual and random distributions, *T. corymbosus* was found in dominant native forest in a lower proportion than expected by chance (Figure 2b), 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 ($D = 0.50$, $P < 0.001$).

<Figure 2 about here>

Pattern causality

We first examined the causal relationships between the gradient of habitat transformation at the three defined spatial scales and a set of response variables relevant for *T. corymbosus'* reproductive success and recruitment (Table 1). Regarding fruit availability, crop size showed a significant variation in function of the spatial structure but was not affected by the amount of native habitat at any spatial scale. Conversely, the visit 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 m and 100-250 m. Regarding pollination, 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. In general, the visit rate of *S. sephaniodes* responded to the spatial structure. Regarding *T. corymbosus'* recruitment, 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 neither by the proportion of native habitat at any scale (Table 1).

<Table 1 about here>

To inquire into the putative causality of the patterns just described, we performed partial correlations between the native habitat proportion at the three defined scales and three microclimate variables (temperature, relative humidity and luminosity). For the three scales measured, relative humidity was significantly correlated to the proportion of native habitat ($P < 0.001$ in all cases), but temperature and luminosity were not. Fitting GAM models using those microclimate features as explanatory variables revealed that crop size and the visitation rates of *D. gliroides* and *S. sephaniodes* were influenced by microclimate conditions and spatial structure. The number of seedling was affected by relative humidity, and the number of plants per tree was not affected by any of the microclimate features measured (Table 2).

<Table 2 about here>

Pattern covariation

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

<Table 3 about here>

Pattern concordance

Finally, examining pattern concordance through pairwise spatial association of the variables examined above, we found significant associations between crop size and the number of mistletoes (SADIE $X_p = 0.27$, $P = 0.01$), and *D. gliroides*' visit rates and the number of seedlings ($X_p = 0.39$, $P < 0.01$). However, the association between crop size and the number of plants lost its significance after a sequential Bonferroni correction ($P_{corr} = 0.11$) but the association between *D. gliroides*' visit rate and the number of seedlings retained its significance after correction ($P_{corr} = 0.01$).

DISCUSSION

Mistletoes were more abundant at transformed forest stands than expected by chance. Such pattern might emerge from the fact that the eucalyptus plantation has less canopy cover and complexity than the native forest, causing favorable conditions for photosynthesis and early fruit ripening due to a greater exposure to sunlight as reported for other mistletoe species in Australia (Bowen *et al.* 2009). The observed spatial pattern might also be explained by the stress gradient hypothesis (Kikvidze *et al.* 2011), which states that habitat disturbance could be beneficial for certain species due to variations on the dominant interaction, resulting in some cases on the reduction of antagonistic interactions (competition, predation). Extrapolating this explanation to our study system, the high activity shown by *D. gliroides* in sites where transformed habitat is dominant might result from a reduced predation pressure by native owls, the main predators of this marsupial at old-growth native forests (Martínez & Jaksic 1996). In this sense, habitat transformation effects could be comparable to those from selective logging described by Schleuning *et al.* (2011), which may favor some mutualisms by restraining dominant competitors, resembling the intermediate disturbance hypothesis (Roxburgh *et al.* 2004).

Mistletoes were more aggregated in places where transformed habitat was dominant. Such aggregation pattern is likely to emerge from (1) the resource concentration of neighboring mistletoes with larger crop sizes (see Figure S1a at Supplementary Material) within the disperser's home range (ranging from 1.0 to 2.2 ha; Fontúrbel *et al.* 2010), and (2) the higher availability of alternative fleshy-fruits from other shadow intolerant species characteristic of early successional stages (e.g., *Aristotelia chilensis*, *Rhaphithamnus spinosus*, *Ugni molinae*), which usually have large fruit displays (dozens to hundreds of fruits per m^2). A study conducted in Argentina showed that *D. gliroides* responded to a greater resource availability by increasing frugivory activity and reducing dispersal distances, causing aggregation patterns at locations with dense fruit neighborhoods (Morales *et al.* 2012). The same pattern has been described for frugivorous birds (Carlo & Morales 2008, Uriarte *et al.* 2011). Concordantly, both crop size and number of seedlings responded to the spatial structure, which is closely related to the observed aggregation patterns.

When we examined pattern causalities between the response variables and the proportion of native habitat cover, we found a significant effect at the smallest scale (0-50 m, depicting the situation in the immediate vicinity of the plant) only for *D. gliroides* visit rates. This result was not surprising as the arboreal marsupial depends on the fine habitat structure to mobilize and reach the plant by climbing through bamboo stems and thin branches. However, contrary to our expectations *D. gliroides* visited more frequently mistletoes 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* would be a forest generalist, able to thrive on second-growth forests as long as they retain its three-dimensional structure and some key elements such as fallen logs, thin branches, and bamboo (Fontúrbel 2012, Fontúrbel *et al.* 2012, Rodríguez-Cabal & Branch 2011). Native habitat cover at the broadest scale (100-250 m) also influenced *D. gliroides* visit 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. Further, *D. gliroides* activity varied at broad and intermediate spatial scales, according to the MEM analyses performed, matching the scales at which resource (i.e., crop size) varied as well. This matching pattern was previously reported in Argentina (García *et al.* 2009).

The activity of the second mutualist involved, *S. sephaniodes*, was significantly affected by native habitat cover at 50-100 m scale, being interactions also more frequent at those locations where transformed habitat were dominant; also there was a opposite but marginally effect at 100-250 m. This result is somewhat expected as this hummingbird has greater mobility capabilities than *D. gliroides* (which depends on fine habitat texture to disperse from one point to another). Surprisingly, no MEM eigenvector covaried with *S.*

sephaniodes activity. This could be explained because hummingbirds may benefit from floral resources provided by species present at the transformed habitat such as *Eucalyptus* flowers and those from the vine *Lapageria rosea*, which has conspicuous and nectar-rich flowers that are scarce at native forest stands but highly abundant at the transformed ones since this plant uses eucalyptus' stems to fasten.

Changes in microclimate conditions have been recognized to affect the probability of fruit consumption in fragmented habitats (Galetti *et al.* 2003). At our study system, the only microclimate factor that was correlated with the gradient of habitat transformation was relative humidity because of the structural simplification involved in the transformation process. Temperature and luminosity also are affected by structural simplification but their influence was weaker than humidity. Most biotic components of our study system were not affected by differences in microclimate excepting crop size, which showed a consistent negative association with temperature, humidity, and luminosity. However, there is 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 host's branches they probably require humidity to maintain embryo's moisture and ensure haustorium development until contacting the host phloematic vessels.

Regarding *T. corymbosus* recruitment, there was no significant effects of the amount of native habitat at any scale on the number of seedlings and the number of plants per tree. However, both responses covaried with MEM eigenvectors at intermediate and fine scales, respectively. Intermediate-scale variation on the number of plants per tree could be covarying to crop size and *D. gliroides* activity at the same scale, whereas number of seedlings covariation at fine scale might be related to safe sites (Bustamante & Canals 1995, Reid 1989). We found spatial concordance between the number of plants per tree and crop size (although it was non-significant after Bonferroni correction), suggesting that hosts with large mistletoe display are more likely to be reinfected (Medel *et al.* 2004). 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 *et al.* 2007, Rodríguez-Cabal & Branch 2011), stressing the role of *D. gliroides* as the sole legitimate disperser of *T. corymbosus* at the southern portion of its distribution range (Amico *et al.* 2011).

Contrarily to our first hypothesis, *T. corymbosus* was more abundant at transformed habitats, and was also found in denser aggregates than plants located at native habitat stands. Mistletoes' distribution is characteristically aggregated due to host and disperser effects (Medel *et al.* 2004, Rawsthorne *et al.* 2011). In this study, transformed habitats offer structural and microclimate condition that favor large crop sizes of *T. corymbosus* and other fleshy-fruited plants that provide a rich mixed neighborhood attractive to frugivores (Carlo *et al.* 2007). It is likely that this spatial pattern reinforces the cyclic process of reduced dispersal distances that cause even more aggregation at the next generation (Morales & Carlo 2006). Contrarily to our second hypothesis, both *D. gliroides* and *S. sephaniodes* increased their interaction rates with *T. corymbosus* as transformed habitats become dominant. Regarding our third hypothesis, habitat transformation had a positive effect on *D. gliroides* and *S. sephaniodes* activity, but they operate at different spatial scales. As expected *S. sephaniodes* response occurred at a broader scale than *D. gliroides*'. Lastly, *D. gliroides* activity was positively associated with *T. corymbosus* recruitment, confirming our forth hypothesis.

In a recent review, McConkey *et al.* (2012) described the effects of many biodiversity threats on seed dispersal. However, habitat transformation was not mentioned among them. Here we presented a particular scenario of anthropogenic disturbance on which both habitat extent and species composition remained constant, but habitat structure has been modified due to a 20-years old replacement of native forest by eucalyptus plantation. It is promising for forest regeneration that such specialized plant-animal interactions still remain functional in this scenario, but under a novel spatial arrangement since transformed habitat is influencing animal's behavior by concentrating resources and driving spatial patterns even more aggregated than it is normal for a mistletoe. Nevertheless, the costs of thriving in such transformed habitats are virtually unknown yet. This novel scenario could be costly for plants (e.g., gene flow reduction, increased foliar and floral herbivory), and we need further research to properly understand the real impact of habitat transformation on plant's life cycle and its ecological interactions.

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TABLES

Table 1. Summary of results of Generalized Additive Model for each response variables measured, contrasted against the proportion of native habitat at three spatial scales, incorporating a spatially explicit non-linear term (X,Y spline). Adjusted R^2 values, Estimates, their standard error (in parenthesis), and P-values are presented. Abbreviations: Dg = *Dromiciops gliroides*, Ss = *Sephanoides sephaniodes*. Significance levels: NS = not significant, † P < 0.1, * P < 0.05, ** P < 0.01.

		Native habitat proportion			
	R^2	Scale 0-50 m	Scale 50-100 m	Scale 100-250 m	X,Y spline
Crop size	0.32	0.02 (0.21)^{NS}	0.07 (0.47)^{NS}	0.98 (0.65)^{NS}	$P < 0.01$
Dg visit rate	0.84	-2.87 (1.05)**	-5.59 (6.77)^{NS}	-53.96 (18.72)**	$P = 0.404$
Ss visit rate	0.69	1.71 (1.90)^{NS}	-5.86 (2.80)*	6.71 (3.85)†	$P < 0.01$
Seedlings	0.99	-1.59 (5.58)^{NS}	4.50 (46.61)^{NS}	11.44 (56.67)^{NS}	$P = 0.02$
N° of plants	0.05	0.16 (0.42)^{NS}	-0.33 (0.85)^{NS}	0.40 (1.02)^{NS}	$P = 0.69$

Table 2. Summary of results of Generalized Additive Model for each response variable analyzed, contrasted against microclimate features (temperature, relative humidity and luminosity), incorporating a spatially explicit non-linear term (X,Y spline). Estimates, their standard error (in parenthesis), and P-values are presented. Abbreviations: Dg = *Dromiciops gliroides*, Ss = *Sephanoides sephaniodes*. Significance levels: NS = not significant, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$.

	Microclimatic variables			
	Temperature	Relative humidity	Luminosity	X,Y spline
Crop size	-0.18 (0.55)^{NS}	-0.03 (0.01)^{NS}	-0.01 (0.01)†	$P < 0.01$
Dg visit rate	-0.07 (0.12)^{NS}	-0.04 (0.03)^{NS}	0.01 (0.01)†	$P < 0.01$
Ss visit rate	0.29 (0.18)^{NS}	-0.02 (0.01)^{NS}	0.01 (0.01)**	$P < 0.01$
Seedlings	-8.25 (7.48)^{NS}	11.18 (5.42)*	0.01 (0.01)^{NS}	$P = 0.72$
N° of plants	0.05 (0.08)^{NS}	0.01 (0.02)^{NS}	0.01 (0.01)^{NS}	$P = 0.93$

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

Scale	Crop size	Dg visit rate	Seedlings	N° of plants
Broad				
MEM vectors	V1	V1, V7		
R^2	0.18	0.19		
P-value	0.024	0.018		
Intermediate				
MEM vectors	V13	V11, V12		V11
R^2	0.18	0.19		0.13
P-value	0.010	0.008		0.028
Fine				
MEM vectors			V18	
R^2			0.40	
P-value			< 0.001	
R^2 total	0.41	0.38	0.49	0.24

FIGURE CAPTIONS

Figure 1. Sampled plants and habitat cover configuration. Light gray areas correspond to native forest and dark gray areas to transformed habitat (eucalyptus plantation with native regeneration in between). In the box, habitat rings at the three spatial scales defined are depicted.

Figure 2. Distribution of the proportion of native habitat at three spatial scales: 0-50 m, 50-100 m, and 100-250 m. (a) Actual distributions measured from aerial imagery and GIS files, (b) random distribution generated with 70 random points along the study area.

FIGURES

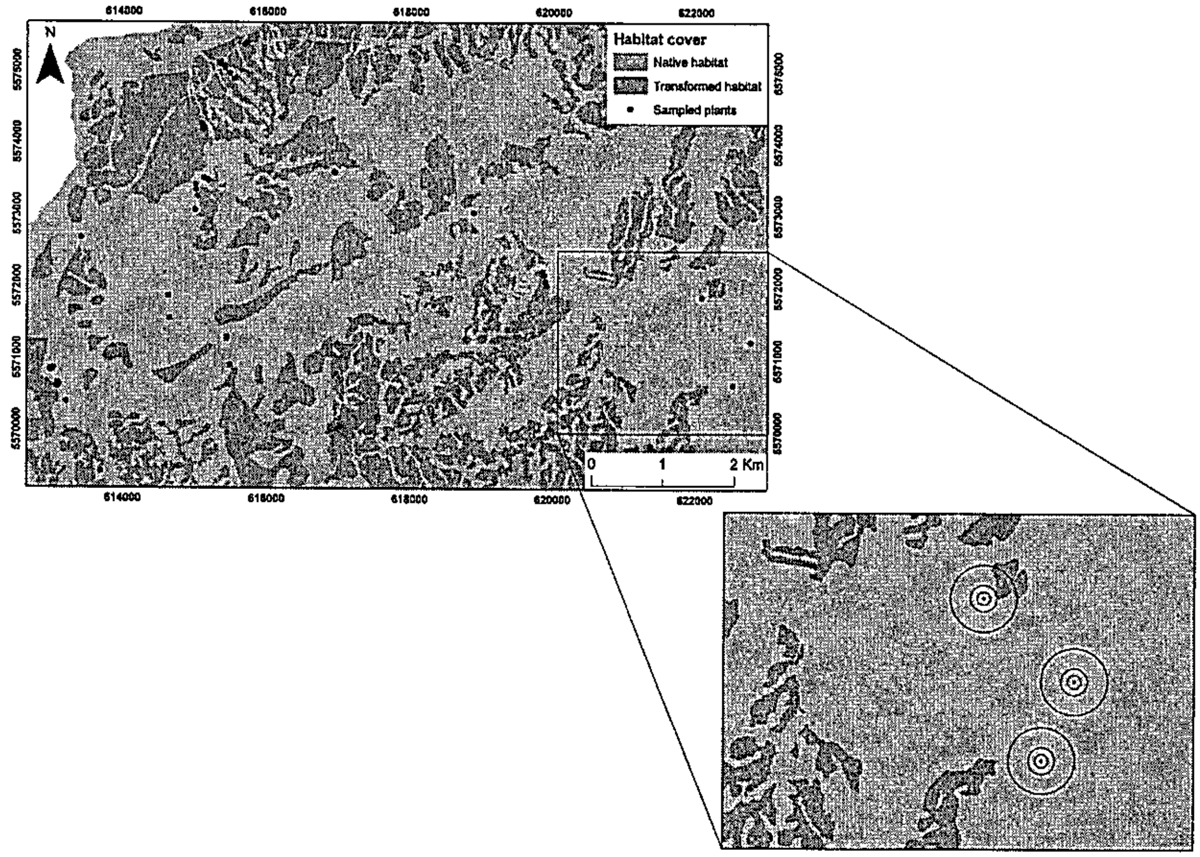
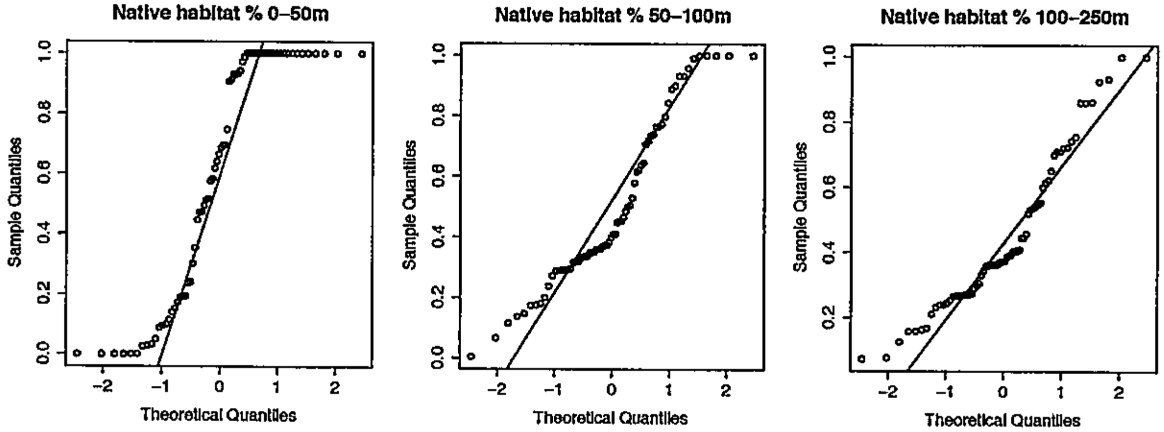


Figure 1

(a) Actual distribution



(b) Random distribution

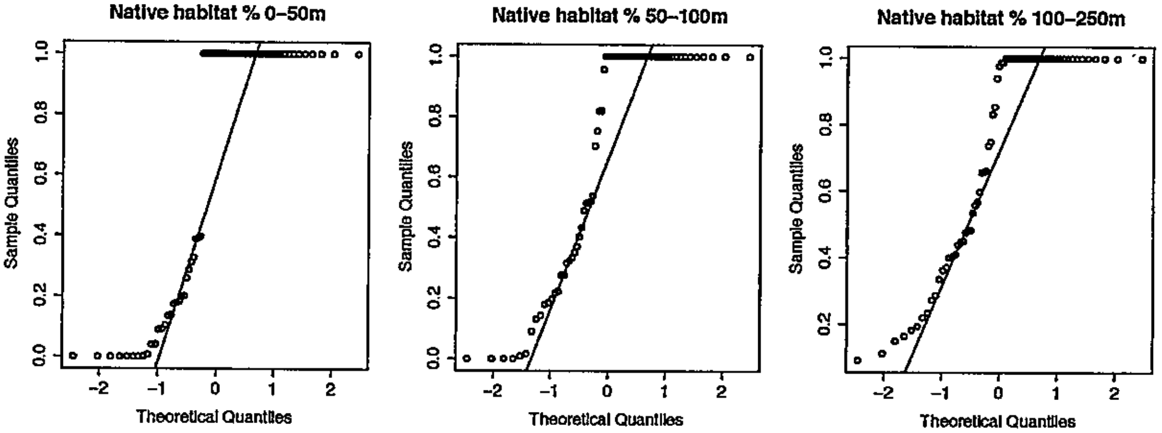
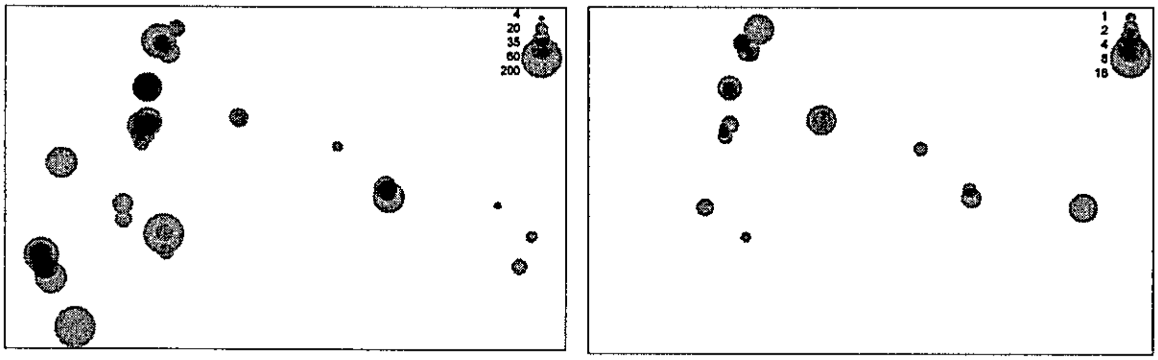


Figure 2

SUPPLEMENTARY MATERIAL

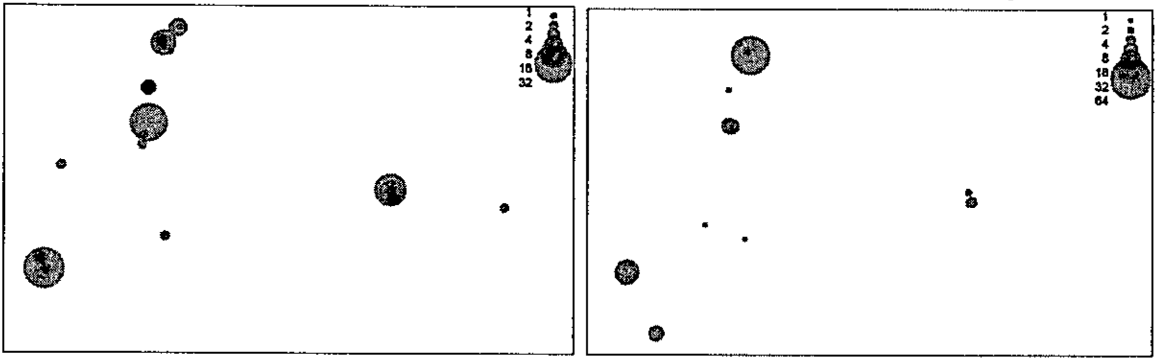
Table S1. Multivariate partial Mantel correlograms results for raw response variables measured. Bonferroni-corrected P-values are shown for each distance class. Bold figures denote significant P-values. Abbreviations: Dg = *Dromiciops gliroides*, Ss = *Sephanoides sephaniodes*.

		P-values after Bonferroni correction				
Class	Distance range (m)	Crop size	Dg visit rate	Ss visit rate	Seedlings	No of plants
1	0.0 - 815.5	**0.015**	**0.014**	0.335	0.173	0.112
2	815.5 - 1630.9	0.528	0.927	0.393	0.942	0.208
3	1630.9 - 2446.5	1.000	0.109	0.564	0.787	1.000
4	2446.5 - 3261.9	0.358	0.360	0.293	0.477	1.000
5	3261.9 - 4077.5	1.000	**0.046**	1.000	1.000	1.000
6	4077.5 - 4892.9	1.000	1.000	1.000	1.000	1.000
7	4892.9 - 5708.5	1.000	1.000	0.489	1.000	1.000
8	5708.5 - 6523.9	**0.029**	1.000	1.000	0.995	1.000
9	6523.9 - 7339.5	1.000	1.000	0.991	1.000	1.000
10	7339.5 - 8154.9	0.713	0.467	1.000	1.000	1.000
11	8154.9 - 8970.4	1.000	1.000	0.981	1.000	1.000
12	8970.4 - 9786.9	1.000	1.000	1.000	1.000	1.000



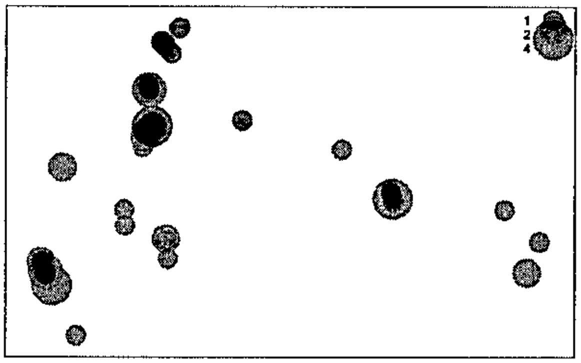
(a) Crop size

(b) *Dromiciops gliroides* visit rates



(c) *Sephanoides sephaniodes* visit rates

(d) Number of seedlings



(e) Number of plants per tree

Figure S1. Spatially explicit bubble plots depicting the variation of the measured response variables: (a) crop size, (b) *Dromiciops gliroides* visit rates, (c) *Sephanoides sephaniodes* visit rates, (d) number of seedlings, and (e) number of plants per tree.

CAPÍTULO 3

PLANT-ANIMAL MUTUALISM EFFECTIVENESS LANDSCAPES ON A HABITAT TRANSFORMATION SCENARIO: A MULTI-SCALE ASSESSMENT OF A HIGHLY SPECIALIZED MISTLETOE

(To be submitted to Journal of Ecology)

ABSTRACT

Most of the temperate flowering plants depend on pollination and seed dispersal for reproductive success. Those plant-animal mutualisms are composed by a quantitative and a qualitative component that together define interaction effectiveness and directly affect plant's reproductive success. Habitat fragmentation is known to disrupt those mutualisms and jeopardize plant reproductive success, but the effects of habitat transformation in this context were poorly explored. Aiming to assess the effects of habitat transformation on the effectiveness landscape of pollination and seed dispersal mutualisms, we studied a highly specialized system (a mistletoe with one pollinator and one disperser), comparing native and transformed stands. Both mutualism effectiveness landscapes were highly variable, especially pollination. When comparing native and transformed habitats, pollination and seed dispersal effectiveness did not differ. However, fruit removal was larger at the transformed habitat. Overall effectiveness did not differ between habitats, being pollination slightly larger at transformed habitat and seed dispersal at the native habitat, and the resulting plant fitness was significantly higher at the transformed habitat. Contrary to other studies conducted on eucalyptus plantations, this highly specialized system has persisted and maintained its functionality at transformed habitats despite structural differences. Perhaps this pattern result from the presence of abundant understory secondary vegetation that provide dispersal pathways and enhance resource availability.(mixed fruit neighborhoods), attracting mutualists and specially seed dispersers that are expected to have a more profound effect on plant's recruitment.

Key words: Chile; *Dromiciops gliroides*; eucalyptus plantation; *Sephanoides sephaniodes*; South American temperate forest; *Tristerix corymbosus*

INTRODUCTION

Plant-animal mutualisms (i.e., pollination and seed dispersal) may have a key role in generating and maintaining biodiversity (Thompson 1994). Over 70% of the temperate flowering plants depend on an animal vector for pollination (Ollerton *et al.* 2011) or seed dispersal services. Plant-animal mutualisms at temperate habitats depend on the spatiotemporal variation of markedly-seasonal resources (Jordano 1987, Jordano 2000). Further, reproductive success of those plants depending on animal vectors for pollination or seed dispersal relies on the effectiveness of the interacting party. Then, mutualism effectiveness results from the combination of two components: a quantitative component (i.e., number of visits and number of flowers pollinated / seed dispersed per visit) and a qualitative component (i.e., quality of the gut treatment and deposition, and the likelihood of transferring pollen to a compatible flower or leaving seeds at safe sites) (Reid 1989, Schupp *et al.* 2010).

Plants are expected to interact simultaneously with more than one mutualist species since this kind of plant-animal interactions often results from diffuse coevolutionary processes (Herrera 1982, Thompson 1994) and are usually asymmetric (Ashworth *et al.* 2004). However, this ecologically redundant species are expected to differ in interaction's quantity and quality (Schupp *et al.* 2010). Usually only a few species within the mutualistic assemblage are effective in terms of plant's reproductive success, by providing high quality dispersal at high interaction frequencies. On the other hand, the interaction with many other species would be little effective as result of poor quality services (e.g., damaged seed after gut passage, seed deposited in unsuitable sites), low interaction frequencies (few visits and / or few seeds removed per visit), or the interaction of both factors. This inter-species variability on interaction quantity and quality could be represented in effectiveness landscapes -which are an abstract representation of the quantitative and qualitative components of the interaction- correlating both components and providing a more accurate representation of the actual contribution of each mutualist to plant's reproductive success.

On the other hand, plant-animal mutualisms are interactions sensitive to anthropogenic disturbance (McConkey *et al.* 2012), which is becoming the most common scenario in a changing world. Human activities cause habitat loss, fragmentation, and degradation, which can affect ecological interactions by altering individual species abundance and assemblage composition. Habitat fragmentation is known to modify mutualist abundance, identity and behavior (Figueroa-Esquivel *et al.* 2009, Gonzalez-Varo 2010), causing reduced plant recruitment or even local extinction (Cordeiro & Howe 2003, Rodríguez-Cabal *et al.* 2007). However, habitat transformation (i.e., total or partial replacement of native vegetation by exotic species) effects on plant-animal interactions have been little studied despite its importance as a biodiversity loss driver (Albert *et al.* 2013). Transformed habitats are expected to differ in structure and microclimate since they are usually less complex than the original ones, and are expected to be dominated by generalist species (Barlow *et al.* 2007). This situation also may lead to altered mutualistic assemblages, altered reproductive success (Murúa *et al.* 2010), and altered pollen / seed limitation patterns (Espinoza *et al.* 2012).

Aiming to test the effects of habitat transformation on plant-animal mutualisms, we used a mistletoe that it is pollinated by one hummingbird species and dispersed by one mammal. Hence, we examined pollination and seed dispersal effectiveness landscapes along a habitat transformation gradient, where *Eucalyptus* plantations have gradually replaced native forest. In this context, we hypothesized that: (1) overall mutualism effectiveness would be lower at transformed habitats; (2) habitat transformation would have a larger effect on the quantitative component of effectiveness; and (3) plant's fitness would decrease at transformed habitats.

METHODS

Study site

This research was conducted at the Valdivian Coastal Reserve (39°57'S 73°34'W), a 50,530-ha private owned protected area managed by The Nature Conservancy (Delgado 2010). This Reserve is one of the largest remnants of temperate rainforest of southern South America. This is also an endemism-rich but highly threatened ecosystem (Mittermeier *et al.* 2005, Myers *et al.* 2000), due to anthropic activities such as extensive exotic-species forestry. This protected area represents a large forest continuum depicting a complex habitat mosaic of old-growth and secondary growth (regenerated after clear-cutting) native stands and transformed stands composed of exotic eucalyptus plantations (12-20 years old) with abundant native regeneration in between.

Study system

The mutualistic system consists of a hemiparasitic mistletoe (*Tristerix corymbosus* (L.) Kuijt, Loranthaceae), which is pollinated almost exclusively by a hummingbird (*Sephanoides sephaniodes*), and dispersed uniquely by an arboreal endemic marsupial (*Dromiciops gliroides*) (Aizen 2003, Amico & Aizen 2000, Amico *et al.* 2011). This mistletoe has a winter-flowering phenology, which is seemingly an adaptation to monopolize pollinator's services during scarcity periods when the availability of alternative flower resources are limiting. *Tristerix corymbosus* has large displays of tubular red flowers from austral fall to early spring, and the fruiting season lasts from late spring to mid-summer, when *D. gliroides* activity reaches its peak because of the warm temperatures. Despite being self-compatible, *T. corymbosus* relies on pollination for flower fertilization and subsequent fruit production (Aizen 2005). There are two known legitimate dispersers of *T. corymbosus*: the Chilean Mockingbird (*Mimus thenca*) at its northern distribution range (30-37°S), and *D. gliroides* at its southern distribution area (37-42°S). Despite *M. thenca* distribution goes as far as 40°S, it shifts its role as disperser below 37°S seemingly due to variation in forest structure and microclimate conditions that influence ripe fruits below 37°S to remain green (in contrast to the red-colored ripe fruits above 37°S), which makes them undetectable by avian dispersers (Amico *et al.* 2011). In terms of reciprocal dependency, this highly specialized system is asymmetric since *T. corymbosus* strictly depends on both mutualists for reproductive success, whereas *D. gliroides* is a generalist consumer that relies on fleshy fruits, insects and eggs (Fontúrbel *et al.* 2012) and *S. sephaniodes* pollinates about 20% of the native flora (Smith-Ramírez 1993).

We examined the coupled effect of two phases of *T. corymbosus* reproductive cycle: (1) pollination success represented in the fruit set, and (2) fruit consumption and the consequent seed dispersal. For both phases, we measured quantitative and qualitative components of each mutualistic interaction (Schupp *et al.* 2010). In both cases, we assessed the quantitative component using visit rates, which has been showed to be a good interaction proxy (Vásquez *et al.* 2005). For pollination, we used fruit set as a quality proxy since this measure represents the proportion of flowers successfully pollinated that resulted in fruits. Regarding seed dispersal, we used fruit removal as a quality proxy representing the proportion of the available fruits that is effectively removed during disperser visits.

Data collection

We sampled 70 *T. corymbosus* plants during the austral summer of 2012 and 2013. For each sampled plant, we recorded the following information: (1) *S. sephaniodes* visit rates, measured using infrared camera-traps (Bushnell Trophy Cam 2011) placed in front of each focal plant for 48 straight hours. Cameras were set in video mode (640x480 pixels resolution, 15 s length, 1 min interval between shots, sensor set at normal level) to determine actual interaction. Rates were expressed as number of visits per hour. (2) Fruit set, estimated by counting the number of flowers (when there were >50 flowers we subsampled by tagging and counting every flower on a branch) during the flowering season (20.2 ± 1.1 flowers tagged on average); then, we returned at the beginning of the fruiting season (about two months later) and counted the number of fruits produced. Fruit set was then expressed as the ratio of the number of fruits and the number of flowers. (3) *D. gliroides* visit rates, measured using camera-traps as described for *S. sephaniodes*; and (4) fruit removal, measured by painting ten random fruits with a non-toxic water-based acrylic paint and counting the number of remaining painted fruits five days later. Seed traps were set below sampled plants for accounting for fallen fruits. Additionally, to get a more precise fitness estimator, we sampled five random fruits per plant and assessed its germination rate in Petri dishes with wet filter paper at room temperature; germination rate was calculated as the proportion of germinated seeds after five days. Plant's fitness was calculated as fruit set * fruit removal * seed germination rate.

Habitat effect

Considering that our study area comprises a mixed landscape of native and transformed habitats, we measured its effect on ecological interactions as the proportion of native habitat at three non-overlapping rings: 0-50 m, 50-100 m, and 100-250 m, describing the immediate environment, the plant's neighborhood, and the foraging patch area of mutualistic species, respectively. We used this approach to avoid multicollinearity among scales (García & Chacoff 2007). Detailed information about the GIS procedures followed to this calculations is available at Chapter 2. As thumb rule, when native habitat cover was >50% the plant was assigned to the 'native' group, whereas when native cover was ≤50% the plant was assigned to the 'transformed' group. This procedure was repeated for each spatial scale.

To have a detailed description of the habitat effect on the studied system, we sampled the following structural variables at a 2.5-m radius from each focal plant: shrub cover (estimated visually), bamboo cover (estimated visually), number of stems, number of fallen logs, number of stumps, and number of natural cavities in the trees. Also we recorded air temperature, relative humidity, and luminosity (using handheld termohygrometer and luxometer devices) below each sampled plant.

Data analysis

We first elaborated effectiveness landscapes for each mutualist species. For the pollinator *S. sephaniodes* we used the number of visits per hour and flower as quantitative measure and the fruit set at qualitative measure. For the disperser *D. gliroides*, we used the number of visits per hour and fruit as quantitative measure and fruit removal rates as qualitative measure. Aiming to express the outcome of each mutualism, we used plant's fitness as bubble plots' size factor. Then, we calculated the overall mutualist effectiveness by multiplying quantitative and qualitative components, and with those values we constructed the overall effectiveness landscape also using plant fitness as bubble plot's size factor.

In a second step, we constructed individual and overall effectiveness landscapes comparing the mean effectiveness at native and transformed habitats at the three spatial scales defined. We tested pairwise differences within each scale (comparing native and transformed habitats) using non-parametric Mann-Whitney tests since our data did not met normality assumption in any case. Then, we compared plant's fitness among habitats and scales using a non-parametric Kruskal Wallis test (since data were not normally distributed) and an *a posteriori* Dunn's test. Values are shown as mean ± 1 SE.

In order to assess the potential effects of habitat structure and microclimate, we used the field measures taken as explanatory variables, and mutualists' effectiveness and plant fitness as response variables. Considering that our response variables did not met normality assumptions, precluding the use of parametric regression methods, we used regression trees to estimate the relative importance of each structural and microclimate features. All statistical analyses were conducted in R 2.15 (R Development Core Team 2012) and the packages 'tree' and 'pgirmess'.

RESULTS

Sephanoides sephaniodes activity ranged from zero (no visit) to 37 visits in a 48-hour monitoring period, with a mean activity of 2.96 ± 1.22 visits. Its activity along the flowering season yielded fruit sets ranging from 55 to 100%, with a mean of 84.44 ± 2.19%. Conversely, *Dromiciops gliroides* activity ranged from zero to nine visits in a 48-hour monitoring period, with a mean activity of 1.19 ± 0.38 visits. Removal trials were very variable, with plants with total removal and others with no removal at all, with a mean of

37.83 ± 6.14% of the fruits removed in five-day trials. With this information, we constructed effectiveness landscapes for both mutualisms (Figure 1). Pollination effectiveness landscape (Figure 1a) shows that most plant received less than 0.02 visit per flower and hour, and those plants had larger fitness values than outlier plants that received >0.025 visits * flower^{-1} * h^{-1}; further, plants with fruit set >70% had larger fitness. On the other hand, seed dispersal effectiveness landscape (Figure 1b) showed that most plants had removal values <60%, and only a 15% of the sampled plant had a fruit removal >80%. When combining pollination and seed dispersal effectiveness in one landscape (Figure 1c), we observed that most plants have low to moderate effectiveness values for both mutualisms, but a few plants had high effectiveness values for pollination or for both pollination and seed dispersal.

<Figure 1 about here>

Habitat transformation had no significant effects on both quantitative and qualitative components of pollination effectiveness (Figure 2a, Mann-Whitney tests, P > 0.12), except for fruit set that is significantly larger at the native habitat at 50-100 m scale (Mann-Whitney test, P = 0.042). There was a great variation on both components at every habitat and scale, but despite most differences were non-significant, both visit rates and fruit sets were larger at the native habitat -except for fruit set at 50-100 m scale. Examining the seed dispersal effectiveness landscape (Figure 2b), no significant difference in visit rates was observed. However, fruit removal rates are significantly greater at 100-250 m scale (Mann-Whitney test, P = 0.02) and marginally significant at 0-50 m (P = 0.08). When the effectiveness of the two mutualistic interactions were combined in a single landscape (Figure 2c), neither pollination or seed dispersal were statistically significant at any scale, but overall effectiveness values were marginally (P = 0.067) larger at the transformed habitat at 0-50 m.

<Figure 2 about here>

Plant fitness was larger at transformed habitats, irrespective of the spatial scale (Kruskal-Wallis test, $\chi^2 = 74.21$, df = 5, P = 0.01, Figure 3). *A posteriori* comparisons showed no significant differences within habitat types among scales (see Table S1 on Supplementary Material), stressing the positive effect of the transformed habitat on plant's fitness. Moreover, examining potential effects of habitat structure and microclimate on this system, regression trees showed that pollinator effectiveness was influenced by shrub cover, and when over than 32.5% is covered the number of stems becomes relevant (Figure 4a). Disperser effectiveness was influenced by a complex cascade of factors (Figure 4b) starting by shrub cover that is subsequently influenced by luminosity when cover >77.5%, but when cover is <77.5% the number of stems becomes relevant and when number of stems is below 5.5 humidity becomes relevant. Finally, plant's fitness was mainly influenced by relative humidity, when this feature exceeds 76.5% luminosity becomes relevant, but when <76.5% the number of stems and temperature are relevant (Figure 4c).

<Figures 3 and 4 about here>

DISCUSSION

Contrary to our initial hypothesis, the overall effectiveness (i.e., the combination of the quantitative and qualitative components of the interaction) of both pollination and seed dispersal was larger when transformed habitat was dominant. Transformed habitats are known to hold a nested subset of native habitat species, but due to strong structural differences between transformed and original habitat, transformed habitats are usually dominated by generalist species, as Barlow *et al.* (2007) have found at eucalyptus plantations in Amazonia. Here we found a situation in which a highly specialized plant-pollinator-disperser system inhabits a 20-year old eucalyptus plantation with abundant native understory secondary vegetation in between. Eucalyptus plantations are often allelopathic and preclude other vegetation to grow by causing water shortage (May & Ash 1990, Zhang *et al.* 2012). This situation is unlikely to occur at our study site since annual rainfall often exceeds 2500 mm (Delgado 2010) which provides enough water for eucalyptus and native plants.

Sephanoides sephaniodes was expected to be less affected by habitat transformation than *D. gliroides*, as this hummingbird has less movement restrictions than the arboreal marsupial, which depends on a dense forest texture for maneuverability and protection accordingly to its body size (Bro-Jørgensen 2008, Fischer *et al.* 2008). *Dromiciops gliroides* has been described as an old-growth forest specialist (e.g., Hershkovitz 1999), restricted to *Nothofagus* spp.- and *Araucaria araucana*-dominated forests. However, there is a growing body of literature demonstrating that this ancient marsupial has a plastic response to habitat disturbance and its capable to thrive in many secondary forests (see Fontúrbel *et al.* 2012 for a review), but until now this species has not been recorded in exotic-tree dominated forests. Even more surprising is the fact that while *S. sephaniodes* is slightly more effective at the native habitat (although differences with the transformed habitat were non-significant), *D. gliroides* is removing significantly more fruits at the transformed habitat than in the native forest (contrarily to what is expected at disturbed habitats; Markl *et al.* 2012), despite visit rates were more frequent (although non-significant) at the native habitat.

Habitat disturbance is assumed to impact on disperser's behavior as forest cover and resource availability are often dramatically modified with respect to undisturbed habitats. This modification usually results in reduced seed dispersal distances (Breitbach *et al.* 2012, Herrera & García 2010). In this particular situation, the vast offer of fleshy fruits from *T. corymbosus* and many other native plants in the transformed habitat is likely to influence *D. gliroides* activity rates at a reduced spatial extent through two mechanisms, (1) the presence of mixed fruit neighborhoods (see Chapter 4 for further details) that turn transformed habitats more attractive for frugivores (Carlo *et al.* 2007), and (2) the low-cost and low-risk gain of visiting closely aggregated plants compared to visit isolated ones that imply a great energy expenditure and exposure to predators (Fedriani *et al.* 2010); such plant aggregation may be also responsible for the increased plant fitness at the transformed habitat. Conversely, old-growth native forest stands have less abundant and diverse fruit displays, characteristics of late successional vegetation where shadow-tolerant plants dominate. Transformed habitats have a less complex canopy, but in contrast they have a well-developed and dense understory where shrubs and bamboo are abundant, resourced known to be associated to *D. gliroides*' presence and abundance (Rodríguez-Cabal & Branch 2011), and serve as movement pathways between foraging and shelter sites.

To our best knowledge, this is the first study examining pollination and seed dispersal effectiveness landscapes simultaneously. Here we saw an advantage by using this highly specialized system because it allowed quantifying the actual effect of each mutualistic interaction on plant's fitness removing confounding effects attributable to other species (e.g., pulp consumers or less effective pollinators) could introduce. Pollinator effectiveness showed to be more variable and heterogeneous, whereas disperser effectiveness was more dependent on fruit removal (qualitative component) than into the visit rate (quantitative component). Plants interacting a great deal only with one kind of mutualist had lower fitness than those that moderately interacted with both mutualists. *Sephanoides sephaniodes* is almost ubiquitous at the study area and the elevated fruit set rates suggest that there is no or little pollen limitation in this system. However, disperser limitation is likely to have a profound effect on plant's fitness since seed dispersal and germination are crucial steps for ensuring recruitment.

In summary, mutualism effectiveness was highly variable but not significantly different between habitats, except at the immediate plant environment (0-50 m scale) where overall effectiveness was slightly higher. Habitat transformation does not disrupted pollination or seed dispersal effectiveness as has been described previously for habitat fragmentation (e.g., Magrach *et al.* 2012, Rodríguez-Cabal *et al.* 2007), on the contrary, despite the overall effectiveness was similar between native and transformed habitats, pollinator visits and fruit removal were greater when transformed habitat was dominant. Further, plant's fitness resulted to be larger at transformed habitats due to increased fruit removal and germination rates.

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FIGURE CAPTIONS

Figure 1. Effectiveness landscapes for: (a) pollination, (b) seed dispersal, and (c) the combination of both mutualisms. Bubble size represents plant's fitness.

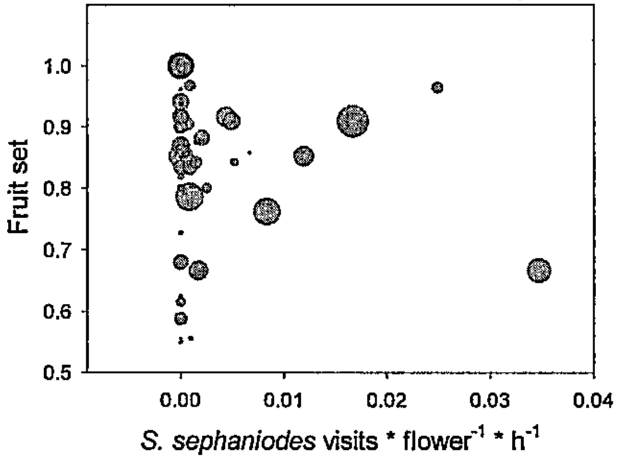
Figure 2. Influence of habitat transformation on effectiveness landscapes (presented as mean ± 1SE) for: (a) pollination, (b) seed dispersal, and (c) the combination of both mutualisms. Filled figures represents native habitat and open ones represent transformed habitat. Circles correspond to 0-50 m scale, triangles to 50-100 m scale, and squares to 100-250 m scale.

Figure 3. Influence of habitat transformation on plant's relative fitness (presented as mean ± 1SE). Filled figures represents native habitat and open ones represent transformed habitat. Circles correspond to 0-50 m scale, triangles to 50-100 m scale, and squares to 100-250 m scale.

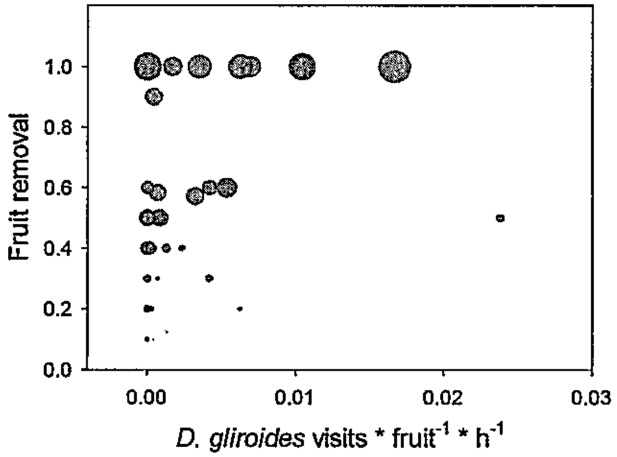
Figure 4. Regression trees of habitat structure and microclimate variables against: (a) pollinator effectiveness, (b) disperser effectiveness, and (c) plant's fitness. Pseudo-R^2 values are show in parenthesis.

FIGURES

(a) Pollinator effectiveness landscape



(b) Seed dispersal effectiveness landscape



(c) Overall effectiveness landscape

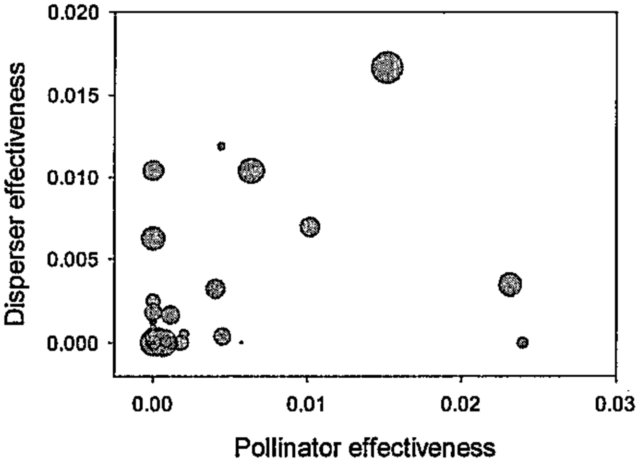
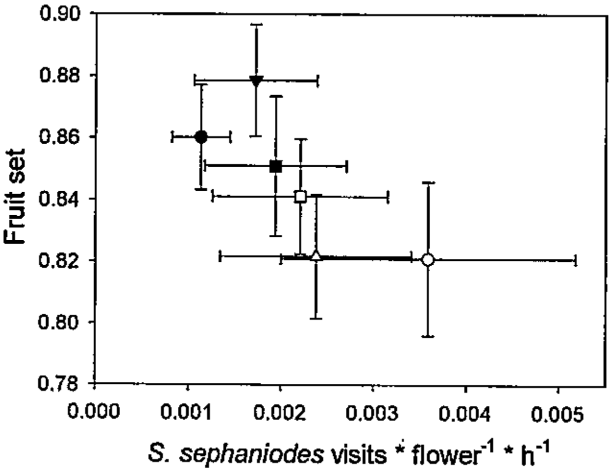
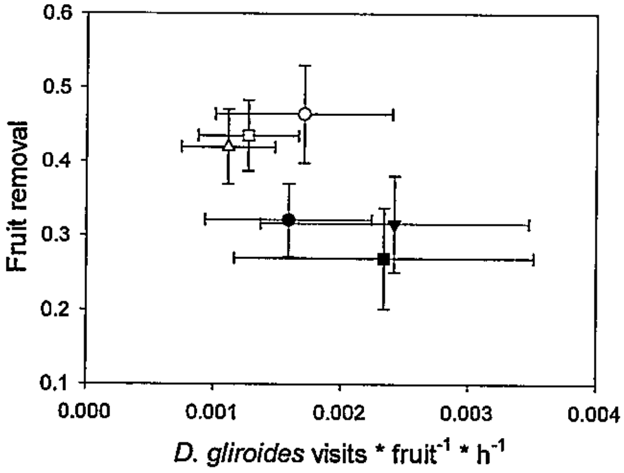


Figure 1

(a) Pollinator effectiveness landscape



(b) Seed dispersal effectiveness landscape



(c) Overall effectiveness landscape

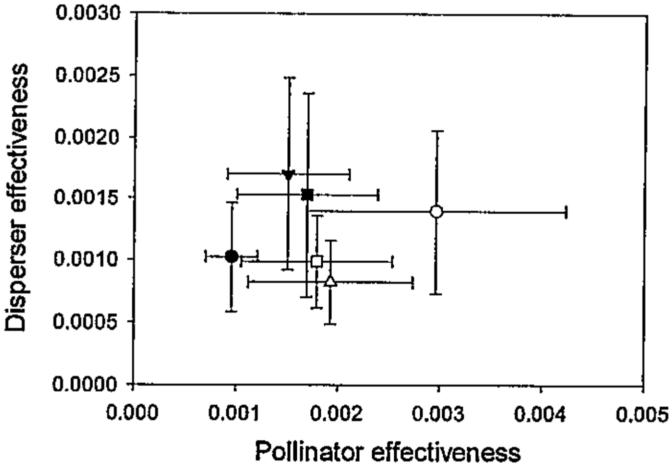
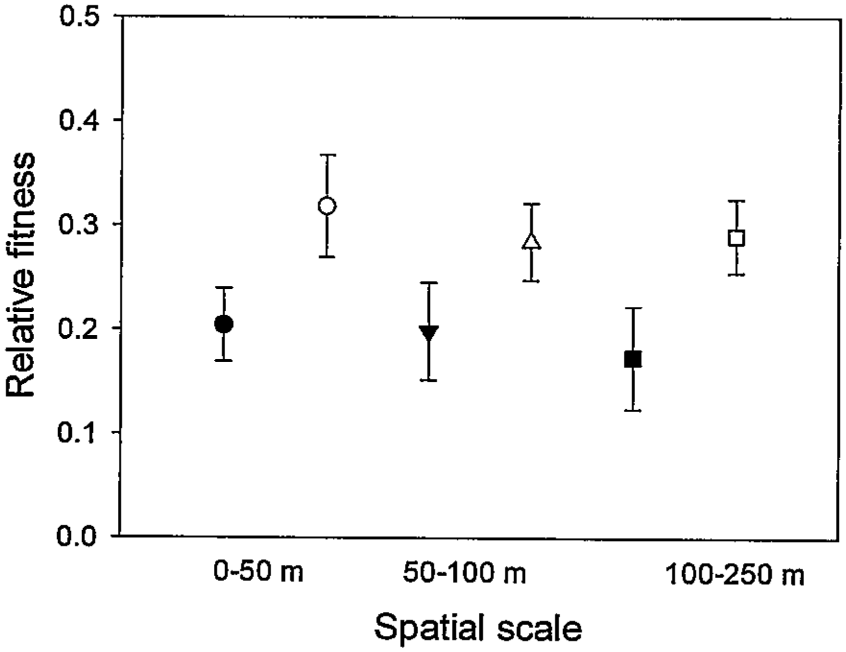


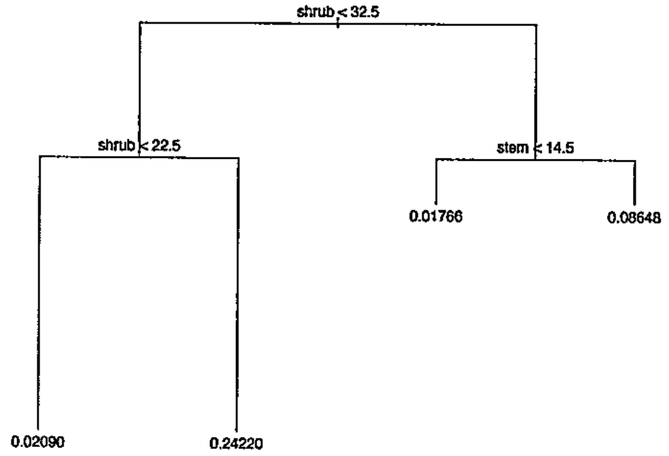
Figure 2



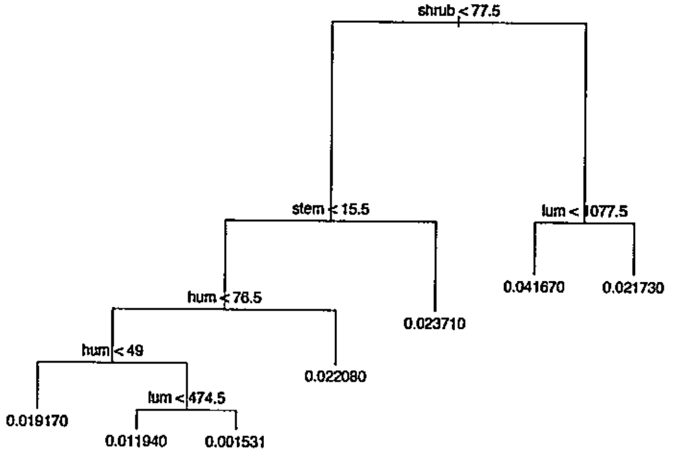
0.5
0.4
0.3
0.2
0.1
0.0
Relative fitness
0-50 m
50-100 m
100-250 m
Spatial scale

Figure 3

(a) Pollinator effectiveness ($R^2 = 0.53$)



(b) Disperser effectiveness ($R^2 = 0.35$)



(c) Plant fitness ($R^2 = 0.55$)

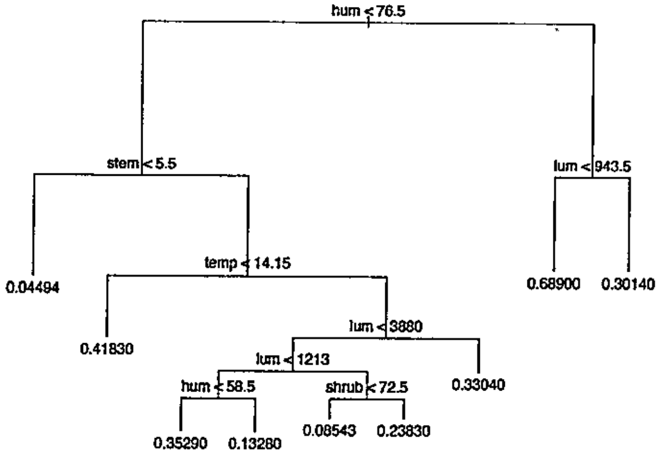


Figure 4

SUPPLEMENTARY MATERIAL

Table S1. *A posteriori* comparisons (based on Dunn's method) of fitness values between native and transformed habitats for each spatial scale assessed.

Contrast scale	Q	P-value
0-50 m	2.102	0.036
50-100 m	1.963	0.050
100-250 m	2.406	0.016

CAPÍTULO 4

RESPONSES OF TWO KEY MUTUALISTS OF THE AUSTRAL TEMPERATE RAINFOREST TO HABITAT STRUCTURE MODIFICATION

(To be submitted to Functional Ecology)

ABSTRACT

Habitat structure is a key factor influencing plant-animal interactions. Habitat fragmentation is known to negatively affect pollination and seed dispersal, but little is known about the effects of habitat transformation on plant-animal mutualisms. We contrasted native and transformed stands (eucalyptus plantation with a dense native understory) at a non-fragmented scenario in order to test the effects of structural modification on the presence, abundance, and activity of two key mutualists (*Sephanoides sephaniodes* and *Dromiciops gliroides*) of the South American temperate rainforest. Despite the strong between-habitat structural and microclimate differences, bird composition was similar as well as *S. sephaniodes* abundances. The endemic marsupial *D. gliroides* was recorded at both native and transformed habitats, which was unexpected since this species was described as an old-growth forest specialist. Structural and microclimate features poorly explained its presence in the two habitats. Regarding their interaction with the mistletoe *Tristerix corymbosus*, both mutualists responded to plant's spatial structure and certain structural features (shrubs, bamboo, moss abundance). Further, *D. gliroides* also responded to the neighborhood of fleshy fruiting species from January to March, when most fruits have ripened. The transformed habitat retained both key mutualistic species and their ecological roles in spite of its structural simplification. This retention could be attributed to the Myrtaceae-dominated understory vegetation, in which flower and fruit resources are diverse and abundant, outweighing the effects of the replacement of native tree species by eucalyptus plantations.

Key words: *Dromiciops gliroides*; fruit neighborhood; habitat structure; *Sephanoides sephaniodes*; southern Chile

INTRODUCTION

Habitat structure has an outstanding role in modulating plant-animal interactions since structural and microclimate features are likely to determine plant's performance, animal's behavior, and interaction probabilities (Breitbach *et al.* 2012, Figueroa-Esquivel *et al.* 2009, Lefevre & Rodd 2009). Structural and microclimate features of natural habitats can be affected by anthropogenic disturbance (e.g., selective logging, fragmentation, replacement of native vegetation with exotic species), and species dwelling on disturbed habitats respond idiosyncratically in function of their habitat requirements, disturbance sensitiveness, and life history traits (Thiollay 1999, Vargas *et al.* 2012). Such idiosyncratic responses are usually mediated by key forest structures such as perching branches and shelter provided by old-tree cavities, which become a limiting at disturbed habitats (Kelm *et al.* 2008, Reem & Lõhmus 2011). Human activities exert non-random modifications on natural habitats, which usually involves the removal of elder trees, canopy structure simplification, and reduction of cryptogam plants and the litter layer (Fontúrbel 2012), impacting most accessible lands first.

While habitat fragmentation effects on ecological interactions result from area and edge effects, habitat transformation (i.e., the total or partial replacement of native for exotic species) effects are poorly known yet despite being a major biodiversity loss driver. As transformed habitats are not necessarily fragmented, predicting species response to structural and compositional changes -when edge and area remains almost stable- is a challenging task. Further, strong structural and microclimate differences between native and transformed habitat are expected to have profound consequences on ecological interactions due to changes in species abundance and composition (e.g., Murúa *et al.* 2010).

Transformed habitats usually have a less complex vegetation structure and composition, and are commonly dominated by generalist species (Barlow *et al.* 2007, Peh *et al.* 2006).

Among ecological interactions, plant-animal mutualisms (i.e., pollination and seed dispersal) may have an important role in plant population dynamics and forest regeneration (Godínez-Alvarez & Jordano 2007). Pollination and seed dispersal interactions are particularly relevant at the South American temperate rainforests (SATF, hereafter) because over 70% of its native flora depends on them for reproducing (Aizen *et al.* 2002). Further, habitat fragmentation process also involves certain degree of habitat degradation (e.g., Fontúrbel 2012), correlated to patch area and the influence of the surrounding matrix. However, the effects of habitat transformation *per se* (i.e., with no fragmentation involved) at the plant-animal mutualisms at this endemism-rich ecosystems has not been assessed yet, despite the high dependence degree of SATF's flora on these key ecological processes.

Aiming to fill this knowledge gap, we assessed the effect of structural modifications related to habitat transformation (replacement of native forest by exotic eucalyptus trees) on two key SATF's mutualist species: *Sephanoides sephaniodes* and *Dromiciops gliroides*, which are known to be negatively affected by habitat fragmentation (Magrach *et al.* 2012, Rodríguez-Cabal *et al.* 2007). Due to the structural differences between native and transformed habitats, we hypothesize that: (1) bird assemblages differ between habitats, being *S. sephaniodes* and frugivorous birds more abundant and diverse at the native habitat; (2) *D. gliroides* is absent in the transformed habitat; (3) *S. sephaniodes* and *D. gliroides* interaction rates relate to structural and microclimate features; (4) *D. gliroides* frugivory interaction is affected by the availability and temporal variation of fleshy.

METHODS

Study site

We conducted this research at the Valdivian Coastal Reserve (39°57'S, 73°34'W), which is private protected area of The Nature Conservancy (Delgado 2010). This Reserve is one of the largest SATF remnants (50,530 ha) in southern Chile. These forests are considered a biodiversity hotspot due to their high degree of endemism and being threatened by human actions as well (Mittermeier *et al.* 2005, Myers *et al.* 2000). There is a complex habitat mosaic at this site, composed by old- and second- growth native stands, and a transformed habitat consisting in exotic eucalyptus plantations (planted 12-20 years ago) with abundant native understory vegetation.

Study species

We focused our research on two SATF's native species: the Green-Backed Firecrown (*Sephanoides sephaniodes*) and the monito del monte (*Dromiciops gliroides*), which have mutualistic roles at these forests. On the one hand, *S. sephaniodes* pollinates about 20% of the native flora of the SATF (Smith-Ramírez 1993). On the other hand, *D. gliroides* is a generalist species that feeds on fruits, eggs, and insects (Fontúrbel *et al.* 2012, Jiménez & Rageot 1979), and it is known to be the legitimate disperser of at least 16 species of native plants (Amico *et al.* 2009). Many plant species interact with both mutualists (i.e., are pollinated by *S. sephaniodes* and then dispersed by *D. gliroides*), but the most striking case is the hemiparasitic mistletoe *Tristerix corymbosus* (Loranthaceae), which reproductive success strictly depends on both mutualists (Aizen 2003).

Data collection

We used two approaches to assess the effect of structural habitat modification on the presence and abundance of *S. sephaniodes* and *D. gliroides*. The first approach consisted in locating and contrasting two 0.16-ha plots of native habitat (corresponding to old-growth forest stands) against two 0.16-ha plots of transformed habitat (corresponding to eucalyptus plantation with native understory). Plot selection was aided by aerial images and field surveys, aiming to locate each plot within each type of habitat without mixing native and eucalyptus stands. At each plot we also conducted eight bird censuses during the austral summer season, in order to quantify the avifauna present, and particularly *S. sephaniodes* and frugivorous birds. Census were made using the point counts of a fixed radius of 40 m for 10 minutes (following Jiménez 2000). Further, at each plot, we set a squared grid of 5X5 sampling points, arranged 10 m away from each other. At each sampling point we placed an infrared camera-trap (Bushnell Trophy Cam 2011), which was set in photo mode and baited with fresh banana slices, a bait highly effective for attracting *D. gliroides* (Fontúrbel & Jimenez 2009). This relative activity technique has the advantage of extensive monitoring periods at low cost, but do not allow recognizing individuals. Cameras were operated from December 2011 to April 2012, and from November 2012 to March 2013, with a total sampling effort of 3,878 camera-days (each camera was operated ca. 27 days on average). During the first sampling period, cameras were located in five random locations during the whole season (austral late spring to early fall); during the second sampling period, cameras were rotated every 28 days within each plot to assess all sampling points. Once camera data were retrieved we constructed (1) a presence-absence matrix for each sampling point, (2) a relative activity matrix for each habitat type replicate.

Additionally, we recorded a set of structural and microclimate variables at a 2.5-m radius from each sampling point. Structural variables measured were: (1) shrub cover (estimated visually); (2) bamboo (*Chusquea quila*) cover (estimated visually); (3) stem density, measured as the number of stems with DBH > 1 cm; (4) the number of fallen logs; (5) the number of stumps; (6) the number of natural cavities; (7) moss abundance (as a categorical variable with three levels: absent, scarce, abundant); and (8) fern abundance (categorical variable: absent, scarce, abundant). Microclimate features measured were: (1) air temperature; (2) relative humidity; and (3) luminosity.

The second approach consisted on examining mutualists' activity at plant's scale, by locating, tagging and georeferencing 70 *T. corymbosus* plants along the study area. We choose this species due to its high specificity for both mutualists, since this mistletoe interacts mostly with *S. sephaniodes* for pollination (althoug bees may provide ~5% of the pollination services; Aizen 2005), and at this latitude is dispersed solely by *D. gliroides* (Amico *et al.* 2011). By choosing this highly specialized plant we avoid confounding effects of other redundant species. At each focal plant, we placed infrared camera-traps, aiming to quantify the number of *S. sephaniodes* and *D. gliroides* visits, each camera was operated by two consecutive days at each location, making a total cumulative effort of 140 camera-days. At each focal plant we measured the same set of structural and microclimate variables measured at the sampling plots. Further, as fruit offer is an important factor in determining *D. gliroides* visit rates (Morales *et al.* 2012), we quantified fruit offer at two levels: (1) *T. corymbosus*' crop size, and (2) the abundance of other fleshy-fruit resources at a 2.5-m radius from each sampling plant. *Tristerix corymbosus*' crop sizes were estimated by counting all ripe fruits during the February-March period, when most fruit ripen and reach its peak abundance. As the other fleshy-fruited plants have distinct phenology patterns, we counted ripe fruits repeatedly from November 2012 to March 2013 on a monthly basis (unripe fruits were counted but not considered in this analysis since they are unlikely consumed).

Data analysis

Between-habitat bird assemblage composition differences was assessed using a one-way analysis of similarity (ANOSIM), which is a non-parametric test based on permutations (Clarke 1993). ANOSIM was conducted using the Bray-Curtis distance, a measurement previously used in comparisons of bird communities (Barlow *et al.* 2007). Complementarily, we used a Similarity Percentage (SIMPER) test to rank species in a decreasing similarity degree of the ANOSIM test. ANOSIM and SIMPER tests were performed using PAST 2.15 (Hammer *et al.* 2001). Structural and microclimate variables related to the presence-absence of *D. gliroides* at the sampling plots were assessed using a logistic regression analysis, including both structural and microclimate features as explanatory variables.

To establish the relationships between *S. sephaniodes* and *D. gliroides* visit rates and the structural and microclimate features measured at plant scale, we fitted Generalized Additive Models (GAM) including an spatially-explicit spline (based on the UTM coordinates of each focal plant) to account for the system's spatial structure, since mistletoes are not regularly distributed along the study area. Mistletoes are usually spatially aggregated, which may compromise independence assumptions in statistical models due to spatial autocorrelation. To overcome this problem, we used this spatially explicit GAM approach (Dormann *et al.* 2007) for all analyses conducted with mistletoe-related data. We also used spatially explicit GAM models to assess the potential effect of *T. corymbosus* and other fleshy-fruited species fruit offer on *D. gliroides* visits. Models were fitted using *T. corymbosus* crop size values for the peak ripening period (February-March), and we used the total number of ripe fruits of other species present at each sampling period in three combinations: (1) *T. corymbosus* crop size only; (2) all monthly fruit counts without *T. corymbosus* crop size data; and (3) individual models with *T. corymbosus* crop size and significant fruiting periods, which were assessed individually to test for potential statistical interaction between *T. corymbosus* fruit offer and the fleshy fruit offer of the other species. All values are presented as mean ± 1SE. Statistical procedures were conducted in R 2.15 (R Development Core Team 2012) unless otherwise indicated.

RESULTS

Native and transformed (i.e., eucalyptus plantation with native understory regrowth) habitats differed in terms of structure and microclimate. We found larger shrub covers and higher air temperatures at transformed habitats, whereas bamboo cover, stem density, fallen logs, natural cavities, and relative humidity were larger at the native habitat; the number of stumps and luminosity did not differ between habitats (for detailed information see Table S1, available in Supplementary Material).

First approach: contrasting plots

Regarding avifauna, we detected 21 species at the whole study area. Twenty-one species were present at the native plots, whereas 20 species were detected at the transformed habitat. The only species absent from the transformed habitat was the Magellanic Woodpecker (*Campephilus magellanicus*). Most abundant species were the Chucao Tapaculo (*Scelorchilus rubecula*) at both native (22.7% and 25.6%) and transformed

(18.4% and 22.2%) plots; the hummingbird *Sephanoides sephaniodes*, with 8 and 13 records at the native habitat plots (9.1% and 15.9%), and 11 and 6 records at the transformed habitat plots (11.2% and 8.3%); and the White-crested Elaenia (*Elaenia albiceps*; 11.4% and 12.2% at native habitat plots, and 17.3% and 18.1% at transformed habitat plots). The White-crested Elaenia is one of the four frugivorous birds registered. We recorded another three frugivorous species in both habitat types: the Chilean Pigeon (*Patagioenas araucana*, 4.5% and 4.9% at native habitat plots and 2.0% and 2.8% at transformed habitat plots), the Austral Thrush (*Turdus falcklandii*, 5.7% and 4.9% at native plots, and 7.1% and 6.9% at the transformed plots), and the Chilean Mockingbird (*Mimus thenca*, 4.5% and 1.2% at native plots, and 0% and 2.8% at transformed plots). A complete list of the avifauna registered is available upon request.

Overall avifauna composition between native and transformed habitats was marginally different (ANOSIM R = 0.061, P = 0.053). There were no composition differences within native (R = 0.032, P = 0. 273) or transformed habitat (R = 0.010, P = 0. 389) plots. Regarding between-habitat composition, the first native plot showed no composition differences with the two transformed plots (R = 0.047, P = 0.235 and R = 0.035, P = 0.280), whereas the second native plot significantly differed in composition with the first transformed plot (R = 0.147, P = 0.035) but not with the second one (R = 0.055, P = 0.213). The species that contributed the most to habitat similarity were *S. rubecula*, *S. sephaniodes*, *E. albiceps*, *Aphrastura spinicauda*, and *Pteroptochos tarnii*, with a cumulative contribution to the between-habitat similarity of 48.4%. On the other hand, *Colaptes pitius*, *Scelorchilus albicollis*, *C. magellanicus*, and *Zonotrichia capensis* were the most dissimilar species between habitat types. Examining only the subset of frugivorous birds, species composition differed substantially between habitats (R = 0.126, P = 0.012), being between-plot differences significant in all cases ($R > 0.140$, $P < 0.043$) but the first native plot and the second transformed plot ($R = -0.024$, $P = 0.567$); there were no composition differences between plots of the same habitat type. *Elaenia albiceps* was the most ubiquitous species, whereas *M. thenca* contributed the most to between-habitat dissimilarity, being more common at native forest plots. Complete SIMPER results are available upon request.

Regarding the activity of *D. gliroides*, we were able to gather data from two austral summer seasons. At the first season (December 2011 to April 2012), we recorded 63 photos at the native habitat (38 and 25 for each plot) and 46 (30 + 16) at the transformed habitat plots. At the second season (November 2012 to March 2013), we recorded 207 (79 + 128) photos at the native habitat plots and 123 (50 + 73) at the transformed habitat plots. In both cases, there was a strong monthly and between-plot variation in activity, which decreases as the summer is over in the transformed habitat but increased at the native habitat (Figure 1). Relating *D. gliroides* response to structural and microclimate features, moss abundance ($\chi^2 = 6.853$, df = 2, $P = 0.033$; goodness-of-fit test, Hosmer-Lemeshow = 6.863, $P = 0.551$) was the only significant variable for explaining presence-absence patterns between habitats. Analyzing native and transformed habitat separately, there was no significant explanatory variable for *D. gliroides* presence at the transformed habitat (goodness-of-fit test, Hosmer-Lemeshow = 13.217, $P = 0.105$), whereas moss ($\chi^2 = 12.187$, df = 2, $P = 0.002$) and fern abundance ($\chi^2 = 7.851$, df = 2, $P = 0.019$), bamboo cover ($\chi^2 = 7.335$, df = 1, $P = 0.007$), stem density ($\chi^2 = 5.413$, df = 1, $P = 0.019$), the number of cavities ($\chi^2 = 6.469$, df = 1, $P = 0.011$), and luminosity ($\chi^2 = 8.393$, df = 1, $P = 0.004$) explained *D. gliroides* presence at the native habitat (goodness-of-fit test, Hosmer-Lemeshow = 7.738, $P = 0.459$).

<Figure 1 about here>

Second approach: plant-scale interaction

From the 70 monitored plants, we have registered 207 *S. sephaniodes* and 83 *D. gliroides* visits. *Sephanoides sephaniodes* and *D. gliroides* visits at the plant scale were influenced by structural and microclimate features, as well as by the spatial configuration (Table 1). Testing the effect of fruit offer on *D. gliroides*' visits, we first examined the effect of *T. corymbosus* crop size, which has no effect on visit rate (GAM estimate = 0.003 ± 0.004, P = 0.392), but it have responded to the spatial structure (spline (X,Y), $\chi^2 = 24.45$, P = 0.034). Then, we examined the effect of the total monthly abundance of other fleshy fruits available nearby focal plants, which have considerably varied across monitoring periods, reaching its peak at February, being *Aristotelia chilensis* and *Rhaphithamnus spinosus* the most abundant species recorded (Figure 2). These fleshy fruits influenced *D. gliroides* visits at February (GAM estimate = $-2.1e^{-3} \pm 8.5e^{-7}$, P = 0.016) and March (GAM estimate = $4.4e^{-3} \pm 2.1e^{-3}$, P = 0.033), and also have responded to the spatial structure (spline (X,Y), $\chi^2 = 35.37$, P < 0.001).

<Table 1, and Figure 2 about here>

Finally, we examined the combined effect of *T. corymbosus* fruits and the fruit offer of accompanying species with fleshy fruits (i.e., fruiting neighborhoods). We tested fruiting neighborhood effects for the peak *T. corymbosus* fruiting period (January - March). During January and February, the fruiting neighborhood has a negative effect on *D. gliroides* visits (i.e., the more fleshy fruits available, the less visits to *T. corymbosus*). Conversely, during March (when overall fleshy fruit offer is beginning to decay), there was a positive interaction between the resource offer of *T. corymbosus* and the other fleshy-fruited species

(Table 2). In all cases, there was a significant effect of the spatial configuration on *D. gliroides* visits.

<Table 2 about here>

DISCUSSION

Native and transformed habitat differed in most structural and microclimate features, as was expected considering that the original canopy composed of a variety emerging species (e.g., *Nothofagus dombeyi*, *Eucryphia cordifolia*, *Fitzroya cupressoides*) was replaced by a single *Eucalyptus* species, resulting in a less complex canopy structure. Conversely, transformed habitats presented a more complex understory composed by several early-successional and shadow-intolerant shrubs (e.g., *Rhaphithamnus spinosus*, *Aristotelia chilensis*, *Ugni molinae*) and sparse spots of the bamboo *Chusquea quila*. This situation is not a commonplace for eucalyptus plantations since this species usually precludes other vegetation to grow by causing water shortage. This is not a limiting factor here since this area receives over 2,500 mm of annual rainfall (Delgado 2010).

Avifauna composition differed in relative abundance terms and not in species richness, as both habitats shared 95% of their species. Similar studies in eucalyptus plantations at Amazonia (Barlow *et al.* 2007) and in rubber tree plantations at Peninsular Malaysia (Peh *et al.* 2006) found reduced bird diversity and abundance in exotic tree-dominated forest systems, in which generalist species were dominant. We found many native forest-dependent species at the transformed habitat, pinpointing the high abundances of two rhynocryptid species with limited dispersal (*Scelorchilus rubecula* and *Pteroptochos tarnii*) and an understory insectivore (*Sylviorthorhynchus desmursii*) that are known to be negatively affected by habitat fragmentation (Castellón & Sieving 2006, Diaz *et al.* 2006).

Further, frugivores' composition difference between habitats was due to their relative abundance, since species richness remained constant. Between-habitat dissimilarity was mainly given by *Mimus tenca*, which is known to be a legitimate disperser of many mistletoe species (Amico *et al.* 2011, Medel 2000).

The generalist pollinator *S. sephaniodes* was present at both habitats in similar abundances, representing ~10% of the total birds detected. Its presence at the transformed habitat might be related to the presence of alternative floral resources than those available at native forests, but especially by *Eucalyptus* and *Lapageria rosea* flowers that are an ubiquitous abundant resource at transformed habitats (FE Fontúrbel, personal observation). *Sephanoides sephaniodes* activity is known to be affected by habitat fragmentation, and particularly by the composition of the surrounding matrix (Magrach *et al.* 2012). In this case, as there are no contrasting non-forested habitats, differences emerged from the spatial configuration of the focal plants assessed -which were more aggregated at Eucalyptus-dominated sites- and its visit rates were influenced by structural and microclimate features related to *T. corymbosus* exposure and physiology (i.e., opener sites receiving more sunlight usually had larger plants with larger floral displays than shadowy below-canopy microhabitats).

On the other hand, *Dromiciops gliroides* presence at the transformed habitat was an unexpected result, given that this species was considered to be restricted to old-growth forests (Hershkovitz 1999), and even more surprising was the fact of recording 169 photos of this species at the eucalyptus-dominated stands (versus 270 photos registered at the native habitat). A recent review on this species (Fontúrbel *et al.* 2012) showed that this marsupial is actually not restricted to old-growth forest stands, but it also is capable to thrive in Myrtaceae-dominated second-growth forests as long they have certain structural complexity. This finding goes beyond, showing that *D. gliroides* is also a common species at this exotic tree-dominated habitat, in which its presence could be determined by a dense understory presenting five Myrtaceae species (*Ugni molinae*, *Luma apiculata*, *Amomyrtus luma*, *Amomyrtus meli*, and *Myrceugenia planipes*), and *Chusquea quila* bamboo, which is used by *D. gliroides* as nesting material (Jiménez & Rageot 1979) and was described to be an important factor influencing its presence (Rodríguez-Cabal & Branch 2011). Based on these evidences, *D. gliroides* seems to be a forest-generalist species.

Dromiciops gliroides' presence / absence between habitats was poorly explained by the structural and microclimate features measured, being moss cover the only significant explanatory variable. This situation is perhaps arising from the great inter-habitat variation, which also might be leading to behavioral responses on *D. gliroides*, since this species is responding to a set of environmental features at the native habitat that are coherent with previous reports on this species (Kelt 2000, Kelt 2006); but it is not responding to any of the measured variables at the transformed habitat. This suggests that, at the transformed habitat, *D. gliroides* may be responding to other environmental variables than those measured in field. Notwithstanding, food resource availability might be playing a more important role at transformed habitats, outweighing the importance of habitat structure. This could explain the pattern observed at the first monitoring season, in which *D. gliroides* relative activity was greater at the transformed habitat up to February and then it has been reduced dramatically and most activity was concentrated at the native habitat, which is coinciding with the ripening of most fleshy-fruited plants (including *T. corymbosus*) at the native habitat. In a fragmentation scenario, habitat degradation had a determinant role in determining *D. gliroides*' presence and abundance (Fontúrbel 2012) since this species is unable to disperse among forest patches by non-forested matrices (Fontúrbel *et al.* 2010).

However, in this scenario resource availability would be determining its presence and interaction strength with *T. corymbosus*.

Beyond *D. gliroides* presence at transformed habitats, the maintaining of its role as seed disperser agent could be a regeneration opportunity since these eucalyptus-dominated habitats are acting as surrogate habitats (Melo *et al.* 2013). Giving that habitat fragmentation at the study area is negligible, *D. gliroides* individuals would be able to establish foraging areas at transformed stands, but concentrate their nesting sites at neighboring native remnants. As has been previously reported in Argentina (Morales *et al.* 2012), there was a strong neighborhood effect on *D. gliroides* activity, reflected by the significant effect of spatial configuration detected accounting for the large *T. corymbosus* aggregations found where transformed habitat is dominant (see Chapter 2 for further details). Mixed neighborhoods (as those from transformed habitats) are more attractive for frugivorous species, and larger fruit availability reduced dispersal distances, driving plant's distribution even more aggregated due to frugivores' behavior (Morales & Carlo 2006).

At this habitat transformation scenario, bird assemblages did not differed greatly between native and transformed habitats, as observed with *S. sephaniodes* and frugivorous birds abundance, contrary to our first hypothesis. In contrast to our second hypothesis, *D. gliroides* was present at the exotic-tree dominated habitat, and its relative activity was ca. 63% of the abundance registered at the native habitat. As expected, both structural and microclimate features influenced *S. sephaniodes* and *D. gliroides* interaction rates with *T. corymbosus*. *Dromiciops gliroides* frugivory was also influenced by fleshy fruit offer, but limited to the January-March period, when most fruits are ripe.

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TABLES

Table 1. Structural and microclimate features affecting *Sephanoides sephaniodes* and *Dromiciops gliroides* visits at 70 *Tristerix corymbosus* plants. Poisson GAM estimates are presented (standard error is shown in parenthesis). Significance codes: ^{NS} not significant, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The spatially explicit term Spline (X,Y) represents the effect of the spatial configuration.

Variable	*Sephanoides sephaniodes*	*Dromiciops gliroides*
Shrub cover	0.025 (0.029)^{NS}	0.025 (0.007)***
Bamboo cover	0.110 (0.046)*	0.001 (0.002)^{NS}
N° of stems	0.215 (0.096)*	-0.001 (0.001)^{NS}
Fallen logs	1.773 (0.712)*	0.069 (0.183)^{NS}
Stumps	1.763 (0.464)***	-1.178 (0.650)†
Cavities	-2.128 (1.553)^{NS}	-83.340 (3e+7)^{NS}
Moss (abundant)	-4.858 (1.542)**	-1.343 (0.686)*
Moss (scant)	-5.203 (1.604)**	0.103 (0.548)^{NS}
Fern (abundant)	-1.511 (3.279)^{NS}	-0.259 (0.599)^{NS}
Fern (scant)	-1.853 (3.366)^{NS}	0.032 (0.520)^{NS}
Temperature	1.271 (0.326)***	-0.256 (0.129)*
Humidity	-0.375 (0.127)**	-0.0089 (0.028)**
Luminosity	7e-5 (9e-5)^{NS}	9e-5 (4e-5)*
Spline (X,Y)	χ^2=75.63***	χ^2=17.67***

Table 2. Effects of fruiting neighborhoods on *Dromiciops gliroides* visits. The effects of the resource offer of the mistletoe (M) *Tristerix corymbosus*, the accompanying fruiting neighborhood (N), and its interaction (M x N) are presented, after fitting Poisson GAM models. P-value significances: * P < 0.05.

Period	Effect	Estimate	P-value
January	M	$2.7e^{-3}$	0.530
	N	$-7.4e^{-3}$	0.044 *
	M x N	$4.3e^{-5}$	0.122
February	M	$1.7e^{-3}$	0.714
	N	$-1.5e^{-3}$	0.048 *
	M x N	$7.5e^{-6}$	0.582
March	M	$-6.3e^{-3}$	0.296
	N	$-4.2e^{-3}$	0.115
	M x N	$8.7e^{-5}$	0.031 *

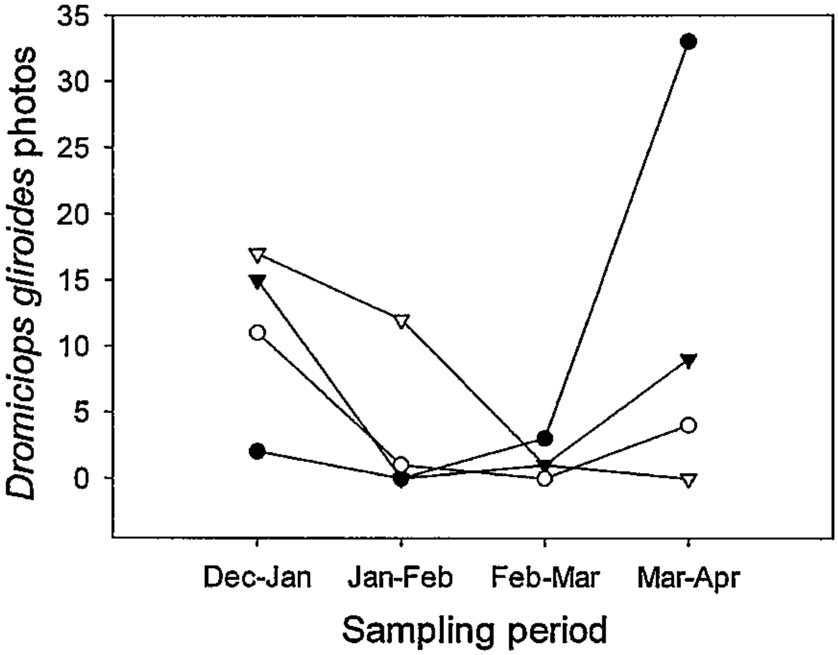
FIGURE CAPTIONS

Figure 1. *Dromiciops gliroides* relative activity from camera-trap monitoring for (a) 2011-2012 austral summer (December to April), and (b) 2012-2013 austral summer (November to March). Closed symbols represent native habitat plots and open symbols transformed habitat plots.

Figure 2. Abundance of fleshy fruits available from November 2012 to March 2013. Values correspond to ripe fruits counted at a 2.5-m radius from each focal *Tristerix corymbosus* plant. Vertical bars represent 1SE.

FIGURES

(a) 2011 - 2012 summer monitoring



(b) 2012 - 2013 summer monitoring

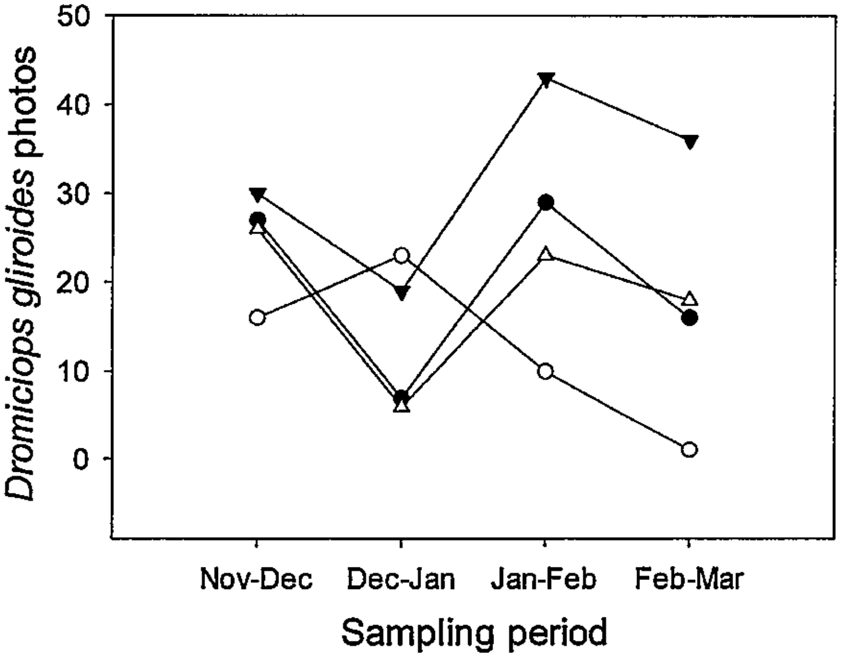


Figure 1

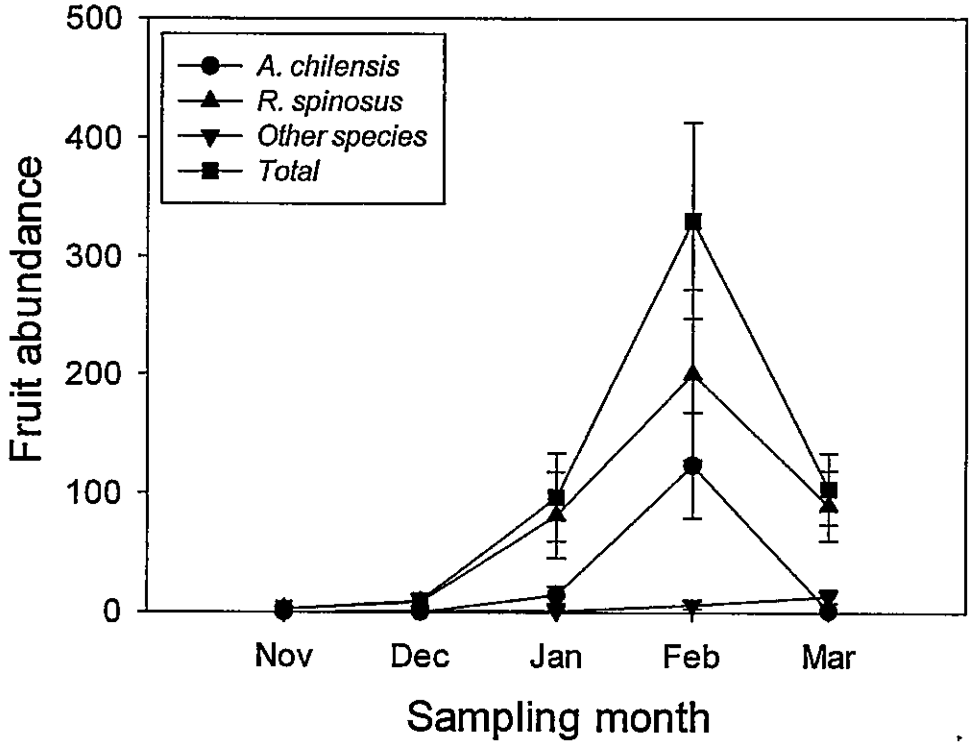

500
400
300
200
100
0
Fruit abundance
A. chilensis
R. spinosus
Other species
Total
Nov
Dec
Jan
Feb
Mar
Sampling month

Figure 2

SUPPLEMENTARY MATERIAL

Table S1. Comparison of structural and microclimate features between native (N) and transformed (T) habitats. Moss and fern abundances are categorical variables measured in three discrete levels: absent (0% cover), scant (<50%), and abundant (≥ 50%).

Category	Variable	Mann-Whitney test		Difference
		U-value	P value	
Structure	Shrub cover	190.5	< 0.001	N < T
	Bamboo cover	629.5	< 0.001	N > T
	Stem density	705.5	< 0.001	N > T
	Fallen logs	421.5	< 0.001	N > T
	Stumps	1231.0	0.884	N ≈ T
	Cavities	1097.0	0.043	N > T
	Moss abundance (categorical)	N: 40% abundant, 54% scant, 6% absent T: 0% abundant, 20% scant, 80% absent		
	Fern abundance (categorical)	N: 26% abundant, 46% scant, 28% absent T: 8% abundant, 20% scant, 72% absent		
Microclimate	Temperature	550.0	< 0.001	N < T
	Humidity	362.5	< 0.001	N > T
	Luminosity	1231.5	0.901	N ≈ T

CAPÍTULO 5

FRUGIVORE-MEDIATED NATURAL SELECTION IN A HABITAT TRANSFORMATION SCENARIO: CONTEXT MATTERS

(To be submitted to Ecology)

ABSTRACT

Plant-animal mutualisms are strong drivers of phenotypic evolution. Frugivore-mediated selection is a little developed topic, since most studies are focused in pollination. Besides, most studies were conducted within the framework of habitat fragmentation. Habitat transformation alters structural and microclimate features, imposing a new scenario for plant-animal interactions that opens new opportunities for natural selection; however, it is a little explored subject. We used a mistletoe (*Tristerix corymbosus*) that is solely dispersed by a marsupial (*Dromiciops gliroides*) to test the effects of habitat transformation along a gradient of native forest replacement by *Eucalyptus*. Transformation gradient affected seed weight and sugar content, which are associated to germination success and frugivore attraction. *Dromiciops gliroides* exerted significant selection forces on those traits, being seed weight relevant as native habitat becomes dominant, and sugar content when transformed habitat was dominant. Plants had larger fitness values at both extreme scenarios, but lower fitness values were registered at those plants thriving at intermediate situations. Habitat structure and microclimate features were correlated to the analyzed traits, depending mainly on the degree of sunlight received. Moreover, spatial structure was significantly influencing natural selection processes, suggesting that the clumped patterns observed at the transformed habitat are determining reproductive success. The response of this plant-frugivore interaction to human disturbance showed to be highly context-dependent, in which extremely transformed habitats would offer new opportunities for natural selection and local adaptation.

Key words: Adaptive landscape; *Dromiciops gliroides*; plant-animal mutualism; seed dispersal effectiveness; spatial structure; *Tristerix corymbosus*.

INTRODUCTION

Plant-animal interactions are strong drivers of phenotypic evolution (Gómez & Perfectti 2010, Sapir & Armbruster 2010), which have coevolved in a non-random way and are capable to maintain their ecological association through time and space (Eklöf *et al.* 2013, Lomáscolo *et al.* 2008, Lomáscolo *et al.* 2010). Such coevolutionary associations emerge from plant traits that are selected by frugivores, imposing selection forces over a subset of characteristics that reinforce the association with a broad spectrum of possible results (Lord *et al.* 2002), known as 'syndromes'. Pollination syndromes have been captured the attention of many scientists since Darwin's times (Darwin 1862) due to the almost-flawless association between flower and pollinator morphology (Gómez & Perfectti 2010). However, seed dispersal syndromes are less evident since fruiting plants tend to interact with a variety of organisms (e.g., birds, small mammals, and bats), making more difficult -although not impossible- to characterize dispersal syndromes in the wild (Lomáscolo *et al.* 2010). In this sense, bird and mammal dispersal syndromes could be recognized from a set of traits such as size, color, and odor (Lomáscolo *et al.* 2008), which could be also interpreted as plant's adaptations to their dispersers (Pizo 2002).

Besides its role as evolutionary force, plant-animal mutualisms (i.e., pollination and seed dispersal) also play a major role in plant's demography and spatial structuration (Jordano *et al.* 2011, Sasal & Morales 2013), since over 75% of the flowering species depend on an animal vector for achieving its reproductive success (Jordano 1987, Ollerton *et al.* 2011). However, this complex eco-evolutionary system is challenged by a world in constant change due to a number of biodiversity loss drivers from anthropic origin (Chapin III *et al.* 2000). Each species respond to habitat disturbance according to their life history traits (Kolb & Diekmann 2005, Vargas *et al.* 2012), which explains the variety of responses observed in nature (McConkey *et al.* 2012, Schleuning *et al.* 2011). In this regard, habitat fragmentation effects have been thoroughly assessed during the past two decades, and most plant-animal interaction consequences could be explained by area and edge effects (Didham *et al.* 2012, Ewers & Didham 2006). However, habitat transformation (i.e., the total or partial replacement of the native vegetation by one or few exotic species) are less obvious and have been little studied despite being recognized as a strong biodiversity loss driver (Albert *et al.* 2013). Comparative studies between native and transformed habitats described changes in pollinator-mediated selection and pollen limitation due to a change in pollinator's composition, which created a brand new eco-evolutionary scenario (Espinoza *et al.* 2012, Murúa *et al.* 2010).

Most studies on plant-animal interaction-mediated selection in anthropogenic habitats dealt with pollination and habitat fragmentation (e.g., Weber & Kolb 2013), being studies on habitat transformation and frugivore-mediated selection very scarce. However, seed dispersers are important selection agents playing a key role on plant's evolutionary dynamics (Jordano 1995, Thorpe *et al.* 2011), and are essential for understanding how seed dispersal shapes plant's spatial and genetic structure according to its actual context (García *et al.* 2009). Moreover, anthropic effects on natural habitats do not occur randomly (e.g., most accessible lands are degraded first), stressing the importance of considering the context when dealing with ecological interactions (Herrera & García 2010). Aiming to fill this knowledge gap, we choose a highly specialized system (a mistletoe with a single disperser species) along a habitat disturbance gradient to test the following hypotheses: (1) given that habitat transformation modifies structural and microclimate habitat features, mistletoes' traits related to seed dispersal would vary along a habitat transformation gradient; (2) given that seed dispersers consume fruits in a non-random way, dispersal-related fruit traits would be selected by frugivores, varying along the habitat disturbance gradient being its effects more pronounced at extreme situations; (3) given that transformed habitats have contrasting structural and microclimate conditions respect to native forests, such environmental features would exert a direct influence on fruit traits under selection; and (4) given that plants -and specially mistletoes- are spatially distributed in a non-random fashion, frugivore-mediated selection would be influenced by a context-dependent spatial structure.

METHODS

Study site and species

This study was carried out at the Valdivian Coastal Reserve (39°57'S 73°34'W), a private protected area owned and administrated by The Nature Conservancy (Delgado 2010). It constitutes one of the largest remnants (50,530 ha) of native temperate rainforest in southern South America, which present many endemic species but is facing increasing levels of anthropogenic disturbance (Mittermeier *et al.* 2005, Myers *et al.* 2000). This area represents a large forest continuum with a complex habitat mosaic comprising old-growth native stands, secondary growth native stands (regenerated after clear-cutting), and exotic *Eucalyptus globulus* plantations (12-20 years old) with a dense native understory regenerated in between.

Tristerix corymbosus (Loranthaceae) is a hemiparasitic mistletoe, considered as a keystone species at the South American temperate rainforests because of its winter-flowering phenology that provides food resources in scarcity periods and sugar-rich fruits during the summer (Aizen 2003). This mistletoe is dispersed only by two species: the

Chilean Mockingbird (*Mimus thenca*) at its northern distribution (30°-37°S), and the arboreal marsupial *Dromiciops gliroides* at its southern distribution (37°-42°S) (Amico *et al.* 2011). Despite *M. thenca* latitudinal distribution extends up to 40°S, its interaction with *T. corymbosus* is apparently precluded by a fruit color polymorphism (Amico *et al.* 2011). Although *T. corymbosus* completely depends on *D. gliroides* for dispersing its seeds, *D. gliroides* has a broad diet including fleshy fruits (of at least 16 species; Amico *et al.* 2009), insects, and eggs (Fontúrbel *et al.* 2012, Jiménez & Rageot 1979). In fact, this marsupial is considered as an obligate generalist since it can't sustain a diet based only on fruits or insects (Cortés *et al.* 2011). This highly specialized mutualistic interaction would allow us to examine the effects of frugivore-mediated selection without the background noise that redundant frugivore species might impose on a plant-frugivore system.

Data collection

Between 2012 and 2013, we surveyed the study area and located 197 *T. corymbosus* plants along the Reserve. From those, 70 plants were accessible and fruiting during the sampling period (January-March, corresponding to the austral summer when most fruits are ripe). We tagged each plant and recorded its location using a Garmin Vista Cx GPS device. We determined each plant's crop size by counting all fruits present. After that, we took two samples of 10 random fruits each from every plant for measuring traits and germination trials. Also 10 random fruits were marked with a water-based non-toxic paint in order to quantify fruit removal, painted fruit were count five days later. Complementarily, aiming to identify potential pulp consumers and quantify *D. gliroides* visits, we set infrared trap-cameras in front of each sampled mistletoe, operated in video mode for 48 straight hours.

Collected fruits were taken to a field-based laboratory, where we measure fruit's diameter (using a 0.01 mm precision caliper) and sugar content (using a handheld brix refractometer). Seeds were dried at room temperature for one week and then weighted using a precision scale once we returned to the city. A second batch of fruits was used for germination trials. We conducted germination experiments in the field facility, since *T. corymbosus* seeds are recalcitrant (i.e., germinate immediately); seeds were placed in Petri dishes with wet filter paper, and were checked daily for five days (but most seeds germinated within the third day).

As habitat structure varied considerably along the study area, we measured the following environmental variables that might be influencing *T. corymbosus* fruit traits in a 5-m buffer from each plant: shrub cover (estimated visually), bamboo (*Chusquea quila*) cover (estimated visually), stem density (the number of DBH ≥ 1 cm) stems within the buffer), air temperature, relative humidity (using a handheld thermohygrometer), and luminosity (using a handheld luxometer). Aiming to establish causal relationships between the measured plant traits used on selection-gradient analyses (fruit diameter, sugar content, and seed dry weight) and habitat structure, we performed a field experiment at the beginning of the fruiting season when all fruits were unripe (early November). We experimentally shaded half mistletoe fruits of three plants at the transformed habitat, and opened a gap (trimming vegetation with gardener scissors) of another three plants at the native habitat, from which we had fruit measurement data from the previous fruiting season. The reduced sample size of this experiment was due to intervention restrictions imposed by protected area regulations. Then, when fruits were ripe, we collected five ripe fruits per treatment, which were measured following the same procedure than the regular samples.

Habitat transformation gradient

As the study area presents a complex and heterogeneous habitat mosaic, assigning each sampled mistletoe to a discrete category was a challenging task. Hence, we used two complementary approaches for overcoming this situation. First, we used the data of the 70 sampled plants and constructed habitat transformation gradient covariates. For doing to, we used the approach of García and Chacoff (2007), defining three non-overlapping buffer rings from each plant: 0-50 m, 50-100 m, and 100-250 m. At each buffer, we determined the proportion of native habitat present using aerial images, digital cartography and field surveys (see Chapter 2 for further details). The second approach consisted in splitting sampled plants in two discrete groups regarding the dominant habitat type (native / transformed). We set an arbitrary threshold of 67% of native habitat (corresponding to the median value of the 0-50 m buffer ring), and plants above this threshold were considered as native habitat, and those below the threshold as transformed habitat. We are aware that this procedure reduced the effective sample size for statistical processing, but also give important comparative information, which should be interpreted with caution.

Data analysis

We calculated seed dispersal effectiveness by multiplying *D. gliroides* visit rates (number of visits per hour; quantitative component) and fruit removal rates (qualitative component) (Schupp *et al.* 2010). Relative fitness was calculated as the product of fruit removal rate and seed germination. First, we correlated raw variables with each other and with habitat covariates, as our data do not met normality assumptions, we used a non-parametric Spearman correlation test.

Raw trait (i.e., fruit diameter, seed dry weight, and sugar content) values, its quadratic (i.e., the squared values) and correlational (i.e., the product of each pairwise trait combination) terms were standardized (to mean = 0 and variance = 1) in order to make them comparable (following Jordano 1995). With those standardized values, we used a modified version of Lande and Arnold (1983) equations to estimate linear and non-linear selection gradients; relative fitness was used as response variable. We made two modifications to the original method: (1) we include the three habitat rings and crop size as covariates, and (2) given that *T. corymbosus*, as most mistletoes, has an aggregated spatial distribution (Rawsthorne *et al.* 2011), we made this analysis spatially explicit by using Generalized Additive Models (GAM) with a spline term containing UTM coordinates of each sampled plant in order to deal with potential effects and independence assumption violations, related to spatial autocorrelation (Dormann *et al.* 2007). Hence, modified equations were expressed as:

(eq. 1) $\hat{w} = \alpha + \sum_{i=1}^{n} \beta_i x_i + CS + \sum_{1}^{s} hab_s + spline(x, y)$

(eq. 2) $\hat{w} = \alpha + \sum_{i=1}^{n} \beta_i x_i + \frac{1}{2}\sum_{i=1}^{n} \sum_{j=1}^{n} \gamma_{ij} x_i x_j + CS + \sum_{1}^{s} hab_s + spline(x, y)$

where $\hat{w}$ = relative fitness, β_i = directional selection gradients, γ_i = stabilizing / disruptive selection gradients, CS = crop size (as covariate), hab_s = proportion of native habitat at each *s* scale (as covariate), spline (x,y) = spatially explicit term.

Selection differentials were calculated as the covariance between trait and fitness values. We also used the spatially explicit GAM approach to assess the relationship between crop size (log-transformed) and relative fitness; crop size was not assessed as trait since it represents a higher hierarchical level of the plant's phenotype and it may lead to misleading interpretations, however it was included as a covariate term in selection gradient equations as described above.

As some data examined had strongly non-normal distributions, we used bootstrapping procedures (with 5,000 iterations) in order to verify each coefficient (except the spatial term) significance level (Jordano 1995, Weber & Kolb 2013). The relationship between relative fitness and habitat ring covariates was assessed by a spatially explicit GAM. Then, we split up our data in two discrete groups (native and transformed habitat) as described above. We first described raw data using the dichotomous criterion defined above, native and transformed habitat values were compared using a non-parametric Mann-Whitney test since our data was not normally distributed. Additionally, aiming to conduct a global comparison between habitat types, we conducted a non-parametric MANOVA test. Then, we have repeated the modified Lande and Arnold equations and bootstrapping procedures in order to estimate selection linear and non-linear gradients, as described above. For those traits that had non-significant directional gradients, we estimate the minimum sample required (MSR) for obtaining statistical significance at $\alpha = 0.05$, which is calculated as $\mathrm{MSR} = (t\sigma / \beta)^2$, where t was set to 1.96, β is the selection gradient and σ its standard deviation (Medel 2000). To compare selection coefficients between habitat types, we performed an analysis of covariance (ANCOVA) including trait values as covariates (Murúa *et al.* 2010); P values were corrected for multiple comparisons using a Bonferroni sequential adjustment. Aiming to relate trait's variation to environmental features, we correlated raw trait values with untransformed structural and microclimate features using a Spearman correlation test. Field experiment data was analyzed using paired t-tests.

Finally, the spatially explicit dimension of the sampled mistletoes was examined by constructing Delaunay triangulations to depict plant's isolation degree (larger triangles indicate more isolated plants), superimposing size plots of *Dromiciops gliroides* visit rates, seed dispersal effectiveness, plant's fitness (Bivand *et al.* 2008), and a kriging map of the proportion of native forest along the study area. This approach allows a visual interpretation of the actual spatial configuration, the plant aggregation level, and the spatial variation of determinant steps of plant recruitment at this scenario. Values are presented as mean ± 1 SE. All statistical procedures were conducted in R 2.15 (R Development Core Team 2012) and external packages (boot, car, deldir, ggplot2, mgcv, spdep, spatstat, vegan).

RESULTS

Plant's crop size was correlated with most of the plant traits and fecundity components, whereas intra-trait correlation was significant only for fruit diameter and seed weight. Also seed weight was positively correlated to germination. The proportion of native habitat was negatively correlated to seed weight and fruit removal at 50-100m and 100-250m scales, and no variable was correlated at 0-50 m habitat scale (Table 1). Along the habitat transformation gradient, seed weight presented a positive significant directional gradient, whereas sugar content had a negative significant gradient. It will be required to increase sample size to 6,232 plants to detect a significant directional gradient for fruit diameter. There were no significant stabilizing or disruptive gradients (Table 2), and crop size covariate was not significant in any case. No correlational gradient was significant (data not shown), and there was no effect of native habitat cover at any spatial scale. However, spatial structure was significant ($P < 0.001$) for both directional and stabilizing /

disruptive selection gradients. Further, sugar content was not correlated with fruit diameter ($r_s = -0.59$, $P = 0.737$) neither with seed dry mass ($r_s = 0.23$, $P = 0.176$).

<Tables 1 and 2 about here>

Crop size was negatively related to relative fitness and was strongly affected by plant's spatial structure (spatially explicit GAM, Estimate = -0.31 ± 0.07, $P < 0.001$; spline (X,Y), $F = 3.72$, $P = 0.002$). Fitness reached its maximum at those plants with small crop sizes that usually have all their fruits removed at the end of the summer. As crop size increase, a greater proportion of the available fruits remain in the plant, decreasing the relative fitness due to a reduction on its quantitative component. Relative fitness decreased with crop size up to 40 fruits, and beyond that value the increase in the number of fruits has no visible effects on plant's relative fitness (Figure 1).

<Figure 1 about here>

For comparative purposes, we arbitrarily separated sampled plants in two groups, aiming to contrast plant's traits and fecundity components between native and transformed types, being only fruit removal and relative fitness significantly different between habitats (Table S1 in Supplementary Material); the global comparison showed no differences between native and transformed habitats (non-parametric MANOVA $F_{1,69} = 0.88$, $P = 0.397$). Examining selection gradients in this between-habitat context, fruit diameter was non significant in any case, requiring to increase sample size to 101 and 800 plants to detect significant directional gradients at native and transformed habitats, respectively. Conversely seed weight presented significant directional and disruptive selection gradients at native habitats, but this trait was not significant at the transformed habitat (Table 3), requiring to increase sample size to 303 for detecting a significant gradient. On the other hand, sugar content was not significant at native habitats (2,525 plants will be required to

detect a significant directional gradient), but it showed significant directional and stabilizing gradients at the transformed habitat. The 'fail safe' numbers calculated indicate that the patterns detected are not a statistical artifact derived from small samples. At the native habitat there was a significant correlational gradient for fruit diameter and seed weight ($\gamma'' = 0.167 \pm 0.071$ (mean ± 1SE), $P = 0.027$), the remaining correlational gradients were not significant (data not shown). In all cases spatial structure was significant, suggesting that plant arrangement explains an important fraction of data variability.
Crop size covariate was significant (with a negative effect) only for directional gradients at native habitat. Comparing selection coefficients between habitats, the ANCOVA model results showed that habitat has an important effect (however marginally significant after Bonferroni correction); sugar content showed to be relevant to explain between-habitat differences in fitness (Table 4).

<Tables 3 and 4 about here>

Fruit diameter was the only trait that does not presented any significant directional selection gradient, having a significant effect only at the correlational gradient with seed weight at the native habitat. Examining the influence of structural and microclimate features, this trait was correlated to bamboo cover. Conversely, seed dry weight and sugar content were positively correlated to luminosity (larger seeds and sugar contents when plants received more sunlight), also sugar content was negatively correlated to shrub cover, which together with the positive correlation with sunlight exposure suggests that less shaded plants tend to produce sweetener fruits (Table 5). Regarding our field experiment, fruit diameter does not varied between shaded and gap mistletoes (paired $t = 0.859$, $P = 0.402$), but seed dry weight (paired $t = -3.071$, $P = 0.007$) and sugar content (paired $t = -4.198$, $P < 0.001$) were significantly larger at gap mistletoes.

<Table 5 about here>

As spatial structure was significant at all spatially explicit GAM models that we fitted, we used a Delaunay triangulation approach to relate the observed *T. corymbosus* fecundity patterns with plants' arrangement (Figure 2). Each sampled plant represents a vertex of the triangle; consequently, larger triangles indicate more isolated plants. *Dromiciops gliroides* visits were more frequent at those sites where *T. corymbosus* plants were densely aggregated (Figure 2a). Seed dispersal effectiveness (Figure 2b) showed a similar trend, but those *T. corymbosus* aggregations located at sites with intermediate native habitat cover (50-60%, confront with Figure 2d) had lower dispersal effectiveness despite having been visited many times by *D. gliroides*. A similar pattern is observer for the resulting plant fitness (Figure 2c), but results suggest a compensatory effect from the qualitative component (i.e., seed germination) that balances plant's fitness along the landscape. On the one hand, those sites with dense plant aggregations, large visit rates and higher effectiveness values had slightly lower fitness values due to reduced germination rates. On the other hand, those sites receiving a few visits and presenting low effectiveness values had slightly larger fitness values due to a higher seed germination rate (confront panels 2a-2c). Finally, comparing panels those results with the abundance of native habitat at landscape level, it is evident that dense plant aggregations and larger relative fitness values occur at those sites dominated whether by transformed or by native habitat, suggesting that frugivory and seed dispersal performed best at either extreme situation but have a lower performance at intermediate situations (Figure 2d).

<Figure 2 about here>

DISCUSSION

This highly specialized mistletoe-disperser system not only persisted at transformed habitats, but also performed even better when comparing extreme situations. As Jordano (1995) found for *Prunus mahaleb*, crop size was negatively correlated to fruit removal and consequently to seed dispersal effectiveness. Mistletoes with less than 30 fruits usually had no remaining fruits left at the end of the austral summer (March-April), whereas those plants with large crop sizes (> 100) had a large proportion of their fruits undispersed, and consequently a lower seed rain contribution. Also, fruits with larger seeds had greater germination rates, which is expected since large seeds have large energy reserves and are capable to maintain the embryo especially in harsh environmental conditions (Chacón *et al.* 1998, Chacón & Bustamante 2001). In this sense, as transformed habitat become dominant, seeds were larger and also were removed in a greater extent, stressing the role of the large seeds as a germination success factor. Despite seed size decisive role to plant's recruitment in disturbed habitats, fruit size had no effect on any situation, concurring to what was found in *P. mahaleb* (Jordano 1995).

Two fruit traits were significantly selected by *D. gliroides* along the habitat disturbance gradient: seed dry weight and sugar content, the former being related to seed survival (Chacón & Bustamante 2001), and the latter to its attractiveness to the disperser in terms of energetic reward (Izhaki 2002). Splitting sampled plants in two discrete groups tear apart this first result, depicting a intriguing scenario, in which seed mass is relevant at the native forest-dominated habitats, where there are less intra- and inter-specific competition (i.e., other fleshy-fruited plants with similar phenologies; see Chapter 4 for further details), but host quality is a limiting factor because the most abundant host species (*Pluchea absinthioides*) experiences high inter-season mortality, compared to early-successional high-quality hosts (e.g., *Aristotelia chilensis*, *Rhaphithamnus spinosus*) found at the native habitat (F.E. Fontúrbel, unpublished data). On the other hand, sugar content becomes relevant at the transformed habitats, where seed size is from little relevance since most seeds are large enough allow high germination rates and host mortality is negligible, but sugar content might play a decisive role in attracting seed dispersers that are strongly influenced by a mixed neighborhood of fruiting species (see Chapter 4); it also was the most relevant inter-habitat factor, according to the ANCOVA model.

Environmental features affected fruit traits under selection in some extent. Despite there are no strong environmental effects, there is a chance that the environment is covarying with plant's phenotype and fitness beyond the trait value itself (MacColl 2011). Introducing environmental variables as covariates in natural selection studies could help cleaning the observed patterns and allow determining what portion of the fitness-trait covariance is related to natural selection in a strict sense, and what proportion is influenced by environmental features (MacColl 2011). This caveat was partially solved with our field experiment, which partially related the seed weight - sugar content spatial variation pattern to a structural effect of canopy complexity and sunlight incidence. Of course, further studies with larger sample sizes are needed to properly establish causal relationships.

Additionally, frugivore-mediated selection significantly depended on mistletoe's spatial structure. Spatial arrangement is that much important as environmental features (Latimer *et al.* 2006), since plant aggregation influences dispersal distance (and thus, gene flow) and originate a positive-feedback loop driving aggregated plant clusters even more aggregated each generation (Carlo & Morales 2008, Morales & Carlo 2006). Aggregated plants have greater chances to be visited and its seed dispersed respect to isolated plants, which are unlikely to be visited due to displacements costs and enhanced predation risks

(Fedriani *et al.* 2010). Our study system shows that frugivore-mediated selection in a habitat transformation scenario is a context-dependent phenomenon, in which extreme situations (on the one hand, the original native habitat, and on the other hand, a transformed habitat with a highly aggregated distribution) are favoring adaptive evolution and the persistence of a highly specialized plant-disperser system, which is known to be disrupted and even driven locally extinct by habitat fragmentation (Rodríguez-Cabal *et al.* 2007). This situation illustrates Fahrig *et al.* (2011) functional landscape heterogeneity definition, showing that this scenario has a neutral -or even somewhat benefic- effect on *T. corymbosus* recruitment and persistence.

Frugivore-mediated selection on *Tristerix corymbosus* showed to be context-dependent, as many pollination systems are (e.g., Cuartas-Hernández *et al.* 2010). As we hypothesized, habitat structure and microclimate influenced seed dispersal and phenotypic selection along a habitat transformation gradient, with a complex combination of spatial arrangement and environmental effects, depicting a scenario in which extreme situations are favoring *T. corymbosus* persistence related to *D. gliroides*' differential response to phenotypic traits of particular relevance at each local context. Human disturbances represent strong sources of evolutionary change (Kinnison & Hairston 2007), and its study in an eco-evolutionary and context-dependent framework is necessary for ensuring the persistence of native species -and more importantly- its ecological interaction (Kinnison *et al.* 2007, Stockwell *et al.* 2003).

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TABLES

Table 1. Spearman correlation coefficients between plant characters (crop size, fruit diameter, seed dry weight, and sugar contents), fecundity components (fruit removal, seed dispersal effectiveness, and seed germination), and the proportion of native habitat (NH) at three progressive spatial scales. Significance levels: * P < 0.05, ** P < 0.01.

Character	Crop size	Fruit diam	Seed wgt	Sugar %	Removal	SDE	Germ
Fruit diam	0.116						
Seed wgt	0.258*	0.628**					
Sugar %	0.514**	-0.003	0.225				
Removal	-0.442**	0.056	0.153	-0.147			
SDE	-0.282*	-0.143	-0.067	-0.143	0.581**		
Germ	0.093	0.277*	0.488**	-0.050	0.100	-0.065	
NH 0-50m	0.098	0.063	-0.158	-0.097	-0,161	0.039	-0.135
NH 50-100m	0.056	-0.142	-0.252*	-0.076	-0.243*	0.094	-0.140
NH 100-250m	0.056	-0.073	-0.293*	-0.092	-0.264*	0.071	-0.104

Table 2. Frugivore-mediated selection coefficients for 70 *Tristerix corymbosus* plants along a habitat disturbance gradient. Directional (β') and stabilizing / disruptive (γ') standardized selection coefficients are presented (standard errors are shown in parentheses). Selection differentials are presented for directional (S_i) and stabilizing / disruptive (S_j) gradients. Significance of each gradient was tested using a bootstrapping procedure; ^{NS} = not significant (confidence interval overlapped zero), * = significant (confidence interval do not overlapped zero).

Plant trait	β'	S_i	γ'	S_j
Fruit diameter	0.016 (0.083)^{NS}	0.012	-0.040 (0.106)^{NS}	0.014
Seed weight	0.085 (0.036)*	-0.058	-0.105 (0.151)^{NS}	0.046
Sugar content	-0.072 (0.030)*	-0.078	-0.152 (0.088)^{NS}	-0.075

Table 3. Frugivore-mediated selection coefficients for 35 *Tristerix corymbosus* plants surrounded by ≥ 67% of native habitat, and 35 plants surrounded by < 67% of native habitat. Directional (β') and stabilizing / disruptive (γ') standardized selection coefficients are presented (standard errors are shown in parentheses). Selection differentials are presented for directional (S_i) and stabilizing / disruptive (S_j) gradients. Significance of each gradient was tested using a bootstrapping procedure; ^{NS} = not significant (confidence interval overlapped zero), * = significant (confidence interval do not overlapped zero).

Plant trait	Habitat	β'	S_i	γ'	S_j
Fruit diameter	Native	0.038 (0.033)^{NS}	0.120	0.045 (0.038)^{NS}	0.121
	Transformed	-0.025 (0.061)^{NS}	-0.042	-0.054 (0.062)^{NS}	-0.048
Seed weight	Native	0.135 (0.032)*	0.145	0.128 (0.031)*	0.149
	Transformed	0.040 (0.060)^{NS}	-0.058	0.043 (0.057)^{NS}	-0.055
Sugar content	Native	-0.006 (0.026)^{NS}	-0.024	-0.016 (0.027)^{NS}	-0.032
	Transformed	-0.118 (0.047)*	-0.142	-0.139 (0.046)*	-0.133

Table 4. Analysis of covariance (ANCOVA) of trait effect on plant's fitness between native and transformed habitats. Degrees of freedom (df), sum of squares (SS), and F values are presented. Significance codes: * $P < 0.05$, $P < 0.01$, [a] coefficients that retained significance after Bonferroni sequential adjustment.

Source	df	SS	F
Habitat (H)	1	0.392	7.134**
Fruit diameter (F)	1	0.012	0.201
Seed dry weight (W)	1	0.175	0.175
Sugar content (S)	1	0.607	11.055**[a]
H x F	1	0.094	1.715
H x W	1	0.167	3.049
H x S	1	0.247	4.497*
Error	62	3.405	

Table 5. Spearman correlation coefficients between *T. corymbosus* fruit traits and environmental structural and microclimate features. Significance levels: * P < 0.05, ** P < 0.01.

Trait	Shrub cover	Bamboo cover	Stem density	Air temperature	Relative humidity	Luminosity
Fruit diameter	-0.078	0.289*	-0.138	0.098	-0.199	0.176
Seed weight	-0.142	0.195	-0.074	-0.179	0.104	0.238*
Sugar content	-0.271*	0.006	-0.020	-0.155	-0.019	0.339**

FIGURE CAPTIONS

Figure 1. Crop size effect on plant's relative fitness. Shaded areas represent 95% confidence intervals.

Figure 2. Delaunay triangulation maps for (a) *Dromiciops gliroides* visits, (b) Seed dispersal effectiveness, and (c) Plant's relative fitness; bubble size is proportional to the magnitude of each variable. Panel (d) shows a kriging map of the proportion of native habitat, warmer colors indicate more abundant native cover.

FIGURES

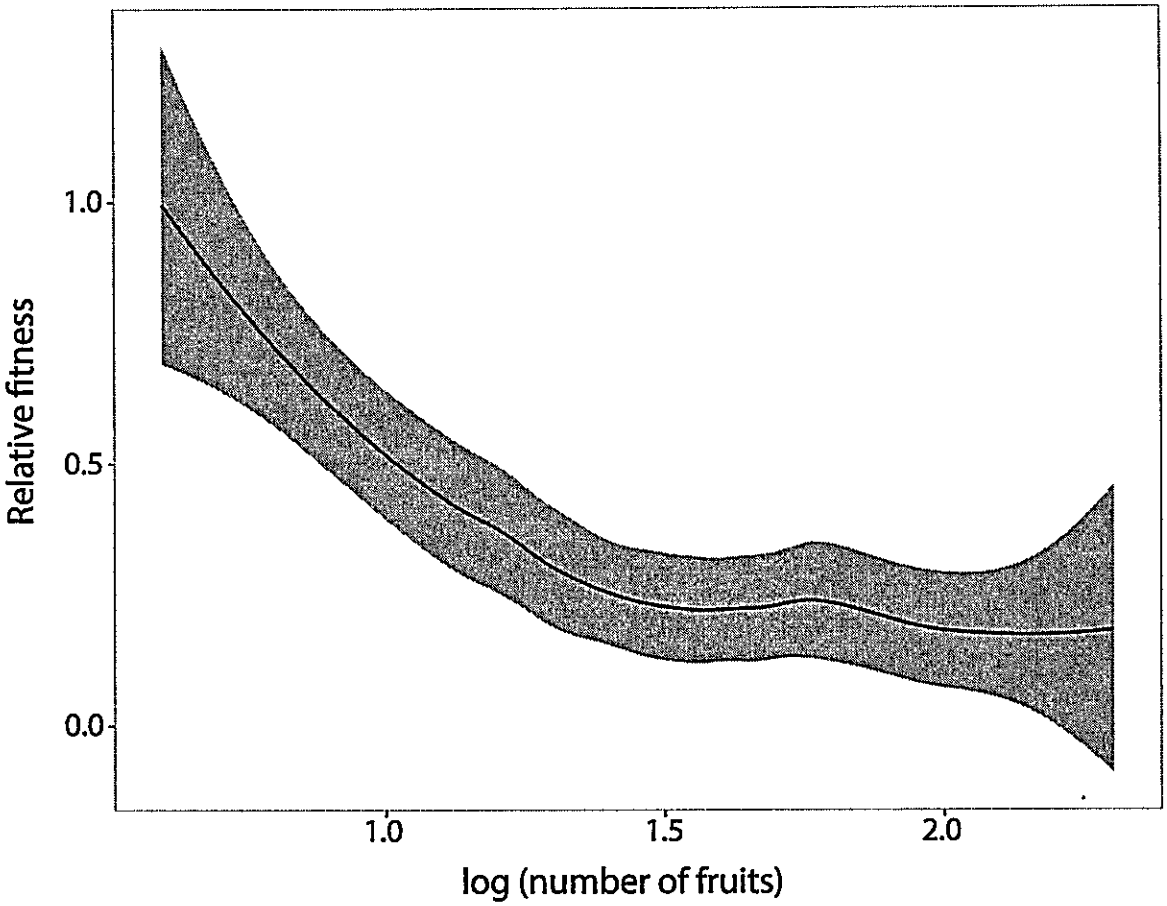
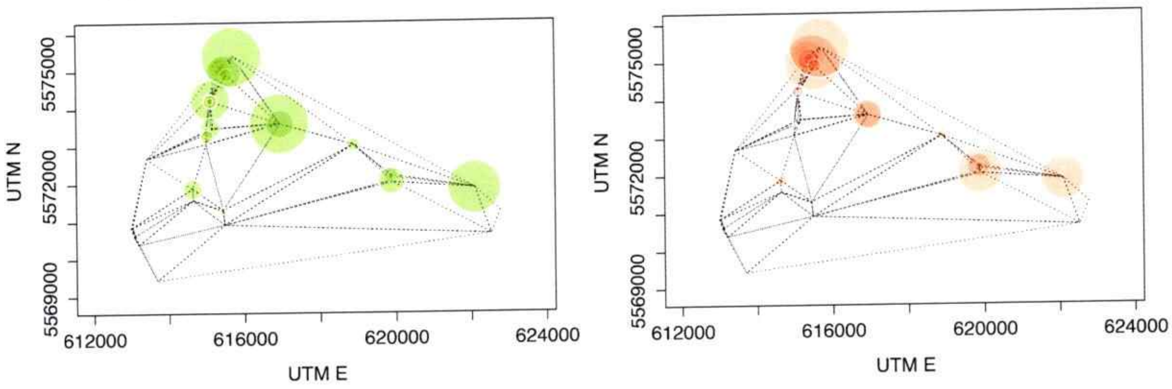
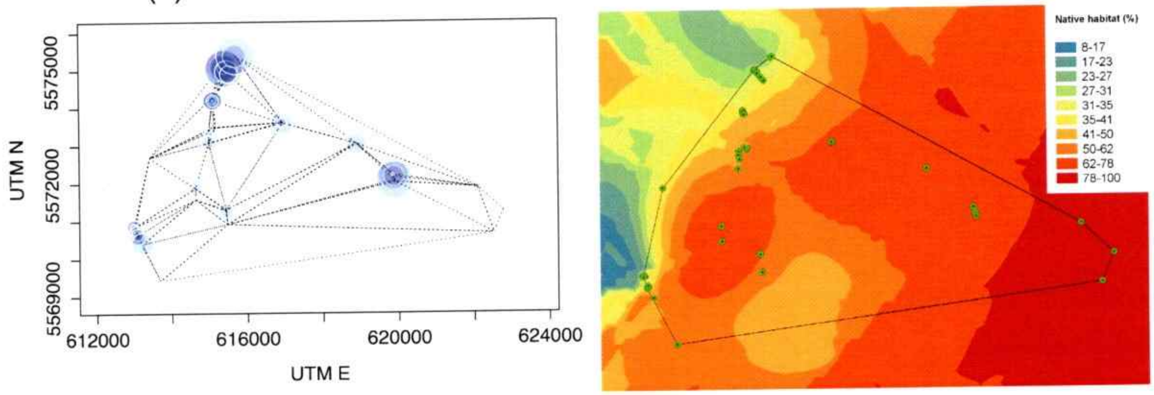


Figure 1



(a) *Dromiciops gliroides* visits

(b) Seed dispersal effectiveness



(c) Plant's relative fitness

(d) Native habitat kriging

Figure 2

SUPPLEMENTARY MATERIAL

Table S1. Between-habitat comparisons for plant (crop size, fruit diameter, seed dry mass, and sugar content) and fecundity (*Dromiciops gliroides* visits per plant, fruit removal, germination, and relative fitness) characteristics. Differences were assessed through non-parametric Mann-Whitney tests. Significance levels: ** P < 0.01

Variable	Habitat		Mann-Whitney
	Native	Transformed	P-value
Crop size	54.46 ± 7.98	44.63 ± 6.71	0.284
Fruit diameter (mm)	5.29 ± 0.08	5.12 ± 0.10	0.385
Seed dry mass (mg)	24.41 ± 1.66	26.66 ± 1.82	0.356
Sugar content (Brix %)	17.10 ± 0.91	17.52 ± 0.89	0.496
D. gliroides visits	1.17 ± 0.39	1.20 ± 0.35	0.937
Fruit removal (%)	24.85 ± 4.18	50.81 ± 6.17	0.003**
Germination (%)	74.91 ± 4.55	79.39 ± 4.26	0.484
Relative fitness	0.19 ± 0.04	0.41 ± 0.05	0.002**

CAPÍTULO 6

DISCUSIÓN GENERAL Y CONCLUSIÓN

DISCUSIÓN GENERAL

La transformación del hábitat mostró tener efectos contexto-dependientes sobre los mutualismos planta-animal, los que no se corresponden con lo descrito para el caso de la fragmentación y pérdida del hábitat (Magrach *et al.* 2012, Rodríguez-Cabal *et al.* 2007). En el caso estudiado, los efectos de área y borde que potencialmente pueden afectar al área de estudio son mínimos, y por lo tanto las variables ambientales (i.e., factores estructurales y de microclima) y los cambios en las interacciones dominantes estarían dando cuenta de una importante proporción de la variabilidad observada en el sistema (Kikvidze *et al.* 2011). Si bien las plantaciones de eucalipto están consideradas como hábitats sub-óptimos dominados por especies generalistas (Barlow *et al.* 2007) debido al efecto alelopático que suelen presentar los eucaliptos al impedir la regeneración y crecimiento de otras plantas, en otras situaciones su carácter sub-óptimo puede no ser tan conspicuo. En el sistema de estudio, el régimen de precipitaciones característico del sur de Chile permite una abundante vegetación de sotobosque entre los eucaliptos, dándole al hábitat transformado una textura fina que usualmente no se encuentra en este tipo de plantaciones, en especial en aquellas plantaciones que se encuentran bajo manejo forestal.

El emplear al quintral (*Tristerix corymbosus*) como modelo de estudio, debido a la especificidad de sus interacciones con una sola especie de polinizador y una sola especie de dispersor, permitió dar cuenta de los efectos escala-dependientes de la transformación del hábitat sobre las interacciones. En este sentido, el picaflor *Sephanoides sephaniodes* se vio ligeramente afectado por la modificación estructural del bosque nativo, donde los cambios se dieron a una escala espacial más amplia las respuestas del dispersor *Dromiciops gliroides*. Esto es esperable debido a las restricciones de movimiento a las que está sujeto *D. gliroides* (i.e., dependencia de ramas, arbustos y bambú para poder desplazarse de un lugar a otro) las que no afectan a *S. sephaniodes* quien es capaz de desplazarse volando de una planta a otra independientemente de la estructura del entorno circundante. Sin embargo, ambas especies respondieron significativamente al arreglo espacial de las plantas, las que fueron más abundantes de lo esperado por azar en hábitats transformados, donde se encontraron formando agregados más densos que en el bosque nativo. Dentro de esta estructuración espacial, la asociación espacial encontrada entre la actividad de *D. gliroides* y el número de plántulas de *T. corymbosus* da cuenta de la importancia de esta interacción para el reclutamiento del quintral (García *et al.* 2009).

La efectividad de la polinización y la dispersión de semillas varió ampliamente a lo largo del gradiente de transformación de hábitat estudiado, siendo mayor la efectividad global de ambas interacciones mutualistas en el bosque nativo. Sin embargo, debido a la mayor remoción de frutos y mayor germinación en los ambientes dominados por bosque transformado, la adecuación biológica global de las plantas de *T. corymbosus* fue mayor que en el bosque nativo. Los paisajes de efectividad de las interacciones mutualistas se componen de dos componentes: uno cuantitativo y otro cualitativo (Schupp *et al.* 2010), por lo que distintas combinaciones de estos componentes pueden dar lugar a resultados similares. En el caso de los mutualismos actuando sobre *T. corymbosus*, el componente cuantitativo fue siempre mayor en bosque nativo, mientras que el componente cualitativo fue mucho más variable, siendo mayor en el bosque nativo para el caso del cuajado de frutos (proxy de calidad del polinizador), mientras que la remoción de frutos (proxy de calidad del dispersor) y la germinación (proxy de calidad de las semillas) fueron mayores en los bosques dominados por eucaliptos. Esta evidencia sugiere que *T. corymbosus* se ve beneficiado de cierta manera por la modificación del hábitat, donde se mantienen sus interactuantes clave a niveles similares de efectividad, pero con mayores oportunidades de remoción de frutos y germinación de sus semillas. Esto concuerda con otras descripciones de muérdagos beneficiados por la perturbación antrópica (e.g., Bowen *et al.* 2009).

Los bosques productivos usualmente presentan una menor diversidad y suelen estar dominados por especies generalistas (Barlow *et al.* 2007, Peh *et al.* 2006). Sin embargo, en este caso particular la presencia de un abundante sotobosque formado por vegetación nativa sombra-intolerante, característica de sucesiones tempranas, parecer ser un factor determinante para la mantención de gran parte de las especies de bosque nativo en los ambientes transformados. Esto se evidencia en la escasa diferenciación a nivel de las aves, donde solo el carpintero magallánico (*Campephilus magellanicus*) estuvo ausente en los bosques transformados, y la diferenciación entre hábitats estuvo dada por cambios de abundancia relativa y no por recambio de especies. Ambos mutualistas (*S. sephaniodes* y *D. gliroides)* estuvieron presentes en ambos tipos de hábitat, en abundancias similares. Esto resulta particularmente interesante para el caso de *D. gliroides*, que estaba descrito como una especie restringida al bosque nativo (Hershkovitz 1999), pero ésta y otras evidencias recientes (Fontúrbel *et al.* 2012) apuntan a que sería un generalista de bosque. Al igual que lo reportado por otros estudios en plantaciones comerciales (e.g., Estades *et al.* 2012, Nájera & Simonetti 2009), la presencia de abundante sotobosque nativo parece ser el factor determinante para la persistencia de las interacciones ecológicas en los bosques dominados por eucalipto, considerando además que la vegetación dominante de éste sotobosque ofrece una gran cantidad y diversidad de recursos (escasos o ausentes en el bosque nativo), a los que los mutualistas -y particularmente *D. gliroides*- responden debido a un efecto de vecindario mixto (Carlo *et al.* 2007, Carlo & Morales 2008, Morales & Carlo 2006). Sin embargo, esta situación no sería esperable en plantaciones manejadas de eucalipto, las que usualmente están desprovistas de vegetación de sotobosque, ya que éste es removido periódicamente.

Por último, pero no menos importante, el contexto provisto por el escenario de transformación de hábitat estudiado influenció la selección natural mediada por el frugívoro. Dos rasgos de los frutos de *T. corymbosus* fueron seleccionados por *D. gliroides*: el peso de la semilla y el contenido de azúcar. El peso de la semilla es un rasgo importante en aquellos sitios dominados por hábitat nativo, donde la baja calidad del hospedero dominante (*Pluchea absinthioides*) hace que una semilla más grande tenga mayores oportunidades de sobrevivir (Chacón & Bustamante 2001), mientras que el contenido de azúcar fue más importante en los ambientes transformados, donde la mayor exposición a la luz solar hace que la mayor parte de las semillas sean grandes y germinen, y donde los hospederos son de buena calidad (e.g., *Rhaphithamnus spinosus*, *Aristotelia chilensis*), pero la alta competencia intra e interespecífica –debida al efecto de vecindario- hace que la atracción de dispersores sea más competitiva (Izhaki 2002, Morales *et al.* 2012).

Este estudio demuestra la importancia del contexto ecológico al estudiar las interacciones planta-animal, y sus consecuencias demográficas y evolutivas en un mundo en constante cambio. Las perturbaciones antrópicas representan fuerzas selectivas excepcionalmente fuertes en los ambientes naturales (Kinnison & Hairston 2007), modificando la dinámica ecoevolutiva de las interacciones ecológicas, donde las consecuencias últimas son claramente contexto-dependientes. Mientras que la fragmentación del hábitat -debido a efectos de área, borde y aislamiento- tiene un efecto muy fuerte sobre el reclutamiento de *T. corymbosus* al provocar la reducción o incluso la extinción local del dispersor (Rodríguez-Cabal *et al.* 2007), la transformación del hábitat parece ser menos disruptiva, permitiendo la persistencia de mutualismos altamente específicos, gracias a la estructura y la provisión de recursos del sotobosque. El escenario estudiado podría actuar como un 'sustituto de hábitat' (Melo *et al.* 2013), ya que aunque no reemplaza al bosque nativo, permite la persistencia de muchas especies nativas –y más importante aún- de las interacciones ecológicas. Finalmente, cabe resaltar el rol que los mutualismos planta-animal tienen en la regeneración de los ambientes perturbados (Corlett 2002, Gomes *et al.* 2008), los que de mantenerse y manejarse adecuadamente representarían una oportunidad de recuperar las zonas perturbadas por la actividad antrópica en el sur de Chile.

CONCLUSIÓN

A pesar de los cambios estructurales y microclimáticos, la transformación del hábitat no tuvo un efecto negativo sobre la abundancia de los quintrales y sus mutualistas, contrariamente a lo hipotetizado inicialmente. Los mutualistas *S. sephaniodes* y *D. gliroides* presentaron abundancias similares a las registradas en el bosque nativo, mientras que *T. corymbosus* fue incluso más abundante en el ambiente transformado. Consiguientemente, la frecuencia y la efectividad de las interacciones ecológicas (i.e., polinización y dispersión de semillas) en las que estas especies participan no se vieron significativamente afectadas.

Contrariamente a lo descrito para la fragmentación del hábitat, los potenciales efectos negativos de la transformación del hábitat pueden ser compensados por la presencia de vegetación de sotobosque abundante y diversa, la que provee de estructura fina y recursos a muchas especies sensibles a la perturbación. Este hecho se verificó en la presente investigación, y confirma lo reportado por otros estudios en sistemas productivos. La regeneración de vegetación nativa secundaria en sistemas degradados puede facilitar -al menos parcialmente- la recuperación de las interacciones ecológicas, acelerando su restauración. La evidencia empírica aquí presentada respalda estas afirmaciones. Consecuentemente, el manejar el sotobosque en las plantaciones comerciales puede incrementar su valor para la conservación de la biodiversidad.

Si bien el escenario descrito en esta investigación sugiere un efecto moderado a neutro de la transformación del hábitat sobre el éxito reproductivo del quintral, por medio de la mantención de los mutualismos con el polinizador y el dispersor, queda pendiente de evaluar –en futuros estudios– el costo a largo plazo que implica el vivir en estos ambientes transformados. Debido a los cambios registrados en el arreglo espacial de las plantas (i.e., una mayor agregación), es esperable que se reduzcan las áreas de forrajeo de los mutualistas y por consiguiente, que se reduzca el flujo génico. A largo plazo, esto podría implicar un costo importante a nivel de depresión por endogamia y mayor estructuración genética en aquellas plantas que habitan en hábitats transformados.

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ANEXOS

ANEXO 1: CO-PATROCINIO DE UN SEMINARIO DE TÍTULO

Efecto positivo indirecto del parasitismo por *Tristerix corymbosus* sobre el éxito reproductivo de *Rhaphithamnus spinosus*

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Profesor patrocinante: Dr. Rodrigo Medel

Co-patrocinante: Francisco E. Fontúrbel

Por definición las plantas parásitas ocasionan efectos negativos en sus hospederos, como lo son la pérdida de nutrientes, menor reproducción, o reducción en sobrevivencia. Dentro de este grupo se encuentra el muérdago hemiparásito *Tristerix corymbosus* (Loranthaceae), que infecta una variedad de hospederos entre los que se encuentra *Rhaphithamnus spinosus* (Verbenaceae), cuyos patrones fenológicos de floración y fructificación están sincronizados parcialmente con los del parásito. En este trabajo se examinó si esta sincronía supondría un beneficio indirecto al hospedero en términos de polinización, dispersión de semillas y reclutamiento, debido a una atracción de mutualistas. Se compararon plantas de *R. spinosus* parasitadas y no parasitadas con *T. corymbosus* para examinar los potenciales efectos del parasitismo sobre las tasas de polinización y dispersión de semillas. Los resultados indican que la tasa de polinización (número y tiempo de visitas por *Sephanoides sephaniodes*) sobre *R. spinosus* no difirió entre plantas parasitadas y no parasitadas. La lluvia de semillas, fue significativamente mayor en las plantas parasitadas. Además, en plantas no parasitadas, el número de frutos colectados en las trampas de semillas estuvo espacialmente asociado con el número de frutos presentes en la planta, mientras que en las plantas parasitadas no se observó tal asociación. Por último, se contabilizaron las plántulas cercanas de *R. spinosus,* encontrándose que bajo las plantas parasitadas hay un mayor reclutamiento, en cantidad y densidad. En este caso particular de parasitismo planta-planta los frutos carnosos del parásito serían responsables de la atracción de un mayor número de dispersores, lo que conllevaría a mayores tasas de remoción de frutos, ocasionando un mayor reclutamiento bajo el dosel, que beneficiaría indirectamente al hospedero en términos de éxito reproductivo.

ANEXO 2: EXTENSION Y DIFUSIÓN DE RESULTADOS

Conjuntamente con el desarrollo de la investigación, estuve realizando actividades de extensión y difusión de la información obtenida con los actores sociales involucrados. Para ello, se organizaron talleres con los niños (8-12 años) de las escuelas rurales de Chaihuín (Figura S1) y Huape (Figura S2), comunidades próximas al área de estudio. En los talleres se les dio a conocer el monito del monte a los niños, utilizando mucho material gráfico para explicarles de dónde viene, su importancia como dispersor de semillas de las plantas nativas, y su amenaza debido a la destrucción de su hábitat (Figura S3). También se compartió la parte metodológica de campo utilizada con ellos, en los seminarios tuvieron la posibilidad de ver, tocar y operar trampas de captura viva, equipo de telemetría y trampas-cámara.

Figura S1. Taller en la escuela rural de Chaihuín (noviembre de 2012).

Figura S2. Taller en la escuela rural de Huape (noviembre de 2012).

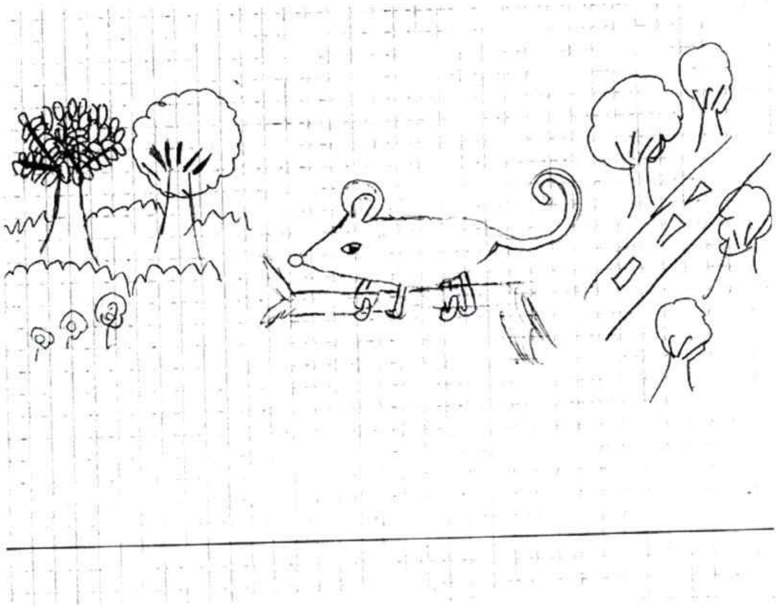

Figura S3. Dibujo de uno de los niños de la escuela rural de Chaihuín, mostrando al monito del monte en su hábitat original y en la situación actual.

También se realizó un taller con jóvenes scouts que visitaron la Reserva (Figura S4), en el que se les explicó la importancia del monito del monte como agente de regeneración de los bosques naturales y se los puso en contexto del sitio al que visitaban, destacando las áreas de mayor presencia del monito del monte, haciendo énfasis en el reclutamiento del quintral, que en ese momento estaba comenzando la floración y era fácilmente visible desde los caminos. Al término del trabajo de campo se realizó un pequeño taller interno con los guardaparques y los funcionarios de la Reserva Costera Valdiviana, a fin de retribuir la colaboración otorgada y restituir la información generada, que es muy pertinente para ellos dado que en este momento se encuentran ejecutando un proyecto de largo plazo que pretende la restauración del área protegida, mediante la eliminación progresiva de los eucaliptos.

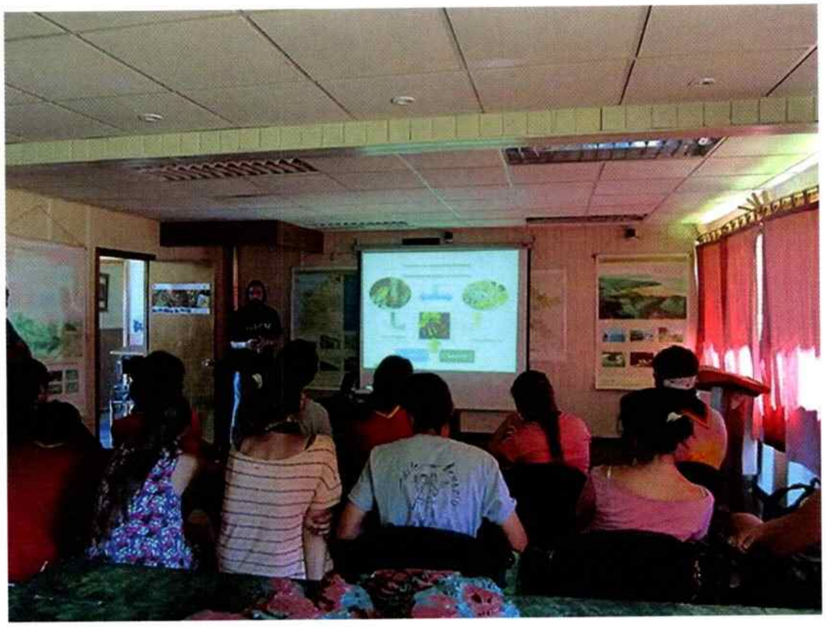

Figura S4. Taller con jóvenes scouts en la Reserva Costera Valdiviana (enero de 2013).

Complementariamente, se preparó un póster educativo (Figura S5) para ser socializado en las escuelas y las comunidades. La finalidad de este material gráfico fue crear un refuerzo visual, con información precisa y altamente relevante, que permita recordar por más tiempo lo aprendido en los talleres y también el compartir los aspectos generales del trabajo con la gente que a la que no se pudo llegar directamente con los talleres.

El monito del monte:
Ayudando a regenerar nuestros bosques

Es un marsupial muy antiguo que lleva millones de años habitando los bosques templados del sur

Ayuda a la regeneración del bosque porque se encarga de dispersar las semillas de muchas especies de plantas nativas

Construye sus nidos con musgo y quila, se mueve por las ramas de los árboles y vive en las copas

Actualmente está amenazado por la destrucción de su hábitat... sin bosques no hay monito del monte

Para mayor información visite:
http://sites.google.com/site/ecoevolutionary

Fotografías: Jaime E. Jiménez - Javiera Malebrán

Figura S5. Poster educativo elaborado a partir del proyecto de tesis.

ANEXO 3: FOTOGRAFÍAS DE LAS ESPECIES DE ESTUDIO

Figura S6. Flores de *Tristerix corymbosus* en invierno. Fotografía: J. Villagra (2010)

Figura S7. Frutos de *Tristerix corymbosus* en verano. Fotografía: F. Fontúrbel (2011)

Figura S8. *Sephanoides sephaniodes.* Fotografía: F. Fontúrbel (2008)

Figura S9. *Dromiciops gliroides.* Fotografía: J. Jiménez (1984)