Endozoochory decreases environmental filtering imposed to seedlings

Laura Cavallero, Marcelo A. Aizen & Estela Raffaele

Cavallero, L. (corresponding author,
laucavallero@yahoo.com.ar/
cavallerol@comahue-conicet.gob.ar),
Aizen, M.A. (marcelo.aizen@gmail.com) &
Raffaele, E. (estelaraffaele@yahoo.com.ar):
Laboratorio Ecotono, Universidad Nacional del
Comahue-CONICET (INIBIOMA), Quintral 1250,
S.C. de Bariloche, 8400, Pcia. de Río Negro,
Argentina

Abstract

Questions: Recruitment microsites are imposed on plants, first by seed dispersal and then by the environment. Different seed dispersal vectors could decrease or increase the environmental filtering imposed on seedlings, depending on their specificity to deliver seeds to suitable microsites for germination and establishment. We addressed the hypothesis that endozoochory reduces the environmental filtering imposed to seedlings to a larger extent than does anemochory. We predicted that seedlings from animal-dispersed species should show a higher degree of environmental coupling.

Location: Northern Patagonian Andean region of Argentina, 40–42° S.

Methods: We characterized the relationship of seedlings, saplings and reproductive individuals to total radiation, air temperature and relative humidity for 16 woody species growing in 25 plant communities of different post-fire ages in temperate forests of southern South America.

Results: Seedlings and saplings of endozoochorous species occurred under more similar environmental conditions than seedlings and saplings of anemochorous species. Basically, the mean difference in plant–environment correlation coefficients between saplings and seedlings was <0.2 for all endozoochorous species and >0.2 for all anemochorous species for all environmental variables. Comparisons between dispersal modes were also statistically significant before and after controlling for phylogenetic relationships among species. Even though saplings and seedlings of endozoochorous species tended to occur under similar environmental conditions, we did not find evidence that saplings and seedlings co-occur in the same physical microsites. Finally, we did not find evidence either that seedlings of endozoochorous species tended to occur differentially underneath reproductive individuals of the same species (i.e. more seeds falling by gravity beneath maternal plants).

Conclusions: The tighter coupling between seedlings of endozoochorous species and environmental factors persisting to the sapling stage suggests that relying on animals for seed dispersal reduces mortality costs during the early stages of recruitment by reducing the environmental filtering imposed on seedlings.

Introduction

Plants, being sessile organisms, do not actively choose habitats; instead, recruitment microsites are imposed on them, first by seed dispersal and then by the environment (Bazzaz 1991; Schupp 1995; Temperton et al. 2004). Seed dispersal, either abiotic or biotic, is the demographic phase most important for plant movement in space and the first sieve that a plant-to-be has to go through (Harper 1977). This spatial process is crucial for decreasing sibling compe-

tition and increasing recruitment of new individuals by enlarging the potential area for seedling establishment (Alcántara et al. 2000; Nathan & Muller-Landau 2000; Wang & Smith 2002; Clark et al. 2005). Spatial patterns of recruitment largely depend on the initial template established by seed dispersal, but are also influenced by successive post-dispersal factors, such as seed predation, germination success and seedling mortality (Herrera et al. 1994; Jordano & Herrera 1995; Wenny 2000; Garcia et al. 2005). Therefore, after landing, a seed has to pass through

a complex of additive and interactive environmental filters that will determine patterns of plant recruitment (Bazzaz 1991; Schupp 1995; Temperton et al. 2004). More specifically, patches where seeds are disseminated differ in abiotic (i.e. light, temperature, water availability, nutrients) and biotic factors (i.e. seed predation, herbivory, competition, allelopathy, plant–pathogen and plant–mycorrhiza interactions). These differences result in a gradient of patch suitability for seed survival and germination, seedling establishment, and plant growth and reproduction (Schupp 1995; Baythavong et al. 2009). Thus, seed dispersal may benefit plants when seed transfer occurs to microsites where conditions favour survival, particularly during critical stages of early plant development when environmental filtering is most severe and mortality rates are highest (Harper 1977; Schupp 1995; Schupp et al. 2010).

Different dispersal vectors could decrease or even increase the environmental filtering imposed on seedlings, depending on their specificity to deliver seeds to more suitable microsites for germination and establishment. There are some empirical examples documenting that animals can deliver seeds to rather specific microsites (e.g. under perches), which are characterized by a narrow range of environmental conditions (Jordano & Schupp 2000; Schupp et al. 2002; Carlo & Morales 2008; Hampe et al. 2008). This relates to the fact that endozoochory (i.e. dispersal by animals that ingest fruits and defecate intact seeds) is assumed to be a mutualistic interaction in which plants, in exchange for food, benefit because their propagules are moved away from the parent plant, and in some cases, enjoy the additional benefit of 'directed dispersal' (*sensu* Howe & Smallwood 1982; see also Alcántara et al. 2000; Nathan & Muller-Landau 2000; Wenny 2001; Herrera 2002; Jordano et al. 2004). On the other hand, dispersal distance for anemochorous species (i.e. wind dispersed) mainly depends on wind speed, propagule traits (e.g. mass, area, wing loading) and maternal traits (e.g. height of seed release) (Augspurger & Franson 1987). Therefore, anemochory relies on a rather unspecific vector that also moves seeds away from the maternal plant but with a limited capacity to carry seeds to specific microsites. We do not know of any comparative test addressing the higher post-dispersal efficiency (i.e. relative higher number of seedlings that could reach the sapling stage) of endozoochory than anemochory, because of the possibility of 'directed dispersal' associated with the first but not the second dispersal mode. Here we take a community approach to address the hypothesis that vertebrate dispersal reduces the environmental filtering imposed on seedlings to a larger extent than does wind dispersal.

Several authors have proposed that abiotic filtering is more important than biotic filtering in determining early plant recruitment in temperate ecosystems, whereas the opposite has been suggested for less seasonal tropical ecosystems (Schemske 2002, 2009; Schemske et al. 2009). Nevertheless, studies on the importance of abiotic filtering in determining patterns of plant recruitment are scarce (Gómez-Aparicio et al. 2005), particularly when compared to the many studies focused on the importance of biotic filtering for the maintenance of high tree species richness in the tropics. Indeed, Janzen-Connell effects (i.e. positive biotically-mediated effects of increasing distance from conspecifics on seed and seedling survival) have been extensively documented (Houle 1995; Schupp et al. 2010). Yet, the relationship between seed dispersal and the abiotic characteristics of the microsite where seeds land could be extremely important to understand patterns of plant recruitment, population and community dynamics, and even the evolution of dispersal mode itself (Schupp et al. 2010).

This study compares the environmental filtering that occurs during the transition from recently emerged seedlings to established saplings between wind- and vertebrate-dispersed species occurring in post-fire, woody plant communities from the temperate forest of southern South America (TFSA). Although wind is the main seed dispersal vector of many temperate woody floras, the TFSA has a high incidence of biotic seed dispersal (Willson 1991; Aizen & Ezcurra 1998; Aizen et al. 2002). More than 50% of the woody genera occurring in these forests produce fleshy fruits, indicating the importance of endozoochory in this biome (Armesto et al. 1996; Aizen & Ezcurra 1998; Aizen et al. 2002). After animals, wind is the second most important seed vector in the flora of the TFSA, with about 26% of woody genera producing wind-borne seeds (Aizen & Ezcurra 1998). Therefore, endozoochory and anemochory represent the two main seed dispersal modes in this biome.

Here we characterized the relation of seedlings, saplings and reproductive individuals with small-scale environmental heterogeneity (Leathwick & Whitehead 2001; Purves et al. 2007) for ten endozoochorous and six anemochorous woody species. We tested the hypothesis of a higher specificity of endozoochory than anemochory in terms of delivering seeds to environmentally suitable microsites. We used saplings as 'microsite quality indicators' (i.e. microsites with established saplings would indicate a higher likelihood of seedling survival) and distribution of recently emerged seedlings as a proxy of previous seed distribution. Even though the distribution of seeds reflected in seedlings can become distorted because of post-dispersal processes such as herbivory or seed predation (Janzen 1970; Connell 1971; Jordano & Herrera 1995; Schupp 1995; Wenny 2000; Garcia et al. 2005), this distortion should not be related to dispersal type. Therefore, under the assumption that the spatial distribution of seedlings reflects, at least in part, the primary seed dispersal

template, we expect that the distribution of anemochorous seedlings would be mostly independent of microsite quality. In contrast, if animals were more efficient vectors in carrying seeds to microsites characterized by a narrower range of environmental conditions that increase seedling survival (Wenny 2001), we would expect a higher degree of plant–environment coupling from the earliest stages of recruitment. In other words, the environmental conditions that characterize microsites where sapling abundance of endozoochorous species peaks would be similar to those where seedlings are also most abundant. Thus, we predict that the difference between the correlation coefficient of microsite environmental variables with saplings and the correlation coefficient of the same environmental variables with seedlings will be lower in species whose seeds are dispersed by animals than in species whose seeds are dispersed by wind (Fig. 1). Our results support the expectation that endozoochory reduces environmental filtering imposed on seedlings to a larger extent than anemochory.

Methods

Study area

The study area is located in the northern Patagonian Andean region of Argentina, at 40–42° S. Soils are mostly derived from volcanic ash (andisols). Due to the rain shadow effect of the Andes on the westerlies, mean annual precipitation declines from >3000 mm at the continental divide to <800 mm only 50 km eastwards. Approximately 60% of the annual precipitation falls in the winter season (from May to August). In the study region, the lowland forests are dominated by tall trees (>40 m) of the evergreen *Nothofagus dombeyi* or mixed with the shorter conifer (ca. 20 m) *Austrocedrus chilensis*. The understorey of the wetter forest types is typically dominated by dense populations of the bamboo *Chusquea culeou* (>2 m tall). Dense, tall shrublands occur at sites that are edaphically unsuitable for forest development or as successional communities after burning. Shrublands are dominated by the small deciduous tree *N. antarctica* accompanied by *C. culeou*, and numerous other small trees and shrubs (*Schinus patagonicus, Embothrium coccineum, Maytenus boaria, Diostea juncea, Lomatia hirsuta* and *Berberis* spp.) occurring from low to high elevations.

The study area extends over ca. 2,520,000 ha including Nahuel Huapi and Lanín National Parks and the adjacent area of Epuyén (40–42° S, and the west–east belt at 71° W). Within this area, we sampled a total of 25 plant communities of different post-fire age (from <1 to 180 yr after fire) during the growing seasons of 2007–2008, 2008–2009 and 2009–2010. These communities varied greatly in plant
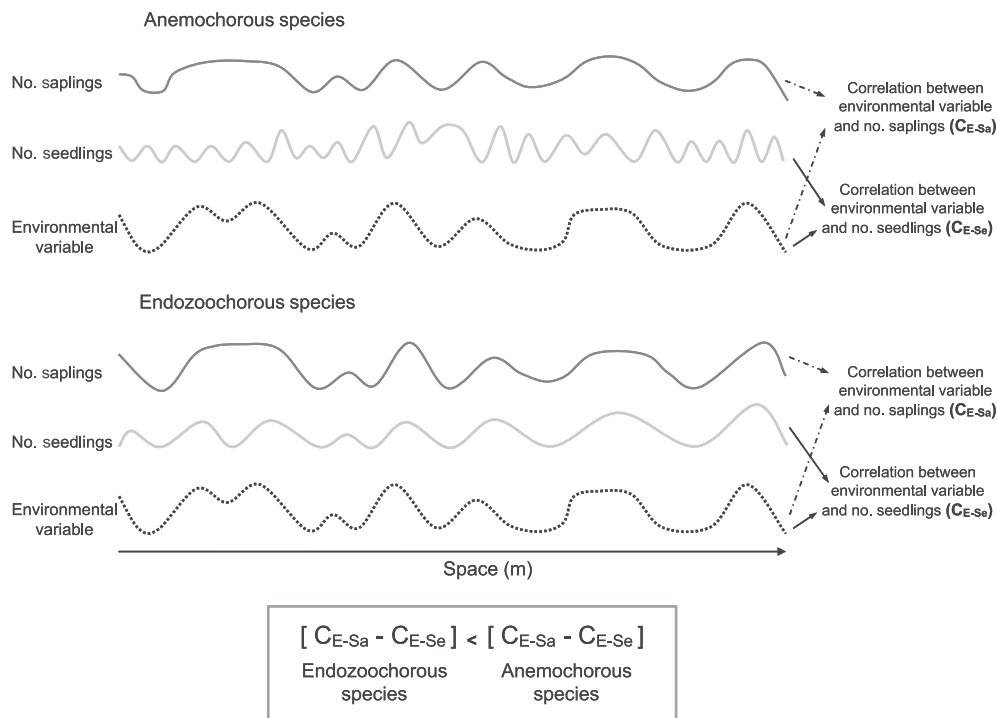

Fig. 1. Proposed hypothesis for the correlation between a given environmental variable and seedling and sapling numbers for anemochorous and endozoochorous species, and expected differences between correlation coefficients for both dispersal modes.

cover, from negligible plant cover to almost negligible bare soil, with the middle communities having plant patches immersed in a bare soil matrix and communities with bare soil patches immersed in a plant cover matrix. Sampling along this gradient of plant cover and environmental heterogeneity increases the generality of our test and conclusions.

Sampling procedure

In each sampled community we set two perpendicular transects of 300 m each, which intersected at the centre. One transect was in a N–S direction and the other in an E–W direction. Every 5 m along each transect we recorded: (1) total, direct and diffuse radiation with hemispherical photographs, taken at a height of 0.20 m using a horizontally levelled digital camera (Coolpix 990; Nikon Corp., Tokyo, Japan) and aimed at the zenith with a fisheye lens of 180° field of view (FC-E8, Nikon); (2) air temperature and relative humidity near ground level with a pocket weather station, averaged over 10 min at each point (Kestrel 3000; Nielsen-Kellerman, Boothwyn, PA, USA) and recorded over 1 d along each transect; and (3) number of seedlings (i.e. individuals recruiting from seed <10 cm, with cotyledons or cotyledon scars) of each woody species in 1-m^2 plots centred at the same points where we recorded radiation, temperature and humidity. We recorded environmental variables at 5-m intervals rather than at shorter intervals, due in part to logistic limitations and because this spacing increased independence between consecutive sampling points. We estimated (4) abundance and (5) cover of each woody species at 1-m

intervals along each transect using the point intercept method (Mueller-Dombois & Ellenberg 1974; Elzinga et al. 2001). Abundance was estimated for plants in the low (<2-m tall) and medium (>2 and <10-m) vegetation layers, whereas cover was estimated for plants in the high layer (>10-m tall) because at that height it was difficult to differentiate individual plants due to overlapping canopies. Individuals of trees and shrubs >0.5-m and <2-m high were recorded as 'saplings' (Panton 1993; Russell-Smith et al. 1993), although this category also included the reproductive individuals of some short shrub species (e.g. Berberis spp.; Table 1). Specifically, abundance was estimated by the number of individuals of each species in the low or medium layer intercepted by a pole positioned perpendicular to the transect, whereas cover (high layer) was estimated by the number or proportion of times that a given species intercepted the vertical projection of the 1-m separated sampling points. Thus, at the transect level, a species had 100% cover when it intercepted all 301 sampling points. For each plant species, abundance and point cover values (i.e. 0 or 1) were summed over consecutive 5-m length segments centred in the middle of the plots where we also recorded the environmental variables and seedling number.

We used Winscanopy^{TM} software for hemispherical image analysis, version 2003b,c (Regent Instruments Inc., Quebec, QC, Canada) to analyse the hemispherical photographs. From each photograph we obtained total site factor (TSF), direct site factor (DSF) and indirect site factor (ISF). TSF is defined as the proportion of direct (DSF) and diffuse (ISF) radiation received below the canopy expressed as a fraction of the radiation received above the canopy (Rich

Table 1. Brief description of woody species studied (species, family, growth form and dispersal mode).

Species	Family	Growth form [max. height (m)]	Dispersal mode	nT [nC]	nTp [nCp]
Austrocedrus chilensis	Cupressaceae	Tree (20)	Anemochorous	15 [8]	23 [13]
Aristotelia chilensis	Elaeocarpaceae	Tree (5)	Endozoochorous	20 [10]	26 [15]
Berberis buxifolia	Berberidaceae	Shrub (1.5)	Endozoochorous	25 [13]	32 [18]
Berberis darwinii	Berberidaceae	Shrub (1.5)	Endozoochorous	9 [5]	21 [12]
Embothrium coccineum	Proteaceae	Tree (15)	Anemochorous	4 [2]	12 [8]
Gaultheria spp.	Ericaceae	Shrub (2)	Endozoochorous	9 [5]	17 [12]
Lomatia hirsuta	Proteaceae	Tree (15)	Anemochorous	6 [5]	30 [18]
Maytenus boaria	Celastraceae	Tree (15)	Endozoochorous	33 [18]	38 [21]
Maytenus chubutensis	Celastraceae	Shrub (1)	Endozoochorous	17 [9]	25 [15]
Mutisia spp.	Asteraceae	Woody climber	Anemochorous	6 [4]	9 [6]
Nothofagus antarctica	Fagaceae	Tree (20)	Anemochorous	10 [6]	21 [14]
Nothofagus dombeyi	Fagaceae	Tree (45)	Anemochorous	22 [11]	22 [11]
Ribes cucullatum	Grossulariaceae	Shrub (1)	Endozoochorous	10 [7]	10 [7]
Ribes magellanicum	Grossulariaceae	Shrub (3)	Endozoochorous	13 [8]	27 [17]
Rosa rubiginosa	Rosaceae	Shrub (2)	Endozoochorous	12 [8]	14 [11]
Schinus patagonicus	Anacardiaceae	Small tree (8)	Endozoochorous	32 [18]	40 [22]

nT, number of transects, and nC, number of communities where we found all three life stages studied (i.e. seedlings, saplings and reproductive adults). nT$_p$, number of transects, and nC$_p$, number of communities where each species was present.

1990). Although we calculated three radiation parameters (TSF, DSF, ISF), we included only TSF in our analysis because the three parameter estimates were highly correlated (Pearson's r >0.80 for all pair-wise correlations), thus providing redundant information.

Following Aizen & Ezcurra (1998), we classified each species considering its primary dispersal mode as either endozoochorous or anemochorous (Table 1). These dispersal modes were assigned based on fruit morphology, although the putative disperser agent was corroborated from field observations and published records (Armesto et al. 1987; Sabag 1993; Amico & Aizen 2005). The studied endozoochorous species are mostly dispersed by birds (mainly by two species, *Elaenia albiceps* and *Turdus falklandii*), although some are also dispersed by other vertebrates such as foxes (e.g. *Lycalopex culpaeus* and *Pseudalopex griseus*) (Armesto et al. 1987). Of a total of 36 sampled woody species, we selected ten endozoochorous and six anemochorous species that met three criteria: (1) sampled in at least three points of each transect; (2) presence in more than two communities; and (3) occurrence of all the three life stages when sampled (seedlings, saplings and adults). Two of the 'species' were actually two genera, *Mutisia* and *Gaultheria*, which included two and three closely related species, respectively. Species within these genera exhibited the same growth form and similar patterns of association with environmental variables, and were grouped together to avoid excessive taxonomic redundancy and because species identification at the seedling stage was not always clear.

Data analysis

Because temperature and relative humidity data were recorded sequentially along each transect, we detrended these environmental variables to avoid temporal autocorrelation. Using linear and quadratic polynomial regressions, we modelled the daily behaviour of each variable in each transect at each community (because each transect was performed on a different day) and subtracted the predicted values from the fitted curve to the raw data to obtain their residuals (R Development Core Team 2006, Vienna, Austria). Residuals of the environmental variables were then used to assess the degree of plant–environment coupling. Total radiation was not detrended because, from each hemispherical photograph, Winscanopy provides a predicted daily mean by modelling radiation for the entire growing season (i.e. November–April).

For each species in each transect at each community, we correlated total radiation and detrended temperature and humidity with both seedling number and sapling abundance. We used polyserial correlations to assess the association between environmental and life-stage variables. Polyserial correlation is used to measure the association between a continuous variable (i.e. radiation, detrended temperature or relative humidity) and an ordinal variable (i.e. seedling number, sapling abundance or tree cover), which is assumed to reflect an underlying continuous variable (Mulaik 2009). Polyserial coefficients vary between −1 and +1 and are interpreted analogously to a Pearson's correlation coefficient. We considered seedling number, sapling abundance and adult cover as ordinal variables because of the many zeros and narrow value ranges. We used the Polycor package (developed by John Fox) from R statistical software to calculate maximum likelihood estimators of polyserial correlation coefficients (Olsson et al. 1982).

To assess the similarity in environmental coupling of seedlings and saplings for each species in each transect at each community, we subtracted the environment–seedling from the environment–sapling correlation coefficient. In this way, we controlled for the large environmental variability existing between the sampled communities, which can cause contrasting associations with the same environmental variable in communities of different post-fire age. For example, we observed that in recently burned communities the relationship between plant occurrence (i.e. either number, abundance or cover) and temperature tended to be negative, whereas the relationship with relative humidity tended to be positive (i.e. plants avoid stress induced by heat and drought). In contrast, in communities burned more than 40 yr ago the relationship between plant occurrence and temperature tended to be positive, whereas the relationship with relative humidity tended to be negative (i.e. plants avoid stress induced by shade and excess moisture).

To evaluate whether seedlings occur in microsites different from those already occupied by saplings, we assessed the degree of coupling between seedlings and saplings. Thus, for each species in each transect at each community, we correlated sapling abundance and seedling number, calculating the maximum likelihood estimators of polychoric correlations (Polycor package developed by John Fox in R). A polychoric correlation is used when both variables are dichotomous or ordinal, but both are assumed to reflect underlying continuous variables (Mulaik 2009). In addition, we discarded the possibility that a larger degree of environmental coupling for endozoochorous than for anemochorous species could be attributed to a higher incidence of seeds falling by gravity beneath maternal plants rather than being dispersed away from maternal plants. To assess this hypothesis, we estimated the degree of coupling between plant abundance or cover, depending on the layer in which we found the reproductive individuals of each species (i.e. the low layer for a few species of short shrubs, medium layer for tall shrubs and treelets, and medium and

high layers for trees), and seedlings. Thus, for each species in each transect at each community, we evaluated the association of reproductive adult abundance and/or cover with seedling number using polychoric correlation. Overall, a low or null association between reproductive individuals and seedlings, independent of dispersal mode, would indicate dispersal away from maternal plants.

In addition to using classic parametric *t*-tests, we compared the different correlation coefficients (the environment–sapling and environment–seedling separately, the difference between these two correlation coefficients, the sapling–seedling and the adult–seedling) between endozoochorous vs anemochorous species by means of phylogenetically independent contrasts. We first reconstructed the phylogenetic relationships among our 16 study species using the Phylomatic database and assembly tools (http://www.phylodiversity.net/phylomatic) and the template of the Angiosperm Phylogeny Group (APG) base tree (Angiosperm Phylogeny Website, http://www.mobot.org/MOBOT/research/APweb; Bremer et al. 2009). The Phylomatic program uses this skeleton tree, which is based on a large number of molecular and morphological studies, to construct a sub-tree representing the phylogenetic relations between input taxa. Second, we performed phylogenetically independent contrasts using Maddison's method implemented in the Mesquite program (http://mesquite-project.org). By controlling for phylogeny, we carried out comparisons of the different environment–plant and plant–plant correlation coefficients between dispersal modes (endozoochory vs anemochory) while avoiding the potential problem of pseudoreplication between species sharing a common ancestor. Specifically, Maddison's method identifies all possible phylogenetically independent species pairs that differ in dispersal type, making no assumptions about ancestral states, branch lengths or evolution mode (Maddison 2000).

Results

The association of seedlings and saplings with total radiation did not show a clear pattern in relation to dispersal mode (Fig. 2a, b). In contrast, both seedlings and saplings of endozoochorous species tended to show, on average, more negative associations with temperature (Fig. 2c, d) and positive associations with relative humidity (Fig. 2e, f), compared to seedlings and saplings of anemochorous species. However, mean correlation values between environmental variables and seedlings or saplings did not segregate completely according to dispersal mode, except for the humidity–sapling association with all the endozoochorous species, showing higher correlations than all the anemochorous species (Fig. 2e). In general, seedlings tended to have weaker mean correlations with

environmental variables (range = [−0.23, 0.13], [−0.28, 0.10] and [−0.13, 0.26] for total radiation, temperature and relative humidity, respectively) than saplings ([−0.39, 0.20], [−0.37, 0.14] and [−0.29, 0.39]). The large standard errors associated with some correlation estimates indicate that the degree and even the direction of the environment-seedling number or environment-sapling abundance associations can vary greatly among communities. For instance, the humidity–sapling correlations shown for *Ribes cucullatum* ranged between −0.88 and 0.91 across the sampled communities.

After accounting for variability among communities (i.e. by subtracting the environment–seedling correlation coefficient from the environment–sapling correlation coefficient of each transect within each community), we found that the degree of environmental coupling of seedlings was consistently higher for endozoochorous than for anemochorous species. Basically, the mean difference in plant–environment correlation coefficients between saplings and seedlings was <0.2 for all the endozoochorous species and >0.2 for all the anemochorous species, for all environmental variables (Fig. 3). Comparisons between dispersal modes were also statistically significant before ($P < 0.0001$ for all environmental variables) and after ($P = 0.0018$, 0.0039 and 0.0005 for total radiation, temperature and relative humidity, respectively) controlling for the phylogenetic relationships among species. Thus, seedlings and saplings of endozoochorous species tended to occur under more similar environmental conditions than seedlings and saplings of anemochorous species. An example is provided in Fig. 4, which shows seedlings and saplings of an animal-dispersed (*M. boaria*) and a wind-dispersed (*N. dombeyi*) tree along the same transect. Whereas seedlings of the former species were found preferentially in wet microsites, seedlings of the latter species occurred in both wet and dry microsites.

Moreover, the tighter association between seedlings and saplings and the environment that we found for endozoochorous than for anemochorous species was site-specific and therefore related to distinct plant recruitment requirements in different communities, as exemplified by a group of well-sampled species (Fig. 5). For instance, seedlings and saplings of endozoochorous species growing in young (<40 yr), open post-fire communities were both negatively associated with radiation, whereas in older, shadier post-fire communities they were both positively associated with radiation (Fig. 5b, d, f). In contrast, the occurrence of seedlings of anemochorous species was mostly unrelated to microsite radiation, no matter the age of the post-fire community, even though saplings of the same species showed changing associations with radiation along the post-fire age gradient (Fig. 5a, c, e). Therefore, the recruitment requirements specific to each species in each community
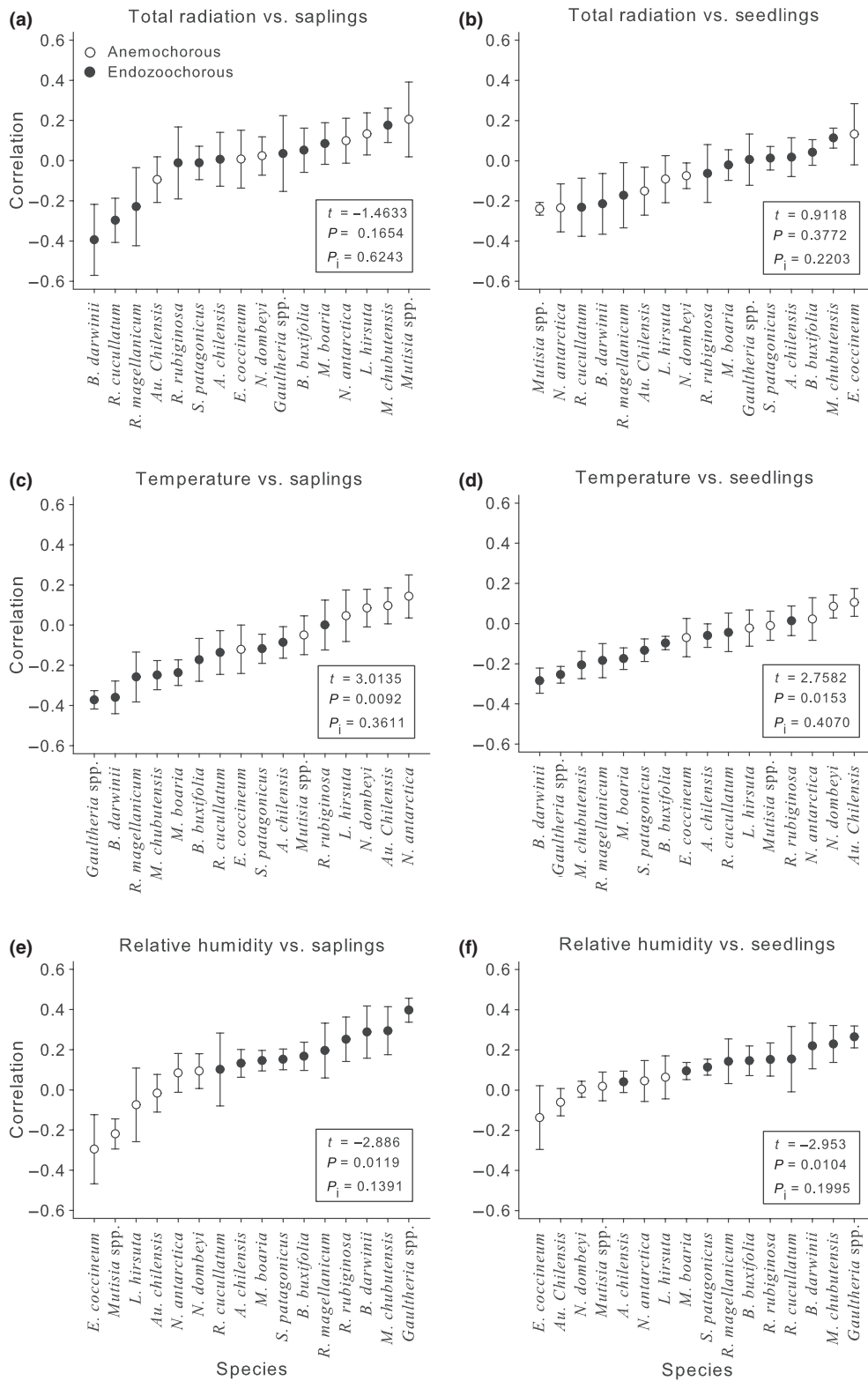
Fig. 2. Mean correlation (±SE) between (**a**) total radiation (TSF), (**c**) temperature, (**e**) and relative humidity and sapling abundance (left column); and mean correlation (±SE) between (**b**) total radiation (TSF), (**d**) temperature and (**f**) relative humidity and seedling number. p = P-value associated with a parametric t-test, and p_i = P-value associated with Maddison's phylogenetically independent contrast.
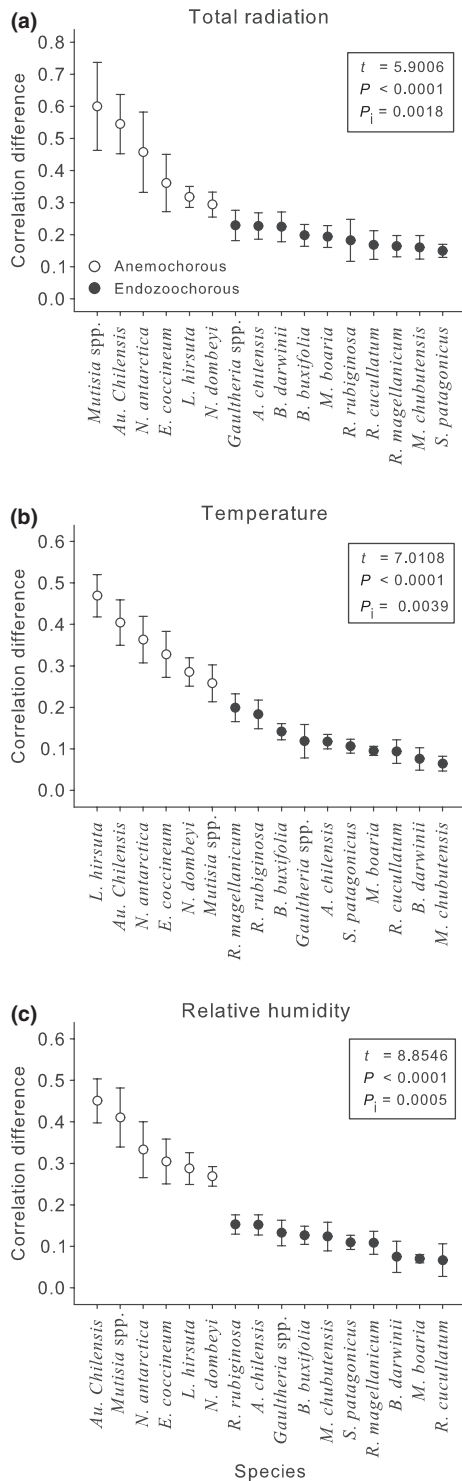
Fig. 3. Mean difference (±SE) of the (**a**) total radiation–sapling minus total radiation–seedling correlation coefficients; (**b**) temperature–sapling minus temperature–seedling correlation coefficients; and (**c**) humidity–sapling minus humidity–seedling correlation coefficients. p = P-value associated with a parametric t-test, and p_i = P-value associated with Maddison's phylogenetically independent contrast.
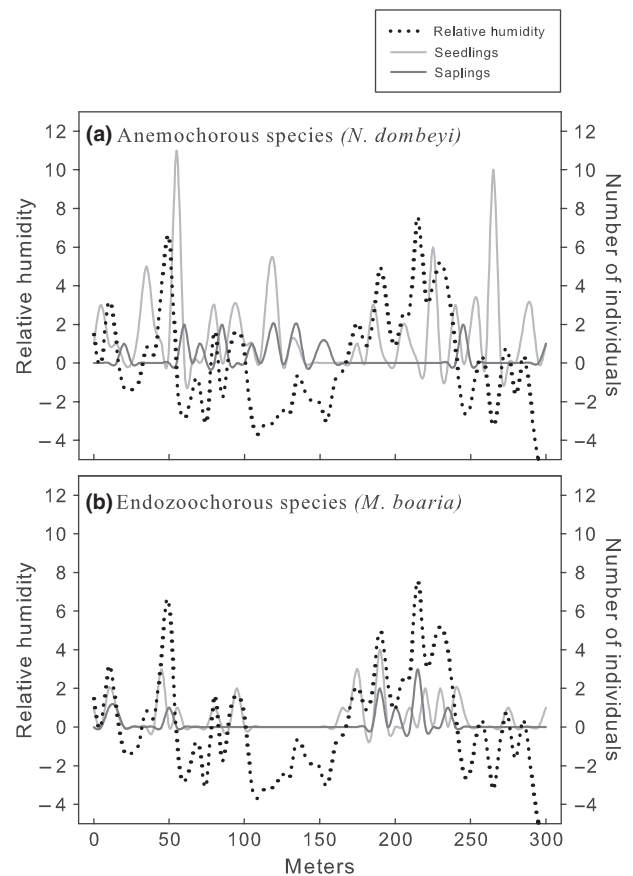

Fig. 4. Spatial variability in relative humidity, seedling number and sapling abundance for (**a**) an anemochorous tree (*Nothofagus dombeyi*) and (**b**) an endozoochorous tree (*Maytenus boaria*) growing in a 40-yr-old post-fire *N. dombeyi* and *Austrocedrus chilensis* mixed forest located near Steffen Lake, Nahuel Huapi National Park (41° 30'45" S, 71° 32'09" W). The curves represent smoothed splines fitted to the data.

were met at an earlier life stage among endozoochorous than anemochorous species.

Although seedlings and saplings of endozoochorous species tended to occur under similar environmental conditions, we did not find evidence that associations between seedlings and saplings were stronger among animal- than wind-dispersed species. In general, mean correlation coefficients between seedling number and sapling abundance were weak for most species (range = [−0.27, 0.53]), and their sign and magnitude were independent of dispersal mode (Fig. 6a). Furthermore, we did not find evidence either that seedlings of endozoochorous species tended to occur differentially underneath reproductive individuals of the same species. Mean correlation coefficients between seedling number and abundance and/or cover of reproductive adults were generally low when positive (maximum = 0.53 corresponding to *R. cucullatum*), with endozoochorous species showing no clear differential
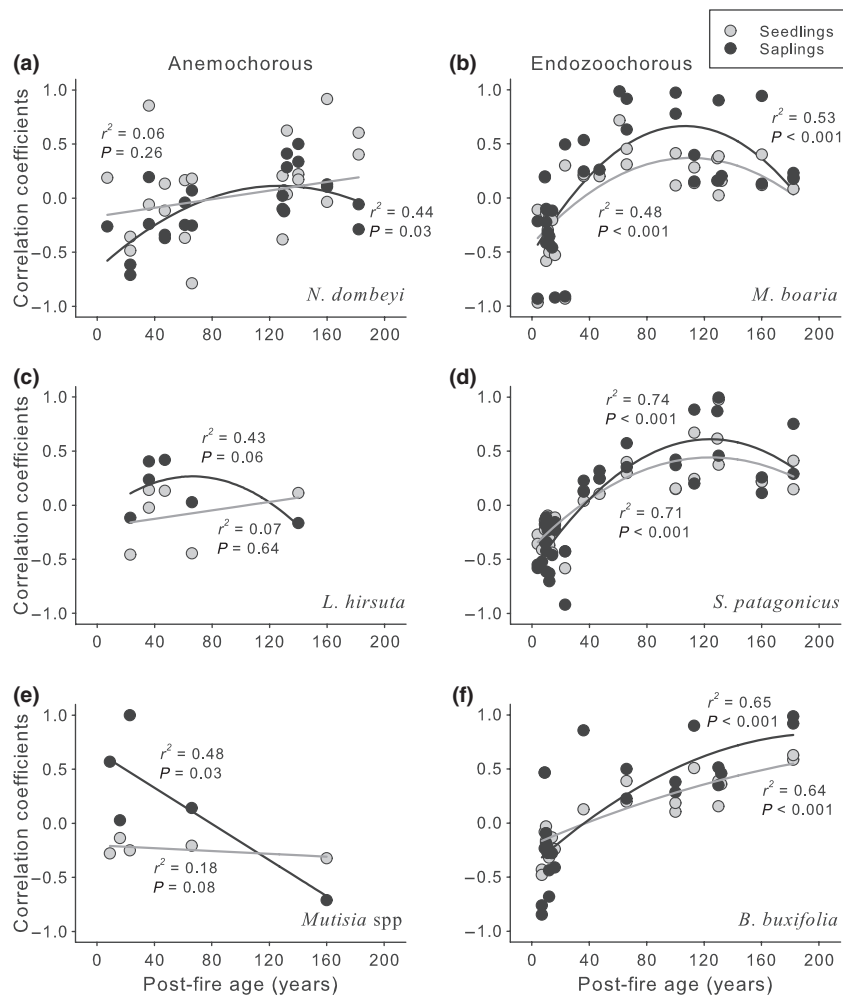
Fig. 5. Radiation–seedling and radiation–sapling polyserial correlation coefficients in relation to community post-fire age for an anemochorous (**a**) seeder tree (*Nothofagus dombeyi*), (**c**) sprouting tree (*Lomatia hirsuta*) and (**e**) woody climber (*Mutisia* spp.); and for an endozoochorous (**b**) sprouting tree (*Maytenus boaria*), (**d**) sprouting small tree (*Schinus patagonicus*) and (**f**) sprouting shrub (*Berberis buxifolia*). The curves represent linear regressions (first and second degree) fitted to the data. r^2 = adjusted coefficient of determination; and p = P-value associated with the fit of the regression model.

seedling–adult associations when compared to anemochorous species (Fig. 6b). Indeed two vertebrate-dispersed species, native *M. boaria* and exotic *Rosa rubiginosa*, provided evidence of strong spatial segregation between seedlings and reproductive individuals (correlations ~−0.9).

Discussion

After passing through the seed dispersal sieve, many seeds and seedlings die due to different post-dispersal abiotic and biotic factors (Harper 1977; Schupp 1995). However, we found that seedlings of endozoochorous species showed a tighter degree of coupling with expectedly favourable micro-environmental conditions that could increase plant survivorship to the sapling stage than seedlings of anemochorous species. Thus, our community-wide survey sup-

ports the view that endozoochory increases the likelihood of seeds being dispersed to more suitable microsites, compared to anemochory (Alcántara et al. 2000; Jordano & Schupp 2000; Nathan & Muller-Landau 2000; Wenny 2001; Herrera 2002; Jordano et al. 2004).

Positive and negative values of environment–seedling and environment–sapling correlation coefficients are the product of the interaction between species-specific habitat requirements and changes in the environmental factors that are most limiting to plant recruitment in each community. In relatively young post-fire communities (<40 yr), we found that species tended to exhibit negative associations with radiation and temperature and positive associations with moisture, recruiting mostly within sparsely vegetated patches immersed in a bare soil matrix. In these shrublands, plant recruitment in open patches is
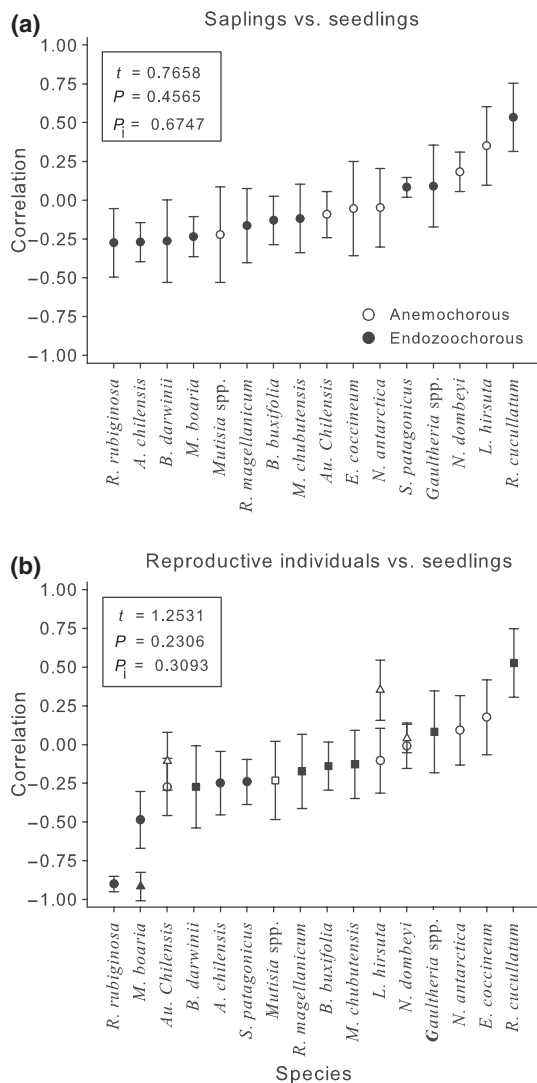
(a) Saplings vs. seedlings

t = 0.7658
P = 0.4565
P_i = 0.6747

○ Anemochorous
● Endozoochorous

(b) Reproductive individuals vs. seedlings

t = 1.2531
P = 0.2306
P_i = 0.3093

Species

Fig. 6. Mean correlation (±SE) between (**a**) sapling abundance and seedling number, and (**b**) abundance/cover of reproductive individuals and seedling number. In (**b**), for comparative tests, we considered the average of the two correlations in the case of species for which reproductive individuals occurred in two of the sampled layers (e.g. *Maytenus boaria* and *Austrocedrus chilensis*). p = P-value associated with a parametric t-test, and p_i = P-value associated with Maddison's phylogenetically independent contrast. In (b) reproductive individuals of the low layer are represented by squares, of the medium layer by circles and of the high layer by triangles.

mainly limited by drought stress (Kitzberger et al. 2000). On the other hand, in old post-fire communities (>40 yr) species showed positive associations with radiation and temperature and negative associations with humidity, recruiting mainly within gaps immersed in a vegetation matrix. In these more forested shady sites, plant recruitment is limited by scarce radiation rather than water availability. Consequently, young individuals of many plant

species are found generally in insolated gaps characterized by relatively high radiation and temperature and low humidity (Kitzberger et al. 2000; Veblen et al. 2003).

Within a species, variation around mean correlations can similarly relate to the fact that environment–plant associations also change across communities according to which factor becomes most limiting (Fig. 5). For instance, recruitment of the native conifer *Au. chilensis* is facilitated by nurse shrubs in communities located towards the eastern forest–steppe ecotone, where water availability greatly limits early survivorship and plant growth, but is restricted to forest gaps in wetter communities where light becomes the most limiting factor (Gobbi & Schlichter 1998; Veblen et al. 2003). *Ribes cucullatum* also provides an example of a ubiquitous species that shows contrasting requirements for establishment, reflected here by the huge variability in the association between relative humidity and sapling abundance, recruiting within vegetated patches in recently burned communities and in open gaps in late-successional communities. Therefore, our results imply that the range of environmental conditions that characterize a suitable microsite is not only species-specific but also context-dependent (Schupp 2007), and suggest that biotic dispersers would be efficient in transporting seeds to different habitat-specific microsites that could increase survivorship to the sapling stage (Fig. 5b, d, f). Interestingly, the behavioural patterns of the most important bird dispersers from the TFSA seem to fit the changing establishment requirements of the studied endozoochorous species in different habitats, perching on sparsely vegetated patches in recently burned communities (Cavallero et al. 2010), trees on forest edges (Armesto et al. 2001), or snapped trunks or branches in tree-fall gaps (Armesto et al. 2001). Therefore, in our study system, endozoochory seems to be a more directed dispersal mode than anemochory, capable of matching microsite abiotic characteristics with the context-dependent requirements for plant recruitment.

Whereas seedlings and saplings of endozoochorous species tended to occur in microsites characterized by similar environmental characteristics, there was no evidence that seedlings and saplings tended to co-occur in the same physical microsites beyond random expectations (Fig. 6a). We found a similar pattern for anemochorous species (Fig. 6). The lack of same microsite incidence also occurred for the relation between seedlings and reproductive individuals (see also Hampe et al. 2008), with two animal-dispersed species, *M. boaria* and *R. rubiginosa* (Fig. 6b) even providing evidence of spatial segregation between these two different life stages. Most importantly, we did not find any significant difference in the average correlation coefficient of seedlings vs reproductive individuals between anemochorous and endozoochorous species, which could indicate a differential incidence of seeds

falling by gravity between the two dispersal modes. In addition, this lack of a differential association suggests that both dispersal modes could contribute to decrease the high density-dependent mortality caused by sibling competition and seed predation near the maternal plant (Janzen 1970; Connell 1971; Wang & Smith 2002).

Weak positive and, particularly, strong negative correlations between young and older life stages can also be produced by post-dispersal biotic filters such as predation, pathogen attack, allelopathy and competition (Janzen 1970; Connell 1971) that could filtered out many seeds and seedlings. After all, we studied 'realized dispersal' by focusing on seedlings that are already filtered individuals, and inferred their fate in those microsites basically by using saplings as 'microsite quality indicators' (Schupp 1995, 2007). Therefore, we acknowledge that our inferences are valid to the extent that seedling distribution reflects the spatial template established by seed dispersal. Indeed, several studies have reported that the spatial pattern of seedling distribution is mainly determined by the spatial pattern of seed distribution generated by the disperser agent, independently of other post-dispersal filters operating between these two life stages (Houle 1995; Jordano & Herrera 1995; Garcia et al. 2005; Hampe et al. 2008; L. Cavallero unpub. data). Moreover, several authors suggest that abiotic filters are more important than biotic filters in determining species recruitment in temperate ecosystems (Schemske 2002, 2009; Schemske et al. 2009). This, coupled with the opposing trends that we found for ten endozoochorous and six anemochorous species studied from a community perspective, suggests that the differences we found should reflect an important inertia generated by the primary dispersal template.

Seed dispersal mode has been associated with growth form, most notably is the high incidence of endozoochory among shrubs (Willson 1991; Herrera 2002). In fact, all the five shrub species included in our sample were endozoochorous (Table 1). Additionally, the other five animal-dispersed taxa were treelets, including a relatively large tree (*M. boaria*) producing arillate seeds. These tree species exhibited the same high degree of environmental coupling since the seedling stage shown by the other endozoochorous species. Also, the high degree of coupling of endozoochorous trees contrasted with the much lower coupling shown by anemochorous trees (Fig. 3). Therefore, the contrasting pattern of environmental coupling we found in relation to dispersal mode is not confounded by differences in life form. Finally, the large variation in the environmental conditions of the communities we surveyed and the relatively high taxonomic diversity represented in our sample of 16 woody species increase the generality of our main conclusion, despite our focus on vegetation of a single biome.

As sessile organisms, plants must invest resources in structures that enhance seed dispersal (e.g. wing- or feather-like structures for wind dispersal, and fleshy and nutritious pulp for animal dispersal). In general, plant investment in food for animal dispersers is considered more energetically costly than non-rewarding ancillary structures that facilitate seed transport by wind or other means (Bawa 1980; De Jong & Klinkhamer 2005). However, the many instances of independent evolution of endozoochory in different extant and extinct lineages of angiosperms and gymnosperms, and the variety of tissues from which nutritious portions have derived indicate that the benefit of relying on animal dispersers has often exceeded the cost of producing fleshy fruits or arillate seeds (Herrera 2002). Because endozoochory increases dispersal distance to parent plant compared to other seed dispersal modes (Clark et al. 2005; Seidler & Plotkin 2006; Jordano et al. 2007), it has been widely documented that this dispersal mode benefits plants by (1) reducing early seed and seedling mortality near the mother plant caused by density-dependent factors (i.e. escape hypothesis) (Clark & Clark 1984; Howe et al. 1985; Martínez et al. 2008); and (2) facilitating the arrival to new areas for seedling establishment (i.e. colonization hypothesis) (Brokaw 1985; Herrera et al. 1994; Van der Meer & Bongers 1996; Ortiz-Pulido & Rico-Gray 2006). The tighter coupling between seedlings and environmental factors persisting to sapling stage associated with endozoochory reported here provides support for a third proposed (Howe & Smallwood 1982; Wenny 2001), but less evaluated, benefit of relying on animals for seed dispersal, which is the transport of seeds to microsites characterized by a narrow range of environmental conditions that could favour plant establishment.

Acknowledgements

References

Aizen, M.A. & Ezcurra, C. 1998. High incidence of plant–animal mutualisms in the woody flora of the temperate forest of southern South America: biogeographical origin and present ecological significance. *Ecologia Austral* 8: 217–236.

Aizen, M.A., Vásquez, D.P. & Smith-Ramirez, C. 2002. Historia natural y conservación de los mutualismos planta-animal del bosque templado de Sudamérica austral. *Revista Chilena de Historia Natural* 75: 79–97.

Alcántara, J.M., Rey, P.J., Valera, F. & Sanchez-Lafuente, A.M. 2000. Factors shaping the seedfall pattern of a bird-dispersed plant. *Ecology* 81: 1937–1950.

Amico, G.C. & Aizen, M.A. 2005. Dispersión de semillas por aves en un bosque templado de Sudamérica austral: quién dispersa a quién? *Ecologia Austral* 15: 89–100.

Armesto, J.J., Rozzi, R., Miranda, P. & Sabag, C. 1987. Plant/frugivore interactions in South American temperate forests. *Revista Chilena de Historia Natural* 60: 321–336.

Armesto, J.J., Smith-Ramirez, C. & Sabag, C. 1996. The importance of plant–bird mutualisms in the temperate rainforest of southern South America. In: Lawford, R.G., Alaback, P.B. & Fuentes, E. (eds.) *High latitude rainforests and associated ecosystems of the west coast of the Americas*. pp. 248–265. Springer, New York, NY.

Armesto, J.J., Díaz, I., Papic, C. & Willson, M.F. 2001. Seed rain of fleshy and dry propagules in different habitats in the temperate rainforests of Chiloé Island, Chile. *Austral Ecology* 26: 311–320.

Augspurger, C.K. & Franson, S.E. 1987. Wind dispersal of artificial fruits varying in mass, area and morphology. *Ecology* 68: 27–42.

Bawa, K.S. 1980. Evolution of dioecy in flowering plants. *Annual Review of Ecology and Systematics* 11: 15–39.

Baythavong, B.S., Stanton, M.L. & Rice, K.J. 2009. Understanding the consequences of seed dispersal in a heterogeneous environment. *Ecology* 90: 2118–2128.

Bazzaz, F.A. 1991. Habitat selection in plants. *American Naturalist* 137: 116–130.

Bremer, B., Bremer, K., Chase, M., Fay, M., Reveal, J., Soltis, D., Soltis, P. & Stevens, P. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* 161: 105–121.

Brokaw, N.V.L. 1985. Gap-phase regeneration in a tropical forest. *Ecology* 66: 682–687.

Carlo, T.A. & Morales, J.M. 2008. Inequalities in fruit removal and seed dispersal: consequences of bird behaviour, neighbourhood density and landscape aggregation. *Journal of Ecology* 96: 609–618.

Cavallero, L., Raffaele, E. & Aizen, M.A. 2010. Birds as mediators of ecological restoration in woodlands of Northwestern Patagonia, Argentina. *VI Southern connection congress*. pp. 86–87. Universidad Nacional del Comahue, Bariloche, AR.

Clark, D.A. & Clark, D.B. 1984. Spacing dynamics of a tropical rain forest tree: evaluation of the Janzen–Connell model. *American Naturalist* 124: 769–788.

Clark, C.J., Poulsen, J.R., Bolker, B.M., Connor, E.F. & Parker, V.T. 2005. Comparative seed shadows of bird-, monkey-, and wind-dispersed trees. *Ecology* 86: 2684–2694.

Connell, J.H. 1971. On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In: Den Boer, P.J. & Gradwell, G. (eds.) *Dynamics of populations*. pp. 298–312. PUDOC, Wageningen, NL.

Correa, M.N. 1969-1997. *Flora Patagónica Parte I–V*. Colección Científica del INTA, Buenos Aires, AR.

De Jong, T.J. & Klinkhamer, P.G.L. 2005. *Evolutionary ecology of plant reproductive strategies*. Cambridge University Press, Cambridge, UK.

Elzinga, C.L., Salzer, D.W., Willoughby, J.W. & Gibbs, J. 2001. *Monitoring plant and animal populations*. Blackwell, Massachusetts, USA.

Garcia, D., Obeso, J.R. & Martinez, I. 2005. Spatial concordance between seed rain and seedling establishment in bird dispersed trees: does scale matter? *Journal of Ecology* 93: 693–704.

Gobbi, M. & Schlichter, T. 1998. Survival of *Austrocedrus chilensis* seedlings in relation to microsite conditions and forest thinning. *Forest Ecology and Management* 111: 137–146.

Gómez-Aparicio, L., Valladares, F., Zamora, R. & Quero, J.L. 2005. Response of tree seedlings to the abiotic heterogeneity generated by nurse shrubs: an experimental approach at different scales. *Ecography* 28: 757–768.

Hampe, A., García Castaño, J.L., Schupp, E.W. & Jordano, P. 2008. Spatio-temporal dynamics and local hotspots of initial recruitment in vertebrate-dispersed trees. *Journal of Ecology* 96: 668–678.

Harper, J.L. 1977. *Population biology of plants*. Academic Press London, UK.

Herrera, C.M. 2002. Seed dispersal by vertebrates. In: Herrera, C.M. & Pellmyr, O. (eds.) *Plant–animal interactions: an evolutionary approach*. pp. 705–727. Blackwell Science, Oxford, UK.

Herrera, C.M., Jordano, P., López-Soria, L. & Amat, J.A. 1994. Recruitment of a mast-fruiting, bird-dispersed tree: bridging frugivore activity and seedling establishment. *Ecological Monographs* 64: 315–344.

Houle, G. 1995. Seed dispersal and seedling recruitment: the missing link(s). *Ecoscience* 2: 238–244.

Howe, H.F. & Smallwood, J. 1982. Ecology of seed dispersal. *Annual Review of Ecology and Systematics* 13: 201–228.

Howe, H.F., Schupp, E.W. & Westley, L.C. 1985. Early consequences of seed dispersal for a neotropical tree (*Virola surinamensis*). *Ecology* 66: 781–791.

Janzen, D.H. 1970. Herbivores and the number of tree species in tropical forests. *American Naturalist* 104: 501–529.

Jordano, P. & Herrera, C.M. 1995. Shuffling the offspring: uncoupling and spatial discordance of multiple stages in vertebrate seed dispersal. *Ecoscience* 2: 230–237.

Jordano, P. & Schupp, E.W. 2000. Seed disperser effectiveness: the quantity component and patterns of seed rain for *Prunus mahaleb*. *Ecological Monographs* 70: 591–615.

Jordano, P., Pulido, F., Arroyo, J., García-Castaño, J.L. & García-Fayos, P. 2004. Procesos de limitación demográfica. In: Valladares, F. (ed.) *Ecología del bosque mediterráneo en un mundo cambiante*. pp. 229–248. Ministerio de Medio Ambiente, EGRAF, Madrid, ES.

Jordano, P., García, C., Godoy, J.A. & García-Castaño, J.L. 2007. Differential contribution of frugivores to complex seed dispersal patterns. *Proceedings of the National Academy of Sciences USA* 104: 3278–3282.

Kitzberger, T., Steinaker, D.F. & Veblen, T.T. 2000. Effects of climatic variability on facilitation of tree establishment in northern Patagonia. *Ecology* 81: 1914–1924.

Leathwick, J.R. & Whitehead, D. 2001. Soil and atmospheric water deficits and the distribution of New Zealand's indigenous tree species. *Functional Ecology* 15: 233–242.

Maddison, W.P. 2000. Testing character correlation using pairwise comparisons on a phylogeny. *Journal of Theoretical Biology* 202: 195–204.

Martínez, I., García, D. & Obeso, J.R. 2008. Differential seed dispersal patterns generated by a common assemblage of vertebrate frugivores in three fleshy-fruited trees. *Ecoscience* 15: 189–199.

Mueller-Dombois, D. & Ellenberg, H. 1974. *Aims and methods of vegetation ecology*. John Wiley & Sons, London, UK.

Mulaik, S.A. 2009. Polychoric correlation and polyserial correlation. In: Mulaik, S. A. (ed.) *Linear causal modeling with structural equations*. pp. 395–414. Chapman and Hall, London, UK.

Nathan, R. & Muller-Landau, H.C. 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. *Trends in Ecology and Evolution* 15: 278–285.

Olsson, U., Drasgow, F. & Dorans, N.J. 1982. The polyserial correlation coefficient. *Psychometrika* 47: 337–347.

Ortiz-Pulido, R. & Rico-Gray, V. 2006. Seed dispersal of *Bursera fagaroides* (Burseraceae): the effect of linking environmental factors. *Southwestern Naturalist* 51: 11–21.

Panton, W.J. 1993. Factors that control monsoon rainforest seedling establishment and growth in north Australian Eucalyptus savanna. *Journal of Ecology* 81: 297–304.

Purves, D.W., Zavala, M.A., Ogle, K., Prieto, F. & Benayas, J.M.R. 2007. Environmental heterogeneity, bird-mediated directed dispersal, and oak woodland dynamics in Mediterranean Spain. *Ecological Monographs* 77: 77–97.

Rich, P.M. 1990. Characterizing plant canopies with hemispherical photographs. *Remote Sensing Reviews* 5: 13–29.

Russell-Smith, J., Lucas, D.E., Brock, J. & Bowman, D. 1993. Allosyncarpia-dominated rain forest in monsoonal northern Australia. *Journal of Vegetation Science* 4: 67–82.

Sabag, C. 1993. El rol de las aves en la dispersión de semillas en un bosque templado secundario de Chiloé (42 S). Master thesis, Facultad de Ciencias, Universidad de Chile, CL.

Schemske, D.W. 2002. Ecological and evolutionary perspectives on the origins of tropical diversity. In: Chazdon, R.L. & Whitmore, T.C. (eds.) *Foundations of tropical forest biology: classic papers with commentaries*. pp. 245–269. The University of Chicago Press, Chicago, IL.

Schemske, D.W. 2009. Biotic interactions and speciation in the tropics. In: Butlin, R.K., Bridle, J.R. & Schluter, D. (eds.) *Speciation and patterns of diversity*. pp. 219–239. Cambridge University Press, Cambridge, UK.

Schemske, D.W., Mittelbach, G.G., Cornell, H.V., Sobel, J.M. & Kaustuv, R. 2009. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution and Systematics* 40: 245–269.

Schupp, E.W. 1995. Seed–seedling conflicts, habitat choice, and patterns of plant recruitment. *American Journal of Botany* 82: 399–409.

Schupp, E.W. 2007. The suitability of a site for seed dispersal is context-dependent. In: Dennis, A.J., Schupp, E.W., Green, R.J. & Westcott, D.A. (eds.) *Seed dispersal: theory and its application in a changing world*. pp. 445–462. CAB International, Wallingford, UK.

Schupp, E.W., Milleron, T. & Russo, S.E. 2002. Dissemination limitation and the origin and maintenance of species-rich tropical forests. In: Levey, D.J., Silva, W.R. & Galetti, M. (eds.) *Seed dispersal and frugivory: ecology, evolution, and conservation*. pp. 19–33. CAB International, Wallingford, UK.

Schupp, E.W., Jordano, P. & Gómez, J.M. 2010. Seed dispersal effectiveness revisited: a conceptual review. *New Phytologist* 188: 333–353.

Seidler, T.G. & Plotkin, J.B. 2006. Seed dispersal and spatial pattern in tropical trees. *PLOS Biology* 4: 2132–2137.

Temperton, V.M., Hobbs, R.J., Nuttle, T. & Halle, S. 2004. *Assembly rules and restoration ecology: bridging the gap between theory and practice*. Island Press Washington, DC, US.

Van der Meer, P.J. & Bongers, F. 1996. Patterns of tree-fall and branch-fall in a tropical forest in French Guiana. *Journal of Ecology* 84: 19–29.

Veblen, T., Kitzberger, T., Raffaele, E. & Lorenz, D. 2003. Fire history and vegetation changes in northern Patagonia, Argentina. In: Veblen, T.T., Baker, W.L., Montenegro, G. & Swetnam, T.W. (eds.) *Fire and climatic change in temperate ecosystems of the western Americas*. pp. 265–295. Springer, New York, NY.

Wang, B.C. & Smith, T.B. 2002. Closing the seed dispersal loop. *Trends in Ecology and Evolution* 17: 379–385.

Wenny, D.G. 2000. Seed dispersal, seed predation, and seedling recruitment of *Ocotea endresiana* (Lauraceae) in Costa Rica. *Ecological Monographs* 70: 331–351.

Wenny, D.G. 2001. Advantages of seed dispersal: a re-evaluation of directed dispersal. *Evolutionary Ecology Research* 3: 51–74.

Willson, M.F. 1991. Dispersal of seeds by frugivorous animals in temperate forests. *Revista Chilena de Historia Natural* 64: 537–554.