

# Decomposing recruitment limitation for an avian-dispersed rain forest tree in an anciently fragmented landscape

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## Summary

**1.** Forest fragmentation may intensify recruitment limitation through impacts on seed production, dissemination and seedling establishment processes. In an ancient mosaic of fog-dependent rain forest patches on coastal hilltops of semi-arid Chile (30 °S), we investigated how aridity-driven habitat fragmentation affected the recruitment of the fleshy-fruited, dioecious tree *Aextoxicon punctatum* (Aextoxicaceae) and the role of birds in seed dispersal across patches.

**2.** We hypothesized that reduced fragment size enhanced recruitment limitation due to constraints on tree fecundity, avian disperser activity and seedling recruitment, caused primarily by strong edge effects in small fragments.

**3.** We mapped adult *Aextoxicon* trees and placed 686 seed traps distributed in 3 small (<0.3 ha) and 3 large patches (> 0.9 ha) in the forest patch mosaic. To identify seed sources, endocarps of 282 dispersed seeds from seed traps were genotyped and compared with 155 genotyped females using nine microsatellite markers. Seed dispersal curves for different forest patches were constructed using maximum likelihood methods. Further, seed addition experiments were conducted in the six fragments to assess recruitment probabilities.

**4.** Lower density and basal areas of reproductive female trees, in addition to shorter fruiting periods and lower seed yields, led to strong source limitation in small patches. Three bird species accounted for the bulk of visits to fruiting trees, but were less active in smaller patches. Dispersal curves were strongly leptokurtic with maximum dispersal distances related to the shape of patches. Important proportions (5–40%) of genotyped seeds in all patches were immigrants. However, seeds arrived mainly at patch edges or below reproductive trees where germination and survival are reduced. Fewer seedlings originated from experimentally added seeds in small patches subjected to greater edge effects.

**5. Synthesis.** In summary, we provide evidence for source, dispersal and establishment limitation of this dioecious tree in an aridity-driven fragmented landscape. Small fragment size and edge effects had negative impacts on fecundity and seedling establishment. Although bird-mediated seed dispersal favoured immigration between patches, recruitment from such seeds will be unlikely because of low quality of deposition sites.

**Key-words:** crop size, dispersal curve, dispersal limitation, endocarp, forest fragmentation, habitat fragmentation, microsatellite markers, relict forest, seed dispersal

## Introduction

Land use changes, and associated habitat loss and fragmentation, are major threats to biodiversity world-wide (Sala *et al.*

2000) and are a major component of human-driven global changes (e.g. Vitousek *et al.* 1997). Fragmentation impacts range from negative effects on genetic diversity and populations persistence (e.g. Young, Boyle & Brown 1996; Fahrig 2003; Lowe *et al.* 2005) to the disruption of key ecosystems functions and services (e.g. Debinsky & Holt 2000; Kremen

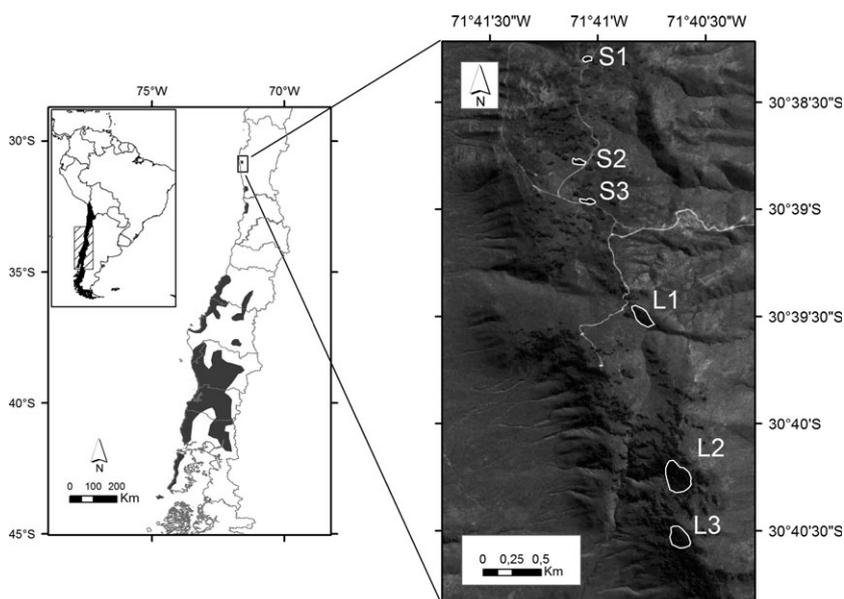
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*et al.* 2007). The impact of fragmentation upon plant species is known to vary across temporal and spatial scales (McEuen & Curran 2004; Vellend *et al.* 2006) and is usually associated with increased recruitment limitation (e.g. Laurance *et al.* 1998a,b; Bruna 2003) through any or all of the following three mechanisms: (i) *Source limitation*, defined as low population-level seed availability (Schupp, Milleron & Russo 2002; Clark *et al.* 2007), might be caused by high mortality of fruiting trees (e.g. Esseen 1994), low per capita fecundity or high fecundity variance within patch in a fragmented landscape (Clark, Macklin & Wood 1998); (ii) *Dissemination limitation*, the failure of the seeds to arrive at all potential recruitment sites (Schupp, Milleron & Russo 2002; Clark *et al.* 2007), might be caused by changes in composition and behaviour of seed dispersers in a fragmented landscape (e.g. Aizen & Feinsinger 1994; Cordeiro & Howe 2003); (iii) *Establishment limitation* occurs when plant population size is constrained by the number and quality of available sites for establishment (Clark, Macklin & Wood 1998; Nathan & Muller-Landau 2000), intensified in forest fragments by unfavourable biotic and abiotic edge effects (Benitez-Malvido 1998; Uriarte *et al.* 2010).

Most studies of recruitment limitation in patchy landscapes have been conducted in forests recently fragmented by humans, especially in the tropics (e.g. Laurance *et al.* 2011), which are probably still in a transient phase or lagged response to fragmentation or extinction debt (Tilman *et al.* 1994; Vellend *et al.* 2006; Vranckx *et al.* 2012). In contrast, this work provides us with the opportunity to evaluate the causes and consequences of recruitment limitation in an ancient and naturally fragmented mosaic of rain forest patches found on coastal hills of Fray Jorge National Park (FJNP hereafter) in semi-arid Chile (30°S). Patches are remnants of an ancient, more extensive forested landscape, gradually segregated from its main range by pronounced aridization in north-central Chile starting in the Pliocene period and continuing today (Troncoso, Villagrán

& Muñoz 1980). Forest patches are predominantly small (< 0.4ha) but have a broad range of sizes (0.01–36 ha). They are dominated by the dioecious fleshy-fruited tree *Aextoxicon punctatum* (Aextoxicaceae) and maintained by maritime fog inundating coastal mountaintops (Fig. 1). High level of genetic differentiation between Fray Jorge and southern temperate *A. punctatum* populations suggests a long period of geographical isolation and aridity-driven fragmentation (Núñez-Ávila & Armesto 2006). In addition to understand the effect of ancient fragmentation on recruitment limitation, this scenario provides a superb natural setting to assess the role of seed dispersal mediated by birds on tree recruitment in a naturally and isolated fragmented landscape, where propagule rain and rescue effects (e.g. Brown & Kodric-Brown 1977; Gotelli 1991) from nearby non-fragmented forest have been minimal or absent for many generations.

In this work, we couple observational, molecular and experimental approaches to assess the effect of long-term fragmentation on the three components of recruitment limitation for the rain forest tree *A. punctatum*. Specifically, we ask (i) How does source limitation affect seedling recruitment and how does it vary with forest patch size? We hypothesized that reduced recruitment may be due to lower fruit production reflecting a lower number of reproductive female trees and low per capita fecundity in small fragments. We tested this hypothesis by quantifying the number of fruit-producing trees and estimating their fecundities in forest patches of different sizes. (ii) How does dissemination limitation vary with forest fragment size? We hypothesized that lower avian visitation and altered composition of bird assemblages in small fragments of forest habitat should lead to stronger seed dispersal limitation. To test this hypothesis, we quantified fruit visitation by birds to fruiting trees and estimated their contribution to the movement of seeds within and between patches using molecular tools. (iii) What is the role of establishment limitation for seedling recruitment of *A. punctatum* in different forest fragments? We hypothesized



**Fig. 1.** Topographic map of the study area showing the location of forest patches (S1-S2 and L1-L3) on coastal mountaintops. Map on the left shows the distribution forests with *Aextoxicon punctatum* in Chile (shaded).

that environmental stress associated with edge effects will constrain seedling recruitment in small fragments. To test this hypothesis, we conducted seed addition experiments in forest patches and assessed seedling emergence.

To our knowledge, this is the first study that explicitly examines recruitment limitation of a dioecious tree in an ancient and naturally fragmented rain forest landscape and compares seed dispersal curves for the same species among patches of different size.

## Materials and methods

### STUDY SITE AND SPECIES

A mosaic of rain forest patches derived from aridization processes are found between 450 and 650 m on coastal mountaintops at FJNP in semi-arid Chile (30 °S), surrounded by dry shrublands (Del Val *et al.* 2006; Gutierrez *et al.* 2008). These patches represent the northernmost extension of South American temperate forests in an area receiving only 147 mm of mean annual rainfall but subsisting on maritime fog inputs (Barbosa *et al.* 2010). According to Necochea (2008), the landscape at FJNP is composed of 470 forest patches ranging in sizes from 0.01 to 32 hectares, with a mean patch size of only 0.36 ha and a median of 0.04 ha, distributed along 9.7 km from north to south in a narrow band parallel to the ocean (Fig. 1).

Forest patches are dominated by the evergreen-broadleaved, dioecious tree *Aextoxicon punctatum* Ruiz et Pavón (1798), which accounts for 80% of the basal area in most forest patches (Del Val *et al.* 2006). The canopy of *A. punctatum* is responsible for intercepting the continuous flow of maritime fog (Barbosa *et al.* 2010), which sustains a rich associated biological community, including other tree

species, vines, epiphytes and cryptogams, with strong floristic affinity with temperate rain forests located 1000 km to the south (Troncoso, Villagrán & Muñoz 1980).

*Aextoxicon punctatum* is a fleshy-fruited tree, characterized as shade tolerant, but often rising to heights of 25 m or more in the forest canopy. In large patches of FJNP, an average density of 550 individuals  $\geq 5$  cm of diameter at breast height (dbh) per hectare has been documented (Del Val *et al.* 2006). No studies are available on *A. punctatum* fruit production in FJNP.

### FIELD DATA

#### Permanent plot sampling

For the purpose of this study, we distinguished two groups of patches based on pronounced differences in patch area, perimeter/area ratio and location within the FJNP (Table 1, Fig. 1). A grid of  $5 \times 5$  m cells was constructed in 3 small patches found on the flat terrace (S1, S2, S3), within an area varying from 0.14 to 0.28 ha (Table 1) and in 3 larger patches found on the hillsides (L1, L2, L3) separated by nearly 5 km from the small patches, within an area varying from 0.9 to 2.4 ha (Fig. 1, Table 1 and Appendix S1).

All *A. punctatum* trees  $\geq 5$  cm dbh (diameter at breast height, 1.3 m above-ground) within the plots were marked using numbered metal tags and their dbh measured. The position of each tree was mapped within each  $5 \times 5$  m grid cell by measuring its  $x$  and  $y$  coordinates in metres.

#### Source limitation

We assessed source limitation in each patch estimating tree densities, basal areas and fecundities for all reproductive female trees. Trees

**Table 1.** Forest patch characterization. Values in parentheses are the relative proportions of female densities and basal areas, with respect to the total number of trees

Patch ID	S1	S2	S3	L1	L2	L3
Area sampled (ha)	0.14	0.28	0.21	0.9	2.4	0.97
P/A ratio (m/ha)*	n.d.	862	1322	469	299	300
Altitude (m)	613	566	529	495	635	639
Slope (%)	15	11	1	35	42	38
Soil water content (gH <sub>2</sub> O per g soil)*	n.d.	0.30 $\pm$ 0.19	0.28 $\pm$ 0.14	0.55 $\pm$ 0.63	1.17 $\pm$ 1.03	0.84 $\pm$ 0.37
No. of all <i>Aextoxicon</i> trees	95	49	52	298	365	432
No. of females trees	16 (17)	13 (27)	12 (23)	64 (21)	41 (11)	24 (5)
No. of reproductive females trees	7 (7)	1 (2)	3 (6)	29 (10)	34 (9)	18 (4)
Tree basal areas (m <sup>2</sup> /ha)	36.9	19.6	26.4	48.4	29.9	44.2
Female basal areas (m <sup>2</sup> /ha)	6.7 (18)	2.9 (15)	6.5 (25)	14.8 (31)	6.6 (22)	7.5 (17)
Reproductive female basal areas (m <sup>2</sup> /ha)	2.6 (7)	0.2 (1)	2.2 (8)	4.2 (9)	5.6 (19)	6.9 (16)
Length of fruiting season	February – May	April	February – May	January – May	January – June	March – July
Numbers of seed traps	61	56	71	160	171	117
Number of fruits collected in traps	47	1	8	980	1211	728
Numbers of seeds dispersed by birds	19	1	3	752	776	594
Seed genotyped	12	1	2	70	122	75
Immigrants seeds (two mismatch)	4	0	2	10	8	12
Immigrants seeds (one mismatch)	5	0	2	12	8	13
Immigrants seeds (non-mismatch)	8	0	2	15	16	23
Fruit production (Mean $\pm$ Standard Error)	9 $\pm$ 51	12	103 $\pm$ 74	766 $\pm$ 182	2864 $\pm$ 612	2618 $\pm$ 980
Range	13–386	12	13–250	25–4438	91–12 003	24–14 981
Total number of fruits/ha	4613	43	1475	24 671	40 568	48 576

\*Data from Barbosa *et al.* 2010.

were identified as male or female by their flowers, the presence of green fruits in their crowns and from the presence of intact fruits on the ground below each tree crown over several consecutive years (2007–2012).

To estimate fecundity per tree, we followed the protocol of Howe & Vande Kerckhove (1980) and other authors (Russo 2003). Briefly, crop sizes were inferred from fruit and seed densities recorded in seed traps placed below each female tree. Between one and four seed traps, depending on crown dimension, were placed directly underneath each tree to capture seed fall. Fruits and seeds inside the traps were counted and classified according to the state of the pericarp: intact fruits were considered fallen by gravity, while clean seeds without pericarp indicated consumption by birds. Crop size was defined as the total number of intact fruits and clean seeds falling in the traps located under the tree crown during the entire fruiting season, divided by the proportion of the crown area sampled (Appendix S4). Although we are aware that this estimate may include some seeds that are not produced by the focal tree, our molecular analysis of dispersed seeds (see below) and dispersal curves suggests that the bulk of seeds comes from the same female tree, and therefore, errors in crop size estimates are rather small.

Seed traps consisted of a circle of stainless steel wire, 0.2 m<sup>2</sup> area, supporting a shallow, open-topped, 1-mm nylon mesh bag mounted over three PVC posts, about 1 m above-ground. The content of seed traps was retrieved monthly from January to December 2009. The position of all traps was mapped within each 5 × 5 m grid cell by measuring its *x* and *y* coordinates in metres (see Appendix S1).

### Dissemination limitation

We estimated bird visitation to randomly selected fruiting trees in each patch. To assess visitation rates, we identified each individual fruit-eating bird species that entered the crown and removed fruits of focal trees during a series of 60-min observation periods, separated by roughly 10-min intervals, starting at 8 AM and ending at 2 PM for a total of 11 days in small patches and 41 days in large patches. Periods of observation were spread over the entire fruiting season (from March

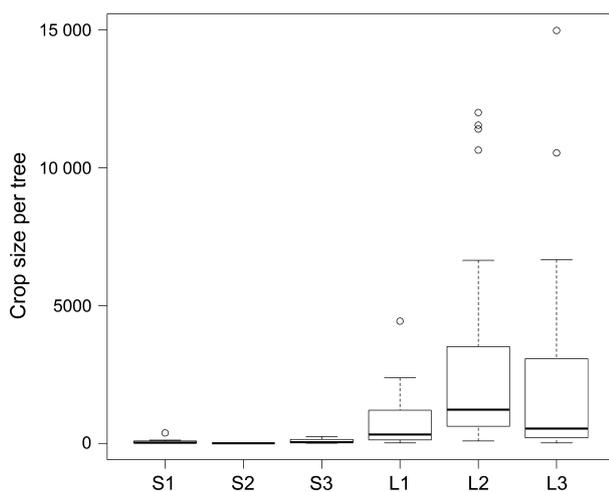
to July). Because of differences in phenology between small and large patches (see below), observations were made in March–April for small patches (40 h) and March–July in large patches (108 h).

To evaluate *distance-restricted seed dispersal* (Schupp, Milleron & Russo 2002), we set up additional seed traps to those placed underneath female tree crowns. These new traps were spread in between reproductive female trees to complete a total number of 636 seed traps in all patches, and their positions were mapped within each 5 × 5 m grid cell (Table 1, Appendix S1). Dispersal distances estimated from the comparison of genotyped females and genotyped seed endocarps could be biased to short (or large) distances if the distribution of seed traps was biased with respect to the distribution of putative seed sources. We constructed a probability distribution of all possible trap-to-female distances to evaluate whether distances were sampled unbiased (Appendix S2). The distribution of actual trap-to-female tree distances was normally distributed implying that the placement of seed traps within the patches was unbiased with reference to distances to reproductive trees and covered the entire range of distances to females inside each patch.

To evaluate distance-restricted seed dispersal within patches, we used microsatellite molecular markers (Núñez-Ávila *et al.* 2011) for comparing endocarp genotypes from each bird-dispersed seed (without pericarp) against the complete set of genotyped reproductive female trees mapped inside patches (Appendix S1). No evidence for scoring errors due to stuttering or large allele dropout was detected; however, one locus (M12) from nine across all six patches showed homozygote excess, which is indicative of a null allele (MICROCHACKER 2.2.3, Van Oosterhout *et al.* 2004). Additionally, to assess the efficiency of the marker set used to discriminate the different female genotypes in each patch, we subsampled loci using a Jack-knife procedure (1000 permutations) in the GenClon 2.0 program (Arnaud-Haond *et al.* 2005). For patch S2 with five loci, it was possible to discriminate genotyped females; for S3, L1 and L2, we discriminated with six locus and in S1 with eight locus, and finally for L3, we discriminated all female genotypes with nine locus (Appendix S5).

Female trees were genotyped using leaf tissue samples (Núñez-Ávila *et al.* 2011). All female trees genotyped had a distinct multilocus genotype ( $P_{ID} = 1.79 \text{ E-}0009$ ); hence, an unambiguous assignment of each seed to its source tree could be made with confidence. This procedure allowed us to link putative mothers to every dispersed seed to construct the dispersal curve (Godoy & Jordano 2001) and to compare dispersal curves among patches. For molecular analyses of endocarps, all dispersed seeds recovered from traps in the 2009 fruiting season were randomly subsampled ( $n = 282$ ), considering at least one seed per trap (when available) to enhance the probability of having all possible trap-to-female distances sampled. The seed endocarp was split open and separated by hand from the seed content. For DNA extraction from seed endocarps and embryos, we used the same protocols as for leaf tissue (Núñez-Ávila *et al.* 2011). To identify the source of dispersed seeds, we used a simple sequence repeat (SSR; microsatellite) loci obtained from the woody seed endocarp of maternal origin (Roth 1977; Godoy & Jordano 2001), which provides a genetic fingerprint of the actual source tree. We tested the accuracy of the method by comparing the genotype of seed endocarps of fruits collected directly from reproductive trees with leaf samples from their mother trees (Appendix S3).

The probability of identity (Waits, Luikart & Taberlet 2001) was used to find matching genotypes from female tree and dispersed seed endocarp genotypes (Godoy & Jordano 2001; Jones *et al.* 2005; Robledo-Amuncio & García 2007), tested using the program CERVUS



**Fig. 2.** Crop size of reproductive female trees of *Aextoxicon punctatum* during the 2009 fruiting season in six forest patches located in Fray Jorge National Park, Chile. Patch sizes mapped are given in Table 1. Numbers of seed traps per tree are given in Appendix S4.

(version 3.0). Matches between seed endocarp and potential seed sources were determined by examining all possible pairwise comparisons in the entire sample of female trees and seeds, with one, two and without any mismatches (Table 1). Seed source assignment is uncertain when several adults share the same multilocus genotype. Given that all female trees in patches yielded different multilocus genotypes, we considered that seed endocarps that remained unassigned to known seed sources within each patch provided an estimate of seed immigration (Godoy & Jordano 2001; Jordano *et al.* 2007).

Euclidean distances between the mapped seed traps that contained genotyped seeds and their identified source trees were calculated for all seeds that could be assigned to a seed source. Only for the three larger patches (L1–L3) we were able to obtain sufficient seed-to-source distance data to construct the probability distribution of seed dispersal distances. Lognormal, Exponential, Weibull and Gamma distributions were all fitted to seed-to-source distance data using a maximum likelihood. We used the Akaike Information Criterion (AIC) to select the distribution that best fitted the data. Statistical analyses were conducted using R language (R Development Team 2008).

### Establishment limitation

To estimate establishment limitation in our study system, we conducted a seed addition experiment in each of the six forest patches. At the end of the seed dispersal season (July 2009), we established 0.25-m<sup>2</sup> quadrats ( $n = 30$  per patch) inside the forest patches. Within the quadrats, we removed the litter and placed 10 *A. punctatum* seeds, obtained from those dispersed by birds, on the ground (300 seeds per patch). After 6 months, we recorded the number of seedlings originated from germinated seeds per quadrat and calculated the proportion of added seeds that became seedlings (Clark, Macklin & Wood 1998; Muller-Landau *et al.* 2002). Differences between small and large patches were evaluated using a non-parametric Kruskal–Wallis test. Statistical analyses were conducted using the R package (R Development Team 2008).

## Results

### SOURCE LIMITATION

Tree densities and basal areas per hectare of *Aextoxicon punctatum* were similar in all patches studied (Table 1), however, from 5.5 to 26% of trees sampled in a given patch were female and just 5–10% of these fruited in 2009 (Table 1 and Appendix S1). Although tree basal areas were similar in all patches, the basal area of reproductive female trees was significantly lower in the small patches ( $F_{(1,4)} = 13.125$ ,  $P = 0.0223$ ; Table 1).

*Aextoxicon punctatum* fruit production in the forest studied extended mainly from April to July (austral fall to winter) (Table 1), although some individual trees were in fruit starting in January in some patches. There were pronounced differences in the length of the fruiting season related to the area of forest fragments. Fruit production ended in May in the three smaller patches and patch L1, but it extended through June and July, late winter, in forest patches L2–L3, respectively (Table 1). Ripe fruit production of *A. punctatum* had a peak in April in all forest patches, but the high fruit produc-

tion tends to continue 1 month later in the three large patches.

Female fecundity was highly variable, ranging from 12 to 386 fruits per tree in small patches and from 24 to 14 981 fruits per tree in large patches in 2009 (Table 1 and Appendix S4). Maximum values of fruit production per tree in small patches were almost 30 times lower than the maximum values in larger patches (Table 1 and Fig. 2).

As predicted, the total number of fleshy fruits per hectare estimated from fallen seed trap data for the entire patch was at least five times lower in the small forest patches than in the larger patches (Table 1), suggesting that seed source limitation of recruitment becomes significantly more intense as patch size declines.

### DISSEMINATION LIMITATION

Seven species of birds visited fruiting *A. punctatum* trees during the period of study (Table 2). These bird species are not forest specialists; they inhabit the mosaic of forest patches and the surrounding shrubland and opportunistically feed on *A. punctatum* trees during the fruiting season. *Mimus thenca* and *Xolmis pyrope*, fruit-eating birds that are most often seen in shrubland habitats (Kelt *et al.* 2012), had the highest frequency of visits to trees per hour in small patches, both with more than 50% of the total visits in the observation period (Table 2). A less frequent disperser of *A. punctatum* seeds in small patches was *Turdus falcklandii*, a common bird in forest habitats (Reid *et al.* 2002). In larger patches, *Xolmis pyrope* had the highest frequency of visits to trees per hour, with 41% of the total visits during the observation period, followed by *Turdus falcklandii* (34%) and *Mimus tenca* (22%). For the last three fruit-eating bird species, visitation rates to large patches more than doubled the number of visits to trees in smaller patches (Table 2), as frugivores concentrated in larger patches where fruits were more abundant. Observed visitation rates by habitat generalist birds indicate that fruits of *A. punct-*

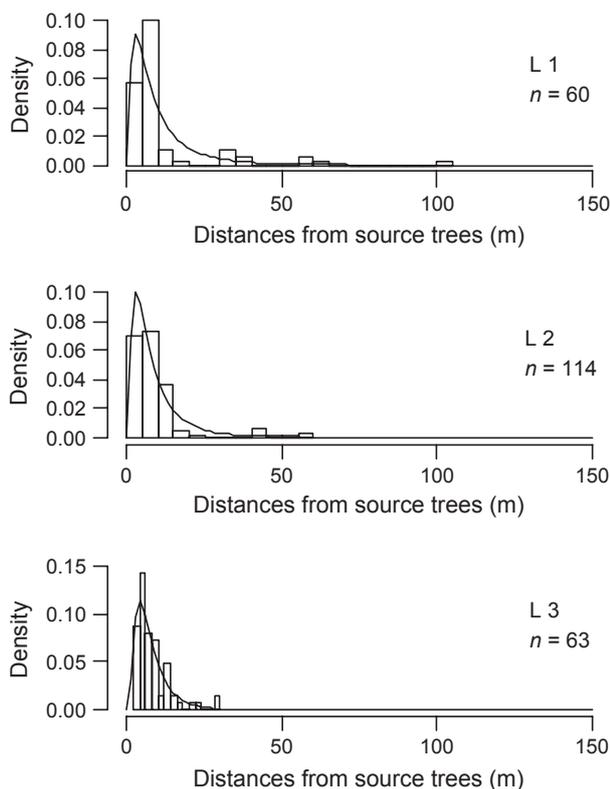
**Table 2.** Total number of visits per hour to fruiting *Aextoxicon punctatum* trees by avian seed dispersers in small (40 h of observation) and large patches (108 h of observation) in Fray Jorge National Park, Chile

Family	Species	Visitation rate in small patches	Visitation rate in large patches
Mimidae	<i>Mimus thenca</i>	0.78 (39)	1.76 (22)
Tyrannidae	<i>Xolmis pyrope</i>	0.45 (23)	3.33 (41)
Turdidae	<i>Turdus falcklandii</i>	0.33 (16)	2.76 (34)
Columbidae	<i>Metriopelia elanoptera</i>	0.30 (15)	0.01 (0)
Columbidae	<i>Patagioenas araucana</i>	0 (0)	0.29 (4)
Fringillidae	<i>Phrygilus gayi</i>	0.13 (6)	0.02 (0)
Emberizinae	<i>Zonotrichia capensis</i>	0.03 (1)	0 (0)

*atum* in remnant fragments can be removed and dispersed outside the patches and be dropped in surrounding shrubland areas or eventually reach other forest patches in the mosaic.

#### Distance-restricted seed dispersal

In an effort to better understand the process of dissemination limitation in the forest patches, we used microsatellite markers to build seed dispersal curves for *A. punctatum*, linking moth-



**Fig. 3.** Observed probability distributions of distances of dispersed *Aextoxicon* seeds to their seed sources (reproductive female trees) in three large forest patches (L1, L2, L3) located in Fray Jorge National Park. Continuous lines show lognormal probability distribution. Parameters of the probability distributions are given in Table 3.

ers to endocarp genotypes of dispersed seeds. Because of small sample sizes of seeds dispersed in the small patches, dispersal curves were constructed only for the three larger forest patches, > 1 ha (L1, L2 and L3). For these forest patches, dispersal curves were strongly leptokurtic, with sharp peaks near the location of source trees and tails related to the patch shape (Fig. 3). The lognormal distributions had the highest AIC value for these patches, and parameters of the fitted curves are shown in the Table 3. The maximum seed dispersal distance observed was 102.9 m in patch L1, but fell sharply to 58.8 m in patch L2 and to 28.5 m in patch L3 (Fig. 3), responding to differences in perimeter/area ratio of these forest patches (Table 1).

The proportion of dispersed seeds that remained unassigned to a known reproductive female in a given forest patch provided an estimate of seed movement between patches in the landscape mosaic of FJNP. In patches L1, L2 and L3, the proportion of immigrant seeds from outside patches was 14.3% ( $n = 70$  total seeds genotyped), 6.5% ( $n = 122$ ) and 16% ( $n = 75$ ), respectively. Due to the low number of seeds collected in seed traps, we pooled all of the genotyped seeds in the small patches. Data showed that 40% of dispersed seeds collected in small patches (6 of 15 genotyped seeds) could not be assigned to trees in the patch and could possibly originate from unidentified trees outside the sampled patches. Regardless of patch size, the location of seed traps from where genotyped seeds were retrieved indicated that bird immigrant seeds fell predominantly in traps located near the edge of forest fragments and/or fell in traps that were associated with reproductive females trees (Appendix S1).

#### ESTABLISHMENT LIMITATION

As hypothesized, the mean percentage of seedlings emerging and surviving after 6 months from experimentally added seeds in each of six forest patches varied greatly depending on patch size. Survival of *A. punctatum* seedlings was significantly lower in the three smaller patches (S1–S3), with a mean ( $\pm$ SE) of  $2.3 \pm 0.2\%$  of seedlings surviving compared with a mean of  $10\% (\pm 0.5)$  in the three larger patches (L1–

**Table 3.** Parameter estimates, negative log-likelihood score ( $-L$ ), Akaike's Information Criterion (AIC) and Akaike weights ( $W_i$ ) for different distributions fitted to seed dispersal distances from reproductive *Aextoxicon punctatum* trees in patches L1, L2 and L3. In bold, the higher  $W_i$

Patch	Probability Distribution	Parameter 1	Parameter 2	$-L$	AIC	$W_i$
L1 ( $n = 60$ )	Lognormal	$\mu = 1.99$ (0.121)	$\sigma = 0.94$ (0.08)	200	405	<b>1.00</b>
	Exponential	$\lambda = 0.079$ (0.010)	NA	212	426	0.00
	Weibull	Shape = 0.93 (0.08)	Scale = 12.1 (1.79)	212	427	0.00
	Gamma	Shape = 1.04 (0.17)	Rate = 0.08 (0.017)	212	428	0.00
L2 ( $n = 114$ )	Lognormal	$\mu = 1.89$ (0.08)	$\sigma = 0.85$ (0.06)	363	730	<b>1.00</b>
	Exponential	$\lambda = 0.101$ (0.009)	NA	378	758	0.00
	Weibull	Shape = 1.09 (0.07)	Scale = 10.29 (0.92)	377	758	0.00
	Gamma	Shape = 1.40 (0.16)	Rate = 0.143 (0.02)	374	753	0.00
L3 ( $n = 63$ )	Lognormal	$\mu = 1.89$ (0.82)	$\sigma = 0.65$ (0.06)	181	367	<b>0.88</b>
	Exponential	$\lambda = 0.121$ (0.015)	NA	195	393	0.00
	Weibull	Shape = 1.56 (0.14)	Scale = 9.20 (0.78)	186	376	0.008
	Gamma	Shape = 2.51 (0.42)	Rate = 0.30 (0.05)	183	371	0.113

L3). This difference was highly significant (Kruskal–Wallis  $\chi^2 = 19.72$ , d.f. = 1,  $P < 0.0001$ ), indicating that small fragments offered less suitable environments for seed germination and survival of *A. punctatum* seedlings.

## Discussion

In naturally or anthropogenically fragmented forests, recruitment limitation may arise due to the failure of trees in fragmented patches to produce and disperse enough seeds or to establish seedlings in the remnant fragments. In this work, we use multiple approaches to investigate the long-term effects of natural forest fragmentation due to a multimillennial trend of increasing aridity, on the recruitment of the avian-dispersed, dioecious rainforest tree *Aextoxicon punctatum*. This is the first study that explicitly assesses recruitment limitation for a dioecious, bird-dispersed tree in naturally fragmented forests, where tree populations have persisted for many generations in a fragmented condition and most probably already paid their extinction debt as proposed by Cornelius, Cofré & Marquet (2000). In this regard, our results provide a unique opportunity to gain insights into the long-term impacts of human-induced forest fragmentation upon tree recruitment.

We showed here that the scarcity of reproductive female trees is a major constraint on seed availability and recruitment in forest patches in this anciently fragmented forest mosaic (Table 1). We do not know whether the depleted proportion of reproductive female trees in the populations studied (less than 10% of the total females on average, Table 1) was due to suboptimal environmental conditions for fruit production in this semi-arid setting, or whether it is a common feature of *A. punctatum* across its entire geographical range. Moreover, female trees in small forest patches had extremely low fecundities (Table 1), further reducing the total seed rain at any site within small patches. In a meta-analysis of habitat fragmentation effects on plant reproduction, Aguilar *et al.* (2006) found evidence for frequent low reproductive success due to animal pollination limitation. Whether habitat fragmentation led to low fruit production due to reduced pollination success in *A. punctatum*, a wind-pollinated and obligate outbred species, remains an open question. Nevertheless, the use of microsatellite molecular markers to assess the contribution of males to seed production could help understand the mechanisms that constrain fecundity in small forest patches. The reduced proportion of reproductive females and low fruit production in the population of *A. punctatum* in small patches was relatively constant over four consecutive years of observations at FJNP and seems to be inherent to this ancient patch mosaic (M. Nuñez-Avila, unpubl. data).

In addition to lower female fecundity, fruiting periods were shorter for trees in smaller patches, which further enhanced seed limitation. Such differences in fruiting phenology may be related to stronger edge effects in smaller compared with large patches, as has been shown for insect species at FJNP (Barbosa & Marquet 2002). Shorter fruit production periods resulted in lower fruit availability for avian seed dispersers, especially towards the end of the reproductive season,

because birds rapidly consumed all available fruits in the trees. Although the three main seed dispersers of *A. punctatum* visited female trees in small patches, their low visitation rate is probably associated with reduced fruit yields that made the trees less attractive to fruit-eating birds. It is possible that constraints on phenology and female fecundity are maximized in this ancient forest patch mosaic due to the extreme reduction in patch size by aridity. In southern South American temperate forest, anthropogenic forest fragmentation often generates a landscape dominated by comparably small patches dispersed in a non-forest matrix (e.g. Wilson *et al.* 1994).

Most seeds in the fragmented forests studied fell directly beneath the crown of reproductive trees, yielding a clumped seed distribution pattern with the greatest seed density below females, which may enhance density-dependent seedling mortality (Cordeiro *et al.* 2009). On the other hand, the tails of the dispersal curves were very different for large patches (> 1 ha). We attributed the different maximum seed dispersal distances estimated for large patches to the shape of patches, where patch L1 had the higher perimeter/area ratio and an elongated shape, while patches L2 and L3 had a more circular shape (Appendix S1). Differences in the shape of patches are therefore important factors regulating the probability of seed movement and therefore the genetic structure of plant populations in fragmented landscapes, especially for animal dispersed species.

From the analysis of unassigned genotyped seeds, frugivorous birds may contribute importantly to the movement of seeds between patches in the forested landscape mosaic. Our findings are coherent with those of Godoy & Jordano (2001), who were unable to identify the source tree for of 17.9% all *Prunus mahaleb* seeds collected in seed traps, and hypothesized that they came from outside the reference population. Other studies have shown that abundant fruit crops in large forest patches can stimulate specialist birds to feed predominantly within one patch rather than fly across patches (Cordeiro & Howe 2003; LeHouck *et al.* 2009). We propose that in FJNP seed movement across the landscape and between forest patches should be favoured because of the generalist habitat use by the main seed dispersers (Reid *et al.* 2002; Kelt *et al.* 2012). Consequently, the extent to which seed dispersal patterns are affected by fragmentation depends largely on the proportion of seed vectors that are habitat specialists, and hence unable to cross the surrounding matrix (Wright & Duber 2001; Galetti *et al.* 2006; Cordeiro *et al.* 2009), in addition to the dispersal syndrome and fruit type, as shown by McEuen & Curran (2004) in northern hemisphere temperate forests.

Once seeds arrive to a new patch, biotic and abiotic effects modulated by fragment size, shape and isolation could affect the number of available safe sites for seed germination and seedling establishment. Such effects could either enhance or depress the chances of seedling recruitment. According to our results and observations, seeds coming from outside patches fell mainly along forest edges (Appendix S1) and always fell below the crown of fruit-producing trees. Therefore, exported seeds may

have low probabilities of recruitment in neighbouring fragments due to the harsh abiotic conditions along the extended edges of small patches (Bruna 1999; Barbosa *et al.* 2010). Therefore, we suggest that in spite of active seed dispersal between patches, establishment of immigrants into new patches would be unsuccessful because of poor habitat quality along edges (Barbosa *et al.* 2010) and high density-dependent mortality under the crown of reproductive trees (Cordeiro *et al.* 2009). However, ongoing population genetic studies (M. Núñez-Ávila, manuscript) suggest that gene flow across patches in this landscape has been significant ( $F_{st} < 0.05$ ) during recent history despite these constraints. This highlights the need for studies about the male contribution to pollen-mediated gene flow.

Edge effects are strongly affected by patch size and perimeter/area ratio (Weathers, Cadenasso & Pickett 2001; Laurance *et al.* 2002). In forest patches in FJNP, soil water content increased markedly with patch area (Barbosa *et al.* 2010), which is consistent with other studies that have shown greater desiccation rates in forest patches with large perimeter to area ratio (Camargo & Kapos 1995; Laurance *et al.* 2000, 2002; Ewing *et al.* 2009). Experimental addition of seeds to presumably suitable germination sites in patches of different sizes showed low germination probabilities (< 3%) in the smaller patches. Limiting factors for seedling recruitment in small patches could be insufficient soil moisture for seed germination (Table 1), associated with enhanced solar radiation, higher temperatures along edges (Barbosa & Marquet 2002; Barbosa *et al.* 2010), where seedlings are highly dependent on fog capture (Del Val *et al.* 2006). Our seed addition experiment demonstrated that in small fragments, in addition to source and dispersal limitation, the establishment of new individuals is strongly limited by edge-associated effects.

We offer for the first time much needed empirical data about the consequences of long-term natural fragmentation on the mechanisms that constrain recruitment for a dioecious rain forest tree species in an ancient forest patch mosaic. However, we examined recruitment limitation during only one reproductive season. Dendroecological studies of *A. punctatum* in FJNP have shown that tree recruitment has occurred fairly continuously over the past 250 years on a decadal scale (Gutiérrez *et al.* 2008). A number of studies have shown strong temporal fluctuations in recruitment in long-lived plant species (Schupp 1990; Connell & Green 2000; Ibañez *et al.* 2007). For instance, interannual differences in regional climate systems, for example ENSO-driven fog and seasonal rainfall in semi-arid Chile (Garraud *et al.* 2008), could potentially lead to large interannual differences in seed production (Wright *et al.* 2005) and seedling recruitment (Ibañez *et al.* 2007). Continuous monitoring over four consecutive years, however, indicates that seed production and dispersal patterns with respect to patches size tend to be consistent with those reported here (M. Núñez-Ávila, unpubl. data). Despite hopeful evidence from the past, the future of dioecious *A. punctatum* in this aridity-driven forest patch mosaic seems uncertain in light of current limits on recruitment and seed dispersal. Under a changing climate, past records cannot assure us that

tree regeneration will continue into the future. Further, demographic work is urgently needed to assess the contribution of the observed seedling recruitment to *A. punctatum* population growth and to assess how much recruitment is necessary for its future persistence (e.g. Bruna 2003). In addition, the strong dependence of these rain forest patches on continuous or enhanced influx of maritime fog (Del Val *et al.* 2006, Barbosa *et al.* 2010) and variable rainfall makes it difficult to predict future tree recruitment. Recent simulations of climate change for Chile (CONAMA 2006) predict enhanced interannual variability in rainfall and greater intervals between extremely wet and dry years. Long-term monitoring of seed rain and seedling recruitment would enable more reliable estimates of probabilities of tree recruitment in small forest patches. As our results suggest, severely limited recruitment in the small patches (<1 ha), which are the most frequent patch sizes in the FJNP mosaic, could put these patches in danger of losing their biological and genetic diversity in the near future. Under this scenario, the chances for generation of new small patches due to enhanced seed dispersal of *Aextoxicon punctatum* seeds by habitat generalist birds to refuges provided by shrubs in the surrounding semi-arid matrix can be of most importance. This type of facilitative succession is the focus of current work in this landscape mosaic.

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## Authorship

MNA, MU, PAM and JJA designed the study. MNA collected field data, samples and generated molecular data, MNA and MU performed statistical analyses. MNA wrote the first draft and all authors contributed substantially to revisions. The authors declare no conflict of interest.

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## Supporting Information

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

**Appendix S1.** Map area of six forest patches (small patches = S1–S3 and large patches = L1–L3, see Table 1) showing the position of all *Aextoxicon punctatum* (Aextoxicaceae) trees 5 cm dbh (open circles) including males and non-reproductive females, reproductive female trees (red circles) during the study period, and the spatial arrangement of all seed traps (grey triangles), coloured as they received one (blue triangle) or two (cyan triangle) immigrants seeds from outside the

patch. Axes labels are lineal distances in metres; each patch was fit to its own spatial scale.

**Appendix S2.** Probability distribution of all female-to-seed trap distances in large patches and the fitted normal distributions using maximum likelihood (ML) method.  $N$  = number of distances. Means and its standard errors are shown for each patch.

**Appendix S3.** Multilocus genotypes for leaf samples, seed endocarp and embryos of *Aextoxicon punctatum* from Fray Jorge rain forest patch mosaic, using microsatellite molecular markers. Alleles for each of nine microsatellite loci are shown for leaf tissue for Female ID 50 from patch L1 and Female 493 from patch L2 together its respective endocarp and embryos genotypes. Five seeds were obtained for each reproductive female tree. Endocarps have in all instances identical genotypes to the leaf tissue. For example, locus M2 for the seed A2 has an heterozygous endocarp (A20) equal to the leaf sample from female 50 (sizes 174/178,) and its embryos A21 is heterozygous with a new allele different to female tree (sizes 174/180). In locus, M9 for the seed B1 has a homozygous endocarp (B10) like the leaf sample from female 493 (sizes 156/156), and its embryo B11 is heterozygous with a new allele (sizes 156/176).

**Appendix S4.** Crop sizes of female trees of *Aextoxicon punctatum* during the 2009 fruiting season in six forest patches (S = small patches, L = large patches, see Table 1) in Fray Jorge National Park, Chile. Columns are patch code name, ID tags of trees, basal areas (m<sup>2</sup>), projected crown areas (m<sup>2</sup>), number of 0.2 m<sup>2</sup> traps placed under each reproductive tree, proportion of crown area (%) sampled by the traps placed under trees, number of fruits fallen in seed traps under each tree and crop size estimate.

**Appendix S5.** Multilocus genotypes (MLGs) identified for all locus combinations from 1 to 9 loci using a Jackknife procedure in order to assess the efficiency of the marker set used to discriminate the maximum clonal diversity present in the sample of *Aextoxicon punctatum* female trees in small (S1–S3) and large (L1–L3) forest patches (Arnaud-Haond *et al.* 2005).