

Spatial heterogeneity of a parasitic plant drives the seed-dispersal pattern of a zoolochorous plant community in a generalist dispersal system

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Summary

1. Biota plays a central role as sources of spatial heterogeneity, having great potential to define ecological processes and patterns in the landscape. Mistletoes are fleshy-fruited parasitic plants that dwell in forest canopies showing a strong aggregated spatial distribution. Parasitized trees potentially concentrate frugivore activity on their canopy, where birds find food, places to perch and protection against predators. Thus, seed-deposition patterns generated from the canopy are expected to reflect the heterogeneity associated with the parasite. This becomes especially important in generalist dispersal systems; however, so far, we do not know the implications of mistletoe spatial heterogeneity on the seed-dispersal pattern of other plants with which they simultaneously fruit.
2. In a Mediterranean pineland, we analyse the impact of *Viscum album* subsp. *austriacum* on the seed-deposition pattern of a zoolochorous plant community, taking into consideration the spatial and temporal variability of environmental factors influencing the frugivore's habitat use, such as fruit availability and forest tree density. For four consecutive years, we studied 55 pairs of trees parasitized and unparasitized by mistletoe, analysing zoolochorous fruit availability, frugivore visits and the zoolochorous seed rain in selected trees.
3. As expected, frugivorous birds responded to mistletoe heterogeneity by visiting parasitized trees preferentially to unparasitized ones, generating a differential deposition of mistletoe seeds on tree branches while dispersing seeds of co-fruiting species under the host canopy. Availability of understory fruits remained similar in patches of parasitized and unparasitized trees, but showed strong temporal fluctuations reflected in the seed rain. On the other hand, mistletoe proved more copious in patches of parasitized trees and their fruit crops varied little between years, making mistletoes reliable food resources likely to lead to consistency in fruit-deposition patterns.
4. In conclusion, mistletoes, by patchily growing on the canopy layer and concentrating zoolochorous seeds underneath, can shape the spatial seed-deposition pattern of fleshy-fruited plants in the forest. Moreover, as seeds constantly reach the same deposition sites over long periods, the soil beneath the host canopy could become hotspots for community regeneration. In degraded areas, such mistletoe effects might be critical, possibly promoting recolonization and vegetation recovery through the frugivore's activity.

Key-words: frugivory, mistletoe, plant–animal interactions, plant–plant interactions, spatial-temporal variability, *Viscum album*

Introduction

Biota constitutes an important agent of spatial heterogeneity, defining ecological processes and patterns in the landscape by interacting with other organisms and by causing

physical changes in the environment. Understanding its role as a source of heterogeneity and the subsequent ecological implications is very useful to gain comprehension of the spatial configuration and functioning of ecosystems (Lovett *et al.* 2005). In this study, we show how the spatial heterogeneity of mistletoes (i.e. hemiparasitic epiphytes) can define the seed-dispersal pattern of a zoolochorous plant

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community by influencing seed-disperser behaviour. Mistletoes are common dwellers of forest canopies that offer abundant and nutritious fleshy-fruited resources, serving as keystone species in ecosystems worldwide (Watson 2001; Watson, McGregor & Spooner 2009; Burns, Cunningham & Watson 2011; Watson & Herring 2012; Ndagurwa *et al.* 2014). They show an extremely patchy distribution, aggregated within specific hosts and neighbourhoods (Aukema 2004) that result in highly heterogeneous forests with clumps of abundant fruit supplies available in the canopy. Mistletoes have great potential to entice frugivores and therefore to concentrate zoochorous seeds.

The patchy distribution of mistletoes arises from the disproportional seed rain that frugivores generate upon already parasitized trees, creating a positive re-infection feedback that enforces mistletoe clumping (Aukema & Martínez del Río 2002; Medel *et al.* 2004; Carlo & Aukema 2005). As mistletoe recruitment is limited to an extremely narrow range of safe sites—requiring seeds to attach on particular branch diameters of specific host species (Reid 1989; Norton & Ladley 1998)—their seed dispersal is frequently carried out by the activity of specialized animals that guarantee an effective dispersal service to the parasite (e.g. Reid 1989; Sargent 1995; Larson 1996; Restrepo *et al.* 2002). Nevertheless, many mistletoe species live where specialists are absent, their seed dispersal depending on generalist birds (e.g. Zuber 2004; Mellado & Zamora 2014a). In these systems, instead of providing directed seed dispersal, frugivores need many attempts to successfully disperse a single mistletoe seed (Mellado & Zamora 2014a). In addition, generalist frugivores feed on multiple fleshy-fruited resources and disperse seeds of different species, commonly under trees that serve as perches or sites to feed (Herrera 1984; Clark *et al.* 2004; Kwit, Levey & Greenberg 2004). In such generalist seed-dispersal systems, mistletoe spatial patchiness may have important implications on the seed-dispersal pattern of other plants with which they simultaneously fruit, as parasitized trees have the potential to concentrate frugivore activity on their canopy, which simultaneously provide food, resting sites and protection against predators. Therefore, seed-deposition patterns generated from the canopy are expected to reflect the heterogeneity associated with the parasite. However, so far, we have no information about the implications of mistletoe patchiness on the seed-dispersal patterns of co-fruiting plants.

To address this question, we need to consider that the attractiveness of parasitized trees (and thus the placement of seed-deposition sites) may depend on many environmental factors influencing frugivore habitat use, such as the availability and distribution of alternative food resources and forest tree density (Carlo 2005; Saracco *et al.* 2005; Blendinger, Blake & Loiselle 2010; Herrera, Morales & García 2011). These factors, in turn, might be subject to temporal changes, being very frequent in certain ecosystems (e.g. Herrera *et al.* 1998; Prasad & Sukumar

2010). On the other hand, mistletoes produce regular fruit crops through time (Larson 1996; Van Ommeren & Whitham 2002), which might permit the maintenance of repeating seed-dispersal patterns over long periods, with zoochorous seeds reaching the same deposition sites time and again.

In this study, we analyse the impact of *Viscum album* subsp. *austriacum* (Wiesb.) Vollmann (*V. a. austriacum*, hereafter) patchiness on the seed-deposition pattern of a zoochorous plant community, taking into consideration the spatial and temporal variability of forest tree density and fruit availability of mistletoe and co-fruiting plants. The study was conducted in a Mediterranean pineland of southeastern Spain, where *V. a. austriacum* coexists with a diverse community of zoochorous understory plants, sharing a broad assemblage of generalist seed dispersers. In this pineland, parasitized trees, constituting the only (or most abundant) nutritive resource offered within the canopy, might be particularly noticeable for frugivorous birds. Under this scenario, we expect frugivorous birds to respond to mistletoe patchiness by visiting parasitized trees preferentially to unparasitized ones, driving a differential deposition of mistletoe seeds towards parasitized trees (Fig. 1). At the same time, while staying on the host canopy, frugivores concurrently disperse seeds of other zoochorous plants, giving rise to multispecies seed-deposition sites underneath parasitized trees (Fig. 1b). Finally, we expect the seed rain to reflect the temporal variability of the environment.

We studied 55 pairs of mistletoe parasitized and unparasitized trees for four consecutive years (2009–2012), analysing fruit availability of mistletoe and co-fruiting species in focal trees and nearby neighbourhoods, frugivore visits and the zoochorous seed rain generated in selected trees, in an effort to answer three main questions: (i) Does mistletoe presence in pine canopies, through its influence on the activity of frugivores, shape the spatial pattern of zoochorous seeds in the forest? (ii) What environmental factors are behind the patterning of seed-deposition? (iii) What is the temporal consistency of mistletoe and understory fruit crops and seed rain at deposition sites?

Materials and methods

STUDY SITE AND EXPERIMENTAL DESIGN

The study was conducted in a Mediterranean pine forest located in the Natural Park of Sierra de Baza (southeastern Spain; 2° 51'W, 37° 22'N). This is a jagged mountain range, mainly calcareous, with minimum altitudes of 1200 m and maximum of 2269 m. The site shows the typical Mediterranean climate, characterized by cold winters and hot summers with pronounced summer drought (June–August), while precipitation concentrates in spring and autumn. Sierra de Baza contains a complex mosaic of plant formations. Dominant tree vegetation is pine forest, mainly Austrian (*Pinus nigra* Arn.) and Scots (*Pinus sylvestris* L.), but also Aleppo (*Pinus halepensis* Mill.) and Maritime (*Pinus pinaster* Ait.) coexisting with oaks (*Quercus ilex* L.) and maples (*Acer opalus* L. ssp. *granatense* Boiss.). The landscape reflects the impact of past human

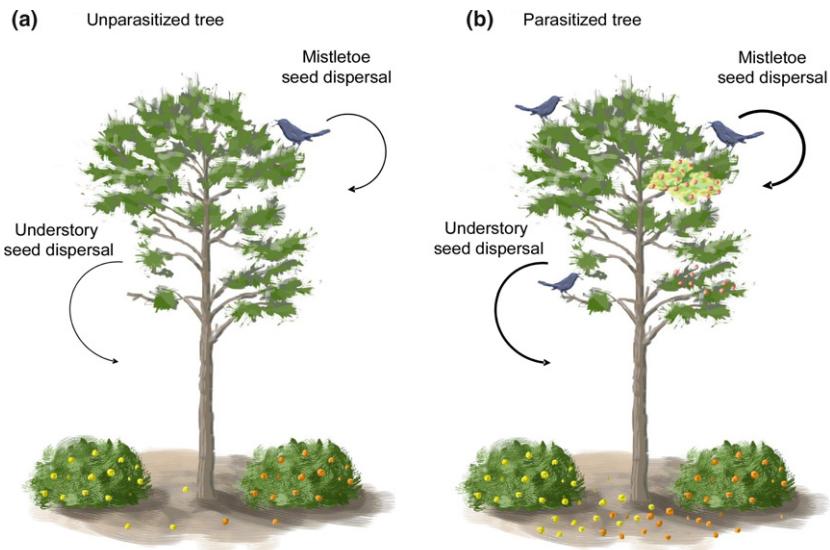


Fig. 1. Hypothesis: In a pine forest, frugivores commonly perch on pine's canopy (a) looking for sites to watch or protect against predators. During this time, they can disperse sporadic quantities of zootochorous seeds. Otherwise, when mistletoes are concentrated in the canopy (b), parasitized pines become highly attractive because they simultaneously offer food, sites to perch, and protection. As a result, frugivores visit parasitized trees preferentially to unparasitized ones, driving a differential deposition of mistletoe seeds towards the canopy of parasitized trees, while carrying along seeds of co-fruiting species, giving rise to multispecies contagious seed-dispersal patterns underneath the canopy of parasitized trees.

activities, having stands of native and naturalized pinelands intermingled throughout the mountain range. A diverse and abundant ensemble of zootochorous fleshy-fruited shrubs accompanies pines, including *Berberis hispanica* subsp. *hispanica* Boiss. & Reut., *Crataegus monogyna* Jacq., *Lonicera arborea* Boiss., *Juniperus oxycedrus* L., *J. communis* L., *Prunus ramburii* Boiss. and *Rosa* spp. Part of the zootochorous plant community is the mistletoe *Viscum album austriacum*, a hemiparasitic, dioecious epiphyte that absorbs water and mineral salts from the xylem of its host trees. This species is widely distributed across Europe parasitizing conifers, *Pinus nigra* and *P. sylvestris* being the most common host species in southern Spain and at the study site (Mellado & Zamora 2014b).

The most usual species of avian seed dispersers in Sierra de Baza include non-migrants and seasonal migrants such as *Sylvia* spp., *Turdus* spp. and *Erithacus rubecula*, which feed on various fruit species during autumn-winter. Thrushes are the main seed dispersers of *V. a. austriacum* (Zuber 2004; Mellado & Zamora 2014a), as well as legitimate dispersers of other zootochorous species of the plant community (Herrera 1984); they swallow entire fruits and defecate or regurgitate intact seeds. Small passerines, such as Robin *Erithacus rubecula* and Blackcap *Sylvia atricapilla* also contribute to the dispersal of both ground and canopy-dwelling fleshy-fruited plants.

We selected 110 *Pinus nigra* (hereafter focal trees), half of which were parasitized by mistletoe, except for the first study year (2009) in which 80 *P. nigra* were used. A parasitized tree was chosen if it hosted at least one fruit-producing mistletoe. The experiment covered a large range of heterogeneity of terrain by choosing focal trees at three altitudes (1300, 1650 and 1850 m) within stands of different tree densities. Trees were randomly selected and spatially paired (one parasitized and one unparasitized). Paired trees were of similar architecture, size (trunk perimeter = 97.5 ± 4.01 cm) and height (6.87 ± 0.23 m), and were located in similar environmental contexts (staying 40–80 m apart). Sites were visited for 4 years from 2009 to 2012.

MISTLETOE AND UNDERSTORY SPECIES FRUIT AVAILABILITY

In all focal pines, we established circular plots of 20 m radius. In these plots, we measured fruit abundance (berries, drupes and functionally analogous structures such as juniper berries) of every fleshy-fruited species, fruit-species richness and tree density

(trees ha^{-1}). Abundance of mistletoe fruit was quantified for all parasitized trees included within the plot, considering all mature female mistletoes as a whole. We visually estimated ripe fruit crops, using binoculars when required. To estimate fruit abundance of understory species, each circular plot was divided into four equal subplots (90° each), covering the whole sampling area. At the beginning of the dispersal season (October), in each subplot we visually counted the quantity of ripe fruits of all fruiting plants that had, at least, 30% of their canopy surface within the subplot. In the study locality, fruiting is quite synchronous among individuals and species, where early- or late-ripening species are rare and most of the ripening period is delimited within 1–2 months (although fruits remain attached to trees for 1–3 additional months). We thus considered that a single sampling to quantify fruit abundance at the beginning of the season would provide an appropriate estimate of the spatial template of fruit resources available for frugivores. For further analyses we differentiated (i) mistletoe fruits available within plots (sum of ripe mistletoe fruits produced in the focal pine and all parasitized pines included in the plot, hereafter 'mistletoe fruits') and (ii) fruits of other zootochorous species (sum of ripe fruits produced by all understory fleshy-fruited species in the plot, hereafter 'understory fruits'). Fruit richness was estimated as the sum of the available fruit species counted within the plot.

FRUGIVOROUS BIRDS

We made direct observations of frugivorous birds visiting parasitized and unparasitized focal pines. Each census was performed between 7:00 to 12:00 a.m., consisting in 5 min of observation per focal pine during different days throughout the dispersal season (from the end of September to the end February for the four study years). During each observation, a trained ornithologist (R. Zamora) covered the area in which focal trees were located, making stops at 20–40 m to get a full image of the whole canopy. Birds were identified to the species level. When the observation period ended, the process was repeated for all other focal trees. At the end of each season, we amassed 12–14 observations per focal pine, for a total of 70 min of observation time per tree per year, resulting in 478 h of observation time for the whole study (80 focal trees during 2009 and 110 trees for the following 3 years). We calculated frugivore-bird abundance per focal pine as the cumulative number of birds watched through the season divided by the total observation time.

MISTLETOE AND UNDERSTORY SPECIES ZOOCHOROUS SEED RAIN

We quantified the seed rain of all zochorous plant species generated from the canopy of all focal pines. Seed traps were used during the complete dispersal season, from October to February, during the four study years. Seed traps consisted of flowerpots (0.125 m^2) covered with an aluminium mesh hanging on the lower branches of the tree canopy, to prevent seed predation by rodents and granivorous birds, while avoiding the intensive wild-boar uprooting that frequently occurs under these trees. Three seed traps hung on three randomly assigned branches of the lower third of the tree height ($c.$ 2 m above the ground level). At the end of February, samples were transferred to the laboratory where seeds of all fleshy-fruited plant species were identified and counted, and seed-species richness calculated. Quantifying the seed rain by this approach directly estimates the seed dispersal of understory species, but not that of mistletoes, as seeds need to stick on tree branches. For this reason, we estimated the relationship between mistletoe seed rain and mistletoe seeds stuck on branches of 20 parasitized focal trees. For statistical analyses, abundance of mistletoe seeds were counted separately (hereafter, ‘mistletoe seeds’), whereas seed abundance of ground-dwelling plant species were all pooled (hereafter, ‘understory seeds’).

STATISTICAL ANALYSES

We performed separate statistical analyses for each year, since the sample size differed; for 2009, 40 pairs of *P. nigra* were used, while for the following 3 years it was increased to 55 pairs. Moreover, during the study period, three parasitized trees died and had to be replaced by others of similar characteristics. Therefore, for 4 years, we compared between parasitized and unparasitized focal pines: (i) total abundance of mistletoe and understory fruits available within the plot (ii) fruit richness, (iii) frugivore visitations to focal pines and (iv) the mistletoe and understory seed rain generated on focal pines. We used generalized linear mixed models (GLMM) with Poisson error distribution and log-link function for fruit and frugivore abundance, negative binomial for seed abundance and linear mixed models (LMM) for fruit and seed richness, including tree condition (parasitized or not) as a fixed factor and paired trees as a random factor.

We analysed the effect of focal-tree condition (parasitized or not) and neighbourhood traits (i.e. mistletoe and understory fruit abundance, fruit richness and tree density) on frugivore visitations, and on the abundance of mistletoe and understory seed rain on focal trees. Models were simplified using a backward stepwise selection analysis, starting with a maximal model that included all predictors and interactions, from which the most parsimonious combination of explanatory variables was identified following a hypothesis-testing approach with the ‘drop1’ command, which drops one explanatory variable in turn and each time applies an analysis of deviance test (Zuur *et al.* 2009). Following an inspection of model residuals and considering dispersion, we chose the most appropriate family of GLMM for each case. For frugivore counts we used Poisson error distribution and the log-link, while for mistletoe and understory species seed abundance, we applied negative binomial distribution and the log-link because the equidispersion assumption of the Poisson model was not fulfilled (Zuur *et al.* 2009). Explanatory variables were standardized (by subtracting the mean and dividing by one standard deviation) for better comparisons.

To analyse temporal variability of mistletoe and understory fruit production and seed rain at deposition sites, we restricted the data to the 40 parasitized *Pinus nigra* trees selected from the beginning of the study (i.e. excluding unparasitized trees and those parasitized that were latter added). Analyses were made with

GLMMs using a log-link function and Poisson error distribution for fruits and a negative binomial distribution for seeds, followed by Tukey’s pairwise comparisons with 95% confidence level. Replicates were included as random factors in the model to account for temporal pseudoreplication while the year was included as a single fixed term.

Analyses were carried out using the open source software Statistical R.2.15.1 (R Development Core Team 2012). GLMMs were run using lmer and glmer functions of the package lme4 (Bates, Maechler & Dai 2008) whereas Negative binomial GLMMs with the glmmadmb function of the ‘glmmADMB’ package (Skaug *et al.* 2012). Tukey’s pairwise comparisons with 95% confidence level were conducted with the ‘multcomp’ package (Hothorn, Bretz & Westfall 2008). Results are presented as mean \pm one standard error, unless otherwise specified.

Results

We recorded 10 plant species with fleshy fruits ripening simultaneously to *V. album*, which made up the diet of frugivorous birds at the study site. These were: *Juniperus communis*, *J. oxycedrus*, *J. sabina*, *Lonicera arborea*, *Crataegus monogyna*, *Berberis vulgaris*, *Sorbus aria*, *Prunus ramburii*, *Hedera helix* and *Rosa* spp. Non-fleshy-fruited trees, which served as perches for frugivores, were also abundant in the vicinity; mainly *Pinus nigra*, *P. sylvestris*, *P. halepensis*, *P. pinaster*, *Quercus ilex* and *Acer granatensis*. The density of trees surrounding focal parasitized and unparasitized trees were quite similar (d.f. = 1, $F_{1, 53} = 10.3$; P -value = 0.38), with ranges of $7.95\text{--}310.35$ trees ha^{-1} (mean \pm SD, 102.29 ± 9.47 trees ha^{-1}) for unparasitized trees and ranges of $9.014\text{--}262.60$ trees ha^{-1} (mean \pm SD, 80.30 ± 9.01 trees ha^{-1}) for those parasitized.

In 478 h of observation, we recorded 285 individuals of eight frugivorous bird species visiting focal trees and consuming fruits of *V. album* and fleshy-fruited species from the understory. Thrushes were the most abundant frugivores, chiefly *Turdus viscivorus* (75.80% of the visits), followed by *T. torquatus* (10.50%), *T. philomelos* (7.70%), *T. iliacus* (1.80%), *T. merula* (1.40%) and *T. pilaris* (0.40%). Other species, *Sylvia atricapilla* (0.18% of the visits) and *Erithacus rubecula* (0.07%), were less frequently recorded.

FRUIT AVAILABILITY, FRUGIVORE VISITS AND SEED-DISPERSAL SPATIAL PATTERNS

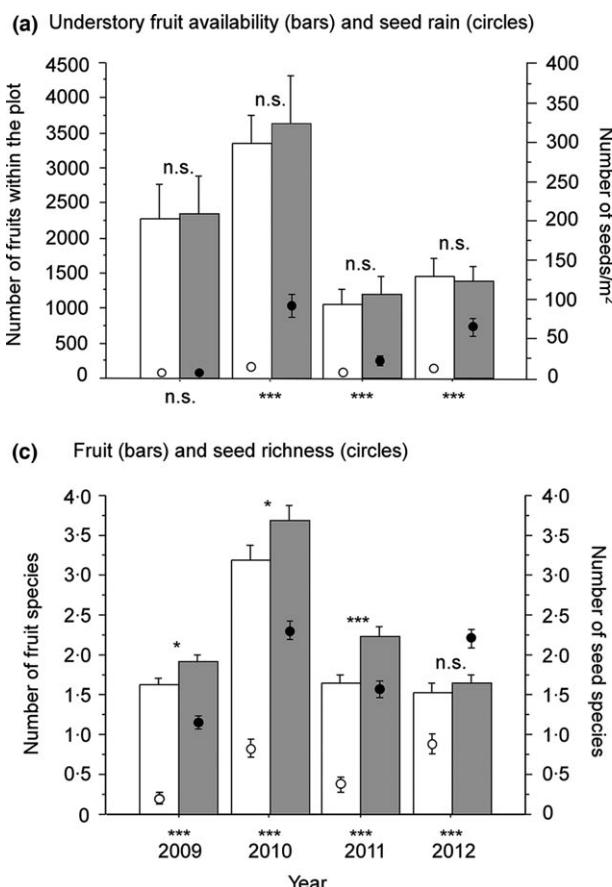
The fruits of understory species were similarly abundant around parasitized and unparasitized trees for all the study years (Fig. 2a) (GLMMs d.f. = 1, 2009: χ^2 , 0.02; P -value = 0.91, 2010: χ^2 , 0.14; P -value = 0.708, 2011: χ^2 , 0.22; P -value = 0.641, 2012: χ^2 , 0.07; P -value = 0.792). In contrast, mistletoe fruit abundance proved more copious in patches of parasitized trees, with significant statistical differences when compared to unparasitized ones (Fig. 2b) (GLMMs d.f. = 1, 2009: χ^2 , 2052; P -value <0.0001, 2010: χ^2 , 4182; P -value <0.0001, 2011: χ^2 , 6447; P -value <0.0001, 2012: χ^2 , 3991; P -value <0.0001). Fruit richness was greater in plots of parasitized trees (Fig. 2c) (LMMs d.f.

= 1, 2009: χ^2 , 8.56; P -value = 0.003, 2010: χ^2 , 8.39; P -value = 0.004, 2011: χ^2 , 27.27; P -value <0.0001, 2012: χ^2 , 0.52; P -value = 0.47). This was later reflected in a richer seed rain (Fig. 2c) (LMMs d.f. = 1, 2009: χ^2 , 101.29; P -value <0.0001, 2010: χ^2 , 95.14; P -value <0.0001, 2011: χ^2 , 78.04; P -value <0.0001, 2012: χ^2 , 74.63; P -value <0.0001). Frugivore visits were more abundant during the first two study years, and pines holding mistletoes were preferably visited (Fig. 2d) (GLMMs d.f. = 1, 2009: χ^2 , 31.64; P -value <0.0001, 2010: χ^2 , 35.88; P -value <0.0001, 2011: χ^2 , 21.87; P -value <0.0001, 2012: χ^2 , 12.16; P -value = 0.0005). Similarly, the zoochorous seed rain was substantially more abundant upon the canopy of parasitized pines, both mistletoe (Fig. 2b) (negative binomial GLMMs d.f. = 1, 2009: χ^2 , 77.24; P -value <0.0001, 2010: χ^2 , 71.509; P -value <0.0001, 2011: χ^2 , 89.26; P -value <0.0001, 2012: χ^2 , 99.68; P -value <0.0001), and understory seeds (Fig. 2a) (negative binomial GLMMs d.f. = 1, 2009: χ^2 , 0.42; P -value = 0.53, 2010: χ^2 , 24.09; P -value <0.0001,

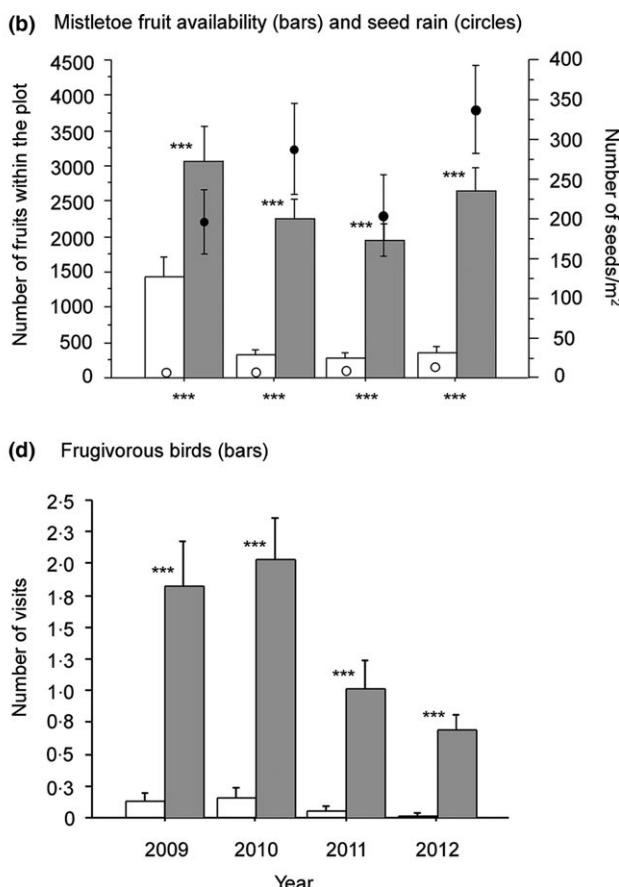
2011: χ^2 , 12.25; P -value = 0.0004, 2012: χ^2 , 22.25; P -value <0.0001). Finally, mistletoe seed rain and seeds attached to pine branches showed a significantly positive relationship (R^2 = 0.32, P -value = 0.009, n = 20), indicating that mistletoe seeds gathered with seed collectors can be a good estimate for mistletoe seed dispersal.

ENVIRONMENTAL FACTORS INFLUENCING SEED-DISPERSAL PATTERNS

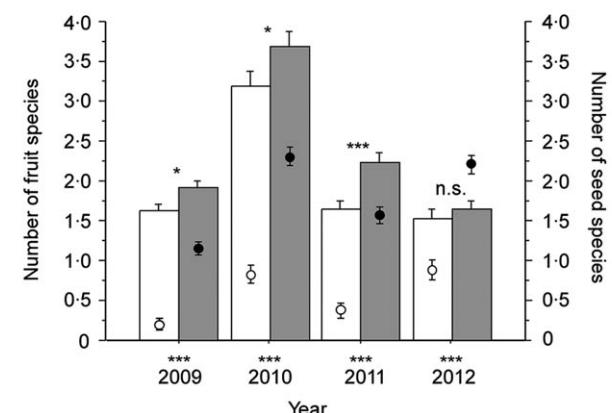
The number of frugivore visits strongly responded to the condition (parasitized or not) of individual hosts, showing weaker relationships with traits of the neighbourhood (i.e. mistletoe and understory fruit abundance, fruit richness, and tree density) (Table 1a). Nevertheless, fruit abundance of mistletoe and understory species was in all cases positively correlated with frugivore visits (Table 1a). Otherwise, high tree densities, when significant, showed negative relationships with frugivores (Table 1a) and dispersed



(a) Understory fruit availability (bars) and seed rain (circles)



(b) Mistletoe fruit availability (bars) and seed rain (circles)



(c) Fruit (bars) and seed richness (circles)

(d) Frugivorous birds (bars)

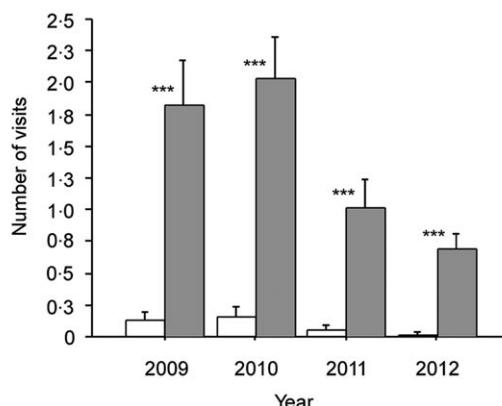


Fig. 2. Spatial pattern of fruit availability, seed rain and frugivore visits. Comparison between unparasitized (white) and parasitized (gray) trees in (a) understory fruit availability within the plot ($r = 20$ m) and the seed rain generated on focal trees; (b) mistletoe fruit availability within the plot and the seed rain generated on focal trees; (c) fruit richness within the plot and seed richness reaching focal trees and (d) number of frugivore visits in focal trees. Generalized linear mixed models with Poisson error distribution were used for frugivore and fruit counts, negative binomial error distribution for seed counts, and linear mixed models for fruit and seed richness, with tree condition (parasitized or not) as the fixed factor and paired trees as the random one. Statistical differences between parasitized and unparasitized trees are indicated as non-significant (n.s.), $P < 0.01$ (*), $P < 0.0001$ (**). Bars represent fruits or frugivores (statistical significance indicated above bars), while circles represent seeds (statistical significance indicated below bars). Results correspond to mean ± 1 SE, $N = 40$ tree pairs for the year 2009 and $N = 55$ tree pairs for the following years.

seeds (Table 1a and b). With regard to seeds, for both mistletoe and understory seed rain (Table 1b and c), the chief influence was the focal pine to be parasitized, while fruiting neighbourhoods exerted different effects on the seed rain in different years. Overall, heterospecific fruit availability positively influenced the seed rain of mistletoe and understory species during years of high fruiting peaks (i.e. 2009 and 2010). An opposite effect was found for years of lower fruit abundances (i.e. 2011 and 2012). For all study years, fruit richness exerted a positive influence on the seed rain of understory species.

TEMPORAL VARIABILITY OF FRUIT AVAILABILITY AND SEED-DISPERSAL PATTERNS

Understory species showed great fruit-crop fluctuations over the study years (Fig. 3b). The mean number of understory fruits produced in 2009 and 2010 was substantially more numerous than those produced in 2011 and 2012, varying significantly between years (Fig. 3b; GLMM, d.f., 3; χ^2 , 31.01; P value < 0.0001). By contrast, milder temporal fluctuations were found in average mistletoe fruit production (Fig. 3a; GLMM, d.f., 3; χ^2 , 18.76; P value, 0.002). Seed rain generated on focal pines varied accordingly to their respective fruit availability (Fig. 3; negative binomial GLMM: understory, d.f., 3; χ^2 , 97.19; P value < 0.0001 and mistletoe, d.f., 3; χ^2 , 19.04, 7.68; P value, 0.004).

Discussion

Our study highlights the important role mistletoe plays as sources of spatial heterogeneity in the forest. In this pine-land, where generalist birds disperse seeds of mistletoe and co-occurring fleshy-fruited plant species, zoochorous seed-deposition patterns clearly reflect the spatial heterogeneity of the parasite.

FRUIT AVAILABILITY, FRUGIVORE VISITS AND SEED-DISPERSAL SPATIAL PATTERNS

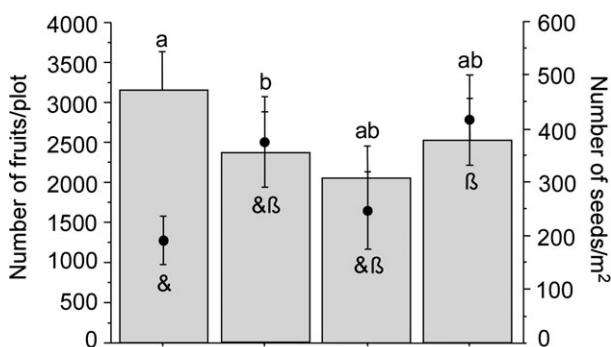
In the study site, parasitized pines constitute outstanding sites for frugivorous birds when compared to unparasitized ones. First, this is because mistletoes transform a conifer into a fleshy-fruited tree, which directly offers abundant fruit rewards in the canopy layer where frugivores can feed at the time they perch. Second, because their nearest environment provides greater fruit supplies, as mistletoe fruit abundance proved more copious in patches of parasitized pines (Fig. 2b). This reflects the typical aggregated disposition of the parasite at the scale of neighbourhoods (Aukema 2004) and increases overall fruit availability within the patch. In contrast, understory fruit availability and forest tree density remain spatially constant for all study years, being quite similar among patches of parasitized and unparasitized focal pines (Fig. 2a). Abundant mistletoe fruit coupled with greater fruit richness (Fig. 2c),

Table 1. Results of generalized linear mixed-model effects for frugivore visitations (with Poisson error, Z-test) and mistletoe and understory seed rain (with negative binomial error, t-test) generated on focal trees as a function of focal-tree condition (parasitized or not) and features the context (mistletoe and understory fruit abundance, fruit richness and forest tree density)

Year	Variable	Estimate	SE	Z/t value	P value
(a) Number of frugivore visits					
2009	Constant	-2.395	0.483	-4.962	<0.0001
	Parasitized tree	2.603	0.481	5.417	<0.0001
	Tree density	-0.4947	0.168	-2.946	0.00322
2010	Constant	-2.349	0.456	-5.152	<0.0001
	Parasitized tree	2.493	0.448	5.564	<0.0001
	Mistletoe neighb.	0.253	0.113	0.133	0.057
	Understory neighb.	0.424	0.424	0.135	0.002
2011	Constant	-3.289	0.631	-5.217	<0.0001
	Parasitized tree	2.740	0.637	4.306	<0.0001
2012	Constant	-4.074	1.035	-3.934	<0.0001
	Parasitized tree	3.585	1.048	3.421	<0.0001
(b) Abundance of mistletoe seeds					
2009	Constant	0.411	1.098	0.370	0.709
	Parasitized tree	6.915	0.817	8.460	<0.0001
	Understory neighb.	0.821	0.273	3.010	0.003
	Tree density	-0.642	0.232	-2.760	0.006
	Fruit richness	-1.110	0.518	-2.140	0.032
2010	Constant	0.695	0.437	1.590	0.111
	Parasitized tree	4.715	0.492	9.580	<0.0001
	Mistletoe neighb.	0.679	0.239	2.850	0.004
2011	Constant	-1.031	0.548	-1.880	0.060
	Parasitized tree	5.814	0.557	10.440	<0.0001
	Mistletoe neighb.	0.521	0.237	2.200	0.028
	Understory neighb.	-0.601	0.290	-2.080	0.038
	Tree density	-0.639	0.283	-2.250	0.024
2012	Constant	0.174	0.515	0.340	0.740
	Parasitized tree	5.708	0.569	10.040	<0.0001
(c) Abundance of understory seeds					
2009	Constant	-1.800	1.920	-0.940	0.350
	Parasitized tree	0.740	1.310	0.570	0.570
	Fruit richness	1.880	1.200	1.570	0.120
2010	Constant	1.997	0.333	6.000	<0.0001
	Parasitized tree	2.274	0.408	5.570	<0.0001
	Understory neighb.	0.659	0.239	2.760	0.006
	Fruit richness	0.502	0.244	2.050	0.040
2011	Constant	1.186	0.411	2.890	0.004
	Parasitized tree	1.362	0.631	2.160	0.031
	Mistletoe neighb.	-0.275	0.282	-0.980	0.329
	Fruit richness	0.694	0.332	2.090	0.037
2012	Constant	2.315	0.231	10.020	<0.0001
	Parasitized tree	1.854	0.332	5.580	<0.0001
	Mistletoe neighb.	-0.452	0.178	-2.540	0.011
	Fruit richness	0.381	0.164	2.32	0.02

In the model analyses, tree condition (parasitized or not), neighbouring mistletoe fruit abundance (Mistletoe neighb.), neighbouring understory fruit abundance (Understory neighb.), fruit richness, and tree density are considered as fixed factors, while tree pairs (see Study site and methods) are considered as the random factor. Models shown are the optimal models found following a hypothesis-testing approach (Zuur *et al.* 2009). $N = 40$ tree pairs for the year 2009 and $N = 55$ tree pairs for the following years. Significance of bold values ($P < 0.05$).

(a) Mistletoe fruit availability (bars) and seed rain (circles)



(b) Understory fruit availability (bars) and seed rain (circles)

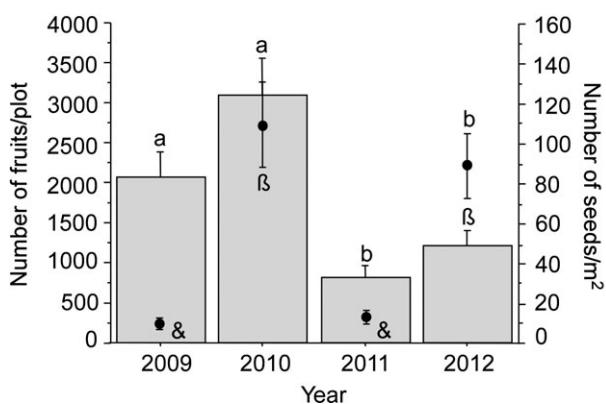


Fig. 3. Temporal variation of mistletoe and understory fruit production and seed rain. Mistletoe fruit production and mistletoe seeds reaching parasitized focal pines remain relatively constant from 1 year to the next (Fruits: d.f., 3; χ^2 , 18.76; P value, 0.002, Seeds: d.f., 3; χ^2 , 19.04; P value, 0.004). On the other hand, understory fruit production sharply fluctuates between years (GLMM, d.f., 3; χ^2 , 31.01; P value <0.0001), as reflected in the seed rain (d.f., 3; χ^2 , 97.19; P value <0.0001). GLMMs with log-link function and Poisson error distribution were used for fruits and negative binomial distribution for seeds, followed by Tukey's pairwise comparisons with 95% confidence level. Replicates were included as random factors in the model to account for temporal pseudoreplication while the year was included as single fixed term. Results correspond to mean \pm 1 SE, N = 40 for the subset of parasitized trees monitored from the beginning of the study. Different Latin letters (a, b) denote significant differences (P < 0.05) among fruit abundance of different years, while different symbols (&, β) refer to such differences for seed abundance of different years.

enhances the attractiveness of the surrounding environment of parasitized pines, where frugivores are likely to optimize their foraging (Carlo & Morales 2008; Morales *et al.* 2012).

Thrushes were major fruit consumers for all study years, the Mistle Thrush (*Turdus viscivorus*) being the most frequent species. These territorial birds frequently use parasitized trees as watchtowers to defend patches of abundant fruit densities from conspecifics or other fruit consumers (Snow & Snow 1984; Skórka & Wójcik 2005). By visiting parasitized trees more preferably (Fig. 2d), frugivores convert these trees into important sites for seed deposition in

the forest landscape. Here, great amounts of mistletoe seeds are deposited on host branches (starting new re-infection loops), while abundant and rich amounts of seeds of understory species reach the soil underneath the canopy (Figs 2 a–c).

ENVIRONMENTAL FACTORS INFLUENCING SEED-DISPERSAL PATTERNS

As expected, forest structure and the availability of other fruit resources influenced frugivore behaviour and the seed rain generated on parasitized trees. In some habitats, especially in those with spatially segregated food resources, frugivorous birds need to optimize their habitat use by compromising the search for fruits or the protection under forest coverage (Howe 1979; Fedriani & Boulay 2006). In our study, however, for the four study years and regardless of other environmental factors, mistletoe presence was the most influential variable for the number of frugivores visiting focal pines (Table 1), perhaps because frugivores find safe sites to feed in parasitized trees as fruits are concentrated in the canopy. Less influential and only for some years, mistletoe and understory fruit abundance of the neighbourhood boosted frugivore activity, whereas forest tree density exerted a negative influence (Table 1), reflecting thrush preferences for freestanding trees (Snow & Snow 1984) and patches of abundant fruit resources (Skórka & Wójcik 2005). Yearly differences in the effects of these environmental factors could be related to the strong temporal fluctuations in fruit availability (Fig. 3b), which changes the environmental context each year.

Due to the wide generalism of frugivorous birds and zoochorous plants (with many plant species sharing seed dispersers), plant–plant interactions mediated by frugivores are likely to emerge (Bascompte *et al.* 2003). In previous studies, facilitative interactions between mistletoes and their fleshy-fruited hosts have been shown, as additional fruit resources of the parasite improve rates of host's seed dispersal (Van Ommeren & Whitham 2002; Carlo & Aukema 2005; Candia, Medel & Fontúrbel 2014). Here, we show that mistletoe–plant interactions, when considering the entire community of zoochorous plants, strongly vary from 1 year to the next, giving rise to a variety of scenarios in which fruiting plants facilitate or hinder the seed dispersal of other zoochorous species. In years of bountiful understory fruit supplies (2009 and 2010), more frugivores visited parasitized pines (Fig. 2) and facilitative interactions (i.e. increased seed dispersal) emerged between mistletoes and understory species (Table 1b and c). Otherwise, when food resources were more scarce (2011 and 2012), zoochorous plants competed for the limited availability of seed dispersers that visited a patch at a given time, with fruiting plants negatively influencing the seed dispersal of other species (Table 1b and c). Nevertheless, regardless of the year, the most influential variable over the seed rain generated on a tree was the fact of being parasitized by mistletoe (Table 1b and c).

TEMPORAL CONSISTENCY OF FRUIT AVAILABILITY AND SEED-DISPERSAL PATTERNS

The attractiveness of parasitized trees remains constant through time as mistletoe fruit-crops vary little from 1 year to the next, making them reliable food resources able to promote consistency in seed-deposition patterns. Such small variation in yearly fruit production is common among mistletoe species and has been recognized as an important phenomenon for the maintenance of frugivore populations at certain sites when other fleshy-fruited species reduce their crops (Larson 1996; Watson 2001; Van Ommeren & Whitham 2002). In contrast, understory species show strong fruit-crop fluctuations between different years (Fig. 3), a common phenomenon for many of the fleshy-fruited species involved in this study, which produce large fruit-crops synchronously at particular time intervals (Herrera *et al.* 1998).

Besides yearly fluctuations of understory fruits and differences in plant–animal and plant–plant interactions, our 4-year data set shows the temporal persistency of seed-deposition sites, with the canopy of parasitized trees consistently receiving large mistletoe seed rain while microsites beneath these trees receive high seed inputs of understory species. In generalist systems, mistletoes, because of their extremely narrow range of safe sites for recruitment (Reid 1989; Sargent 1995; Norton & Ladley 1998; Mellado & Zamora 2014b), and understory species because of their high seed- and seedling-mortality rates (Zamora *et al.* 2010; Matías, Zamora & Castro 2012), have to withstand heavy seed wastage in order to find a single successful recruitment event. Thus, abundant and repeated seed-dispersal events are necessary to ensure a small fraction of recruits on the host canopy (Mellado & Zamora 2014a) and understory recruits in soil microsites (Howe & Mirith 2004; Hampe *et al.* 2008). Moreover, as seeds constantly reach the same deposition site over long time periods, these sites could become hotspots for community regeneration within the landscape (Hampe *et al.* 2008).

Concluding remarks

In conclusion, by patchily growing in the canopy layer and concentrating zoothorophous seed rain in parasitized trees, mistletoes can shape the spatial deposition pattern of zoothorophous seeds in the forest, which may be reflected in the future configuration of the plant community. In degraded areas, this ability of mistletoes to concentrate zoothorophous seeds might be of prime importance, as it could promote recolonization and revegetation recovery through frugivore activity (Watson 2009). In the case of pine plantations, mistletoes could allow their conversion into mixed forests, as many Mediterranean woody species have a comparatively higher probability to persist in the understory of plantations, benefitting from the mild microclimate generated by the canopy (Gómez-Aparicio 2009; Mendoza, Zamora & Castro 2009; Zamora *et al.* 2010). This has

far-reaching ecological implications when we consider the large area covered by pine plantations in the European continent, characterized by a homogeneous spatial structure and monospecific composition. In these scenarios, mistletoes introduce diversity of zoothorophous species with the seed rain and heterogeneity in their spatial distribution, breaking the prevailing species monotony of most pine-lands while introducing new assembly rules within the plant community. As a final remark, it bears mentioning the geographic breadth of the system under consideration, with *Viscum album* and Mistle thrushes expanding from Northern to Southern Europe (Snow & Snow 1984; Zuber 2004), frequently accompanied by other fleshy-fruited plant species. Thus, it would not be fanciful to speculate that mistletoe-mediated seed-dispersal patterns of fleshy-fruited plant communities may be occurring at large geographical scales, driving, with each set of regional peculiarities, many aspects of the community and ecosystem dynamics. For future advancements, it would be of key interest to delve into the influence that mistletoe spatial heterogeneity exerts over more complex plant-frugivore systems (such as tropical forests), using long-term and multispecies approaches.

Acknowledgements

We are especially grateful to Ramón Ruiz Puche for his excellent work in the field, David Watson and an anonymous reviewer for their comments on the article, David Nesbitt for checking the English of this paper, and Enrique Mellado for making Fig. 1. We thank the Consejería de Medio Ambiente (Junta de Andalucía) and the Direction of the Sierra de Baza Natural Park for granting permission to conduct the field work. This study was supported by the Spanish MICINN Projects MUERDAME CGL2008-04794 and CLAVINOVA CGL2011-29910 and a FPI grant BES-2009-023219 to AMG from the Spanish Ministry of Science and Innovation (MICINN).

Data accessibility

Data deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.3m342> (Mellado & Zamora 2015).

References

- Aukema, J.E. (2004) Distribution and dispersal of desert mistletoe is scale-dependent, hierarchically nested. *Ecography*, **27**, 137–144.
- Aukema, J.E. & Martínez del Río, C. (2002) Where does a fruit-eating bird deposit mistletoe seeds? Seed deposition patterns and an experiment. *Ecology*, **83**, 3489–3496.
- Bascompte, J., Jordano, P., Melián, C.J. & Olesen, J.M. (2003) The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences*, **100**, 9383–9387.
- Bates, D., Maechler, M. & Dai, B. (2008) The lme4 Package. <http://lme4.rforge.r-project.org/>
- Blendinger, P.G., Blake, J.G. & Loiselle, B.A. (2010) Composition and clumping of seeds deposited by frugivorous birds varies between forest microsites. *Oikos*, **120**, 463–471.
- Burns, A.E., Cunningham, S.A. & Watson, D.M. (2011) Arthropod communities in tree canopies: an ordinal comparison between assemblages on mistletoes and their eucalypt hosts. *Australian Journal of Entomology*, **50**, 221–230.
- Candia, A.B., Medel, R. & Fontúrbel, F.E. (2014) Indirect positive effects of a parasitic plant on host pollination and seed dispersal. *Oikos*, **123**, 1371–1376.
- Carlo, T.A. (2005) Interspecific neighbors change seed dispersal pattern of an avian-dispersed plant. *Ecology*, **86**, 2440–2449.

- Carlo, T.A. & Aukema, J.E. (2005) Female-directed dispersal and facilitation between a tropical mistletoe and a dioecious host. *Ecology*, **86**, 3245–3251.
- Carlo, T.A. & Morales, J.M. (2008) Inequalities in frugivory and seed dispersal: consequences of bird behavior, neighbourhood density and landscape aggregation. *Journal of Ecology*, **96**, 609–618.
- Clark, C.J., Poulsen, J.R., Connor, E.F. & Parker, V.T. (2004) Fruiting trees as dispersal foci in a semi-deciduous tropical forest. *Oecologia*, **139**, 66–75.
- Fedriani, J.M. & Boulay, R. (2006) Foraging by fearful frugivores: combined effect of fruit ripening and predation risk. *Functional Ecology*, **20**, 1070–1079.
- Gómez-Aparicio, L. (2009) The role of plant interactions in the restoration of degraded ecosystems: a meta-analysis across life-forms and ecosystems. *Journal of Ecology*, **97**, 1202–1214.
- 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.
- Herrera, C.M. (1984) A study of avian frugivores, bird-dispersed plants and their interaction in Mediterranean scrublands. *Ecological Monographs*, **54**, 1–23.
- Herrera, J.M., Morales, J.M. & García, D. (2011) Differential effects of fruit availability and habitat cover for frugivore-mediated seed dispersal in a heterogeneous landscape. *Journal of Ecology*, **99**, 1100–1107.
- Herrera, C.M., Jordano, P., Guitián, J. & Traveset, A. (1998) Annual variability in seed production by woody plants and the masting concept: reassessment of principles and relationship to pollination and seed dispersal. *American Naturalist*, **152**, 576–594.
- Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. *Biometrical Journal*, **50**, 346–363.
- Howe, H.F. (1979) Fear and frugivory. *American Naturalist*, **114**, 925–931.
- Howe, H.F. & Miriti, M.N. (2004) When seed dispersal matters. *BioScience*, **54**, 651–660.
- Kwit, C., Levey, D.J. & Greenberg, C.H. (2004) Contagious seed dispersal beneath heterospecific fruiting trees and its consequences. *Oikos*, **107**, 303–308.
- Larson, D.L. (1996) Seed dispersal by specialist versus generalist foragers: the plant's perspective. *Oikos*, **76**, 113–120.
- Lovett, G.M., Jones, C.G., Turner, M.G. & Weathers, K.C. (2005) *Ecosystem Function in Heterogeneous Landscapes*. Springer, New York.
- Matías, L., Zamora, R. & Castro, J. (2012) Rare rainy events are more critical than drought intensification for woody recruitment in Mediterranean mountains: a field experiment simulating climate change. *Oecologia*, **169**, 833–844.
- Medel, R., Vergara, E., Silva, A. & Kalin-Arroyo, M. (2004) Effects of vector behavior and host resistance on mistletoe aggregation. *Ecology*, **85**, 120–126.
- Mellado, A. & Zamora, R. (2014a) Generalist birds govern the seed dispersal of a parasitic plant with strong recruitment constraints. *Oecologia*, **176**, 139–147.
- Mellado, A. & Zamora, R. (2014b) Linking safe sites for recruitment with host-canopy heterogeneity: the case of a parasitic plant, *Viscum album* subsp. *austriacum* (Viscaceae). *American Journal of Botany*, **101**, 1–8.
- Mellado, A. & Zamora, R. (2015) Data from: spatial heterogeneity of a parasitic plant drives the seed-dispersal pattern of a zochorophyte plant community in a generalist dispersal system. *Dryad Digital Repository*, doi:10.5061/dryad.3 m342.
- Mendoza, I., Zamora, R. & Castro, J. (2009) A seeding experiment for testing tree-community recruitment under variable environments: implications for forest regeneration and conservation in Mediterranean habitats. *Biological conservation*, **142**, 1491–1499.
- Morales, J.M., Rivarola, M.D., Amico, G.C. & Carlo, T.A. (2012) Neighborhood effects on seed dispersal by frugivores: testing theory with a mistletoe-marsupial system in Patagonia. *Ecology*, **93**, 741–748.
- Ndagurwa, H.G., Dube, J.S., Mlampo, D. & Mawanza, M. (2014) The influence of mistletoes on the litter-layer arthropod abundance and diversity in a semi-arid savanna, Southwest Zimbabwe. *Plant and Soil*, **383**, 291–299.
- Norton, D.A. & Ladley, J.J. (1998) Establishment and early growth of *Alepis flavidula* in relation to *Nothofagus solandri* branch size. *New Zealand Journal of Botany*, **36**, 213–217.
- Prasad, S. & Sukumar, R. (2010) Context-dependency of a complex fruit-frugivore mutualism: temporal variation in crop size and neighborhood effects. *Oikos*, **119**, 514–523.
- R Development Core Team. 2012. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org
- Reid, N. (1989) Dispersal of mistletoes by honeyeaters and flowerpeckers: components of seed dispersal quality. *Ecology*, **70**, 137–145.
- Restrepo, C., Sargent, S., Levey, D.J. & Watson, D.M. (2002) The role of vertebrates in the diversification of New World mistletoes. *Seed dispersal and frugivory: ecology, evolution and conservation* (eds D.J. Levey, W.R. Silva & M. Galetti), pp. 83–98. CABI, Wallingford, Oxfordshire.
- Saracco, J.F., Collazo, J.A., Groom, M.J. & Carlo, T.A. (2005) Crop size and fruit neighborhood effects on visitation to fruiting trees. *Biotropica*, **37**, 80–86.
- Sargent, S. (1995) Seed fate in a tropical mistletoe: the importance of host twig size. *Functional Ecology*, **9**, 197–204.
- Skaug, H., Fournier, D., Nielsen, A., Magnusson, A. & Bolker, B. (2012) Generalized Linear Mixed Models using AD Model Builder. R package version 0.7.2.12
- Skórka, P. & Wójcik, J.D. (2005) Population dynamics and social behavior of the Mistle Thrush *Turdus viscivorus* during winter. *Acta Ornithologica*, **40**, 35–42.
- Snow, B.K. & Snow, D.W. (1984) Long-term defense of fruit by Mistle Thrushes (*Turdus viscivorus*). *Ibis*, **126**, 339–349.
- Van Ommeren, R.J. & Whitham, T.G. (2002) Changes in interactions between juniper and mistletoe mediated by shared avian frugivores: parasitism to potential mutualism. *Oecologia*, **130**, 281–288.
- Watson, D.M. (2001) Mistletoe—a keystone resource in forests and woodlands worldwide. *Annual Review of Ecology and Systematics*, **32**, 219–249.
- Watson, D.M. (2009) Parasitic plants as facilitators: more Dryad than Dracula? *Journal of Ecology*, **97**, 1151–1159.
- Watson, D.M. & Herring, M. (2012) Mistletoe as a keystone resource: an experimental test. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 1–8.
- Watson, D.M., McGregor, H.W. & Spooner, P.G. (2009) Hemiparasitic shrubs increase resource availability and multi-trophic diversity of eucalypt forest birds. *Functional Ecology*, **150**, 889–899.
- Zamora, R., Hódar, J.A., Matías, L. & Mendoza, I. (2010) Positive adjacency effects mediated by seed disperser birds in pine plantations. *Ecological Applications*, **20**, 1053–1060.
- Zuber, D. (2004) Biological flora of Central Europe: *Viscum album* L. *Flora*, **199**, 181–203.
- Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R*. Springer, New York.

Received 2 February 2015; accepted 20 July 2015

Handling Editor: Jenny Watling