

Host compatibility interacts with seed dispersal to determine small-scale distribution of a mistletoe in Xishuangbanna, Southwest China

Yahuang Luo^{1,2}, Yi Sui^{1,2}, Jianmin Gan¹ and Ling Zhang^{1,*}

¹ Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan 666303, China

² University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing 100049, China

*Correspondence address. Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China. Tel: +86-691-8715948; Fax: +86-691-8715070; E-mail: zhangl@xtbg.org.cn

Abstract

Aims

Mistletoe infection between intra- and interspecific hosts can be restricted by seed dispersal, host–mistletoe compatibility and other factors, yet few studies have linked seed dispersal and seedling establishment together for understanding mistletoe plant distribution and demography together in different anthropogenic disturbance forest types at a local scale. The objectives of this study were to examine how three factors—seed disperser behavior, post-dispersal host compatibility and canopy cover—affect the spatial distribution of a generalist mistletoe *Dendrophthoe pentandra* (Loranthaceae) in plantation and rainforest within Xishuangbanna, Southwest China.

Methods

We observed mistletoe *D. pentandra* infection patterns at the scale of individual trees and sixteen 400-m² forest plots in adjacent plantation and rainforest within Xishuangbanna. To elucidate what determines infection patterns at different scales and in different forest types, we observed the behavior of major avian seed dispersers and carried out a seed inoculation experiment to examine how post-dispersal compatibility and light incidence affect the infection of different hosts.

Important Findings

Dendrophthoe pentandra displayed an aggregated distribution and infected 10 species in our study site, with a significantly

higher infection prevalence and intensity in the plantation than in the tropical forest. Different seed dispersers provided contrasting initial mistletoe templates: the specialist frugivore *Dicaeum concolor* (plain flowerpecker) preferred to fly between mistletoes in infected trees in the plantation and likely intensified existing infections. In contrast, the dietary generalist *Pycnonotus jocosus* (red-whiskered bulbul) was more likely to visit uninfected trees, thereby establishing new infections. Thus, seed dispersal appears to be an important determinant of the mistletoes distribution, with deposition patterns providing an initial distribution template and determining small-scale patterns. However, post-dispersal and abiotic factors revealed that different host compatibilities and levels of light incidence in different habitats affected the survival of *D. pentandra* seedlings. Hence, our findings suggest that seed dispersal interacts with host compatibility and canopy cover to determine establishment success, survival and the observed distribution patterns.

Keywords: aggregation, frugivory, establishment, seedling survival, host–parasite interaction

Received: 6 August 2014, Revised: 18 January 2015, Accepted: 23 January 2015

INTRODUCTION

Mistletoes are common aerial-stem hemiparasites that frequently depend on frugivores for seed dispersal and their host plants for water and nutrients (Reid *et al.* 1995). They

are important components of most plant assemblages and affect the dynamics, diversity and structure of plant communities (March and Watson 2007; Watson 2009a; Watson *et al.* 2011). Mistletoes provide a link between their hosts, pollinators and frugivores and mediate a series of direct and

indirect competitive and facilitative effects on ecological processes (Watson 2009a). Therefore, issues such as the spatial distribution of mistletoes, their occurrence in plantations and interactions with hosts and vectors and overall relationship with habitats are important to understanding forest dynamics, especially given profound changes in structure due to habitat loss, degradation and fragmentation (Norton and Reid 1997).

Mistletoes exhibit wide variation in the degree of host specificity with some parasitizing only one or a few congeneric host species, while others show very little specificity, even at the family level (Norton and Reid 1997). Host specificity of mistletoes differs between geographic regions (Aukema and Martínez del Río 2002a; Norton and Carpenter 1998), even at the community level (Gibson and Watkinson 1989; Norton and Delange 1999), possibly reflecting differences in host compatibility between different regions (Snyder *et al.* 1996).

Despite the wide distribution of mistletoes in habitats ranging from tropical rainforests and mangroves to arid shrublands (Shaw *et al.* 2004), most research on distribution patterns and dispersal has focused on semiarid habitats, largely neglecting tropical regions (Rist *et al.* 2011; Watson 2001). In species-rich tropical forests, the density of individual host species is likely to be lower than in temperate or arid regions, so relative host density or host specificity may differ, with implications for mistletoe distributions (Norton and Carpenter 1998; Real and McElhany 1996). The habitat diversity and structure may be major factors influencing the distribution pattern of mistletoes, affecting behavior of mistletoe dispersers, where birds move preferentially among sites and have different host preferences in different forest types. With the increase of plantations in tropical areas, mistletoe distribution patterns may change because differences in plant community structure may influence the behavior and diversity of dispersers at local scales. Therefore, we need a broader understanding of mistletoe dispersal and establishment patterns across habitat types in different plant communities including plantations and at small scales. However, very few studies have linked seed dispersal and seedling establishment for understanding mistletoe plant distribution and plant demography together in different anthropogenic disturbance forest types at a local scale, viewing the seed dispersal process as a continuous loop (Wang and Smith 2002).

To investigate how changes in host compatibility, seed disperser behavior, post-seed dispersal processes and forest structure influence the distribution of mistletoes, we chose the generalist mistletoe *Dendrophthoe pentandra* (Loranthaceae) in Xishuangbanna, Southwest China, as a study system. In particular, we addressed the following questions: (i) Do the distribution patterns of *D. pentandra* in plantations and tropical forests differ? (ii) Do host characteristics (host abundance, diameter at breast, height and crown diameter) affect the infection pattern of *D. pentandra*? (iii) How do different seed dispersal and post-seed dispersal processes influence host infection of *D. pentandra* across different forest types at the local scale?

MATERIALS AND METHODS

Study area and species

We conducted our study in the experimental field area of Xishuangbanna Tropical Botanical Garden, Menglun (21°56'N, 101°15'E, 580 m asl), Yunnan Province, Southwest China. In this region, mean annual rainfall is 1500 mm, and the mean annual temperature is 21.8°C. Xishuangbanna has a tropical monsoon climate, which is divided into three seasons: the foggy cool season (November–February), the dry hot season (March–April) and the rainy season (May–October). The predominant vegetation types are tropical rainforest, tropical seasonal rainforest, evergreen broadleaved monsoon forest and bamboo tree mixed forest (Zhu *et al.* 2006). *Dendrophthoe pentandra* is a predominantly tropical mistletoe species and is common in Southeast Asia and Australia. In Xishuangbanna, they parasitize up to 360 host species, belonging to 223 genera and 71 families (Xiao and Pu 1988) and flower from January to February with fruits ripening from the middle of March to June. *Dendrophthoe pentandra* fruits are 12.41 ± 1.20 mm in length and 6.87 ± 0.49 mm in width ($n = 30$) and contain a single seed surrounded by viscin.

Spatial distribution pattern

To determine the distribution of *D. pentandra*, four 1-ha blocks (each 100 m × 100 m) 100–300 m apart were selected in Xishuangbanna Tropical Botanical Garden. In each block, four plots (each 20 m × 20 m) were established ca. 60 m apart. Blocks 1 and 2 were located in a plantation, composed of *Mangifera indica*, *Lucuma nervosa*, *Citrus maxima* and *Adenanthera pavonina*. Blocks 3 and 4 were located in a tropical forest ~35–40 years following indigenous selective cutting, where the most common species in Block 3 were *Phoebe lanceolata*, *Syzygium jambos*, *Pittosporopsis kerrii*, *Millettia leptobotrya* and in block 4 were *Paramichelia bailonii*, *Anogeissus acuminata*, *M. leptobotrya* and *Olea europaea*. In each plot, we identified all host trees taller than 2 m, because mistletoe does not grow on smaller host trees, and then determined which trees were infected by *D. pentandra*. We recorded three size parameters of the hosts: tree height, tree DBH (diameter at breast height) and tree crown diameter. We then calculated the infection prevalence and intensity, where 'infection prevalence' was defined as the proportion of trees of that species infected, while 'infection intensity' was the number of mistletoe plants on each host tree (Aukema 2004). The overall level of aggregation was also determined by calculating the variance to mean ratio of mistletoe numbers per host (Young and Young 1998). The canopy cover, diversity and evenness of the entire plant community were also calculated.

Seed dispersal and deposition

We employed the point count method (Bibby *et al.* 2000) to observe mistletoe frugivory in each of the four blocks during the fruiting season (mid-March to June) in 2011 and 2012. A point count station was located at the central point of each

block. To survey the bird community, point counts of 20 min were conducted during times of high bird activity (once between 08:30 and 11:00 h and once between 15:00 and 18:00 h for a point station). After finishing one point count station, we moved to a new station; when arriving at a point count station, we waited 1–2 min for bird activity to resume and then recorded all bird species and the number of individuals seen within a 50-m fixed radius from the point station, not including flying birds. The order of visiting the points was regularly rotated to minimize possible surveying order and time biases on bird presence and absence at each point, and each point was repeated on 10 clear days. To understand the relationship between the bird dispersers and the mistletoe, we recorded the infection status of trees (infected or not infected) on which birds perched during the point counts.

Then using the focal point observation method (Bibby et al. 2000), we observed bird foraging behaviors within our study block for 6 clear days, from 08:30 to 18:30 h. To do this, we followed individual birds using 10×50 binoculars for as long as possible within the study block. We recorded the number of instances they visited an infected host tree, the time they spent foraging on mistletoe fruits and the locations of bird-visited trees within our view. We compared the two most common frugivorous bird species, *Dicaeum concolor* (plain flowerpecker) and *Pycnonotus jocosus* (red-whiskered bulbuls), in their tendencies to visit infected or uninfected host trees and in the time they spent in the host tree.

To examine variation in the number of seeds deposited on interspecific hosts, we selected five common host species infected by *D. pentandra*, ensuring that there were no significant differences in tree height or crown diameter. We recorded the number of deposited mistletoe seeds on four infected trees and four uninfected trees for each species, by climbing each tree, using ladders when necessary. We counted all mistletoe seeds on all branches, cross-checking between at least two people, yielding a total mistletoe seed count for each tree.

Seed inoculation experiment

To investigate the post-dispersal role of the host species and environmental heterogeneity in determining the distribution of *D. pentandra*, we conducted a seed inoculation experiment, focusing on eight dominant host species and eight species that are low in abundance in the plant community. Seven (four dominant) of the 16 species were parasitized by *D. pentandra*, and nine species were unparasitized by *D. pentandra* in the study area (Supplementary Table S1). Hereafter, we use the term ‘susceptible’ or ‘non-susceptible’ to describe those tree species which do or do not act as hosts for mistletoes, following Watson (2009b). We collected 960 fresh mistletoe seeds from three *M. indica* trees, removed the exocarp to stick seeds in place with a natural viscin (Lamont 1983; López de Buen and Ornelas 2002) and then inoculated branches of the focal trees that were 3–5 m tall in our study plots. We applied sets of 20 seeds on each of three branches (1.0–1.5 cm diameter) of uninfected trees, with each set of experimental seeds being

arranged linearly along the branch and separated from each other by at least 2 cm. All seeds were ‘planted’ in this manner in May 2011 and seed fate was then monitored for 1 year every second day during the seed adhesion and germination stages (1–2 weeks) and at weekly intervals thereafter.

According to Lamont’s (1983) method, *D. pentandra* has four post-dispersal phenological phases: seed adhesion, seed germination, seedling establishment and seedling survival. Adhesion was estimated as the percentage of the number of seeds planted, germination as the percentage of the number of seeds that adhered, seedling establishment as the percentage of the seeds that germinated and seedling survival from 12 to 52 weeks as the percentage of the seedlings that established. Seed germination was defined as seeds with a plumule >0.5–1 cm long, seedling establishment as seedlings in which the haustorium had penetrated the host vascular tissue and seedling survival as established seedlings that continued to live and grow normally.

Statistical analyses

We used a Poisson-distributed generalized linear model (GLM) to examine the relationship between infection prevalence and intensity of *D. pentandra* and the variance to mean ratio of mistletoe numbers per species in relation to relative abundance, average height and crown diameter. Analysis of variance was used to determine whether there was any difference in the number of seeds deposited on host trees of a species or among different host species. To investigate whether avian dispersers differed in their perching rates on infected and uninfected trees in the four study blocks, we used non-parametric chi-square methods. Spearman correlation was used to test whether there was a relationship between the deposition of seeds and the infection intensity of each tree and between the mistletoe infection pattern (prevalence and intensity) in the community and the mistletoe seedling survival, and particular tree canopy cover. All the above analyses were conducted using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Differences in the adhesion, germination, establishment and survival of *D. pentandra* seeds between different host species (potential susceptible/non-susceptible) and forest types (plantation/forest) were analyzed using GLM, and host compatibility was nested within forest types; this analysis was performed in R 3.1.0 (R Development Core Team 2011).

RESULTS

Mistletoe infection pattern

The species richness and tree densities of plants in the forest (88/477) were much higher than in the plantation (19/94) (Table 1). However, the number of infected species and trees was higher in the plantation (7/50) than in the forest (3/14), with the plantation having consequently a much higher infection rate (43.53%) than the forest (4.11%) (Table 2).

In the plantation, the average infection intensity was 2.50 ± 0.64 , ranging from 0 to 5.43, and mistletoes were

aggregated within trees (variance: mean ratio = 6.42 ± 2.86). In contrast, in the forest, the average infection intensity (0.6 ± 0.5) and variance (0.99 ± 0.93) was very low. Canopy cover in the plantation was much lower than in the forest (Table 1).

The results of the GLM showed that the infection intensity, prevalence and variance to mean ratio of mistletoes were significantly related to relative abundance of each host species in the plantation ($n = 10$, $r_1 = 0.749$, $P_1 = 0.0003$; $r_2 = 0.719$,

$P_2 = 0.0077$; $r_3 = 0.8018$, $P_3 = 0.00006$) (Fig. 1), but no relationship with DBH, height and crown diameter of host trees was found.

Infected host trees received a significantly greater number of mistletoe seeds than uninfected trees (Fig. 2), and within these trees, the number of deposited seeds was correlated with infection intensity of adult mistletoes ($n = 40$, $r_2 = 0.904$, $P < 0.001$) (Fig. 2). We also found a large difference between host species in the number of seeds deposited ($F_{4,35} = 9.69$, $P < 0.001$), ranging from 0 to 347 seeds.

Table 1: host tree density, canopy cover and the infection prevalence and intensity of *Dendrophthoe pentandra* in the study plots

	Plantation	Forest
Plot characteristics		
Number of plots	8	8
Plant species richness	3.5 ± 0.9	16.5 ± 2.2
Number of trees	11.8 ± 2.2	59.6 ± 9.4
Shannon index	0.87 ± 0.20	2.27 ± 0.18
Plant community evenness	0.80 ± 0.05	0.83 ± 0.04
Infection pattern		
Number of infected host species	1.5 ± 0.4	0.4 ± 0.2
Number of infected host individuals	6.3 ± 1.67	1.8 ± 1.5
Infection intensity	2.5 ± 0.6	0.6 ± 0.5
Infection prevalence (%)	43.53 ± 11.29	4.11 ± 3.50
Variance:mean ratio	6.42 ± 2.86	0.99 ± 0.93
Canopy cover (%)	36.3 ± 4.9	78.5 ± 3.9

'Infection prevalence' was defined as the proportion of trees of that species infected, while 'infection intensity' was defined as the number of mistletoe plants on each host tree. All results were showed as mean \pm SE.

Table 2: infection parameters of the mistletoe *Dendrophthoe pentandra* in two types of forests at Xishuangbanna tropical botanical garden, China

Host species	Family	Height (m)	Crown diameter (m)	Plantation		Forest	
				Prevalence (%)	Intensity	Prevalence (%)	Intensity
<i>Mangifera indica</i>	Anacardiaceae	5.8 ± 1.3	3.2 ± 1.1	80.0 (24/30)	4.4 ± 1.8	NA	NA
<i>Citrus maxima</i>	Rutaceae	3.4 ± 1.0	1.8 ± 0.8	61.1 (11/18)	2.5 ± 0.5	NA	NA
<i>Adenanthera pavonina</i>	Leguminosae	9.3 ± 3.9	3.3 ± 1.5	60.0 (6/10)	2.8 ± 0.5	NA	NA
<i>Lucuma nervosa</i>	Sapotaceae	5.0 ± 0.9	3.4 ± 0.9	100 (5/5)	4.8 ± 1.5	NA	NA
<i>Alstonia scholaris</i>	Apocynaceae	3.8 ± 1.2	4.1 ± 0.8	33.3 (2/6)	2.0 ± 0	NA	NA
<i>Castanopsis mekongensis</i>	Fagaceae	7.3 ± 0.9	2.8 ± 0.5	33.3 (1/3)	13	0 (0/5)	0
<i>Melastoma candidum</i>	Melastomaceae	3.1 ± 0.6	1.1 ± 0.2	33.3 (1/3)	1	NA	NA
<i>Paramichelia bailonii</i>	Magnoliaceae	14.5 ± 4.8	4.1 ± 1.4	NA	NA	46.15 (12/26)	4.4 ± 0.8
<i>Machilus bombycina</i>	Lauraceae	11.9 ± 6.8	4.5 ± 2.0	NA	NA	33.33 (1/3)	1
<i>Ficus superba</i>	Moraceae	3.5	4	NA	NA	100 (1/1)	1
Total				50/94		14/477	

The prevalence of infection for each host species and the number of mistletoe per parasitized (mean \pm SE) host tree are exhibited. NA: not applicable.

Seed disperser behavior

We recorded eight frugivorous or nectarivorous bird species in our four observation sites during the focal observation period, including *D. concolor*, *P. jocosus*, *Pycnonotus aurigaster*, *Megalaima asiatica*, *Dicaeum ignipectus*, *Dicaeum cruentatum*, *Zosterops japonica* and *Aethopyga siparaja*, with 236 visits in total, of which 146 visits were to infected host trees (the remaining 90 visits were in uninfected trees) and 94 to mistletoe plants. *Dicaeum concolor* (plain flowerpecker) was the main consumer of *D. pentandra* (in total 142 visits/120 visiting infected host trees/80 visiting mistletoe plants) and *P. jocosus* (red-whiskered bulbuls) were the secondary consumers (in total 67 visits/19 visiting infected host trees/11 visiting mistletoe plants).

Both these two frugivores were observed swallowing mistletoe seeds after separating and discarding fruit peels, but these two frugivores exhibited different movement behavior relative to the mistletoes. *Dicaeum concolor* individuals were most frequently encountered perching in infected host trees (84.7% infected vs. 15.3% uninfected, $n = 249$) and spent more time visiting infected host trees (mean = 147 ± 82 s) than uninfected trees (66 ± 30 s) ($F_{1,247} = 45.82$, $P < 0.001$). More than 80% of *D. concolor* individuals that were observed flew

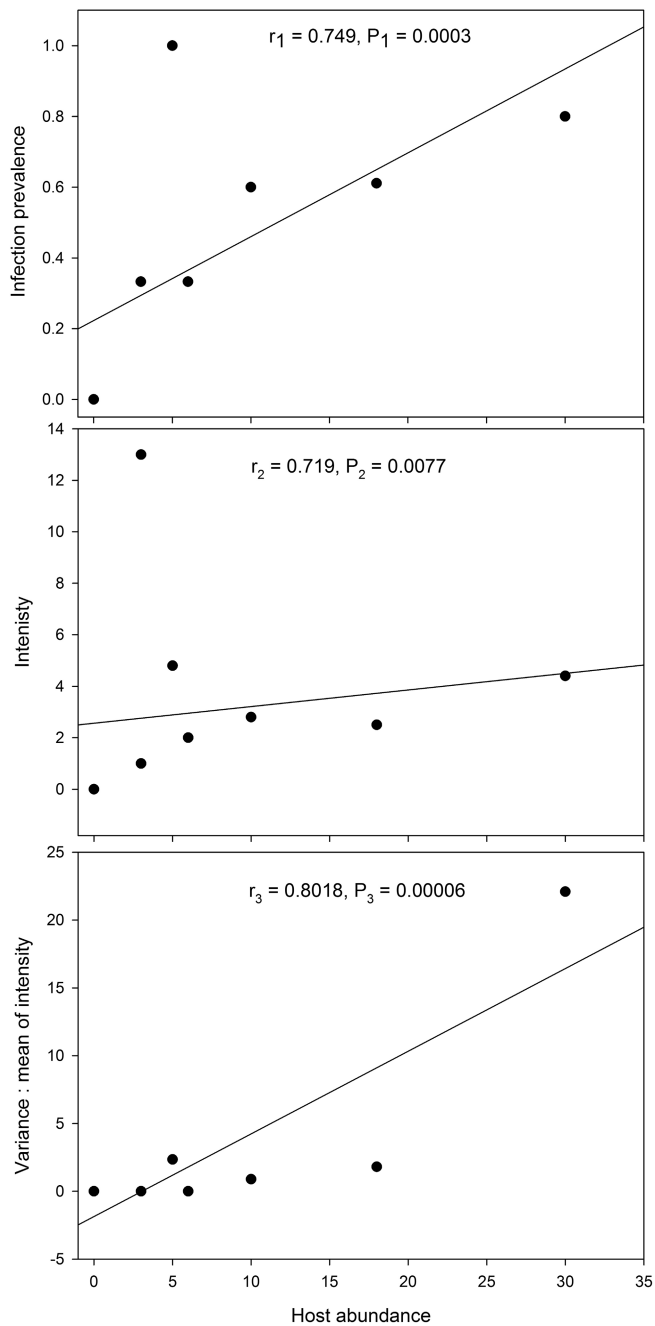


Figure 1: scatter plot of the relationship between infection prevalence, intensity and variance: mean of intensity and host abundance in the plantation.

around *D. pentandra*-infected host trees, and visitation rate was not correlated with the abundance of tree species either in the plantation ($\chi^2 = 39.94$, $df = 9$, $P < 0.001$) or in the forest ($\chi^2 = 12.14$, $df = 5$, $P = 0.033$). Most individuals visited trees infected with mistletoe for <2 min (56.23% of the visits) with a mean length of a visit as 69.0 ± 14.4 s. We also found that the estimated abundance of *D. concolor* varied widely between plots and was greatest in the plantation ($\chi^2 = 34.797$, $df = 3$, $P < 0.001$; Fig. 3). In contrast, *P. jocosus* were most frequently

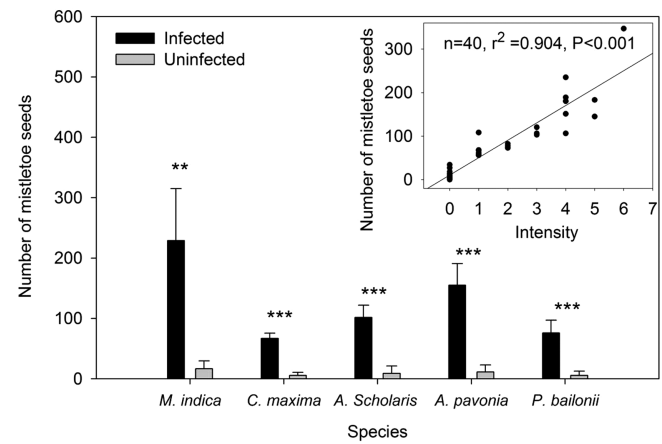


Figure 2: number of *Dendrophthoe pentandra* seeds deposited on five common host species (mean \pm SE, $n = 4$), and the correlation between infection intensity and the number of seeds deposited (** $P < 0.01$, *** $P < 0.001$).

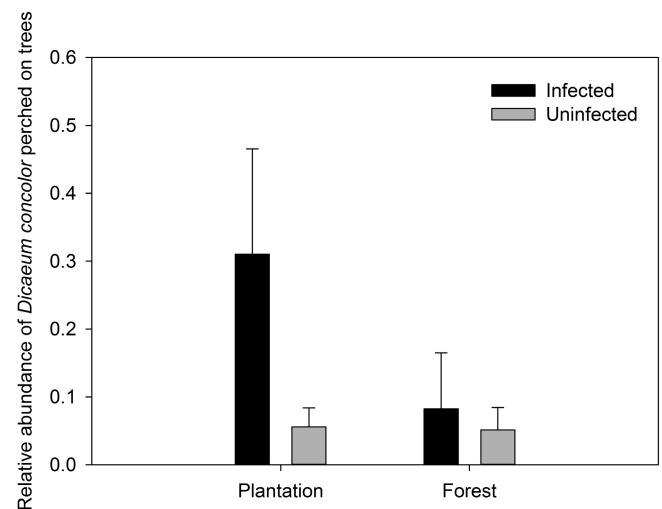


Figure 3: relative abundance of the main disperser, *Dicaeum concolor* ($n = 142$), perching on infected and uninfected trees in the two study forest types.

encountered in uninfected trees (35.8% infected vs. 64.2% uninfected, $n = 159$). However, when *P. jocosus* did visit a mistletoe, it tended to stay a long time (216 ± 79 s mean visitation length), with 40% of their visits to trees infected with mistletoe being longer than 4 min. This species did not show a difference between the amount of time it stayed in infected trees and the time it spent in uninfected trees (207 ± 101 s) ($F_{1,157} = 1.013$, $P = 0.722$).

Host compatibility of different species

In total, $56.0 \pm 14.5\%$ ($n = 16$) of seeds adhered per branch (Fig. 4A). Our results showed that the adherence rate of mistletoe seeds was affected by forest type, with more seeds successfully attaching in the plantation ($61.1 \pm 11.2\%$) than in the forest ($50.8 \pm 15.8\%$) ($F = 6.199$, $P = 0.017$). The

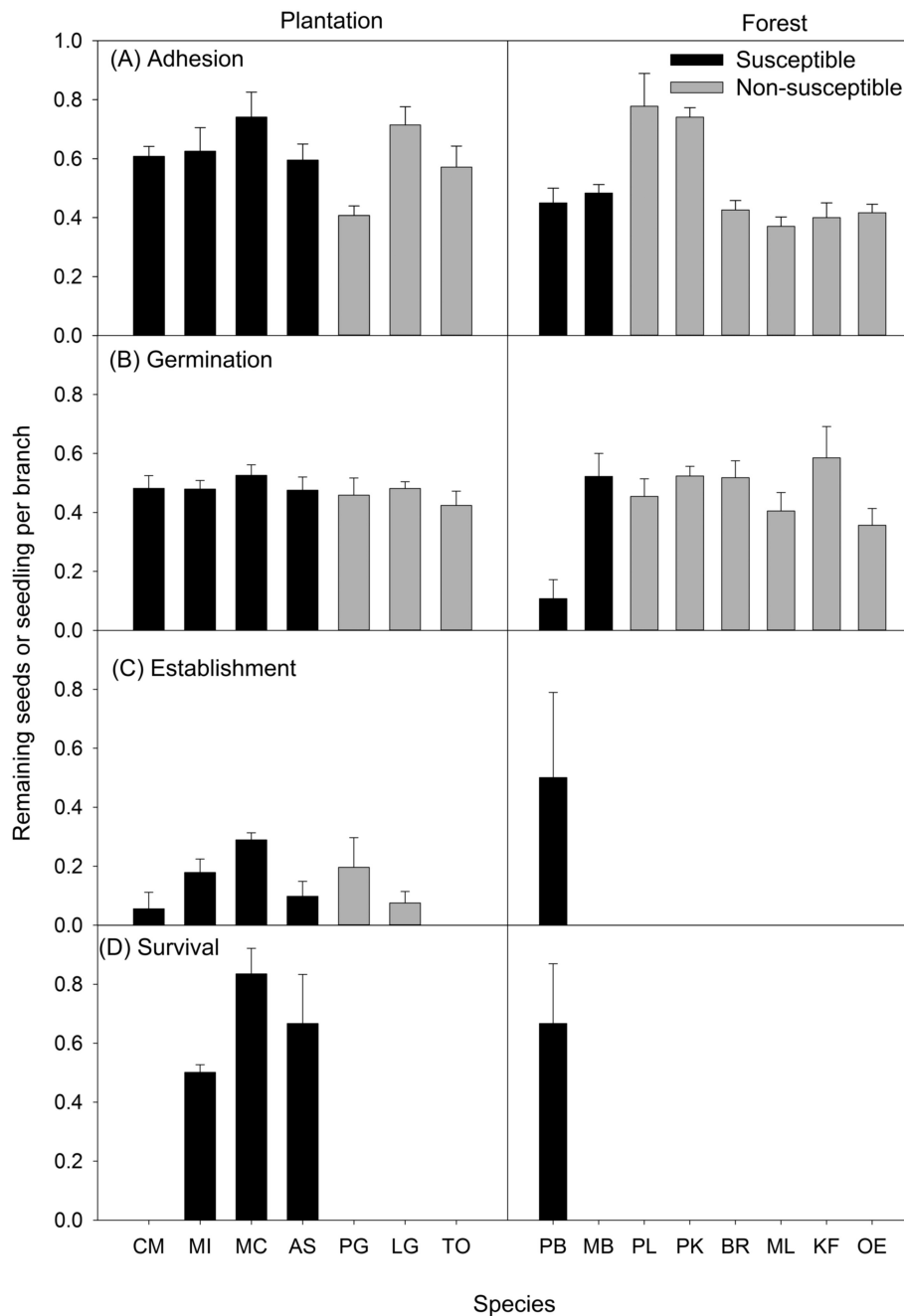


Figure 4: the percentage of seeds or seedlings of *Dendrophthoe pentandra* on each branch following seed inoculation experiments on 16 tree species in the plantation and forest, showing (A) seed adhesion (percentage of the number of seeds planted), (B) seed germination (percentage of the number of seeds adhered), (C) seedling establishment (percentage of germinated seeds) and (D) seedling survival (percentage of seeds established). In the plantation, CM = *Citrus maxima*, MI = *Mangifera indica*, MC = *Melastoma candidum*, AS = *Alstonia scholaris*, PG = *Psidium guajava*, LG = *Litsea glutinosa* and TO = *Tsoongiodendron odorum*. In the forest, PB = *Paramichelia bailonii*, MB = *Machilus bombycina*, PL = *Phoebe lanceolata*, PK = *Pittosporopsis kerrii*, BR = *Baccaurea ramiflora*, ML = *Millettia leptobotrya*, KF = *Knema furfuracea* and OE = *Olea europaea*.

seed inoculation experiment showed no significant difference in the percentage of seed adherence between susceptible and non-susceptible tree species within each forest type ($F = 1.209$, $P = 0.308$) (Table 3).

The average percentage of seed germination across species was $45.5 \pm 13.3\%$ ($n = 16$) (Fig. 4B). The GLM showed that

there was significant difference between susceptible and non-susceptible species in each forest type ($F = 3.843$, $P = 0.029$), with susceptible species fostering higher seed germinations. However, there was no significant difference in seed germination between the plantation ($47.6 \pm 7.0\%$) and forest ($43.4 \pm 17.4\%$) ($F = 3.796$, $P = 0.058$).

Table 3: results of GLM investigating the effect of forest and host compatibility on mistletoe *Dendrophthoe pentandra* seed adhesion, germination, seedling establishment and survival

	df	SS	MS	F	P
Adhesion					
Forest type	1	0.115	0.115	6.199	0.017
Compatibility (forest type)	2	0.045	0.022	1.209	0.308
Germination					
Forest type	1	0.059	0.059	3.796	0.058
Compatibility (forest type)	2	0.12	0.6	3.843	0.029
Establishment					
Forest type	1	1.21E6	1.21E6	4.44E5	0.995
Compatibility (forest type)	2	0.309	0.154	5.661	0.006
Survival					
Forest type	1	0.07	0.07	0.712	0.403
Compatibility (forest type)	2	1.911	0.955	9.7	<0.001

Host compatibility was divided into potential susceptible and non-susceptible hosts, which was nested within forest type. Forest type was divided into plantation and tropical forest. Values in bold are significant ($P < 0.05$).

Seedling establishment rate decreased quickly after 12 weeks, with only 9.83% of germinated seeds penetrating the haustorium into host tissue (Fig. 4C). Forest type had no significant effect on seedling establishment ($F = 4.44E5$, $P = 0.995$), but more seedlings established on susceptible species ($F = 5.661$, $P = 0.006$) (Table 3).

Mistletoe seedling survival declined significantly after 12 months. The analysis of forest survival was not significant ($F = 0.712$, $P = 0.403$). However, host compatibility had a significant effect on seedling survival ($F = 9.7$, $P < 0.001$) (Table 3), with more seedlings surviving on susceptible host species. Only 15 mistletoe seedlings survived on susceptible host trees and no mistletoe seedlings survived on non-susceptible species after 12 months (Fig. 4D).

Mistletoe seedlings established and survived better on host trees growing in areas where the canopy was more open. Our results showed that infection prevalence of *D. pentandra* was correlated with both seedling survival ($r = 0.853$, $P < 0.001$) and canopy cover ($r = -0.526$, $P = 0.036$) of the trees, and the intensity was also correlated with both seedling survival ($r = 0.798$, $P < 0.001$) and canopy cover ($r = -0.513$, $P = 0.042$) of the trees in our study site.

DISCUSSION

The investigations of spatial distribution and post-seed dispersal of a generalist mistletoe from southwest China indicated that mistletoes aggregated more within trees in a plantation, with infection prevalence and number of host species infected by mistletoes significantly higher in the plantation than in the forest at tree and plot scale, despite there being many more species and tree individuals in the forest. Two seed dispersers provided different initial distribution templates,

thus influencing spatial patterns at the tree and plot scales. However, additional elements such as post-dispersal and abiotic factors may also be important in different forest communities affecting the survival of *D. pentandra* seedlings during the mistletoe's early life. Below we will discuss how these features influenced the spatial distribution patterns in the two forest types in more detail.

Infection pattern: forest type and hosts

Aggregated distributions at multiple spatial scales have been documented in many other species of mistletoe (Aukema 2004; Aukema and Martínez del Rio 2002a, 2002b; Kelly 1998; Overton 1994, 1996; Rist et al. 2011). Mistletoes have been shown to have low host specificity in heterogeneous tropical rainforests (Norton and Carpenter 1998), where there tends to be greater species richness but a lower relative abundance of any one potential host species. Host community composition can fundamentally influence the establishment and prevalence of mistletoes, and many investigations have illustrated that mistletoe distribution is correlated with the abundance (number of individuals) of host species in the plant community (Aukema and Martínez del Rio 2002b; Norton and Carpenter 1998; Press and Phoenix 2005). Some studies have showed that overall seedling survival may not differ at varying local seed densities and suggested that mistletoes do not have a dense 'seedling shadow' under the parent mistletoe (e.g. Kelly et al. 2007).

Our data demonstrated a relationship between the abundance of individual hosts and mistletoe infection in the plantation but not in the forest. Although *D. pentandra* is regarded as a generalist mistletoe, having been reported to infect more than 360 host species (Xiao and Pu 1988), only 7 out of 19 species in the plantation and 3 out of 88 species in the tropical forest were infected by *D. pentandra* in our study, which suggests that host species richness and abundance could not explain infection prevalence at the plot scale.

The prevalence and intensity of mistletoes on intra- and interspecific hosts differ across temporal and spatial scales (Aukema 2004; Norton and Carpenter 1998; Yan 1993), due to variation in disperser behavior (Aukema and Martínez del Rio 2002b; Roxburgh and Nicolson 2005), the capability of mistletoes to use hosts (López de Buen and Ornelas 2002; Norton and Carpenter 1998) and even tolerance to the environment (Aukema 2004). We found that the generalist mistletoe *D. pentandra* preferred to use particular host species, with the pattern of host use differing between the two forest types investigated. It seems that the host–mistletoe compatibility, bird preference and seed dispersal directly influenced the spatial distribution of *D. pentandra*, as explained below.

Infection patterns driven by mistletoe seed disperser intensity

Many studies have demonstrated that frugivore foraging and dispersal behaviors could cause aggregation in mistletoe infection at a range of scales (Aukema and Martínez del Rio 2002a,

2002b; Overton 1994; Ward and Paton 2007). Mistletoe specialists and generalists may both be effective seed dispersers (Carlo and Aukema 2005; Davidar 1983; Rawsthorne *et al.* 2011, 2012). Some studies have found that specialists can intensify the infections within previously infected hosts (Aukema and Martínez del Río 2002a, 2002b; Overton 1994; Watson 2013), while dietary generalists (e.g. *Acanthagenys rufogularis*, Meliphagidae) can transport seeds well beyond the boundaries of an existing mistletoe infection (Rawsthorne *et al.* 2011).

Dendrophthoe pentandra fruits are consumed by few bird species, and *D. concolor* and *P. jocosus* were the major important seed dispersers in the study area according to our observations. The mistletoe specialist *D. concolor* (Davidar 1983; Rawsthorne *et al.* 2011; Watson 2001) was mainly active in the plantation, and preferred to visit and spend more time feeding on infected trees, resulting in the infected trees gaining more seeds and intensifying existing infections within the plantation. Similar underlying mechanisms have been highlighted in other systems (Aukema 2004; Aukema and Martínez del Río 2002a, 2002b; Rawsthorne *et al.* 2012; Reid 1991). Specialist flowerpeckers usually have small body sizes (our data: 6–8 g) and have relatively shorter gut passage times (e.g. *Dicaeum hirundinaceum*, 7.5–11 g, Dunning 2008; mean seed retention times of 14 min, Murphy *et al.* 1993) than honeyeaters (e.g. *Acanthagenys refogulari*, 39–57 g, Dunning 2008; mean seed retention times of 41 min, Murphy *et al.* 1993) and yellow-vented bulbuls (*Pycnonotus xanthopygus*, 31–43 g, Dunning 2008; mean seed retention times of 19.3 min, Green *et al.* 2009), and *D. concolor* are also territorial around clumps of fruiting mistletoes, restricting their feeding to these areas (Davidar 1983). Consequently, this species may have a short dispersal range and may contribute to the smaller scale of aggregation in the plantation.

In contrast, the dietary generalist *P. jocosus* (26–43 g, Linnebjerg *et al.* 2009) is more likely to visit uninfected trees for social interactions and self-maintenance activities, such as preening, but tend to spend more time foraging on mistletoe fruits and usually fly long distances. These dietary generalists are more likely to establish new infections through long distance seed dispersal. Thus, it is likely that mistletoe specialist (*D. concolor*) and generalist (*P. jocosus*) species provide different quantity and the quality of dispersal for mistletoe *D. pentandra*. Since different dispersers vary in their effectiveness of mistletoe seed dispersal (Montaño-Centellas 2013; Murphy *et al.* 1993; Okubamichael *et al.* 2009), further detailed study of this dispersal system is needed to understand the dispersal potential of these species.

Infection patterns driven through mistletoe–host compatibility

Mistletoe–host compatibility is a function of both host susceptibility to infection and the infectivity of mistletoe (Yan 1993), which is one of the most important forces driving intraspecific variation in mistletoe infection rates (López de Buen and

Ornelas 2002). Both host compatibility and environmental variation may modify the post-dispersal distribution of mistletoe (Bach *et al.* 2005; Rödl and Wade 2002). The four post-dispersal stages of mistletoe infection (seed adhesion, seed germination, seedling establishment and seedling survival) can be used to investigate host–mistletoe compatibility and distribution patterns.

Our results showed that there was no difference in seed adhesion between infected and uninfected hosts that occurred in the plantation or the forest; therefore, this stage was not crucial determinants of mistletoe compatibility with the host. Forest type had an effect on seed adhesion, with adhesion rates higher in the plantation than the forest. However, these mean adherence rates were not very different from each other (61% versus 51%), and it should be noted that such a difference, although statistically significant, may not be that biologically meaningful. Species compatibility did not influence adhesion. However, in our inoculation experiment, we did find that more *D. pentandra* seeds became attached to rough-barked tree species (such as *P. lanceolata*, *Melastoma candidum* and *Litsea glutinosa*) than smooth-barked species (e.g. *Psidium guajava*), and therefore, we speculate that bark smoothness may be a major cause of early seed loss.

In our inoculation experiment, only 45.5% of *D. pentandra* seeds germinated in the field. These rates are quite low compared to previous studies. For example, Norton and Ladley (1998) got germination of 82% (nearly all the rest fell off, so germination of those adhering was nearly 100%), and Norton *et al.* (2002) got 96–97% germination. Buen and Ornelas also got high germination (80–90%) of *Psittacanthus schiedeianus*, whereas Ladley and Kelly (1996) got 69–82% germination of five different mistletoes species. In our study site, there were not a lot of susceptible species in the plantation, and this may be one of the reasons for the low germination (Fig. 4). Our previous seed germination experiments have also showed that study site temperature and light conditions may have influenced mistletoe seed germination (Luo and Zhang 2013). In addition, Norton *et al.* (2002) suggested that host provenance can be a factor influencing the germination of mistletoe in some host–mistletoe systems. In summary, the reasons for the low germination rates we present here are still not entirely clear, and further studies about the mechanism of low germination are needed.

Due to the variation in penetration and resistance ability of *D. pentandra* on different tree species, significantly more seedlings established on susceptible species than non-susceptible species in our study. Several factors may contribute to seedling establishment on hosts, including host compatibility, the density at which seeds are deposited and the light incidence (López de Buen and Ornelas 2002; López de Buen *et al.* 2002; Norton *et al.* 2002; Rödl and Wade 2002; Sargent 1995). Our results indicated that the seedlings survived better on trees with more open canopies in the plantation (13–49%), implying light incidence may limit the post-dispersal growth of this species, which is consistent with other previous studies

(López de Buen and Ornelas 2002; Sargent 1995). The seedling survival of *D. pentandra* was significantly higher on susceptible trees than on non-susceptible trees, suggesting that both host compatibility and canopy cover could explain the pattern of *D. pentandra* infection prevalence in our study site. However, there was a tendency ($P = 0.058$) for germination to be higher in the plantation than the forest, and it should be noted that there was only one species of susceptible species in the plantation, making it unclear which factors drove these results or whether chance events may explain them. Our seedling data allow us to follow the full process of seed dispersal, what Wang and Smith (2002) described as ‘the seed dispersal loop’, from the production of the seeds through to seedling recruitment.

Other factors, such as bark thickness, density of seed clumping, canopy cover and some herbivore predators, can affect seedling survival and establishment (López de Buen and Ornelas 2002; Sargent 1995; Yan 1993). However, in our inoculation experiment, we did not find any mistletoe seedlings that were predated by herbivores. Yet only a few seedlings survived on the infected host species (also see Kelly et al. 2007); this suggests that other variables may be attributed to failed mistletoe establishment, and a longer-term study is needed to better understand the mechanisms behind this pattern. Our findings suggest that the variation in post-dispersal processes on different host species is the key factor determining the degree of host specialization in mistletoes, and that this specificity is variable in both space and time, and affects the spatial distribution in different plant communities. However, we need to study more mistletoe species to really understand host specialization pattern in mistletoes.

In short, this study indicated that the generalist mistletoe *D. pentandra* aggregated more in infected host trees in the plantation than in the tropical forest. This finding suggests that the seed deposition process (including seed production) provides an initial distribution template, with the specialist disperser *D. concolor* intensifying existing infections and generalist dispersers, such as *P. jocosus*, potentially establishing new infections. Moreover, additional post-dispersal processes, such as different host compatibilities and canopy cover in different forest communities, affected the survival of *D. pentandra* seedlings during the species’ early life. Our results demonstrate that seed dispersal and plant demography are inextricably linked in affecting the observed distribution patterns of *D. pentandra* (Wang and Smith 2002). We need to consider these two processes together in order to understand important ecological issues, such as parasite–host–vector relationships, directed dispersal, the relationship between genetic diversity and host specificity and mistletoe population size, and hence develop effective management strategies.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

FUNDING

National Science Foundation of China (31170406); the key project of the Chinese Academy of Science (KSCX2-EW-Q-17); Queensland—Chinese Academy of Sciences (QCAS) Biotechnology Fund (GJHZ1130).

ACKNOWLEDGMENTS

The authors thank Mr Qiaoshun Li from Xishuangbanna Tropical Botanical Garden for field assistance; Prof. Jin Chen, Prof. Xiaodong Yang and Dr Eben Goodale from Xishuangbanna Tropical Botanical Garden for constructive discussion and comments and Ms Pelin Kayaalp for improving English.

Conflict of interest statement. None declared.

REFERENCES

- Aukema JE (2004) Distribution and dispersal of desert mistletoe is scale-dependent, hierarchically nested. *Ecography* **27**:137–44.
- Aukema JE, Martínez del Rio C (2002a) Where does a fruit-eating bird deposit mistletoe seeds? Seed deposition patterns and an experiment. *Ecology* **83**:3489–96.
- Aukema JE, Martínez del Rio C (2002b) Variation in mistletoe seed deposition: effects of intra- and interspecific host characteristics. *Ecography* **25**:139–44.
- Bach CE, Kelly D, Hazlett BA (2005) Forest edges benefit adults, but not seedlings of the mistletoe *Alepis flavida* (Loranthaceae). *J Ecol* **93**:79–86.
- Bibby CJ, Burgess ND, Hill DH, et al. (2000) *Bird Census Techniques*, 2nd edn. London, UK: Academic Press.
- Carlo TA, Aukema JE (2005) Female-directed dispersal and facilitation between a tropical mistletoe and a dioecious host. *Ecology* **86**:3245–51.
- Davidar P (1983) Birds and neotropical mistletoes: effects on seedling recruitment. *Oecologia* **60**:271–3.
- Dunning JB (2008) *CRC Handbook of Avian Body Mass*, 2nd edn. Boca Raton, FL: CRC Press.
- Gibson CC, Watkinson AR (1989) The host range and selectivity of a parasitic plant: *Rhinanthus minor* L. *Oecologia* **78**:401–6.
- Green AK, Ward D, Griffiths ME (2009) Directed dispersal of mistletoe (*Plicosepalus acaciae*) by Yellow-vented Bulbuls (*Pycnonotus xanthopygus*). *J Ornithol* **150**:167–73.
- Kelly D (1998) Spatial clumping of *Tupeia antarctica* at Wainui. *Canterbury Bot Soc J* **32**:62–5.
- Kelly D, Ladley JJ, Robertson AW (2007) Is the pollen-limited mistletoe *Peraxilla tetrapetala* (Loranthaceae) also seed-limited? *Austral Ecol* **32**:850–7.
- Ladley JJ, Kelly D (1996) Dispersal, germination and survival of New Zealand mistletoes (Loranthaceae): dependence on birds. *New Zeal J Ecol* **20**:69–79.
- Lamont B (1983) Germination of mistletoes. In Calder M, Bernhardt P (eds). *The Biology of Mistletoes*. Sydney, Australia: Sydney Academic Press, 129–43.
- Linnebjerg JF, Hansen DM, Olesen JM (2009) Gut passage effect of the introduced red-whiskered bulbul (*Pycnonotus jocosus*) on germination of invasive plant species in Mauritius. *Austral Ecol* **34**:272–7.

- López de Buen L, Ornelas JF (2002) Host compatibility of the cloud forest mistletoe *Psittacanthus schiedeana* (Loranthaceae) in Central Veracruz, Mexico. *Am J Bot* **89**:95–102.
- López de Buen L, Ornelas JF, García Franco JG (2002) Mistletoe infection of trees located at fragmented forest edges in the cloud forests of Central Veracruz, Mexico. *For Ecol Manage* **164**:293–302.
- Luo YH, Zhang L (2013) Germination characteristics of *Dendrophthoe pentandra* seeds. *Plant Divers Resour* **35**:73–80.
- March WA, Watson DM (2007) Parasites boost productivity: effects of mistletoe on litterfall dynamics in a temperate Australian forest. *Oecologia* **154**:339–47.
- Montaño-Centellas FA (2013) Effectiveness of mistletoe seed dispersal by tyrant flycatchers in a mixed Andean landscape. *Biotropica* **45**:209–16.
- Murphy SR, Reid N, Yan Z, *et al.* (1993) Differential passage time of mistletoe fruits through the gut of honeyeaters and flowerpeckers: effects on seedling establishment. *Oecologia* **93**:171–6.
- Norton DA, Carpenter MA (1998) Mistletoes as parasites: host specificity and speciation. *Trends Ecol Evol* **13**:101–5.
- Norton DA, Delange PJ (1999) Host specificity in parasitic mistletoes (Loranthaceae) in New Zealand. *Funct Ecol* **13**:552–9.
- Norton DA, Ladley JJ (1998) Establishment and early growth of *Alepis flavida* in relation to *Nothofagus solandri* branch size. *New Zeal J Bot* **36**:213–7.
- Norton DA, Ladley JJ, Sparrow AD (2002) Host provenance effects on germination and establishment of two New Zealand mistletoes (Loranthaceae). *Funct Ecol* **16**:657–63.
- Norton DA, Reid N (1997) Lessons in ecosystem management from management of threatened and pest Loranthaceae mistletoes in New Zealand and Australia. *Conserv Biol* **11**:759–69.
- Okubamichael DY, Rasheed MZ, Griffiths ME, *et al.* (2009) Avian consumption and seed germination of the hemiparasitic mistletoe *Agelanthus natalitius* (Loranthaceae). *J Ornithol* **152**:643–9.
- Overton JM (1994) Dispersal and infection in mistletoe metapopulations. *J Ecol* **82**:711–23.
- Overton JM (1996) Spatial autocorrelation and dispersal in mistletoes: field and simulation results. *Vegetatio* **125**:83–98.
- Press MC, Phoenix GK (2005) Impacts of parasitic plants on natural communities. *New Phytol* **166**:737–51.
- R Development Core Team (2011) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Rawsthorne J, Watson DM, Roshier DA (2011) Implications of movement patterns of a dietary generalist for mistletoe seed dispersal. *Austral Ecol* **36**:650–5.
- Rawsthorne J, Watson DM, Roshier DA (2012) The restricted seed rain of a mistletoe specialist. *J Avian Biol* **43**:9–14.
- Real LA, McElhany P (1996) Spatial pattern and process in plant-pathogen interactions. *Ecology* **77**:1011–25.
- Reid N (1991) Coevolution of mistletoes and frugivorous birds? *Aust J Ecol* **16**:457–69.
- Reid N, Stafford Smith M, Yan Z (1995) Ecology and population biology of mistletoes. In Lowman MD, Nadkarni NM (eds). *Forest Canopies*. San Diego, CA: Academic Press, 285–310.
- Rist L, Uma Shaanker R, Ghazoul J (2011) The spatial distribution of mistletoe in a southern Indian tropical forest at multiple scales. *Biotropica* **43**:50–7.
- Rödl T, Wade D (2002) Host recognition in a desert mistletoe: early stages of development are influenced by substrate and host origin. *Funct Ecol* **16**:128–34.
- Roxburgh L, Nicolson SW (2005) Patterns of host use in two African mistletoes: the importance of mistletoe-host compatibility and avian disperser behaviour. *Funct Ecol* **19**:865–73.
- Sargent S (1995) Seed fate in a tropical mistletoe: the importance of host twig size. *Funct Ecol* **9**:197–204.
- Shaw D, Watson D, Mathiasen R (2004) Comparison of dwarf mistletoes (*Arceuthobium* spp., Viscaceae) in the western United States with mistletoes (*Amyema* spp., Loranthaceae) in Australia—ecological analogs and reciprocal models for ecosystem management. *Aust J Bot* **52**:481–98.
- Snyder MA, Fineschi B, Linhart YB, *et al.* (1996) Multivariate discrimination of host use by dwarf mistletoe *Arceuthobium vaginatum* subsp. *cryptopodum*: inter- and intraspecific comparisons. *J Chem Ecol* **22**:295–305.
- Wang BC, Smith TB (2002) Closing the seed dispersal loop. *Trends Ecol Evol* **17**:379–86.
- Ward MJ, Paton DC (2007) Predicting mistletoe seed shadow and patterns of seed rain from movements of the mistletoe bird, *Dicaeum hirundinaceum*. *Austral Ecol* **32**:113–21.
- Watson DM (2001) Mistletoes—a keystone resource in forests and woodlands worldwide. *Annu Rev Ecol Syst* **32**:219–49.
- Watson DM (2009a) Parasitic plants as facilitators: more Dryad than Dracula? *J Ecol* **97**:1151–9.
- Watson DM (2009b) Determinants of parasitic plant distribution: the role of host quality. *Botany* **87**:16–21.
- Watson DM (2013) The relative contribution of specialists and generalists to mistletoe dispersal: insights from a Neotropical rain forest. *Biotropica* **45**:195–202.
- Watson DM, McGregor HW, Spooner PG (2011) Hemiparasitic shrubs increase resource availability and multi-trophic diversity of eucalypt forest birds. *Funct Ecol* **25**:889–99.
- Xiao LY, Pu ZH (1988) An investigation on the harm of the Loranthaceae in Xishuangbanna, Yunnan. *Acta Bot Yunn* **10**:69–78.
- Yan Z (1993) Germination and seedling development of two mistletoes, *Amyema preissii* and *Lysiana exocarpis*: host specificity and mistletoe–host compatibility. *Aust J Bot* **18**:419–29.
- Young LJ, Young J (1998) *Statistical Ecology: A Population Perspective*. Boston, MA: Kluwer Academic Publishers.
- Zhu H, Cao M, Hu HB (2006) Geological history, flora, and vegetation of Xishuangbanna, Southern Yunnan, China. *Biotropica* **38**:310–7.