

Uncorrelated mistletoe infection patterns and mating success with local host specialization in *Psittacanthus calyculatus* (Loranthaceae)

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Abstract Mistletoe infection between conspecific and interspecific hosts can be restricted by seed dispersal, host-mistletoe compatibility and abiotic factors, yet no studies have linked mistletoe infection patterns and pollination together for understanding mistletoe distribution at a local scale. *Psittacanthus calyculatus* (Loranthaceae) is a hemiparasitic plant with a broad host range across its geographic distribution. The potential for local host adaptation has been shown using cross-inoculation experiments, in which plants of mistletoe seeds collected from a given host are more likely to survive when they are inoculated on conspecific host trees compared with those inoculated on other host provenances. Here we evaluate host adaptation by describing the local patterns of infection (prevalence and intensity) of *P. calyculatus* mistletoes on three native host tree species (*Alnus acuminata*, *Quercus crassipes*, *Salix bonplandiana*) and one introduced species (*Populus alba*) and carried out cross-pollination experiments to examine how pollination affects infection patterns of different host species. Mistletoe infection prevalence (proportion of infection) and infection intensity (mean number of mistletoes per tree) were in general disproportional with respect to the availability of native host tree species but higher to that of non-native host tree species. Cross-pollination experiments showed higher mating success on the native host tree species, suggesting higher local adaptation to specially *Q. crassipes*. The observed spatial distribution of host tree species and mistletoe infection along with the non-random mating could contribute to local genetic structuring of mistletoe populations.

Keywords *Psittacanthus* · Spatial distribution · Host availability · Cross pollination · Host adaptation · Host range · Mexico

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Introduction

Parasites that complete their life cycle on a single host individual are suitable systems to study speciation resulting from a differential use of host species (Price 1980; Norton and Carpenter 1998; Poulin 2007). Race formation through host specialization is one avenue to eventually species formation in sympatry because host specialization to particular host species selects for barriers to reproduction (see Norton and Carpenter 1998; Poulin 2007). Alternatively, non-overlapping ecologies between sympatric host species could isolate groups of parasites leading to speciation (Perkins 2001; McCoy et al. 2003; Criscione et al. 2005). There are several cases reported in the literature of incipient sympatric speciation occurring via the evolution of host specialization, particularly in insects (e.g., Bush 1969; Knerer and Atwood 1972; Phillips and Barnes 1975; Jermy 1984; Craig et al. 2001) and mites (e.g., Magalhães et al. 2007). Studies on parasitic plants exploring the existence of host-specific relationships, using cross-inoculation experiments (e.g., Clay et al. 1985; Glazner et al. 1988) or molecular markers (e.g., Jerome and Ford 2002a, b), have highlighted host adaptation as the initial step of differentiation in the speciation process of sympatric mistletoes via host switching.

Parasitic plants account for 1 % of angiosperm species (c. 4000) within c. 270 genera and more than 20 plant families (Nickrent et al. 1998). Mistletoe parasitism of Lorantheaceae constitutes a continuum of host specificity, ranging from specialist mistletoes typically restricted to a single host species to generalist mistletoes that use various host species without apparent preference (broad host range) (Reid 1989; Monteiro et al. 1992; Reid et al. 1995; Norton and Carpenter 1998). Mistletoe host specificity usually declines as new hosts are colonized along its range (Reid et al. 1995; Poulin 2007) or host preference changes within different areas of their geographic range (Norton and de Lange 1999; Mathiasen et al. 2008). In many cases, mistletoes tend to be host generalists in regions with a high diversity of tree species and host specialists in those of low diversity (Kavanagh and Burns 2012). Although most parasitic mistletoes seem to behave as host generalists, they can show host specialization at a regional scale (Norton and Carpenter 1998).

According to the ‘host specialization’ hypothesis (Norton and Carpenter 1998), mistletoes infect the most abundant tree species in a given community because of the advantages to interact more profitably with a frequently encountered host (Norton and Carpenter 1998; Kavanagh and Burns 2012). These include an increased mistletoe’s efficiency in capturing resources from the host, the location of potential mates, a reduction in the probability of local extinction, or the potential for pollination and seed dispersal (Norton and Carpenter 1998). Also, host tree specialization might occur when mistletoes interact locally with suboptimal hosts that improve its fitness (e.g., Ward 1992). Under all these scenarios, co-occurring host tree species would show disproportional mistletoe infection in relation to their abundance in a given area (Press and Phoenix 2005).

Mistletoe-host specialization has been usually investigated from the perspective of the mistletoe, i.e. host selection by the parasite is mediated by compatibility mechanisms between the parasite and the host (e.g., host structure and wood characteristics, allocation of resources provided by the host; Lamont 1983; Clay et al. 1985; Sargent 1995; Cocolletzi et al. 2016). However, mistletoe host selection is also determined by ecological factors such as the spatial distribution of potential hosts, local host tree diversity and relative abundance of host tree species (López de Buen et al. 2002; Aukema and Martínez del Río 2002a; Medel et al. 2002; Arruda et al. 2006; Teodoro et al. 2010; Kavanagh and Burns 2012; Roura-Pascual et al. 2012), or by those derived from the interaction between mistletoes and their pollinators and seed dispersers (Bennetts et al. 1996; Martínez del Río

et al. 1996; López de Buen and Ornelas 1999; Aukema and Martínez del Río 2002b; Arruda et al. 2006; Roxburgh and Nicolson 2005; Troncoso et al. 2010; Lemaitre et al. 2012). Some researchers have evaluated the mistletoes' host preferences along the generalization/specialization continuum by describing mistletoe infection patterns, and mixed results had been found in support of the 'host specialization' hypothesis explaining host specificity patterns (e.g., Monteiro et al. 1992; Yan and Reid 1995; López de Buen and Ornelas 1999, 2002; Norton and de Lange 1999; Azpeitia and Lara 2006; Teodoro et al. 2010; Fadini 2011; Arce-Acosta et al. 2016).

Several mistletoe species described as host generalists have been shown to be specialists at the local level, in which the mistletoe species use a large number of host species over its geographic distribution but are specific to only a few hosts in any one area (Clay et al. 1985; Norton et al. 1995; Norton and de Lange 1999). The occurrence of local host specificity suggests that differences in host utilization might play an important role in mistletoe speciation via the two-steps *host-switching* process (mistletoe race formation; Norton and Carpenter 1998). First, the mistletoe must include a new host species in its host range, typically a phylogenetic or ecologically similar to those already utilized. Second, the parasite has to adapt to the new host by restricting itself from previous hosts (Norton and Carpenter 1998; Poulin 2007). Therefore, mistletoe race formation might occur with an initial decrease in host specificity and subsequent increase in host specificity as specialization to the new host occurs (Norton and Carpenter 1998). Nonetheless, the host-switch leading to mistletoe race formation could also occur if the abundance of the host species changes or if the mistletoe expands its range into an area where relative host abundance differ to those normally encountered (Norton and Carpenter 1998). Lastly, mistletoe speciation can also occur in response to host speciation if both populations of the mistletoe and its hosts became geographically isolated, regardless of levels of host specificity (allopatric speciation; Norton and Carpenter 1998).

The existence of host-specific races has been suggested through cross-dispersal experiments for a number of mistletoe species (e.g., May 1971; Clay et al. 1985; Overton 1997; Norton et al. 2002; Lara et al. 2009; Ramírez and Ornelas 2009, 2012; Okubamichael et al. 2014). These experiments have shown that seedling development is greatest when mistletoe seeds are placed on their source host species, regardless of gut processing by seed dispersers (Clay et al. 1985; López de Buen and Ornelas 2002; Rödl and Ward 2002; Lara et al. 2009; Ramírez and Ornelas 2009, 2012; Okubamichael et al. 2014). Using genetic markers, the distinct host races identified were geographically isolated by some distance (Glazner et al. 1988; Nickrent and Butler 1990, 1991; Nickrent and Stell 1990; Linhart et al. 2003; Jerome and Ford 2002a, b; Amico and Nickrent 2009; Zuber and Widmer 2009; Yule et al. 2016) or the mistletoe phylogeographic structure was best explained by geographic isolation linked to past geological events and climate changes (Amico et al. 2012; Lira-Noriega et al. 2015; Ornelas et al. 2016). Therefore, these studies indicate that the potential effects of geographic isolation on host race formation are difficult to distinguish from those linked to local differences in host utilization. When mistletoes infect different host species, their mating, dispersal, and establishment can be random with respect to host species (permeable barriers to gene flow), and the accumulated genetic differences are vanished in a panmictic mistletoe population. However, gene flow can be interrupted or diminished if mating, dispersal, and establishment occur only among mistletoes adapted to specific hosts (i.e., reproductive isolation), in which genetic differences between groups of mistletoe populations (host races) would accumulate, sharpening boundaries between them, and leading to evolutionary divergence and eventually speciation of each mistletoe lineage (Rieseberg and Wendel 2004; Rieseberg and Willis 2007).

The purpose of this study is to investigate the potential for host specialization of the host generalist mistletoe *Psittacanthus calyculatus* (DC.) G. Don (Loranthaceae) to host tree species in sympatry, by assessing its spatial host infection pattern and host adaptation through evaluation of its mating success. Specifically, the following questions were asked: (1) is the pattern of mistletoe infection and abundance related to host availability as predicted by the host specialization hypothesis? (2) Are spatial patterns of mistletoe infection explained by the local distribution and infection of host species? And (3), is there a potential for local host adaptation through mating success differences of the mistletoes growing on different host species? According to the host specialization hypothesis, the most abundant host species is expected to be the most infected by the mistletoes but local host adaptation is not necessarily expected as infection patterns are mediated by the foraging and defecation behaviors of seed-dispersal vectors. Second, if potential for host adaptation exists then mating success would be higher among mistletoe individuals within any host species with respect to random mating.

Materials and methods

Study species

Psittacanthus calyculatus is a mistletoe characteristic of the temperate forest edges along the Trans-Mexican Volcanic Belt, from sea level up to 2600 m above sea level (Kuijt 2009). The self-compatible, bright orange-to-red flowers of these mistletoes produce large amounts of nectar, last five days, and are mainly pollinated by hummingbirds (Azpeitia and Lara 2006; Díaz Infante et al. 2016), and its ripe purplish-black, fleshy (one-seed) fruits are consumed by a variety of bird species (Lara et al. 2009; Díaz Infante et al. 2016). Inflorescences produce 100–200 flowers that open asynchronously from July to November (Azpeitia and Lara 2006; Díaz Infante et al. 2016). The current distribution of *P. calyculatus* is potentially related to the availability of suitable host trees species and its distributional range historically influenced by geographical host range changes. This hemiparasite lives on several host tree species, with more than 50 tree species reported to be parasitized, including species of diverse genera such as *Quercus*, *Acacia*, *Populus*, *Salix*, *Prunus*, *Prosopis*, *Celtis*, *Bursera*, *Fraxinus*, *Persea*, *Pseudospondingium*, and *Ulmus* (Kuijt 2009; Lara et al. 2009; Zuria et al. 2014; Arce-Acosta et al. 2016; Díaz Infante et al. 2016). Despite the broad host range of *P. calyculatus*, cross-infection experiments have suggested local adaptation of these parasites to their hosts, in which germination and seedling establishment rates of bird gut-processed mistletoes were more likely when mistletoes were inoculated on their source host trees (Lara et al. 2009). However, evaluation of local host adaptation through specifically designed cross-pollination experiments (i.e. female reproductive success) had not been performed.

Study site

The study was conducted in July 2013 at the ex-Hacienda Santa Agueda, W slope of the La Malinche volcano at Tetlatlahuca, Tlaxcala, Mexico (19°10'56.3"N, 98°17'25.40"W and 19°14'04.7"N, 98°16'26.2"W; at 2200 m above sea level). The study area has a mean annual temperature of 16 °C, ranging between 7.2 and 24.3 °C, and a mean annual precipitation of 762 mm, with a minimum of 6.3 mm in February and a maximum of 165 mm

in June (Instituto Nacional para el Federalismo y Desarrollo Municipal, <http://www.inafed.gob.mx/work/enciclopedia/EMM29tlaxcala/municipios/29032a.html>, latest access 16 July 2015). The study area (780 ha) is characterized by remnants of riparian vegetation composed of *Salix bonplandiana* Kunth and *Alnus acuminata* Kunth syn. *A. jorullensis* var. *acuminata* (Kunth) Regel, immersed in a matrix of secondary vegetation (*Crataegus mexicana* DC., *Populus alba* L., *Prunus serotina* var. *capuli* (Cav.) McVaugh, *Schinus molle* L., *Casuarina equisetifolia* L.) and crop fields (*Zea mays* L., *Medicago sativa* L.) under natural irrigation, with edge roads, riparian vegetation, natural fences and wind-breaks (Instituto Nacional para el Federalismo y Desarrollo Municipal, <http://www.inafed.gob.mx/work/enciclopedia/EMM29tlaxcala/municipios/29032a.html>, latest access 16 July 2015).

In Tlaxcala, 17 angiosperm species are known to be parasitized by *P. calyculatus* including *Alnus acuminata*, *Salix bonplandiana*, *S. babylonica*, *Populus* sp., *Quercus* sp., *Crataegus mexicana*, *Persea americana*, *Prunus serotina*, and *Malus domestica* (Azpeitia and Lara 2006). The availability, spatial distribution (host tree species and infection) and intensity of *P. calyculatus* infection were quantified for two native, *Alnus acuminata* and *Salix bonplandiana*, and one introduced species, *Populus alba*. An additional host tree species, *Quercus crassipes* Humb. & Bonpl., which is found in the surroundings (<3 km), at Rancho La Herradura, San Andres Coamilpa, Tetlatlahuca, Tlaxcala, Mexico (19°15'38.7"N, 98°16'00.4"W; at 2300 m above sea level) was also included to assess spatial distribution of host and infection, and to test host adaptation. This species does not occur in the plots where the other tree species were found and, therefore, it was not included in evaluations of host availability and preference patterns of infection.

Host availability and infection preference

To assess host availability of *A. acuminata*, *P. alba* and *S. bonplandiana*, the 40 ha at the ex-Hacienda Santa Agueda were divided into a grid of 50 × 50 m plots and numbered from 1 to 160. A subsample of 17 non-adjacent plots was then randomly chosen and, in each of these plots, trees with a diameter at breast height (DBH) >10 cm were identified. Infection (binary response) and the number of mistletoes plants per tree in each of the chosen plots were recorded. Availability of host and prevalence of infection (the percentage of infected host trees per species) in the study area were estimated (Norton and de Lange 1999; Dzerefos et al. 2003). Because prevalence and availability of hosts is not equivalent to parasite's preferences, the Manly's α preference index (α_i) was used to assess mistletoe's host preference (Manly et al. 1972).

Local spatial patterns of hosts and infection

To test whether the infection distribution of the parasites follows a random spatial pattern or reflects the host availability and distribution, a sampling scheme with a T-square methodology (Hines and Hines 1979) was performed in both the ex-Hacienda Santa Agueda for three of the host species (*A. acuminata*, *P. alba*, *S. bonplandiana*) and Rancho La Herradura for *Q. crassipes*. For that purpose, 15 coordinates were randomly selected in each study area (O) and the distance (x_i) from each coordinate (O) to the nearest organism (P) was registered. Afterwards, distance (r_i) from the organism (P) to its nearest neighbor (Q) that fulfill the condition OPQ angle >90°, was registered. Spatial distribution of host trees and infected host trees were assessed by pooling data of all host species and by species.

Infection prevalence and infection intensity

With data obtained from the ex-Hacienda Santa Agueda's plots and from the two additional randomly selected 50 × 50 m plots at Rancho La Herradura, infection prevalence and infection intensity (mean number of mistletoe individuals per infected host) per host species were analyzed to assess potential parasite adaptation and/or preference for a given host species. DBH values were included in the analysis as a predictor of mistletoe intensity because trees with larger diameter have been in the area for a longer time and, therefore, they have higher incidence of parasitism (Overton 1994; Aukema 2004).

Pollination success and host local adaptation

Female reproductive success among mistletoes growing on different hosts was assessed to test host local adaptation. For that purpose, we conducted an experiment of manual cross-pollination in emasculated flowers and bagged with mesh bags (1 mm mesh) to exclude floral visitors. Pollination treatments were applied 48 h after opening to flowers emasculated and growing on four host species (*A. acuminata*, N = 8; *P. alba*, N = 10; *Q. crassipes*, N = 10; *S. bonplandiana*, N = 8; hereafter "fate"). A mistletoe pollen donor for each of the host species was selected (hereafter "origin"). To compare mating success for each origin × fate combination, we manually cross-pollinated 554 flowers for a total of 16 possible combinations per host species (Treatment 1: cross-xenogamy; *A. acuminata*, n = 134; *P. alba*, n = 146; *Q. crassipes*, n = 155; *S. bonplandiana*, n = 119). An additional treatment with 136 flowers open to pollinators was performed at the same time (Treatment 2: open pollination; *A. acuminata*, n = 35; *P. alba*, n = 31; *Q. crassipes*, n = 36; *S. bonplandiana*, n = 34).

Floral morphology and nectar standing crops

To assess whether morphological differences and nectar reward are involved in the mistletoe reproductive effort (or resource allocation by host species), mistletoes growing on *A. acuminata* (N = 10 trees, n = 190 flowers), *P. alba* (N = 9, n = 225), *Q. crassipes* (N = 10, n = 200), and *S. bonplandiana* (N = 10, n = 244) trees were selected for measurements of floral traits and nectar standing crops. Traits involved in attraction and pollination success, such as pedicel length (mm), corolla tube length (mm), style length (mm), filament length from ovary to the tip (mm), filament length from attachment to petal to the tip (mm) and anther length (mm), were measured for fully developed flowers. Nectar standing crops in flowers, from 0800 to 1000 h, were quantified to test for differences between mistletoes growing on different hosts. Nectar was extracted from flowers using calibrated micropipettes (10 µL) and column of nectar was measured with a digital vernier (error 0.1 mm). Sugar concentration (percentage of sucrose) was measured with a pocket refractometer (VEEGEE BTX-1, range of concentration 0°–32° BRIX scale), and sugar amount produced was expressed as milligrams of sugar per ml of solution, after Kearns and Inouye (1993).

Statistical analyses

The abundance of available host trees and prevalence of infection (response variable) by species (fixed factor, 3 species) was analyzed with a generalized linear model (GLM) using

R (R Core Development Team 2014) and simple contrasts were used to compare mean values between species pairs in the model (Pinheiro and Bates 2006). Then, the proportion of infection by host species was compared with a GLM and a logit link function including infection (binary response variable) and host species as fixed effects factor (categorical variable).

Infection host preference was evaluated with Manly's index (α_i), which is defined as $\alpha_i = (O_i/P_i)/(1/\sum_{j=1}^n O_j/P_j)$; where O_i is the percentage or proportion of trees infected by species i and P_i is the percentage or proportion of trees available of species i . When selective preference of host does not occur, $\alpha = 1/n$ (n , total number of host types). In this study, availability of host species was estimated from a sample (plots included in a sample of the area), and could be a mismatch between proportion of host available censused and available in the environment. In this situation, G test is recommended to provide statistical significance to α values testing the hypothesis of no selection of the host species by the parasites (Manly et al. 1972).

To test the null hypothesis of random pattern, the following equation was used (Hines and Hines, 1979): $h_t = 2n[2 \sum(x_i^2 + \sum(r_i^2))/(\sqrt{2} \sum x_i + \sum(r_i))^2]$. Under the null hypothesis of a random spatial pattern, h is distributed as F with degrees of freedom $2n = 30$ (n = number of random coordinates O in both numerator and denominator). Decision rules are for $\alpha = 0.05$, and a two-tailed test. If observed h value is >1.4209 the pattern is clumped, and if observed h value are <1.1479 the pattern is uniform. Otherwise, the null hypothesis of randomness is not rejected.

Infection and intensity of infection were analyzed in relation with DBH values of host species as a surrogate of host age (Overton 1994). Thus, a GLM was performed in R with a binomial error and a logit link function including infection as binary response variable, host species as fixed effects factor (categorical variable), DBH (continuous variable) and their interaction. To test for differences in infection intensity (response variable) between hosts species (categorical variable) related to DBH values (continuous variable), a linear model (ANCOVA) was performed in R to test main effects (host species, DBH) and their interaction.

Data from the two treatments of the manual pollination experiment were analyzed separately. For treatment 1, host specialization was assessed by testing for differences in pollination success between cross-pollinated flowers in all of the origin \times fate combinations (treatment 1, 16 combinations). A generalized linear mixed-effect model (GLMER) with a binomial family (logit link function) was used including origin, fate, and their interaction as fixed effects factors, plant identity as random effects, and pollination success (fruit production) as the binary response variable. The full model was simplified using Akaike Information Criterion (AIC) to select the final model (Akaike 1981). A second analysis was carried out in R with fruit production data from the open pollination experiment (treatment 2), with a GLMER including the host species factor as fixed effects, plant identity as random effects and fruit production as the binary response variable (pollination success).

Lastly, measurements of flower traits, nectar volume and sugar content (response variable), were analyzed in R using linear mixed-effects models (LME). The models included plant identity as random factor and host species as the fixed effects factor (Laird and Ware 1982; Pinheiro and Bates 2006). Simple contrasts were performed to compare means between pairs in all models (Pinheiro and Bates 2006).

Results

Host availability and infection preference

Of a total of 154 trees of three host species with a DHB >10 cm were registered at the ex-Hacienda Santa Agueda, 117 of which were infected with *P. calyculatus* (75.9 %). Tree abundance ($F = 8.510$, $df = 2, 48$, $p < 0.001$) and prevalence of infection ($\chi^2 = 19.08$, $df = 2$, $p < 0.001$) were significantly different among host species. *Populus alba* was the most abundant host species in the area and had the highest prevalence (Table 1). Differences in tree abundance between *A. acuminata* and *S. bonplandiana* were not statistically different ($t = 0.56$, $df = 48$, $p = 0.57$). *Populus alba* ($\chi^2 = 18.39$, $df = 1$, $p < 0.001$) and *S. bonplandiana* ($\chi^2 = 4.52$, $df = 1$, $p < 0.05$) were more infected than *A. acuminata*. Although Manly's index indicated that *P. alba* and *S. bonplandiana* were more parasitized than expected and *A. acuminata* was less infected than expected, host preference differences were not statistically significant (G test: $\chi^2 = 0.082$, $df = 2$, $p > 0.05$; Table 1).

Local spatial patterns of hosts and infection

The null hypothesis of a random pattern of spatial distribution for both host availability and infection was tested by host species. Whereas a clumped spatial pattern of host availability was observed for *P. alba* ($h = 2.82$), *Q. crassipes* ($h = 2.43$) and *S. bonplandiana* ($h = 2.13$) or when all species were pooled ($h = 2.62$), *A. acuminata* trees were randomly distributed across space ($h = 2.05$). A random spatial distribution of the infection was also observed when species were pooled ($h = 2.02$) and individually for *A. acuminata* ($h = 2.00$) and *S. bonplandiana* ($h = 2.06$), whereas for *P. alba* and *Q. crassipes* the spatial distribution of infested trees was clumped ($h = 2.67$ and 2.08 , respectively).

Infection prevalence and infection intensity

Prevalence of mistletoe infection among host species was significantly different, mistletoe infection increased with DBH, and the infection/DBH relationship varied significantly among host species. Infection of *P. alba* and *A. acuminata* was significantly different ($\chi^2 = 4.53$, $df = 1$, $p < 0.033$), and between *P. alba* and *Q. crassipes* were also different ($\chi^2 = 6.35$, $df = 1$, $p = 0.011$; Table 2, Fig. 1). The probability of infection in *P. alba* increased from 30 % at lowest DBH value (10.19 cm) to 99.9 % at highest (79.58 cm); in *A. acuminata* from 13 % at lowest DBH (11.14 cm) to 70 % at highest (60.48 cm); and in *Q. crassipes* from 14 % at lowest DBH (40 cm) to 26 % at highest (100 cm). In *S. bonplandiana*, the probability to infection at lowest DBH (10.19 cm) was zero and almost 100 % at the highest DBH value (135.6 cm).

Mean number of mistletoes per tree (intensity of infection) was significantly different among host species ($F = 29.437$, $df = 3$, $p < 0.001$); *A. acuminata* and *Q. crassipes* had fewer mistletoe plants per tree than *P. alba* and *S. bonplandiana* (Table 2). The intensity of mistletoe infection increased positively with DBH ($F = 110.936$, $df = 1$, $p < 0.001$), and the increase of infection in relation to DBH was different among host species ($F = 33.688$, $df = 3$, $p < 0.001$). The increase of intensity of mistletoe infection in relation to DBH was higher on *P. alba* than it was on *A. acuminata* ($t = -6.181$, $df = 197$, $p < 0.001$), *Q. crassipes* ($t = 8.473$, $df = 197$, $p < 0.001$) or *S. bonplandiana* ($t = 7.614$, $df = 197$, $p < 0.001$; Table 2; Fig. 2).

Table 1 Host abundance and host preference of *Psittacanthus calyculatus* infection at ex-Hacienda Santa Agueda

	<i>Alnus acuminata</i>	<i>Populus alba</i>	<i>Salix bonplandiana</i>	<i>p</i> Value
Host abundance (mean \pm SD)	1.47 \pm 1.64	6.64 \pm 1.26	0.94 \pm 1.86	0.00068
Prevalence of infection (%)	40	84	75	0.00007
Manly's index	0.2	0.42	0.37	>0.05

Pollination success and host local adaptation

The GLMER full model was simplified in two steps because the origin \times fate interaction ($F = 1.7280$, $df = 9, 36$, $p = 0.1061$) and the fate fixed factor ($F = 1.5152$, $df = 3, 36$, $p = 0.2271$) were not significant ($AIC = 743.59$). First, the origin \times fate interaction was removed ($AIC = 741.95$, $p = 0.06$). Secondly, the resulting model was compared with a model without the fate fixed factor ($AIC = 742.54$, $p = 0.09$). Accordingly, the final model included origin as fixed factor and plant identity as the random effects factor.

Fruit set was significantly different among flowers pollinated with pollen of mistletoe donors growing on different host tree species (*A. acuminata* 37 %, *P. alba* 31 %, *Q. crassipes* 52 %, *S. bonplandiana* 48 %; $F = 5.0193$, $df = 3, 336$, $p = 0.005$; Fig. 3a). Flowers pollinated with pollen from mistletoe flowers growing on *Q. crassipes* or *S. bonplandiana* produced more fruits (52 %, $z = -3.432$, $p = 0.0033$; 48 %, $z = -2.688$, $p = 0.0362$, respectively) than those pollinated with pollen from mistletoes growing on *P. alba* (Fig. 3a).

Fruit set of flowers exposed to open pollination was statistically different among flowers of mistletoes growing on different host species (fate fixed factor, $F = 4.8937$, $df = 3, 34$, $p = 0.006$). Flowers of mistletoes growing on *Q. crassipes* produced significantly more fruits (95.27 %) than those of mistletoes growing on *P. alba* (38.99 %, $z = -3.596$, $p = 0.00166$) and *S. bonplandiana* (65.66 %, $z = 2.587$, $p = 0.04618$), but fruit set differences between *Q. crassipes* and *A. acuminata* (70.17 %) were not statistically different ($z = -2.368$, $p = 0.0804$; Fig. 3b).

Floral morphology and nectar standing crops

Floral measurements yielded no significant differences among flowers of mistletoes trees growing on different host tree species, except for anther length ($F = 3.734$, $df = 3, 37$, $p = 0.0193$; Table 3). Anthers of flowers growing on *Q. crassipes* were smaller and statistically different from those of flowers of mistletoes growing on *A. acuminata* ($t = 2.092$, $df = 37$, $p < 0.044$), *P. alba* ($t = -2.9718$, $df = 37$, $p < 0.005$) or *S. bonplandiana* ($t = -2.8378$, $df = 37$, $p < 0.007$). Variation in the amount of nectar available to floral visitors (nectar standing crops) on mistletoe flowers growing on the different host species was not statistically significant according to the best LME model (Table 3).

Discussion

Our results showed that *P. calyculatus* performed differently among host tree species at the ex-Hacienda Santa Agueda. Mistletoe infection did not increase with host tree abundance as predicted by the host 'specialization' hypothesis. Both clumped spatial patterns of hosts

Table 2 Infection prevalence (a) and intensity of infection (b) of *Psittacanthus calycularis*

	<i>df</i>	Deviance (χ^2)	Res. <i>df</i>	Res. Dev.	Pr ($>\chi^2$)	<i>Alnus acuminata</i>	<i>Quercus crassipes</i>	<i>Populus alba</i>	<i>Salix bonplandiana</i>
<i>(a)</i>									
Null			204	273.32					
Host	3	72.009	201	201.31	1.59E-15***	0.4	0.19	0.84	0.75
DBH	1	29.508	171.8	171.8	5.57E-08***	36.80 ± 3.20	67.82 ± 2.31	26.70 ± 1.31	47.78 ± 8.08
Host × DBH	3	30.773	141.03	141.03	9.49E-07***				
<i>(b)</i>									
	<i>df</i>	Sum Sq	Mean Sq	F	Pr ($>F$)	<i>Alnus acuminata</i>	<i>Quercus crassipes</i>	<i>Populus alba</i>	<i>Salix bonplandiana</i>
Host	3	1307.3	435.76	29.437	9.06E-16***	1.32 ± 0.43	0.86 ± 0.28	6.25 ± 0.65	6.00 ± 1.33
DBH	1	1642.2	1642.21	110.936	<2.2e-16***	36.80 ± 3.20	67.82 ± 2.31	26.70 ± 1.31	47.78 ± 8.08
Host × DBH	3	1496.1	498.69	33.688	<2.2e-16***	0.1078 ± 0.033x	-0.755 ± 0.024x	-3.811 ± 0.377x	2.7020 ± 0.069x
Residuals	197	2916.2	14.8						

*** $p < 0.0001$

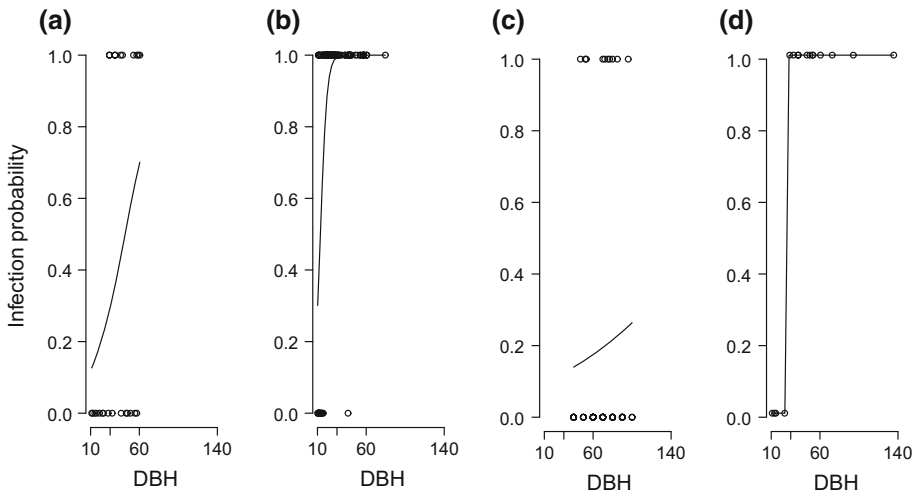
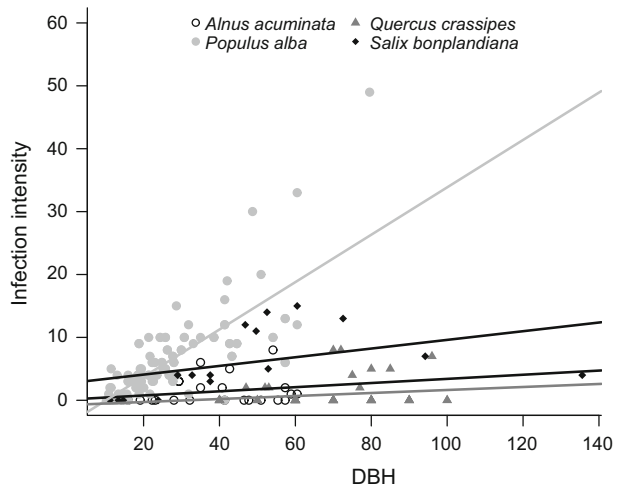


Fig. 1 Probability of infection by *Psittacanthus calyculatus*. Each point represents an individual host tree and the line is the slope of the relationship. **a** *A. acuminata*, **b** *P. alba*, **c** *Q. crassipes*, **d** *S. bonplandiana*

Fig. 2 Relationship between intensity of mistletoe infection and DBH values by host species. Each point represents an infected tree and the line is the slope of the relationship

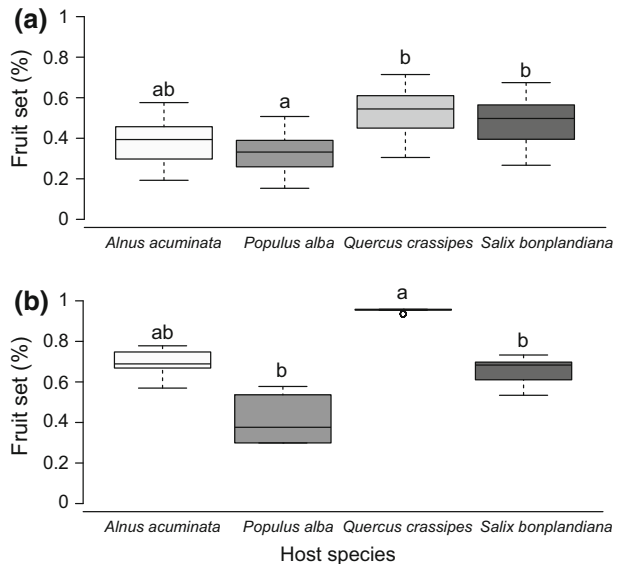


and infection seem to explain the intensity of mistletoe infection on *P. alba* and *S. bonplandiana*, and the abundance of infected hosts had an impact on the infection intensity of *P. alba*. Lower mistletoe fitness measured as seed production in the cross-pollination experiments was observed when flowers were pollinated with pollen from mistletoes growing on the non-native *P. alba*, whereas seed production was higher when flowers were pollinated with pollen from mistletoes growing on the native host species, presumably preferred hosts to which *P. calyculatus* had been interacting for a longer time.

Host availability and infection preference

Mistletoes parasitize a subset of potential hosts at the local scale and frequently show specificity for the most common host trees (Norton and Carpenter 1998; Norton and de

Fig. 3 Female reproductive success among *P. calyculatus* mistletoes growing on different host tree species. **a** Manual cross-pollination (treatment 1). **b** Open pollination (treatment 2). Contrasts between pairs are marked with *small letters*



Lange 1999; Arruda et al. 2006; Fadini 2011). This apparent host specialization might result from frequent encounters between mistletoe bird-dispersed seeds and the commonest host tree species. Several studies have shown that perch preferences and foraging decisions taken by birds might either result in a pattern that concentrates mistletoe seeds on the most abundant trees (López de Buen and Ornelas 1999), or even on the less abundant ones (Aukema and Martínez del Río 2002b; Roxburgh and Nicolson 2005). Other studies have argued that non-random perch preferences of seed dispersers are also important for shaping the patterns of mistletoe infection prevalence (proportion of infected) and, ultimately, determining host specificity (e.g., Monteiro et al. 1992). Alternatively, mistletoe-host compatibility might explain mistletoe host use patterns and one of the primary causes of mistletoe host specificity (May 1971; Calder 1983; Monteiro et al. 1992; Yan and Reid 1995; López de Buen and Ornelas 2002; Fadini 2011). Mistletoe-host compatibility, the result of the genetically and, perhaps, environmentally determined processes (biochemical, mechanical, physiological) including mistletoe infectivity and host susceptibility to seed germination, seedling development and penetration and formation of haustoria (Yan and Reid 1995; Cocolletzi et al. 2016), may counteract or amplify the effects of seed rain on the observed distribution of mistletoes.

According to the host 'specialization' hypothesis, the most abundant host tree species is expected to be the most infected. Contrary to this prediction, studies on mistletoe-host interactions have found that the intensity of mistletoe infection does not correlate with resource availability (Aukema and Martínez del Río 2002b; Medel et al. 2002; Roxburgh and Nicolson 2005; Fadini 2011; Lemaitre et al. 2012). In these studies, the observed disproportional usage of available host tree species was explained as to be the result of their spatial distribution, perch preferences and foraging and defecation behaviors of seed dispersers, and host-mistletoe compatibility mechanisms. Our results showed that the proportion of mistletoe infection (prevalence) was disproportionate in relation to host availability (e.g., *S. bonplandiana*) though higher on the most abundant and non-native host tree species (*P. alba*). Given that mistletoe preference to any particular host species

Table 3 Morphological flower measurements and nectar standing crops for individuals of *Psittacanthus calycularis* by host species

	<i>Abnus acuminata</i>			<i>Quercus crassipes</i>			<i>Populus alba</i>			<i>Salix bonplandiana</i>			<i>p</i> Value
	Mean \pm SD		N	Mean \pm SD		N	Mean \pm SE		N	Mean \pm SE		N	
	n			n			n			n			
<i>Flowers</i>													
Pedicel length	10	190	13.93 \pm 0.55	10	200	13.27 \pm 0.54	9	225	12.29 \pm 0.52	10	244	14.20 \pm 0.54	0.0674
Floral tube length			9.01 \pm 0.55			8.85 \pm 0.55			8.68 \pm 0.52			8.49 \pm 0.55	0.9192
Style length			39.57 \pm 1.89			39.63 \pm 1.89			36.84 \pm 1.80			37.19 \pm 1.89	0.5872
Filament length (a)			36.03 \pm 1.31			36.43 \pm 1.31			35.20 \pm 1.24			34.10 \pm 1.30	0.6023
Filament length (b)			13.09 \pm 0.73			13.46 \pm 0.73			12.73 \pm 0.70			11.91 \pm 0.73	0.4876
Anther length*			3.80 \pm 0.13			3.41 \pm 0.13			3.94 \pm 0.12			3.92 \pm 0.13	0.0193
<i>Nectar standing crop</i>													
Volumen (μ l)	10	190	2.54 \pm 0.45	10	200	1.60 \pm 0.43	11	226	2.05 \pm 0.41	10	244	2.35 \pm 0.44	0.4577
Sugar content (mg/ μ l)			0.47 \pm 0.08			0.23 \pm 0.08			0.32 \pm 0.07			0.38 \pm 0.08	0.1709

Size measurements are expressed in millimeters (mm). N, mistletoe individuals; n, flowers. (a) Measurement taken from ovary to the tip. (b) Measurement taken from petal attachment to the tip

* $p < 0.05$

was not evident, host adaptation or any ecological factors could explain the observed host use pattern.

Local spatial patterns of hosts and infection

The spatial distribution of host species is crucial to determine the structure and dynamics of the parasite population and, indirectly, the interactions between the hosts, mistletoes, and the seed dispersers (Poulin 2007). Using simulations on how the spatial pattern and seed dispersal affect plant population dynamics, Morales and Carlo (2006) found that the dispersal distance is reduced as the plant's spatial aggregation increased, but gut passage time, foraging behaviors and densities of bird seed-dispersers affected the linear relationship. Carlo and Morales (2008) also assessed how bird's density, landscape aggregation and neighborhood effects (local scale pattern) modified fruit removal rates and seed dispersal, in a way that the aggregation of plants promoted seed dispersal distances by decreasing fruit removal rates. Moreover, cross-seed inoculation experiments have shown that success for survival of *Psittacanthus* mistletoe seedlings depends on the interaction between host provenance and host fate of gut-processed seeds (Overton 1994; Lara et al. 2009; López de Buen and Ornelas 2002; Ramírez and Ornelas 2012), as mistletoe seeds collected from a given host were more likely to survive when they were inoculated, gut-processed or not, on conspecific host trees compared with those inoculated on other host provenances. The results of these seed inoculation experiments suggest the potential for local host adaptation.

Given the spatial conditions of host congeners, seed dispersal of *P. calyculatus* would be less compromised if the seed-dispersal vector reaches patches of *P. alba* and *S. bonplandiana* (even patches of *Q. crassipes* at Rancho La Herradura) as distance to congeners were smaller due to host clumped distribution. Even in the absence of local host adaptation, decreases in dispersal distances between host individuals in a given patch might lead to future aggregation of infection (Overton 1994; Yan and Reid 1995). Accordingly, several studies have shown that mistletoe aggregation at small scales results from the high rates of infection and reinfection attributed to perch preferences and territorial behavior of mistletoe consumers (Overton 1994; Martínez del Río et al. 1996; López de Buen and Ornelas 1999; Aukema and Martínez del Río 2002b; Medel et al. 2002; Aukema 2004; Rist et al. 2011). At the ex-Hacienda Santa Agueda, the spatial distribution of *P. calyculatus* mistletoe infection was clumped on the most common species (*P. alba*), while the overall infection was randomly distributed on the other two species (*S. bonplandiana* and *A. acuminata*). These results have two important implications: (1) random distribution of overall infection in the area suggests no preference for any of the evaluated host tree species (i.e. the Manly's index results), and (2) *P. alba* clumped infection vs. *S. bonplandiana* random infection coming from the same underlying host distribution (clumped) could be explained by the patchiness and unequal host densities in the area. If resources are clumped, aggregation will be maintained by reinfection as bird movements among trees in a dense patch (*P. alba*) would be shorter and foraging time would be longer. Conversely, few individuals in a patch (i.e. *S. bonplandiana*, *A. acuminata*) facilitate dispersal to other patches and infection probably does not remain within individuals of the patch (Morales and Carlo 2006; Carlo and Morales 2008). Studies contrasting whether the main seed dispersers, *Tyrannus vociferans* (Cassin's Kingbird) and *Ptilogonys cinereus* (Gray silky-flycatchers), are attracted by fruit abundance on individuals or by tree characteristics (Sargent 1990, 1995; Carlo and Morales 2008) and whether they defend neighboring infected trees of the main host tree species differentially are further needed.

Infection prevalence and infection intensity

A size-occupancy relationship is predicted in mistletoes when larger trees are disproportionately infected (Overton 1994; Roxburgh and Nicolson 2005; but see Fadini 2011). Overton (1994) proposed that the prevalence of *Psittacanthus sonora* would increase with host tree size because larger trees are older and the probability to be infected over time would be accumulated year after year. Roxburgh and Nicolson (2005) found that tall trees of *Acacia sieberana* presented higher probability to be infected by *Phragmanthera dschallensis* (Loranthaceae). Our results indicated that trees with higher DBH values were more likely to be infected by *P. calyculatus*. Also, younger individuals of the native host species were not infected, suggesting that they are not attractive to seed dispersers or that mistletoe survival is compromised in young individuals (Mathiasen et al. 1990; Overton 1994; Donohue 1995; López de Buen et al. 2002). Besides, *A. acuminata* and *Q. crassipes* individuals were either infected or not at higher DBH values, suggesting that some individuals have the ability to resist parasitic infection or that the probability to encounter those individuals by the vector is lower. According to models of parasite population dynamics successful parasite species evolve to be harmless to their hosts and some individuals have the ability to resist parasitic infection because of genetics as well as environmental factors (e.g., Anderson and May 1982).

It has been proposed that a high aggregation of parasites on a small proportion of host trees reduces the deleterious effect of the parasites on the host population (Anderson and May 1978; May and Anderson 1978; Jaenike 1996; Shaw et al. 1998). At the local scale, researchers have attributed the aggregated pattern of mistletoe infection to be the result of prior infection and intensity of mistletoe infection, bird abundance, diversity of seed dispersers, preferences and foraging behavior of seed dispersers, and host resistance traits (Overton 1994; Martínez del Río et al. 1996; Aukema and Martínez del Río 2002b; Medel et al. 2002; Aukema 2004). In addition, different host susceptibility to mistletoe infection results in prevalence or aggregation at a slower rate. According to this, studies of several mistletoe species have found a weak positive relationship between intensity of mistletoe infection and host tree DBH values but the relationship turned non-significant when non-infected individuals were removed from the analysis (Roxburgh and Nicolson 2005 and references therein). Contrary to previous results, we showed that the intensity of mistletoe infection/DBH relationship remained strong and significant after removing non-infected individuals from the analysis, suggesting that DBH is a good predictor of infection intensity at least for *P. alba*. These results should be treated with caution because the intensity of mistletoe infection in *P. alba* might be affected by the architecture of tall individuals, and therefore, DHB variation might not be sufficient for predicting the intensity of infection on this species. Although strikingly tall individuals were not encountered in the study area, it is possible that the higher intensity of infection on *P. alba* taller trees is not only the accumulation of infections as trees age but also the result from differential dispersal of mistletoe seeds and seedling survival to taller trees (Roxburgh and Nicolson 2005). Furthermore, *P. alba*, an exotic man-cultivated species, does not invade naturally, can form locally extensive clonal stands but in the study area *P. alba* trees are planted in line as wind fences, which represents an unusual distribution different to other naturally-distributed species under study. DBH was chosen as a surrogate of host tree height and host age to predict intensity of infection over the years (probability of being infected and re-infected; Overton 1994; Roxburgh and Nicolson 2005), but likely it does not explain full variation in rates of infection and aggregation because the linear DBH-tree

height relationship might change as both DBH and tree height continuously increases for years (Sumida et al. 2013 and references therein).

Local host adaptation

Outcomes of mistletoe host interactions vary across geography, thus local specialization is possible (Norton and de Lange 1999), and interactions with a particular host species should improve mistletoe fitness (Norton and Carpenter 1998). Our results indicated no local adaptation of *P. calyculatus* to any of the hosts included in the manual pollination experiment. However, seed production was higher when mistletoe individuals were pollinated with pollen from plants growing on the three native species (pollen provenance) in the manual cross-pollination experiments. We did not confirm if any of the studied host species provided better resources to the mistletoe while pollen is produced, but these results suggest that the quality or/and viability of pollen grains varied among *P. calyculatus* individuals growing on different host species. In the open pollination experiment, seed production was higher in mistletoe plants growing on *Q. crassipes* at Rancho La Herradura as compared to those growing on *S. bonplandiana* and *P. alba* at the ex-Hacienda Santa Agueda. These results are generally consistent with those from the manual pollination experiment, though a confounding site effect should not be discarded.

Flower traits did not vary among flowers on different host species, thus possible effects of floral traits involved in pollinator's attraction could be excluded, except for anther size that was significantly shorter on plants growing on *Q. crassipes*. Hummingbirds are the main pollinators of *Psittacanthus calyculatus* flowers, and pollinators' bill length is linked to effective pollen removal and pollen deposition (Azpeitia and Lara 2006). Thus, differences in nectar production and pollinator assemblages between the areas where flowers were left open to natural pollination should be considered in future studies. Although sugar content differences were not statistically significant among species, nectar standing crops (nectar volume and sugar content) were about half smaller in mistletoe flowers growing on *Q. crassipes* than those growing on the other host tree species. These results can primarily suggest differences in environmental conditions and/or hummingbird visitation rates in both study sites. For example, *A. acuminata*, *P. alba*, and *S. bonplandiana* are planted as a barrier to fields, particularly along irrigation canals, which provides them with a constant source of humidity at the ex-Hacienda Santa Agueda. Also, this site is located at lower altitude (~100 m above sea level) compared to the location where *Q. crassipes* was studied (Rancho La Herradura). Although sugar concentration and sugar production tend to decrease with increased altitude in some plant species (see also Stiles and Freeman 1993; Ornelas et al. 2007), which suggests that nectar concentration in hummingbird flowers decreases to compensate for the higher viscosity at lower temperatures, and/or the result of differences in water and carbon resources, i.e. at high altitudes sugar production may be more costly, the significant negative correlations between sugar concentration and altitude and between sugar production and altitude are removed when phylogeny is accounted for (Ornelas et al. 2007). On the other hand, nectar standing crops fall when rates of removal exceed secretion rate as often happens when foragers are numerous. Hence the standing smaller nectar volume and sugar content in mistletoe flowers growing on *Q. crassipes* suggest higher hummingbird-visitation rates in this species compared to other host trees, which in turn might increase rates of pollen movement. These alternatives require further testing.

Conclusions

Our results revealed that infection by *P. calyculatus* varied significantly among host tree species, and demonstrated that mistletoe infection did not increase with host tree abundance. While host availability is thought to explain local mistletoe/host compatibility patterns, the proportional relationship between host abundance and infection as expected by the host ‘specialization’ hypothesis (Norton and Carpenter 1998) is not always significant. Spatial distribution of host and infection should be considered to explain the mistletoe/host pattern. Finally, host specialization is believed to be the initial step of differentiation among species of mistletoes using several host species (Norton and Carpenter 1998), mainly through differences in their fitness. In this regard, mating success was higher on both, mistletoe plants manually-pollinated with pollen from donors growing on *Q. crassipes* and those growing on *Q. crassipes* exposed to natural pollination. Thus, our study is first to show that mating success was higher among mistletoe individuals within a host species with respect to random mating and, therefore, that host adaptation might be higher with those to which mistletoes likely share the oldest evolutionary history.

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