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Critical importance of large native trees for conservation of a rare Neotropical epiphyte

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Summary

1. The distribution of rare plants may be limited by environmental or density-dependent factors that reduce population growth and persistence. The relative importance of environmental limitations vs. the degree to which conspecifics influence recruitment may determine optimal management strategies for plants of conservation concern.

2. We sowed seeds of a rare epiphytic orchid in trees from agricultural Costa Rican landscapes to ask how recruitment is influenced by established conspecific plants and the environment. We hypothesized that recruitment is positively influenced by conspecific plants. Conspecific adults were expected to be associated with favourable microhabitats and mycorrhizal fungi for germinating seeds, without creating unfavourably competitive conditions. Alternatively, we hypothesized that recruitment varies due to environmental differences among study sites or host trees, irrespective of adult proximity.

3. We experimentally added 240 packets of seeds from 2 source populations into four naturally established populations. Germination was evaluated after five months. We used mixed models and conditional inference trees to evaluate results.

4. Proximity to conspecific adults neither increased nor decreased germination. Instead, large native trees and microsites with more closed canopies supported significantly greater germination than smaller cultivated trees and microsites with more open canopies.

5. *Synthesis*. Landscape changes that replace large native trees with a more homogenous array of cultivated species may not only reduce rare epiphyte populations directly, but also limit their ability to colonize disturbed secondary habitats. Because the habitat is the prime determinant of recruitment, large and often isolated native trees that act as refuges for rare epiphytes in disturbed landscapes should be a top priority for local conservation efforts.

Key-words: dispersal, habitat fragmentation, mycorrhiza, Neotropics, Orchidaceae, plant population and community dynamics, symbiosis

Introduction

Persistence of rare plant populations in fragmented landscapes depends on their ability to reproduce and disperse seeds into safe recruitment sites. Rare plants are defined as having restricted geographical distributions, narrow habitat specificity and/or small population sizes (Rabinowitz 1981), and rarity is often attributed to a limited availability of suitable environments (Shipley, Vile & Garnier 2006). However, density-dependent processes that influence population growth may also contribute to rarity, including Allee effects (Stephens & Sutherland 1999) and intraspecific competition or facilitation (Brooker *et al.* 2008). Deforestation degrades habitats and isolates populations, substantially reducing biodiversity (Gardner *et al.* 2009). Potential biodiversity loss is greatest in the tropics, where plant diversity and endemism levels are highest (Gentry 1988). Vascular epiphytic plants comprise a large proportion of this diversity and are among the most likely growth forms to experience population declines due to the loss of populations, host trees and microhabitats with deforestation (e.g. > 68% of a Singaporean epiphyte community extirpated; Sodhi *et al.* 2008). Epiphytes with restricted geographical distributions and narrow climatic tolerances are the most vulnerable to human-mediated environmental change (Köster *et al.* 2012). Knowledge about recruitment of rare epiphytes will be useful in anticipating and addressing such population declines.

Symbiotic mycorrhizal fungi can enhance plant recruitment via positive feedbacks if adult symbionts also favour seedlings (Bever *et al.* 2010). Interactions with mycorrhizal fungi

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may influence rarity in the highly diverse and predominantly epiphytic Orchidaceae (≥ 24 000 species; 70% epiphytes; Dearnaley 2007). Orchids produce many tiny seeds capable of long-distance wind dispersal (Arditti & Ghani 2000). Following dispersal, the tiny seeds completely depend on resources from mycorrhizal fungi for establishment and these symbioses often persist in mature plants (Dearnaley 2007). Most evidence indicates that this is a parasitic relationship, in which orchids digest fungal tissue to extract resources without providing resources in return (Dearnaley 2007). Symbiont diversity varies among orchid species, but is usually narrowly circumscribed (Dearnaley 2007). Recruitment of orchids that specialize on specific fungi may not be buffered against declines in the population of their symbiont (Bascompte & Jordano 2007).

The distribution of mycorrhizal fungi may limit the distribution of some orchids, but may not be the primary factor influencing safe site distributions (Swarts *et al.* 2010; Phillips *et al.* 2011). Some epiphytic orchids often recruit near conspecific plants, possibly because established plants are associated with favourable microhabitats and mycorrhizal fungi (Trapnell, Hamrick & Nason 2004). Alternatively, negative density-dependent recruitment due to competition or the accumulation of natural enemies may reduce recruitment near conspecifics, although this has not been reported for orchid populations (Diez 2007). Local adaptations to environments or mycorrhizal fungi (Otero, Bayman & Ackerman 2005) as well as fine-scale variation in abiotic factors such as water and substrate (Diez 2007; McCormick *et al.* 2012) may also influence recruitment patterns. A previous *in situ* epiphytic orchid germination study found low germination compared to similar studies of terrestrial orchids, suggesting high heterogeneity in recruitment success (Zettler *et al.* 2011).

Diverse old-growth tropical forests are being replaced with more homogenous suites of native and non-native trees that are disturbance tolerant and/or cultivated (Tabarelli, Peres & Melo 2012). Remnant or reintroduced epiphyte populations in disturbed habitats may be insufficient to sustain populations in these areas. Interactions between epiphytic orchids and their host trees may influence recruitment and mycorrhizal symbioses, particularly if different types of trees maintain unique microhabitats or fungal communities (Gowland *et al.* 2011). Because seed germination is a key recruitment stage, associations between seed germination and particular ecological factors could strongly influence the population dynamics of epiphytes. Thus, identification of microhabitats that favour epiphytic orchid seed germination could facilitate the conservation of a plant family that constitutes a major proportion of tropical biodiversity.

We asked whether the recruitment of an epiphytic orchid *Epidendrum firmum* Rchb.f. 1866 is limited by the local environment or the distribution of established conspecific plants. An *in situ* seed sowing experiment was used to compare germination rates among isolated trees across four Costa Rican pastures. Germination success was hypothesized to increase in close proximity to established adults, due to locally favourable microhabitats and mycorrhizal fungi. An alternative hypothesis was that recruitment decreases near established adults, due to competition for resources or accumulation of host-specific enemies. Our final hypothesis was that recruitment varies with microenvironment, irrespective of adult proximity. If recruitment is consistently associated with adult proximity, conservation efforts should focus on preserving current populations to maintain safe sites for seedlings. If not, conservation efforts require broader understanding of seed and environmental limitations in canopy habitats.

Materials and methods

STUDY SPECIES

The epiphytic orchid *Epidendrum firmum* Rchb.f. 1866, in the subtribe Laeliinae, occurs on the Pacific slope of Central American mountains from Nicaragua to Panama (Morales 2009). Its narrow band of habitat is identified as humid premontane (bmh-P) and lower montane wet forest (bmh-MB) by the Holdridge life-zone system (TRK, pers. obs.; herbarium records), which are among the most highly fragmented habitats in Costa Rica (Sánchez-Azofeifa, Harriss & Skole 2001). Although populations can be locally abundant, the species is considered rare and vulnerable due to its limited geographical distribution and climatic niche (Rabinowitz 1981; Köster *et al.* 2012). Individuals grow in dense, 10- to 35-cm-tall clusters that can have ≥ 60 stems but often many fewer. Plants occur on tree trunks and twigs in the canopy of primary forests and highly disturbed areas. Each stem bears two to four flowers during the wet season from May to August. Capsules dehisce and release tiny wind-dispersed seeds during the dry season, from February to March.

STUDY SITES

We tested the hypothesis that germination success is higher in proximity to conspecific plants. Study sites included four pastures near Monteverde, Costa Rica, that each had naturally established *E. firmum* populations. Three sites (ALO, CAB and EFR) were at the University of Georgia Research Station (N 10°16.989, W 84°47.778) with pairwise distances of 0.35–0.45 km and an elevation of 1080–1130 m above sea level (mASL). The fourth (REF) was at the Refugio Vida Silvestre (N 10°19.521, W 84°49.548), 5.48 km away, at 1395–1415 (mASL). All *E. firmum* individuals within a site are considered a population, and these individuals occurred on ≥ 5 trees per site (Table 1). *Epidendrum firmum* naturally occurs in many tree species, most of which are evergreen although some are deciduous in the dry season. The most frequent host in this study is *Sapium glandulosum* (Euphorbiaceae), a dry-season deciduous species that is often planted in local pastures. *Acnistus arborescens* (Solanaceae) is another common evergreen host that is often planted in local pastures and has become naturalized. *Cupressus lusitanica* (Cupressaceae) is an evergreen native to Central America from Mexico to Honduras that is often planted in pastures as windbreaks. This study included some large native and evergreen remnant trees, including *Cupania glabra* (Sapindaceae) and *Nectandra membranacea* (Lauraceae). Other large native trees, most of which are evergreen, may have naturally colonized pastures, including *Inga punctata* (Fabaceae), *Erythrina berteroana* (Fabaceae; deciduous) and *Ehretia latifolia* (Boraginaceae).

Table 1. Characteristics of each study tree and the microsites within it. Study site, host species, tree type (native remnant tree, native secondary forest tree, native and cultivated tree or non-native and naturalized as a cultivated tree species), of each tree used in the experiment. For each tree, ranges include the minimum and maximum branch diameter (cm), range of canopy openness values, range of the number of stems in each adult orchid cluster and range of the percentage of mycorrhizal root cross sections in each adult orchid cluster. Moss indicates the fraction of microsites in which seed packets were placed on a tree that were predominantly mossy

Site	Species	Tree type	Branch diameter	Canopy openness	Adult stems	Myco (%)	Moss
EFR	*Acnistus arborescens* (Solanaceae)	Naturalized	7.8–10.3	11.97–20.17	4–12	0–33†	5/6
EFR	*Inga punctata* (Fabaceae)	Native secondary	12.7–23.8	9.31–11.97	4–34	71–100	0/6
EFR	*Acnistus arborescens* (Solanaceae)	Naturalized	4.2–11.1	8.44–9.58	12–14	0–63	2/4
EFR	*Acnistus arborescens* (Solanaceae)	Naturalized	9.5–9.5	11.98–12.52	11	75	1/2
EFR	*Sapium glandulosum* (Euphorbiaceae)*	Native cultivated	6.1–8.9	29.88–39.00	2–42	22–100	4/6
EFR	*Sapium glandulosum* (Euphorbiaceae)*	Native cultivated	4.1–9.0	22.64–29.15	3–6	50–66	3/6
CAB	*Sapium glandulosum* (Euphorbiaceae)*	Native cultivated	11.0–20.1	18.49–23.74	2–5	0††	0/6
CAB	*Sapium glandulosum* (Euphorbiaceae)*	Native cultivated	9.0–15.0	12.89–32.61	2–12	29–67	1/6
CAB	*Sapium glandulosum* (Euphorbiaceae)*	Native cultivated	7.9–21.0	25.76–29.33	5–22	44–86†	3/6
CAB	*Sapium glandulosum* (Euphorbiaceae)*	Native cultivated	4.0–9.3	7.68–16.13	2–18	17††	2/6
CAB	*Acnistus arborescens* (Solanaceae)	Naturalized	3.5–6.6	5.86–14.91	6–18	17–33†	1/6
ALO	*Cupania glabra* (Sapindaceae)	Native remnant	2.5–4.0	12.89–25.70	4–16	83–89	3/6
ALO	*Erythrina berteroana* (Fabaceae)*	Native secondary	7.6–23.7	15.31–19.98	2–3	67–71†	4/6
ALO	*Nectandra membranacea* (Lauraceae)	Native remnant	3.9–5.3	10.98–22.86	11–23	17–100	3/6
ALO	*Ehretia latifolia* (Boraginaceae)	Native secondary	3.6–5.6	1.45–4.91	10–14	60–89	5/6
ALO	*Ehretia latifolia* (Boraginaceae)	Native secondary	7.4–16.3	29.09–37.35	5–10	50–100	3/6
REF	*Cupressus lusitanica* (Cupressaceae)	Naturalized	21.0–23.0	7.73–10.12	1–2	44–63	6/6
REF	*Sapium glandulosum* (Euphorbiaceae)*	Native cultivated	5.0–15.0	13.06–20.50	5–13	33–63	6/6
REF	*Cupressus lusitanica* (Cupressaceae)	Naturalized	9.2–23.7	6.01–11.96	2–9	0–50†	6/6
REF	*Cupressus lusitanica* (Cupressaceae)	Naturalized	28.0–29.4	5.86–11.47	5–67	38–100	6/6
REF	*Cupressus lusitanica* (Cupressaceae)	Naturalized	28.0–28.0	6.58–9.24	1–3	75–100	6/6

*Indicates dry-season deciduous tree species.
†Indicates one cluster for which no healthy root section could be obtained.
†† Indicates two clusters for which no healthy root section could be obtained.

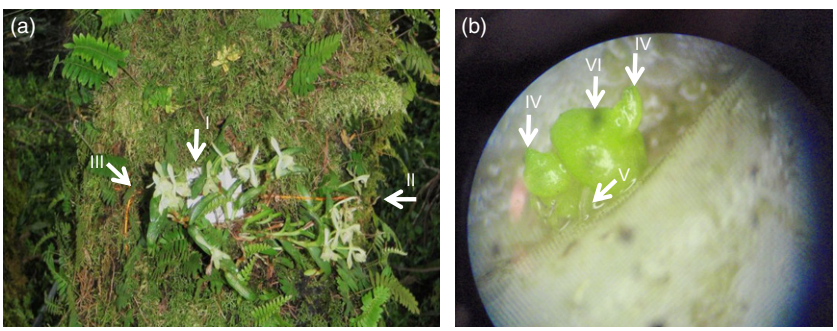

Fig. 1. Photographs of seed packets and germinating seedlings. (a) A flowering *E. firmum* from the experiment is shown. A slide case containing two 50-μm nylon mesh seed packets from the 2 seed sources is placed in close proximity to the adult and upon its roots (I). A second slide case is placed in a similar microenvironment between 0.2 and 0.5 m away from the adult, out of view (II). The slide case is secured to the tree using floral wire and nylon cord (III). (b) Stage 3+ germinating protocorms, including leaf (IV) and root (V) primordia and the initiation of mycorrhizal symbiosis (VI), viewed through a dissecting microscope.

EXPERIMENTAL DESIGN

Seeds were collected in February 2011 from two populations (ALO and REF). Because of negligible fruit set in 2010 and 2011, no seeds were collected from EFR or CAB. One capsule was collected from each of 18 plants/population, just prior to dehiscence. Capsules were surface-sterilized, seeds were extracted, and seeds from the 18 capsules/population were mixed to produce a homogenized sample from that population. Because we could not independently assess seed quality at the outset of the experiment, seeds were pooled from 18 fruits to test population-level hypotheses without the confounding influence of unusually high- or low-quality fruits. Approximately 250–400 seeds/population were sealed in separate 50-μm nylon mesh packets, placed side-by-side within 120 plastic slide cases and stored in a cool, dry, dark location for 1–2 days before transfer to the field (Rasmussen & Whigham 1993).

Slides were secured to tree branches near and away from established adult *E. firmum*, including many from which seeds were collected, using floral wire and nylon cord (Fig. 1a). Each study site was allocated 30 slide cases (i.e. 60 seed packets with two seed packets per slide case) to be distributed across five trees. Trees represented a broad range of habitat characteristics (Table 1) and were selected based on the criteria that they host *E. firmum* adults, had limited risk of interference by humans or livestock and allowed safe access for

investigators. Within each tree, a slide case was set atop the live roots of three adult *E. firmum* plants ('near' treatment). The one exception was in EFR, where only 4 trees had ≥ 3 adult *E. firmum*, and thus, the final three *E. firmum* adults were located in two different trees. Thus, a total of 21 experimental trees were selected in ALO (five trees), CAB (five), EFR (six) and REF (five). In each tree, an equal number of slide cases were also placed in a similar microhabitat between 0.2 and 0.5 m away from the adult ('away' treatment). This distance range allowed the slide case to be placed a distance beyond the root zone of the adult, but close enough to maintain relatively similar microenvironments. The full experiment included four study sites, two seed sources, two levels of adult proximity (near and away) and 15 adults/site, totalling 120 slide cases and 240 seed packets. Seeds remained in the field for 5 months, spanning the transition from dry to wet seasons (see Fig. S1 in Supporting Information). A pilot study found that 5 months is sufficient for germination under these conditions. Seeds were not monitored during the 5 months to avoid introducing bias.

Seeds were recovered in July 2011, when we recorded microsite characteristics and quantified germination and adult mycorrhizal symbiosis. We recorded substrate (mossy or not) and branch diameter and measured canopy openness using hemispherical photographs and WinSCANOPY Pro (Regent Instruments Inc., Quebec, QC, Canada). We scanned seed packets under a dissecting microscope to assess germination success (Fig. 1b). Orchid germination is often categorized by developmental stages (Ramsay, Silvasithamparam & Dixon 1986), which we adapted: stage 0 = no germination; stages 1–2 = swelling green embryos, including seeds with cracked testa; stage 3+ = leaf and root growth with mycorrhizal pelotons occasionally visible. While some stage 0 seed packets may have contained viable but inactive seeds, these packets often contained visibly deformed or damaged seeds. Packets with germinating seeds were snap-frozen in liquid nitrogen and transported to the University of Georgia, where we used DNA barcodes to identify fungi associated with 5–10 bulked seeds/packet (see Appendix S1). The goal of this barcoding effort was to identify mycorrhizal fungi facilitating germination of stage 3+ seedlings; however, some seeds that may have asymbiotically reached germination stages 1–2 were analysed for comparison. Bulking seeds was necessary due to their small size. Thus, fungal sequences could represent either mycorrhizal symbionts or more general components of the asymbiotic fungal community within seed packets. The roots of adults in contact with seed packets were cross-sectioned at 1-cm intervals over 3–9 cm/plant to observe the proportion of sections with mycorrhizal pelotons under a compound microscope. We sampled healthy roots from beneath and beyond seed packets wherever possible without causing undue harm to plants. Because *E. firmum* roots are small and delicate, it was impractical to compare the levels of mycorrhizal colonization at different distances from the base of the plant.

STATISTICAL ANALYSIS

We tested for an effect of adult proximity, seed source and study site on germination, accounting for random host tree effects. We used an ordinal response variable that considered seed packets failing to germinate (stage 0), beginning to germinate (stages 1–2) and containing advanced protocorms (stage 3+). A cumulative link mixed model (Agresti 2002) was fit with germination stage as a function of adult proximity, seed source and study site as fixed factors and each tree as a random factor. Significance of model terms was assessed using likelihood ratio tests ($\alpha = 0.05$), and models were compared with Akaike Information Criterion (AIC). Models were fit using *clmm2* in the *ordinal* package in R v. 2.15.1 (R Core Development Team 2010; Christensen 2011a). The condition number of the Hessian was used to assess model fit (Christensen 2011b). The preferred fitted model was used to predict the probability that seeds reach each germination stage in each site–source comparison.

If both germination rates and adult mycorrhizal symbioses are greatest in certain microsites, these microsites represent favourable habitats. We assessed how host trees and microhabitats are associated with germination and adult mycorrhizal colonization using conditional inference trees (see Appendix S2). Conditional inference trees offer appropriate methods for identifying ecological patterns in a data set containing a large number of potentially informative variables that may interact hierarchically (Olden, Lawler & Poff 2008). Conditional inference trees take a data set of observations (germination or mycorrhizal colonization) and a set of potentially informative variables (habitat characteristics), then split the data set into two groups based on a predictor variable that has the strongest association with the observations. Relationships between the two smaller data sets and the remaining predictor variables are recursively tested until no statistical support is found for additional splits. Although splits in the data are identified *post hoc*, patterns can be particularly useful as *a priori* hypotheses in future experiments. We constructed conditional inference trees to consider seed source, study site, proximity to adult, host tree species, substrate type and canopy openness using *ctree* in the R package *party* (Hothorn, Hornik & Zeileis 2006; R Core Development Team 2010).

Results

Our seed sowing method was successful, with 60% of the 240 seed packets containing germinated seedlings. Eighty-one (34%) packets contained seeds reaching a maximum of stages 1–2, and 62 (26%) contained stage 3+ seedlings (Fig. 2). Seeds appeared to have desiccated or decayed in 97 (40%) seed packets (Fig. 2). Seeds from both sources reached each stage of germination in each of the four study sites (Fig. 2).

Germination significantly differed between seed sources, among study sites and among host trees, but was unrelated to adult proximity (Table 2 and Fig. 3). Across sites, ALO and REF seeds had a similar predicted probability of reaching stages 1–2 (ALO = 28.1–42.8%; REF = 23.0–42.8%), but ALO had a greater probability of reaching stage 3+ (ALO = 21.2–63.5%; REF = 6.2–30.0%; Fig. 3). Local adaptation cannot account for seed source differences because the number of germinating ALO seed packets exceeded REF at all sites (Fig. 2) and ALO seeds had a greater predicted probability of reaching stage 3+ at both the ALO site (interquartile probability: ALO = 53.4–72.6%; REF = 22.0–39.5%) and the REF site (ALO = 15.0–29.0%; REF = 4.2–9.1%; Fig. 3).

Canopy openness and host tree species were associated with variation in germination rate. Conditional inference trees underscored the significant germination differences between seed sources ($P < 0.001$; Fig. 4). Significantly more ALO seed packets reached more advanced stages of germination in less open canopies ($P = 0.031$; Fig. 4). Significantly more REF seed packets reached more advanced stages on the native remnant trees *Cupania glabra* and *Nectandra membranacea* and the native secondary growth species *Inga punctata, Ehretia latifolia* and *Erythrina berteroana* than on the cultivated

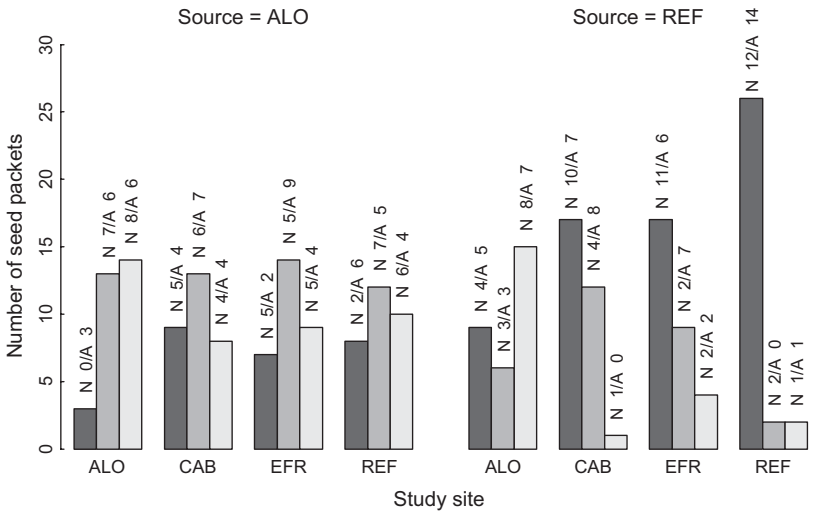

Fig. 2. The number of seed packets failing to germinate (black bars), reaching stages 1–2 (light grey bars) and reaching stage 3+ (dark grey bars) differs between seed sources (ALO, REF) and among study sites (ALO, CAB, EFR and REF). The overall number of seed packets that were placed near (N) and away (A) from an adult is listed above each bar. The total number of seed packets of each source at each study site is 30.

Table 2. Likelihood ratio tests indicate a significant effect of seed source, study site and host tree, but not adult proximity. The full model appears in row 1. Each subsequent row (2–5) compares the full model to a model with one dropped term. The effective degrees of freedom, Akaike Information Criterion value (AIC; lowest value corresponds to the preferred model), Hessian value ($> 10^4$ would indicate an ill-defined model) and likelihood ratio tests are shown for each model comparison. The fourth model, which drops the term for adult proximity, is the preferred model and is the only comparison that does not result in a significant likelihood ratio test

Model # and comparison	The full model and each dropped term	d.f.	AIC	Hessian	Likelihood ratio test
1	Response variable: germination stage Fixed terms: seed source + study site + adult proximity Random term: tree	8	478.72	61.31	
2 vs. 1	Dropped term: study site	5	483.28	14.09	d.f. = 3, LR = 10.56, $P = 0.014$
3 vs. 1	Dropped term: seed source	7	505.99	54.77	d.f. = 1, LR = 29.27, $P < 0.001$
4 vs. 1	Dropped term: adult proximity	7	476.77	60.02	d.f. = 1, LR = 0.06, $P = 0.814$
5 vs. 1	Dropped term: tree	7	482.83	38.50	d.f. = 1, LR = 6.11, $P = 0.013$

species *Sapium glandulosum*, *Acnistus arborescens* and *Cupressus lusitanica* ($P < 0.001$; Fig. 4). This occurred even though REF seeds were collected from a robust population on *C. lusitanica* and *S. glandulosum*.

As expected, most adults associated with mycorrhizal fungi, but the proportion of mycorrhizal root sections varied among environments. Healthy root tissue was obtained from 85% of adults, of which 92% contained mycorrhizal pelotons. The median proportion of colonized root sections was 0.625 (Fig. 5). Significant mycorrhizal colonization differences occurred among host tree species, with plants on cultivated *A. arborescens* and *S. glandulosum* having significantly less than others (median = 0.444; $P = 0.030$; Fig. 5). Adult plants on substrate without moss on other host species had significantly more colonization (median = 0.889 vs. 0.667; $P = 0.017$; Fig. 5).

We identified fungi associated with 10 protocorm samples in six seed packets, representing 4.2% of packets containing seedlings. Two seed packets containing stage 3+ seedlings contained Cantharellales, an order including orchid mycorrhizal taxa (see Table S1). Other orchid associates from the Hypocreales and Pleosporales associated with protocorms in one packet containing stage 3+ seedlings and three packets containing stage 1–2 seeds, respectively (Table S1). Such potential orchid mycorrhizal fungi and other common orchid associates were observed in seed packets placed both near and away from adults (Table S1). Multiple taxa, including orchid associates, saprotrophs and parasites, occurred within two ALO seed packets (018 = stage 3+ and 043 = stages 1–2; Table S1). We obtained too few sequences to compare fungi across seed sources, study sites or habitats. Pelotons were often visible in stage 3+ seedlings under the microscope, but some stage 3+ seedlings may not have formed mycorrhizas, yielded sufficient fungal DNA or associated with taxa amplified by our primers.

Discussion

We asked whether recruitment of an epiphytic orchid is limited by microenvironmental conditions or the distribution of established conspecific plants, and found that microhabitats influence seed germination but adult proximity does not. Consistent with previous findings, large native trees have a central role in the conservation of tropical plant diversity in fragmented habitats (Nadkarni & Haber 2009).

Low adult density in disturbed areas may not limit recruitment if adults do not provide favourable microsites. Adults

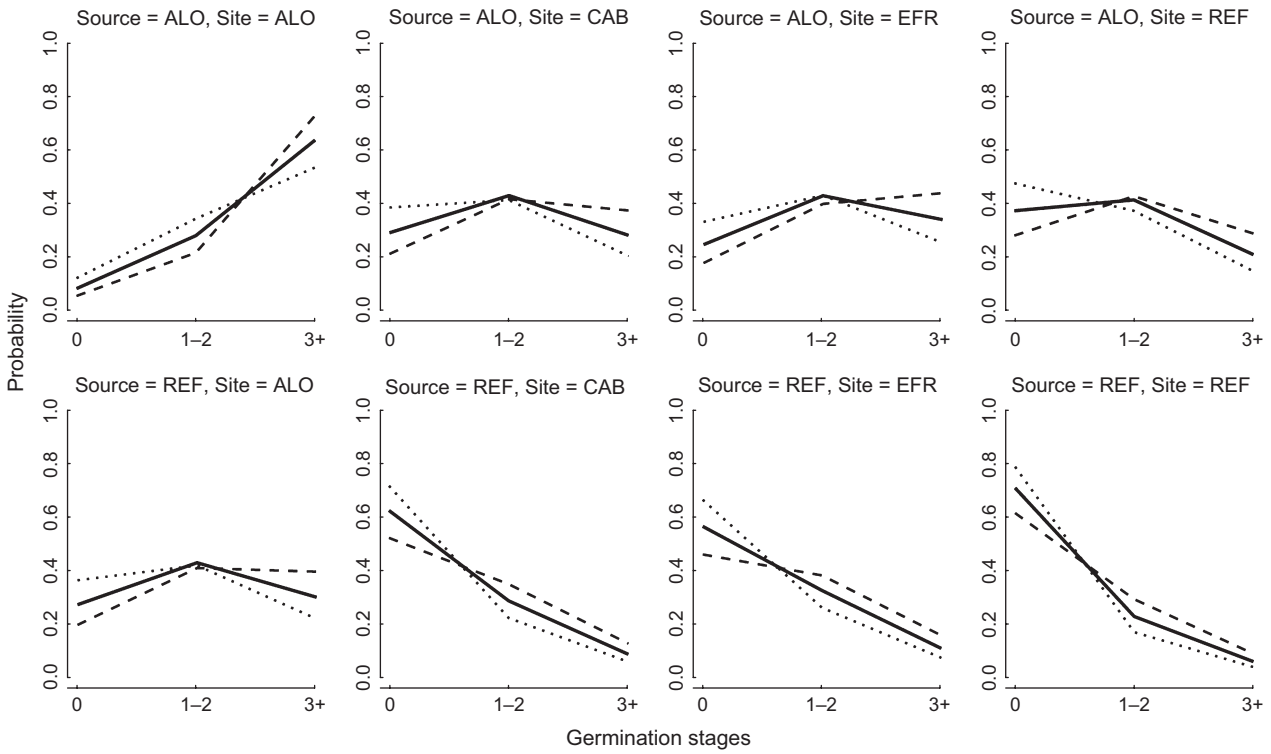

Fig. 3. The predicted probability of reaching germination stages differed between seed sources (top = ALO; bottom = REF) and among study sites (columns). To show the random effect of trees in the fitted model, solid lines indicate the predicted probability of seed packets reaching each stage on the average tree for each site–source combination, while dotted and dashed lines represent the predicted probabilities for trees across the middle quartiles of the model (25th and 75th percentile, respectively). Together, these fitted models show significant differences in the fate of seeds based on the predicted probability that at least one seed per packet survives and advances through the observed germination stages in each site–source combination.

may be expected to provide favourable microsites if they are associated with an abundance of the mycorrhizal fungi that seedlings require. However, mycorrhizal fungi may be more broadly distributed in the canopy than in established plants, as is true for some terrestrial orchids (e.g. Bonnardeaux *et al.* 2007; Phillips *et al.* 2011). Additionally, adult orchids may not harbour an abundance of the specific fungi seedlings require, since non-photosynthetic germinating orchid seeds may rely on mycorrhizal fungi that provide carbon, while photosynthetic adults may not Dearnaley 2007). Indeed, some *E. firmum* seed packets contained fungal genera that commonly associate with orchids and are known to facilitate *in vitro* germination of both terrestrial and epiphytic orchids as well as temperate and tropical orchids, including *Ceratobasidium sp.* and *Fusarium sp*. (Hadley 1970; Vujanovic *et al.* 2000). Other packets contained saprotrophic Pleosporales and Hypocreales, which are among the most common non-mycorrhizal fungi in orchid roots and which may indirectly improve nutrient access to orchids by decomposing local substrates (Herrera, Suárez & Kottke 2010). These mycorrhizal and non-mycorrhizal orchid associates occurred near and away from adults. While we did not measure abundance of fungal taxa, symbiont abundance can influence orchid germination patterns (McCormick *et al.* 2012). Finally, the relatively rapid rate with which epiphytic orchid seeds absorb water, germinate and begin photosynthesis suggests that they may not experience the extended period of physiological dependence on mycorrhizal fungi that terrestrial orchids do (Yoder *et al.* 2010; Rasmussen 2011). Nevertheless, some seedlings detectably associated with fungi that form orchid mycorrhizas, suggesting that seedlings can benefit from mycorrhizal symbioses but that these symbioses are not spatially aggregated around conspecific adults.

In this experiment, there is a chance that some mycorrhizal hyphae extend 20–50 cm from adult orchids into the vicinity of 'away' seeds. This small distance range away from adults was selected in order to establish similar spacing between adults and seed packets within microenvironments, while allowing available seeds to be disseminated among many adults and microenvironments. We considered the evaluation of many microenvironments important due to the low germination rates reported in previous epiphytic orchid work (e.g. Zettler *et al.* 2011). Since terrestrial orchid seed germination rates and mycorrhizal abundance can decrease with distance from adults (Diez 2007), we encourage future investigations into distance-dependent relationships in epiphytes.

Germination patterns suggest that populations experience seed and microenvironmental limitation. Germination occurred in microsites with and without adults, indicating that greater seed rain should increase recruitment. Similar seed limitation was found in experiments that increased seed rain by manipulating epiphytic orchid fruit set (Ackerman, Sabat & Zimmerman 1996) and epiphytic bromeliad seed availability

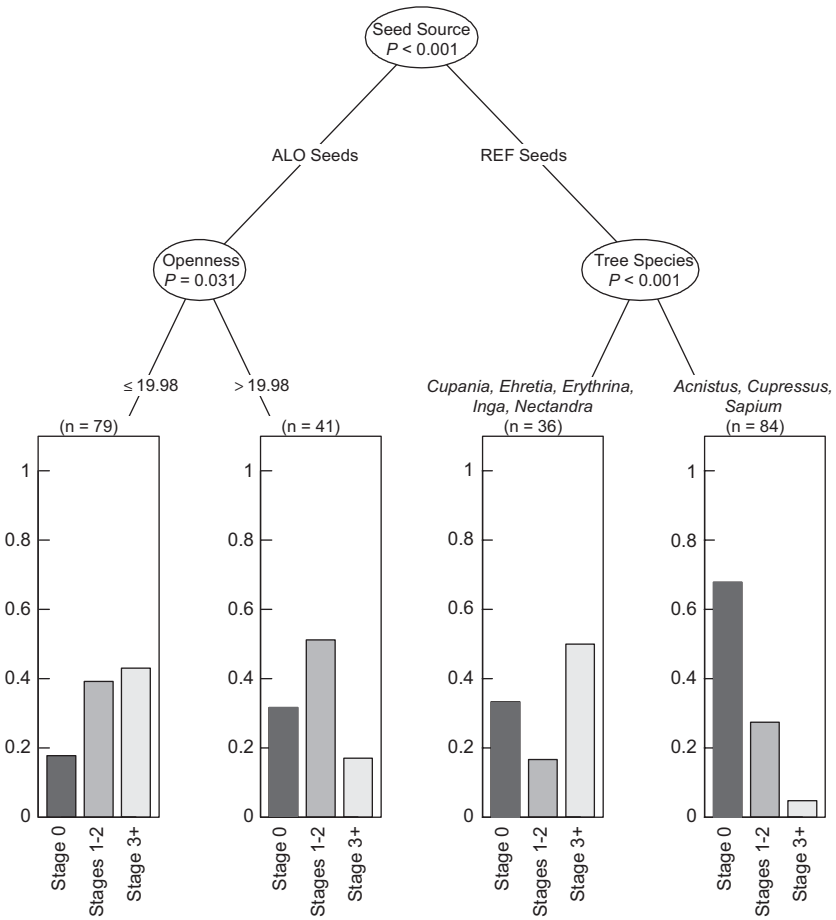

Fig. 4. Proportion of germinating seed packets varies with different environmental factors for each seed source. The conditional inference tree shows a significant difference between seed sources ($P < 0.001$). Greater germination to stage 3+ by ALO seeds is associated with less canopy openness. The conditional inference tree algorithm determined the optimal *post hoc* value of openness at which to split the data is 19.98 ($P = 0.031$). Greater germination to stage 3+ by REF seeds is shown *post hoc* to be associated with a group of remnant and naturally established native tree species (listed by genus; $P < 0.001$). The number of observations in each node is indicated above each bar chart (n).

(Cascante-Marin *et al.* 2008). Compounding such seed limitations, some microsites are clearly more favourable for recruitment than others. Closed canopies can minimize harmful exposure to sun, wind or drought, favouring germination, growth and survival (Zotz & Hietz 2001), while broad canopies harbouring abundant epiphytes stabilize temperature and humidity (Stuntz, Simon & Zotz 2002). Such microenvironmental influences may have influenced *E. firmum* germination patterns. For example, germination may have been low in cultivated *S. glandulosum* trees because they are deciduous and germination may have been delayed until canopy closure at the onset of the rainy season. However, epiphytic orchid seed banks that would allow such temporal variation in germination are likely small because the tiny seeds may rapidly perish (Rasmussen 2011), as was true in 40% of our packets (ALO seed = 22.5%; REF seed = 57.5%). Significant differences between seed sources could result from variation in seed quality or genetic differences pertaining to how rapidly seeds germinate in particular habitats. It is possible that seed quality varied among cohorts and that germination success was not related to genotype by environment interactions. Staining seeds to visually detect differences in seed viability could be useful for evaluating spatial and temporal variation in seed quality among many seed sources (Vujanovic *et al.* 2000). However, if differences in germination stimuli intrinsically vary among seed sources, habitats with patchy safe site distributions may differentially limit recruitment by seeds from some sources, influencing the ability of these sources to facilitate colonization or gene flow.

Conspecific adults do not produce safe sites for seedling recruitment, but some host tree characteristics favour both recruitment and adult mycorrhizal symbiosis. High adult mycorrhizal colonization occurred on most of the tree species where seed germination rates were high (Figs 4 and 5). This is consistent with studies of terrestrial orchids showing that both recruitment and the abundance of mycorrhizal fungi are aggregated in safe sites where the environment favours both orchid and fungal growth, even though the saprotrophic fungi are ordinarily distributed independently of orchids (Jersáková & Malinová 2007). Moss did not have the positive influence on either germination success or adult plant mycorrhizal colonization that would have been expected based on previous studies of epiphyte recruitment (Nadkarni 2000; Cascante-Marin *et al.* 2008) and epiphytic orchid mycorrhizal symbiosis (Osorio-Gil, Forero-Montana & Otero 2008). While epiphytes and their symbionts may vary in preference for mossy substrate, moss occurrence was not associated with *E. firmum* germination success and adult *E. firmum* on some moss-free substrates had significantly less mycorrhizal colonization. It is possible that increased mycorrhizal colonization on moss-free substrates is necessary to overcome moisture or nutrient limitations of adult roots on nutrient-poor bark subject to

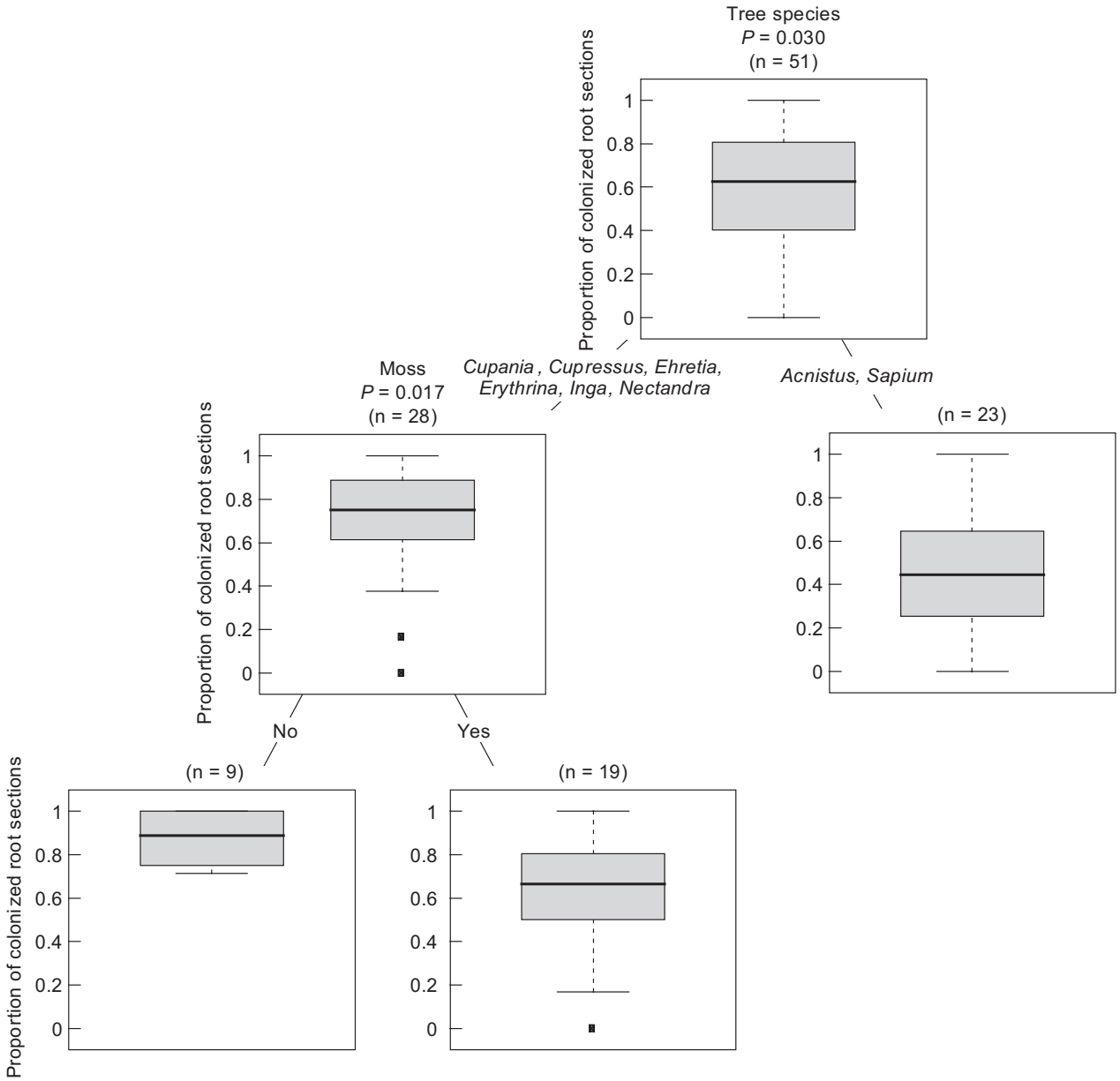

Fig. 5. Adult mycorrhizal colonization is shown *post hoc* to be higher on host tree species (listed at the genus level) that do not include two cultivated pasture tree species (*Acnistus arborescens* and *Sapium glandulosum*; $P = 0.030$) and occur in microsites without moss ($P = 0.017$). Mycorrhizal colonization of adult orchids on the two cultivated trees spans the full range of values. The sample size (n) is shown for terminal nodes, where boxplots show the median (dark line), middle quartiles (grey boxes), the range of the data within 1.5× the inner-quartile range (whiskers) and observations that fall outside of this range (points).

desiccation (Suárez *et al.* 2008). Spongy velamen tissue surrounding roots of certain orchid species may also enhance mycorrhizal growth in the absence of moss by concentrating water and nutrients on otherwise dry surfaces (Herrera, Suárez & Kottke 2010; Zotz & Winkler 2013). Better understanding of epiphytic orchid mycorrhizal symbiosis could clarify why some trees favour both recruitment and adult symbioses.

To our knowledge, this is the first seed baiting experiment to compare influences on epiphytic orchid seed germination in natural populations. Epiphytes may recruit into suboptimal habitats, such as small, cultivated tree species, but possibly at a rate that is insufficient to maintain populations. It remains unclear how canopy habitats influence the presence, abundance or diversity of fungi. Studies pairing *in vitro* and *in situ* experiments that consider a variety of seeds and fungi, including controls to characterize germination under optimal conditions, could provide key knowledge for epiphyte conservation. Because epiphytes are a major component of tropical biodiversity, work should continue to assess how different habitats impact epiphyte populations. Our results suggest that landscape changes resulting in the replacement of large native trees with a more homogenous array of cultivated species may not only reduce epiphyte populations directly, but also limit their ability to colonize disturbed secondary habitats.

Because habitat quality may often be the prime determinant of recruitment, large and often isolated native trees that act as refuges for epiphytes in disturbed landscapes should be a top priority for local conservation efforts.

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Supporting Information

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

Appendix S1. Detailed description of genetic barcoding of seedling-associated fungi.

Appendix S2. Detailed description of conditional inference trees.

Table S1. A high diversity of seedling-associated fungi was identified through genetic barcoding and BLAST searches.

Figure S1. Weather data during the study period measured at the University of Georgia's San Luis Weather Station.