Benefits of seed dispersal for plant populations and species diversity

Evan Fricke

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Reading Committee:
Joshua Tewksbury
Janneke Hille Ris Lambers
Jeffrey Riffell

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Abstract

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Evan Fricke

Chair of the Supervisory Committee:
Joshua Tewksbury
Department of Biology

Seed dispersal influences plant diversity and distribution, and animals are the major vector of dispersal in the world's most biodiverse ecosystems. Defaunation occurring at the global scale threatens a pervasive disruption of seed dispersal mutualisms. Understanding the scope of this problem and developing predictions for the impact of seed disperser loss on plant diversity requires knowledge of the ways in which dispersers benefit their plant mutualists and how the loss of these benefits influence plant population dynamics. The first chapter explores novel benefits of seed dispersal in a wild chili from Bolivia caused by the reduction of antagonistic species interactions via gut-passage by avian frugivores. The second chapter measures how movement away from parent plants influences species interactions for three tree species in the Mariana Islands, assessing the source of distance-dependent mortality. The third chapter quantifies demographic impacts of density-dependent mortality in the forest at Barro Colorado Island, Panamá. The last chapter uses network concepts and information of the benefits of mutualisms to improve coextinction predictions within plant-animal mutualistic networks.

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When condition trumps location: seed consumption by fruit-eating birds removes pathogens and predator attractants

Evan C. Fricke[1], Melissa J. Simon[1], Karen M. Reagan[1], Douglas J. Levey[2], Jeffrey A. Riffell[1], Tomás A. Carlo[1,3], and Joshua J. Tewksbury[1,4]

[1] Department of Biology, University of Washington, Seattle, WA 98195;
[2] Division of Environmental Biology, National Science Foundation, Arlington, VA 22230;
[3] Biology Department, The Pennsylvania State University, University Park, PA 16802;
[4] Current address: The Luc Hoffmann Institute, WWF International, 1196 Gland, Switzerland.

Email addresses: ECF, ecfricke@u.washington.edu; MJS, melissa_simon@nps.gov; KMR, sphitz@u.washington.edu; JDL, dlevey@ufl.edu; JAR, jriffell@u.washington.edu; TAC, tac17@psu.edu; JJT, jtewksbury@wwfint.org

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Correspondence:
Evan C. Fricke
UW Biology
Box 351800
Seattle, WA 98195
E-mail: ecfricke@u.washington.edu
Telephone: +1 406.270.4726

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Abstract

Seed ingestion by frugivorous vertebrates commonly benefits plants by moving seeds to locations with fewer predators and pathogens than under the parent. For plants with high local population densities, however, movement from the parent plant is unlikely to result in “escape” from predators and pathogens. Changes to seed condition caused by gut-passage may also provide benefits, yet are rarely evaluated as an alternative. Here we use a common bird-dispersed chili pepper (*Capsicum chacoense*) to conduct the first experimental comparison of escape-related benefits to condition-related benefits of animal-mediated seed dispersal. Within chili populations, seeds dispersed far from parent plants gained no advantage from escape alone, but seed consumption by birds increased seed survival by 370% – regardless of dispersal distance – due to removal during gut passage of fungal pathogens and chemical attractants to granivores. These results call into question the preeminence of escape as the primary advantage of dispersal within populations and document two overlooked mechanisms by which frugivores can benefit fruiting plants.

INTRODUCTION

Vertebrates are the dominant vectors of dispersal for virtually all the world's most diverse plant communities, and seed dispersal by vertebrates is considered one of the key innovations in angiosperm radiation (Jordano 2000; Smith 2001; Tiffney 2004). The most commonly cited advantage of seed dispersal is deposition of seeds away from the parent plant, where the probability of survival is generally high because predators and pathogens are usually most abundant near the parent plant (Janzen 1970; Connell *et al.* 1971; Bell *et al.* 2006; Mangan *et al.* 2010; Swamy *et al.* 2011). However, vertebrate consumption also alters seed condition, potentially changing seed conspicuousness, attractiveness, and vulnerability to predators and pathogens (Herrera 2002). By consuming seeds, vertebrates may thus mediate interactions with predators and pathogens via changes to both location and condition, yet most studies addressing the benefits of this mutualism for plants have focused solely on location-related benefits (Nathan & Muller-Landau 2000; Harms *et al.* 2000; Wenny 2001; Howe & Miriti 2004; Schupp *et al.* 2010).

Perhaps escape is considered the primary benefit of vertebrate seed dispersal because dispersal benefits have been most thoroughly studied in canopy trees of tropical forests (Hille Ris Lambers *et al.* 2002). In these species, escape from long-term, high-density seed shadows may be critical, and seed movement beyond the canopy of the parent tree typically results in seeds being deposited in environments away from conspecifics (i.e., individuals of the same species), as the tree diversity is high and the density of any one species in the community is generally low. Because the probability of being dispersed under a conspecific adult increases as the density of a species increases, rare species should benefit most often from dispersal away from the parent (Schupp

1992). For locally common species, however, escape from conspecifics is much less likely and the benefits of dispersal *per se* are presumably less important; indeed, negative density dependent survival (i.e., the decrease in survival caused by close proximity to others of the same species) is not apparent for many common tree species (Comita *et al.* 2010; Mangan *et al.* 2010; Johnson *et al.* 2012).

The reduction of escape-related benefits of dispersal should apply to any species, from trees to shrubs, as their density increases, and these benefits should disappear entirely where species become common enough that their predator and pathogen communities become functionally uniform across the landscape. For such species, condition-related benefits of gut-passed seeds may better account for advantages of vertebrate seed dispersal than might dispersal itself. To our knowledge, no previous study has experimentally compared spatial (escape) and condition-related benefits of seed consumption by frugivorous vertebrates. Doing so is important because intertwined escape-related and condition-related benefits constrain our ability to understand biological mechanisms that underlie the ecological and evolutionary interactions between fruiting plants and frugivores.

Here, we use a common understory plant species to evaluate three non-mutually exclusive hypotheses about how frugivore consumption of seeds may alter seed fate by affecting antagonistic interactions between seeds and their predators and pathogens. The ‘escape hypothesis’ posits increased seed survival resulting from movement away from parent plants (Janzen 1970). The ‘chemical camouflage hypothesis’ posits that gut-passed seeds are either less detectable or less attractive to seed predators because gut passage removes or alters chemicals on the seed coat that would otherwise increase the risk of seed predation. Finally, the 'pathogen removal hypothesis' posits that passage through a vertebrate's gut increases seed survival by removing pathogens from the seeds (Janzen 1977). The first hypothesis is a restatement of the traditional location-related benefit, whereas the second two hypotheses address condition-related benefits that are broadly applicable to all fleshy-fruited plant species, but are rarely considered as potential benefits of seed consumption (Travaset *et al.* 2007).

We test these hypotheses in *Capsicum chacoense* (Solanaceae), a species of wild chili native to Bolivia, Paraguay, and Argentina (McLeod *et al.* 1982). Fruits of this long-lived shrub are dispersed only by birds (Levey *et al.* 2006). The primary cause of seed mortality prior to dispersal is infection by *Fusarium* fungi, conveyed to seeds by fruit-piercing hemipteran insects (Tewksbury *et al.* 2008a). After dispersal has taken place, ants are the primary cause of seed mortality. To compare escape- versus condition-related benefits of avian seed consumption, we tested three predictions, each specific to one of the three hypotheses: (i) seeds deposited far from conspecific *C. chacoense* plants will experience lower predation rates than seeds deposited near conspecifics (escape hypothesis), (ii) gut-passed seeds will have a lower probability of detection by seed predators than will seeds taken directly from fruit (chemical camouflage hypothesis), and (iii) seeds passed though the guts of birds will have reduced *Fusarium* fungal load and increased survival relative to unpassed seeds (pathogen removal hypothesis). To explore the mechanism for the second prediction, we also assessed whether volatile emissions from chili seeds contain olfactory cues to seed predators.

MATERIAL AND METHODS

Species and Study Sites

Capsicum chacoense plants are long-lived and produce between 5 and 500 fruits per season from February through March; each fruit contains ~18 seeds (Tewksbury *et al.* 2008b). Canopies are 0.5 to 1.5 m in diameter and up to 1.5m in height. Ants were the major contributor to seed removal in exclosure experiments that isolated the impacts of insects, mammals, and birds on removal of seeds (unpubl. data). Given that we frequently observed consumption of seeds in place by ants, that the genera of ants that removed seeds (*Pheidole* and *Solenopsis*) are commonly considered granivorous, and that we looked for but did not find chili seeds on ant colony refuse piles, we assume that seed removal is equivalent to seed predation. Seed germination occurs from November to January after a 5-7 month dry season. We studied two populations, one at Rancho San Julian (-19.769° -62.700°) and one at Rancho Tres Aguadas (-21.520° -63.781°) in the Gran Chaco region of southeast Bolivia.

Escape Hypothesis

Because populations of chili plants can extend for hundreds of meters, most seeds defecated by birds likely fall within several meters of adult plants in these patches. To test the escape hypothesis within populations, we monitored predation rates of seeds placed near and far from parent plants. We used seeds passed through the gut of captive *Elaenia parvirostris*, the most common consumer of chilies at our study sites (Levey *et al.* 2006). All birds were maintained on a standardized fruit-based diet (Denslow *et al.* 1987), and all readily consumed *C. chacoense* fruits (Tewksbury *et al.* 2008b).

At both study sites, we placed groups of 10 gut-passed seeds directly on the ground at marked locations and counted remaining seeds every 48 hours for 14 days. For 35 plants at each study site, we placed one group of 10 seeds under the canopy of a *C. chacoense* plant (25 cm from the plant stem; "near" treatment) and a second group far from any conspecific adult (5 m from the stem of the focal plant, and >5 m from any other chili plant; "far" treatment). At Tres Aguadas, data from day 10 were excluded from analysis because a rainstorm on day 9 washed seeds away; remaining seeds were used to assess survival rates for subsequent periods. Average between-plant distances are 2.0 m in San Julian and 0.9 m in Tres Aguadas, as estimated by 40 x 50 m stem mapping plots placed randomly within each population. Although our far treatment is only 5 meters from the focal adult plant, this distance is 5 or 2.5 times greater than the average distance between plants at San Julian and Tres Aguadas, respectively, and is thus further away from a conspecific adult than the great majority of seeds would fall.

To compare per-seed rates of daily seed predation between near and far treatments, we used a Cox-regression mixed-effects model in the R package *coxme* (R Core Development Team 2012). Treatment (near or far) was included as a fixed effect, and pile ID, nested within site, was included as a random effect.

Chemical Camouflage Hypothesis

To test whether gut passage reduces detectability or attractiveness of *C. chacoense* seeds by seed predators, we first conducted a field test comparing predation of gut-processed seeds versus seeds taken directly from fruits. Next, we conducted chemical analyses of the volatiles emitted from seed over time to determine if attractant emissions could explain differences observed in the field. Volatile analyses were conducted because seed volatiles can attract ants, and attractiveness depends in part on the concentrations of emitted volatiles (Youngsteadt *et al*. 2008; Blatix & Mayer 2010; Youngsteadt *et al.* 2010).

For the field experiment, we established 100 plots (each 10 x 15 m) arrayed at 50-m intervals along a transect at each of the two sites. Each plot was marked with a 3 x 4 grid, thus creating 12 points, each 5 m from its nearest neighbor. We randomly chose 2 locations in each plot and placed 10 control seeds extracted from fruit at one, and 10 seeds passed by *E. parvirostris* at the other. Control seeds were extracted from fruit at the same time that fruits were passed by *E. parvirostris,* to control for differences in exposure time between treatments. Average seed retention time is about 40 minutes (Tewksbury *et al.* 2008b). We counted the number of seeds remaining in each pile every 48 hours for 14 days. To compare rates of seed predation between control and gut-processed treatments, we used Cox regression mixed-effects models. Treatment (gut-processed or control) was the fixed effect, and pile ID, nested within site, was the random effect.

To identify *C. chacoense* seed volatiles and determine their emission rate over time, we used solid phase microextraction fibers (SPME) (Pawliszyn 1998; Vas & Vekey 2004). Seeds were collected from ripe fruits harvested from *C. chacoense* plants grown in a glasshouse. We divided the seeds into three groups of six samples, each containing ten seeds. Volatiles were collected on the first, second, and fourth days after seeds were removed, corresponding to Days 0, 1, and 3 of the field experiments. Seeds were kept in a chamber with a 31°C/21°C day/night cycle prior to sampling to replicate field temperature conditions.

Samples were placed in 10-mL glass screw vials with ultraclean screwcaps with teflon septa for SPME sampling (Agilent Technologies, Palo Alto, CA, USA). After 1 h equilibration, a SPME fiber (black; 75 μm Carboxen-PDMS; Supelco Analytical, Bellefonte, PA, USA) was inserted into the vial and exposed for 1 h. The adsorbed volatiles were injected to a gas chromatograph-mass spectrometer (GCMS) by desorption at 200°C for 2 min in the injector (splitless mode). The GCMS analysis was done on HP 7890A GC and a 5975C Network Mass Selective Detector (Agilent Technologies, Palo Alto, CA, USA). A DB1 GC column (J&W Scientific, Folsom, CA, USA; 30m, 0.25mm, 0.25μm) was used, with helium as carrier gas at constant flow of 1 cc/min. The initial oven temperature was 50°C for 4min, followed by a heating gradient of 10°C/min to 250°C, which was held isothermally for 10min. Chromatogram peaks were identified tentatively with the aid of the NIST mass spectral library (ca. 120,000 spectra) and verified by chromatography with authentic standards (when available). Peak areas for each compound were integrated using ChemStation software (Agilent Technologies, Palo Alto, CA, USA) and are presented in terms of nanograms per 10-seed sample per hour.

Pathogen Removal Hypothesis

We used two experiments to assess how gut passage affects fungal pathogen load and seed survival. The first isolated the mechanism under laboratory conditions and tested whether gut passage alters pathogen load. The second determined the consequences of gut passage on seed survival under natural conditions when post-dispersal seed predators are excluded.

The first experiment used the seed infection scoring system developed by Tewksbury *et al.* (2008a) to compare severity of fungal infection for gut-processed seeds versus those removed directly from fruit. Fungus is typically present at the time of dispersal, but grows, becomes visually apparent, and can be scored several months after dispersal but before germination. Each seed received a score of 0 to 5 on each side of the flat seeds. A score of zero represents no sign of fungal infection and a score of five represents complete coverage of fungus on the seed; scores were summed across the two sides for each seed to yield a score between 0 and 10. Seed survival probability is negatively related to fungal infection score (Tewksbury *et al.* 2008a). To obtain paired gut-processed seeds and unprocessed seeds, we first harvested ripe *C. chacoense* fruits from local plants. We removed three to five seeds directly from each fruit through a small incision. We then presented the remainder of the fruit (typically containing 13-15 seeds) to one of 9 randomly assigned captive *E. parvirostris* (see Tewksbury *et al.* 2008b for feeding protocols). We retrieved passed seeds from feces, stored all seeds outside in a cage that allowed air flow but blocked rain and pests, and scored seeds after 3 months. We compared fungal infection on unprocessed and gut-processed seeds from 12 trials with a paired t-test, which controls for individual differences among fruits.

In the second experiment, we assessed the consequences of gut passage on seed survival in the field over the 5 to 6-month dry season, between dispersal and germination. We collected fruits from local chili plants, fed half of them to captive *E. parvirostris*, and removed seeds directly from the remaining fruit. We then placed seeds, in groups of 5, in small (30 mm diameter) plastic cups filled with local soil with drainage holes in the bottom. Individual cups contained seeds of one of the two treatments, and in total, 108 cups had gut-processed seeds and 108 cups had seeds taken directly from fruit (N = 1080 seeds in total). To exclude seed predators, and thereby isolate impacts of fungal pathogens, all cups were covered with fine nylon mesh, secured with a rubber band, and buried so that the lip of the cup was ~2 mm above the soil surface. Cups were placed in the field during the fruiting season in March and retrieved after the end of the dry season. When cups were removed, germinated seeds were easily recognized due to the persistence of the hypocotyl and the presence of the open seed coat, which was often found attached to the tip of the hypocotyl. Ungerminated seeds were cut in half and stained with tetrazolium chloride to determine viability (Cottrell 1947). Survival data of seeds were analyzed using general linear mixed models with a binomial error distribution in the R package *lme4*. Treatment (gut processed or control) was the fixed effect, and cup ID and plot ID were random effects; time in the field (number of days) was included as a covariate. We were unable to test both the impact of gut passage on fungal load and the impact of gut passage on field survival in the same experiment because discoloration from soil on seeds in the cups precluded accurate fungal scoring.

RESULTS

Escape. Removal rates of seeds placed far from adult plants were higher than removal rates of seed placed under adult plants (Cox regression, p = 0.028; Table 1), providing no support for the escape hypotheses at the scale of the populations tested. By the end of 14 days, 72% of seeds placed near adult plants and 79% of seeds far from adult plants were removed. Although sites differed in per-seed daily predation rates, these rates peaked in the first days of the experiment; the magnitude of the difference between near and far was small and relatively constant over time (Fig. 1a-b).

Chemical Camouflage. Seeds passed through the guts of birds were much less likely to be removed than were seeds taken directly out of fruits (55% vs. 78% after 14 days; Table 1; Cox regression, $p < 0.00001$). This doubling of survival was generated by differences in removal rates during the first two days of the experiment (Fig. 1c-d), indicating that seeds not gut-passed were more detectable or attractive. Although the effect was transient (i.e., rates of per-seed survival between treatments did not differ after the second day), the difference it generated in survival of passed and non-passed seeds persisted for the duration of the experiment (Fig. 1c-d). This effect may be caused by gut passage removing volatile compounds of seeds that attract predators. Indeed, our collection of volatile compounds under laboratory conditions revealed a 100-fold decrease in emission rates from the first to third day post-removal (496 ng/h vs. 4ng/h; t-test: t=2.36, $p < 0.05$), corresponding to the time when seeds in both treatments began experiencing similar predation rates. The volatile chemicals from unprocessed seeds included aromatic and aliphatic compounds that are known attractants to *Pheidole* and *Solenopsis* ants (Fig. S1, Table S1).

Pathogen Removal. In the first experiment, which used seeds stored in sheltered conditions for three months, gut passage reduced fungal load on seeds by 31% relative to control seeds taken from the same fruit (paired $t = 2.79$, $df = 11$, $p = 0.018$; Fig. 2a). In the second experiment, which used seeds placed on soil in natural conditions for six months, gut-passed seeds had twice the survival of control seeds taken directly from fruits (likelihood ratio test, χ^2 =3.86, p = 0.049; Fig. 2b); 3.9% of gut-passed seeds survived, whereas only 1.7% of control seeds survived. These results indicate that seed ingestion reduces *Fusarium* load and that this reduction doubles seed survival in nature.

DISCUSSION

We assessed the mechanism and consequence of both escape- and condition-related benefits of seed consumption by frugivores, and found that the benefits derived from changes to seed condition in *C. chacoense* outweigh the traditionally oriented benefits of dispersal within populations. Fruit consumption greatly reduced seed predation by removing volatile chemicals known to attract granivorous ants, and decreased seed mortality due to pathogens (Tewksbury *et al*. 2008a) by reducing fungal pathogen load. Together, these condition-related benefits of gut passage increased seed survival by 370%. Whereas changes in seed location are broadly recognized to affect species interactions, studies examining the effects of changes in seed condition typically focus on intrinsic characteristics such as germinability (Traveset *et al*. 2007; Schupp *et al*. 2010). We show strong experimental support that changes to condition alter species interactions.

Because our study focused on dispersal within established populations of *C. chacoense*, we cannot assess the relative importance of escape-related benefits that might apply to dispersal at larger scales (Nathan 2006). As in most studies of dispersal benefits, we did not quantify benefits of escape that may apply outside of populations, or the fraction of seeds to which such benefits might apply. Still, we are able to conclude that distances that are relatively far from conspecifics within populations – several times greater than mean between-plant distances – are not sufficient for location-related benefits in this species. Research to assess the distance necessary to gain location-related benefits, and how these distances vary with species abundance, would provide greater insight into the nature of dispersal benefits for plants species of differing local abundance.

The interaction modifications caused by frugivory alone can produce strong benefits of the plant-frugivore mutualism, and condition-related advantages of frugivory are likely common in seed dispersal mutualisms. For example, other studies have reported benefits of pulp removal, even when seeds are not consumed or dispersed (Meyer & Witmer 1998; Lambert *et al.* 2001; Fedriani *et al*. 2012). Condition-related benefits may be particularly important in common species, for which the traditional escape related benefits of animal-mediated seed dispersal may be small, or may apply to only a very small fraction of seeds (Comita *et al.* 2010; Mangan *et al.* 2010; Johnson *et al.* 2012). Consumption effects on seed condition have the potential to change many post-dispersal interactions, and the lack of strong escape-related benefits of seed consumption does not necessarily indicate that the mutualism between these fruiting plants and their fruit consumers is any less important for these species. Even without escape-related benefits in *C. chacoense*, consumption of seeds by frugivorous birds resulted in large reductions in interactions with predators and pathogens, and the mechanisms were entirely due to changes in seed condition.

Consumption of seeds may reduce interactions with predators and pathogens by several mechanisms. Our results support the hypothesis that gut passage removes pathogens on seeds (Janzen 1977). Although the effects of gut passage on intrinsic physical seed properties that influence germinability and the effects of gut passage on species interaction, like in many studies, cannot be distinguished, reduction of fungi on *C. chaocense* seeds is likely important because fungal pathogens are a major cause of mortality (Tewksbury *et al*. 2008a). Further, impacts of gut-passage on intrinsic physical seed properties are unlikely to increase survival because greater physical breakdown of the seed coat during gut passage reduces survival in this species (Tewskbury *et al*. 2008b). More generally, studies that test for impacts of scarification using gut-passed and un-passed seeds may inadvertently quantify benefits of pathogen removal. We also provide evidence that seed passage alters cues used by granivorous ants. In particular, *C. chacoense* seeds taken directly from fruits emit alkaloid volatiles that also serve as olfactory cues for ants (Table S1; Vander Meer *et al*. 2010; Sonnet 1972; Morgan 2009)

These findings of strong, spatially-independent benefits of animal-mediated seed dispersal have implications for the role of dispersal in the maintenance of plant species diversity. Many authors have argued that natural enemies promote diversity through conspecific negative density dependence (Adler & Muller-Landau 2005; Freckleton & Lewis 2006; Terborgh 2012). To test for density dependence, it is typical to compare rates of mortality, predation, or infection as a function of distance from a parent tree or of conspecific density. Greater per-seed rates of mortality at locations near conspecific trees or in locations with high seed density are considered evidence for advantage-when-rare benefits caused by negative density dependence.

We suggest this approach overestimates the advantage-when-rare benefits of escape. Seed condition varies systematically between areas near and far from conspecifics (or areas of high and low seed density); seeds that fall near parent trees (or in areas of high conspecific seed density) are less likely to have been dispersed by a vertebrate. In contrast, for most animal-dispersed species, seeds falling far from parent trees are almost always processed by vertebrates before arrival. Our results indicate that greater survival far from parent plants is likely due at least in part to density-independent benefits of gut processing rather than negative density dependence. Indeed, if our study had ignored seed condition and half of our 'near' seeds were not gut-passed, while 'far' seeds were all gut-passed (a situation that is common in many tropical species) movement away from the parent plant would appear to nearly double the chance of survival. Such a result would be interpreted as strong support for the advantage-when-rare benefits of escape. However, density-independent benefits of gut-passage actually account for all of these differences. Changes to condition provide a second axis along which impacts of dispersal should be considered.

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TABLES AND FIGURES:

Table 1. Analysis of distance (escape hypothesis) and gut passage (chemical camouflage hypothesis) on seed survival using Cox proportional hazard models with mixed effects.

Hypothesis	Parameter	Coefficient	SE	*z*	*P*
Escape	Distance	0.295	0.134	2.2	0.028
Camouflage	Gut Passage	-1.356	0.130	-10.4	<0.00001

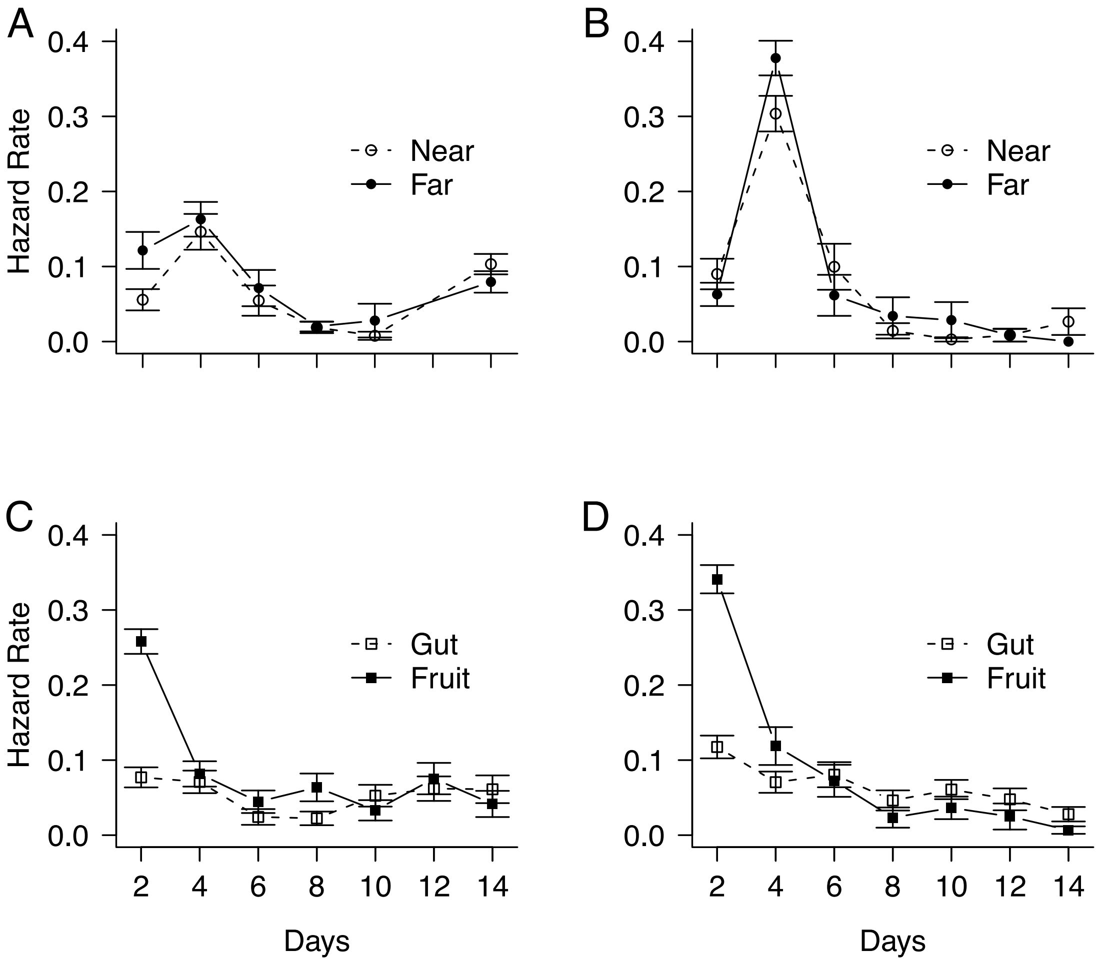

Fig. 1. Impact of distance and gut passage on hazard rate (daily per-seed probability of predation) at Rancho San Julian (a,c) and Rancho Tres Aguadas (b,d) study sites. Escape Hypothesis (a-b): predation rates were greater for seeds placed 5 meters from any *Capsicum chacoense* plant ('Far') than for seeds placed under the canopy ('Near') (Cox regression, $p = 0.028$), contrary to the escape hypotheses. Chemical Camouflage Hypothesis (c-d): seeds taken directly from fruit ('Fruit') suffered greater predation rates than gut-passed seeds ('Gut') (Cox regression, $p < 0.00001$), particularly in the first two days of the experiment.

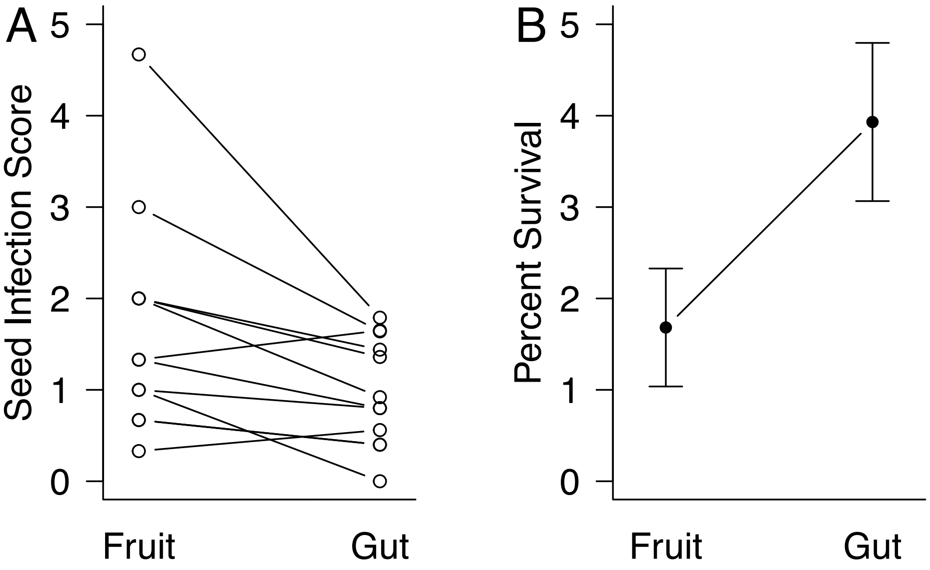

Fig. 2. Effect of gut passage on seed infection score and survival in the field. (a) Seeds from 12 *C. chacoense* fruits were taken directly from fruit ('Fruit') or passed though the gut of birds ('Gut'). Gut passage reduces fungal load of seeds relative to control seeds taken directly from fruit (mean effect size = 0.313; paired t = 2.79, df = 11, p = 0.018). (b) Survival through the dry season for gut-passed and unprocessed seeds. Gut passage increased probability of survival (likelihood ratio test, χ^2 =3.86, p = 0.049). Error bars indicate ± 1 SE.

SUPPORTING INFORMATION

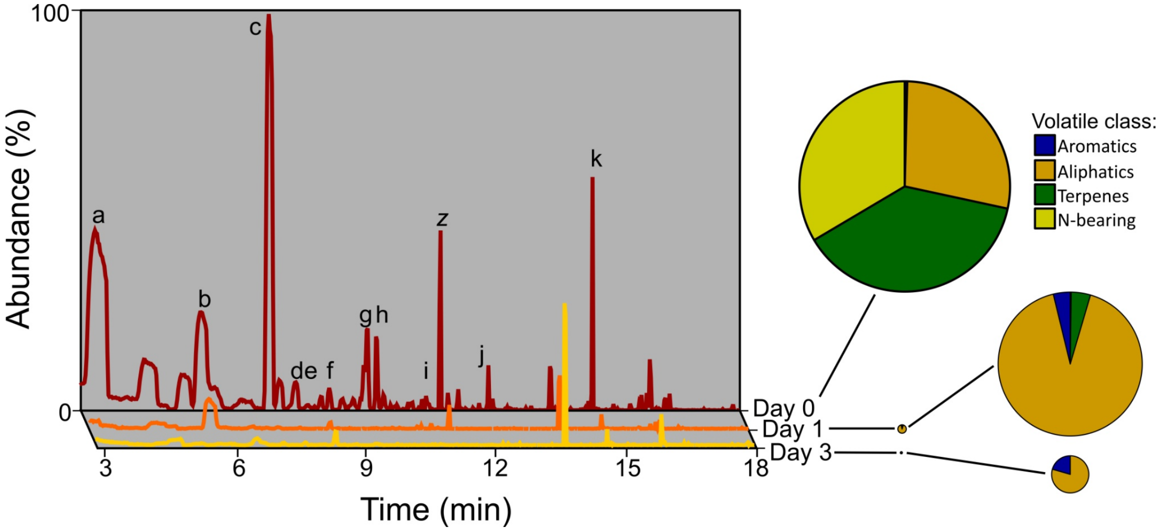

Figure S1. GCMS analysis of the headspace volatiles from chili seeds. Constituents of chili seed headspace shown in the total ion chromatogram for day 1 (red trace), 2 (orange trace), and 4 (yellow trace). Individual constituents include nitrogen-bearing alkaloids: 1-methyl pyrrole (a), 2-methoxy-3-pyrazine (e), tetramethylpyrazine (i), and 2-isobutyl-3-methoxypyrazine (j); monoterpenes tricyclene (c), camphene (d), *β*-myrcene (f), eucalyptol (g), and *cis*-*β*-ocimene (h); and aliphatics 2-hexenal (b), and 2-methyl-tridecane (j). *z* denotes a contaminant. Over the course of 4 d total emission rates decreased from 496 ng/h (± 207 ng/h SEM) to 4.2 ng/h (± 0.7 ng/h SEM). In addition, the composition of the headspace volatiles changed over the course of the experiment, with the composition being dominated by nitrogen-bearing pyrazines and pyrroles – known pheromone attractants of ants – at day 0, to the composition dominated by aliphatics at day 3. Pie charts to the right of the chromatogram are the absolute amounts on the same scale, and pie charts to the far right are those for day 1 and 3 at an increased scale but relative to one another. Constituents are classified by chemical class: aromatics, aliphatics, terpenes, and nitrogen-bearing (N-bearing).

Table S1. GCMS analysis of the *Capsicum chacoense* seed volatiles over the course of a four-day time period (N=6 replicates of 10 seeds for each time period).

	Treatment		
	Day 0	Day 1	Day 3
Emission rate (ng/h)*	496.0 (207.6)	19.8 (4.4)	4.2 (0.8)
Compound (ng/h)			
1-methylpyrrole	161.2 (67.5)	nd	nd
hexanal	35.6 (14.9)	2.8 (0.6)	nd
2-hexenal	52.0 (21.8)	14.8 (3.3)	nd
tricyclene	134.4 (56.2)	0.2 (0.1)	tr
camphene	8.5 (3.5)	nd	nd
2-methoxy-3-methylpyrazine	1.78 (0.7)	nd	nd
***β*-thujene**	0.3 (0.1)	nd	nd
***β*-myrcene**	4.0 (1.6)	nd	nd
eucalyptol	20.4 (8.5)	0.2 (0.1)	nd
***cis*-*β*-ocimene**	9.5 (4.0)	nd	nd
tetramethylpyrazine	0.8 (0.3)	nd	nd
2-isobutyl-3-methoxypyrazine	0.2 (0.1)	nd	nd
tetradecane	0.4 (0.2)	tr	nd

**t*-test: *P*<0.05

Compounds in bold are those verified by synthetic standards

nd. denotes volatile not detected by the MS

tr. denotes trace levels of the volatile

Multiple natural enemies cause distance-dependent mortality at the seed-to-seedling transition

Evan C. Fricke[1], Joshua J. Tewksbury[1,2], Haldre S. Rogers[3]

[1] Department of Biology, University of Washington, Seattle, WA 98195;
[2] Current address: The Luc Hoffmann Institute, WWF International, 1196 Gland, Switzerland;
[3] Department of Ecology and Evolutionary Biology, Rice University, Houston, TX 77005.

Email addresses: ECF, ecfricke@u.washington.edu; JJT, jtewksbury@wwfint.org; HSR, haldre@rice.edu

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Correspondence:
Evan C. Fricke
UW Biology
Box 351800
Seattle, WA 98195

E-mail: ecfricke@u.washington.edu
Telephone: +1 406.270.472

Statement of Authorship: ECF, JTT, and HSR designed field experiments. ECF collected field data, analyzed data, and wrote the first draft of the manuscript. All authors revised the manuscript.

Abstract

Specialized natural enemies maintain forest diversity by reducing tree survival in a density- or distance-dependent manner. Fungal pathogens, insects, and mammals are the enemy types most commonly hypothesized to cause this phenomenon. Still, their relative importance remains largely unknown, as robust manipulative experiments have generally targeted a single enemy type and life history stage. Here, we use fungicide, insecticide, and physical exclosure treatments to isolate the impacts of each enemy type on two life history stages (germination and early seedling survival) in three tropical tree species. Distance dependence was evident for five of six species-stage combinations, with each enemy type causing distance dependence for at least one species-stage and their importance varying widely between species and stages. Rather than implicating one enemy type as the primary agent of this phenomenon, our field experiments suggest that multiple agents acting at different life stages collectively contribute to this diversity-promoting mechanism.

INTRODUCTION

Density- or distance-dependent mortality plays a large role in maintaining diversity (Janzen 1970; Connell 1971; Wright 2002) and determining species abundance (Klironomos 2002; Comita *et al.* 2010; Mangan *et al.* 2010). Field studies in tropical and temperate forests show the widespread significance of this phenomenon (Harms *et al.* 2000; HilleRisLambers *et al.* 2002; Comita *et al.* 2010; Johnson *et al.* 2012). These findings have resulted in an increased research focus on determining the agents responsible (Packer & Clay 2000; Klironomos 2002; Bell *et al.* 2006; Bradley *et al.* 2008; Swamy & Terborgh 2010). Fungal pathogens, mammals, and insects are often hypothesized to be the agents of distance- or density-dependent mortality (Janzen 1970; Connell 1971; Terborgh 2012). An understanding of the relative importance of different natural enemy types is limited, as research that has tested the impacts of certain enemy types individually (e.g., Packer & Clay 2000; Bell *et al.* 2006; Mangan *et al.* 2010; McCarthy-Neumann & Kobe 2010; Liu *et al.* 2012) is poorly equipped to assess which types of natural enemy types contribute most to this diversity-promoting mechanism.

The majority of studies focused on the agents causing distance- and density-dependent mortality has targeted fungal pathogens, finding strong evidence for their involvement using a variety of approaches. Observational studies have found an increased prevalence of wilting or discoloration in seedlings near conspecific adults or at high conspecific density (e.g., Augspurger 1984; Alvarez-Loayza & Terborgh 2011). Other studies have shown survival of seedlings grown under greenhouse conditions to be greater in soil taken far from conspecifics than in soil taken near conspecifics (e.g., Packer & Clay 2000; Nijjer *et al.* 2007; Magnan *et al.* 2010; McCarthy-Neumann &

Kobe 2010; Liu *et al.* 2012). Field manipulations have used fungicide to show that fungal pathogens contribute to distance or density dependence (e.g., Hood *et al.* 2004; Kotanen 2007; Bell *et al.* 2006; Bagchi *et al.* 2010). When distance- or density-dependent mortality has been observed, all of the above studies have implicated fungal pathogens as a cause of the phenomenon, giving rise to the suggestion that fungal pathogens are a primary driver of distance- and density-dependent mortality (Bell *et al.* 2006; Bever *et al.* 2010). However, we argue that this conclusion may be premature, as uneven research effort across enemy types and methodological concerns associated with testing a single enemy type at a time limit the generality of existing studies.

Although fewer studies isolate effects of mammals or insects, both enemy types have been confirmed as agents of distance- or density-dependent mortality. Studies focused on mammals have reported distance- and density-dependent attack by mammals by observing signs of mammal predation (e.g., Wright & Duber 2001) and manipulating mammal access (e.g., Paine & Beck 2007; Hautier *et al.* 2010; Wotton & Kelly 2011) near and far from conspecifics or in locations of varying adult abundance. Other tests show no impact of mammals using similar observational (e.g., Visser *et al.* 2011) and manipulative approaches (e.g., Clark *et al.* 2012). Studies focused on insects include observational studies that show distance- and density-dependent attack by recording bore holes, mines, and herbivory (e.g., Sullivan 2003; Norghauer *et al.* 2006a; Mangan *et al.* 2010; Visser *et al.* 2011). Manipulations have demonstrated distance-dependent impacts of insects at nonvolant stages in one study (Sullivan 2003) and have shown that large insects were not a cause of distance dependence in another (Swamy & Terborgh 2010). At least two experimental manipulations have targeted all insects using an insecticide, but either did not find survival to be distance-dependent (Hammond *et al.* 1999) or did not attribute density dependence observed in one species to insects (Gripenberg *et al.* 2013).

Simply comparing the number of single-enemy studies that show distance- or density-dependent impacts of each enemy type is not sufficient to assess the relative importance of natural enemy types. Because single-enemy studies do not measure impacts of unmanipulated – and potentially more important – enemy types, this approach could overestimate the importance of an enemy type if its impacts are widespread but weak and could underestimate impacts of enemy types that are rarely tested. Even in field studies where natural enemy exclusion appears to remove all distance or density dependence, it may be unsafe to assume that the excluded enemy is the sole agent. Studies that compare impacts of more than one enemy type within the same ecosystem are, in principle, much better equipped to determine the relative importance of enemy types. Simultaneous comparisons of multiple enemy types have been done using observational approaches (Yamazaki *et al.* 2009; Mangan *et al.* 2010; Alvarez-Loayza & Terborgh 2011). However, attributing the source of distance or density dependence using observations has been criticized due to the difficulty in detecting the actual cause of mortality without manipulations (Freckleton & Lewis 2006).

Experimental approaches that manipulate multiple enemy types provide the most robust approach for determining the relative importance of different natural enemies, as they make unambiguous comparisons between survival of plants exposed to different combinations of natural enemies. Indeed, several studies have used two or more size-based exclosure treatments. These studies effectively quantify the impact of larger natural enemies – usually a defined group of organisms such as mammals (Norghauer *et al.*

2006b; Wotton & Kelly 2011) and in at least one case larger insects (Swamy & Terborgh 2010) – versus a group of smaller organisms including small insects and fungal pathogens. This approach gets to a part of the issue, but additional pesticide treatments are necessary to distinguish between fungal pathogens and insects (Freckleton & Lewis 2006). Approaches that isolate taxon-specific impacts are likely to provide more generalizable conclusions than are approaches that isolate impacts based on size alone, as individual species in taxonomically-based groups are more likely to share similar life cycles and dispersal abilities, traits relevant to their role in distance dependence (Beckman *et al.* 2012). Gripenberg *et al.* (2013) may be the first study to independently target insects and fungal pathogens with pesticides, and yet in their study, density-dependent mortality was not observed in six of seven species-stage combinations, and the cause of density dependence was not attributed to either enemy type in the seventh. Existing studies have simply not separated impacts of fungal pathogens, insects, and mammals to determine their relative importance.

Knowledge of differences in the source and strength of distance- and density-dependent mortality between life history stages is also limited, and this reduces our ability to model community dynamics and predict the consequences of seed disperser loss (Terborgh 2013). Mortality at seed and seedling stages is a key driver of plant demography (Wills *et al.* 1997; Harms *et al.* 2000), yet most studies of the source of distance and density dependence focus on only one of these life history stages, with most targeting seedlings alone (but see Wotton & Kelly 2011; Gripenberg *et al.* 2013). Existing single-enemy studies suggest that fungi may be more important and mammals less important at the seedling stage than at the seed stage (reviewed in Terborgh 2012).

However, the difficulties involved with comparing disparate single-enemy studies also limit the ability to determine the relative importance of enemy types across life stages. From a demographic perspective, approaches that use data from a single life stage or enemy type (Mangan *et al.* 2010) to predict impacts on population or community dynamics may yield misleading results if the strength of distance dependence observed by one enemy type at one stage is not predictive of patterns observed across the full range of enemies and at other life stages.

In this study, we determined the relative importance of fungal pathogens, mammals, and insects on mortality in the field at both seed and seedling stages for three forest species. We used seed additions, combined with fungicide, insecticide, and physical exclosures to attribute sources of distance-dependent mortality at each stage.

METHODS

Study site and species

This study was conducted at three forest sites on the island of Saipan in the Mariana Island chain in the Western Pacific. The island experiences an average temperature of 27°C with little annual variation, and receives approximately 2 m of precipitation per year with pronounced wet and dry seasons (Lander 2004). Experiments were conducted in three areas of limestone karst forest, the primary native forest type on the island, during the peak fruiting period in the early wet season. The three forest areas were separated by at least 500 m. We focused on three native, moderately common, fleshy-fruited forest tree species, *Aglaia mariannensis* Merr. (Meliaceae), *Morinda citrifolia* L. (Rubiaceae), and *Premna obtusifolia* R.Br. (Verbenaceae). The fruits of *A.*

mariannensis contain one or two arillate seeds inside a fibrous pericarp, *M. citrifolia* has fruits containing one to two hundred seeds, and *P. obtusifolia* produces single-seeded drupes.

Seed additions and exclusion experiments

We collected ripe fruits from at least 10 trees per species from several limestone forest sites, removed pulp from fruits, and thoroughly mixed seeds prior to planting. Fruits were collected within three days of planting and seeds were removed from fruits within 24 hours of planting. All seeds of a species were planted at all sites on the same day.

We planted seeds into plots either under adult conspecific canopies or under heterospecific canopies greater than 10 m from conspecifics. Placing ‘far’ plots further from conspecifics would have reduced the number of replicates because the focal species are relatively abundant across the forest. All plots were placed within 60 m x 60 m areas where tree locations have been mapped, therefore we are confident that far plots have no conspecifics within 10 m. Across the three forest sites, we established 36 plots each for *A. mariannensis* and *P. obtusifolia*, and 30 plots for *M. citrifolia*, with half under the focal tree canopy and half far from conspecifics. To account for differences in light availability between plots, we used a spherical crown densiometer to record canopy density at each plot.

Within each plot, we established six circular 0.05 m^2 subplots separated from each other by at least 0.5 m, and randomly assigned treatments to each subplot. In five of the six subplots, we added seeds, and applied one of the following treatments: exclusion of fungal pathogens (fungicide), exclusion of insects (insecticide), vertebrate exclusion (physical exclusion), exclusion of all three mortality agents, or control (no exclusion) (treatments described in detail in *Natural enemy exclusion treatments* below). For *A. mariannensis*, *M. citrifolia*, and *P. obtusifolia* we added 20, 40, and 50 seeds per subplot, respectively, to achieve seed densities scaling inversely with seed size. These densities are observed locally under adults; we matched these densities in far plots to isolate effects of distance, and used small subplots to minimize the area of high seed density far from conspecifics. In the sixth subplot we did not add seeds but applied all treatments to measure maximum recruitment from background seed rain. We found a total of two seedlings across all species in these 'seed addition control' subplots and thus do not include data from these subplots in the analysis. In all plots, we marked any preexisting seedlings to exclude these from our analysis; all species had fewer than five total preexisting seedlings across all plots. During weekly checks, we recorded germination, marked all germinants, and recorded survival of previously marked germinants. Germination of *A. mariannensis*, *M. citrifolia*, and *P. obtusifolia* began after approximately 4, 6, and 1 weeks, respectively, and we monitored seedling survival for an additional 10 weeks for *A. mariannensis* and 5 weeks for *M. citrifolia* and *P. obtusifolia*.

Natural enemy exclusion treatments

For the exclusion of fungal pathogens, we used Ridomil Gold (Syngenta, Basel, Switzerland), a selective fungicide that has been used successfully in other experiments (Bell *et al.* 2006; Bagchi *et al.* 2010). We applied the fungicide weekly, in 50 mL of aqueous solution, at a concentration of 0.25 g/m^2.

For insect exclusion we used esfenvalerate (FenvaStar, LG International, Englewood Cliffs, NJ, USA). This pyrethroid insecticide, or its stereoisomer fenvalerate, has been used widely and existing evidence suggests that it does not impact plant survival (Carson & Root 2000; Mitchell 2003). We applied esfenvalerate bi-weekly, also in 50 mL of aqueous solution, at a concentration of 0.006 g/m^2. All other subplots were treated with an equal volume of water whenever treatments were applied.

To exclude rodents, we used closed-top cylindrical 1.3 x 1.3 cm mesh exclosures staked into the soil. As the wire mesh exclosures could influence the light environment, the subplots that did not have the rodent exclosure treatments were also covered with equally sized wire mesh exclosures, but with 13 x 13 cm openings cut into the side of the cylinder to allow rodent access.

Analysis

To analyze impacts of each natural enemy type on germination and seedling survival, we used generalized linear mixed effects models with a binomial error distribution in the R package *lme4* (Bates *et al.* 2013). In separate models for each species and each of the two stages, the response variable was the portion of seeds that germinated ("germination" stage) or the portion of germinants that survived to the end of the study period ("seedling survival" stage). The fixed effects included distance (under canopy or far) and each of the three exclosure treatments individually; canopy density was included as a covariate and site as a random effect. We derived estimates from a full model containing canopy density and interactions between distance and each exclosure treatment. The presence of an interaction between an exclosure treatment and distance would indicate that the agent causes distance-dependent mortality. The size of this interaction indicates how much more the treatment improves survival near conspecifics than it does far from conspecifics, or the distance-dependent effect of the agent that was excluded. A treatment effect without an interaction with distance would indicate that the agent is responsible for distance-independent mortality, or causing equally large impacts on mortality near and far from parent trees.

RESULTS

We observed distance-dependent mortality, with germination or survival greater far from conspecific canopies, in all but one life stage-tree species combination (Fig. 1, Table S1); no overall distance dependence was observed at the seedling stage of *Morinda citrifolia*. Insects caused distance-dependent mortality in germination of *Premna obtusifolia* and for seedlings of *Aglaia mariannensis* and *M. citrifolia* (Fig. 2). Rodents contributed to distance-dependent morality of seeds for *P. obtusifolia* and *M. citrifolia*. Fungal pathogens caused distance-dependent mortality only for seeds of *M. citrifolia*. Fungi had the opposite impact on seedling survival of *M. citrifolia*, they appear to compensate for distance-dependent mortality caused by insects such that seedling survival of *M. citrifolia* in control plots was independent of distance. In two instances, the source of observed distance-dependent effects was not captured by our experimental treatments. For seeds of *A. mariannensis* and seedlings of *P. obtusifolia*, distance-dependent mortality was observed – “total” distance dependence was non-zero (Fig. 2) – but no exclusion treatments increased survival near conspecifics more than they did in far plots. Survival was greater under more open canopies for *M. citrifolia* and *P. obtusifolia,* whereas canopy openness had little effect on survival for *A. mariannensis* (Table S1).

DISCUSSION

Our manipulative field experiments provide evidence that multiple types of natural enemies are important agents of distance-dependent mortality, which we observed in both germination and seedling survival stages of two tropical forest species and in the germination stage of the third species. Of six species-stage combinations, fungal pathogens were an agent of distance dependence in one species-stage, insects in three species-stages, and mammals in two species-stages. Although determining the relative importance of these enemy types in general will require the study of many more species and forests, these findings do not support the suggestion that fungal pathogens are the primary cause of this phenomenon (Bell *et al.* 2006; Bever *et al.* 2010). These results highlight the complexity of the mechanism underlying distance-dependent mortality and suggest a need for increased caution when integrating data on distance dependence across studies or life stages to model community dynamics.

This experimental approach demonstrated that multiple natural enemy types operate simultaneously to create distance-dependence, and that natural enemy types can differ across life history stages. Insects were the most prevalent cause of distance-dependent mortality in this study; we suggest that their impacts may be underestimated in the literature because few manipulative experiments have targeted insects (but see Hammond *et al.* 1999; Gripenberg *et al.* 2013). Mammals caused more distance dependence at the seed stage than at the seedling stage. This followed our predictions because the mammalian natural enemies in this system are rodents most commonly associated with seed predation, and supported previous suggestions based on observational, single-enemy, or single-stage studies (reviewed in Terborgh 2012). In both species-stages in which mammals caused distance dependence, however, they played a supporting role – either fungal pathogens or insects were a stronger cause of distance-dependent mortality. Further research using similar methods to determine the relative importance of these enemy types may reinforce the suggested trend that mammals are weaker agents of distance and density dependence (Terborgh 2012). Finally, we were unable to attribute a source of distance dependence for two species-stages in which we found distance-dependent mortality. These patterns could be interpreted as indirect evidence for compensation among types of natural enemies (e.g., when fungal pathogens are excluded, the effects of insects may increase, masking some of the distance-dependent effects of fungal pathogens) or they could suggest that an even broader set of mortality agents is at work. For example, we did not manipulate competition, allelopathy, or certain less-often studied natural enemies such as bacteria and gastropods. These enemy types are seldom manipulated, although the impacts of both groups have been examined individually (Bradley *et al.* 2008, Pigot & Leather 2008).

Our results, showing large differences in the source and strength of distance dependence between species and between life history stages of the same species, have implications for the collection and use of distance- or density-dependent survival data. Specifically, our findings suggest that the strength of distance dependence at one life stage or caused by one enemy does not predict the strength of distance dependence at other life stages or caused by other enemies. Because of this, we caution against using distance-dependent survival data from experiments that target a single enemy type or a short temporal window when predicting the consequences of seed disperser loss (Terborgh 2013) or linking distance dependence to population-scale dynamics (Mangan *et al.* 2010). Distance- or density-dependent survival data obtained by recording survival in the field (Harms *et al.* 2000; Comita *et al.* 2010) may be more appropriate for these purposes than are data obtained from manipulations that quantify single enemy types. These experiments indicate that, even when removal of one enemy type appears to completely remove the distance dependence observed in control plots, other enemy types can simultaneously contribute to distance dependence; this provides a further caution against attempting to determine the relative importance of natural enemy types by manipulating a single enemy type. The observed large differences in the strength of distance dependence between life stages reinforce the suggested need for more complex demographic models or long-term monitoring to link recruitment patterns to adult abundances (McConkey *et al.* 2012).

Testing the relative importance of natural enemy types provides information relevant for research that considers how spatial recruitment patterns and natural enemy specialization impact diversity maintenance. Characteristics of the agents that cause distance dependence can greatly influence patterns of survival, and thus the probability that these dynamics maintain diversity (Adler & Muller-Landau 2005; Beckman *et al.* 2012). Natural enemies that specialize on certain plant species are thought to cause density dependence, and insects and fungi exhibit high specialization (Gallery *et al.* 2007; Beckman & Muller-Landau 2011; Sedio & Ostling 2013). Although some have suggested that mammals are usually generalists and less likely to produce distance dependence

(Hammond & Brown 1998), mammals do have a role in generating distance and density dependence (Paine & Beck 2007; Hautier *et al.* 2010; Wotton & Kelly 2011), and did so for two species in this study.

Assessing the sources of mortality over the seed-to-seedling transition helps fill gaps in our understanding of the source and strength of factors limiting recruitment in tropical forests. In the first experiments testing impacts of multiple enemy types on predispersal seed mortality for multiple tropical plants, Beckman and Muller-Landau (2011) note that the relative contribution of invertebrates, vertebrates, and pathogens are poorly known at early life stages. Our results show that the source and severity of mortality at the seed-to-seedling transition is characterized by variability; the contribution of each enemy type varies widely by species, stage, and between locations near and far from conspecifics.

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FIGURES

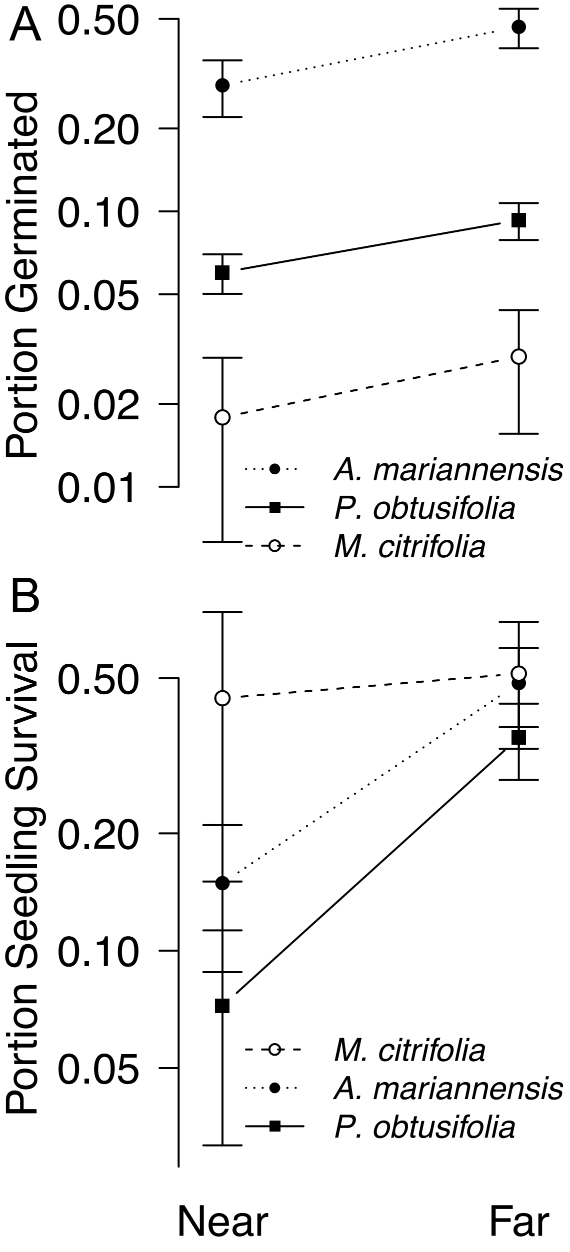

Fig. 1. Distance-dependent survival of *Aglaia mariannensis*, *Morinda citrifolia*, and *Premna obtusifolia* at germination (A) and early seedling (B) stages. Survival was greater far from adult conspecifics for each species and stage, except during the seedling stage of *M. citrifolia*. Survival data shown from untreated plots only. Error bars indicate ±1 S.E.

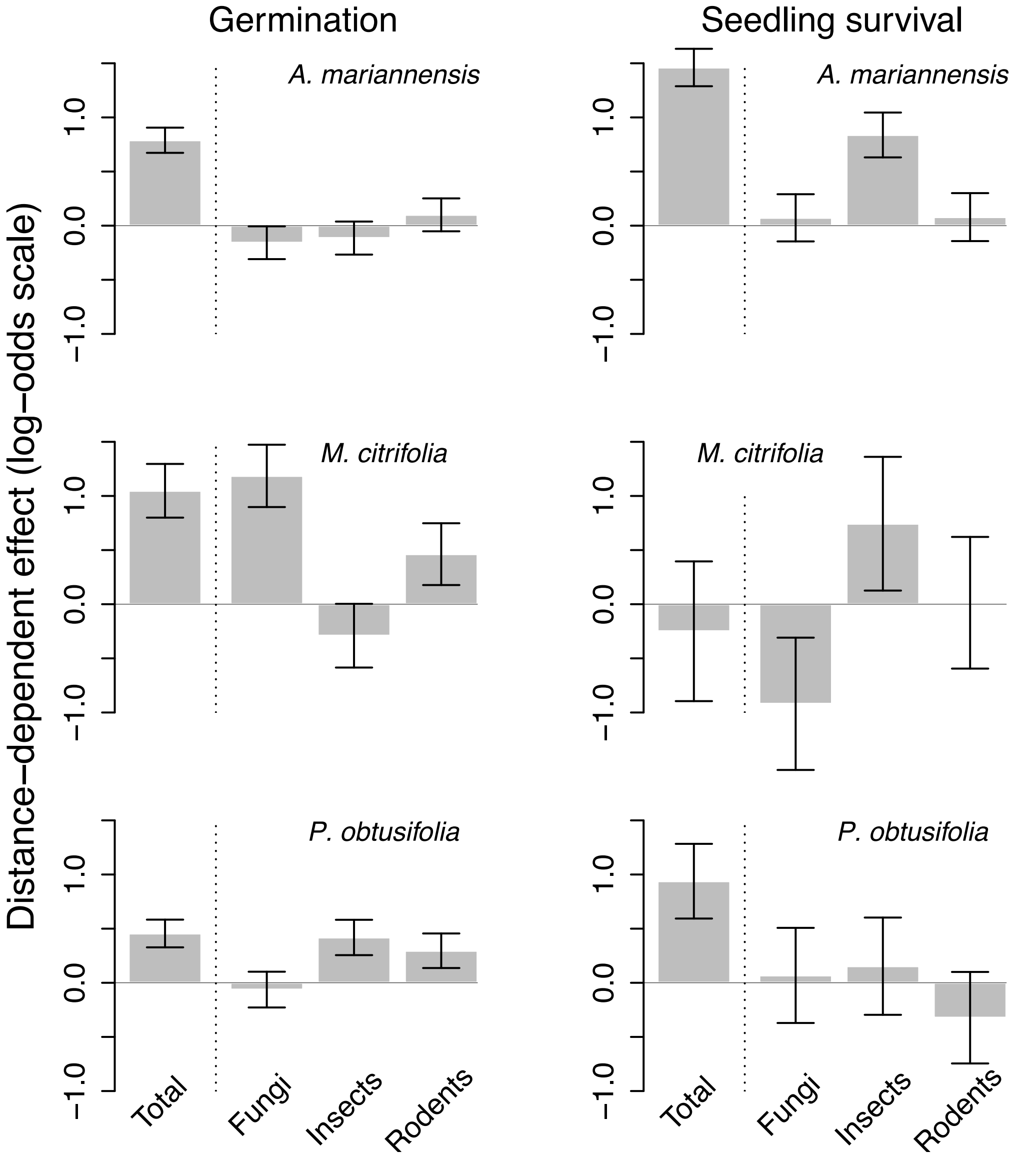

Fig. 2. Source and strength of distance-dependence for three tree species at germination and early seedling survival stages. Estimated effect sizes ("distance-dependent effect"; log-odds scale) indicate greater mortality near conspecific adults than far from adults when positive, the reverse when negative. Individual bars show the total observed distance-dependent impacts ("Total", left of dotted line), and the distance-dependent impacts attributable to fungal pathogens, insects, and rodents, assessed using fungicide, insecticide, and physical exclosure treatments (right of dotted line). Error bars indicate ±1 S.E.

SUPPLEMENTARY INFORMATION

Table 1. Coefficient estimates (β) for fixed effects in models describing the impact of fungicide (Fung.), insecticide (Ins.), and rodent exclosures (Rod.) on germination (Germ) and early seedling survival (Surv) of *Aglaia mariannensis*, *Morinda citrifolia*, and *Premna obtusifolia*. Distance-dependent variables indicate interaction terms between distance (Dist; near or far) and each treatment effect, whereas distance-independent variables show impacts of the treatment on germination or survival independent of distance.

			Density-independent				Density-dependent		
		Dist.	Fung.	Ins.	Rod.	Canopy	Fung.	Ins.	Rod.
Aglaia mariannensis									
Germ	β	0.789	-0.343	-1.086	0.096	-0.021	0.158	0.114	-0.100
	SE	0.116	0.110	0.113	0.110	0.016	0.152	0.153	0.152
Surv	β	1.461	-0.277	0.218	0.282	0.003	-0.073	-0.838	-0.079
	SE	0.173	0.146	0.138	0.146	0.002	0.218	0.207	0.222
Morinda citrifolia									
Germ	β	1.048	0.302	-0.039	-0.039	0.472	-1.185	0.291	-0.463
	SE	0.248	0.210	0.201	0.201	0.040	0.288	0.294	0.285
Surv	β	-0.250	-0.917	-0.428	0.193	-0.053	0.920	-0.744	-0.013
	SE	0.387	0.495	0.497	0.481	0.089	0.611	0.617	0.608
Premna obtusifolia									
Germ	β	0.455	0.017	0.146	-0.112	0.069	0.063	-0.419	-0.296
	SE	0.128	0110	0.111	0.109	0.018	0.381	0.163	0.160
Surv	β	0.938	-0.003	0.377	-0.395	0.301	-0.068	-0.153	0.322
	SE	0.345	0.242	0.254	0.239	0.050	0.439	0.449	0.423

Title: Measuring the demographic impact of conspecific negative density dependence

Evan C. Fricke[1]*, Stefan A. Schnitzer,[2,3] S. Joseph Wright[3]

[1]Department of Biology, University of Washington, Seattle, WA 98195 USA
[2]Department of Biological Sciences, University of Wisconsin, PO Box 413, Milwaukee, WI 53201, USA
[3]Smithsonian Tropical Research Institute, Apartado 0843-03092, Balboa, Panama
[*]Correspondence author. E-mail: ecfricke@u.washington.edu

Running headline: Demographic impact of density dependence

Key-words: conspecific negative density dependence, diversity maintenance, coexistence, Janzen-Connell hypothesis, seed dispersal, advantage-when-rare, community compensatory trend

Summary

1. Conspecific negative density dependence (CNDD) has been observed to be stronger among rare species in plant communities. All else equal, this will cause a disadvantage among rare species relative to more abundant species that appears to be inconsistent with the role of CNDD as a diversity maintenance mechanism.
2. The resolution to this apparent paradox is for lower species abundance to decrease the demographic impact of CNDD sufficiently to outweigh the disadvantage of stronger CNDD in rare species.
3. Whether this occurs in natural systems remains untested because existing metrics do not isolate the demographic impact of CNDD and is particularly uncertain for tropical trees because rare species tend to be more spatially aggregated, which will intensify the demographic impact of CNDD.
4. We develop a new metric, effective density dependent mortality (EDDM), to quantify the portion of a population's individuals that are killed by density-dependent effects. EDDM increases with greater local conspecific densities and stronger CNDD.
5. In a long-term study of seed fall and recruitment at Barro Colorado Island, Panama, more abundant species experienced greater local conspecific seed densities and had more clumped distributions of seed density. Although stronger CNDD tended to increase EDDM, less abundant species experienced less EDDM. Spatial aggregation had no relationship with EDDM.
6. *Synthesis:* We show that lower species abundance reduces the frequency of conspecific interaction and, consequently, mortality caused by CNDD. This mechanism allows rare species to avoid the demographic disadvantage-when-rare that

would, all else equal, result from stronger CNDD in rare species. Our work provides empirical support for a resolution to the apparently paradoxical recent findings that rare species experience stronger CNDD, and clarifies how CNDD can determine abundances and contribute to species coexistence.

Introduction

The mechanisms that maintain diversity and determine species abundance are fundamental to ecology. Conspecific negative density dependence (CNDD) appears to play a key role in both, particularly among tropical forest trees (Wright 2002; Comita *et al.* 2010; Mangan *et al.* 2010). In theory, CNDD gives locally rare species an advantage that enables their persistence and thereby maintains diversity (Chesson 2000). In field studies, rare plant species are rare because they suffer stronger CNDD in grasslands and temperate and tropical forests (Klironomos 2002; Comita *et al.* 2010; Mangan *et al.* 2010; Johnson *et al.* 2012). This suggests a possible paradox. If greater CNDD strength were to cause more negative demographic impacts among rare species, CNDD might impose a disadvantage on rare species that would limit rather than facilitate species coexistence.

Recent simulations help resolve this apparent paradox. Stronger CNDD stabilizes species at lower abundance and allows rare species to persist when stochastic population fluctuations would otherwise cause extinction (Chisholm & Muller-Landau 2010; Yenni, Adler & Ernest 2012; Mack & Bever 2014). These studies also suggest how the apparent paradox is avoided. Lower abundance reduces the frequency of conspecific interactions and the demographic impact of CNDD. However, the spatially implicit simulations assume population-level abundance controls the responses of individuals to CNDD (Chisholm & Muller-Landau 2011; Yenni, Adler & Ernest 2012). Among tropical forest trees, strong interactions are restricted to local scales defined by immediate neighbors (Harms *et al.* 2000; Comita *et al.* 2010; Uriarte et al. 2010; Lebrija-Trejos et al. 2014) and there is a strong inverse relationship between spatial aggregation and population-level abundance (Condit *et al.* 2000). Greater aggregation will increase local conspecific density experienced by the average individual and intensify the demographic impact of CNDD. How the counteracting effects of greater spatial aggregation and lower population-level abundance influence local conspecific densities will determine how the demographic impact of CNDD varies with abundance.

Existing metrics do not measure the population-level consequences of CNDD. For tropical forests, population-level tests of a rare species advantage have focused on a ‘community compensatory trend’, predicting that individuals of rare species should have greater survival probabilities (Connell, Tracey & Webb 1984; Webb & Peart 1999; Queenborough *et al.* 2007; Comita & Hubbell 2009; Chen *et al.* 2010). In contrast, studies that relate the strength of density dependence to abundance extract a CNDD-strength parameter from the relationship between density at one life stage and survival to a subsequent stage (Klironomos 2002; Comita *et al.* 2010; Mangan *et al.* 2010; Johnson *et al.* 2012; Bagchi *et al.* 2014). Neither metric directly quantifies how CNDD itself influences demography. The survival probabilities used to test for a community compensatory trend do not isolate the impact of CNDD from other density-independent influences on survival. Likewise, CNDD strength does not provide information on interspecific differences in the frequency of conspecific interaction that are likely to determine how CNDD acting among near neighbors scales up to population-level demographic outcomes. A new metric that isolates the demographic impact of CNDD is needed.

To improve our ability to quantify the demographic impact of CNDD on populations, we develop a metric that estimates the proportion of deaths attributable to density dependent effects. We call this new metric effective density dependent mortality (EDDM). EDDM compares the number of individuals that actually survive to the number of individuals estimated to survive under a density-independent scenario. To quantify EDDM for a population using data on local conspecific seed density and seedling recruitment, we first fit a model of density-dependent survival where local seed density (*S*) is related to local recruit density (*R*) as $R = a\, S^{b}$. The a-value is per-capita survival at low conspecific density and the b-value is the CNDD strength term, with $b = 1$ and $b < 1$ indicating density-independence and CNDD, respectively (Harms *et al.* 2000; Wright *et al.* 2005a; Bagchi *et al.* 2014). We then estimate recruitment if there were no CNDD by setting b to 1 and using the same fitted a-value. Finally, we compare this estimate of density-independent recruitment to density-dependent recruitment using the fitted a- and b-values (although observed recruitment could be used instead). In practice, we measure the local density of conspecific seeds and recruits across locations (*j*) and estimate the number of recruits for density dependent (Rdd_i) and density independent (Rdi_i) scenarios and EDDM as follows:

$$R_{dd} = \sum_{j} aS_j{}^{b}$$

$$R_{di} = \sum_{j} aS_j{}^{1}$$

$$EDDM = (R_{di} - R_{dd})/R_{di} = 1 - \sum_{j} S_j{}^{b} \Big/ \sum_{j} S_j{}^{1}$$

Note that the a-values, which are equal, cancel from the summation. This removes the impact of species-specific, density-independent recruitment probabilities that would otherwise complicate interspecific comparisons. As a result, EDDM isolates the demographic impact of CNDD on the population and can be compared among species. EDDM increases with stronger CNDD, higher mean seed density, and more clumped seed distributions (Fig. 1C). A species with stronger CNDD (*Sp3*, *b* = 0.25) can have a lower proportion of death attributable to CNDD than a species with weaker CNDD (*Sp2*, *b* = 0.5) if its individuals tend to experience lower conspecific densities (Fig. 1B).

Here, we use data from a long-term study of seed fall and recruitment on Barro Colorado Island, Panama to measure the demographic impact of CNDD and the factors that influence its severity. We assess how abundance and adult spatial aggregation impact CNDD strength and the distribution of conspecific seed density, and then examine relationships between EDDM and abundance, adult aggregation, and CNDD strength. To compare to one previous approach for measuring population-scale demographic impacts of CNDD, we also test for a community compensatory trend in survival across the seed-to-seedling transition.

Materials and methods

Seed production and seedling recruitment

Our seed production and seedling recruitment data are from censuses conducted in the 50 ha Forest Dynamics Plot at Barro Colorado Island, Panama from 1993 to 2009 (Wright *et al.* 2005a). Weekly censuses recorded the number of seeds of each species in 200 0.5-m^2 seed traps. Annual censuses recorded the number of seedling recruits in 600 1-m^2 plots located 2 m from three sides of each seed trap. We refer to each seed trap and its associated seedling plots as a station. We summed seeds and the resulting recruits for each year and station, associating recruits with the appropriate year of seed production by incorporating species-specific germination lags (Garwood 1983). We include 29 tree and liana species with more than 100 recruits recorded over the study period.

The strength of CNDD

We used maximum likelihood estimation in the R package *bbmle* (Bolker 2013) to model the relationship between seeds and recruits per square meter as $R = a\,S^{b}$ with a negative binomial error distribution (Wright *et al.* 2005a). When seed density was less than recruit density, we assumed that seed density was equal to recruit density (Harms *et al.* 2000; HilleRisLambers, Clark & Beckage 2002). Because this might bias estimates of *b* and recruitment upwards (Wright et al. 2005a), we tested our ability to use fitted parameters and the original, unmanipulated seed densities to predict observed recruitment. Estimated recruitment summed across station-years for each species strongly predicted observed recruitment (linear regression, $R^2 = 0.99$, $P < 0.0001$), suggesting that this assumption does not bias our estimates of recruitment. Another potential bias could result from space limitation; some stations had more seeds than could recruit because of spatial constraints. Excluding from the analysis data from station-years that had conspecific seed densities above the maximum observed conspecific seedling density, 71 seedlings m^{-2} (Harms *et al.* 2000), we found qualitatively similar results, suggesting that our conclusions are not simply the result of space limitation.

Abundance, aggregation, and conspecific seed densities

To measure adult spatial aggregation, we calculated $\Omega_{0\text{-}10}$ for adults of each species (Condit *et al.* 2000). The $\Omega_{0\text{-}10}$ index standardizes the density of adult conspecifics located within 10 m of an adult by the overall adult density observed for the 50-ha plot. To determine whether individuals were adults, we used species-specific estimates of the dbh threshold at which individuals are reproductive for trees (Wright *et al.* 2005b), and we included all rooted stems >5 cm dbh for lianas (Kurzel et al. 2006, Ledo & Schnitzer 2014). We also used size thresholds of 3 and 4 cm to identify adult lianas, and our results are robust to the size threshold used. As measures of abundance, we used basal area (m^2 ha^{-1}) and population density (total number of individuals ≥1 cm dbh ha^{-1}) (Hubbell, Condit & Foster 2005; Schnitzer *et al.* 2012). To describe the local conspecific seed densities that seeds of each species experience, we fitted negative binomial distributions to seed density data using only those station-year combinations that received seeds for each species. By omitting station-year combinations with zero seeds, we captured the relevant distribution of seed densities experienced by seeds of each species. The negative binomial parameters are mean conspecific density (μ) and the clumping parameter (κ) (Muller-Landau *et al.* 2008). We used the glm.nb function in the MASS package to fit negative binomial distributions (Venables and Ripley 2002).

Effective density dependent mortality

We used the seed density data from each of the 3,400 station-year combinations and the species-specific parameter indicating survival at low density (*a*-value) from the power law model to estimate recruitment in the density-independent scenario (summed across stations as *Rdi*, as described in the Introduction). For recruitment in the density-dependent scenario, we used observed seedling density data (summed across station-years as *Robs*). The use of observed recruit density data in place of *Rdd* differs slightly from the description of EDDM in the Introduction, which used fitted parameters to calculate *Rdd*. Results are consistent whether *Rdd* or *Robs* is used. For each species *i*, we calculated effective density dependent mortality as $EDDM_i = (Rdi_i - Robs_i) / Rdi_i$

Analyses of EDDM and the community compensatory trend

We first analyzed relationships between EDDM and the factors hypothesized to influence the severity of EDDM, mean seed density (μ), the clumping parameter (κ), and CNDD strength (b) (Fig. 1). The logit-transformation of EDDM (Warton & Hui 2011) was the dependent variable in a generalized linear model with μ, κ and b as fixed effects. We next analyzed relationships between μ, κ and b and factors that may in turn influence them. We included aggregation ($\Omega_{0\text{-}10}$), abundance, and growth form (tree or liana) as fixed effects. Gamma error distributions were used in models with μ and κ as dependent variables because only positive values are possible for the dependent variables, and we used the normal error distribution in the model with b as the dependent variable. Finally, we evaluate relationships between EDDM as the dependent variable and adult spatial aggregation, abundance, growth form, and CNDD strength as independent variables. We assume that each effect is present and interpret estimates and their standard errors from full models (Bolker et al. 2008). We repeated each analysis that included abundance as an independent variable using basal area or population density as the measure of abundance. Because lianas have lower basal area relative to trees, basal area may have different effects for trees and lianas. We include interaction terms between growth form and abundance that improve AIC values by 2 units.

We tested for the existence of a community compensatory trend in the relationship between abundance and the logit transformation of the recruit to seed ratio, calculated for each species as the mean ratio of recruit to seed density across all station-year combinations. Results are consistent when the recruit to seed ratio is calculated as recruit density summed across all station-years to seed density summed across all station-years.

Results

Our analysis of 29 tree and liana species included 233 759 seeds and 27 232 recruits. A density-dependent recruitment model strongly improved model fit over a linear model for 26 of the 29 species ($P < 0.001$, Table S1). The three exceptions were extreme heliophiles, with recruitment limited to tree fall gaps and the three smallest a values ($a \leq 0.009$, meaning that 1 000 seeds generated 9 or fewer first-year seedling recruits) (Table S1). Parameter estimates for these three species are dominated by spatial variation in light availability and do not reflect density-dependent recruitment dynamics, and thus we remove them from further analysis. Negative binomial distributions provided strong fits describing the distribution of conspecific seed densities experienced by seeds (Table S1).

EDDM strongly increased with greater mean conspecific seed density (higher values of μ; 0.042 ± 0.004; estimate ± 1 S.E.), more clumped distributions of conspecific seed density (lower values of κ; -1.62 ± 0.24), and stronger CNDD (lower values of b; -1.95 ±) (Table 1). Mean conspecific seed density (μ) was greater in species with greater basal area and for lianas, but did not increase with adult spatial aggregation (Table 1).

Larger clumping parameters were present among lianas with greater basal area (Table 1). A positive trend related CNDD strength (*b*) and basal area, but the estimate was not two standard errors from zero (Table 1, Figure S1).

EDDM increased with greater basal area and with stronger CNDD, but was not significantly related to adult aggregation (Table 2, Figure 2). EDDM decreased with abundance for lianas. We found similar results in all analyses when population density was included as the measure of abundance (Table S2 and Table S3).

To test for the existence of a community compensatory trend, we analyzed the relationship between abundance and the recruit-to-seed ratio. The recruit-to-seed ratio was not significantly related to basal area (linear regression, $R^2 = 0.001$, $P = 0.90$; Figure S2A) or to population density (linear regression, $R^2 = 0.04$, $P = 0.33$; Figure S2B).

Discussion

We develop a new metric, effective density dependent mortality (EDDM), to measure the demographic impact of conspecific negative density dependence (CNDD) using field data. EDDM estimates the portion of individual deaths associated with density-dependent effects. EDDM should increase with greater local conspecific densities and with stronger CNDD. Interspecific differences in these characteristics should cause variation in EDDM among species within a community. We measured EDDM at the seed-to-seedling transition for 26 tree and liana species at Barro Colorado Island, Panama. We expected greater species abundance to increase local conspecific densities and consequently increase EDDM at the population scale. We found that more abundant species had higher local conspecific seed densities and that EDDM increased with abundance. We expected greater adult spatial aggregation to increase EDDM by a similar process. However, we found that adult aggregation did not increase local conspecific density or EDDM for the species tested. We also found support for the expectation that EDDM would increase with stronger CNDD.

An apparent paradox exists between the roles of CNDD in determining species abundance and in maintaining species diversity. To achieve its role in determining abundance, CNDD is thought to cause rare species to suffer more from conspecifics and thus to have fewer opportunities to establish. Indeed, rare plant species experience stronger CNDD in both tropical and temperate communities (Klironomos 2002; Comita *et al.* 2010; Mangan *et al.* 2010; Johnson et al. 2012). All else equal, stronger CNDD in rare species should cause a demographic disadvantage on rare species, which would be inconsistent with the role of CNDD as a diversity maintenance mechanism (Chesson 2000). Yet simulation studies provide support that CNDD can achieve both outcomes; species with varied CNDD strength do stabilize at different abundances (Mangan *et al.* 2010; Chisholm & Muller-Landau 2011; Yenni, Adler & Ernest 2012; Mack & Bever 2014). In these simulations, the paradox is resolved because the demographic impact of CNDD decreases as abundance decreases. However, in natural communities the demographic impact of CNDD may not respond to abundance sufficiently to outweigh the disadvantage of stronger CNDD. If the local conspecific densities that individuals experience in the field are unrelated to abundance, rare species may be unable to avoid the disadvantage-when-rare. Furthermore, rare species tend to be more aggregated in tropical forests (Condit *et al.* 2000). This should increase local conspecific densities, worsen the demographic impact of CNDD for rare species, and further limit the ability of CNDD to maintain diversity.

EDDM allows us to address this apparent paradox by quantifying the empirical demographic impact of CNDD for species of varied abundance. We found that individuals do experience greater local conspecific densities as species abundance increases and, in turn, EDDM increases with abundance. When acting on individual populations, this constitutes the stabilizing mechanism necessary for diversity maintenance (Chesson 2000). This advantage of rarity was present despite heterogeneity in local conspecific density and was not outweighed by any effect of adult spatial aggregation on local conspecific seed density. These results provide empirical support for the mechanism that allows rare species to avoid a disadvantage that would otherwise result from the stronger CNDD they experience, and thus for the resolution to the paradox. Stronger CNDD in rare species can be balanced by less frequent conspecific interaction to avoid a demographic disadvantage-when-rare.

We found no evidence of a community compensatory trend (CCT) in survival probabilities among these species. For two reasons we question whether the CCT can provide a strong test of the demographic rare species advantage hypothesized to be necessary for diversity maintenance (Connell, Tracey & Webb 1984). First, the survival probabilities used to test the CCT are shaped by confounded density-dependent and density-independent influences. This should increase variability around the predicted CCT relationship or, if density-independent influences are correlated with abundance, can cause spurious CCTs (Wright 2002). Second, we question whether the CCT is necessary for the diversity-maintaining outcome of CNDD. The CCT should tend to make rare species increase in abundance and common species decrease (Connell, Tracey & Webb 1984). Unless other factors correlated with abundance balance this effect (Wright 2002), all species should stabilize toward the same abundance. If any processes do stabilize species at different abundances, it seems unlikely that species abundance alone would strongly predict whether a species is currently above or below the abundance at which it is stable, and therefore unlikely that a CCT would be evident. Still, we did find a rare species advantage to the extent that rare species tended to have lower EDDM. Whether this positive relationship between EDDM and abundance is necessary for diversity maintenance is not resolved by this study. On first principles, it may be more appropriate to expect the lack of a relationship between abundance and EDDM among species; perhaps species should stabilize toward abundances that result in similar EDDM across species.

One previous approach has incorporated information on the frequency of conspecific interaction to better quantify demographic impacts of CNDD (Kobe & Vriesendorp 2011; Lin *et al.* 2012). These studies have quantified ‘potential NDD’ for each species by multiplying CNDD strength by the maximum conspecific density at which seedlings of a species were observed in the field. This index is proportional to the maximum potential demographic impact of CNDD on a population, but may not be a strong measure of the actual demographic impact if a species exhibits variation in local conspecific density. For example, the potential NDD metric would overestimate the demographic impact if local conspecific density were nearly always low but very high at a single measured location, or underestimate demographic impacts relative to other species if observed local conspecific densities were often near the maximum conspecific density. We suggest that approaches like EDDM that account for heterogeneity in local conspecific density, incorporating densities recorded across all measured plots to make demographic predictions, provide a stronger ability to link local, heterogeneous survival dynamics to community-scale outcomes of CNDD.

In one case the observed relationships differ from the framework that our results otherwise support. Trees and lianas were inconsistent in their responses to species abundance. Lianas as a group tended to have low abundance and low EDDM, consistent with our predictions and the relationship observed across all species. Yet among liana species alone, greater abundance did not increase local conspecific densities or EDDM as it did for trees. Weaker support for our predictions among lianas is consistent with recent work indicating that disturbance, rather than CNDD, best explains liana spatial distribution and diversity (Ledo and Schnitzer 2014).

We have structured our discussion of CNDD as though there are two distinct outcomes of CNDD, as existing research has generally focused on either the abundance-determining or diversity-maintaining outcomes separately, employing distinct predictions and metrics to test each. Yet as our results demonstrate, it may be more appropriate to consider both outcomes to be the result of the single role of CNDD as a stabilizing mechanism. By stabilizing species abundance, CNDD maintains diversity, and because the strength of this stabilization varies, CNDD determines species abundance. Effective density dependent mortality provides an approach to measure in the field how CNDD influences demography to achieve both outcomes.

Acknowledgements

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Figures

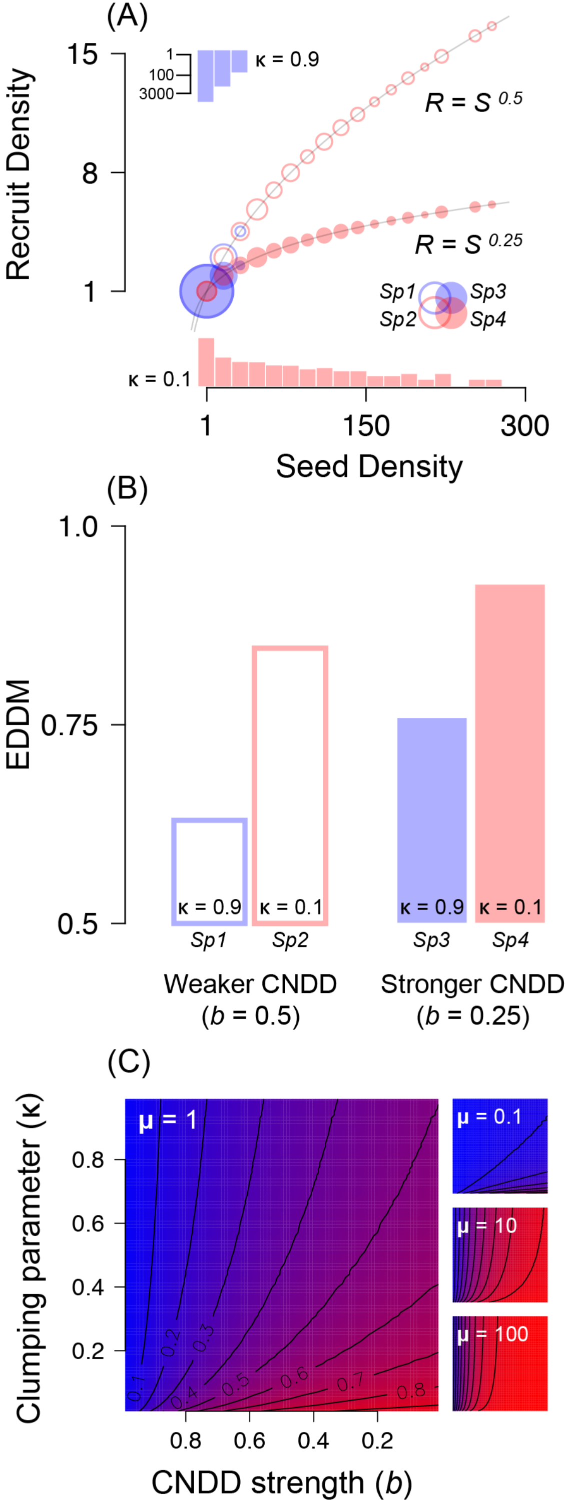

Figure 1. Effective density dependent mortality (EDDM) during the seed-to-seedling transition depends on the strength of CNDD (b), the mean conspecific seed density (μ), and the clumping parameter of the seed distribution (κ). Let recruit density (R) be related to local conspecific seed density (S) by a power function $R = a\,S^{\,b}$ (here, $a = 1$), and let the distribution of local conspecific seed densities be described by the clumping

parameter of the negative binomial distribution (κ). Panel A shows distributions of local seed densities (histograms, blue $\kappa = 0.9$ and red $\kappa = 0.1$) and relationships between R and S (scatter plots, with symbol size proportional to the number of seeds at each seed density) for four hypothetical species characterized by $b = 0.5$ and $\kappa = 0.9$ (Sp1), $b = 0.5$ and $\kappa = 0.1$ (Sp2), $b = 0.25$ and $\kappa = 0.9$ (Sp3), $b = 0.25$ and $\kappa = 0.1$ (Sp4) and a single mean seed density ($\mu = 5$). Panel B presents EDDM values calculated for the four hypothetical species in panel A. EDDM is greater with stronger CNDD (smaller b values) and for the species whose seeds are more frequently found at greater conspecific seed density (smaller κ values). In panel C, the heat maps show how EDDM varies with CNDD strength (b) and the clumping parameter of the negative binomial distribution (κ) for four values of mean seed density (μ). Red indicates higher EDDM and blue lower EDDM. EDDM increases with stronger CNDD, more clumped distributions of seed density, and higher mean seed density.

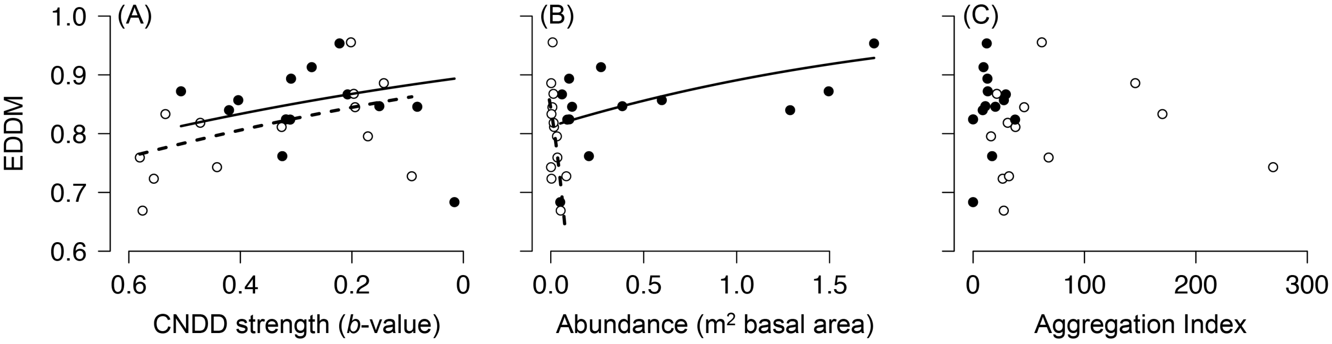

Figure 2. Relationships between effective density dependent mortality (EDDM) and CNDD strength (panel A), abundance (B) and adult spatial aggregation (C). EDDM was positively related to CNDD strength and to species abundance for trees (filled points and solid lines) and positively related to CNDD strength and negatively related to species abundance for lianas (open points and dashed lines). Note the inverted *b*-value scale for CNDD strength, which is negatively related to *b*-values. Each panel uses mean values for the remaining independent variables.

Tables

Table 1. Effects of adult spatial aggregation, abundance, growth form and the interaction of abundance and growth form on local conspecific seed density (μ), the negative binomial seed clumping parameter (κ) and the strength of density dependence (b). Coefficient estimates (± 1 S.E.) are from generalized linear models with μ, κ, or b as the dependent variable.

Dependent variable	Aggregation (Ω_{0-10})	Abundance (Basal area m^2 ha^{-1})	Liana	Abund:Liana
μ	0.00002 ± 0.00017	-0.054 ± 0.017	-0.029 ± 0.029	
κ	-0.0037 ± 0.0023	0.54 ± 0.36	0.62 ± 0.38	-19.6 ± 5.4
b	0.00051 ± 0.00061	0.11 ± 0.08	0.094 ± 0.081	

Table 2. Effects of adult spatial aggregation, abundance, and CNDD strength on effective density dependent mortality (EDDM). Coefficient estimates (± 1 S.E.) are from a linear model with logit-transformed EDDM as the dependent variable.

CNDD strength (b)	Aggregation (Ω_{0-10})	Abundance (Basal area m^2 ha^{-1})	Liana	Abund:Liana
-1.35 ± 0.65	0.0009 ± 0.0021	0.63 ± 0.25	0.50 ± 0.33	-15 ± 7

Supplementary Information

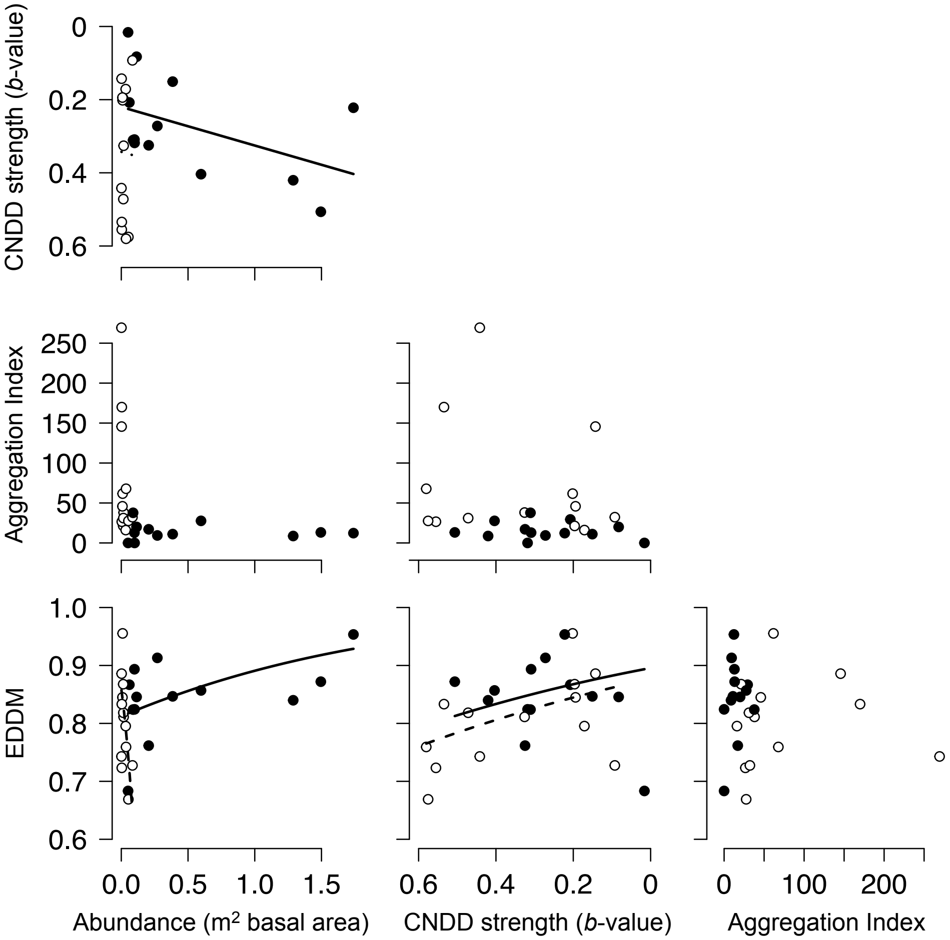

Figure S1. Pairwise relationships between effective density dependent mortality (EDDM), abundance (expressed as basal area), CNDD strength, and the aggregation index ($\Omega_{0\text{-}10}$, Condit et al. 2000). Lines represent model predictions using mean values for other predictor variables among trees (filled circles and solid lines) and lianas (open circles and dashed lines). Note the inverted *b*-value scale for CNDD strength, which is negatively related to *b*-values. The bottom row of figures is also presented in text Figure 2 and is included here for completeness.

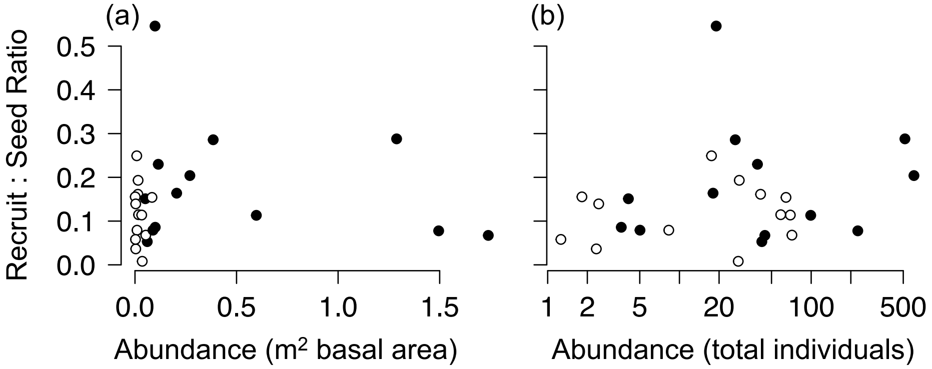

Figure S2. A community compensatory trend, a negative relationship between recruitment and abundance, was not present for abundance in terms of basal area (a) or population density (individuals 50 ha^{-1}) (b). Tree and liana species are represented by filled and open symbols, respectively.

Table S1. Fitted parameters for recruitment models and negative binomial distributions describing local conspecific seed densities. Recruitment models link seed density (S) to recruit density (R) using a power law ($R = a S^{b}$) or a linear model ($R = a S$). Likelihood ratio tests assessed whether power law models improved fit over linear models. Negative binomial parameters describe the mean conspecific seed density (μ, m^{-2}) surrounding focal seeds for station-years where seeds were present and the clumping parameter (κ).

		Power law		Linear	LRT	Neg. Bin.		
Species	Liana	a	b	a	P	μ	κ	P
Chrysophyllum cainito	0	0.131	0.318	0.056	<0.001	8.0	0.41	<0.001
Cordia alliodora	0	0.009	0.794	0.005	0.497			
Coussarea curvigemmia	0	0.081	0.208	0.026	<0.001	9.2	0.43	<0.001
Doliocarpus major	1	0.297	0.197	0.109	<0.001	9.3	0.43	<0.001
Doliocarpus multiflorus	1	0.169	0.202	0.047	<0.001	32	0.29	<0.001
Doliocarpus olivaceus	1	0.167	0.326	0.065	<0.001	8.1	0.47	<0.001
Eugenia oerstediana	0	0.419	0.083	0.159	<0.001	6.2	0.47	<0.001
Faramea occidentalis	0	0.542	0.42	0.219	<0.001	13	0.38	<0.001
Heisteria concinna	0	0.258	0.325	0.108	<0.001	5.6	0.52	<0.001
Hiraea reclinata	1	0.192	0.171	0.068	<0.001	5.2	0.65	<0.001
Hiraea faginea	1	0.267	0.442	0.100	0.002	8.2	0.67	<0.001
Hiraea grandifolia	1	0.344	0.194	0.170	<0.001	7.3	0.35	<0.001
Hybanthus prunifolius	0	0.498	0.272	0.132	<0.001	20	0.43	<0.001
Jacaranda copaia	0	0.001	0.713	0.000	0.468			
Macfadyena unguis-cati	1	0.154	0.555	0.065	<0.001	11	0.37	<0.001
Mascagnia hippocrateoides	1	0.357	0.472	0.139	<0.001	15	0.43	<0.001
Mascagnia nervosa	1	0.053	0.534	0.014	<0.001	26	0.42	<0.001
Ocotea puberula	0	0.237	0.016	0.113	0.001	2.2	0.86	<0.001
Paragonia pyramidata	1	0.099	0.575	0.047	0.019	7.7	0.6	<0.001
Pithecoctenium crucigerum	1	0.089	0.143	0.022	<0.001	9.8	0.43	<0.001
Pouteria reticulata	0	0.424	0.151	0.186	<0.001	6.8	0.34	<0.001
Prionostemma aspera	1	0.275	0.093	0.105	<0.001	3.0	0.87	<0.001
Quararibea asterolepis	0	0.159	0.222	0.039	<0.001	37	0.36	<0.001
Randia armata	0	0.797	0.309	0.447	<0.001	13	0.18	<0.001
Tabebuia rosea	0	0.008	0.635	0.004	0.148			
Tetragastris panamensis	0	0.178	0.404	0.072	<0.001	15	0.35	<0.001
Thinouia myriantha	1	0.012	0.58	0.003	<0.001	19	0.68	<0.001
Trichilia tuberculata	0	0.143	0.506	0.052	<0.001	31	0.35	<0.001
Triplaris cumingiana	0	0.133	0.311	0.037	<0.001	8.7	0.55	<0.001

Table S2. Effects of adult spatial aggregation, abundance, growth form and the interaction of abundance and growth form on local conspecific seed density (*μ*), the negative binomial seed clumping parameter (*κ*) and the strength of density dependence (*b*). Coefficient estimates (± 1 S.E.) are from generalized linear models with μ, κ, or *b* as the dependent variable.

Dependent variable	Aggregation ($\Omega_{0\text{-}10}$)	Abundance (individuals ha^{-1})	Liana	Abund:Liana
μ	0.00001 ± 0.00026	-0.016 ± 0.009	-0.10 ± 0.06	0.031 ± 0.016
κ	-0.0078 ± 0.0034	0.23 ± 0.12	2.28 ± 0.87	-0.72 ± 0.23
b	0.00083 ± 0.00071	0.024 ± 0.024	0.048 ± 0.074	

Table S3. Effects of adult spatial aggregation, abundance, and CNDD strength on effective density dependent mortality (EDDM). Coefficient estimates (± 1 S.E.) are from a linear model with logit-transformed EDDM as the dependent variable.

b-value	Aggregation	Pop. dens.	Liana	Pop. dens.:Liana
-1.22 ± 0.72	-0.0019 ± 0.0030	0.20 ± 0.10	1.1 ± 0.7	-0.39 ± 0.18

Mutualistic strategy tradeoffs structure networks and increase robustness to coextinction

Authors: Evan Fricke[1], Joshua Tewksbury[1,2], Elizabeth Wandrag[3], Haldre Rogers[4]

Affiliations:

[1]Department of Biology, University of Washington, Seattle, WA 98195, USA

[2]Luc Hoffmann Institute, WWF International, 1196, Gland, Switzerland

[3]Department of BioSciences, Rice University, Houston, TX 77005, USA

Abstract: The structure of mutualistic networks, and the position of species within networks, determines their robustness to coextinction. Yet the mechanisms currently used to explain network assembly and predict coextinction do not consider how species vary in their dependence on mutualisms for reproduction and survival. Among plants in a highly resolved seed dispersal network, we show that a tradeoff between mutualistic and non-mutualistic strategies explains investment in and benefits of the mutualism, and species' network position. The tradeoff is present among animals in 11 seed dispersal networks and greatly increases network robustness, yielding 90% fewer simulated coextinctions compared to current models assuming obligate mutualisms. Our work strongly reorients predictions for the species most vulnerable to coextinction and shows that strategy tradeoffs buffer mutualistic networks from coextinction.

Main Text: Plant-animal mutualistic interactions generate and maintain biodiversity (Stebbins 1970, Bascompte et al. 2006, Bastolla et al. 2009, Thébault and Fontaine 2010), but their ongoing disruption may cause cascading coextinctions globally (Koh et al. 2004, Tylianakis et al. 2010). Mutualistic interaction networks share a nested structure, in which generalists interact with both specialists and a generalist core (Bascompte et al. 2003). The nested architecture of mutualistic networks makes ecological communities robust to coextinction cascades (Tylianakis et al. 2010). The mechanisms that assemble nested networks are therefore critical to our understanding of the diversity and stability of mutualist communities, as well as our predictions of community responses to species extinction, the species most vulnerable to coextinction, and the magnitude of the current extinction debt.

Current models proposing mechanisms for the assembly of nested networks are limited in their predictive capacity, as they fail to consider how species vary in their intrinsic dependence on the mutualism – the degree to which species are facultative or obligate mutualists. Two general mechanisms are thought to contribute to observed network structure (Vásquez et al. 2009, Kaiser-Bunbury et al. 2014): neutral mechanisms, where interaction probability depends on partner abundance (Vásquez et al. 2007, Blüthgen et al. 2008) and trait matching, where physical or temporal constraints yield "forbidden links" (Jordano et al. 2003). Without predictions regarding interspecific differences in intrinsic dependence, simulations employed to study robustness and coextinction use a null expectation of equivalent intrinsic dependence across all species. The prevailing assumption is that all species depend on the mutualism entirely for reproduction or survival (Fortuna and Bascompte 2006, Rezende et al. 2007, Thébault and Fontaine 2010, Pocock et al. 2012, Vieira and Almeida-Neto 2014). This assumption is not only violated by empirical evidence (Blüthgen et al. 2008), it also neglects the possibility that a systematic difference among species in intrinsic dependence on the mutualism determines observed network structure.

We propose that the nested structure of mutualistic networks results from a tradeoff between mutualistic strategies and non-mutualistic strategies for reproduction and survival. Tradeoffs are fundamental aspects of ecological strategy that allow species to maximize fitness under a spectrum of strategies (Agrawal et al. 2010), and underlie the niche differences that facilitate species coexistence (Tilman 2004). Mutualistic strategies exist along a spectrum; plants and animals that participate in mutualisms also employ non-mutualistic strategies (e.g. self-fertilization, vegetative reproduction, survival of undispersed seed, use of resources other than mutualistic rewards) that to varying degrees reduce their intrinsic dependence on a mutualism (Bond 1994). The costs and benefits of a mutualism should influence where species exist on the spectrum between obligate mutualistic and non-mutualistic strategies. Species that invest most in mutualistic strategies should derive larger benefits from the mutualism (Bronstein 2001), and species with greatest dependence on the mutualism should have a more diverse set of mutualistic partners (Bond 1994). Following predictions regarding resource use breadth in animals (Schoener 1971), we expect species that invest little in the mutualism to interact with the most generalized mutualistic partners, which themselves possess traits that facilitate interactions with the broadest set of mutualists. The tradeoff should result in the assembly of networks with nested architectures and create a positive relationship between species' intrinsic dependence on the mutualism and both their interaction frequency and partner diversity.

Mutualistic networks assembled by the tradeoff between mutualistic and non-mutualistic strategies should respond differently to coextinction than should networks whose species do not vary systematically in intrinsic dependence on the mutualism. Although species with greater intrinsic dependence are most vulnerable to complete mutualist loss, these species are also the most likely to have developed redundant interactions that create a buffer from coextinction (Memmott et al. 2004). In contrast, species that invest little in the mutualism should derive little benefit and have few mutualistic partners. Although these species have less mutualist redundancy, their lower intrinsic dependence on mutualistic strategies should buffer them from coextinction. This strategy tradeoff should assemble networks that are more robust to coextinction than would be predicted under current models.

We first evaluate the prediction that a tradeoff between mutualistic and non-mutualistic strategies causes systematic differences in the costs and benefits of the mutualism among species. We measured costs and benefits of seed dispersal for plant species in a seed dispersal network we developed on the island of Saipan in the Mariana Island chain. Seven animal species consumed 6304 diaspores during 1320 hours of observation of six tree species (Fig. 1A). Interaction frequency was standardized as the portion of fruit crop removed per hour, which varied widely among species. To evaluate the direct cost of attracting frugivores, we measured the dry mass pulp to seed ratio (Howe and Vande Kerckhove 1981, Obeso 2004). We use species strength, a quantitative version of species degree (the number of mutualists), as a measure of the structural position of species within the network. Species with larger investment in mutualist attraction (pulp:seed ratio) had greater species strength ($P = 0.006$, Fig. 1B), indicating that they interact more frequently and with a greater diversity of partners. Species with the greatest species strength (*Carica*, *Pipturus*, and *Premna*) invested nearly twice as much mass in mutualistic rewards as they did in seeds, whereas the species with lowest species strength (*Aglaia*) invested only 10% as much in mutualistic rewards as in seeds. To measure benefits of the mutualism, we used seed and seedling experiments to compare survival of seeds and seedlings in dispersed and non-dispersed scenarios (we define the ratio of survival in these scenarios as the dispersal benefit ratio). These experiments measured three of the key benefits of seed dispersal within populations: movement away from the area of high mortality near conspecifics, movement to favorable high light microhabitats, and handling of fruits by frugivores. We found that the benefits of dispersal increase with species strength ($P = 0.05$; Fig. 2), with the number of mutualists ($P = 0.03$), and with total interaction frequency ($P = 0.03$). We found larger benefits of dispersal in species with greater species strength in 6 out of 7 experiments (Fig. 2). These findings provide support that a tradeoff determines the costs of the mutualism, its benefits, and the structural position of species within a mutualistic network.

To assess the generality of a positive relationship between species strength and intrinsic dependence among animals and in other seed dispersal networks, we turn to 11 globally distributed empirical quantitative seed dispersal networks (available at www.web-of-life.es). We compare the structural position of animal species, all of which were birds, to their intrinsic dependence on the mutualism, measured using estimates of the portion of the bird's diet that is comprised of fruit in a global bird functional trait database (Wilman et al. 2014). We find the predicted positive relationship among these species; species of lower species strength (more peripheral to network structure) tend to have lower intrinsic dependence on the mutualism (*P* = 0.001; Fig. 3A). Although the species with few interactions would typically be interpreted as specialists in network studies, they instead tend to be generalists that opportunistically eat fruit. Conversely, bird species that have the most generalized interactions with plants within the network are likely to be more specialized on fruit. Facultative and opportunistic frugivory is pervasive, with 15% of the animals in seed dispersal networks estimated to eat fruit as <5% of their diets (Fig. 3A). The tradeoff between mutualistic and non-mutualistic strategies explains the structural position of animals in mutualistic networks.

Empirical support for the existence of a tradeoff in mutualistic strategy, where the species that are most dependent on the mutualism also have the most diverse and frequent interactions, reorients predictions regarding the species most vulnerable to coextinction. Under assumptions currently used for coextinction predictions, a species with a single mutualist is considered most vulnerable because it is assumed to depend entirely on a single mutualist for reproduction (Fortuna and Bascompte 2006, Rezende et al. 2007, Thébault and Fontaine 2010, Pocock et al. 2012, Vieira and Almeida-Neto 2014). Without redundant interactions, a single extinction could cause the coextinction of that species. For example, one tree species in our network, *Aglaia*, has only one mutualist and would be expected to be most vulnerable under these assumptions. However, we found that it derives relatively small benefits from the mutualism. These small benefits, and thus the small detriment of losing them, suggest that *Aglaia* is least likely among the vertebrate-dispersed species we tested to face coextinction after losing all mutualists. Based on the benefits of dispersal we measured, species such as *Carica* and *Premna* that have many mutualists and frequent interactions should, if they were to lose all mutualists, be much more likely to face extinction. And yet these are the species most buffered from coextinction by mutualist redundancy and should therefore be robust to individual mutualist extinctions. These dynamics should reduce expected coextinctions across mutualistic networks; species with low species strength are buffered from extinction by low intrinsic dependence on the mutualism and species with high species strength are buffered by mutualist redundancy.

We next assessed how the existence of the tradeoff influences predictions for coextinction. Using 31 empirical quantitative seed dispersal and pollination networks, we simulate coextinctions with the stochastic simulation model developed by Vieira and Almeida-Neto (2014). In this model the probability of coextinction of a focal species after partner extinction depends on the focal species' intrinsic dependence on the mutualism (R_i, values between 0 and 1) and the portion of its observed interactions that were with the partner. After a randomly chosen species goes extinct and coextinctions are allowed to cascade through the network, the portion of species that experience coextinction is recorded. We first compare the difference in coextinction between a tradeoff scenario, where intrinsic dependence is assigned to species based on their species strength, versus an obligate scenario, where all species are assumed to be obligate mutualists. The portion of species that experience coextinction should depend on the strength of the tradeoff, or the slope of the relationship between species strength and intrinsic dependence, which we describe as Tradeoff Strength. We therefore simulate coextinctions for Tradeoff Strength values between 0 ($R_i = 1$ for all species) and 1 (R_i varies linearly between $R_i = 0$ when species strength = 0 and $R_i = 1$ at maximum species strength; see inset panels in Fig. 3B). The strongest tradeoffs tested reduced predicted coextinctions over 90% relative to predictions assuming obligate mutualists (Fig. 3B). Because increasing Tradeoff Strength necessarily decreases the intrinsic dependence assigned to species and should decrease coextinctions for that reason alone, we next compared to a null model that controls for this decrease in intrinsic dependence. This null model assigns the same intrinsic dependence to all species using a flat relationship between intrinsic dependence and species strength that integrates to the same value as the relationship for the corresponding value of Tradeoff Strength (see inset panels in Fig. S1). The tradeoff confers greater robustness than does the simple decrease in intrinsic dependence (Fig. S1).

How does the existence of the tradeoff between mutualistic and non-mutualistic strategies influence predictions for coextinction in real networks? To constrain how the tradeoff influences robustness to coextinction in empirical networks, we assign intrinsic dependence for plant and animals in 11 seed dispersal networks using the empirical relationship between species strength and intrinsic dependence, measured as the animal species' degree of frugivory (Fig. 3A). Relative to predictions based on the assumption that all species are obligate mutualists, the mean empirical relationship results on average in 87% fewer extinctions (nested scenario, Fig. 3C). Does nested network architecture still confer robustness when species exhibit the tradeoff? When all species are assumed to be obligate mutualists, the probability of coextinctions in empirical networks was on average 18% lower than in null networks modified to be non-nested (Fig. 3C). This result recapitulates findings of previous studies showing the benefit of nestedness for network stability. However, when the empirical tradeoff relationship was used to assign species' intrinsic dependence, this benefit to robustness decreases, with only 5% fewer extinctions in nested networks than in non-nested networks (Fig. 3C). Our simulations show that the decrease in coextinction attributable to nested network architecture falls to zero for the strongest tradeoffs tested (Fig. S2). The tradeoff between mutualistic and non-mutualistic strategies confers greater robustness to mutualistic networks than does nestedness, and it limits the degree to which nestedness improves network robustness.

Our findings support a novel explanation for the mechanism generating mutualistic network structure. We have shown that a tradeoff between mutualistic and non-mutualistic strategies, resulting in interspecific differences in investment and benefits of the mutualism, explains the position of species within networks. Species that depend, benefit, and invest most in the mutualism interact more frequently with a larger diversity of partners, and species that invest little are most likely to interact with generalists that invest most in maintaining interactions. Together these processes assemble networks with nested architectures. Another explanation, trait matching, is thought to result from coevolution between interacting partners (Guimaraes et al. 2011). Yet trait matching alone cannot explain nested network assembly; when coevolution of traits is allowed to influence network structure, the mechanism of trait matching produces anti-nested networks (Nuismer et al. 2013). We do expect species traits to predict the structural position of species, but we propose that this is not a result of pairwise coevolution, but instead because the traits reflect fundamental tradeoffs in mutualistic strategies. Our hypothesis for the assembly of nested networks draws stronger links between the study of mutualistic networks and fundamental theory in community ecology regarding tradeoffs and coexistence.

Examples of coextinction after contemporary mutualist loss are few (Dunn et al. 2009). This may result from the time lags that give rise to an extinction debt (Tilman et al. 1994), or the low intrinsic dependence on mutualistic strategies that species possess. Our framework predicts that the species most likely to lose all mutualists – those with few or infrequent interactions – include those most likely to possess non-mutualistic strategies for reproduction and survival. Thus the existence of few examples of mutualist coextinction may reflect the robustness of mutualistic networks to low-level extinctions. However in more severely degraded networks, including those in increasingly common defaunated ecosystems (Dirzo et al. 2014) or those with multiple stressors (Brodie et al. 2014), our framework predicts that, above some threshold of network degradation, the species most central to network structure will face rapid declines resulting from their greater intrinsic dependence on the mutualism. This nonlinear response, causing a tipping point in network disassembly, may fundamentally reduce the resilience of mutualistic networks to recover interactions after anthropogenic stressors cause their loss.

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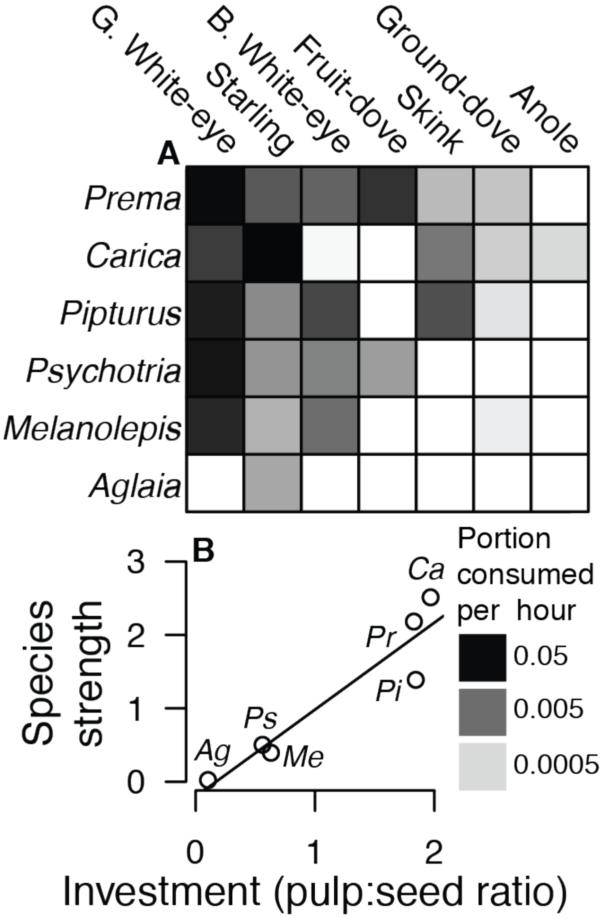

Fig. 1. Seed dispersal network on Saipan, with rows representing plants and columns animals (**A**). Shading indicates the portion of the ripe diaspores consumed per hour. Species with greater dry mass pulp-to-seed ratios had greater species strength, a quantitative network metric giving the sum of interaction strengths between a species and its mutualistic partners (**B**). Points jittered slightly to reduce overlap.

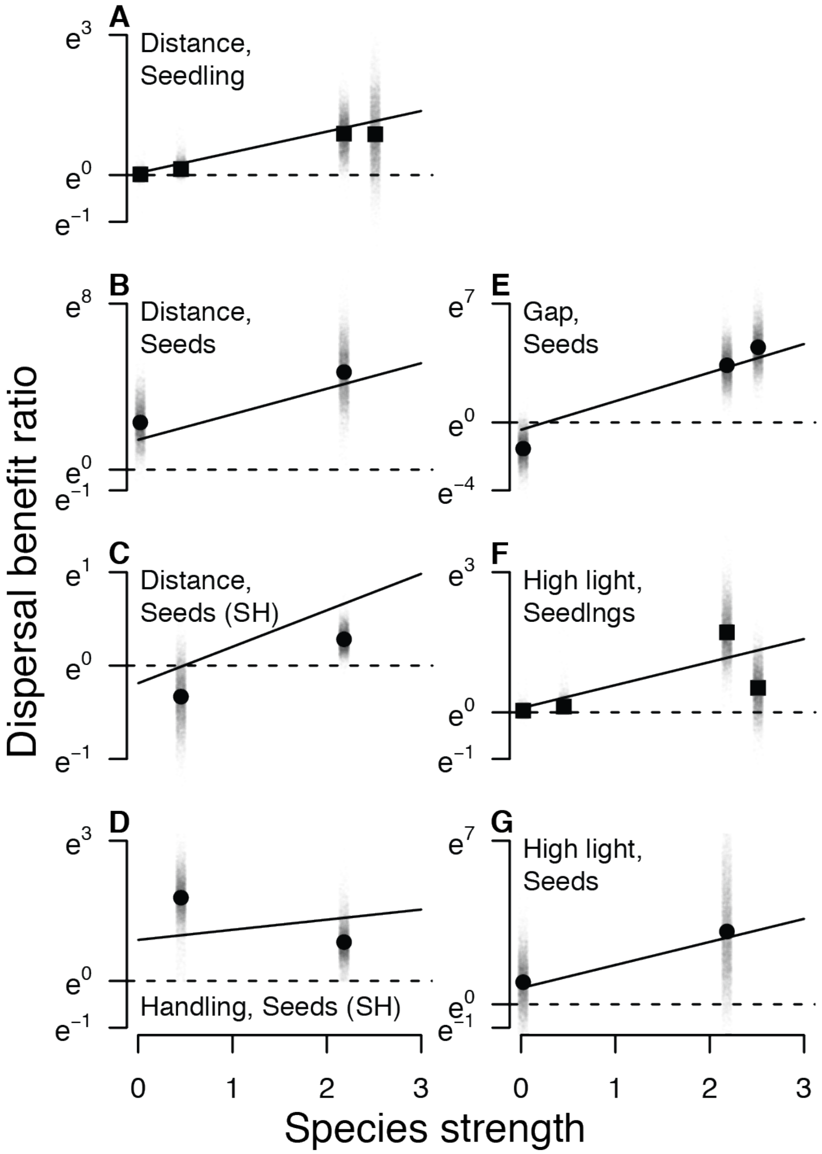

Fig. 2. Benefits of seed dispersal increase with species strength for trees in the Mariana Islands. The dispersal benefit ratio (ratio of survival of dispersed seeds or seedlings to survival of undispersed seeds or seedlings) was quantified for experiments measuring benefits associated with movement away from adult conspecifics (Distance; **A-C**), handling of seeds by frugivores (Handling; **D**), and movement to canopy gaps (Gap; **E**) or high light microsites (High light; **F-G**). Experiments were conducted by sowing seeds (Seeds; circles; **B-E,G**) or planting seedlings (Seedlings; squares; **A,F**). Experiments performed in a shadehouse are indicated with "SH"; experiments were otherwise performed in the field. Grey bars show posterior estimates for the dispersal benefit ratio, points indicate the mean dispersal benefit ratio, and lines represent the model fit.

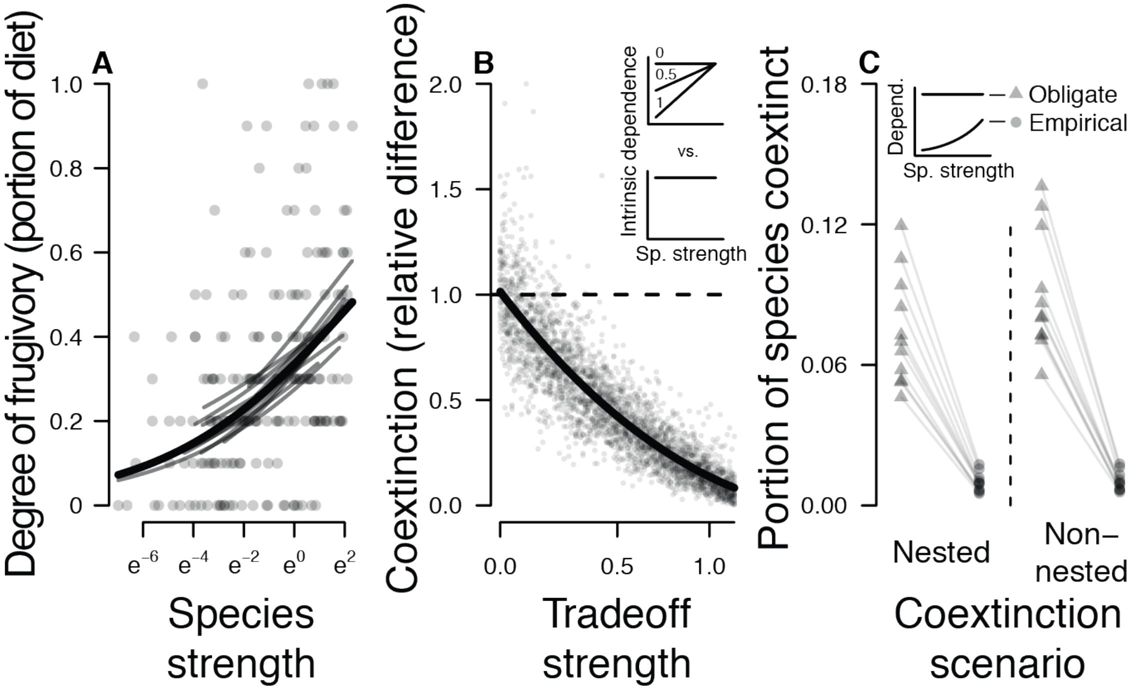

Fig. 3. Bird species with a higher degree of frugivory have greater species strength in 11 empirical seed dispersal networks (**A**). Thin lines represent model fits for each network and the thick line represents the mean model fit. Relative to a scenario where all species are assumed to be obligate mutualists (flat relationship between species strength and intrinsic dependence on the mutualism in the bottom inset panel), predictions for coextinction decrease strongly under greater values of Tradeoff Strength (greater positive relationships in top inset panel) when simulated within 31 empirical seed dispersal and pollination networks (**B**). When the empirical relationship between degree of frugivory and species strength is used to assign species intrinsic dependence on the mutualism in coextinction simulations, use of the empirical relationship (circles) predicts fewer species coextinctions than using the assumption of obligate mutualists in each of the 11 seed dispersal networks whether coextinctions are simulated using empirical network structure (Nested) or using networks modified to lack nestedness (Non-nested) (**C**).

Supplementary Materials:

Materials and Methods

Study System

We study interactions between trees and seed dispersers, and the demographic benefits of dispersal for trees, in the Mariana Island chain of the western Pacific Ocean. Our observations and experiments were conducted in native limestone karst forest, the primary forest type across the islands. The islands receive 2.0-2.5 m of rain annually with a dry season from January to June. The forest canopy is short (~8-15 m) due to frequent typhoons and species richness is relatively low (~40 tree and shrub species in this forest type). The forests have a similar portion of tree species that are dispersed by vertebrates (~80%) as in other tropical forests (Jordano 2000). Experiments and observations were conduced across the inhabited Mariana Islands of Guam, Rota, Tinian, and Saipan, and we developed a seed dispersal network on the island of Saipan, which possesses the most intact assemblage of native birds among the inhabited Mariana Islands (Craig 1996). Birds are the primary seed dispersing species in this system. A single fruit bat species (*Pteropus mariannus*) is rare across these islands, and its extremely low population densities on Saipan suggest the absence of a functional role in seed dispersal (McConkey and Drake 2006). We therefore limit our analysis of the seed dispersal mutualism to diurnal interactions.

Seed Dispersal Network

We conducted frugivory observations on Saipan from May to August in 2013 and 2014. These months encompass the beginning of the wet season and the time of peak fruiting. Observations were conducted in three forest sites across the island. We recorded frugivory through extended direct observation of focal fruiting trees (Schleuning et al. 2011). Observers surveyed study sites for representative fruiting trees and rotated among sites and available tree species. Using binoculars, observers recorded the number of diaspores manipulated, distinguishing between interactions where the diaspore was consumed, those where the diaspore was dropped, or those where pulp was removed without dispersal. We analyze only data on the number of diaspores consumed. We recorded the number of ripe fruits visible on each focal tree. For species with multiple diaspores per fruit we multiplied the number of visible fruits by the mean number of diaspores per fruit to estimate number of ripe diaspores. Our analysis covers six tree species, *Aglaia mariannensis* (Meliaceae), *Carica papaya* (Caricaceae), *Melanolepis multiglandulosa* (Euphorbiaceae), *Pipturus argenteus* (Urticaceae), *Premna serratifolia* (Lamiaceae), and *Psychotria mariana* (Rubiaceae), hereafter referred to by genus. These species are those that we observed during both years and whose seeds we observed to be dispersed by vertebrates. For one species, *Carica*, we used video recordings as well as direct observations to record frugivory events. Species recorded consuming seeds were the Golden White-eye (*Cleptornis marchei*), Bridled White-eye (*Zosterops conspicillatus*), Micronesian Starling (*Aplonis opaca*), White-throated Ground-dove (*Gallicolumba xanthonura*), Mariana Fruit-dove (*Ptilinopus roseicapilla*), Emerald Tree Skink (*Lamprolepis smaragdina*), and Green Anole (*Anolis carolinensis*).

Observations were conducted between 6:00 AM and 2:00 PM. We recorded data on an average of 23 individuals per species (range 13-43 individuals). Observation periods lasted between 2 and 8 hours and the total duration of observation per species averaged 220 hours (range 102-386 hours). We standardize interaction frequency as the portion of ripe diaspores removed per hour. The use of a continuous metric (portion ripe diaspores consumed/hour) to describe interaction frequency differs from the discrete interaction counts that are typically used in quantitative networks (Vásquez et al. 2012). However, our continuously quantitative approach still allows the calculation of species-level quantitative network metrics, many of which are only sensitive to relative, rather than absolute, differences.

Distance- and light-dependent survival: field seed additions

We measured survival at the seed to seedling transition for *Aglaia* and *Premna* using seed additions near and far from conspecific adults on Saipan. These experiments are described in detail in Fricke et al. (2014). We added seeds in circular 0.05 m^2 plots, with six near and six far plots per species at each of the three forest sites. Seeds were collected from at least 10 individuals and were sown at densities of 20 seeds per plot for *Aglaia* and 50 seeds per plot for *Premna*. Because these plots were control plots used in experiments testing natural enemy impacts on survival, they were partially surrounded by wire mesh that allowed for a consistent light environment with the other experimental plots but that did not influence natural enemy access. We measured survival of seedlings 5 weeks after germination began for *Premna* and 10 weeks after germination began for *Aglaia*. We recorded canopy openness above each plot using a spherical densitometer.

Distance- and light-dependent survival: field seedling additions

We measured survival at the seedling stage for *Aglaia*, *Carica*, *Psychotria*, and *Premna* using seedling additions near and far from conspecifics on Saipan, and the nearby Mariana Islands of Tinian, Rota, and Guam. These experiments are described in detail in Rogers et al. (*in prep*). We transplanted seedlings grown in shadehouses into circular ~2 m^2 plots, with four near (under canopy) and four far (typically > 7m away from conspecifics) plots per species at each forest site where conspecifics were present.

Saipan, Tinian, and Rota each had three forest sites and Guam had five forest sites. We planted seedlings at a density of 10 per plot. We controlled for any effect of introduced ungulates that are present on Guam and Rota using 1 m tall poultry netting fences, and used fences around plots on all islands. We measured seedling survival after 4-7 months, depending on species identity and island. We recorded canopy openness above each plot using a spherical densitometer.

Distance-dependent survival: shade-house experiments

We compared survival over the seed to seedling transition for *Premna* and *Psychotria* seeds grown with soil taken either under the canopy of adult conspecifics or 30 m away from the nearest conspecific. Soil for each treatment was collected from at least three locations, mixed thoroughly, and passed through a 1 mm mesh that removed any conspecific seeds. Each seed was sown in individual cells containing one part field soil and three parts of a 1:1 perlite and peat moss mix. Each species by treatment combination had a minimum of 274 seeds. We measured seedling survival 10 weeks after sowing seeds, although some viable seeds would likely have germinated later. Germination began approximately one week after seeds were sown for *Premna* and approximately 6 weeks for *Psychotria*.

Frugivore handling: shade-house experiments

We compared germination of *Premna* and *Psychotria* seeds that were either handled or not handled by frugivores in a shadehouse experiment. These experiments are described in detail in Rogers et al. (*in prep*). Seed traps were placed under fruiting trees on Saipan, Tinian and Rota to collect falling fruit and seeds. Handled seeds were those that did not have pulp remaining on the seed. We planted uningested fruits and ingested seeds in individually labeled cells. Each species by treatment combination had a minimum of 117 seeds. Plants were watered and checked for germination daily for six months, but no new germinants were recorded after four months and we assume all viable seeds had germinated.

Gap survival: field seed additions

We compared survival in natural canopy gaps and in nearby closed-canopy areas for *Aglaia*, *Carica*, and *Premna* using seed additions. We performed these experiments on Guam because canopy gaps are more prevalent on Guam than the other islands, allowing sufficient replication, and because germination from background seed rain away from conspecifics is likely minimal due to the functional extirpation of frugivorous birds on Guam. We systematically searched the area surrounding our mapped forest sites for canopy gaps that were 5-10 m^2 in area, the gap size typical of canopy gaps in the Marianas. Four forest sites had three or more suitable gaps, and we randomly selected three as experimental plots. To obtain a paired closed-canopy plot for each gap plot, we chose a random compass bearing, went 10 m from the edge of the canopy gap, and established a closed-canopy plot if the substrate of the potential closed-canopy plot was qualitatively similar to that in the gap plot. If not, the process was repeated with another random compass bearing. Each ~7 m^2 plot was fenced to exclude non-native ungulates. We established 0.25 m^2 seed addition subplots and randomly assigned each species to one per plot. Seeds were sown at a density of 75 per plot for *Aglaia*, 125 per plot for *Carica*, and 150 per plot for *Premna* to approximately scale densities inversely with seed size. We recorded the number of germinants in each plot using weekly checks up to 20 weeks.

Seed and fruit measurements

We measured pulp-to-seed ratios from fruit collected on Guam, where frugivorous birds are functionally extirpated, to avoid bias caused by removal preferences of frugivores. We analyze fruit traits on Guam because the traits of ripe fruits collected from trees on Guam, where frugivorous birds are functionally extirpated, are unlikely to be biased by preferential removal of fruits with certain traits by frugivores. Still, intraspecific variation in fruit and seed measurement between islands in trial measurements were much smaller than interspecific variation, making it unlikely that any potential short-term. We collected ripe fruits from multiple individuals at multiple forest sites. A minimum of 24 diaspores per species were used to obtained seed and pulp data. Pulp is fruit tissue excluding seeds and any fruit tissue that frugivores were observed not to consume. Seed and pulp samples were dried at 65C to a constant mass. We report the mean pulp-to-seed ratio calculated as mean pulp mass per diaspore / mean seed mass per diaspore.

Analysis

To quantify the benefit of seed dispersal, we calculate a ‘dispersal benefit ratio’ that is the ratio of survival for seeds or seedlings in a ‘dispersed’ scenario (far from conspecifics, in canopy gaps, etc.) versus in an ‘undispersed’ scenario (near conspecifics, under closed canopy, etc.). We obtain survival predictions by fitting generalized linear mixed effects models using the R package MCMCglmm (Hadfield 2010) with binomial error distributions and uninformative priors. For distance-dependent seedling mortality, the response variable was the portion of seedlings surviving out of the seedlings planted. The fixed effects were distance (near or far) and canopy openness. The random effects were site and island. For distance-dependent mortality at the seed to seedling transition, the response variable was the portion of seedlings surviving out of seeds sown. The fixed effects were distance and canopy openness. The random effects were nearest grid point within each mapped forest site. For shadehouse distance-dependent survival, the response variable was survival as a Bernoulli response. The fixed effect was soil type (near or far). The random effect was the parent tree. For shadehouse frugivore handling, the response variable was germination as a Bernoulli response. The fixed effect was handling treatment (handled or unhandled). The random effects were site and island. For gap-dependent survival, the response variable was the portion of germinants out of seed sown. The fixed effect was light environment (gap or closed canopy). The random effects described pairs of gap and closed-canopy plots. To obtain species-specific estimates, all models had interactions by species with each of the fixed effects. We considered dispersed versus undispersed scenarios as far from conspecifics versus near conspecifics, in canopy gap versus under closed canopy, handled by frugivores versus not handled, and in high light microsites representing the 90th percentile of recorded canopy openness values versus the 10th percentile of canopy openness. The dispersal benefit ratio was calculated as the log-transformed ratio of posterior estimates of survival from each of the dispersed vs. undispersed scenarios.

To assess relationships between network indices and benefits of dispersal, we use a linear mixed effects model, again with the MCMCglmm package, with the mean dispersal benefit ratio as the dependent variable and species strength as the fixed effect. Treating each benefit type-by-species stage combination (distance dependence at the seed stage, distance dependence at the seedling stage, etc.) as a random effect, we allow random slopes and intercepts. The presence of a positive effect of species strength would indicate that, even with variation in the magnitude of the benefit or the slope of its relationship, species with greater species strength have greater benefits of dispersal.

All analyses used uninformative inverse Gamma priors (Hadfield 2010) priors for the residual variance, setting the mean of the inverse Wishart distribution to 1 and the degree of belief parameter to 0.002 for random effects and residuals. We fix unit-level variance to 1 only in models with a Bernoulli response variable (distance dependence and handling shadehouse experiments) because unit-level variances cannot be estimated in those cases. For all models, we performed 300,000 iterations, used a thinning interval of 50, and discarded the first 10,000 iterations as burn-in. We report pMCMC (Hadfield 2010) as a Bayesian p-value.

Simulated Coextinctions

Empirical networks: We test the influence of a tradeoff between mutualistic and non-mutualistic strategies on the robustness of networks by simulating coextinctions within globally distributed empirical quantitative seed dispersal and pollination networks (available at www.web-of-life.es; see Table S1 for references and network description). These are the available quantitative networks that report species-specific data on observed interactions. These networks allow us to simulate extinctions using networks possessing empirical variation in network properties but they do not provide information on species' intrinsic dependence on the mutualism; we assign intrinsic dependence to species as described below.

Simulation model: We use the stochastic coextinction model developed by Vieira and Almeida-Neto (2014). This model contrasts with topological coextinction models that assume that a species only experiences coextinction when it loses all its mutualists, and thus that a single partner can maintain the species, however infrequently they are observed to interact. Instead, coextinction is determined stochastically. The probability of coextinction of each species is given by the portion of a focal species' interactions that were observed to be with the now-extinct mutualist multiplied by the intrinsic dependence of the focal species on the mutualism as a whole (R_i, values 0-1). Each iteration of the simulation begins with the extinction of a randomly chosen species. Primary coextinctions are allowed to occur as the result of the initial extinction, and then coextinctions of progressively higher order occur (e.g., secondary coextinctions that result from primary coextinction, etc.) until no further. The number of coextinctions is recorded, and another iteration of the simulation begins.

Manipulating the tradeoff and nestedness: We modify the approach developed by Vieira and Almeida-Neto (2014) to allow variation between species in their intrinsic dependence on the mutualism as a function of species strength. The tradeoff should create a positive relationship between species strength and intrinsic dependence on the mutualism. For consistency across networks, we calculate relative species strength that varies between 0 and 1 by dividing the species strength of plants and animals by the species strength of the plant and animal species with the highest species strength. To consider a range of possible relationships, we vary "Tradeoff Strength," which defines the slope of a linear relationship between species strength and R_i. A value of 0 for Tradeoff Strength equates to the assumption that all species are obligate mutualists (R_i = 1 across all values of relative species strength). For greater values of Tradeoff Strength, we allow the slope to increase while bounding R_i to 1 when relative species strength equals 1 (slope increases by decreasing the y-intercept in the relationship between R_i and intrinsic dependence). We consider values of Tradeoff Strength between 0 and 1. Increasing values of Tradeoff Strength should reduce coextinctions simply because the area under the curve of the R_i-relative species strength relationship decreases with increasing Tradeoff Strength. To control for this, we compare simulations under each combination of Tradeoff Strength to simulations using a horizontal relationship between species strength and R_i that integrates to the same value. We describe the scenarios lacking any slope between species strength and intrinsic dependence as "tradeoff null" (Null_T) scenarios.

To assess the influence of nested structure on coextinction, we either used empirical networks, possessing any nestedness, to empricial networks that were randomized to, on average, lack any nestedness present in the empirical networks. The approach we use maintains the total number of interactions per species but not nestedness (Patefield 1981). We use the r2dtable function in the bipartite package in R (Dormann et al. 2008). We describe the scenarios with networks randomized to lack nestedness as "non-nested" (Null_N) scenarios.

Coextinction scenarios: We perform different sets of coextinction simulations to assess how the tradeoff between mutualistic and nonmutualistic strategies and nestedness each influence the probability of coextinction in mutualistic networks, and how coextinction predictions differ when species intrinsic dependence is either determined by the empirical tradeoff or the assumption that all species are obligate mutualists.

Our first simulations record the number of coextinctions under six scenarios that allow us to explore how the relationship between species strength and intrinsic dependence and nestedness influence predictions for coextinction. In the "obligate" scenarios, $R_i = 1$ for all species. In the "tradeoff" scenarios, a linear relationship relates relative species strength and R_i, with this linear relationship is increasingly positive with larger values of Tradeoff Strength. In the "tradeoff null" scenarios, a horizontal relates relative species strength and R_i, with the area under the curve integrating to the area under the "tradeoff" curve at the same value of Tradeoff Strength. For each of the obligate, tradeoff, and tradeoff null scenarios, we perform simulations using either empirical "nested" networks or "non-nested" networks modified to lack nestedness. For each iteration of the simulation, a random species is chosen for extinction and a randomized network is saved. The number of resulting coextinctions is recorded in each of the six scenarios. For each network, we performed 200 iterations of the simulation at each of 100 values of Tradeoff Strength between 0 and 1. Where we present the relative difference in coextinction between two scenarios, we take the ratio of the number of coextinctions averaged across the 200 iterations between the two scenarios at each value of Tradeoff Strength, fitting a spline for a fit line. Where we present the portion of species experiencing coextinction, we used a generalized linear mixed effects model with the number of coextinctions out of the number of species in the network as the response variable, tradeoff strength as the predictor variable, network ID as a random effect, and the binomial error distribution in the MCMCglmm package. Equivalent priors were used as those specified above.

We next compare simulated extinctions within the 11 seed dispersal networks under an "obligate" scenario or a scenario where the strength of the tradeoff is constrained by empirical data on bird diets. Again, in the obligate scenario, $R_i = 1$ for all species. In the tradeoff scenario the relationship between species strength and intrinsic dependence for both plants and animals is given by the mean relationship between species strength and the degree of frugivory exhibited by birds in those 11 networks. We compare the obligate scenario to the single empirical tradeoff scenario, and perform these simulations either using empirical nested networks or non-nested networks, with 5000 iterations for each of the four network-by-scenario combinations.

Supplementary Figures and Tables

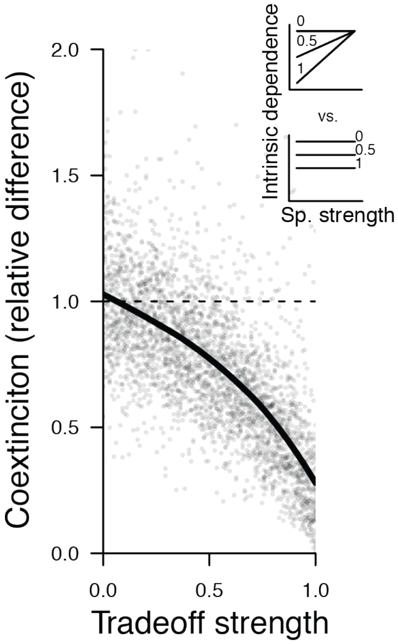

Fig. S1. The tradeoff confers resilience when compared to a null model that accounts for the overall decrease in intrinsic dependence that greater Tradeoff Strength causes. We record the number of simulated coextinction using 31 empirical seed dispersal and pollination networks. At each value of Tradeoff Strength, coextinctions in the tradeoff scenario (species intrinsic dependence assigned based on a positive relationship between species strength and intrinsic dependence) are compared to coextinctions simulation performed after assigning intrinsic dependence based on a horizontal relationship between intrinsic dependence and species abundance. For each value of Tradeoff Strength, this horizontal relationship is defined as the relationship whose area under the curve is equal to the area under the curve for the corresponding tradeoff scenario. Relative to equivalent simple decreases in intrinsic dependence, the tradeoff scenario results in less coextinction.

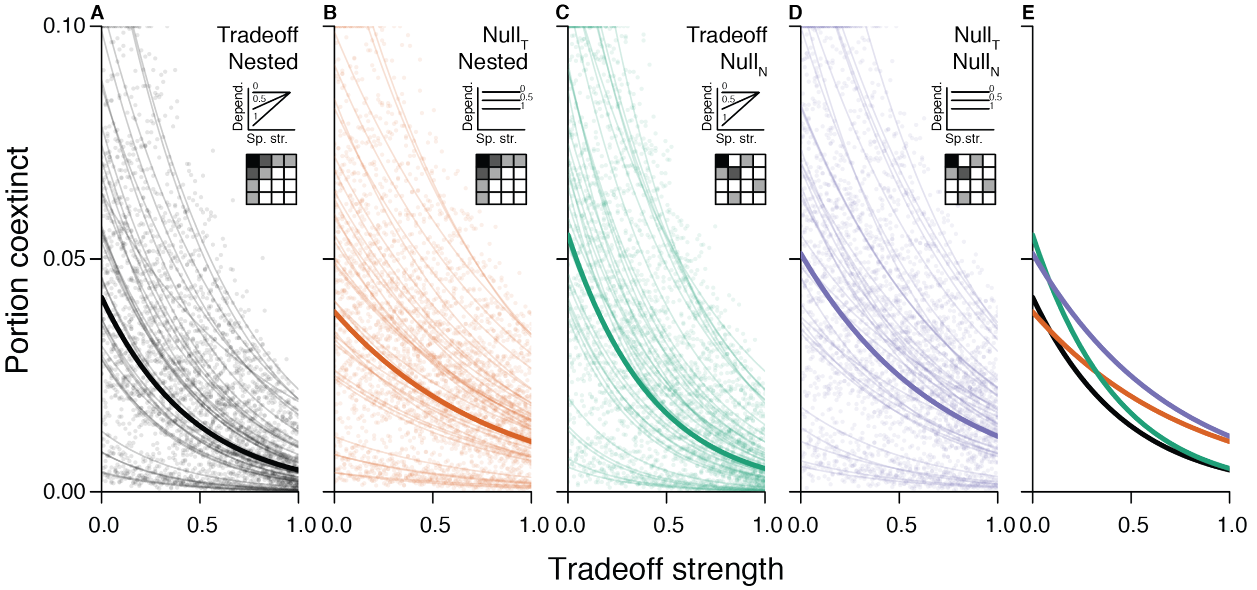

Fig. S2. Portion of species experiencing simulated coextinction after a random extinction in 31 empirical seed dispersal and pollination networks. Four scenarios (panels a-d) represent each combination of the presence or absence of a tradeoff in ecological strategy and of nestedness. Inset line plots show how Tradeoff Strength relates to the relationship between species strength (Sp. Str.) and intrinsic dependence on the mutualism (Depend.) in either the “tradeoff” or “tradeoff null” (Null$_T$) scenarios. Inset bipartite plots illustrate either empirical “nested” or “non-nested” (Null$_N$) scenarios. Points are the number of extinctions in a network in a given iteration of the simulation, with values greater than 0.1 plotted at 0.1. Thin lines are model fits for each of the 31 empirical networks and the thick line in each panel is the mean relationship across all networks, each of which is overlaid in panel e. The existence of a tradeoff increases robustness over that provided by nestedness alone (a vs. b), even in non-nested networks (c vs. d). Nestedness increases robustness in the presence (c vs. a) or absence (d vs. b) of a tradeoff in ecological strategy.

Table S1. References and network description for 31 networks included in coextinction simulations. Descriptors include a unique network identification from the Web of Life database (ID), number of plant and animal species in the network (Spp), total number of interactions (Int), connectance (Con), mutualism type (P = pollination, SD = seed dispersal), the reference, locality, latitude, and longitude.

ID	Spp	Int	Con	Type	Reference	Locality	Lat	Long
M_PL_004	114	167	0.14	P	Barrett, S. C. H., and K. Helenurm. 1987. The Reproductive-Biology of Boreal Forest Herbs.1. Breeding Systems and Pollination. Canadian Journal of Botany 65:2036-2046.	Central New Brunswick, Canada	46.55	-66.07
M_SD_001	28	50	0.34	SD	Baird, J.W. 1980. The selection and use of fruit by birds in an eastern forest. Wilson Bulletin 92: 63-73.	Princeton, Mercer, New	40.33	-74.67

						Jersey, USA		
M_SD_002	40	119	0.43	SD	Beehler, B. 1983. Frugivory and polygamy in birds of paradise. Auk, 100: 1-12.	Mount Missim, Morobe Prov., New Guinea	-7.27	146.7
M_SD_003	41	68	0.17	SD	Carlo et al. (2003) Avian fruit preferences across a Puerto Rican forested landscape: pattern consistency and implications for seed removal. Oecologia 134: 119-131	Caguana, Puerto Rico	18.3	-66.78
M_SD_004	54	95	0.14	SD	Carlo et al. (2003) Avian fruit preferences across a Puerto Rican forested landscape: pattern consistency and implications for seed removal. Oecologia 134: 119-131	Cialitos, Puerto Rico	18.26	-66.54
M_SD_005	38	49	0.15	SD	Carlo et al. (2003) Avian fruit preferences across a Puerto Rican forested landscape: pattern consistency and implications for seed removal. Oecologia 134: 119-131	Cordillera, Puerto Rico	18.17	-66.59
M_SD_006	36	51	0.16	SD	Carlo et al. (2003) Avian fruit preferences across a Puerto Rican forested landscape: pattern consistency and implications for seed removal. Oecologia 134: 119-131	Fronton, Puerto Rico	18.31	-66.56
M_PL_006	78	146	0.14	P	Dicks, LV, Corbet, SA and Pywell, RF 2002. Compartmentalization in plant-insect flower visitor webs. J. Anim. Ecol. 71: 32-43.	Hickling, Norfolk, UK	52.76	1.58
M_PL_007	52	85	0.15	P	Dicks, LV, Corbet, SA and Pywell, RF 2002. Compartmentalization in plant-insect flower visitor webs. J. Anim. Ecol. 71: 32-43.	Shelfanger, Norfolk, UK	52.41	1.1
M_SD_009	25	38	0.3	SD	Galetti, M., Pizo, M.A. 1996. Fruit eating birds in a forest fragment in southeastern Brazil. Ararajuba, Revista Brasileira de Ornitologia, 4: 71-79.	Santa Genebra Reserve T1. SE Brazil	-22.82	-47.1
M_SD_010	64	234	0.33	SD	Snow, B.K., Snow, D.W. 1971. The feeding ecology of tanagers and	Tropical rainforest.	10.72	-61.3

					honeycreepers in Trinidad. Auk, 88: 291-322.	Trinidad.		
M_PL_013	65	103	0.2	P	Ollerton, J., S. D. Johnson, L. Cranmer, and S. Kellie. 2003. The pollination ecology of an assemblage of grassland asclepiads in South Africa. Annals of Botany 92:807-834.	KwaZulu-Natal region, South Africa	-29.62	30.13
M_SD_012	64	146	0.14	SD	Galetti, M., Pizo, M.A. 1996. Fruit eating birds in a forest fragment in southeastern Brazil. Ararajuba, Revista Brasileira de Ornitologia, 4: 71-79.	Santa Genebra Reserve T2. SE Brazil	-22.82	-47.1
M_PL_017	104	299	0.15	P	Memmott J. 1999. The structure of a plant-pollinator food web. Ecology Letters 2:276-280.	Bristol, England	51.57	-2.59
M_PL_019	125	264	0.08	P	Inouye, D. W., and G. H. Pyke. 1988. Pollination biology in the Snowy Mountains of Australia: comparisons with montane Colorado, USA. Australian Journal of Ecology 13:191-210.	Snowy Mountains, Australia	-36.45	148.27
M_PL_024	29	38	0.19	P	Mosquin, T., and J. E. H. Martin. 1967. Observations on the pollination biology of plants on Melville Island, N.W.T., Canada. Canadian Field Naturalist 81:201-205.	Melville Island, Canada	75	-114.97
M_PL_025	57	143	0.25	P	Motten, A. F. 1982. Pollination Ecology of the Spring Wildflower Community in the Deciduous Forests of Piedmont North Carolina. Doctoral Dissertation thesis, Duke University, Duhram, North Carolina, USA; Motten, A. F. 1986. Pollination ecology of the spring wildflower community of a temperate deciduous forest. Ecological Monographs 56:21-42.	North Carolina, USA	36.08	-79
M_PL_033	47	141	0.32	P	Small, E. 1976. Insect pollinators of the Mer Bleue peat bog of Ottawa. Canadian Field Naturalist 90:22-28.	Ottawa, Canada	45.4	-75.5
M_PL_040	72	114	0.09	P	Ingversen TT (2006). Plant-pollinator interactions on Jamaica and Dominica: The centrality, asymmetry and modularity of	Windsor, The Cockpit Country,	18.35	-77.65

					networks. Msc thesis (Univ of Aarhus, Aarhus, Denmark).	Jamaica		
M_PL_041	74	145	0.11	P	Ingversen TT (2006) MSc thesis (Univ of Aarhus, Aarhus, Denmark). (MSc, 2006, Tanja Toftemark Ingversen, Plant-pollinator interactions on Jamaica and Dominica: The centrality, asymmetry and modularity of networks)	Syndicate, Dominica	15.52	-61.47
M_PL_044	719	1125	0.02	P	Kato M (2000). Anthophilous insect community and plant-pollinator interactions on Amami Islands in the Ryukyu Archipelago, Japan. Contr Biol Lab Kyoto Univ 29:157-252.	Amami-Ohsima Island, Japan	28.38	129.49
M_PL_045	43	63	0.14	P	Lundgren R, Olesen JM (2005). The Dense and Highly Connected World of Greenland's Plants and Their Pollinators. Arc Antarc Alp Res 37:514-520.	Uummannaq Island, Greenland	71	-52
M_PL_051	104	164	0.13	P	VÃ¡zquez DP. 2002. Interactions among Introduced Ungulates, Plants, and Pollinators: A Field Study in the Temperate Forest of the Southern Andes. Ph.D. Dissertation. Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville.	Nahuel Huapi National Park, Argentina	-41.08	-71.53
M_PL_054	431	773	0.02	P	Kakutani, T., T. Inoue, M. Kato and H. Ichihashi (1990) Insect-flower relationship in the campus of Kyoto University, Kyoto: An overview of the flowering phenology and the seasonal pattern of insect visits. Contribution from the Biological Laboratory, Kyoto University, 27: 465-521.	Kyoto City, Japan	35.03	135.78
M_PL_055	259	431	0.03	P	Kato & Miura (1996). Flowering phenology and anthophilous insect community at a threatened natural lowland marsh at Nakaikemi in Tsuruga, Japan. Kyoto University, Vol. 29: 1-48	Nakaikemi marsh, Fukui Prefecture, Japan	35.65	136.08
M_PL_056	456	871	0.03	P	Kato et al. 1993. Flowering Phenology and Anthophilous Insect Community in the Cool-Temperate	Mt. Kushigata, Yamanashi	35.58	138.38

					Subalpine Forests and Meadows at Mt. Kushigata in the Central Part of Japan. Contributions from the Biological Laboratory, Kyoto University 28: 119-172	Pref., Japan		
M_PL_057	997	1920	0.02	P	Inoue et al 1990. Insect-flower Relationship in the Temperate Deciduous Forest of Kibune, Kyoto: An Overview of the Flowering Phenology and the Seasonal Pattern of Insect Visits.	Kibune, Kyoto, Japan	35.17	135.87
M_SD_020	58	150	0.18	SD	P. Jordano, unpubl.	Nava Correhuelas. S. Cazorla, SE Spain.	37.93	-2.87
M_SD_023	23	38	0.32	SD	Noma, N. 1997. Annual fluctuations of sapfruits production and synchronization within and inter species in a warm temperate forest on Yakushima Island, Japan. Tropics, 6: 441-449.	Yakushima Island, Japan	30.33	130.5
M_PL_058	113	319	0.12	P	Bartomeus, I., Vilá , M. & Santamaria, L., 2008. Contrasting effects of invasive plants in plant-pollinator networks. Oecologia 155: 761-770.	Parc Natural del Cap de Creus	42.3	3.24
M_PL_059	26	71	0.42	P	Bezerra ELS, Machado ICS, Mello MAR. 2009. Pollination networks of oil-flowers: a tiny world within the smallest of all worlds. Journal of Animal Ecology 78:1096-1101.	Parque Nacional do Catimbau	-8.51	-37.2