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Experimental evidence for an intraspecific Janzen-Connell effect mediated by soil biota

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Abstract. The negative effect of soil pathogens on seedling survival varies considerably among conspecific individuals, but the underlying mechanisms are largely unknown. For variation between heterospecifics, a common explanation is the Janzen-Connell effect: negative density dependence in survival due to specialized pathogens aggregating on common hosts. We test whether an intraspecific Janzen-Connell effect exists, i.e., whether the survival chances of one population's seedlings surrounded by a different conspecific population increase with genetic difference, spatial distance, and trait dissimilarity between them. In a shade-house experiment, we grew seedlings of five populations of each of two subtropical tree species (*Castanopsis fissa* and *Canarium album*) for which we measured genetic distance using intersimple sequence repeat (ISSR) analysis and eight common traits/characters, and we treated them with soil material or soil biota filtrate collected from different populations. We found that the relative survival rate increased with increasing dissimilarity measured by spatial distance, genetic distance, and trait differences between the seedling and the population around which the soil was collected. This effect disappeared after soil sterilization. Our results provide evidence that genetic variation, trait similarity, and spatial distance can explain intraspecific variation in plant–soil biotic interactions and suggest that limiting similarity also occurs at the intraspecific level.

Key words: *Canarium album*; *Castanopsis fissa*; density dependence; functional traits; genetic distance; Heishiding Nature Reserve, south China; intraspecific dissimilarity; Janzen-Connell effect; pathogens; seedling survival; subtropical forest.

INTRODUCTION

Understanding the mechanisms that regulate abundances and coexistence of different species in forest communities is a central question in ecology. The Janzen-Connell hypothesis (Janzen 1970, Connell 1971) predicts that offspring located close to adult conspecifics suffer increased mortality caused by host-specific herbivores and pathogens. Therefore, offspring of heterospecific species have a competitive advantage, leading to higher diversity at the stand level. Many observational and experimental studies have provided evidence for the Janzen-Connell effect (e.g., Augspurger and Kelly 1984, Condit et al. 1992, Webb and Peart 1999, Packer and Clay 2000, Bell et al. 2006, Mangan et al. 2010, Liu et al. 2012), but some have failed to find support for the distance-dependent prediction of the theory (Hyatt et al. 2003). In addition to negative feedbacks, positive plant–soil interactions have also been found mediating the home-field advantage (Ayres et al. 2009, Strickland et al. 2009, Miki 2012), such that offspring may benefit more from the microhabitat near the adult than farther away,

and/or locally adapted microbial communities may decompose home leaf litter faster than foreign litter, and hence, positively impact local nutrient cycles and plant performance.

While most studies detecting the Janzen-Connell effect considered the species or community level but not individual variation (e.g., Bever 1994, Klironomos 2002, Kulmatiski et al. 2011), many other studies argue that individual-level variation can have significant effects on community dynamics and species coexistence (see reviews in Whitham et al. 2006, Johnson and Stinchcombe 2007, Hughes et al. 2008, Bailey et al. 2009). Individuals may vary substantially and are rarely identical (see Plate 1), and conspecific individuals can differ in antipredator defenses, parasite resistance, abiotic tolerances, resource use, or competitive ability (Bolnick et al. 2011). Some studies found tree to tree variation in plant–soil pathogen interactions, which causes density- and distance-dependent mortality (Augspurger and Kelly 1984, Hood et al. 2004, Reinhart and Clay 2009). They reported considerable variation in the strength of the distance-dependent effect among individual trees and sites (Reinhart and Clay 2009) and evidence that seedlings were more vulnerable to disease when establishing around their parent tree than around

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other conspecific trees (Augsburger and Kelly 1984). However, little is known about the underlying mechanisms driving these intraspecific differences in host–pathogen interactions among different individuals and populations. We identify various correlates of these intraspecific differences and argue that they inform us about the mechanisms.

Intraspecific genetic diversity has a large effect on ecological processes such as interspecific interactions, community structure, and predator–prey or host–parasitoid systems (reviewed by Hughes et al. 2008). Susceptibility to disease seems to vary in relation to genetic variation in host populations; the idea that higher genetic diversity in hosts can provide greater disease suppression is supported by studies on foliar pathogens (Zhu et al. 2000, Gilbert and Webb 2007), but specific evidence for trees is mixed. The importance of genetic diversity hinges heavily on enemies being host-specialists, which may be overly simplified and not always the case, especially for soil-borne pathogens (van West et al. 2003, Augspurger and Wilkinson 2007, Thomidis et al. 2008). Because these effects are contingent upon genetic diversity being related to the magnitude of variation in functional traits (McGill et al. 2006), recent studies used host functional variation to explain foliar disease risk (e.g., Garrett et al. 2009), and they found strong evidence for greater disease reduction when host trait variation was larger.

Studies of interspecific and intraspecific variation in plant traits have shown that trait variation has significant ecological effects (reviewed by McGill et al. 2006, Bolnick et al. 2011). Several key traits have been proposed to play a key role in host susceptibility and defense to pathogen infection, including physiological and morphological traits such as specific leaf area (SLA), wood density, dry seed mass, leaf nitrogen concentration (LNC), and photosynthetic rate (see Appendix A for detailed descriptions of how these traits influence host–pathogen interactions).

Considering that pathogens may cause density-dependent mortality (Augsburger and Kelly 1984, Bell et al. 2006, Bagchi et al. 2010) and intraspecific genetic variation and trait variation can influence pathogen reproduction and spread, as well as host resistance (Garrett et al. 2009, Tack et al. 2014), a question that arises is whether density-dependent effects vary among individuals and how this variation correlates with genetic differences, spatial distance, and trait dissimilarities. We study the role of genetic differences, as well as trait dissimilarities, among different populations in a comprehensive set of morphological, physiological, and biogeographical characteristics in the Janzen-Connell effect driven by soil biota. As we do not know exactly which gene or trait determines pathogen defense, we took characteristics that measure dissimilarity among populations, including a genetic characteristic, which targets hundreds of fragments throughout the genome, and functional traits, which are plausible proxies for

pathogen defense based on life-history trade-off theory. We also included local abundance of conspecific individuals within each population and spatial distance among different populations, as they are important factors for the Janzen-Connell effect (Augsburger and Kelly 1984, Webb and Peart 1999).

We propose the conspecific dissimilarity hypothesis, which states that the Janzen-Connell effect experienced by one population's seedlings surrounded by a different conspecific population decreases with increasing dissimilarity, measured by genetic variation, trait similarity, and spatial distance between the two populations. In a shade-house experiment, we studied seedling survival of two subtropical tree species (*Castanopsis fissa* and *Canarium album*), where, for each species, the seedlings from each of five different populations were treated with soil biota collected around their parent tree and around the four other conspecific populations. We analyzed genetic distances among populations based on 17 intersimple sequence repeat (ISSR) primers and measured eight characters of these populations, i.e., spatial distance, local abundance of conspecific adults, SLA, LNC, photosynthetic rate, wood density, seed mass, and diameter at breast height (dbh) to test whether genetic distance and trait-based dissimilarity could explain the variation in negative soil biota effects among different populations within the same species.

MATERIALS AND METHODS

Study site and species

We conducted fieldwork at Heishiding Nature Reserve (111°53' E, 23°27' N; 150–927 m altitude), Guangdong Province, in south China. The reserve consists of approximately 4200 ha of subtropical evergreen broad-leaved forest and has a subtropical moist monsoon climate. Mean annual temperature is 19.6°C, with the lowest mean monthly temperature of 10.6°C in January and the highest of 28.4°C in July. Annual precipitation is 1743.8 mm on average, occurring mainly between April and September (79% of annual rainfall), and a pronounced dry season lasts from October to March. The study area has relatively high species richness containing 240 tree species in total and approximately 125 tree species per 1-ha plot.

We selected two tree species, *Castanopsis fissa* Rehd. et Wils (Fagaceae) and *Canarium album* (Lour.) Rausch. (Burseraceae), which are both common but not dominant, so that they have clear discrete populations (sufficient numbers of individuals and, at the same time, sufficiently large distances among different populations, i.e., at least 1 km apart). We hereafter refer to these species by their genus names as they belong to different genera. *Castanopsis* is a heliophilous species and *Canarium* is a late-successional and shade-tolerant species. Both species are indeciduous and monoecious; their seeds are mainly gravity dispersed, although *Canarium* seeds may sometimes be dispersed by rodents and other animals. These features provide comprehen-

sive trait ranges (see Plate 1) and allow testing on whether variation in the strength of plant–soil feedback among populations is correlated with these characteristics.

For each species, we chose five populations that were at least 1 km away from each other and had no other conspecific trees among them (called A, B, C, D, and E, respectively; see Appendix B for relative spatial locations of these populations). The mean numbers of individuals for these populations were 11.4 and 2.2 for *Castanopsis* and *Canarium*, respectively. Each population had at least one adult individual that produced a sufficient number of seeds in 2011. Even though some of the sites (populations C and E for *Canarium* and D for *Castanopsis*) have only one adult individual, we still refer to them as populations and treat the local abundance as a population character. We measured genetic variation based on intersimple sequence repeat polymerase chain reaction (ISSR PCR) analysis and eight characteristics for each focal population to investigate the genetic and trait dissimilarities among populations.

Shade-house experiment

Flowering occurs from April to June for both study species, they are pollinated by insects, and fruits/seeds ripen from late September to December. We collected fruits and seeds under the canopy of each focal population during autumn and winter 2010. We were able to distinguish the maternal tree for each seed, while evidently seeds from the same maternal tree may be half-siblings because the pollen could have come from different paternal trees. We labeled the seeds by their specific populations, surface-sterilized (70% ethanol for 1 min, 2.625% NaOCl for 3 min, 70% ethanol for 1 min, and sufficiently rinsed with distilled water), and stored them at 4°C until April 2011. Then seeds were left to germinate in plastic boxes filled with sterilized sand.

To determine the intraspecific variation of the Janzen-Connell effect driven by soil biota, we conducted a shade-house experiment with a full reciprocal design, where we planted newly germinated seedlings into autoclaved field soil and treated with soil material collected around their parent tree and around other conspecific trees. Experimental treatments consisted of two tree species, five seed sources, five soil biota sources, and three soil treatments. For each species, we collected soil material from each of the five populations and divided them into three soil biota treatments (field soil, sterile soil, and sterile soil infected with soil filtrate). Newly germinated seedlings from each of the five populations received 15 different treatments, including five field soil treatments (one from each population), five filtrate treatments, and five sterile treatments. Hence, an entire treatment unit contained 75 pots for each study species. We chose the adult trees that provided seeds as pathogen inoculum sources. We collected three soil cores of 10 cm in diameter and 30 cm depth within a

distance of 0–2 m in three different directions from these adult trees in late April 2011, after which they were thoroughly mixed for each population. In addition to using untreated field soil as inocula, we also extracted soil filtrate from these soil samples using a wet-sieving method (Klironomos 2002) to exclude arbuscular mycorrhizal fungi (AMF) spores that may enhance seedling performance (Smith and Read 1997). For each extraction, 50 g soil was blended with 100 mL tap water for 30 s. We then washed the liquid suspension through 250- μ m and 45- μ m analytical sieves with tap water and used the suspensions that passed through the 4- μ m sieve as pathogen inocula. By testing the AMF colonization and pathogen infection rates after inoculating, this filtrate approach has been confirmed as an effective way to separate AMF and fungal pathogens (Liang et al., *in press*). We admit that although we aimed to test the effect of soil-borne pathogens, both field soil and soil filtrate approaches are somewhat crude ways for interpreting pathogen effects, because the field soil treatment actually measures effects of whole soil biota, and the soil filtrate treatment measures effects of filtrate without AMF and other biota greater than 45 μ m.

We transferred newly germinated seedlings to sterilized field soil. Field soil was collected from a common understory with no *Castanopsis* and *Canarium* trees within 50 m at the field site, thoroughly mixed, and autoclaved for 3 h at 121°C followed by a 2-d incubation and a second autoclaving. We filled plastic pots (10 cm in diameter, 12 cm in height) with this soil, and then planted seedlings of each population into these pots (one randomly selected seedling for each pot). We added 50 g original soil cores (field soil treatment) or 50 mL nonsterilized filtrate of soil cores (soil filtrate treatment) or 50 mL filtrate that was autoclaved for 30 min at 121°C (sterile treatment) to the pots using a full reciprocal design, i.e., seedlings from each of the five populations received 15 different treatments, five field soil treatments (one from each population), five filtrate treatments, and five sterile treatments. We then covered each pot with a layer of 2 cm sterile soil after inoculating. The experimental units consisted of 30 blocks (replicates) for each species, each block containing an entire treatment unit (i.e., 75 pots). We randomly arranged and separated all blocks by a distance of 0.5 m and all pots by 0.2 m in the shade house to limit the potential pot effluent and pathogen dispersal via water. We regularly watered the seedlings and monitored seedling survival every 2 weeks. All seedlings were allowed to grow for 6 months and then harvested to determine their biomass.

ISSR PCR examination for population genetic variation

For each focal population, we randomly selected 1–6 individuals (depending on how many adults there were in each population, but always including the focal adults that provided seeds and soils) and sampled fresh leaf materials directly from upper branches, using silica gel

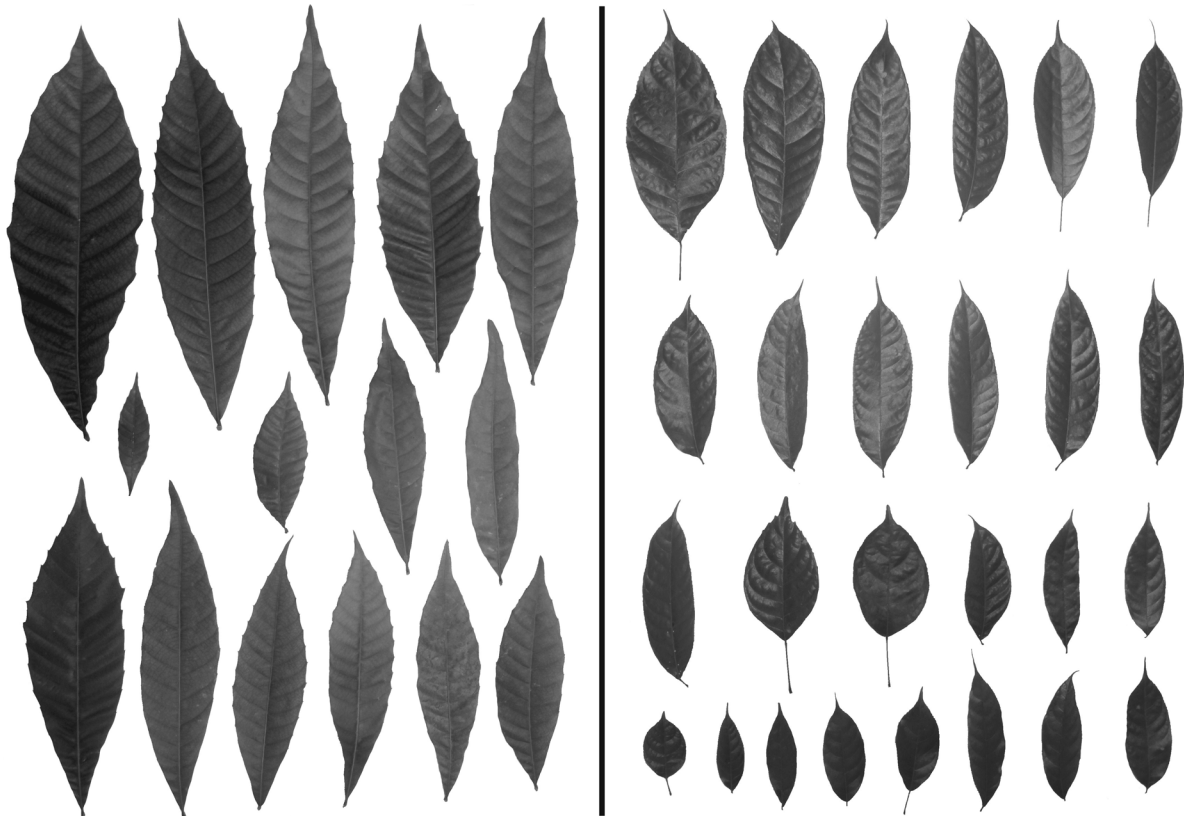


PLATE 1. Leaf shape and size are variable among different individuals for both *Castanopsis fissa* (left) and *Canarium album* (right). Photo credit: X. Liu.

to dry and preserve samples separately for each individual. Genomic DNA was isolated using the modified cetyltrimethyl ammonium bromide (CTAB) protocol (Su et al. 2005). A total of 100 ISSR primers from the Biotechnology Laboratory of University of British Columbia (Vancouver, British Columbia, Canada) were initially screened and 17 of the primers that produced clear and reproducible banding patterns for both species were used for the final ISSR analysis (Appendix C: Table C1). These commonly used primers targeted hundreds of gene segments that widely spread throughout the whole genome of the focal species. PCR products were separated using electrophoresis on 1.8% agarose gels stained with ethidium bromide, then digitally photographed under UV light (see Appendix C for detailed DNA isolating and PCR procedures). Based on the presence/absence of each segment for each population, the ISSR-PCR approach can effectively detect the genetic diversity among populations (Esselman et al. 1999, Wang et al. 2012). We checked that individuals within the same population had much smaller genetic distances than individuals from different populations for both study species (Appendix F). As both focal species are monoecious and entomophilous and the populations had relatively long distances from each other, the genetic variation among the adult trees

providing seeds should therefore be a good proxy for population genetic variation and seedling genetic variation.

Individual characteristics and traits

For each focal population that provided seeds and soil inoculums, we used a comprehensive set of morphological, physiological, and spatial characteristics as predictors of intraspecific variation of the Janzen-Connell effect, including specific leaf area (SLA), wood density, seed mass, photosynthetic rate, leaf nitrogen concentration (LNC), local abundance, individual size, and spatial distance. We measured the traits from leaves and trunks of the focal adult trees for each population (only one individual per population that provided seeds and soils was sampled) in the summer of 2011 (See Appendix D for detailed descriptions on how each trait was measured). SLA and photosynthetic rate were also measured for the seedlings in the shade house.

Data analysis

To test whether the survival rates of seedlings treated with soil material collected around their parent tree were different from those treated with soil beneath other conspecific trees, we combined all seedlings infected by soil biota from their own population (own soil, $n = 5$ for

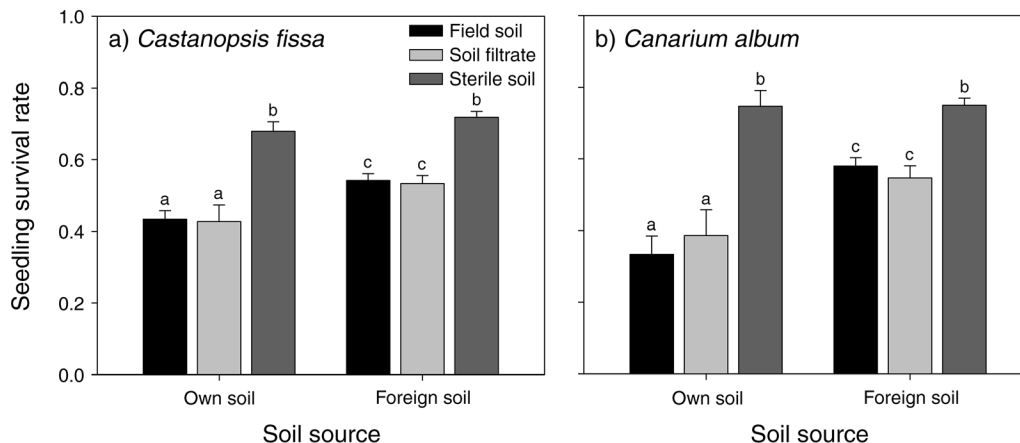


FIG. 1. Effect of soil biota on the survival (mean \pm SE) of (a) *Castanopsis fissa* and (b) *Canarium album* seedlings in a shade-house experiment at the field site. Bars show mean survival rates combining all seedlings planted in soil collected from their own population (own soil, $n = 5$ for all treatments) or from other four populations (foreign soil, $n = 20$). Different lowercase letters above the error bars denote significant differences ($P < 0.05$) based on one-way ANOVA. Error bars represent standard errors.

all treatments) or from the other four populations for each species (foreign soil, $n = 20$). We confirmed homogeneity of variances by performing Levene's test, and then conducted a one-way analysis of variance (ANOVA) to test the effects of inoculum sources (own soil vs. foreign soil) and soil treatments (infected with field soil, soil filtrate, or sterile) on seedling survival.

We scored ISSR banding patterns as presence, 1, or absence, 0, for each DNA sample, excluding the smeared and weak ones, and input the binary data matrix into POPGENE 1.32 (Yeh et al. 1999). We estimated genetic differentiation among individuals by Nei's genetic distance (Nei 1972) and used pairwise genetic distance between individuals to construct UPGMA (unweighted pair group method using arithmetic average) dendrograms.

For a given functional trait i , the dissimilarity between population A and B was defined as

$$d_{iAB} = \frac{|x_{iA} - x_{iB}|}{\max\{x_i\} - \min\{x_i\}} \quad (1)$$

with \mathbf{x} being the matrix of trait values of five populations, and x_{iA} and x_{iB} being the values of trait i for population A and B, respectively, $\max\{x_i\}$ and $\min\{x_i\}$ are the maximum and minimum values of trait i of all five populations (Gower 1971). Therefore, the dissimilarity of trait i for seedlings from population A treated with pathogen filtrate from population B was denoted as d_{iAB} in the subsequent analysis. This definition ensures that, for all functional traits, d_{iAB} always ranges between 0 (when seedlings and soil inocula were collected from the same population) and 1.

To determine the effect of soil biota on seedling survival, we calculated the log survival odds ratio comparing seedling survival in field soil pots vs. sterile pots and soil-filtrate-treated pots vs. sterile pots with the same seedling source and the same soil source. We used

odds ratio regression to determine whether the log odds ratio (i.e., the relative survival) was significantly correlated with genetic distance or trait dissimilarity (d_{iAB}) among populations. Odds ratio regression, a convenient way to model and estimate the probability of failure or survival during a specified time period (Prentice and Farewell 1986), uses a maximum likelihood approach where the distribution of the log odds ratio is assumed to be approximately normal (Jewell 2004, Liu et al. 2012) and where a likelihood ratio test checks for significant relationships with explanatory variables.

Odds ratio regression models included terms for genetic distance, all eight trait dissimilarities, and their interaction terms. We selected the best-fit multivariate model based on the corrected Akaike's information criterion (AIC_C) value and backward stepwise algorithm. We then conducted a principal component analysis (PCA) based on all nine characteristic dissimilarities for each species to disentangle the dissimilarity among different populations. We performed odds ratio regression using the first two principal components as explanatory variables to test whether the log odds ratio was correlated with them. We also conducted multivariable model selections with all the principal components. All statistical analyses were performed using R 2.15.1 (R Development Core Team 2012).

RESULTS

Seedling survival rates varied substantially among different seedling and soil combinations for both plant species (Appendix E: Fig. E1), while the sterilized treatment generally showed higher survival of seedlings than field soil and filtrate treatments for a plant's own soil as well as foreign soil pots (Fig. 1). When combining all seedlings infected by own soil or foreign soil, the mean survival rate (\pm SE) of own soil pots was

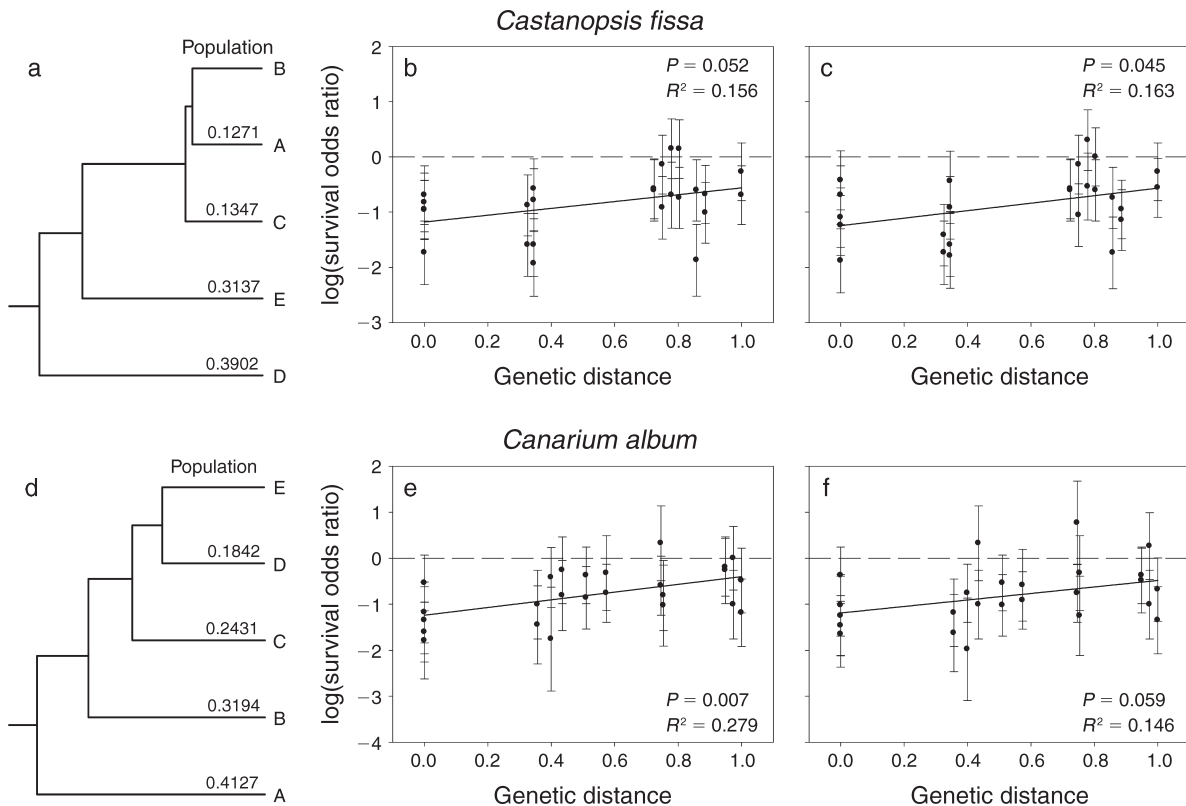


FIG. 2. The intraspecific Janzen-Connell effect in relation to genetic distance for (a–c) *Castanopsis fissa* and (d–f) *Canarium album*. Panels (a, d) are UPGMA (unweighted pair-group method using arithmetic average) dendrograms of *Castanopsis* and *Canarium* populations, respectively, based on ISSR (intersimple sequence repeat) analysis; numbers show Nei's (1972) genetic distance. Panels (b, c, and e, f) show odds ratio regressions between survival log odds ratio and genetic distance. Log odds ratios were calculated by comparing seedling survival in field soil vs. sterile soil for panels (b) and (e) and filtrate-treated soil vs. sterile soil for panels (c) and (f), where $n = 30$ seedlings per treatment. Log-transformed survival odds ratios above 0 indicate positive effects and log odds ratios that are significantly lower than 0 indicate negative effects. Best-fit odds ratio regression lines are shown; P values indicate significant departure from no relationship with genetic dissimilarities. Error bars represent standard errors.

significantly lower than foreign soil pots in the field soil treatment and the soil filtrate treatment, but there was no significant difference for the sterilized treatment (Fig. 1).

UPGMA dendrograms of individuals based on ISSR analysis showed that individuals within the same population had much smaller genetic distances than individuals from different populations for both study species (Appendix F). In the odds ratio regression models, we obtained similar results from the field soil treatment and the soil filtrate treatment (Figs. 2 and 3). The survival odds ratio comparing seedling survival in field soil treatment or soil filtrate treatment vs. sterile soil treatment increased with increasing genetic distance for *Castanopsis* (Fig. 2a–c), as well as *Canarium* (Fig. 2d–f).

Although the PCAs of characteristic dissimilarity had different component loadings for the two species, all nine characteristics were strongly correlated on PC1 (Appendix G: Fig. G1), and the survival log odds ratios were significantly correlated with PC1 for both *Castanopsis* and *Canarium* (Fig. 3). The best-fit multivariate

models also included PC1 as the only significant explanatory variable for the survival odds ratios comparing seedling survival in field soil treatment vs. sterile soil treatment (Appendix H: Table H1) and in soil filtrate treatment vs. sterile soil treatment (Appendix H: Table H2) for both species.

For many of the measured characteristics, the log odds ratio comparing seedling survival in field soil treated or soil filtrate treated pots vs. sterile soil pots increased with increasing characteristic dissimilarity for *Castanopsis* (Appendix H: Fig. H1), as well as *Canarium* (Appendix H: Fig. H2). In particular, among the five *Castanopsis* populations, the survival odds ratio was positively related with dissimilarities of DBH, SLA, photosynthetic rate, and wood density (Appendix H: Fig. H1), while for the *Canarium* populations it was positively correlated with dissimilarities in spatial distance, DBH, SLA, and wood density (Appendix H: Fig. H2). Using the two trait dissimilarities that were measured on seedlings (SLA and photosynthetic rate), we found that the results were similar to those based on adult traits. The log odds ratio comparing seedling

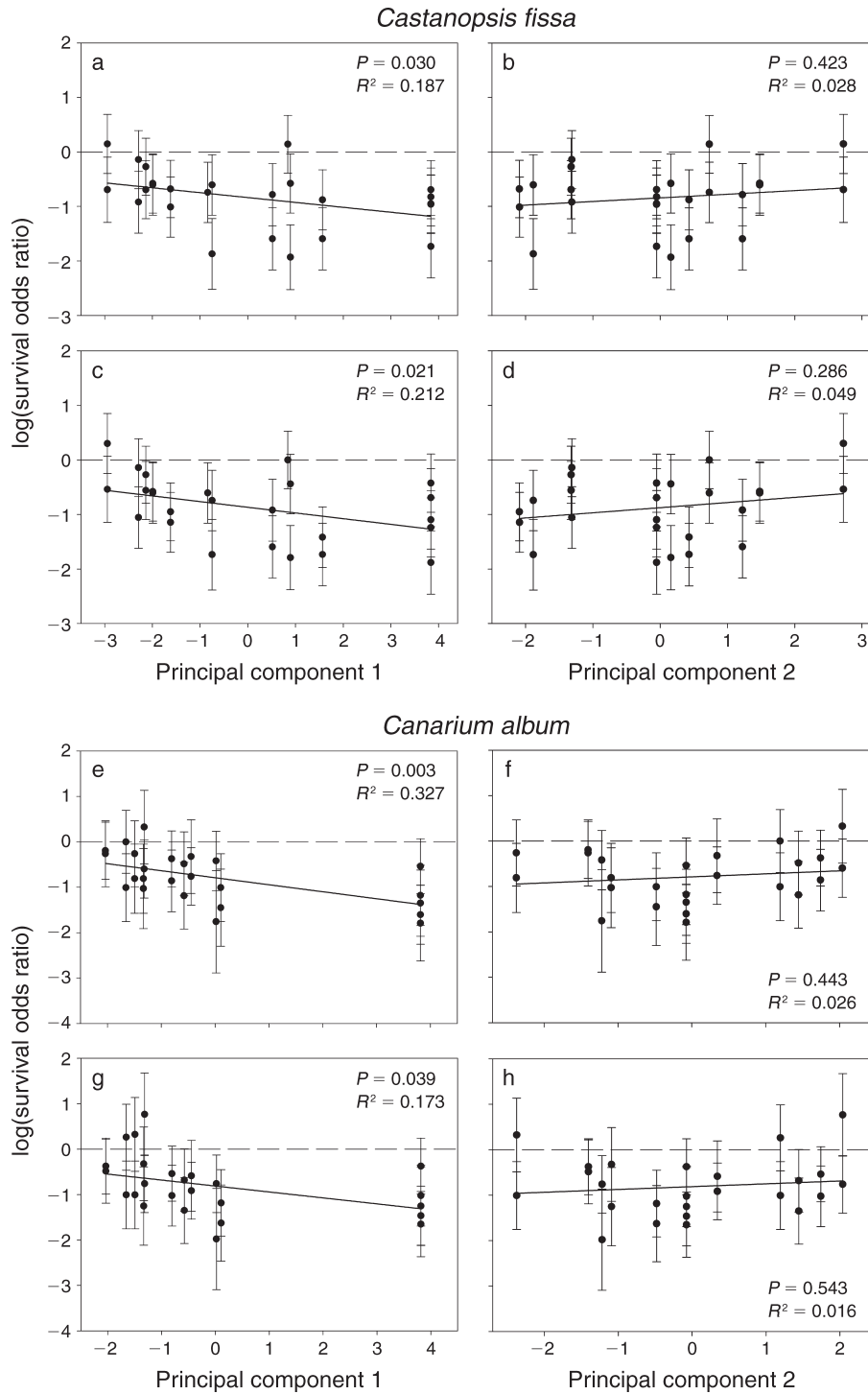


FIG. 3. The intraspecific Janzen-Connell effect and the relation to dissimilarity measured by spatial distance, genetic distance, and trait differences for (a–d) *Castanopsis fissa* and (e–h) *Canarium album*. Odds ratio regressions are shown between survival odds ratio and principal component 1 (left column) or principal component 2 (right column). Log odds ratios were calculated by comparing seedling survival in field soil vs. sterile soil for panels (a, b, e, f), and filtrate treated soil vs. sterile soil for panels (c, d, g, h). Error bars represent standard errors.

survival in soil biota treated pots vs. sterile soil pots increased with increasing trait dissimilarity (Appendix H: Fig. H3).

DISCUSSION

Though numerous hypotheses have been presented to explain forest diversity (Palmer 1994), we focus on the Janzen-Connell effect, which provides a potentially ubiquitous explanation of diversity at both the interspecific and the intraspecific level (Janzen 1970, Connell 1971, Webb and Peart 1999, Packer and Clay 2000, Klironomos 2002, Liu et al. 2012, but see Hyatt et al. 2003). Observational and experimental evidence suggests that among different phylogenetically related species, the Janzen-Connell effect is strongest for closely related species and decreases with increasing evolutionary distance (Webb et al. 2006, Liu et al. 2012). We experimentally investigated the effect of intraspecific variation and demonstrated that seedlings suffer higher mortality when growing in soil collected around their parent trees than around conspecific trees from other populations (Fig. 1). When seedlings were treated with soil biota from other conspecific trees, the negative effect of the soil biota, likely from pathogens, on seedling survival decreased with increasing dissimilarity among populations (Fig. 2; Appendix H).

Unlike previous studies that focused on leaf litter decomposition or local habitat and detected evidence for the home-soil advantage (Ayres et al. 2009, Strickland et al. 2009, Miki 2012), our results demonstrated an away-field advantage (i.e., a case of fouling the nest): the soil conditioned by a local population decreased the performance of conspecific seedlings, and this negative plant-soil feedback decreased with increasing spatial distance (Appendix H). This may be caused by the design of our shade-house experiment, which excluded the leaf litter and used fertile understory soil as culture medium, and all seedlings were placed in a relatively homogeneous environment to eliminate the heterogeneity of soil nutrients and focus exclusively on soil biota effects. Hence, newly germinated seedlings may benefit less from home-field advantage in decomposition and microhabitat and suffer more from disease caused by pathogenic fungi and bacteria. While both home-soil advantage and away-soil advantage depend on the specialization of soil biota communities to local host individuals, our study suggests that this specialization is different due to intraspecific plant variation.

The Janzen-Connell hypothesis describes a density- and/or distance-dependent self-inhibition effect on tree species, which can prevent a certain species from becoming too dominant in the local community and hence maintain diversity at the stand level. Consistent with many previous studies (e.g., Augspurger and Kelly 1984, Packer and Clay 2000, Gilbert 2002, Hood et al. 2004, Bell et al. 2006, Mangan et al. 2010), our experiment clearly demonstrates that Janzen-Connell effects caused by soil biota exist even among conspecific

individuals (Fig. 1). Moreover, although individual comparisons may deviate, we detected a general tendency that negative soil biota effects become less severe (i.e., more neutral) with greater dissimilarity among different populations (Fig. 3). Therefore, a high relative abundance of individuals with similar traits is prevented by an intensive intraspecific Janzen-Connell effect. By dispersing to other populations or communities where they undergo weaker inhibition effects from conspecific adults, a seed or seedling will have an increased probability to reach maturity. Our finding that intraspecific variation as a component of the Janzen-Connell hypothesis provides empirical support for the Red Queen hypothesis (Van Valen 1973), which describes the evolutionary arms race where hosts and their specific parasites constantly evolve with each other to reach some sort of uneasy balance. Within local populations, soil biota appear to have specialized on common host genotypes and cause negative frequency-dependent selection on these hosts (Clay et al. 2008). Rare tree genotypes may then temporally have a fitness advantage: avoiding regulation by negative frequency-dependent effects that act on common genotypes as predicted by the Red Queen hypothesis. While rarity at the species level within communities has been correlated to negative soil feedback effects (e.g., Klironomos 2002, Mangan et al. 2010, McCarthy-Neumann and Ibanez 2013, but see Reinhart 2012), we interpret that negative frequency-dependent effects may take many forms and have effects on both population and community levels.

Previous studies have detected tree to tree variation in plant-soil biotic interactions (Hood et al. 2004, Reinhart and Clay 2009), and explained it by local environmental factors such as light conditions (e.g., McCarthy-Neumann and Kobe 2008). We also detected intraspecific variation in plant-soil biota interactions and were able to attribute 14.6–27.9% to genetic variation (Fig. 2) and 23.0–47.8% to trait variation (Appendix H: Tables H1, H2). This suggests that various factors may explain variability in plant-soil biotic interactions in nature.

Having confirmed that intraspecific genotype similarity was correlated with negative soil biota effects, we also explored whether trait similarity (which is likely correlated with genotypic differences) also explained variation in soil biota effects. As predicted, we observed that the negative effects of soil biota on seedling survival decreased with increasing trait dissimilarities among populations. Although the functional traits we measured have been found to have direct or indirect influences on pathogen spread as well as host susceptibility (Garrett et al. 2009), our main intention in using these characteristics was to quantify the dissimilarities among the five populations of each species. These traits may vary due to genotype, environment, and age and represent genetic and acquired dissimilarity among individuals.

Our results suggest that limiting similarity among coexisting individuals may not only occur at the interspecific level, but also at the intraspecific level.

The concept of limiting similarity refers to the maximum level of niche overlap between two species allowing their coexistence (MacArthur and Levins 1967): morphologically and/or ecologically similar species will differ enough in shape, size, or other variables to minimize competition (community-wide trait overdispersion). We detected a higher negative soil biota effect among conspecific individuals within the same population than among different populations (Fig. 1), suggesting that individuals should exhibit trait differences greater than those expected by chance (population-wide trait overdispersion; see Plate 1). The strength of the Janzen-Connell effect gradually changes with changing genetic distance among conspecific populations (as shown in the current study) and among different phylogenetically related species (Liu et al. 2012), implying that pathogens may coevolve with their preferred host and host-pathogen coevolution may be parent-specific and site-specific (Augsburger and Kelly 1984) and may occur at a broad range of spatial scales (Burdon and Thrall 2000).

In summary, our results indicate that host genetic and functional differences moderate the Janzen-Connell effects (i.e., negative soil biota effects). This study and our previous research on the phylogenetic Janzen-Connell effect among different related species (Liu et al. 2012) indicate that soil biota effects may be moderated by host similarities within and among species. We cautiously interpret that our soil biota effects may be driven by pathogens that are highly specialized and possibly coevolved with their hosts. Validating the role of soil pathogens, however, will require more detailed experiments that isolate soil pathogens and then perform pathogenicity trials to elucidate whether the pathogenic effects of isolates vary by host genotype. Despite this, our study indicates that negative soil biota effects vary by genetic difference, spatial distance, and trait dissimilarity and add to existing literatures showing the prevalence of negative soil feedbacks among interspecific groupings of plant species (Bever 1994, Klironomos 2002, Kulmatiski et al. 2011). Our results coupled with others indicate that negative soil biota effects may be influenced by a series of factors ranging from genotype and trait variations within a host species, (phylogenetic) differences among host species, and abiotic factors. This study, to our knowledge, is the first to connect the Janzen-Connell effect variation to intraspecific dissimilarity based on genetic distance and functional traits and suggests an experimentally supported mechanism for how intraspecific variation influences individual fitness and performance and promotes local diversity.

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SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A–H are available online: <http://dx.doi.org/10.1890/14-0014.1.sm>