Maternal microbes complicate coexistence for tropical trees

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How can hundreds of tree species coexist in a single hectare of tropical forest when the environmental conditions, as well as the species' basic requirements, appear so similar? A leading explanation, particularly in tropical forests, is called the Janzen–Connell hypothesis after the two ecologists who proposed it in the early 1970s (1, 2). In this mechanism, specialized predators and pathogens concentrated near adult plants differentially reduce survival and growth of conspecific offspring relative to seedlings of other species. These natural enemies limit regeneration of common species and confer a relative advantage to rare species because they have more enemy-free regeneration sites. This has driven decades of empirical work to understand whether enemies are specialized enough in nature to cause this pattern and allow coexistence by this mechanism, and a recent metaanalysis supports the prediction that seed or seedling survival is greater away from conspecific adults (3). In PNAS, Eck et al. (4) show that natural enemies can be even more specialized, specializing on individual genotypes within a wild population. Through simulation models exploring the theoretical implications of this field result, Eck et al. (4) show that this greater specialization may weaken the stabilizing effects of natural enemies on species coexistence but also may select for greater dispersal over evolutionary time.

The body of theory developed since the original formulation of the Janzen–Connell hypothesis has primarily asked (*i*) How specialized must the natural enemies be? and (*ii*) At what scale must these patterns operate? With regard to natural enemies, a longstanding conclusion is that species specificity of natural enemies allows coexistence. If a rare plant species shares a generalist enemy with an abundant plant species, then this abundant generalist enemy could extirpate the rare species from the community. With regard to patterns, the spatial scale matters—both in terms of enemy attack and of seed dispersal away from the maternal plant. A plant would experience a rare-species advantage if the abundance of its specialist enemy declines as the plant becomes rare, or if many seeds disperse away from areas of high specialist enemy abundance near conspecific plants. Although this body of theory emphasizes specialization, these models have only considered specialization down to the species level.

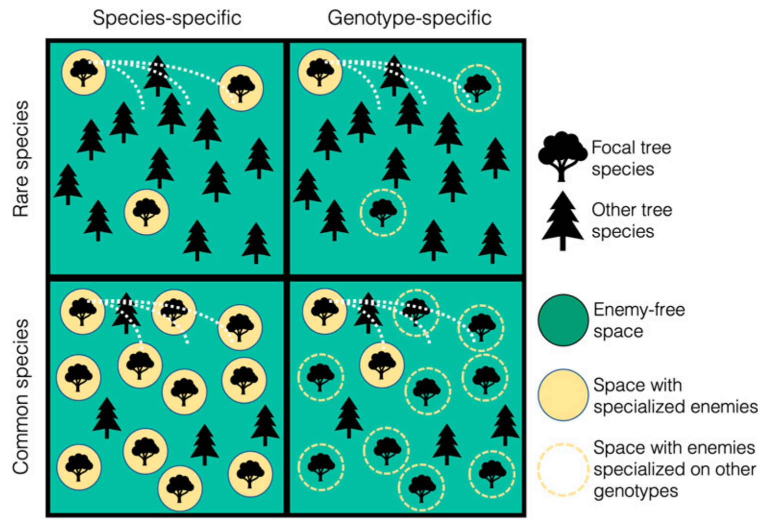

Fig. 1. For rare species, the benefit of escape from pathogens is similar regardless of whether the pathogens are species specific or genotype specific, whereas common species benefit more when pathogens are genotype specific because a larger proportion of available space is free from specialized enemies. This mechanism reduces the rare-species advantage, weakening the stabilizing effects of specialized natural enemies.

While coexistence theory that is focused on natural-enemy specialization stops at the species level, empirical work supports a role for specialization within species. In agricultural systems, there is increasing recognition that microbes can specialize on particular genotypes (5, 6). Several studies on plants in the wild have hinted at an advantage associated with escaping one's mother plant, with rare genotypes showing an advantage over common genotypes (7), greater survival away from close relatives (8), and the general finding that seedlings underneath a tree typically are not the

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offspring of that tree. Liu et al. (9) elegantly demonstrated that the Janzen–Connell effect exists between populations more than a kilometer apart, and Eck et al. (4) show that Janzen–Connell effects could operate on the scale that most seed dispersal occurs—tens to hundreds of meters.

Eck et al. (4) conducted an experiment with a dioecious bird-dispersed canopy tree, *Virola surinamensis*, to test whether seedlings perform more poorly when exposed to the soil microbes from underneath their mother tree versus those from other mothers. *V. surinamensis*, a wild nutmeg, is a relatively common, large-seeded (~2 cm) species with a fleshy aril that attracts monkeys as well as large birds such as toucans and motmots that then disperse the seeds (10). On Barro Colorado Island, Republic of Panama, the authors collected seeds and soil samples below fruiting trees for a shadehouse plant–soil feedback experiment. Seeds were surface sterilized, and the resulting seedlings were transplanted into a planting medium inoculated with soil microbes from their own mother, a different *V. surinamensis* mother, or a different species. Monitoring growth of the resulting seedlings over the course of eight months, Eck et al. (4) found that seedlings exposed to the maternal microbial environment grew less compared to seedlings exposed to microbes from a different mother of the same species. Differences in nutrients and mycorrhizae across samples do not seem to explain these patterns. The experiments support a mechanism in which natural enemies accumulate on individual trees depending on that plant's susceptibility. The shared susceptibility of their offspring makes those seedlings perform worse under their mother than in the microbial environment of another mother. This is evidence for microbial enemies specializing below the species level in a wild population, causing genotype-specific patterns of natural-enemy attack.

The finding that enemies can specialize below the species level is likely to spur a renewed examination of results from previous studies and emphasizes the importance of genetic diversity in plant restoration projects. Studies that have monitored survival and growth of seeds sown or seedlings planted near conspecific adults, but randomly with respect to maternal source, may underestimate the effect of natural enemies because the experimental plants are less likely than under natural dispersal to share the susceptible genotypes of the nearby mother. On the other hand, studies of plant–soil feedback that mix soil sampled from multiple adult plants may overestimate the effect of the conspecific soil environment; mixing makes it more likely that the soil inoculant contains microbes that can attack the focal-plant genotype. These factors might simply quantitatively shift estimates without impacting study conclusions, introduce a bias that qualitatively impacts conclusions drawn in analyses across taxa and sites, or affect statistical results by artificially reducing variability across experimental units. In addition to guiding study design and interpretation, the Eck et al. (4) study has implications for real-world applications such as restoration efforts. The use of genetically diverse seeds and seedlings should limit the susceptibility of restored populations to be wiped out by genotype-specific enemies, echoing similar potential concerns of agricultural monoculture.

The empirical results showing that natural enemies are more specialized than thought necessary for coexistence poses a key conceptual question regarding the role of natural enemies. How does specialization at the genotypic level impact coexistence? The authors paired the shadehouse experiment with a theoretical model to explore the impacts of an intraspecific Janzen–Connell effect on coexistence. In this simulation, the forest is represented by many regeneration sites that can each be occupied by an adult. When an adult dies, it is replaced from among the seeds that have dispersed to the site. Which seed wins depends on its susceptibility to the natural enemies at the site. The simulation results show that genotype-specific natural enemies favor species coexistence, yet less strongly than in a scenario in which natural enemies are species specific. When enemies are genotype specific and when many genotypes exist, seeds of both common and rare species dispersed away from their mothers are likely to fall in areas without their genotype-specific enemies. As a result, genotype-specific natural enemies make per capita reproduction of common and rare species more similar, weakening the "advantage when rare" mechanism that most effectively stabilizes coexistence (Fig. 1).

The simulations by Eck et al. show that genotype-specific enemies increase selection for greater dispersal compared with species-specific enemies.

The study by Eck et al. (4) is likely to catalyze a new generation of plant–soil feedback studies. One class of questions focuses on which natural enemies cause these genotype-specific impacts. In the present study, the pathogens that cause the growth differences are unknown. Are they caused by different genotypes of a single microbe species or by different microbial species? Or perhaps they are caused by abundance or diversity differences within a community of microbes. Pointing toward the last scenario, DNA barcoding of pathogens found on seeds of multiple tree species at the same study site has demonstrated specialization within the suite of microbes that coinfect seeds (11). Further mechanistic insight would be possible with omics data on plants and enemies to assess genotype associations or the biochemical mechanisms of defense and attack. A second class of questions focuses on which species are impacted most. Do rare and common species have similar patterns of genotype-specific attack? How is this related to life history traits? Does heavy investment in defense of offspring drive selection for greater enemy specialization? Does dispersal mode, and thus the proportion of seeds likely to escape the parent, relate to the prevalence of genotype-specific natural enemies? A third class of questions could focus on specialization in relation to local environmental or latitudinal gradients. Are these patterns of enemy hyperspecialization unique to diverse tropical systems or are they a global phenomenon?

The final component of the Eck et al. (4) study was to understand how genotype-specific enemies shape the evolutionary ecology of seed dispersal. Escape from natural enemies near adult plants is considered a key driver of seed dispersal evolution (12). For animal-dispersed fleshy-fruited species such as *V. surinamensis*, the benefits of escape trade off with the costs of attracting dispersers and the costs of lower germination and increased predator and pathogen attack associated when seeds are left undispersed (13). However, the benefit of enemy escape within populations theoretically depends on plant species abundance—dispersed seeds of common species are more likely to fall near a member of their same species and thus benefit less from dispersal. The simulations by Eck et al. (4) show that genotype-specific enemies increase selection for greater dispersal compared with species-specific enemies. This is because (in the case of genotype-specific enemies) any seed moved away from the maternal environment is more likely to land in an area without the enemies to which they are susceptible, regardless of species abundance. For this species of wild nutmeg, the presence of genotype-specific pathogens may favor seeds that are more attractive to seed-dispersing birds (14). Phrased another way, a seedling's susceptibility to its mother's microbes may underlie a toucan's taste for nutmeg.

This study uses a compelling example of the extraordinary biological complexity of tropical ecosystems to expand the scope of theory linking natural enemies to plant diversity. This coincides with the recent shift in plant community ecology away from characterizing each population within a community based on its mean values and toward a recognition that intraspecific variation can be functionally important, impacting higher-level ecological processes and evolutionary dynamics (15). By narrowing in on processes below the species level, this study shows that empirical results and theoretical implications change when the experimental designs and models used in community ecology research are premised on the existence of intraspecific variation. Specific to research on natural enemies and coexistence, this study is indicative of a conceptual shift from seeing the strength of plant–soil feedbacks as a fundamental property of a plant species to a recognition that these patterns are a result of a dynamic web of interactions between plants and their natural enemies that fluctuates over space and time and is shaped by evolutionary processes. Empirical and theoretical work to understand how coexistence emerges from these dynamic interactions will be critical in the continuing quest to understand how so many tree species can coexist.

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