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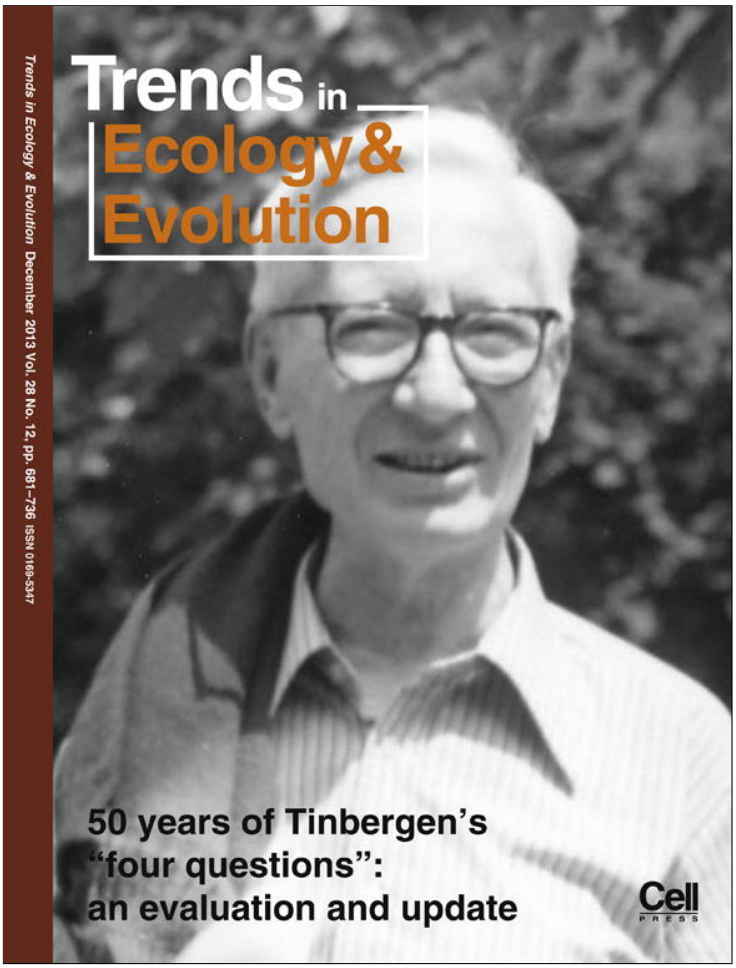

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Opinion

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Pathogen regulation of plant diversity via effective specialization

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The Janzen–Connell (JC) hypothesis, one of the most influential hypotheses explaining forest diversity, is inconsistent with evidence that tree species share the same natural enemies. Through the discussion of seedling diseases from a pathogen-centered perspective, we expand the JC hypothesis to tie in host–pathogen–environment interactions at three levels: local adaptation, host specificity of the combined effect of multiple infections, and environmental modulation of disease. We present evidence from plant pathology, disease ecology, and host–parasite evolution relevant to (but not commonly associated with) forest species diversity maintenance. This expanded view of the JC hypothesis suggests ways to direct new experiments to integrate research on pathogen local adaptation, co-infection, and environmental effects on infection by using high-throughput molecular techniques and statistical models.

Pathogen regulation of plant diversity: current theory

The JC hypothesis [1,2] describes a mechanism by which plant enemies regulate tree community structure and diversity through negative density- and distance-dependent (NDD) regulation. Under JC, natural enemies promote host diversity by preferential attack on juveniles of specific hosts where and when the host is at high density, benefitting nonhost competitors. In the presence of a host-specific enemy for each host species with strong NDD effects [3], no host species can dominate. Local host rarity and limited enemy dispersal provide host individuals with the opportunity to escape enemy attack. The JC hypothesis considers all natural enemies, including bacterial, viral, and fungal and oomycete (fungus-like protists) plant pathogens along with herbivores and seed predators [4–6]. An increasing amount of evidence supporting the JC hypothesis has been described in various communities from both observational and experimental studies, including tropical forests [7–9], temperate forests [10–13], and grasslands [14]. Similar patterns of distance-dependent survival have even been described in spawning corals [15].

Despite ample evidence of NDD in multiple plant communities, the JC hypothesis is conflicting with data that many plant enemies inflict damage to more than one host species [16–18]. This issue becomes confounded by the challenges surrounding the definition of host specialization. Host specialization is often measured as the number of host species used by a parasite or the phylogenetic diversity of its host range [18], and it implies higher parasite performance [19]. The degree of host specialization of a parasite can be considered along a continuum from highly specialized to nonspecialized [19]. Given that the JC hypothesis assumes a unique host-specific enemy for each host species [3,20], emphasis should shift to explaining impacts on host diversity when multiple multihost enemies simultaneously attack individual host plants, and how effective specialization (see Glossary) of multihost plant enemies results in host-specific effects of parasitism. Here, we synthesize evidence of the mechanisms contributing to effective specialization that may provide for the regulation of host diversity by multihost pathogens within the context of JC.

Observations that JC often operates on the juvenile stages of hosts motivate scrutiny of plant enemies contributing to seedling mortality. The biotic agents contributing to plant mortality and their degree of host specialization have been less commonly studied than the JC phenomenon itself. Several potentially important fungal and oomycete forest pathogens have been revealed by recent studies [11,17,20–25]. Studies focused on phylogenetically distant

Glossary

Co-infection: infection of an individual seedling by different pathogens. Infection can be simultaneous or sequential and can be detected in one or more tissue types; it does not imply a particular interaction among co-infecting pathogens.
Disease: malfunctioning of host physiology as a result of infection with a pathogen.
Effective specialization: specialization of the effect of a pathogen observed on a host population, which results from the particular host–pathogen–environment interaction.
Effector: molecules secreted by plant pathogens during the course of infection, which aid in the process of infection and can result in deployment of host resistance.
Local adaptation: adaptation of a population of a particular species to its local environment as compared with other environments.
Pathogen virulence: the degree of damage caused by a plant pathogen to its host, which could result in reduction of host fitness.

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plant hosts have presented an apparent dominance of multihost enemies [21,22,25]. In principle, organisms from multiple taxonomic groups (bacteria, viruses, nematodes, and foliar fungi) could also be important contributors to NDD and JC. However, most published work focuses on belowground pathogens, testing effects of soil sterilization and fumigation [14,26], reciprocal transplants [8,27], and, in a few instances, effect of individual fungal and oomycete pathogens [11,22,23,25]. Thus, we focus our discussion on soil-borne fungal and oomycete pathogens.

Effective specialization as a framework for JC

We argue that multihost pathogens have the capacity to regulate plant diversity through effective specialization. We define 'effective specialization' as the specialization of the effect of a pathogen observed on a host population, which results from the particular host–pathogen–environment interaction. If attack by the same pathogen species results in differences in host survival, then NDD could operate despite the multihost nature of pathogens currently described in JC studies.

Combining pathogen local adaptation and cryptic specialization increases the number of ways in which multihost pathogens might regulate multiple host species. Host adaptation within a local pathogen population can change the virulence of a pathogen population to its host species over time and space [28]. Multiple outcomes of infection with the same pathogen species on different hosts could be expected. Thus, NDD regulation by fungal pathogens could operate at the intraspecific level, with N pathogen populations, as opposed to N pathogen species, regulating N hosts.

The diversity of pathogen species that can be isolated from a symptomatic seedling indicates the potential for interactions among co-infecting pathogens [22]. The impact of a pathogen on a host may depend on its infection status by other pathogens. The assumption that the effects of co-infecting pathogens are additive is not warranted, because pathogens within a host might facilitate, inhibit, or act independently of one another [29–32]. Interactive effects of multiple pathogens could be positive or negative. If a tree seedling could host P different pathogens, we have to consider 2^P possible infection combinations, each with a unique set of interactions. For N host species, the host–pathogen network increases to $N \times 2^P$ [22,33]. Thus, the shift from host specificity of individual pathogens to host specificity of co-infection reduces the total number of pathogens required to achieve N combinations of ways in which species could be regulated in a species-specific fashion. For $N = 100$ hosts, a host-specific effect of co-infection could be achieved by as few as $P = 7$ pathogens, provided that co-infection effects play out differently in different hosts.

Host and pathogen species respond to environmental variation in different ways, hence we expect the outcome of infection to be context dependent [22,34,35]. For example, if both pathogens and their seedling hosts benefit from warm, moist conditions, pathogens can become more efficacious, hosts better defended, or both, hence enabling more unique interactions and opportunities for effective specialization.

To summarize, there are at least three not mutually exclusive mechanisms that result in effective specialization: (hypothesis I) populations of a single pathogen species differ in virulence and adaptation to local hosts; (hypothesis II) co-infection by combinations of pathogens have host-specific effects; and (hypothesis III) interactions between pathogen and host genetics and environmental conditions make pathogen impacts context specific. Concepts related to hypothesis I, here termed the 'local adaptation' hypothesis, have been widely tested in forestry and agriculture, most often for particular host–pathogen combinations [6,28,36,37], although this concept has been examined experimentally in only a few cases within the context of JC [20,25]. Although simultaneous infection by multiple pathogens is widely observed, hypothesis II, here termed the 'co-infection interaction' hypothesis, has, to our knowledge, only been experimentally tested in the context of JC in a single study [22]. In that study, host-specific effects on survival of co-infection combinations were consistent with effective specialization of JC [22]. Although field studies linking co-infection to diversity maintenance in plants are rare [28], several studies [29,30,38] document nonadditive effects of co-infection in plants. Nonadditive effects of co-infection in animal hosts are well established [39,40]. Hypothesis III, here termed the 'environmental modulation' hypothesis, results from the large number of interactions between host, pathogen, and environment. In itself, this concept is not novel, but the novelty arises as we present it here as a mechanism that allows the possibility of multihost pathogens causing NDD effects in plant communities.

To illustrate how each of the hypothesized mechanisms contributes to effective specialization and NDD, we present supporting evidence from plant pathology, disease ecology, and host–parasite evolution that has not been fully integrated into the discussion of plant community diversity regulation.

Janzen–Connell and the local adaptation hypothesis

Under the local adaptation hypothesis, multihost pathogens can regulate tree species diversity, provided that different populations of the same pathogen affect host species differently. A given multihost pathogen varies genetically as a result of interactions with different host individuals.

Pathogen local adaptation occurs when a pathogen population exhibits greater fitness in a local host compared with a nonlocal host [41,42]. Adaptation can be driven by the most abundant host species, individuals of which become reservoirs for the variant population and a source of infection to other conspecific hosts [37]. Hence, as host diversity increases, the opportunity for pathogen adaptation decreases as the host becomes rare, although the relatively long lifespan of many tree species may still provide sufficient opportunity for adaptation. Pathogens have shorter generation times than their hosts, and gene flow is most effective at high host density, therefore contributing to local adaptation [41,42]. Local adaptation is generally measured as a differential of fitness traits on local compared with nonlocal environments [41].

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For plant pathogens, different traits have been used to demonstrate local adaptation to sympatric hosts, including genotype frequency over time [43], gene flow and recombination [44], and pathogen performance [45]. Pathogen performance describes the ability of a pathogen to infect and colonize tissue, increase in biomass and sporulation, and cause disease. The relation between pathogen local adaptation, fitness, and virulence is not clear. Plant pathogen virulence tends to be measured in terms of host fitness, and depends on pathogen performance as well as plant responses to infection [45]. Plant disease and symptom development results from the interaction of pathogen signal molecules (effectors) with plant cells. Host specialization in many plant pathogens often results from the variety and number of effectors expressed by the pathogen genome [46,47]. Barrett and Heil [46] review concepts associated with molecular aspects of host specificity in plant enemies.

Under the local adaptation hypothesis, pathogen-mediated regulation of tree diversity relies on differences in pathogen performance in a locally abundant host. Better pathogen performance could increase pathogen fitness through greater contribution of the pathogen to the total gene pool [43,45]. Pathogen local adaptation could then result in differential expression of host resistance phenotypes upon infection [25]. Plant resistance phenotypes are diverse, and also depend on both the environment and pathogen genetics [36,48,49].

Experimental studies of pathogen local adaptation, including cross-inoculations or transplantation assays, examine adaptation to sympatric versus allopatric host populations, to local populations of different host species, and within tissues of a single host individual [43,44,50]. Most examples of host specialization and local adaptation by multihost pathogens come from agriculture and intensively studied pathosystems in nonmanaged ecosystems, and are reviewed elsewhere [28,36,41,42,45]. Several studies evaluate local adaptation of forest pathogens in the context of JC: Augspurger *et al.* [20] observed differences in host susceptibility and mortality to strains of *Pythium* causing damping-off in tropical forests; Konno *et al.* [25] observed host specialization in strains of *Colletotrichum anthrisci* causing damping-off in Japanese forests; and Liu *et al.* [23] demonstrated that NDD observed for a legume tree was caused by the presence of a host-specific *Fusarium* species. In addition, Gilbert and Webb [17] showed that specialization of multihost foliar pathogens occurs within phylogenetically related host taxa. Considering the fungal and oomycete genera reported in association with forest seedling diseases [11,21–24], we know little of the potential adaptations and/or specializations of these pathogens to their hosts.

Some of the elements required to demonstrate the efficacy of the local adaptation hypothesis as a contributor to JC are in place. Pathogen adaptation to local hosts has been demonstrated by several studies [28,41,42]. That this adaptation might occur most commonly for locally abundant hosts is plausible; however, that this adaptation would necessarily result in lower fitness of abundant host species is less clear. Challenges associated with determining the efficacy of local adaptation as a JC mechanism include the identification of pathogen fitness traits and how those traits change within different host species, understanding how adaptation affects host fitness, and quantifying effects of environmental variables on host–pathogen interactions. Counterexamples where the presence of tolerant hosts can promote highly virulent pathogen populations [51] should also be considered.

Janzen–Connell and the co-infection hypothesis

Molecular tools are expanding the list of fungal taxa that associate with plant hosts [52] and at the same time providing information on individual plants being simultaneously infected by multiple fungi [29,30,46,52]. Hence, the assumption of 'one pathogen per host' could be expanded to 'one-pathogen-combination per host'. In studies that identified potential forest seedling pathogens [22,33], all fungi infected many host species, but each had a different effect on host survival depending on other fungi that were present in the host and their interactions with environmental conditions. The extent of pathogen co-infection and the degree of host adaptation to co-infection remains unknown for most fungal plant pathogens.

Pathogen co-infection has been studied in multiple host–pathogen systems and from different perspectives, including host and pathogen fitness and evolution, host defense responses and signaling, and host symptom development [25,53,54]. In plants, co-infection has been particularly studied for viruses, herbivores, and combinations of different phyla of parasite. For viruses in particular, different *in planta* interactions have been described, including synergism, complementation, and antagonism [29,38]. These result from specific virus–virus interactions or modulation of plant defense responses [19]. Synergism has also been described in nonviral systems; for example, root-knot nematode colonization can alter resistance in chickpea to *Fusarium* wilt development [55].

The outcome of co-infection interaction may also depend on the timing and the extent to which each pathogen colonizes the host. Multiple infections could be tissue specific and occur simultaneously or sequentially. For example, early infection of tomato with Tomato rugose mosaic virus (ToRMV) negatively interferes with systemic infection by Tomato yellow spot virus (ToYSV), whereas in later stages of infection, ToRMV facilitates systemic infection by ToYSV [56]. Alternatively, host-specific co-infection with endophytic or mycorrhizal fungal species could result in host-specific pathogen suppression [57]. Co-infecting pathogen interactions depend on many factors, including host and pathogen genetics, the combination of co-infecting pathogens, and environmental variables, such as temperature and soil moisture [38,58]. Considering a spectrum of interactions between potential pathogens within hosts broadens the mechanisms by which multihost pathogens exert host-specific effects of infection.

In animal disease ecology, the importance of within-host interactions among parasites is also becoming increasingly apparent [40]. The effects of these interactions can be diverse; for example, immune suppression by nematode infection facilitates bovine tuberculosis infection in African buffalo [59], whereas increased trematode diversity in Pacific tree frogs reduced infection success for individual

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parasites [60]. Similarly, analyses of multiparasite communities in rabbits [31] and field voles [32] revealed both positive and negative interactions between co-infecting parasite species. The adaptation and evolution of co-infecting pathogens depends on the outcome of the co-infection interaction, the host defense responses, and the potential for genetic recombination between pathogens [40,54]. Therefore, differences in infection patterns, virulence, and local adaptation could be expected for a particular pathogen as a single or co-infecting pathogen within the same host [54,61]. The consequences of these differences in virulence and adaption of co-infecting pathogens for host survival have direct implications on the JC hypothesis.

Janzen–Connell and the environment-modulation hypothesis

The outcome of a host–pathogen interaction results from a combination of the host and pathogen genotypes as well as environmental conditions [28,36]. The contributions of environmental variables to disease have been intensely quantified in agricultural systems and are used to predict the outcome of an epidemic and as a tool for disease management [62]. The effects of light availability and soil moisture content are commonly quantified in relation to survival and/or disease development in forest trees [22,48,63]. For instance, *Fagus* damping-off was observed to have greater effects on host survival in closed stands compared with gap areas [48], whereas high light resulted in increased disease in a tropical tree [63]. High soil moisture also determines the outcome of co-infection combinations [22].

The host–pathogen–environment effect on disease and host survival can result from different levels of interaction. First, changes in environmental variables can result in differences in host and pathogen physiological status, as well as host and pathogen dispersal and establishment in the field. For instance, host phenology determines which tissues are available for infection, and the resistance phenotype might change through host development [36,45]. From the pathogen perspective, variation in environmental conditions can also trigger changes in developmental stage (e.g., mycelial growth and sporulation) [45,63]. Light intensity has been documented to change the lifestyle from endophytic to pathogenic in *Diploidia mutila* [63].

Within a coevolutionary context, adaptation of both pathogen and host plant appears to be responsive to environmental variation. Environmental variation diversifies host resistance to infection and influences the ability of the pathogen to infect [28,36]. For environmental modulation to contribute to the JC hypothesis, it is important to recognize spatial variation in environmental variables within a forest. Topographic variation has been shown to affect mortality differentially in seedlings across different habitats [64] and, as Burdon and Thrall [65] suggest, environmental variation could result in 'hot and cold spots' of pathogen infection.

Experimental considerations for refining pathogen-mediated JC effects

At least three nonexclusive extensions to the JC hypothesis may contribute to the understanding of pathogen-mediated NDD regulation of plant species diversity. Evidence from other fields suggests how these mechanisms contribute to the JC hypothesis. However, further research is required to test their actual contribution in forests. We describe four considerations when testing and refining pathogen-mediated JC in the field: (i) understanding pathogen life-history strategies contributing to dispersal, infection, and disease; (ii) testing for host specificity; (iii) evaluating pathogen co-infection and co-infection effects; and (iv) considering epidemiological and environmental drivers of infection. Figure 1 illustrates contributions of these aspects to observations of host survival and infection.

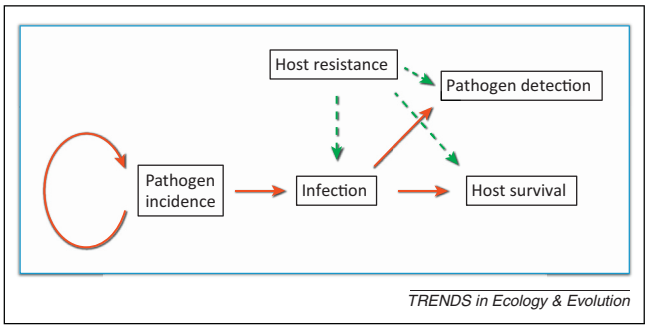

Figure 1. Pathogen–host–environment contributions to host survival and pathogen detection. Graphical representation of an 'extension' of a 'co-infection of multiple hosts by multiple pathogens' modified from [33]. The figure incorporates effects from pathogen × host × environment interactions. Red and green arrows represent pathogen and host biology characteristics, respectively, that contribute to infection, disease development, and plant host survival. Pathogen biology, including aspects of pathogen dispersal and overwintering capacity, will determine pathogen spatial and temporal distribution in relation to the host. Initial pathogen infection results from pathogen–host recognition and attachment. Pathogen colonization and establishment in the plant host is determined by the expression of pathogen effectors and host resistance mechanisms. Pathogen infection could be detected by screening of individual isolations from symptomatic tissue, molecular analyses of pathogen communities in infected plant tissue, and/or or host symptom development and severity in infected seedlings. The outcomes of the host–pathogen interaction are determined by the degree of host specialization of the host and interactions with the environment. Differences in host survival are used to predict pathogen-mediated effects on plant community dynamics. Interactions across pathogen, host, and environmental conditions provide information on the mechanisms contributing to the differences in host survival.

From the pathogen perspective, there is a need for understanding the etiology and natural history of forest seedling diseases. The probability of seedling survival can be inferred from the observation of infection or co-infection for a particular host species [22,33]. Infection is possible when an infective pathogen propagule is present; however, disease is a function of pathogen infection ability and host responses to infection (Figure 1). Pathogen incidence depends on pathogen dispersal and overwintering capacity. Certain pathogens could initially establish as endophytes or cause latent infections, and the pathogenic life style could be triggered by environmental cues [63,66]. Measures of primary inoculum and pathogen latent infection period provide information about the temporal and spatial distribution of the pathogen in relation to the host. Host symptom development, pathogen sporulation on hosts, and disease progress add to analyses of host–pathogen adaptation and fitness. In the same manner as these variables are measured to predict the course of an epidemic and evaluate disease control measures [62,67], they can contribute to the understanding and prediction of pathogen-mediated JC processes.

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Methods to assess quantitatively the effect of single or combined pathogen infection in various hosts should be incorporated in studies of NDD. Host specialization is a continuum that could be measured at structural, individual, and geographical levels [19,46]. Host genetics, physiology, and phenology [28,36,45] also contribute to pathogen specialization and local adaptation. Host specialization can be quantified through the analysis of pathogen genotype diversity, where host-specialized pathogen genotypes increase the frequency of infection of a particular host at a greater rate than do nonspecialized genotypes [43]. Studies of the diversity of pathogen effectors and differential effector expression *in planta* can also provide quantitative evidence of pathogen host specialization [46]. From the host perspective, host specialization could be analyzed based on the expression of host resistance traits, as well as the severity and rate of symptom development during infection by different multihost pathogens [25,43]. High-throughput sequencing methodologies could contribute to addressing these questions by generating genetic, genomic, and phylogenetic information at the individual, population, and community level.

An alternative to host specialization of individual pathogens is host specialization arising from pathogen co-infection. The specificity of co-occurrence of infecting pathogen taxa, as well as patterns of spatial and temporal distribution of co-infection in the field, can be evaluated through correlation [60], network analyses [68], or hierarchical modeling of observational and experimental data [22], including analyses of pathogens identified from infected tissue or nucleic acid-based community analyses via high-throughput sequencing of pathogen-related markers. However, the relevance of co-infection and the outcome of mixed infections for a particular host should be further investigated through laboratory manipulations [60]. Quantitative molecular methods can also provide insight into the relative amount of infection of each co-infecting pathogen at the individual plant or tissue level. A potential challenge in assessing effects of co-infection is the diversity of the nontarget microbial community (microbiome) associated with the plant host. Nonpathogenic endophytes, mycorrhizae, bacteria, or virus populations all contribute to plant health and trigger host defense responses, potentially confounding fungal pathogen effects [57,66,69].

Quantification of the local adaptation and co-infection hypotheses cannot ignore environmental heterogeneity. The concept of triangulation, where information of a pathosystem is gathered from field and laboratory observations, historical records, and model predictions, is especially relevant for the dynamics of complex system [67]. Field and laboratory experiments should consider inclusion of different levels of environmental variable into their design. The complexity of these interactions places the epidemiology of JC within the realm of ecological genomics, high-throughput sequencing, and bioinformatics. Although the inclusion of different interacting variables scales up the design of experiments to be assessed, the mentioned molecular techniques enable the processing of diverse and numerous sets of samples and the generation of molecular data with strong statistical power. Bioinformatics tools continue to improve our ability to infer the role of interactions in high-dimensional networks [70]. This extended JC hypothesis brings all the challenges of inference where not only are the strengths of the relationships unknown, but also the topology is uncertain. There are many host–pathogen–environment interactions, most of which vary in strength and sign in space and time. At least one effort to confront the co-infection challenge of JC reduced the complexity of $>10^3$ co-infection combinations down to a handful of combinations that had important effects on host survival [22,33]. This approach exploited the advantages of hierarchical modeling and reversible jump Markov Chain Monte Carlo algorithms to select combinations with important effects, confronting the large variable selection challenge. The high dimensionality of the hypothesis described here may require continued innovations that can provide insight into which of the many potential pathogen effects are most important in a range of settings.

Concluding remarks

Although the JC hypothesis requires host specificity, that same degree of host specificity could be achieved through this revised version of JC that incorporates the efficacy of multihost pathogens. Here, we have presented aspects of the complexity of plant diseases and host–pathogen–environment interactions that are important for understanding pathogen regulation of plant species diversity. Some of the concepts and literature presented, such as co-infection effects, have not been widely considered in JC-type studies, despite their relevance to forest diversity. The challenge is to link successfully aspects of pathogen biology and host–pathogen–environment interactions with the ecology of forest community dynamics. A combination of state-of-the-art molecular tools and statistical models is required to approach the scale of complexity of these questions. Given that these tools are currently available, we can begin to answer questions related to the mechanisms that drive the widely described NDD phenomenon. Confronting the complexity of this system, from multihost pathogens to co-infection, will help the field continue to progress from attributing plant mortality to a black box of pathogens to a deeper understanding of the ecology and biology of the interactions between pathogens, hosts, and environment, and the contribution of these to the maintenance of diversity.

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