Individuals and the Variation Needed for High Species Diversity in Forest Trees

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hypothesis that upon rhizobial infection, the plant machinery for processing secretory proteins is mobilized coordinately (Fig. 5E).

In *dnf1* mutants, bacterial release into the host cell is apparently normal, but the subsequent differentiation of the bacteria is blocked. Such a phenotype suggests that among the substrates of the DNF1 complex are important host determinants of symbiosome development, such as the processed NCR peptides described by Van de Velde *et al*. (*11*). The NCR proteins are found only in legumes such as *Medicago* spp. that subject their microbial partners to terminal differentiation; however, it is possible that the DNF1 apparatus is present even in legume species that lack NCR type proteins, such as *Lotus* or bean (*18*). If so, it will be interesting to test whether disrupting function of a DNF1 homolog affects symbiosome function in these legumes. Such a result would imply that there are other substrates for the DNF1 SPC, a possibility that can be empirically tested. Potential substrates include the A1b/leginsulin family proteins ENOD8, ENOD16, and nodulin-25, all of which are proteins with a signal peptide, are up-regulated during nodulation, and in some cases are shown to localize to the symbiosome (*13*, *20*, *21*). Some of these proteins are conserved in *Lotus japonicus* and soybean, suggesting that they may be processed by a common mechanism in a variety of legume species. Alternatively, the *DNF1* and coexpressed signal peptidase genes may have coevolved with the *NCR* genes within the clade of legumes that show terminal bacteroid differentiation as a specialized means to control bacterial proliferation and function within the host cells.

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Individuals and the Variation Needed for High Species Diversity in Forest Trees

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In the past, explanations for high species diversity have been sought at the species level. Theory shows that coexistence requires substantial differences between species, but species-level data rarely provide evidence for such differences. Using data from forests in the southeastern United States, I show here that variation evident at the individual level provides for coexistence of large numbers of competitors. Variation among individuals within populations allows species to differ in their distributions of responses to the environment, despite the fact that the populations to which they belong do not differ, on average. Results are consistent with theory predicting that coexistence depends on competition being stronger within than between species, shown here by analysis of individual-level responses to environmental fluctuation.

The paradox of low diversity predicted by theory and the high diversity in nature has been recognized for a half-century (*1*). Forest trees compete intensely for a small number of resources, including light, water, and several nutrients (*2*, *3*). Models of competition for few resources predict low diversity and precise parameter trade-offs that limit the strength of competition among those species that do coexist. It has become increasingly apparent that mean demographic rates or responses to resources in the few dimensions that can be measured are not significantly different among many species (*4*–*7*). The weak trends in data are inconsistent with the precise parameter relations and trade-offs needed to explain coexistence in models; yet there is abundant evidence for long-term coexistence of competitors in nature and rapid return to previous densities after disturbance (*8*). In summary, there are apparently few dimensions along which species can partition the resources for which they compete intensively. Evidence is lacking for the species differences required for coexistence, but there is pervasive evidence for long-term persistence of competitors at high diversity.

Recent demonstration that variation within tree species exceeds the differences in species-level averages (*4*–*6*) motivated the present analysis of how variation in many dimensions might provide the explanation for high biodiversity, made possible by extensive data and alternative methods for analysis. Six to 18 years of annual, individual-level demographic estimates on 11 forests in three regions in the southeastern U.S. include 33 tree species, >22,000 trees, and >226,000 tree years (*9*, *10*). The data have sufficient detail to resolve responses to environmental fluctuations at high frequency (annual or less) and sufficient duration to permit inference at the individual level. This combination of annual resolution and long duration is not available from previous studies, which are either short- (a few years or less) or long-term, but with 5- to 10-year resolution. A hierarchical Bayesian analysis quantifies the structure of variation among individuals within populations and over time. From observations on tree diameter, canopy status, reproductive status, survival, remote sensing of canopy stature, and seed rain, we inferred annual demographic estimates of growth, fecundity, and survival risk for every individual in 11 forest stands (*9*, *10*). The structure in these estimates of demographic rates can be used to evaluate how species compare at the level of individual responses to environmental variation.

The analysis of individual variation expands on two relations demonstrated from theory and simulation. First, analytical models have long shown that coexistence is promoted when intraspecific competition is stronger than interspecific

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competition (*11–13*). Simulation studies confirm low diversity of competitors and the prediction that coexistence depends on resource partitioning (*13–16*). Such criteria for coexistence can be met by trade-offs among species, such as negative correlations in consumption of different resources, colonization versus competitive ability, and low-light survival versus high-light growth (*2*). The problem is that most studies do not find such trade-offs. Although trends in data are sometimes consistent with the assumption of trade-offs for a few species within some communities, we still require an explanation for the persistence of large numbers of competing species that do not possess such trade-offs. None of the trade-offs traditionally postulated for trees is evident in the extensive, long-term data sets analyzed here (*4*, *9*).

Second, coexistence is promoted when species differ in how they respond to fluctuations (*17*). Negative correlation between species in response to environmental fluctuation can promote coexistence (*5*, *18*, *19*). For example, a trade-off between growth potential and low-resource tolerance may allow desert annual species to partition variation, with different species benefiting in different years (*20*). However, many competitors do not show such temporal partitioning. *Acer rubrum* and *Nyssa sylvatica*, examples from this analysis, illustrate a general pattern. These species have coexisted in shaded understories throughout the eastern U.S. at least since the early Holocene, but their growth responses are positively correlated across years (Fig. 1A). The responses broadly overlap, with no indication that one experiences high growth when the other does not; at the species level, there is no evidence of partitioning environmental fluctuations in the form of negative correlation. The positive correlation is not surprising for species limited, on average, by the same few resources. This is one example of a large number of species that show no tendency to trade off (*9*). Thus, the high diversity of southeastern U.S. forests analyzed in this study is not consistent with the prediction that species are negatively correlated in their responses, at least when applied at the species level.

Despite small differences in species means, there is substantial variation within species, and the structure of variation shows that species partition environmental variation in higher dimensions. This can be the case if competition between individuals of the same species is stronger than with individuals of other species, even if there is no apparent trade-off in species mean traits in a few dimensions and also if there is positive correlation in species means over time. To illustrate this concept, I now shift focus from species to individuals. Consider individual variation in a demographic rate, such as growth or fecundity. Individuals responding to environmental variation in similar ways show positive correlation and vice versa (Fig. 1B). Clearly, the negative correlation between individuals shown in the lower-left panel of Fig. 1B is evidence that individuals are responding differently. In the lower-right panel of the same figure, they are responding similarly. However, to promote coexistence, the question is not whether correlations are negative, but rather if correlations are lower when comparing different species. The latter indicates that intraspecific competition will be stronger than interspecific competition. For n_A and n_B individuals of two species (denoted by A and B, respectively), there will be $n_A n_B$ interspecific and $\frac{1}{2}[n_A(n_A - 1) + n_B(n_B - 1)]$ intraspecific comparisons. For the example in Fig. 1B, histograms of comparisons between individuals of the same species in the same neighborhoods tend toward positive correlation, reflecting similar resource requirements and physiological constraints as the environment varies over time and among sites. If individual-level variation promotes coexistence, correlations involving individuals of different species (*Acer:Nyssa*) are lower than those between individuals of the same species (*Acer:Acer* and *Nyssa:Nyssa*). In more general terms, the strength of competition experienced by an individual i from a neighbor of its own species A exceeds that from a neighbor of species B if $R_{iA} > R_{iB}$, where R_{iA} and R_{iB} are the correlations between individual i and others of species A and i and others of species B, respectively (*10*). The more similarity existing between individual i and its own species A (large R_{iA}), the more likely it is to coexist with species B, because it experiences stronger competition when its own species A is abundant. Competition can be stronger within than between species, even where correlations between species mean responses are positive (Fig. 1A). This basic relation can be extended to spatial neighborhoods of multiple individuals $\mathbf{S}_{iA}\mathbf{S}_{AA}^{-1}\mathbf{S}_A > \mathbf{S}_{iB}\mathbf{S}_{BB}^{-1}\mathbf{S}_B$, where $\mathbf{S}_{iA}$ and $\mathbf{S}_{iB}$ are the vectors of covariances between growth of individual i and those of n_A and n_B competitors of the two species, $\mathbf{S}_{AA}$ and $\mathbf{S}_{BB}$ are the growth covariance matrices for A and B, and $\mathbf{S}_A$ and $\mathbf{S}_B$ are the vectors of growth standard deviations for neighbors of A and B (*10*).

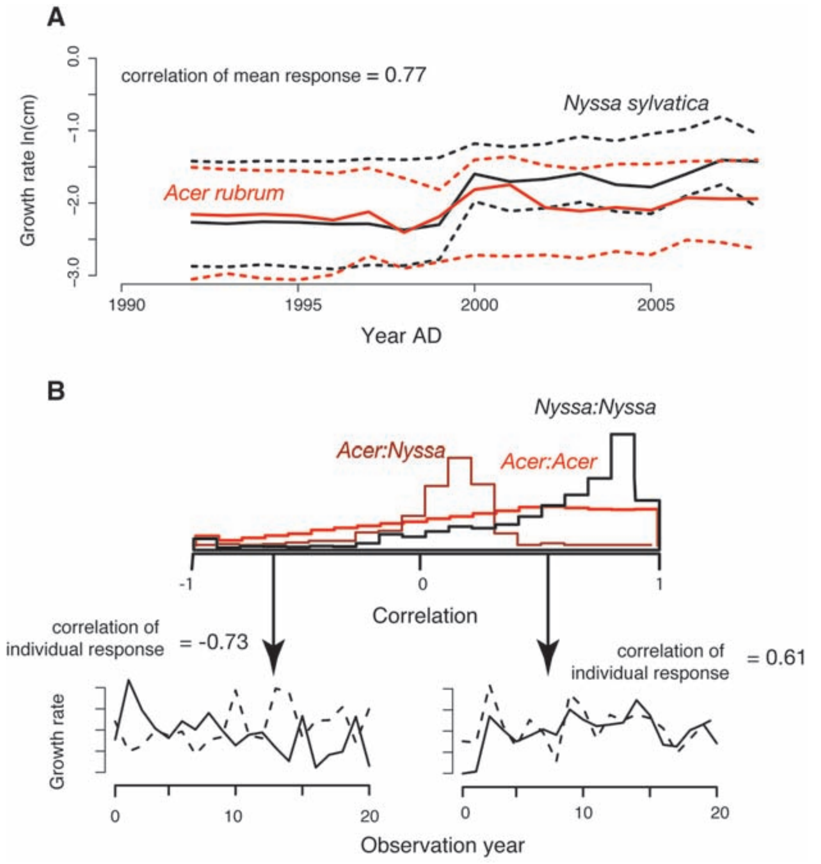

Fig. 1. (**A**) At the species level, growth responses over time overlap broadly [included are median (solid lines) and 95% variation (dashed lines) among individuals], as shown for the example of *A. rubrum* and *N. sylvatica*. The correlation between mean species response is high (0.77). (**B**) Frequency distribution of correlations for intra- (*Nyssa:Nyssa*, *Acer:Acer*) and interspecific (*Acer:Nyssa*) comparisons of growth rate for individuals that occur in the same neighborhoods, defined as 20 m in radius. Although the correlation between species means is high (A), the mean correlations for interspecific comparisons is lower than is the mean for intraspecific comparisons. The lower panels in (B) show examples of low (left) and high (right) correlation between the growth of two individuals.

To determine if the relations between individuals of different species contribute to coexistence, I evaluated correlation and covariance structure for all individuals of all 33 co-occurring species in the 11 forest stands. These species are light-limited in shaded understories and occur on plots that experience annual moisture deficits:

They compete both above- and belowground. Individual-level estimates of year-to-year growth and fecundity are available for each tree. The correlation and covariance was determined for every pair of individuals occurring in local neighborhoods of radii 20 m for growth and 60 m for fecundity. These distances represent approximate interaction neighborhoods for growth, which determine resource capture through their effect on tree size, and dispersal, which determines capture of recruitment sites. The qualitative results reported here also hold for much larger neighborhoods.

The analysis confirms that individual responses contribute the species differences that are lacking in species-level comparisons. The vertical axis in Fig. 2 shows the fraction of species-level comparisons in which mean correlations (solid lines) and mean growth or fecundity deviations (dashed lines) were greater when comparing individuals of the same versus different species. Correlations between individuals of different species were lower than those for the same species, for both growth (Fig. 2A) and fecundity (Fig. 2B). As the environment fluctuates, individuals respond in different ways, depending on fine-scale variation in resources such as moisture, nutrients, and light and their genotypic differences. Because individuals are responding in many dimensions, but with more similarity to individuals of the same species than to individuals of different species, intraspecific comparisons have higher correlation than interspecific comparisons. More similar responses translate to stronger competition: Growth influences long-term competitive ability by changing size (and, thus, resource capture), and fecundity determines capture of new recruitment sites.

Although trade-offs evident at the species level do not explain coexistence, species partition the environment without showing species-level correlations. Responses can differ between species, even if species mean responses in a few observable dimensions do not differ (Fig. 1A). If individuals of each species exploit variation in many dimensions in different ways, then species-level analysis misses the factors responsible for coexistence. The individual level dominates species interactions, because the few environmental axes that are measured account for only a small fraction of the variation between species (*4*). For example, two species may be limited by moisture, but they differ in their capacity to exploit high moisture versus tolerate extended drought (*21*). Species-level comparisons can show both species increasing in wet years (positive correlation), whereas each is actually competing more with individuals of its own species, depending on moisture variation in space and time. On average, both species benefit in wet years, but it is not the same individuals that are benefiting and not to the same degree on sites with differing moisture

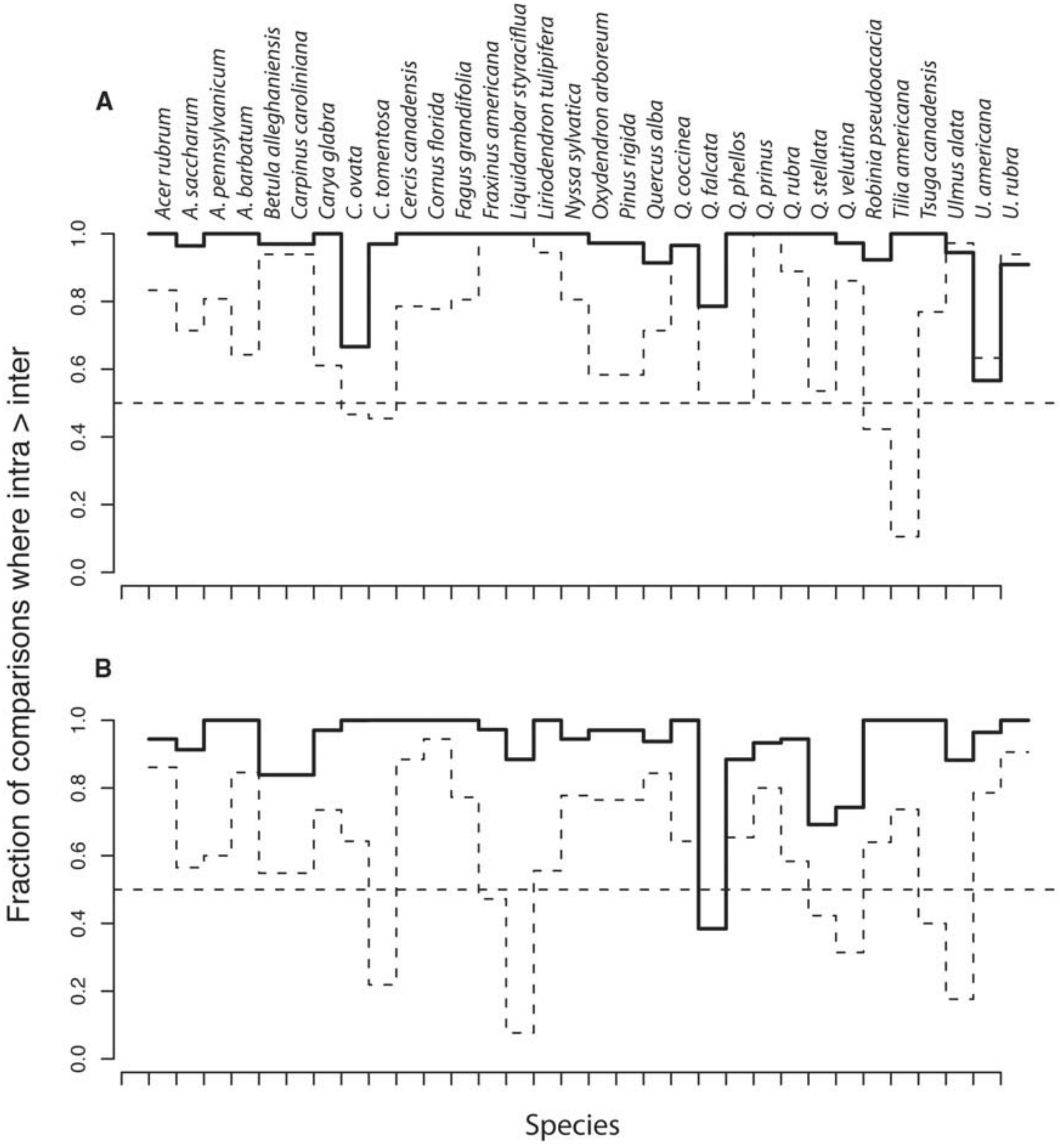

Fig. 2. Fractions of comparisons consistent with promoting coexistence at the individual level, including correlations ($R_{iA} > R_{iB}$) (solid lines) and conditional responses ($\mathbf{S}_{iA}\mathbf{S}_{AA}^{-1}\mathbf{S}_{A} > \mathbf{S}_{iB}\mathbf{S}_{BB}^{-1}\mathbf{S}_{B}$) (dashed lines). For both growth rate (**A**) and fecundity (**B**), the majority of values greater than 0.5 indicates that individuals respond more like individuals of their own species, thus focusing intraspecific competition in space and time. Interspecific correlations are weaker, thus providing opportunity for partitioning the environment.

status. Variation within populations, be it genetic or not, can differ between species in many dimensions that are not documented in species-level data. Species-level analyses fail to capture variation that contributes to diversity if variation among individuals in many dimensions is species-specific. This analysis shows that the massive variation within populations documented in previous studies (*4*–*6*) is structured in such a way that it contributes to coexistence.

The explanation that diversity depends on individual variation is consistent with the lack of evidence that individuals recognize the species identities of their competitors. Most competition theory is based on interaction coefficients specific to each species pair, often termed the "community matrix." This concept has prompted substantial study to quantify interaction strength between species pairs. Neutral theory (*22*, *23*) and the belief that all species are functionally equivalent was partly motivated by the implausibility that plant competition operates differently for each species pair. The individual-level maintenance of biodiversity shown here involves only differences in the ranges of response to the environment and the tendency to respond like conspecifics, and not species-by-species recognition. Those differences translate into differences in individual growth, which determines resource capture, and fecundity, which determines capture of new sites. In contrast to neutral theory, which posits no difference, high diversity is possible because species differ in so many ways. The mechanism demonstrated here is also more general than a rare-species advantage, which invokes host-specific natural enemies (one to control each species when it becomes abundant) and disappearance of the effect when the host becomes rare (*24*, *25*), or *N* natural enemies to explain coexistence of *N* hosts. The tendency to respond like other individuals of the same species can promote coexistence independent of local frequency or density and does not require a large number of host-specific enemies.

The biodiversity paradox of many coexisting competitors in which the number of limiting resources seems low can be resolved at the individual level. Just as variation among individuals is required to maintain species by natural selection, providing a means for adaptive evolution in response to many factors in many dimensions, variation at the individual scale is also needed to explain why large numbers of intensely competing species coexist. Individual-level variation need not be genetic (although genotypic variation can be large), but species do need to differ in how individuals respond in many dimensions. In the absence of precise information on the many dimensions in which species differ, individual-level data provide evidence for species differences.

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Generating a Prion with Bacterially Expressed Recombinant Prion Protein

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The prion hypothesis posits that a misfolded form of prion protein (PrP) is responsible for the infectivity of prion disease. Using recombinant murine PrP purified from *Escherichia coli*, we created a recombinant prion with the attributes of the pathogenic PrP isoform: aggregated, protease-resistant, and self-perpetuating. After intracerebral injection of the recombinant prion, wild-type mice developed neurological signs in ~130 days and reached the terminal stage of disease in ~150 days. Characterization of diseased mice revealed classic neuropathology of prion disease, the presence of protease-resistant PrP, and the capability of serially transmitting the disease; these findings confirmed that the mice succumbed to prion disease. Thus, as postulated by the prion hypothesis, the infectivity in mammalian prion disease results from an altered conformation of PrP.

Transmissible spongiform encephalopathies (TSEs or prion disease) are infectious neurodegenerative disorders. The prion hypothesis (*1*) proposes that the infectious agent is an aberrant conformational isoform of the normal PrP ($\mathrm{PrP^{C}}$), a glycosylphosphatidylinositol (GPI)–anchored glycoprotein. By virtue of its self-perpetuating characteristic, the aberrant isoform ($\mathrm{PrP^{Sc}}$) converts host $\mathrm{PrP^{C}}$ into the $\mathrm{PrP^{Sc}}$ conformation and leads to neurodegeneration (*2*–*4*). Despite strong supporting evidence (*5*–*11*), a crucial prediction derived from the prion hypothesis—that an infectious prion can be generated with bacterially expressed recombinant PrP (recPrP)—remains unfulfilled (*2*, *12*), leaving lingering doubts about the prion hypothesis (*13*).

Recombinant PrP has been folded into various forms similar to $\mathrm{PrP^{Sc}}$, but none of them fully recapitulates the characteristics of the infectious agent (*2*, *12*). The amyloid fiber of a recPrP fragment (recPrP89-230) causes prion disease in transgenic mice overexpressing PrP89-231 (*10*), but a prolonged incubation time in mice overexpressing PrP has led to uncertainty about whether the infectivity is indeed derived from recPrP89-230 amyloid fibers (*2*, *12*). The difficulty in creating a recombinant prion is likely due to the lack of proper facilitating factors (*14*). Polyanions, particularly RNA, have been found to facilitate PrP conversion and promote de novo prion formation (*9*, *15*–*17*). We investigated lipid as a potential facilitating factor because GPI-anchored $\mathrm{PrP^{C}}$ is in the vicinity of lipid membranes and the interfacial lipid bilayer region strongly influences protein structure (*18*). Encouraged by the findings that lipid interaction converts recPrP to a $\mathrm{PrP^{Sc}}$-like form (*19*), we applied protein misfolding cyclic amplification (PMCA) (*8*) to study recPrP

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