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Why Are There So Many Species of Herbivorous Insects in Tropical Rainforests?

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Despite recent progress in understanding mechanisms of tree species coexistence in tropical forests, a simple explanation for the even more extensive diversity of insects feeding on these plants has been missing. We compared folivorous insects from temperate and tropical trees to test the hypothesis that herbivore species coexistence in more diverse communities could reflect narrow host specificity relative to less diverse communities. Temperate and tropical tree species of comparable phylogenetic distribution supported similar numbers of folivorous insect species, 29.0 ± 2.2 and 23.5 ± 1.8 per 100 square meters of foliage, respectively. Host specificity did not differ significantly between community samples, indicating that food resources are not more finely partitioned among folivorous insects in tropical than in temperate forests. These findings suggest that the latitudinal gradient in insect species richness could be a direct function of plant diversity, which increased sevenfold from our temperate to tropical study sites.

Large numbers of herbivore species in the Tropics relative to temperate communities might reflect differences in (i) host plant species diversity, (ii) numbers of herbivore species per host, and/or (iii) host specificity, the number of plant species hosting each insect species. The tropical maximum in plant species richness is well documented. For instance, there are 5 to 10 times as many plant species per 10,000 km^2 in tropical than in temperate areas (*1*), and woody plant species richness per hectare in the Tropics is on average six times as high as that in temperate forests (156.8 ± 63.6 and 25.2 ± 19.7 species with diameter at breast height ≥10 cm; fig. S1). However, latitudinal differences in host specificity and numbers of insect species per host plant species are more difficult to assess (*2*, *3*).

A recent proliferation of quantitative studies on tropical insect herbivores that include feeding and rearing experiments (*4*–*9*) have not been matched by comparable activity in temperate forests (*10*, *11*), perhaps because patterns of host use are believed to be well documented for temperate herbivores. Much qualitative data on host associations of herbivores accumulated during the past two centuries, particularly in Great Britain and Central Europe, are not directly comparable to recent, quantitative studies in the Tropics (*12*). A temperate-tropical comparison of herbivore communities is further complicated by differences in the phylogenetic diversity of the vegetation. Temperate forests are dominated by a relatively small number of woody plant lineages as compared to tropical forests (*13*).

We compared temperate and tropical communities of folivorous insects using identical sampling protocols and phylogenetically comparable sets of local tree species (*14*). All externally feeding folivorous insects were hand collected from the foliage of 14 woody plant species in a lowland floodplain forest in Moravia, Central Europe, and 14 species in a lowland hill forest in Madang, Papua New Guinea. Caterpillars (Lepidoptera) were also collected from eight woody species in an oak-hornbeam forest in Slovakia, Central Europe, and compared with caterpillars from eight tree species in Papua New Guinea (Madang). Samples of tree species from the local vegetation included both close relatives (i.e., congeneric species) and distantly related plant lineages (i.e., multiple familes and orders) at each site (table S1). Molecular phylogenetic relationships among species sampled at each locality were compiled from the recent literature, and branch lengths were estimated from the large subunit of ribulose-1,5-bisphosphate carboxylase-oxygenase (rbcL) gene sequences. The diverse vegetation of lowland New Guinea provided an opportunity to select subsets of tree species with phylogenetic patterns closely matching those of temperate forest tree

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communities (Fig. 1). Highly concordant and correlated branch lengths permitted the comparison of host specificity and herbivore community structure given a nearly identical phylogenetic distribution of food plants. Controlling for the effect of vegetation on phylogenetic diversity enabled a direct comparison of herbivore specificity between these different tropical and temperate communities.

Adult herbivores were experimentally tested for feeding, and larvae were reared to adults. Our analysis included 26,970 feeding records of herbivorous insects representing 850 species (appendices S1 and S2). Folivorous communities included larval and adult feeders of Lepidoptera, Coleoptera, Hymenoptera, and orthopteroids (Orthoptera and Phasmatodea). Larval Lepidoptera dominated both temperate and tropical communities, followed by adult Coleoptera, whereas larval Coleoptera were of marginal importance (Fig. 2).

Although Hymenoptera were limited to temperate samples and orthopteroids were only encountered in the Tropics, tree species in both regions supported similar overall species diversity of leaf-chewing insect species per unit area of foliage (Table 1). The occurrence of more speciose assemblages of insect herbivores in tropical forests as compared to temperate forests therefore cannot be attributed to finer partitioning of foliar resources among herbivore species feeding on the same plant species. Comparable overall species diversity of herbivores resulted from opposing trends in species diversity of larval and adult folivores, being maximally diverse in Central Europe and New Guinea, respectively. Despite considerable differences in the taxonomic composition of

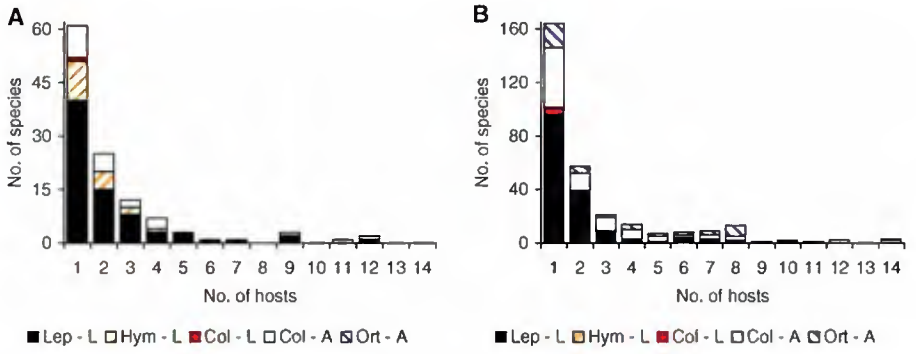

Fig. 2. Host specificity of folivorous insects on (**A**) temperate and (**B**) tropical trees. The number of hosts among the 14 studied tree species (Fig. 1A) is shown for larvae (L) and adults (A) from Lepidoptera, Hymenoptera, Coleoptera, and Orthopteroids. The number of hosts was not significantly different between temperate and tropical folivores, Lepidoptera larvae, and Coleoptera adults (Mann-Whitney test, $P > 0.05$).

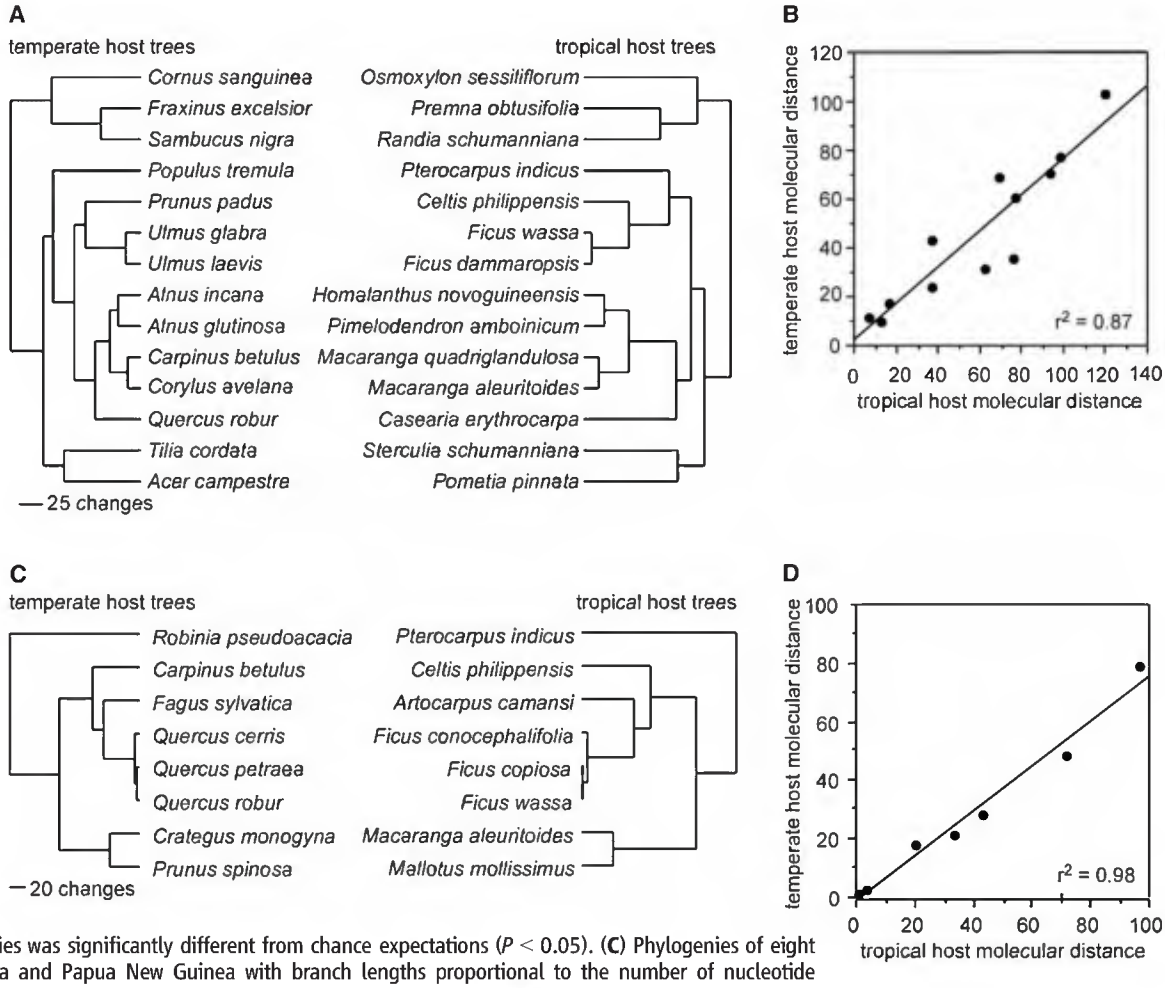

Fig. 1. Phylogenetic relationships and molecular divergence of temperate and tropical trees selected for the comparison of insect herbivore communities. Temperate and tropical plant species spanning the continuum between close relatives and distantly related lineages were paired to control for differences between communities in the phylogenetic distribution of plant resources. Branching order and branch lengths were matched as closely as possible between the temperate and tropical sets of tree species from different clades. (**A**) Phylogenies of 14 tree species from Moravia and Papua New Guinea with branch lengths proportional to the number of nucleotide substitutions in rbcL sequences. (**B**) The correlation of molecular phylogenetic distances between ancestral and descendant nodes for 14 pairs of temperate and tropical tree species was significantly different from chance expectations ($P < 0.05$). (**C**) Phylogenies of eight tree species from Slovakia and Papua New Guinea with branch lengths proportional to the number of nucleotide substitutions in rbcL. (**D**) The correlation of molecular phylogenetic distances between ancestral and descendant nodes for eight pairs of temperate and tropical tree species ($P < 0.001$).

Table 1. Numbers of insect species and individuals per unit area of foliage in larval and adult taxon guilds of folivorous insects reared from temperate and tropical trees. The mean (±SE) values for the density of species and individuals were calculated for insect herbivores from *N* species of study trees. Rows 1 to 8 refer to the Moravia–New Guinea comparison, row 9 to the Slovakia–New Guinea comparison. The temperate-tropical differences were evaluated by *t* test (*$P < 0.05$). Comparative analyses accounting for the statistical nonindependence of tree species yielded results identical to those obtained with *t* tests (supporting online text). N.S., not significant.

No.	Taxon	Guild	Species/100m² foliage		*t* test	Individuals/100m² foliage		*t* test	*N*
			Temperate	Tropical		Temperate	Tropical		
1	Lepidoptera	Larvae	19.9 (1.9)	9.1 (1.3)	*	66.6 (13.5)	36.7 (5.9)	N.S.	14
2	Hymenoptera	Larvae	1.8 (0.4)	0 (0)	*	3.8 (1.1)	0.0 (0.0)	*	14
3	Coleoptera	Larvae	0.1 (0.1)	0.3 (0.1)	N.S.	6.4 (6.3)	2.7 (2.7)	N.S.	14
4	Coleoptera	Adults	5.5 (0.7)	9.5 (0.7)	*	42.9 (12.7)	24.4 (2.6)	N.S.	14
5	Orthopteroids	Adults	0.0 (0.0)	4.2 (0.4)	*	0.0 (0.0)	7.5 (1.3)	*	14
6	All	Larvae	23.5 (2.1)	9.4 (1.3)	*	84.4 (13.4)	40.4 (6.1)	*	14
7	All	Adults	5.5 (0.7)	13.7 (0.9)	*	42.9 (12.7)	31.9 (3.1)	N.S.	14
8	All	All	29.0 (2.2)	23.5 (1.8)	N.S.	127.2 (15.6)	75.2 (5.8)	*	14
9	Lepidoptera	Larvae	19.3 (3.6)	9.2 (2.0)	*	82.5 (18.0)	45.5 (5.9)	N.S.	8

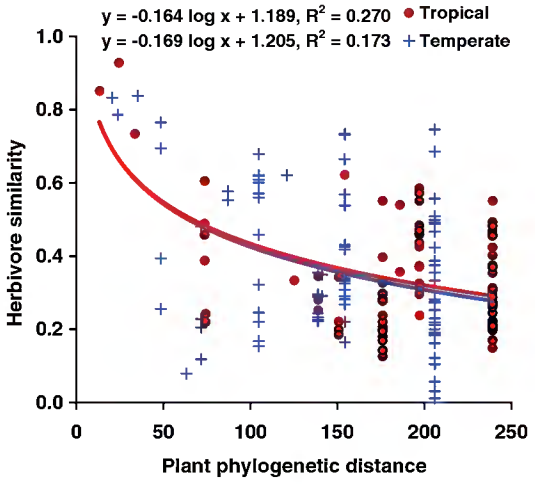

Fig. 3. Similarity of folivorous communities between pairs of host species versus the phylogenetic distance between the hosts in a temperate (crosses) and a tropical (circles) forest. Herbivore similarity was estimated as the proportion of shared species according to the Chao-Sorensen index (*27*); phylogenetic distance was estimated from Fig. 1A as the absolute number of pairwise differences in rbcL sequences from trees listed in table S1. The negative correlation between community similarity and phylogenetic distance was significant in both data sets ($P < 0.05$, Mantel test).

tropical and temperate communities, overall estimates of herbivore species diversity per host plant are of similar magnitude in tropical forests (*4*–*7*, *15*) and temperate forests (*10*, *16*). The absence of a latitudinal trend in the ratio of butterfly to plant species is also consistent with this observation (*17*).

Temperate trees supported a higher overall density of folivores than did tropical trees (Table 1). Lepidoptera and Coleoptera larval densities tended to be higher on temperate trees, but only the density of Hymenoptera larvae was significantly different. The relatively low abundance of larvae on tropical foliage is attributed to high predation, particularly by ants, in the Tropics (*18*, *19*). Predation pressure on tropical trees at our study sites was 18 times as high as that on temperate trees, as measured by the proportion of live insect baits attacked by predators (mostly ants) during 30 min of exposure on the foliage (28 ± 27% in the Tropics and 1.6 ± 0.1% on temperate vegetation; table S2).

The two most important taxon guilds in terms of species numbers and abundance, namely Lepidoptera larvae and Coleoptera adults, as well as the entire folivorous community, showed no difference in host specificity between temperate and tropical trees (Fig. 2). Lepidoptera larvae on temperate trees in Slovakia were less host-specific than were those on the tropical trees, but the mean difference in host range was small (fig. S2), averaging a single host per herbivore in tropical samples versus two hosts per herbivore in temperate samples. The similarity of folivorous communities on any pair of hosts decreased as the phylogenetic distance of hosts increased. The slope of the relation was not significantly different between temperate and tropical tree species, also suggesting a common pattern of host specificity (Fig. 3).

Our findings reject the hypothesis that greater host specificity of tropical herbivores accounts for the greater insect species diversity. Other studies also suggest that there is no difference in host specificity between temperate and tropical communities of insect herbivores. Fiedler (*20*) found no such difference in butterflies, although particular lineages may be more (e.g., Lycaenidae: Polyommatini) or less (e.g., Papilionidae) (*21*) specialized in temperate than in tropical regions. Bark beetles (Coleoptera: Curculionidae) (*22*) and treehoppers (Hemiptera: Membracidae) (*23*) were more specialized in temperate than in tropical regions, whereas a community of temperate caterpillars (*10*) exhibited lower host specificity than was reported from the Tropics (*6*, *8*). However, none of these studies has controlled for the phylogenetic diversity of the vegetation.

There are a few caveats to our conclusions. In particular, our species diversity estimates per 100 m² of foliage may not be representative of those for larger areas of foliage, because tropical communities are known to include numerous rare species that can be detected only with large sample sizes (*24*). The upper-canopy foliage, which was undersampled in this study, can provide additional microhabitats for specialized herbivores, particularly in the Tropics (*25*). Tropical vegetation can also include additional resources that are rare or absent in temperate forests, such as woody climbing plants (*7*).

Despite these caveats, our analysis suggests that the latitudinal gradient in species diversity of herbivorous insects is to a large extent driven by the parallel increase in plant diversity (fig. S1). There was a sevenfold increase in plant diversity from our temperate to tropical study sites, with 21 tree species per hectare with diameter at breast height ≥5 cm in Moravia, as compared to 152 species in Madang (*26*). Our sample of 14 tree species represented 85% of the standing timber in a temperate forest, whereas a phylogenetically comparable subset of tropical forest represented less than 20% of the local vegetation. Greater phylogenetic diversity of tropical vegetation compared to temperate forests rather than greater host specificity of tropical herbivores is the more probable explanation for the extraordinary diversity of tropical insect communities.

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Supporting Online Material

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Brassinosteroids Regulate Dissociation of BKI1, a Negative Regulator of BRI1 Signaling, from the Plasma Membrane

Xuelu Wang and Joanne Chory*

Brassinosteroids, the steroid hormones of plants, are perceived at the plasma membrane by a leucine-rich repeat receptor serine/threonine kinase called BRI1. We report a BRI1-interacting protein, BKI1, which is a negative regulator of brassinosteroid signaling. Brassinosteroids cause the rapid dissociation of BKI1–yellow fluorescent protein from the plasma membrane in a process that is dependent on BRI1-kinase. BKI1 is a substrate of BRI1 kinase and limits the interaction of BRI1 with its proposed coreceptor, BAK1, suggesting that BKI1 prevents the activation of BRI1.

There are more than 400 serine/threonine receptor–like kinases predicted in the *Arabidopsis* genome (*1*). BRI1, the major brassinosteroid receptor of *Arabidopsis* (*2–4*), has been studied using loss-of-function mutants, overexpression, and biochemical analyses to identify the activation and specificity of plant receptor–like kinases (*5*). Brassinosteroids control physiological and developmental processes such as stem elongation, vascular differentiation, seed size, fertility, flowering time, senescence, and resistance to biotic and abiotic stresses (*2*, *6*, *7*). Direct binding of brassinolide (BL), the most active brassinosteroid, to the extracellular domain of BRI1 activates a preformed homo-oligomer. Auto- or trans-phosphorylation of the C terminus of BRI1 then enhances kinase activity and the affinity of BRI1 for BAK1, its proposed coreceptor (*8–11*). A version of BRI1 lacking the 41 C-terminal amino acids is a more active receptor but cannot be fully activated, suggesting that other factors are also required to regulate BRI1 activity.

Downstream from BRI1 and BAK1, BIN2, a glycogen synthase kinase-3 family member (*12*), negatively regulates brassinosteroid signaling by phosphorylating members of a plant-specific family of transcriptional regulators, defined by the *BES1* and *BZR1* genes (*13–16*). In the presence of brassinosteroids, BIN2 is inhibited by an unknown mechanism, leading to the dephosphorylation of BES1 and BZR1. Dephosphorylated BES1 and BZR1 then homodimerize or cooperate with other transcription factors, which allows DNA binding and regulation of hundreds of brassinosteroid-responsive genes (*15–17*).

To investigate the signaling events between the plasma membrane and transcriptional responses, we searched for proteins that interact with BRI1 using yeast two-hybrid screens with a cDNA library from *Arabidopsis* shoot apical meristems. We repeatedly identified two proteins that interacted with the intracellular domains of wild-type or kinase-inactive BRI1: a transthyretin-like protein (TTL), which is a negative regulator of brassinosteroid-related plant growth (*18*), and an expressed protein of unknown function, At5g42750. We designated At5g42750 as *BKI1* for *BRI1 Kinase Inhibitor 1*. A simple modular architecture research tool [(SMART), http://smart.embl-heidelberg.de] predicts *BKI1* to encode a protein of 337 amino acids with two separate Ser-rich domains and an Asn-rich region (Fig. 1A). BLAST searches of the predicted BKI1 amino acid sequence identified a similar gene in rice, as well as multiple expressed sequence tags (ESTs) from other angiosperms, which in several cases appear to contain the entire predicted coding region (Fig. 1B and table S1). The rice protein was previously reported to interact with the kinase domain of rice BRI1, although its function is unknown (*19*). No other similar sequences similarities were identified in other species, which suggests that *BKI1* may be angiosperm-specific.

Sequence alignments indicated that the C-terminal domain of BKI1 is the most conserved region [about 32% identity in the C-terminal region (residues 253 to 337)]. The C terminus was both necessary and sufficient to bind the kinase domain of BRI1 (BRI1-KD) (Fig. 1C). BKI1 associated specifically with the kinase domain of BRI1 and not with TTL, BIN2, or kinase domains of other receptor-like kinases tested, including BAK1 and NIK1, another member of the BAK1 subfamily (Fig. 1D). BKI1 did not interact with CLV1, a leucine-rich repeat receptor-like kinase (LRR-RLK) involved in shoot apical meristem development (*1*), nor did it interact with BRI1's closest relatives, BRL1 and BRL3 (*20*) (fig. S1), indicating that the interaction of BKI1 with BRI1 is highly specific. Glutathione *S*-transferase (GST) pull-down experiments using GST-BRI1-KD and ^{35}S-Met–labeled BKI1-6XHIS further indicated that BKI1 interacts with the kinase domain of BRI1 (Fig. 1E). Immunoprecipitation experiments confirmed that endogenous BRI1 interacted with a BKI1-FLAG fusion protein in vivo (Fig. 1F).

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