Trait Evolution and Species Coexistence in the Hyperdiverse Tropical Tree Genus *Psychotria*

by

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For Mom and Dad, for instilling in me a love of nature and a belief that dreams are things that can be achieved through hard work.

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Table of Contents

List of Figures

Figure

List of Tables

Table

Abstract

Understanding how tree species coexist in the world's most diverse tropical forests in the face of intense competition for resources has proved an enduring challenge to ecology. This challenge is only exacerbated when one recognizes that an inordinate number of species in the most diverse tropical forests are comprised of a small number of exceptionally species-rich genera. Such genera, however, may provide the key to understanding the origins and maintenance of tropical forest diversity, because they allow for the explicit examination of trait and niche differences between species that are otherwise likely to share important similarities owing to their shared ancestry. This dissertation aims to identify the processes responsible for the diversification of tropical forest understory shrubs in the genus *Psychotria* (Rubiaceae), one of the most species-rich plant genera in the world, and for their coexistence in species-dense communities such as that found on Barro Colorado Island (BCI), Panama. I have examined the phylogenetic and community patterns exhibited by physiological traits associated with drought and shade tolerance as well as secondary compounds and physical leaf traits thought to function as anti-herbivore defenses. I have sought to account for the influence of historical biogeography on these patterns by explicitly examining the relationships between species' traits, microhabitat preferences, and biogeographic origins, and have furthermore examined the theoretical implications of plant interactions with shared enemies for stable coexistence. The results of my dissertation work suggest that hydraulic traits that dictate species' tolerance of drought strongly influence species distributions, both regionally and locally. Species that exploit similar hydraulic environments on BCI are likely to be physiologically similar and closely related, and to have migrated from the same biogeographic center of origin. The lack of phylogenetic signal exhibited by secondary compounds, on the other hand, coupled with the even distribution exhibited by *Psychotria* anti-herbivore defenses and insect herbivores, is consistent with the view that secondary chemistry has played an important role in the evolutionary diversification of the *Psychotria* lineage and the coexistence of species-rich assemblages by allowing even closely related species to reduce the extent to which they share natural enemies.

Chapter 1: Introduction

A relatively small number of species-rich genera contribute disproportionately to the number of species in tropical forests worldwide. The five largest genera of trees and shrubs on Barro Colorado Island, Panama (out of ca. 330 genera) comprise nearly 22% of those species (Croat 1979). In the western Amazon, 22 species of *Pouteria* (Sapotaceae) can be found in a single hectare, and the same number of *Inga* (Mimosoideae) can be encountered in only 1/6 ha (Valencia *et al.* 1994). Gentry (1982, 1989) referred to genera such as these as "species swarms" owing to their ecological similarity and exceptional species richness at fine spatial scales.

How plant species coexist in the world's most species-rich tropical forests is an enduring question in ecology, and one that has stimulated a large number of hypotheses proposing mechanisms that might preclude the erosion of diversity through competitive exclusion or ecological drift (reviewed by Wright 2002; Leigh et al 2004). All plants require water, light, CO_2, and a number of shared nutrients, which begs the question concerning along which resource dimensions species can differentiate themselves to minimize competition (Silvertown 2004). In addition to resources, plants compete for the services of mutualists such as pollinators, seed dispersers, and mycorrhizal fungi. Plants may also compete through their interactions with shared antagonists such as herbivores, pathogens, or seed predators ("apparent competition" sensu Holt 1977). Classical niche theory maintains that for species to stably coexist, they must occupy distinct positions along axes defined by their resource requirements and their interactions with other species (Gause 1934, Hutchinson 1959, MacArthur & Levins 1967). Species differences with respect to moisture, light, and nutrient requirements represent important niche axes and explain differences in species composition over environmental gradients (Wright 2002, Leigh *et al.* 2004). And yet soil moisture, edaphic, and regeneration niches rarely account for the stable coexistence of more than a small fraction of the species richness found in most tropical forests (Wright 2002).

Locally-diverse "swarms" of congeneric species only add to the challenge of understanding coexistence, since species with recent common ancestry are likely to share ancestral traits and hence environmental tolerances and other aspects of the niche. In fact, the relationship between phylogeny and competition did not go unnoticed by Charles Darwin (1859), who mentioned in *The Origin of Species* that:

> As species of the same genus have usually, though by no means invariably, some similarity in habits and constitution, and always in structure, the struggle will generally be more severe between species of the same genus, when they come into competition with each other, than between species of distinct genera.

Because species with recent common ancestry are likely to share ancestral aspects of the niche, Darwin (1859) and later Hutchinson (1959) supposed that they would be likely to exclude one another from local communities. On the contrary, recent studies of the phylogenetic structure of forest communities have found that co-occurring species are often more closely related than by chance (i.e. are phylogenetically clustered), as would be expected if traits that dictate species distributions relative to environmental or habitat variation are more conserved than those that might partition niches more locally (Silvertown 2001, Kembel & Hubbell 2006, Swenson *et al.* 2007, Kraft & Ackerly 2010). Species swarms therefore offer an excellent opportunity to answer important questions concerning both the ultimate cause of lineage diversification as well as the ecological mechanism or mechanisms by which species with recent common ancestry coexist in species-rich assemblages. A trait that evolves by random drift (Brownian motion; Pagel 1999) is likely to exhibit phylogenetic signal. That is, unless natural selection has caused a departure from a random pattern, more closely related species are likely to be similar in their traits and hence in the niches that those traits define. As a result, identifying the traits and niche dimensions that depart from the expectation under a model of Brownian Motion can help identify which niche dimensions have played a role in the evolutionary diversification of a genus, while the spatial organization of plants with respect to traits and phylogeny can indicate which niche dimensions are likely to facilitate coexistence. If the evolution of traits that influence species' tolerance to broad climatic or environmental conditions have caused or otherwise been associated with recent bursts of speciation in species-rich genera, then we can expect those traits to exhibit an absence of phylogenetic signal. Likewise we would expect distant relatives within the genus to co-occur locally, as they would avoid competition with their closest relatives. Alternatively, if traits that influence niche dimensions that may be partitioned locally, including those that mediate trophic interactions, have been associated with speciation over the course of the diversification of the genus, then we would expect close relatives to exploit similar habitats while differentiating themselves locally. Only by simultaneously examining the distribution of traits with respect to phylogeny and community structure can we understand along which niche dimensions evolutionary divergence has been associated with speciation, and the consequences for community structure and coexistence.

Study System: the Psychotria *of Barro Colorado Island, Panama*

In order to identify how evolutionary history has shaped the community assembly and coexistence of a species-rich tropical forest woody plant genus, I endeavored to examine multiple facets of the evolutionary ecology of one of the "species swarms" that originally captivated Gentry (1982): the genus *Psychotria* L. (Rubiaceae). *Psychotria*, as Gentry, myself, and many taxonomists originally understood it, is now recognized to represent two closely related hyper-diverse genera: *Psychotria* L. and *Palicourea* Aubl., which together comprise ca. 2,300 tropical woody plant species, including ca. 1000 in the Neotropics (Taylor 1996), making it the third most species-rich plant genus by some estimates (Andersson 2002). For the purposes of this manuscript I will refer simply to *Psychotria*. In Ch. 3, however, for the sake of clarity in a study of a much larger sample of species spanning a wide swath of the Neotropics, I employ "*Psychotria*" to refer to *Psychotria* subg. *Psychotria* and "*Palicourea*" to refer to *Palicourea* plus *Psychotria* subg. *Heteropsychotria* (i.e. *Palicourea s. lat.*).

Barro Colorado Island (BCI) is one of the most intensively studied tropical forests in the world, as it was established as a research station by the Smithsonian Institution in 1924. *Psychotria* is exceptionally abundant on BCI, with nearly 10 million stems in the islands 15.9 km^2, or roughly 0.66 stems m^{-1} (see Ch. 2). The island hosts 19 species of *Psychotria* (including *Palicourea guianensis*), as well as the closely related *Carapichea* *ipecacuanha*, a small, mostly herbaceous plant formerly known as *Psychotria ipecacuanha*.

The *Psychotria* of BCI represent two large clades. These were previously recognized as subgenera *Psychotria* and *Heteropsychotria*, though my colleague Charlotte M. Taylor is in the process of reorganizing the taxonomy such that the *Heteropsychotria* will be combined with *Palicourea*. In any event, these two clades, be they subgenera or genera, last shared a common ancestor 35.6 Ma (Bremer & Eriksson 2009, see Ch. 3). As a result, *Psychotria*, unlike other species swarms that represent exceptionally recent radiations such as *Inga* (Richardson *et al.* 2001), exhibits a wide range of variation in phylogenetic branch lengths, or time since recent common ancestry, among species. The combination of unrivalled species richness and variation in phylogenetic branch lengths within *Psychotria* provides an excellent opportunity to distinguish alternative hypotheses, as well as processes that may have occurred at different stages of the diversification of the lineage.

Finally, *Psychotria* appears to be quite a prolific oceanic disperser, a feature that is no doubt related to its ability to regenerate asexually. The *Psychotria* of the Hawaiian Islands descend from a single dispersal event that most likely originated in Asia and provide a fascinating case-study of the role of biogeography in speciation (Nepokroeff *et al.* 2003; Ree & Smith 2008). Likewise, the *Psychotria* of the Neotropics have experienced a complex biogeographic history that I explore in more detail below after first considering phylogenetic structure and community assembly in the more confined locality of BCI.

Microhabitat Segregation and Phylogenetic Community Structure

One way in which the study of niche evolution can inform our understanding of lineage diversification and coexistence is by identifying the relative timing of the differentiation of related species. Ackerly *et al.* (2006), for example, found that recent speciation of *Ceonothus* shrubs has been associated with divergence in species habitat preferences with respect to temperature and elevation, such that particular sites along an elevational transect include a member of each of two early-diverging clades in the genus. In a similar vein, Cavendar-Bares *et al.* (2004) found that co-occurring *Quercus* were less closely related than by chance (i.e. phylogenetically overdispersed; “even” sensu Vamosi *et al.* 2009) as a result of convergent evolution of tolerance to alternative habitats defined by precipitation and fire frequency in three oak lineages. At least one species-rich genus of tropical tree follows a pattern broadly similar to that of *Ceanothus* and *Quercus*. Adaptation to nutrient-poor white sand Amazonian soils entails an increase in allocation to defense at the expense of growth rate (Fine *et al.* 2004). The adaptations necessary to tolerate white sands have evolved independently in several phylogenetic lineages within the genus *Protium*, such that sympatric *Protium* represent a phylogenetically even distribution of distantly related species that have converged on a common habitat syndrome (Fine *et al.* 2004, 2005).

Alternatively, closely related species might coexist despite their functional similarities if they occur in environments where the potential for competitive exclusion is limited (Hubbell 2001). Likewise, close relatives might coexist in shared, ancestral habitats if they have diverged with respect to other niche axes that can be partitioned locally sufficiently to stabilize coexistence (Silvertown 2004). Niche axes that may contribute to the coexistence of species that share microhabitat preferences may include interactions with mutualists, natural enemies, or aspects of reproduction and regeneration (Wright 2002, Silvertown 2004).

In Chapter 2, I take advantage of a large dataset pertaining to the microhabitat preferences of the BCI *Psychotria* as well as an experimental examination of drought and shade tolerance conducted during the El Niño of 1996 in order to understand how functional traits influence the phylogenetic structure of the community. Both hydraulic and photosynthetic traits are strong predictors of species distributions relative to soil moisture and light intensity in the field, but only hydraulic traits exhibit significant phylogenetic signal. Species responses to experimental manipulation of water availability likewise demonstrate phylogenetic signal. Environmental filtering according to spatial variation in soil moisture, mediated by phylogenetically conserved hydraulic traits, therefore appears to result in the phylogenetic clustering exhibited by the BCI *Psychotria*, wherein species co-occurring within 3 m radius plots are more closely related than by chance. This pattern suggests that while drought tolerance exerts a strong influence on species distributions and community composition, that particular niche dimension is unlikely to have contributed strongly to the diversification of *Psychotria*. Furthermore, the coexistence of the closest relatives that share important physiological traits at a spatial scale of only 3 m raises two important questions. What means of community assembly might account for such phylogenetic conservatism in traits and habitat occupancy? And, along which other niche dimensions might co-occurring, close relatives have diverged that might stabilize their coexistence?

The Influence of Biogeographic History on Trait Evolution and Community Assembly

Stephens and Wiens (2004) highlight two alternative mechanisms by which niches can be filled in a local community. Native lineages can adapt to exploit unoccupied niches in what is termed *in situ* evolution, or new taxa that are preadapted to conditions found in the local community can disperse from another locality, in what Stephens and Wiens call ecologically conservative dispersal.

The biogeographic history of Neotropical forests has been dominated by two geologically recent events: the uplift of the northern Andean cordilleras from 25-10 Ma, and the closure of the Isthmus of Panama roughly 3 Ma (Coates & Obando 1996). The first established an important barrier to dispersal, eventually terminating in the dry forest-savanna mosaic in Venezuela and separating the Amazon Basin to the east from the forests of the Chocó-Darién in the west to create two distinct zones of lowland moist forest in South America. The latter made possible a massive interchange of organisms between continents known as the Great American Biotic Interchange. Together, these recent rearrangements to the biogeographic landscape of the Neotropics have resulted in continuous change in the distributions and co-occurrences of organisms. What is more, the centers of diversity of many of the species-rich genera that Gentry (1982, 1989) identified as "species swarms" happen to lie in northwestern South America where they are likely to have been significantly affected by both events. Gentry (1982) himself speculated that isolation in Andean valleys may have promoted allopatric speciation of otherwise ecologically similar populations, resulting in swarms of physiologically similar congeners after secondary contact. In Chapter 3, I ask, is trait evolution in *Psychotria* best explained by divergence associated with speciation or conservative evolution associated with geography? And, has *in situ* evolution or ecologically conservative dispersal played a more significant role in the assembly if *Psychotria* communities?

In reconstructing the biogeographic histories of Neotropical *Psychotria,* I found that Central American moist forests were much more strongly influenced by recent immigrants from South America than were South American forests, even those west of the Andes. This confirms a pattern Gentry (1982) noted in the broader Neotropical flora. Yet what few could have anticipated is the significance of the asymmetry of the Great American Biotic Interchange for the phylogenetic and functional diversity of Central American forests, in which unoccupied hydraulic niches were filled by immigrants from South America. In a striking example of the role of ecologically conservative dispersal in shaping community structure, 50% of the variation in the soil moisture habitats and hydraulic traits of the BCI *Psychotria* is explained by geographic region in which the species originated. Not only are species living within 3 m of each other more closely related, they dispersed to central Panama from the same biogeographic center of origin.

Even though *Psychotria* community assembly appears to be little influenced by *in situ* evolution of hydraulic traits, lineages with long histories of shared climatic selection pressure may nevertheless have experience disruptive selection favoring divergence along other important ecological dimensions, including mutualistic and antagonistic interactions with insects, fungi, microbes, and vertebrates. These I discuss below.

Competition for the Services of Mutualists

One dimension along which interspecific differentiation has been thought to promote species coexistence is that associated with mutualistic trophic interactions. In a study of seed dispersal among sympatric congeners, Poulin *et al.* (1999) gave emetic to over 2,000 birds of 103 species in Soberanía National Park on the mainland adjacent to BCI. Of 292 *Psychotria* fruits representing 13 species, 92% were dispersed by just six bird species: three native manakins and three migratory thrushes. Thus, sympatric *Psychotria* do not appear to differentiate with respect to seed dispersers. Trees of the genus *Miconia* were similarly dispersed by a largely shared community of frugivorous birds, but sympatric species appear to reduce competition for shared avian seed dispersers by fruiting at non-overlapping times during the wet season. Sympatric *Psychotria*, however, fruit in synchrony (Poulin *et al.* 1999). Unlike the much larger, subcanopy trees of *Miconia*, it is likely that *Psychotria* shrubs benefit from an indirect, facilitative interaction among one another as they collectively attract avian seed dispersers into the forest understory. The prospect of facilitative interactions among co-occurring *Psychotria* is intriguing, but to find an ecological dimension along which sympatric *Psychotria* might differentiate, we turn our attention to another kind of trophic interaction—between plants and their insect herbivores.

Apparent Competition: Interactions with Insect Herbivores

Negative density-dependent fitness imposed by specialist natural enemies has long been thought to be an important mechanism by which species diversity is maintained in tropical forests (Janzen 1970; Connell 1971). And while environmental tolerances frequently show evidence of phylogenetic conservatism (Wiens & Graham 2005; Sedio *et al.* 2012), trophic interactions represent an ecological dimension along which even closely related species partition niche space by differentiating over evolutionary time. Indeed, defensive traits are often quite divergent among congeneric plants (Agrawal & Fishbein 2006; Becerra 1997; Kursar *et al.* 2009), and evidence of negative density-dependence is widespread in tropical forests (e.g. Harms *et al.* 2000). Yet despite these patterns, large-scale community surveys reveal broad overlap among the herbivore and pathogen communities of congeneric or even confamilial plants (Novotny *et al.* 2002; Gilbert & Webb 2007; Weiblen *et al.* 2006). This apparent contradiction raises important questions bearing on the relevance of plant-insect interactions for the maintenance of species richness in tropical forests and for our understanding of biodiversity in general. Is there sufficient evidence that herbivorous insect host-use is influenced by the variation in defensive traits among congeneric plants? Are insect herbivores specialized enough to facilitate coexistence among sympatric congeners?

In Chapter 4, I characterize host-use among the insect herbivores of 21 sympatric understory shrubs of the genus *Psychotria* on Barro Colorado Island (BCI), Panama through the use of a novel DNA barcode method. I exploit these data through a hierarchical Bayes model to answer three principal questions: i) how specialized are the herbivores of a single, diverse plant genus within a community? ii) is host-use within the genus *Psychotria* predicted by species differences in alkaloid secondary compounds, mechanical properties, or host-plant phylogeny? and iii) are *Psychotria* communities assembled in such a way as to minimize species interactions with shared herbivores?

Can nonspecialist enemies facilitate coexistence?

Out of the large number of hypotheses that have been advanced to explain coexistence, one that has gained a measure of consensus regarding its efficacy and prevalence as a mechanism is the rare-species advantage associated with density dependent mortality imposed by specialist natural enemies, first identified by Janzen (1970) and Connell (1971). Forty years of investigation have provided substantial evidence in support of the prediction that seedling survival and recruitment is inhibited at high density and in the vicinity of conspecific adults (see Terborgh 2012), a phenomenon critical to the rare-species advantage conferred by the mechanism. Nevertheless, the herbivorous insects and fungal and other pathogens thought to be responsible for the mechanism have proved, in general, far less specialized than assumed by Janzen and Connell and subsequent authors (e.g. Novotny *et al.* 2002). It remains unclear how sensitive the Janzen-Connell mechanism is to varying host-specificity, or to what extent enemy-mediated competition enhances plant species richness in observed plant-enemy communities.

In Chapter 5, I examine the influence of host-specialization on diversity maintenance and the community structure of defensive traits using a simulation model. I show that only models in which plant enemy similarity declines rapidly as a function of pairwise defensive trait distance support greater species richness than models of neutral drift. Nonetheless, a wide range of variation in enemy host-specificity results in the dispersion, or exclusion, of defensively similar plant species, indicating that enemy-mediated competition may increase defense trait diversity without enhancing species richness. This result has important implications for the role of nonspecialists in selecting novel defensive phenotypes—a key prediction of Ehrlich and Raven's (1964) classic hypothesis linking defensive trait innovation with lineage diversification.

In addition, I parameterize the model using published empirical studies of herbivorous beetles and pathogenic fungi in Panama, as well as a large-scale survey of caterpillars in Papua New Guinea. All three types of enemy demonstrate sufficiently narrow host-ranges to substantially enhance both plant species richness and defensive trait diversity in those communities. These theoretical results, combined with my extensive analysis of trait evolution and community structure of the BCI *Psychotria*, highlight the importance of plant-enemy interactions for both the evolutionary origins of diverse clades of tropical forest woody plants, and also for the stabilization of species coexistence in the most diverse communities on the planet. My conclusions, in Chapter 6, further suggest that important advances in evolutionary ecology will be made through a more detailed examination of the evolution of plant enemies and plant defenses and their influence on whole communities.

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Chapter 2: Trait evolution and the coexistence of a species swarm in the tropical forest understory

Abstract

A small number of species-rich plant genera make a substantial contribution to the α diversity of tropical forests. These "species swarms" (Gentry 1982) challenge a view of community assembly that maintains that niche overlap, and hence the likelihood of competitive exclusion, should increase with phylogenetic affinity. Related species may, however, occupy different microhabitats within a forest stand or, alternatively, differ ecologically in ways that allow them to co-exist syntopically.

To explore the relationship between phylogenetic history and niche differentiation among sympatric, congeneric species, we performed phylogenetic analyses of microhabitat preferences, photosynthetic and hydraulic traits, and experimental responses to light and water availability for 20 species of *Psychotria* (Rubiaceae) from Barro Colorado Island (BCI), Panama.

The *Psychotria* present in small (3-m radius), circular plots were more closely related than expected by chance. Photosynthetic and hydraulic traits were both associated with species habitat distributions, but only hydraulic traits were conserved phylogenetically. Experimental responses to water availability were also conserved

phylogenetically. Functional trait analyses revealed little or no evidence of niche partitioning within sites.

We conclude that *Psychotria* species' responses to both light and moisture availability determine their microhabitat distributions on BCI, and that evolutionarily conserved hydraulic traits lead to phylogenetic clustering of co-occurring species.

The evolutionary conservation of hydraulic traits related to soil moisture tolerance largely explains phylogenetic clustering in the local assembly of the hyperdiverse genus *Psychotria*. We suggest that close relatives are unlikely to exclude one another from shared habitats because resource availability is determined largely by asymmetric competition with the overstory, rather than by competition with neighboring understory plants. In light of the recent biogeographic admixture in central Panama, the phylogenetic niche conservatism exhibited by *Psychotria* on BCI raises the possibility of an association between local microhabitats and the ancestral climatic regimes under which major *Psychotria* lineages evolved before arriving in sympatry.

Introduction

A handful of species-rich genera contribute disproportionately to the species diversity of woody plants in tropical forests. For example, the five largest out of ca. 500 woody plant genera comprise 22% of the species of trees and shrubs on Barro Colorado Island (BCI), Panama (Foster & Hubbell 1990). Gentry (1982, 1989) marveled at the local species diversity and apparent ecological similarity within “species swarms” of *Miconia* (Melastomataceae), *Piper* (Piperaceae), *Psychotria* (Rubiaceae), and other understory genera in southern Central America and northwestern South America, but the phenomenon is not limited to the understory. A 1-ha plot in Ecuador harbored 22 species of canopy trees in the genus *Pouteria* (Sapotaceae), while just 0.16 ha contained the same number of species of *Inga* (Mimosoideae) (Valencia *et al.* 1994).

How large numbers of woody plant species coexist in the face of intense competition for resources remains a fundamental challenge to community ecology (Wright 2002, Silvertown 2004). All plants require CO_2, light, water, and a common set of nutrients, which seemingly limits the number of niche dimensions along which species can evolve to avoid competition (Silvertown 2004). Plants also compete for the services of mutualists, such as pollinators, seed dispersers, and mycorrhizal fungi, and for enemy-free space as they interact with pests and pathogens (apparent competition; Holt 1977). Classical ecological theory maintains that for competing species to coexist stably, they must occupy distinct niches, broadly defined by the way they interact with the abiotic environment and all other species (Gause 1934, Hutchinson 1959, MacArthur & Levins 1967). While niche differences with respect to light intensity, soil moisture, and regeneration have been identified, they rarely explain the coexistence of more than a small fraction of the plant species in tropical forests (Wright 2002). What is more, coexisting species often must pass through habitat filters that select for physiological similarity (Weiher & Keddy 1995, Baraloto *et al.* 2012), further limiting the scope for differentiation among syntopic species, or those that co-occur in a habitat (Rivas 1964).

Locally-diverse genera such as Gentry's (1982, 1989) species swarms further challenge our understanding of coexistence, since phylogenetically related species share recent common ancestry and therefore a recent ancestral niche. For closely related species to coexist stably, they must either have diverged from their ancestral niche in some important way that allows them to partition niche space syntopically, or else have diverged with respect to traits that allow them to exploit distinct environments. Species differences in both of these niche dimensions may distinguish populations that are broadly sympatric (Rivas 1964), and thereby facilitate their coexistence. Do phylogenetic lineages tend to diversify over environmental gradients or retain habitat preferences over time? Which niche axes play a role in the coexistence of sympatric congeners? Community-level patterns of trait and phylogenetic structure depend on the answers to these questions.

If tropical species swarms behave similarly to temperate genera, three studies may provide particular insight. Closely related *Quercus* trees (Cavender-Bares *et al.* 2004) and *Ceanothus* shrubs (Ackerly *et al.* 2006) were found to exploit distinct habitats defined by fire frequency and soil moisture and by temperature and elevation, respectively. In both of these cases and in a study of South African sedges (Slingsby & Verboom 2006), syntopic assemblages were composed of relatively distantly related species derived from separate early-diverging clades within the genus, indicating evolutionary convergence of habitat preference and phylogenetic evenness (i.e. overdispersion sensu Cavender-Bares *et al.* 2004) of coexisting species.

Similar patterns might apply to Gentry's species swarms, with closely related species having diverged functionally to replace one another over fine scale environmental gradients and more distantly related congeners having converged functionally to coexist syntopically. On the other hand, closely related species might coexist syntopically despite their functional similarity, if they occur in environments where the potential for competitive exclusion is limited (Hubbell 2001, Adler *et al.* 2007). This would result in phylogenetically clustered local communities. Phylogenetic clustering might also occur if species differences that do not reflect habitat preferences are evolutionarily labile and effectively stabilize coexistence (Silvertown 2004, Adler *et al.* 2007, Kursar *et al.* 2009a). Examining the evolution of ecologically important traits and their distributions among sympatric congeners may thus help elucidate the processes that generate and maintain tree diversity in tropical forests.

In the present study we investigate the influence of trait evolution on the coexistence of 20 woody species in the genus *Psychotria* (Rubiaceae) on Barro Colorado Island (BCI), Panama. In addition to distributional and functional trait data, we take advantage of a factorial transplant experiment performed during a severe El Niño year (1993–1994; previously unpublished data) to investigate species' microhabitat niches and responses to potential environmental filters. Through a phylogenetic analysis of traits, habitat distributions, and experimental responses to light and moisture availability, we address the following questions: (i) Are species assemblages of *Psychotria* at small (3-m radius) spatial scales nonrandom with respect to phylogeny? (ii) Is there evidence of environmental filtering among the 20 congeneric *Psychotria* species with respect to light and moisture availability? (iii) Is there evidence that functional traits are evenly distributed among species with similar microhabitat preferences, such as might indicate within-site niche differences? (iv) Do those traits that are associated with microhabitat or within-site niche differences exhibit phylogenetic signal? Finally, we ask how the distributions and phylogeny of the *Psychotria* of BCI inform our understanding of the role of trait evolution in the diversification of the genus and coexistence in species-dense local communities.

Materials and Methods

Study Site

BCI (9^{o} 9' N, 79^{o} 51' W) has a tropical monsoon climate under the Köppen system of climatic classification and supports semi-deciduous tropical forest with a mature canopy height of 35 m. Mean monthly temperatures are 27^{o}C in April and 26^{o}C otherwise. Annual rainfall averages 2,600 mm. A four-month dry season begins in December or occasionally November and ends in April or occasionally May. Median rainfall is 84 mm over 90 days between 1 January and 31 March. Croat (1978), Windsor *et al.* (1990) and Leigh (1999) provide further descriptions of BCI.

Unusually long and severe dry seasons are sometimes associated with El Niño events on BCI (Leigh *et al.* 1990). This was the case during the dry season of 1991–1992 when just 71.7 mm of rain fell over 140 consecutive days. Gravimetric soil water content and plant water status were evaluated during this harsh dry season.

Psychotria

Psychotria is globally one of the largest plant genera with some 1,650 species distributed throughout the tropics and subtropics (Taylor 1996). The genus is comprised almost exclusively of shrubs and small trees of the forest understory. Along with several other species-rich genera, *Psychotria* inspired Gentry (1982, 1989) to coin the term "species swarm" to describe genera comprised of seemingly ecologically undifferentiated species contributing to high alpha diversity. Because of the Andean-centered distribution of such genera, Gentry (1989) suggested that topographic heterogeneity associated with the Andean uplift may have caused their allopatric divergence (and subsequent remixing) without notable ecological differentiation. Sympatric *Psychotria* are indeed similar with regard to pollinators (Sakai & Wright 2008), seed dispersers (Poulin *et al.* 1999) and growth form. In the present study we more carefully examine microhabitat differences.

Psychotria are exceptionally abundant on BCI. Mean density summed over all *Psychotria* was 0.66 stems m^{-2} in this study (methods described below). Extrapolating from this figure, 15.9 km^2 BCI supports approximately 10,000,000 *Psychotria* stems. BCI is home to 19 species in two well-defined subgenera, *Psychotria* subgenus *Psychotria* (9 species on BCI) and *Psychotria* subgenus *Heteropsychotria* (10 species on BCI), as well as *Carapichea ipecacuanha* (formerly *Psychotria ipecacuanha*). The two subgenera diverged approximately 40 Ma (Paul *et al.* 2009).

Microhabitat Distributions

All *Psychotria* stems were identified, measured (diameter at 5 cm and 20 cm heights) and tagged in 134 circular plots (3 m radius; 28.3 m^2 area) between 18 June and

9 August 1992. Plots were located 20 m to the south of permanent 100-m trail markers along a 6.2 km transect (62 plots) and arbitrarily along eight streams (52 plots) and in a seasonally inundated area (20 plots). Soils at the latter sites remain relatively moist through the dry season.

Moisture and light availability were determined for each of the 134 plots. Canopy photographs were used to calculate incident global radiation and gravimetric soil moisture was measured during the final seven weeks of the El Niño dry season. A canonical correspondence analysis was performed using the R package ‘vegan’ (R Development Core Team 2006, Oksanen *et al.* 2009) to relate *Psychotria* species composition to the two environmental variables. Soils at 40-cm depth in the seasonal swamp were obviously wet late in the 1992 dry season, yet gravimetric soil water content was indistinguishable for plots in the swamp (0.242 ± .031; mean ± s.d.) and along the 6.2-km transect (0.255 ± .035). Consequently, the analysis was restricted to the 15 *Psychotria* species represented by a total of 20 or more stems in the 114 well-drained plots.

Physiological Measurements

Photosynthetic Properties. Leaf traits vary on a global axis thought to reflect strategies that extend from leaves that are long-lived and tolerant to leaves that are highly productive but unprotected and short-lived (Wright *et al.* 2004). We measured leaf respiration rates, light compensation points (LCP), quantum yields and light saturated photosynthetic rates ($A_{\max}$) using an oxygen electrode (Hansatech, Norfolk, UK). Leaf absorptance was determined using an LI-1800 radiometer (Li-Cor, Lincoln, NE).

Hydraulic traits. Tolerance of low leaf water potential is a strong determinant of drought performance and distributions among tropical woody plants (Kursar *et al.* 2009b), whereas stem conductivity exhibits a tradeoff with susceptibility to xylem cavitation and hence drought performance (Markesteijn *et al.* 2011). Leaf water potentials (ψ) were measured in March 1992 with a portable pressure chamber (PMS Instruments, Model 1000, Corvallis, OR) and a steady state porometer (Li-Cor, Model 1600, Lincoln, NE), respectively. Pressure-volume curves were constructed to determine turgor-loss point water potentials (Koide & Schreiner 1992). Leaf-specific native stem conductivity (K_l) was assessed for 17 species in November 2010. Turgor-loss leaf water potential (ψ_t) and K_l were used in subsequent analyses. Specific leaf area (SLA) reflects a tradeoff between thin leaves with high metabolic rates per unit mass, on the one hand, and thick leaves with enhanced nutrient and water-use efficiency on the other (Wright *et al.* 2004, Ackerly & Cornwell 2007), and exhibits a strong negative association with rainfall globally (Wright *et al.* 2004). Here, we consider SLA among hydraulic traits.

Common Garden Experiment

A factorial experiment conducted in 1993–1994 assessed responses to light environment (recent tree fall gaps vs. shaded understory) and dry-season moisture availability (irrigated vs. control) for 17 *Psychotria* species. Seedlings were germinated under high and low light conditions and were transplanted into 20 naturally occurring gaps and 20 arbitrarily chosen understory sites, respectively, in square grids at 25 cm intervals. Ten randomly selected plots from each light environment were irrigated weekly with water from oligotrophic Gatun Lake. Survival and stem height were measured in April, August, and December 1994. Because of the high rates of survival in the irrigated gap plots (see Table 2.4), we did not use a fully factorial calculation of performance in the experiment. Drought performance in shade ($P_{drought}$) was calculated as 1 minus the difference between the fraction surviving in control understory plots and in irrigated understory plots. Shade performance without irrigation (P_{shade}) was 1 minus the difference between survivorship in control understory relative to control gap plots.

Phylogenetic Reconstruction

The nuclear internal transcribed spacer region (ITS) and the chloroplast *psbA-trnH* spacer region were chosen for use as phylogenetic markers due to their variability and ease of amplification in the study species (lab methods provided in Appendix S1). After a partition homogeneity test conducted in PAUP* 4.10b (Swofford 2002) confirmed that the gene sequences could be combined for phylogenetic analysis (Farris *et al.* 1994), the ITS and *psbA-trnH* datasets were concatenated and analyzed through 6 million MCMC generations in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) under a mixed model with independent estimation of parameters for the GTR+I model of DNA sequence evolution as selected by a likelihood ratio test implemented in MrModeltest 2.3 (Nylander 2004). The two-locus Bayesian consensus tree was pruned to include only those species representing the BCI species pool. This community phylogeny (Fig. 2.1) was used in subsequent analyses of community structure.

Phylogenetic signal in the evolution of physiological traits and responses to experimental treatments was evaluated using the R package ‘picante’ (R Development Core Team 2006, Kembel *et al.* 2008), which calculates Blomberg’s K statistic of phylogenetic signal using generalized least squares (Blomberg *et al.* 2003). Because Blomberg's test loses power below about 20 taxa (Blomberg *et al.* 2003), this test was corroborated by a Mantel test of the correlation between the phylogenetic distance matrix and the pairwise Euclidean distance matrix for each trait (implemented in R; R Development Core Team 2006).

Revell (2010) has shown that phylogenetic generalized least squares (PGLS) linear regression is unnecessary when the residual error of the regression model does not exhibit phylogenetic signal, even if the independent variable does show signal, and furthermore that ordinary least squares (OLS) linear regression outperforms PGLS in those circumstances. Because residual errors did not exhibit phylogenetic signal in any of our regression models (Figs. 2.4 and 2.5) we examined the relationships between species distributions, functional traits, and experimental responses using OLS linear regression. Phylogenetic signal and linear regression analyses were conducted on species means.

Community Structure

Phylogenetic community structure was measured by calculating the mean phylogenetic distance (MPD) and mean nearest taxon distance (MNTD; Webb 2000) between *Psychotria* species surviving in experimental plots as well as those within natural communities sampled by the 104 census plots with two or more species present. MPD represents the mean phylogenetic branch length measured between every pairwise combination of species found in a local community or census plot, while MNTD includes only the branch lengths between each species and the most closely related species with which it co-occurs and thus represents community structure at a more terminal phylogenetic scale. The MPD and MNTD metrics were then calculated for 9,999 null communities generated using an independent swap algorithm in which the number of species per community sample and the frequency of occurrence of each species across samples were held constant while the particular species that co-occur in each sample was randomized (Gotelli & Entsminger 2003). Finally, we calculated two standardized effect size metrics. The net-relatedness index (NRI) and nearest-taxon index (NTI) equal the difference between observed and mean simulated values of MPD and MNTD, respectively, standardized by the standard deviation of phylogenetic distances in simulated null communities (Webb *et al.* 2002). Significance was tested using a Wilcoxon rank sum test.

To evaluate potential differences in phylogenetic structure between habitats, NRI and NTI were calculated for subsets of plots located in the seasonal swamp (n = 17 plots with ≥ 2 spp.) as well as for plots within the upper 30% quantile (n = 32) and lower 30% quantile (n = 25) of non-swamp plots with respect to soil moisture. All community phylogenetic metrics were calculated using ‘picante’ (Kembel *et al.* 2008).

In addition to phylogenetic structure, we also measured four metrics of the functional trait structure of local communities relative to the BCI species pool following Kraft & Ackerly (2010). The range and variance of trait values are sensitive to trait clustering caused by habitat filtering (Kraft & Ackerly 2010). The standard deviation of neighbor (SDND) and nearest-neighbor (SDNN) trait distances can be used to test for a signature of limiting similarity, such as that caused by within-site niche differentiation or competitive exclusion, when compared to a null model of community assembly (Ricklefs & Travis 1980). Dividing SDND and SDNN by the trait range within each sample may allow these metrics to detect within-site trait evenness even against a background of habitat filtering (Kraft & Ackerly 2010). Standardized effect sizes were calculated by comparing trait range, variance, SDNNr and SDNDr to their distributions for 999 null communities assembled by choosing *Psychotria* species at random, but weighted by their occurrence frequency in the pool of census plots (Table 2.1).

Results

Microhabitat Distributions

Microhabitat differences among species contributed to the near ubiquity of the genus on BCI (Fig. 2.2). There were 2,553 stems of potential reproductive size (total area sampled = 0.38 ha) and at least one species was present in 128 of 134 small 3-m radius permanent plots (Fig. 2.6). Levels of habitat overlap and coexistence were exceptionally high, with ≥ 5 *Psychotria* species present in 12% of the plots (Fig. 2.6). Two plots contained nine species, whereas just six plots lacked *Psychotria*.

Most plots were in the shaded understory. Median global radiation estimated from hemispheric canopy photographs was 0.64 ± 0.31 Mjoules m^{-2} d^{-1} (mean ± 1 s.d.), and global radiation exceeded 1 Mjoule m^{-2} d^{-1} in just 14% of the plots. Global radiation and gravimetric soil water content were uncorrelated ($R^2 = 0.018$, $n = 134$).

The first canonical correspondence analysis (CCA) axis was defined by gravimetric soil water content (canonical coefficients, axis 1: soil moisture 0.986, light intensity -0.159); and the second by global radiation (canonical coefficients, axis 2: soil moisture 0.167, light intensity 0.987). Eigenvalues were 0.219 and 0.077 and species-environment correlation coefficients were 0.610 and 0.412 for axes 1 and 2, respectively (Fig. 2.2).

The *Psychotria* include two widespread species (*P. horizontalis*, *P. marginata*) found in 94 and 61 of the 134 sites, respectively, one drought-tolerant species (*P. capitata*) whose distribution is restricted to the driest parts of BCI, five moisture-sensitive species (*P. brachiata*, *P. chagrensis*, *P. cyanococca*, *P. grandis*, and *P. psychotriifolia*) whose distributions are limited to moisture-retaining microsites, and two light-demanding species with greater abundance (*P. limonensis*) or largely limited (*P. micrantha*) to microsites with relatively large values of global radiation (Fig. 2.2). The two widespread species, *P. horizontalis* and *P. marginata*, are relatively closely related (Fig. 2.1). The five moisture-sensitive species comprise two sets of closely related taxa. Within the subgenus *Psychotria*, *P. chagrensis* and *P. psychotriifolia* are nearest relatives and also share a close relationship with *P. grandis* (Fig. 2.1). Among the *Heteropsychotria*, *P. brachiata* and *P. cyanococca* are closely-related, while their other closest relatives, *P. poeppigiana* and *P. pubescens*, are rare gap specialists and thus do not contribute strongly to the community pattern (Fig. 2.1). The two remaining light-demanding, gap dependent species, *P. limonensis* and *P. micrantha*, are in the subgenus *Psychotria*, but are not closely related to one another (Figs 2.1 and 2.2).

Phylogenetic structure of syntopic species

The phylogenetic structure of syntopic *Psychotria* was significantly more clustered than were null communities generated using an independent swap algorithm

(Gotelli and Entsminger 2003), whether measured in terms of MPD (mean –NRI = -0.2273, $P < 0.02$) or MNTD (mean –NTI = -0.3358, $P < 0.0001$; Fig. 2.3).

Phylogenetic structure varied with soil moisture. Figure 1.3 displays the phylogenetic structure of all 134 plots relative to subsets of plots located in the seasonal swamp ($n = 17$ plots with ≥ 2 spp.) and the wettest and driest 30% of the remaining well-drained plots. Phylogenetic clustering was significant for plots located in the seasonal swamp (mean –NRI = -0.5819, $P < 0.01$) and in the wettest 30% of plots (mean –NRI = -0.4019, $P < 0.005$) but not for the driest 30% (mean –NRI = 0.0414, $P = 0.69$; Fig. 2.3a). The closest relative of each species in each plot was also more closely related than by chance in swamp (mean –NTI = -0.7559, $P < 0.002$) and wet (mean –NTI = -0.4764, $P < 0.001$) plots, but not significantly so in dry plots (mean –NTI = -0.1756, $P = 0.27$; all one-sided Wilcoxon rank sum tests; Fig. 2.3b).

In the common garden experiment, nonrandom mortality led to phylogenetic clustering when measured in terms of NRI (mean –NRI = -0.6687, $P < 0.01$; mean –NTI = 0.0015, $P = 0.52$, one-sided Wilcoxon rank sum test). The eight most strongly phylogenetically clustered plots were in the understory; five were irrigated and three were control plots.

Photosynthetic Traits, Performance, and Distribution

There was no evidence for phylogenetic signal among the photosynthetic traits or for performance in shade (P_{shade}) in the common garden experiment (Table 2.1). Both $A_{\max}$ and LCP exhibited significant trait clustering in the 134 small, circular plots with respect to one of the two metrics used, but no photosynthetic traits exhibited evidence of trait evenness (Table 2.1).

The positions of species along the light intensity gradient are represented by the perpendicular projection of each species onto the light intensity axis in the ordination. $A_{\max}$ showed a weak but not significant correlation with position along the light intensity gradient (Fig. 2.4a). In the common garden experiment, shade performance (P_{shade}) was significantly associated with $A_{\max}$ (Fig. 2.4e). Despite demonstrating the greatest shade tolerance of all the species analyzed, *P. limonensis* was surprisingly abundant in high-light environments (Fig. 2.4d; grey square). The relationship between P_{shade} and species positions on the light intensity gradient was significant when *P. limonensis* was excluded ($R^2 = 0.44$, $P < 0.02$), but insignificant otherwise (Fig. 2.4d).

Hydraulic Traits, Performance and Distribution

One of the two metrics employed for each of the three hydraulic traits showed significant phylogenetic signal. In the common garden experiment, there was also significant phylogenetic signal for drought performance in understory plots (Table 2.1).

The range and variance of traits observed for syntopic species within the 134 small, circular plots provided evidence of significant trait clustering for all three hydraulic traits (Table 2.1). Only SLA revealed significantly nonrandom SDNDr, suggesting that SLA of co-occurring species was not as similar as it might be (Kraft & Ackerly 2010). Neither ψ_t nor K_l exhibited trait evenness (Table 2.1).

The positions of species along the soil moisture gradient are represented by species' perpendicular projections onto the soil moisture axis in the ordination (Fig. 2.2).

Psychotria species that tolerated lower ψ_t were associated with drier microhabitats (Fig. 2.5a), as were species with lower K_l (Fig. 2.5b). SLA, however, was not significantly related to species distributions relative to soil moisture. Despite the associations between ψ_t and K_l and species native distributions, none of the hydraulic traits exhibited a significant relationship with $P_{drought}$ as assessed in the common garden experiment. $P_{drought}$ was also unrelated to the position of a species along the soil moisture gradient (Fig. 2.5d).

Tolerance of low leaf and stem water potentials is thought to involve a trade-off with hydraulic efficiency, resulting in a negative correlation between drought performance and ψ_t or K_l (Kursar *et al.* 2009b, Markesteijn *et al.* 2010). However, the ratio of species mortality in control versus irrigation treatments in our experiment was not clearly related to either hydraulic traits (Fig. 2.5e-g) or soil moisture habitat distribution (Fig. 2.5d). Indeed, rather than suffering high mortality in control plots during the dry El Niño year, moisture-loving species exhibited high survivorship across treatments, while species found in dry microsites in the forest tended to die in the irrigated treatment (Table 2.4).

Discussion

The phylogenetic structure of communities, when combined with analyses of trait evolution and community trait composition, can reveal which aspects of the niche have differentiated over the course of a clade's evolutionary history. Several genera of temperate plants appear to have diversified with respect to traits that determine climatic and environmental tolerances, such that close relatives exploit distinct habitats and replace each other over environmental gradients (Cavender-Bares *et al.* 2004, Ackerly *et al.* 2006, Slingsby & Verboom 2006). Is there evidence that a similar history of evolutionary diversification with respect to understory light and moisture environments might characterize *Psychotria* and thereby facilitate the maintenance of high local species richness?

Community assembly of the *Psychotria* was clearly influenced by light availability. Photosynthetic rate ($A_{\max}$) was associated with species distributions relative to light intensity in the understory (Fig. 2.4a) and exhibited lower than expected variance in census plots (Table 2.1). Furthermore, species responses to gap and understory environments in the common garden experiment were concordant with their photosynthetic capacities ($A_{\max}$; Fig. 2.4e) and their microhabitat distributions with the exception of one species (Fig. 2.4d). The absence of phylogenetic signal among photosynthetic traits (Table 2.1) means that the contribution of habitat filtering along this axis to the observed community phylogenetic structure was probably minimal. In addition, these findings suggest that responses to light environments are evolutionarily labile among these understory plants.

A consideration of species soil moisture distributions with respect to hydraulic traits supports a role for habitat filtering along the soil moisture environmental gradient. Leaf water potential at turgor loss (ψ_t) and leaf-specific stem conductivity (K_l) were both correlated with species distributions along the soil moisture gradient (Fig. 2.5a,b), and displayed evidence of trait clustering within census plots (Table 2.1). Unlike the photosynthetic traits measured, both ψ_t and K_l displayed phylogenetic signal (Table 2.1). The retention of phylogenetic signal among hydraulic traits and the influence of soil moisture environment on the degree of phylogenetic clustering (Fig. 2.3b) indicate that soil moisture environments are a strong abiotic filter for physiologically similar and closely related species of *Psychotria*.

Species' performances in experimental drought treatments were not consistent with habitat preferences in the forest (Fig. 2.5d) or with measured physiological traits (Fig. 2.5e-g). Despite the implementation of the experiment during a strong El Niño, the control, non-irrigated plots did not prove intolerable to species that, based on hydraulic traits and distributions, would be expected to suffer higher mortality in that treatment. Indeed, moisture-loving species exhibited high survivorship across treatments, while species found in dry microsites in the forest tended to die in the irrigated treatment, suggesting a correlation between moisture and pathogen load may have played a role (Givnish 1999). However, despite the ambiguity concerning the relationship between drought performance and hydraulic traits, drought performance itself exhibited phylogenetic signal (Table 2.1) and mortality in common garden plots resulted in phylogenetically clustered species assemblages. The common garden experiment showed that subjecting unbiased species assemblages of seedlings to different light and moisture environments was sufficient to generate phylogenetically clustered assemblages. On the other hand, the ambiguous relationship between $P_{drought}$ and either species hydraulic traits or habitat distributions suggests that seedling survival during a harsh El Niño year cannot be extrapolated to adult habitat distributions in the field.

The contribution of phylogeny to community ecology is illustrated by comparing the present study to those of Cavender-Bares *et al.* (2004) and Ackerly *et al.* (2006). In all three studies, trait clustering and trait associations with abiotic habitats suggested that environmental filtering influenced species composition at the scales of analysis. Local communities of *Quercus* exhibited functional traits associated with local soil moisture and fire regimes (Cavender-Bares *et al.* 2004), while syntopic *Ceanothus* exhibited functional similarities related to temperature and elevation (Ackerly *et al.* 2006). *Psychotria* likewise showed evidence of environmental filtering mediated by both photosynthetic and hydraulic traits. Only by examining the three genera with respect to phylogeny does one come to the conclusion that the environmental gradients in question were important axes of relatively recent evolutionary differentiation among closely related oaks (Cavender-Bares *et al.* 2004) and *Ceanothus* shrubs (Ackerly *et al.* 2006). In contrast, our results suggest that species distributions are phylogenetically conserved with respect to soil moisture, and therefore that differentiation along this niche axis has not played a strong role in the recent diversification of the genus, despite the fact that soil moisture is an important determinant of species distributions in tropical forests generally (Engelbrecht *et al.* 2007) and with respect to *Psychotria* in particular (Figures 2.2, 2.5a).

One Neotropical species swarm, the rain forest tree genus *Inga*, varies in its community phylogenetic structure in Panama and Peru (Kursar *et al.* 2009a). Yet in both locations, nearby individuals are significantly less similar with respect to secondary chemistry than by chance (Kursar *et al.* 2009a), suggesting that trophic interactions may distinguish close relatives in ways that allow them to coexist. The name *Psychotria* is a testament to its potent secondary compounds (some species yield hallucinogenic, or psychotropic, compounds), yet it remains unclear whether co-occurring or closely related *Psychotria* differ in ways that limit the extent to which they share natural enemies.

Assembly of Tropical Forest Understories

That the community structure of *Psychotria* understory shrubs contrasts with that of genera in temperate forest and chaparral communities may not be incidental. Several features of tropical forest understory plants may act to reduce resource competition. These include: (i) low population densities, (ii) dispersal limitation, and (iii) stochasticity imposed by sunflecks and canopy gaps

Understory plants, unlike canopy trees, may not compete with each other directly for light, water, and soil nutrients if their densities are low enough that their crowns and root systems (i.e. zones of influence of resource use) do not overlap (Wright 2002, Paine *et al.* 2008, Svenning *et al.* 2008). There is evidence that the low stem densities of understory shrubs may be caused by asymmetrical competition with the overstory and by herbivore pressure. Experimental trenching experiments that eliminate root competition with overstory trees lead to increased growth rates in the understory (Coomes & Grubb 1998, 2000, Lewis & Tanner 2000, Barberis & Tanner 2005, Tanner & Barberis 2007), and even shade-tolerant species typically perform better in full sunlight (Barberis & Tanner; this study). Understory plants have been found to respond with increased growth when protected from mammalian (Dirzo & Mirando 1991, Terborgh & Wright 1994) and insect (Sagers & Coley 1995) herbivores. In contrast, understory removal and competition experiments have consistently failed to find a measurable effect on performance (Marquis *et al.* 1986, Brown & Whitmore 1992, Paine *et al.* 2008, Svenning *et al.* 2008). The growth of understory plants is clearly limited by the availability of light and water. However, the limits on the availability of these resources imposed by other understory plants pales in comparison to the burden imposed by the forest overstory.

From a resource perspective, membership in the understory appears to be largely determined by environmental tolerance to understory conditions rather than competitive trade-offs in resource utilization.

A species is said to be dispersal-limited if its propagules fail to arrive at suitable sites in sufficient numbers to become established (Nathan & Muller-Landau 2000). Seed-addition experiments and studies using seed traps on BCI have found dispersal limitation to be nearly ubiquitous for canopy trees (Hubbell *et al.* 1999, Wright *et al.* 2003, Svenning & Wright 2005, Muller-Landau *et al.* 2008) and it may be even more acute among understory plants because of their reduced fecundity and their dependence on low density populations of understory birds for seed dispersal (Givnish 1999). In addition to the effects of small size and limited physical resources on fecundity, the heterostylous *Psychotria* are all obligate outcrossing species. If the relatively small floral displays are not effective at attracting pollinators, then their fecundity may also be pollen limited (Sakai & Wright 2008). Fecundity and dispersal limitation can limit the potential for direct competitive interactions.

Chance variation in light is critical to the success of understory plants. Sunflecks contribute from 32 to 65% of the daily carbon gain of understory plants in closed canopy forests (Chazdon & Pearcy 1991). In addition, treefalls temporarily release understory plants from light limitation by opening gaps in the forest canopy. The elevated resources available in such gaps may be critical to the reproductive success even of shade-tolerant understory species (Levey 1988, Denslow *et al.* 1990, Dirzo *et al.* 1992, Schnitzer *et al.* 2008). The chance, ephemeral nature of both sunflecks and treefall gaps imposes stochasticity on the success of understory plants that may further mitigate against competitive exclusion.

In sum, the overstory and herbivore suppression, stochasticity imposed by gaps and sunflecks, and dispersal limitation common to tropical forest understories may facilitate unstable coexistence by slowing the rate of competitive exclusion relative to random drift (Hubbell 2001) or regional assembly processes (Ricklefs 2004). These considerations may apply to a large proportion of tropical forest plant diversity. Nearly 70% of the free-standing woody plant species of central Panama attain a maximum height below that of the canopy (King *et al.* 2006). While many of those species are substantially larger than *Psychotria*, they most likely face many of the same factors that attenuate competitive exclusion in the understory.

Broader Biogeographic Perspectives

The Isthmus of Panama is a zone of biogeographic interchange wherein lineages from Mesoamerica, South America and the Caribbean contribute to local floras (Croat & Busey 1975). Within the Isthmus, BCI is situated in the center of a north-south precipitation gradient, which varies from < 2000 mm yr^{-1} on the Pacific coast to > 3000 mm yr^{-1} near the Caribbean (Pyke *et al.* 2001). This gradient influences tree species distributions (Engelbrecht *et al.* 2007) and results in greater species turnover (β diversity) in Central Panama than across much greater distances in Amazonia (Condit *et al.* 2002). BCI contains rain forest species that are generally restricted to the Caribbean slope, as well as dry forest species more characteristic of the Pacific coast (Pyke *et al.* 2001, Condit *et al.* 2002). Because of its location in the path of the Great American Biotic

Interchange and its intermediate level of rainfall with microsite variation in soil moisture, BCI is a zone of historical and regional floristic admixture, both of which may contribute to the local pattern of community assembly.

If clades of *Psychotria* that originated in climatically different regions, such as wet and dry forest biomes, are slow to evolve away from the edaphic and soil moisture adaptations of their ancestors, then conserved physiological traits may influence the habitat preferences of members of the clade long after they migrate to new geographic localities. We hypothesize that environmental differences among regional biogeographic centers of speciation contribute to the phylogenetic signal observed in *Psychotria* habitat preferences, and ultimately result in the observed phylogenetic clustering as members of the regional species pool filter into local assemblages according to phylogenetically conserved soil moisture niches. This hypothesis linking biogeographic origins and environmental filtering at the stand level can be tested with the aide of an ancestral area analysis (Ronquist 1997) across a more intensively sampled *Psychotria* phylogeny.

Phylogenetic niche conservatism has been found to characterize the assembly of continental and regional biotas (Wiens & Donoghue 2004, Donoghue 2004, Donoghue 2008, Ricklefs & Renner 2012). Here we show that niche conservatism may influence local community assembly as species filter into distinct microhabitats. Although competitive interactions are expected to be most severe between relatives with similar niches, the absence of resource competition among understory dwellers described above may allow habitat preferences derived from shared biogeographic histories to be retained at fine spatial scales.

Conclusion

Psychotria is one of a handful of genera that contribute disproportionately to the species richness of tropical forests. Phylogenetic signal among physiological traits and along environmental gradients within these genera would suggest that niche evolution has occurred along trait axes not quantified in our study, such as defensive traits related to herbivory (Ehrlich & Raven 1967). The role of environmental filters and the reduced importance of resource competition in the understory may have important implications for community assembly of Gentry's (1982, 1989) Neotropical "species swarms," and even for canopy trees as they pass through life-history stages in the understory.

Acknowledgements

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Tables

	Trait Clustering		Trait Evenness		Phylogenetic Signal	
	Range	Variance	SDNNr	SDNDr	K	Mantel
$A_{\max}$	0.09 ± 0.99	0.04 ± 0.99*	-0.04 ± 0.94	-0.02 ± 0.89	0.2332	-0.0271
Respiration	0.09 ± 0.89	0.03 ± 0.93	-0.01 ±1.05	-0.10 ± 0.64	0.2494	-0.0242
LCP	0.14 ± 0.92*	0.09 ± 0.91	-0.01 ± 0.85	0.05 ± 0.68	0.1771	-0.0070
P_{shade}	-	-	-	-	0.2388	0.0601
ψ_t	-0.13 ± 0.95	-0.20 ± 0.85**	-0.04 ± 1.01	-0.05 ± 1.12	0.4360*	0.1187
K_l	-0.21 ± 0.78**	-0.19 ± 0.74**	-0.16 ± 1.09	-0.07 ± 0.78	0.3261	0.2823**
SLA	-0.05 ± 0.93*	-0.05 ± 1.00**	0.05 ± 0.88	-0.07 ± 1.37**	0.4020	0.0640
$P_{drought}$	-	-	-	-	0.3735*	0.1743

Table 2.1. Trait community structure and phylogenetic signal. For each community structure metric we report the mean standardized effect size (SES) ± 1 standard deviation. P-values < 0.05 are shown in boldface. Significance is indicated by asterisks (*,$P < 0.05$; **, $P < 0.01$). Traits are: maximum photosynthetic capacity ($A_{\max}$), leaf respiration, light compensation point (LCP), leaf water potential at turgor loss (ψ_t), leaf-specific stem conductivity (K_l), and specific leaf area (SLA). Experimental shade and drought performance are included.

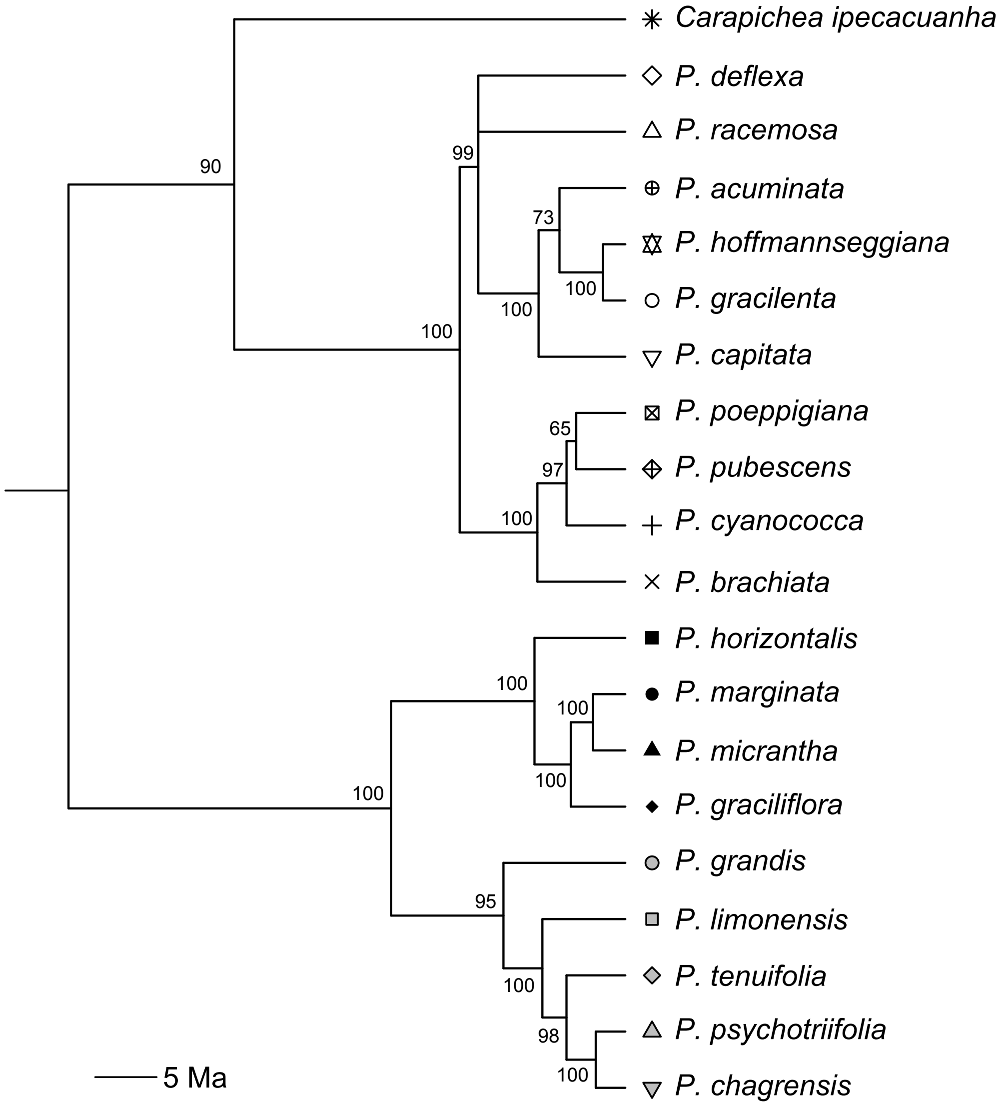

Figure 2.1. Community Phylogeny of the BCI *Psychotria.* A phylogram of 20 *Psychotria* representing the BCI community was pruned from a Bayesian consensus tree contructed from 25 *Psychotria* of central Panama as well as the outgroups *Coussarea curvigemmia* and *Faramea occidentalis*. Concatenated and partitioned nuclear ITS and cp *psbA-trnH* spacer DNA sequence was analyzed in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). The pruned community phylogeny shown here was used in subsequent analyses. Taxon bipartition labels represent Bayesian posterior probabilities. Species symbols relate to Figs 2.2, 2.4 and 2.5. Filled black and grey symbols indicate *Psychotria* subgenus *Psychotria*.

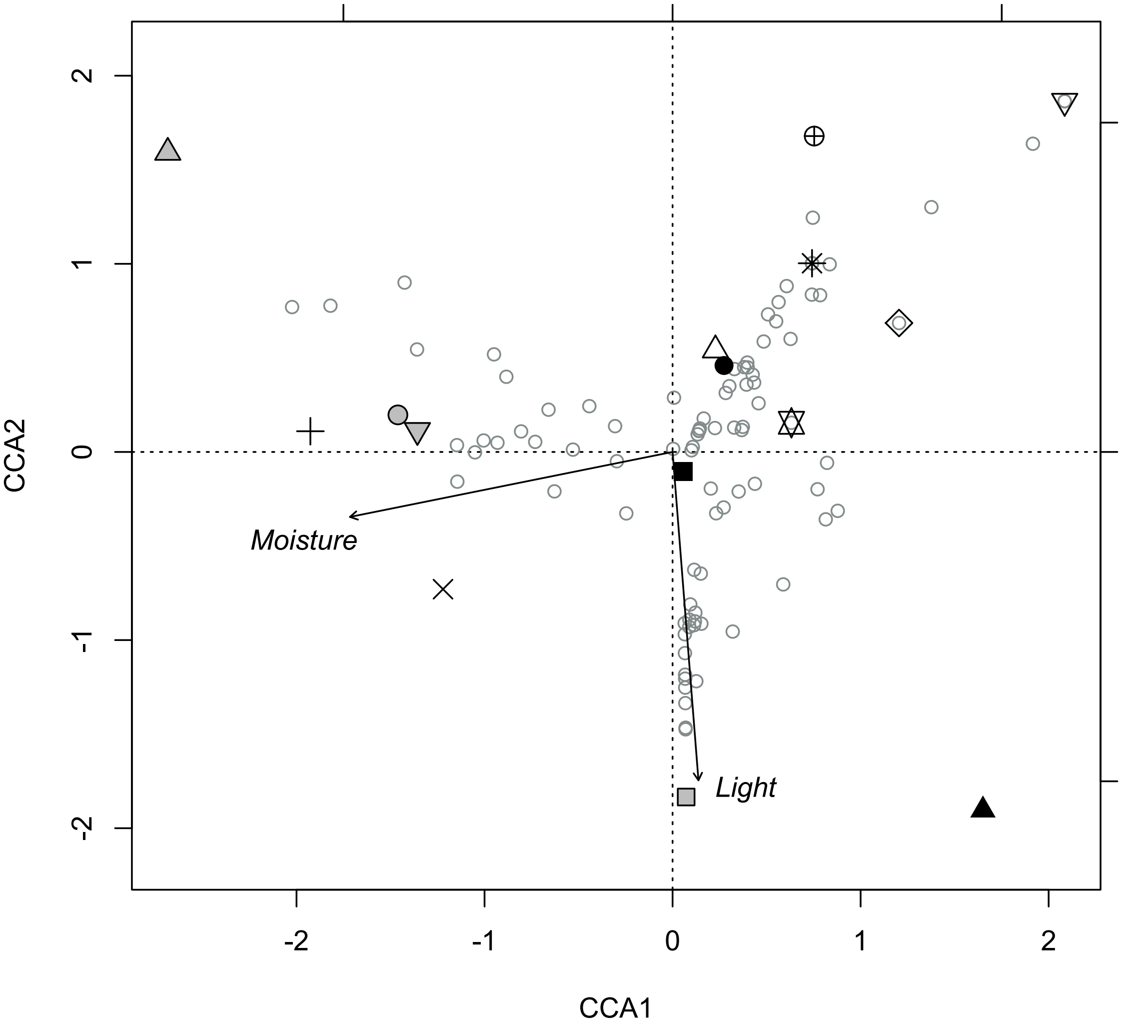

Figure 2.2. Ordination diagram representing the distributions of 15 *Psychotria* species along gradients of moisture and light availability on BCI (a) and phylogeny (b). Arrows represent environmental variables. Species symbols relate to Fig. 2.1. Filled black and grey symbols indicate *Psychotria* subgenus *Psychotria*.

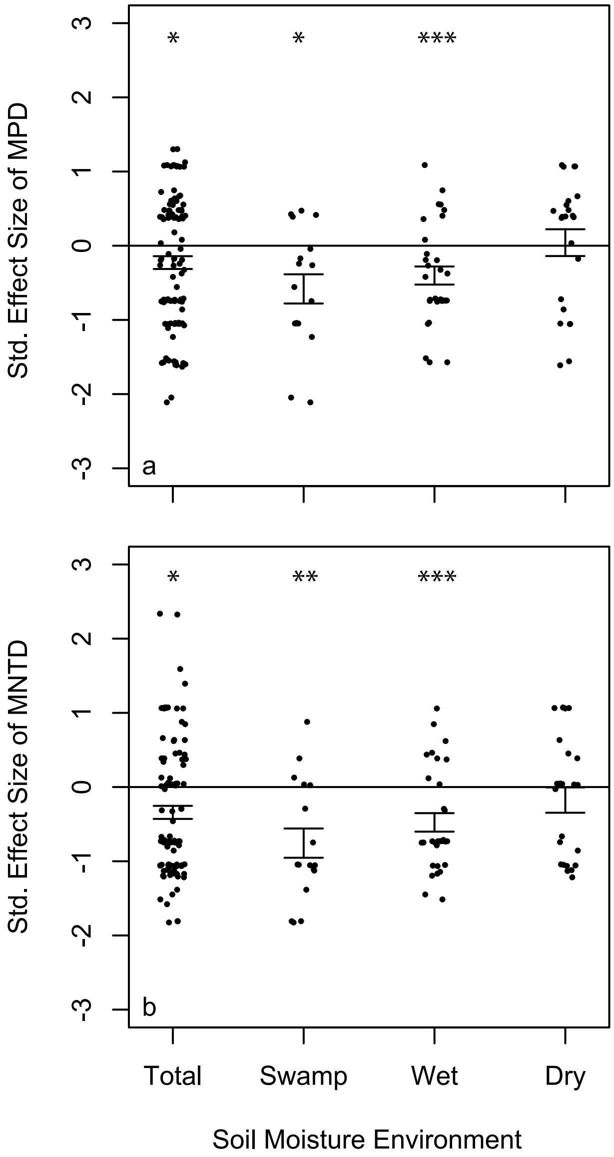

Figure 2.3. Phylogenetic structure of BCI *Psychotria* communities sampled in 3-m radius census plots. Points represent the standardized effect size of the mean phylogenetic distance (MPD) or the mean nearest-taxon distance (MNTD). Bars indicate the mean ± 1 standard error. Values presented here have the opposite sign of Webb's (2000) Net Relatedness Index (NRI) and Nearest Taxon Index (NTI), such that positive values indicate phylogenetic evenness, and negative values indicate phylogenetic clustering. Deviation from the null was tested using one-sided Wilcoxon rank sum tests (*, $P < 0.02$; **, $P < 0.01$; ***, $P < 0.005$). MPD and MNTD are shown with respect to all plots ($n = 104 \geq 2$ spp.) and subsets of plots located in the seasonal swamp ($n = 17$), and the wettest and driest 30% of the remaining well-drained plots. All analyses are limited to the 104 plots with two or more species.

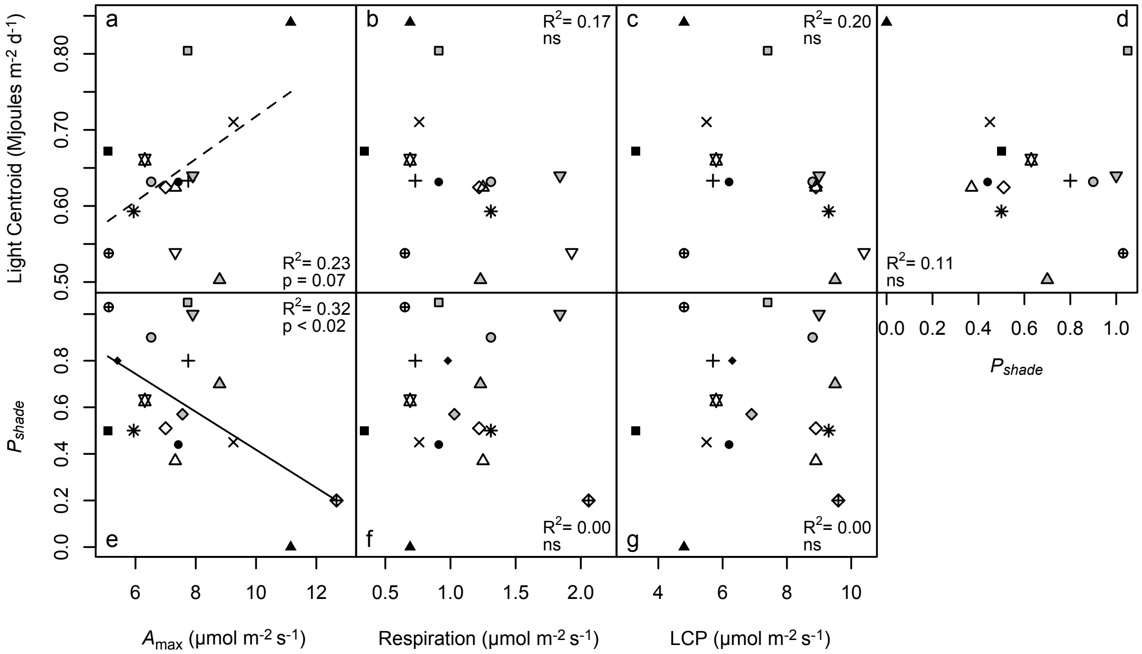

Figure 2.4. Scatter plots of species distributions (panels a-d) and experimental responses to gap and understorey environments (panels e-g) versus photosynthetic traits. Significant and marginally significant linear regressions are represented by solid and dashed lines, respectively. R^2 values and significance levels are given for each pairwise relationship (ns = not significant). The relationship in panel d is significant with the exclusion of *P. limonensis* ($R^2 = 0.44$, $P < 0.02$). Light centroid is the least squares estimate of each species weighted average distribution with respect to light intensity (Oksanen *et al.* 2009). Species symbols can be found in Fig. 2.1.

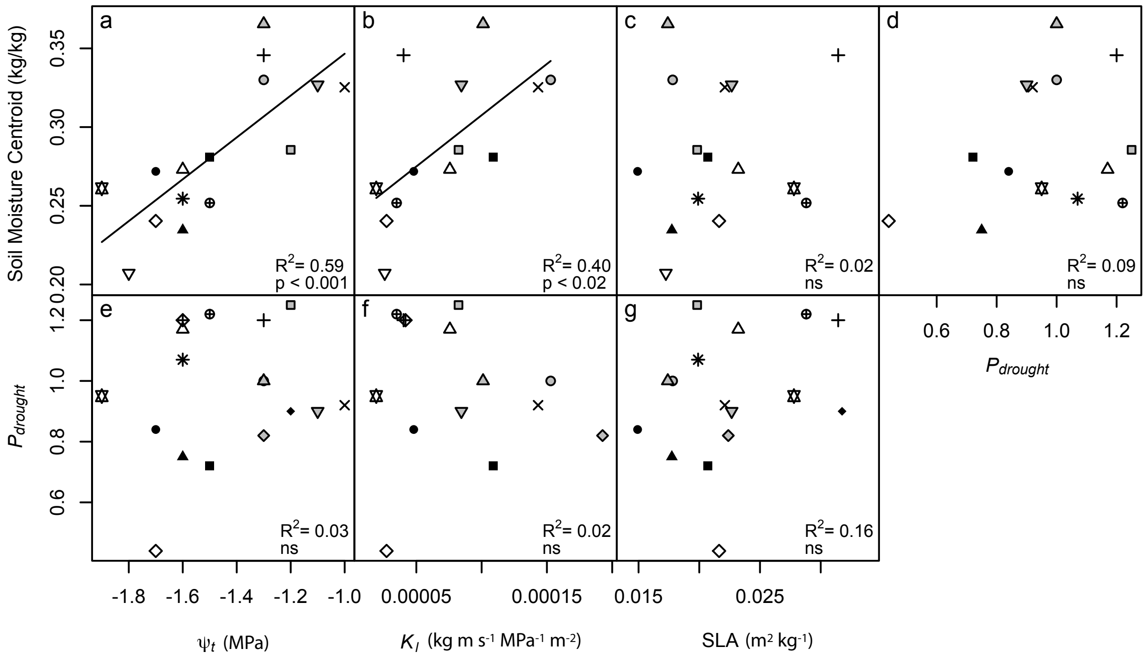

Figure 2.5. Scatter plots of species distributions (panels a-d), and experimental responses to control and irrigation treatments (panels e-g) versus hydraulic traits. Significant linear regresssions are represented by solid lines. R^2 and significance levels are given for each linear relationship (ns = not significant). Soil moisture centroid is the least squares estimate of each species weighted average distribution with respect to soil moisture (Oksanen *et al.* 2009). Species symbols can be found in Fig. 2.1.

Appendix S1: Phylogenetic Methods

The nuclear internal transcribed spacer region (ITS) and the chloroplast *psb*A-*trn*H spacer region were used as phylogenetic markers because of their variability and effective amplification in the study species. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA). The ITS locus was amplified using the LEU1 (Nepokroeff *et al.* 1999) and ITS4 (White *et al.* 1990) primers and a touchdown PCR protocol. The *psb*A-*trn*H locus was amplified using the *trn*Hf and *psb*A3f primers (Sang *et al.* 1997) and cleaned with ExoSAP-IT (USB Corporation, Cleveland, OH). Sequencing was performed for a total of 27 rubiaceous taxa in order to construct a single phylogeny that included several other species sampled in central Panama (*P. elata*, *P. glomerulata*, *P. nervosa*, *P. suerrensis*, and *Palicourea guianensis*) as well as *Coussarea curvigemmia* and *Faramea occidentalis*, two closely related genera within the Rubiaceae. Electropherograms were edited using Sequencher 4.6 (GeneCodes Corp., Ann Arbor, MI) and sequences were aligned using ClustalX 2.0.10 (Larkin *et al.* 2007) and Se-Al 2.0 (Rambaut 2007). After a partition homogeneity test conducted in PAUP* 4.10b (Swofford 2002) confirmed that the gene sequences could be combined for phylogenetic analysis (Farris *et al.* 1994), the ITS and *psbA-trnH* datasets were concatenated and analyzed through 6 million MCMC generations in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) under a mixed model with independent estimation of parameters for the GTR+I model of DNA sequence evolution as selected by a likelihood ratio test implemented in MrModeltest 2.3 (Nylander 2004). The ITS locus yielded 226 parsimony-informative characters out of 716, while the *psbA-trnH* locus yielded 93 parsimony-informative characters out of 496 total nucleotides, for a total of 319 parsimony-informative characters in the 1212 bp combined dataset. Log-likelihood values stabilized after 323,000 MCMC generations. The resulting Bayesian consensus tree was pruned to include only those species representing the BCI species pool. This community phylogeny (Fig. 2.6) was used in subsequent analyses of community structure.

Table 2.2: Photosynthetic traits measured for 18 *Psychotria* of BCI.

Species	Absorptance	Maximum Absorptance (μmol m^{-2} s^{-1})	Respiration (μmol m^{-2} s^{-1})	Quantum Yield (mol mol^{-1})	LCP (μmol m^{-2} s^{-1})
P. chagrensis	90.1	7.90	1.84	0.120	9.0
P. psychotriifolia	90.9^{b}	8.79	1.23	0.107	9.5
P. tenuifolia	87.7	7.56	1.03	0.124	6.9
P. limonensis	87.6	7.73	0.91	0.109	7.4
P. grandis	90.9^{b}	6.52	1.31	0.117	8.8
P. horizontalis	88.0	5.08	0.34	0.096	3.3
P. graciliflora	83.9^{a}	5.40	0.98	0.093	6.3
P. marginata	88.0	7.42	0.91	0.098	6.2
P. micrantha	90.2^{b}	11.15	0.91	0.112	4.8
C. ipecacuanha	89.1	5.94	1.31	0.096	9.3
P. poeppigiana	-	-	-	-	-
P. pubescens	90.0	12.67	2.06	0.100	9.6
P. cyanococca	86.5	7.75	0.73	0.100	5.7
P. brachiata	90.1	9.25	0.76	0.119	5.5
P. deflexa	91.4^{b}	7.00	1.22	0.105	8.9
P. racemosa	90.7	7.32	1.25	0.116	8.9
P. capitata	90.7^{b}	7.32	1.93	0.119	10.4
P. acuminata	90.0^{b}	5.11	0.65	0.108	4.8
P. gracilenta	-	-	-	-	-
P. hoffmannseggiana	88.9	6.31	0.69	0.100	5.8

Note: Means followed by different letters are significantly different ($p < 0.05$, Tukey's HSD). Sample size exceeds three for each species except *P. pubescens* for which sample size was 1.

Table 2.2. Photosynthetic traits measured for 18 *Psychotria* species of BCI. Means followed by different letters are significantly different ($p < 0.05$, Tukey's HSD). Sample size exceeds three for each species except *P. pubescens* for which the sample size was 1.

Table 2.3. Hydraulic traits measured for 20 BCI *Psychotria*.

	Leaf Water Potential (MPa)				
Species	Turgor Loss Point	Pre-Dawn	Mid-Day	Stem Conductivity (kg m s^{-1} MPa^{-1} m^{-2})	SLA (m^2 kg^{-1})
P. chagrensis	-1.1	-0.9	-1.5	8.45 X 10^{-5}	0.0227
P. psychotriifolia	-1.3	-0.4	-0.7	10.11 X 10^{-5}	0.0177
P. tenuifolia	-1.3	-1.8	-2.1	19.26 X 10^{-5}	0.0224
P. limonensis	-1.2	-0.5	-1.1	8.23 X 10^{-5}	0.0198
P. grandis	-1.3	-0.1	-0.5	15.29 X 10^{-5}	0.0178
P. horizontalis	-1.5	-1.8	-2.6	10.90 X 10^{-5}	0.0207
P. graciliflora	-1.2	-0.4	-1.1	-	0.0318
P. marginata	-1.7	-1.8	-2.4	4.80 X 10^{-5}	0.0149
P. micrantha	-1.6	-0.9	-1.4	-	0.0177
C. ipecacuanha	-1.6	-2.8	-3.2	-	0.0199
P. poeppigiana	-	-	-	5.21 X 10^{-5}	0.0271
P. pubescens	-1.6	-1.2	-1.9	4.17 X 10^{-5}	0.0499
P. cyanococca	-1.3	-1.0	-1.8	4.02 X 10^{-5}	0.0314
P. brachiata	-1.0	-0.2	-0.7	14.32 X 10^{-5}	0.0221
P. deflexa	-1.7	-3.1	-3.7	2.72 X 10^{-5}	0.0216
P. racemosa	-1.6	-2.4	-2.9	7.57 X 10^{-5}	0.0232
P. capitata	-1.8	-2.7	-3.4	2.58 X 10^{-5}	0.0173
P. acuminata	-1.5	-1.6	-2.7	3.49 X 10^{-5}	0.0288
P. gracilenta	-	-	-	5.08 X 10^{-5}	0.0251
P. hoffmannseggiana	-1.9	-1.6	-2.7	1.93 X 10^{-5}	0.0278

Note: Sample size exceeds 2 for K_l for each species except *P. tenuifolia*, *P. brachiata*, and *P. gracilenta*.

Table 2.3. Hydraulic traits measured for 20 BCI *Psychotria*. Sample size exceeds 2 for each species except *P. tenuifolia*, *P. brachiata*, and *P. gracilenta*.

Table 2.4: Survival and herbivory measured in four factorial experimental treatments

	Survival (percent surviving)				Herbivory (mean percent leaf damage)			
Species	Gap-Ctl	Gap-Irr	Und-Ctl	Und-Irr	Gap-Ctl	Gap-Irr	Und-Ctl	Und-Irr
P. chagrensis	0.80	1.00	0.80	0.90	0.060	0.010	0.012	0.010
P. psychotriifolia	0.70	0.90	0.40	0.40	0.125	0.062	0.027	0.024
P. tenuifolia	1.00	1.00	0.57	0.75	0.114	0.131	0.004	0.059
P. limonensis	0.70	1.00	0.75	0.50	0.106	0.026	0.018	0.047
P. grandis	0.70	1.00	0.60	0.60	0.095	0.091	0.011	0.036
P. horizontalis	1.00	1.00	0.50	0.78	0.097	0.045	0.040	0.013
P. graciliflora	0.70	1.00	0.50	0.60	0.132	0.057	0.015	0.115
P. marginata	1.00	1.00	0.44	0.60	0.051	0.079	0.011	0.037
P. micrantha	1.00	1.00	0.00	0.25	0.053	0.017	0.037	0.000
C. ipecacuanha	0.70	1.00	0.20	0.13	0.050	0.005	0.000	0.000
P. poeppigiana	-	-	-	-	-	-	-	-
P. pubescens	1.00	0.89	0.20	0.00	0.138	0.112	0.077	-
P. cyanococca	0.70	1.00	0.50	0.30	0.034	0.025	0.033	0.116
P. brachiata	0.80	0.91	0.25	0.33	0.033	0.041	0.000	0.047
P. deflexa	0.63	1.00	0.14	0.70	0.042	0.027	0.033	0.012
P. racemosa	0.80	1.00	0.17	0.00	0.138	0.064	0.006	0.000
P. capitata	-	-	-	-	-	-	-	-
P. acuminata	0.30	0.70	0.33	0.11	0.120	0.027	0.000	0.160
P. gracilenta	-	-	-	-	-	-	-	-
P. hoffmannseggiana	0.70	1.00	0.33	0.38	0.026	0.010	0.002	0.003

Note: Percent survival was measured after one year of growth, from December 1993 until December 1994. Herbivory data are included only for species represented by at least seven individuals in December 1993. Species mean level of herbivory was measured as a proportion of leaf area of most-recently flushed, mature leaves from three censuses of the experiment.

Table 2.4. Survival and herbivory measured in four factorial experimental treatments. Percent survival was measured after one year of growth, from December 1993 until December 1994. Herbivory data are included only for species represented by at least seven individuals in December 1993. Species mean level of herbivory was measured as a proportion of leaf area of most-recently flushed, mature leaves from three censuses of the experiment.

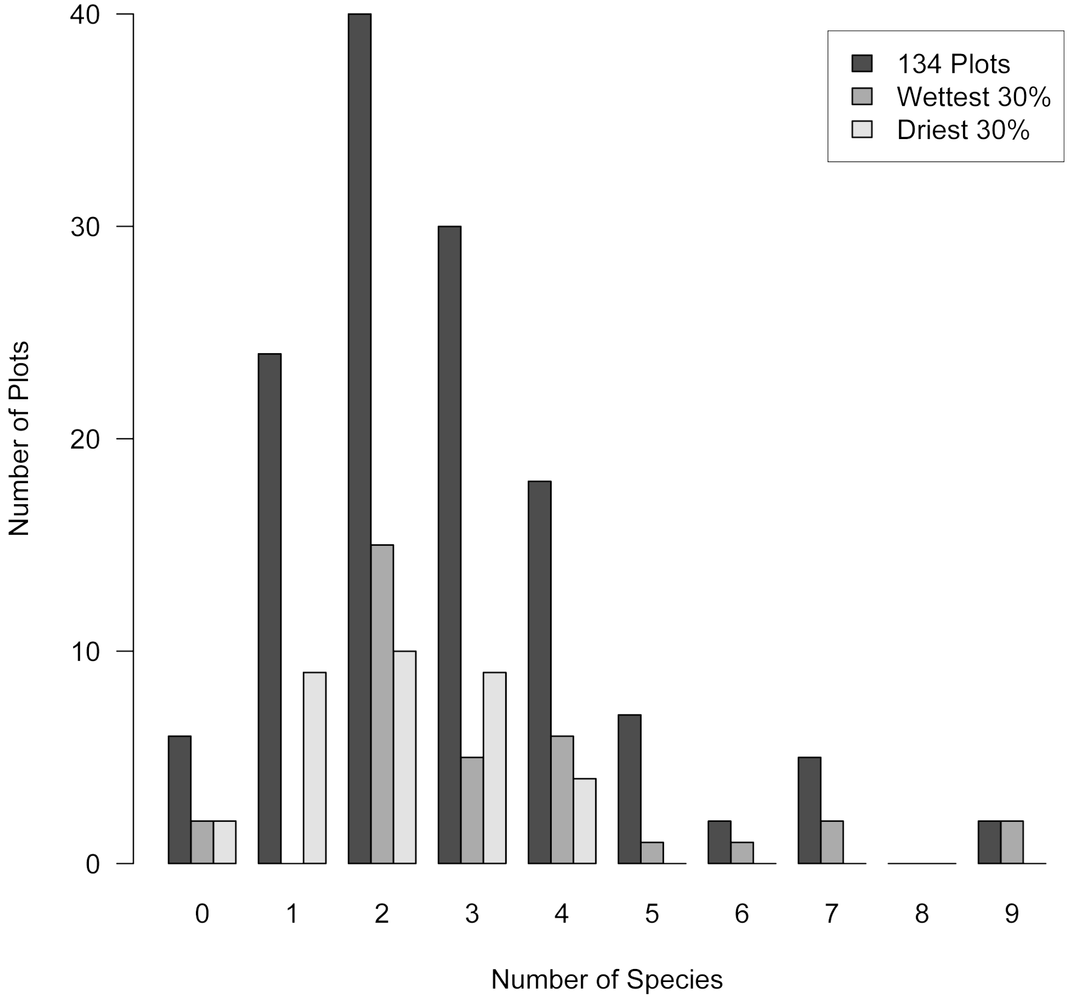

Figure 2.6. Species density of *Psychotria* on BCI, represented by the frequency histogram of number of randomly located, 3-m radius plots including different numbers of *Psychotria* species. The 34 wettest and 34 driest plots represent those above the 70% and below the 30% quantiles with respect to soil moisture, excluding plots in the seasonal swamp.

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Chapter 3: Fine-scale niche structure of Neotropical forests reflects legacy of the Great American Biotic Interchange

Abstract

The tendency of species to retain their ancestral niches may link processes that determine community assembly with biogeographic histories that span geological time scales. Biogeographic history is likely to have had a particularly strong impact on Neotropical forests due to the influence of the Great American Biotic Interchange, which followed emergence of a land connection between North and South America ca. 3 Ma. Here we examine the community structure, ancestral niches and ancestral distributions of the related, hyperdiverse woody plant genera *Psychotria* and *Palicourea* (Rubiaceae) in Panama. We find that 49% of the variation in hydraulic traits, a strong determinant of community structure, is explained by species' origins in climatically distinct biogeographic regions. Niche evolution models for a regional sample of 152 species indicate that ancestral climatic niches are associated with species' habitat distributions, and hence local community structure and composition, even millions of years after dispersal into new geographic regions.

Introduction

Recent advances in systematic biology have revealed a closer relationship between ecological and biogeographic processes than previously assumed (Ricklefs 2004; Wiens & Donoghue 2004; Ricklefs 2008; Wiens *et al.* 2010). The ancestral geographic distributions of organisms might exert lasting influences as they assemble into communities because of a phenomenon known as phylogenetic niche conservatism (PNC; Wiens *et al.* 2010), in which related taxa retain important aspects of the niche of their common ancestors. Exceptions to this general rule are not uncommon, but major evolutionary departures from ancestral niches typically involve instances of geographic or environmental isolation, in which alternative niches are uncontested (Baldwin & Sanderson 1998; Donoghue 2008). In the absence of such isolation or, conversely, in the presence of suitable migration corridors, Donoghue (2008) and others (Ackerly 2003; Wiens & Donoghue 2004) have suggested that "it is easier to move than to evolve." That is, it is more likely for organisms to migrate to habitats for which they are already adapted than to adapt to marginal or novel conditions *in situ*. This hypothesis has proved useful in explaining broad-scale patterns of biodiversity, such as the latitudinal gradient in diversity (a.k.a. the tropical conservatism hypothesis; Wiens & Donoghue 2004) and the recent radiation of cold-adapted Laurasian, even Himalayan, plant lineages in the Andean cordilleras (Bell & Donoghue 2005). Niche conservatism might also allow geographic history to influence the assembly of ecological communities over shorter time periods and at finer spatial scales (Stephens & Wiens 2004; Moen *et al.* 2009; Dexter *et al.* 2012), if the habitat affinities of species comprising contemporary ecological communities are similar to ancestral ecological niches that evolved in response to environments experienced elsewhere.

The biogeographic history of Neotropical forests has been dominated by two geologically recent events (Antonelli *et al.* 2009; Hoorn *et al.* 2010). The uplift of the northern Andean cordilleras (25-3 Ma; Parra *et al.* 2009) increased the climatic diversity of northwestern South America (Hoorn *et al.* 2010), while the subsequent emergence of the Isthmus of Panama at ca. 3 Ma (Coates & Obando 1996) made possible the Great American Biotic Interchange (GABI), which brought species with a history of selection in climatically distinct geographic regions into contact with novel environments and into competition with natives as well as co-immigrants.

If functional traits have tended to evolve *in situ* in response to selection pressure exerted by the local environment and community, taxa are likely to have departed from ancestral niches and show little evidence of PNC. Alternatively, if species tend to track preferred micro-environments after dispersal to new regions, then local species composition may reflect the geographic history of species or lineages prior to the interchange. These alternative assembly mechanisms have important implications for our understanding of species coexistence in tropical forests, which has proved an enduring challenge to ecological theory. Neutral Theory posits that niche differences are unimportant relative to stochastic drift and immigration (Hubbell 2001). Counter examples point to subtle but pervasive niche differences as evidence that communities are structured by competition, character displacement, and limiting similarity (Kraft *et al.* 2008). If such niche differences are contingent upon historical geographic mixing rather than *in situ* character evolution, it may suggest that the stabilizing effect of those niche differences plays a smaller role in community assembly than commonly assumed.

Here we address three questions concerning the influence of biogeographic history on the assembly of communities. Do species distributions in an environmentally heterogeneous locality reflect the geographic origins of member taxa? Was niche evolution significantly influenced by the geographic history of phylogenetic lineages? And finally, has the GABI enhanced phylogenetic and functional trait diversity in Neotropical tree communities?

To address these questions, we examine the biogeographic history, trait evolution, and community structure of members of two closely related and hyper-diverse genera *Psychotria* L. and *Palicourea* Aubl. (Rubiaceae), which comprise ca. 2,300 tropical woody plant species, including ca. 1000 species in the Neotropics (Taylor 1996). We find that ca. 50% of the variation in hydraulic traits, a strong determinant of community structure, is explained by species' origins in climatically distinct biogeographic regions. Niche evolution models for a regional sample of 152 species indicate that ancestral climatic niches are associated with species' habitat distributions, and hence local community structure and species composition, even millions of years after dispersal into new geographic regions.

Results

The community structure of Barro Colorado Island reflects biogeographic history

We have shown previously that assemblages of *Psychotria* and *Palicourea* found co-occurring within 134 small, 3-m radius plots on Barro Colorado Island (BCI), Panama, are more closely related than expected by chance (i.e. are phylogenetically clustered; Sedio *et al.* 2012). This pattern appears to be driven by habitat-filtering mediated by phylogenetically-conserved hydraulic traits that influence a species' tolerance of seasonal drought (Sedio *et al.* 2012).

To determine if local microhabitat patterns reflect biogeographic history, we first determined the ancestral geographic distributions (Ree & Smith 2008) of all nodes in a 203-taxon phylogeny of the tribe Psychotrieae (Fig. 3.1, see Methods). Nearly all Neotropical *Psychotria* found in Central and northern South America descend from a single lineage that diversified in Central America. *Palicourea* originated in northern South America, and shows evidence of several mid-Miocene (12-15 Ma) dispersal events into the Northern Andes and into the trans-Andean biogeographic region comprising the Chocó of western Colombia and the Panamanian Darién (Fig. 3.1a). In addition, some Andean *Palicourea* appear to have dispersed into Central America as long ago as 8 Ma. Many of the extant taxa we analyzed are widespread (Fig. 3.4); their geographic origins were inferred from the distributions of less widespread relatives (Ree & Smith 2008).

Comparing geographic origins with local microhabitats, we find that nearly 50% of the variation in species soil moisture distribution on BCI is explained by differences in the biogeographic area of origin of the study taxa (Fig. 3.1b and c). Likewise, biogeographic origin explains 51% of the variation in leaf water potential at turgor loss (ψ_t) among the BCI species (Fig. 3.1c). These associations suggest that by filtering species with similar hydraulic traits, fine-scale soil moisture environments on BCI effectively segregate species of *Psychotria* and *Palicourea* with shared biogeographic histories.

In order to further examine the association between geographic history and microhabitat preferences, we investigated broad climatic niche differences among species that may, in turn, reflect climatic differences between Neotropical geographic regions. We conducted a principal component analysis (PCA) of species distributions with respect to 20 climatic variables (see Methods). Four principal components (PC's) explain 94% of the variation in climatic niche (Table 3.1). PC1, defining 46% of the variance, is largely defined by elevation and associated temperature variables, while PC2, comprising 27% of climatic niche variance, largely reflects a latitudinal gradient in temperature seasonality (Table 3.1).

The PC3 axis is largely explained by precipitation and precipitation seasonality, comprising a gradient between dry, seasonal climates and wetter, less seasonal ones (Table 3.1). Although PC3 defines only 12% of the variance in species regional distributions, it provides the most insight regarding microhabitat distributions on BCI. Species that tolerate very negative leaf water potentials (ψ_t) and occur in dry microsites on BCI have significantly more positive scores along PC3 (Fig. 3.1c). This result suggests that species distributions with respect to regional climatic gradients, such as those defined by precipitation, are representative of their distributions with respect to local environmental variation, such as soil moisture heterogeneity (Engelbrecht *et al.* 2007), providing a potential causative link between geographic history and local community composition.

The influence of geographic history on hydraulic niche evolution

To test whether geographic history has constrained niche evolution of *Psychotria* and *Palicourea*, we compared the relative fit of eight alternative models of climatic niche evolution (Butler & King 2004). Three models represent scenarios in which geographic regions have had no influence on species' climatic niches (Fig. 3.2a-c). Alternatively, if geographic history has left a signature, it may be reflected in the parameter θ, the "optimum" niche toward which species are selected in a model of random walk with stabilizing selection (Butler & King 2004). If climatic niches are strongly conserved, broad clades may be fit by a single value of θ that reflects the climate of the region in which lineage diversification took place ("Clade History" models Fig. 3.2d-f). Finally, if climatic niche evolution has occurred on a timescale commensurate with that of the changing geographic history of the taxa, climatic niches may have evolved with respect to a θ defined by the region in which each species originated (the "Taxon History" model, Fig. 3.2g). We also examined a variant of the "Taxon History" model in which the only meaningful regional differences were between Andean and non-Andean regions (the "Andes History" model, Fig. 3.2f).

Niche evolution with respect to PC3 is best characterized by the "Taxon History" model (Fig. 3.2g) in which the selection optimum reflects the region of origin of each taxon in the phylogeny. Broad climatic differences between major regions of the Neotropics appear to influence the niche evolution of *Psychotria* and *Palicourea*, and to continue their association with the habitat distributions of widespread species after dispersal to new regions.

Community assembly and the Great American Biotic Interchange

To assess the generality of the patterns observed on BCI, we examined the geographic origins of the *Psychotria* and *Palicourea* assemblages documented at thirteen sites across the Neotropics (Fig. 3.3). The seven South American sites are dominated by lineages with South American origins, while the four Central American moist forests are inhabited by lineages with a long history in Central America, and by relatively recent immigrants from South America (Fig. 3.3a). Central American dry forests appear species poor and largely lacking in *Palicourea*.

The contribution of immigration within the last 3 Ma to the phylogenetic diversity of each site differs significantly by region (Fig 3.3b). The diversity of the four Central American moist forests is substantially more affected than that of sites east of the Andes ($p < 0.0001$; t-test), or in South America in its entirety ($p < 0.0001$). The effect on functional diversity as reflected in species climatic niches relative to PC3 is less substantial (Fig. 3.3c). Recent immigrants to sites east of the Andes appear to be functionally redundant with natives, whereas the effect varies widely among the four Central American moist forests, perhaps due to the influence of South American immigration prior to the closure of the land bridge (Fig. 3.1a, 3.3a).

Discussion

Our results indicate that the hydraulic traits and microhabitat niches of the *Psychotria* and *Palicourea* of BCI are associated with the region of origin of species prior to dispersing to central Panama during the Great American Biotic Interchange. This result suggests that the filtering of species with similar hydraulic traits over fine-scale soil moisture gradients on BCI not only results in phylogenetically clustered assemblages (Sedio *et al.* 2012), but effectively selects species of *Psychotria* and *Palicourea* with a shared biogeographic and climatic history.

An examination of the geographic origins of the *Psychotria* and *Palicourea* recorded in thirteen Neotropical forests suggests that the contribution of the GABI to local community composition has been widespread in humid Central America, but much less pervasive in South American forests, even those west of the Andes (Fig. 3.3), a pattern noted by botanists for the broader Neotropical flora (Gentry 1982). What has not been recognized previously is that recent biogeographic admixture has likely increased the phylogenetic and functional diversity of Central American forests relative to areas less influenced by the GABI. These results suggest that, due to the prevalence of ecologically conservative dispersal in community assembly, the phylogenetic and functional structure of local communities depend on the phylogenetic and climatic diversity of the source pool from which the communities were assembled, making community structure and function a product of biogeographic exchange (Coates & Obando 1996; Antonelli *et al.* 2009; Dexter *et al.* 2012).

The spatial scale at which community structure reflects the geographic history of species of *Psychotria* and *Palicourea* on BCI is striking. Associations between biogeographic history and large-scale climatic or macrohabitat preferences have been observed in plants in the Valerianaceae (Bell & Donoghue 2005) and in Emydid turtles (Stephens & Wiens 2004). In contrast, the hydraulic niches that characterize *Psychotria* and *Palicourea* with a common center of origin differentiate species on a scale of only a few meters in the forest understory (Sedio *et al*. 2012) and therefore bring biogeographic assembly into a spatiotemporal scale that is commensurate with competition and ecological coexistence.

Understanding the coexistence of hundreds, even a thousand, species of woody plants in some tropical forests has proved an enduring challenge and has stimulated some of the most influential, and controversial, theories of coexistence and community assembly (Wright 2002, Leigh *et al.* 2004). Hubbell's (2001) Neutral Theory posits that the stabilizing effect of species niche differences is less important than demographic stochasticity and immigration in the assembly of communities. Meanwhile, evidence of pervasive, though subtle, trait dispersion in communities may support a role for resource- and regeneration-based niche differences in stabilizing species coexistence (Kraft *et al.* 2008). If the hydraulic niches considered here were strongly governed by interactions that limit the similarity of co-occurring species or otherwise favored the exploitation of unoccupied niche space, *in situ* character evolution would be expected to erase the signature of a biogeographic interchange that took place 3 Ma. In contrast, our results suggest that hydraulic niche differences and their prevalence in tropical forest communities may be strongly influenced by historical contingency, making community structure and the niche differences of co-occurring species a product of biogeographic processes such as changes in dispersal barriers and corridors that connect regions with unique climates. If the patterns exhibited by two hyperdiverse genera of Rubiaceae pertain to other species rich plant genera (Gentry 1982), it would suggest that hydraulic niches, and possibly other easily-measured trait dimensions, contribute relatively little to the stabilization of coexistence in tropical forest communities (Wright 2002; Leigh *et al.* 2004). On the other hand, other niche dimensions may strongly stabilize coexistence, including those pertaining to interactions with natural enemies (Kursar *et al.* 2009). It remains unclear to what extent the major resource- and enemy-defined niche dimensions of tropical trees are characterized by *in situ* evolution or PNC.

Our results indicate that the comparatively slow rate of niche evolution relative to changes in geographic distribution has left a notable historical signature on the community structure of one of the most diverse plant clades on the planet, particularly where biogeographic interchange has been recent and pervasive as a result of the GABI. The importance of geographic mixing for the niche structure of Neotropical communities further suggests that widespread niche differences among co-occurring tropical trees may owe as much to the history of dispersal as to selection for niche segregation imposed by competitive interactions.

Methods

Taxonomy versus Clades

The taxonomy of the Psychotrieae does not yet reflect recent findings in molecular phylogenetics, which indicate that the generic name *Psychotria* comprises a polyphyletic group of species (Taylor 1996, Davidse *et al.* 2012). Here, we employ "*Psychotria*" to refer to the *Psychotria* subg. *Psychotria* clade and "*Palicourea*" to refer to the clade comprised of *Palicourea* plus *Psychotria* subg. *Heteropsychotria* (i.e. *Palicourea s. lat.*). These two groups represent 208 and 803 species, respectively, in the Neotropics (www.tropicos.org).

Molecular Methods and Phylogenetic Reconstruction

We compiled 247 internal-transcribed spacer (ITS) sequences from Psychotrieae and outgroup species for analysis. These ITS sequences included all Psychotrieae species represented in GenBank as of October 30, 2011, as well as close relatives used for outgroups, species studied by Paul *et al.* (2009), and 26 new Psychotrieae ITS sequences. New sequences were generated following protocols described in the Supplementary Information and sequenced in both directions at the University of Chicago Cancer Research Center. We discarded a subset of GenBank sequences that could not be reliably attributed to a verified species.

We aligned the 273 sequences using the MUSCLE algorithm (Edgar 2004) executed in Geneious 5.1.7 (Drummond *et al.* 2011). The resulting 856 bp alignment was then used to reconstruct the phylogeny in a Bayesian framework based on a general time reversible (GTR) model of molecular evolution, with gamma distributed rate variation and a proportion of invariable sites. We ran two concurrent runs of four chains on MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003), via the CIPRES web portal (Miller *et al.* 2011)[32], for 5×10^6 generations, sampling parameters every 100 generations and a burn-in of 5×10^4. A chronogram was generated using a PATHd8 (Britton *et al.* 2007) age-estimation of the 50% majority rule Bayesian consensus tree with the most recent common ancestor (MRCA) of the Psychotrieae alliance fixed at 48.7 Ma, and the crown node of the Psychotrieae fixed at 35.6 Ma (Bremmer & Eriksson 2009).

Ancestral Area Reconstruction

We reconstructed the evolution of geographic ranges in the Neotropical Psychotrieae using the maximum likelihood dispersal-extinction-cladogenesis model of Ree & Smith (2008) as implemented in “Lagrange”. We employed an unconstrained but temporally stratified model in which the Andes region was not available for dispersal until the oldest estimate of their origination around 25 Ma (Parra *et al.* 2009). Dispersal probabilities between regions were assumed to be symmetric.

The following five biogeographic regions were used in the analysis: a) Central America and the Caribbean, b) the Trans-Andes lowland forest comprised of the Chocó region of western Colombia and Ecuador, the Darién region of eastern Panama, and Magdalena Valley in Colombia; c) the Andes at elevations > 1500 m; d) the Amazon Basin, Guyana Highlands, and eastern Brazil; and e) Paleotropics, encompassing Asia, Africa, and Pacific islands (Fig. 3.1, inset). Alternative analyses in which distinct northern and central Andes regions were separated at the Huancabamba gap following Gentry (1982) differed little from results shown here because only a single species in our sample is restricted to the central Andes. The Andean region is separated here from the Amazonian and Chocó-Darién regions at ca. 1500 m based on recent, extensive floristic study of Rubiaceae in this region (Taylor 1996, 1999, 2004). Because the lower limits of montane forest vary locally, species are characterized as Andean or lowland based on the predominant area of their occurrence. Lastly, lowland savanna regions of northern Colombia and Venezuela and lowland xeric environments of coastal Peru and Chile (gray areas in Fig. 3.1, inset) were ignored in our analyses.

Climatic Data

A total of 21,115 georeferenced herbarium specimens pertaining to 152 species catalogued at the Missouri Botanical Garden (www.tropicos.org) were mapped using ArcCatalog 10.0 (ESRI, Redlands, CA; mean = 140 records/species, range = 3-1263). For each record, we extracted values for elevation and 19 climatic variables from the WorldClim database at 30-s resolution (Hijmans *et al.* 2005). The species mean for each of the 20 variables was calculated and a principal component analysis performed on 152 species pertaining to the Neotropical *Psychotria* and *Palicourea*, or ca. 15% of the combined hemispheric diversity of the two genera (Taylor 1996). PC1-PC4 explained more variation than expected based on a broken-stick distribution (Jackson 1993) for a total of 94%, and so were used in subsequent analyses. The first three PC's appear to describe geographic gradients in elevation, latitude, and precipitation, respectively, based on the loadings of climatic variables (Table 3.1).

Phylogenetic Comparative Analysis and Climatic Niche Evolution

We compared a series of eight alternative models that reflect alternative hypotheses concerning the influence of geographic history on niche evolution: a Brownian motion model (Felsenstein 1985), an Ornstein-Uhlenbeck (OU) model with a single selection optimum θ, a "white noise" model in which species niches are independent of phylogeny (Harmon *et al.* 2008), and five additional OU models in which θ was allowed to undergo a shift at phylogenetic nodes associated with historic dispersal events (Fig. 3.2; Butler & King 2004). The nodes subtending each of four clades with broadly distinct biogeographic histories were identified as candidates for optimum-shifts, so that the most complex model included a distinct θ parameter for each of the four geographic regions plus the parameters σ^2 (strength of drift) and α (strength of selection) for a total of six parameters. Evolution models were fit independently for each of the four PC axes using the R package 'ouch' (Butler & King 2004) and compared using size-corrected AIC weights. Evolution models for PC1-PC4 can be found in Table 3.3.

Statistical Analysis

We employed phylogenetic generalized least squares (PGLS) linear regression to examine the relationship between species PC factor scores and hydraulic traits and soil moisture distributions among the 20 species occurring on BCI using the 'caper' package in R (Revell 2010, Orme 2012). Pagel's (1999) λ was estimated for PC3 with respect to the regional phylogeny of 147 taxa, and the ML value 0.063 was used for subsequent PGLS linear regression of PC3 on local soil moisture distribution among the BCI species. PGLS ANOVA with simultaneous maximum likelihood estimation of Pagel's λ was used to analyze the proportion of variation in local soil moisture preferences and hydraulic traits explained by geographic center of origin for the *Psychotria* and *Palicourea* found on BCI.

Community Structure and the Great American Biotic Interchange

To assess the generality of the patterns observed on BCI, we examined the geographic origins of the *Psychotria* and *Palicourea* assemblages documented at thirteen sites across the Neotropics (Fig. 3.3, Table 3.3). We measured the contribution of immigration within the last 3 Ma to phylogenetic diversity at each site by comparing Faith's (1992)

PD with respect to the native flora to a distribution of expected PD pertaining to samples of equal species richness of the extant flora of each site using the R package ‘picante’ (Kembel *et al*. 2008). In addition, we estimated the contribution of recent South American immigrants to the functional diversity of each site by measuring the variance of native species factor scores relative to PC3 with the variance expected from samples of equal species richness of the extant flora of each forest site. We more directly measured the influence of the GABI on the functional diversity of BCI by conducting the same procedure with respect to species soil moisture habitat distributions and hydraulic traits. In both types of analysis, we interpreted the negative of the standardized effect size as a measure of phylogenetic or functional diversity.

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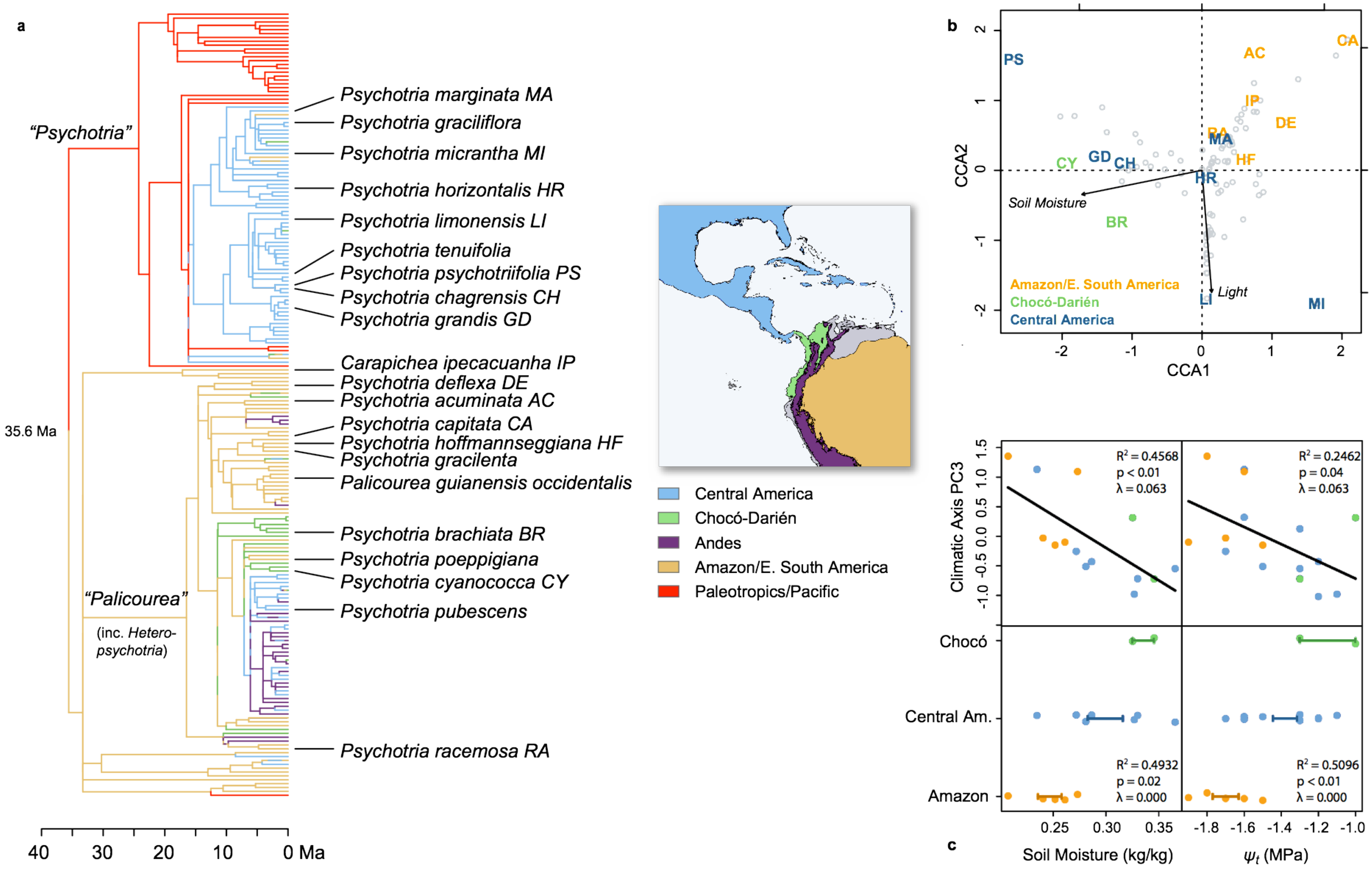

a
"Psychotria"
"Palicourea"
(inc. Hetero-psychotria)
35.6 Ma
40
30
20
10
0 Ma
Psychotria marginata MA
Psychotria graciliflora
Psychotria micrantha MI
Psychotria horizontalis HR
Psychotria limonensis LI
Psychotria tenuifolia
Psychotria psychotriifolia PS
Psychotria chagrensis CH
Psychotria grandis GD
Carapichea ipecacuanha IP
Psychotria deflexa DE
Psychotria acuminata AC
Psychotria capitata CA
Psychotria hoffmannseggiana HF
Psychotria gracilenta
Palicourea guianensis occidentalis
Psychotria brachiata BR
Psychotria poeppigiana
Psychotria cyanococca CY
Psychotria pubescens
Psychotria racemosa RA
Central America
Chocó-Darién
Andes
Amazon/E. South America
Paleotropics/Pacific
b
CCA1
CCA2
PS
CA
AC
IP
DE
RA
MA
HF
CY
GD
CH
HR
Soil Moisture
BR
Light
LI
MI
Amazon/E. South America
Chocó-Darién
Central America
-2
-1
0
1
2
c
Climatic Axis PC3
R² = 0.4568
p < 0.01
λ = 0.063
R² = 0.2462
p = 0.04
λ = 0.063
R² = 0.4932
p = 0.02
λ = 0.000
R² = 0.5096
p < 0.01
λ = 0.000
Chocó
Central Am.
Amazon
-1.0
-0.5
0.0
0.5
1.0
1.5
0.25
0.30
0.35
-1.8
-1.6
-1.4
-1.2
-1.0
Soil Moisture (kg/kg)
ψt (MPa)

Figure 3.1. Biogeographic history and community structure of *Psychotria* and *Palicourea*. The relationship of habitat distribution and hydraulic traits is indicated with respect to climatic niche and biogeographic region of origin among 20 *Psychotria* and *Palicourea* of BCI. (see Methods for discrepancy between clades and taxonomic nomenclature) (a) Ancestral area reconstruction. Branch color indicates the geographic region in which the branch originated. (b) Canonical correspondence analysis of the habitat distributions of the 15 most abundant species censused in 134 3-m radius plots on BCI (Sedio *et al.* 2012) showing soil moisture and light as important correlates of species composition. Abbreviations for species follow complete names provided in Fig. 3.1a and their color indicates the species' geographic region of origin (c) Phylogenetic generalized least squares linear regression of species climatic niche with respect to PC3 and PGLS ANOVA of geographic origin vs. species weighted mean distribution with respect to soil moisture on BCI and leaf water potential at turgor loss (ψ_t); λ indicates phylogenetic signal.

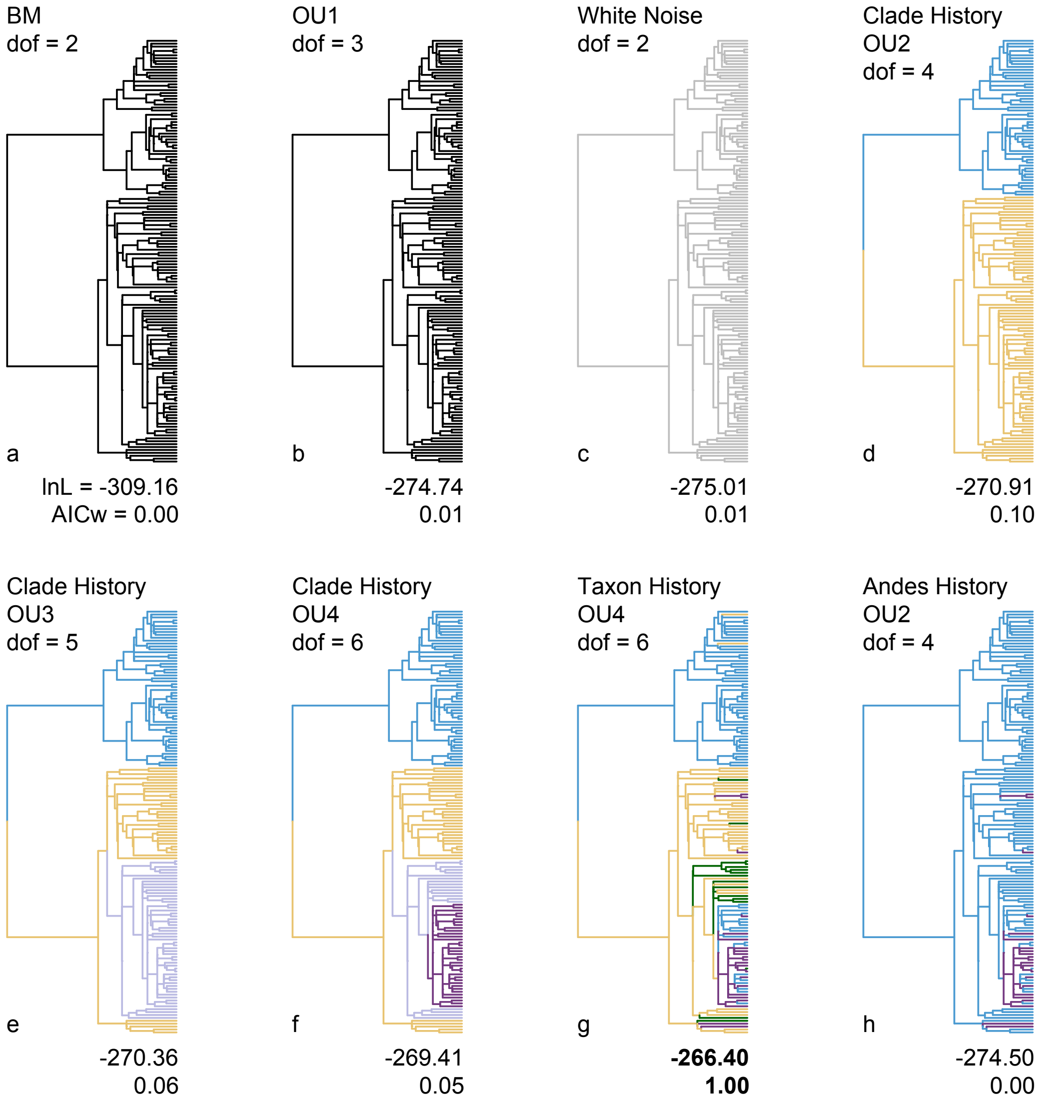


Figure 3.2. Comparison of eight alternative models of climatic niche evolution. Log-likelihoods and size-corrected AIC weights are shown for models pertaining to climatic niche axis PC3. Complete results for all four PC's are found in Table 2. (a) Brownian Motion model in which species randomly drift from the niche of their ancestors (Felsenstein 1985), (b) Ornstein-Uhlenbeck model of random drift with stabilizing-selection in which selection increases as species drift further from a single optimal climatic niche (Butler & King 2004), (c) a "white noise" model of evolution, in which species' climatic niches are drawn from a normal distribution without regard to their phylogenetic relationships or geographic history (Harmon *et al.* 2008), (d-f) random drift

with stabilizing selection toward one of several optimal niches that differ between clades with distinct geographic histories, (g) model in which species climatic niches evolve with respect to an optimum niche defined by the geographic region in which each taxon originated and (h) a variant of G in which the only meaningful differences are between Andean and non-Andean regions. Model G is the best-fit model.

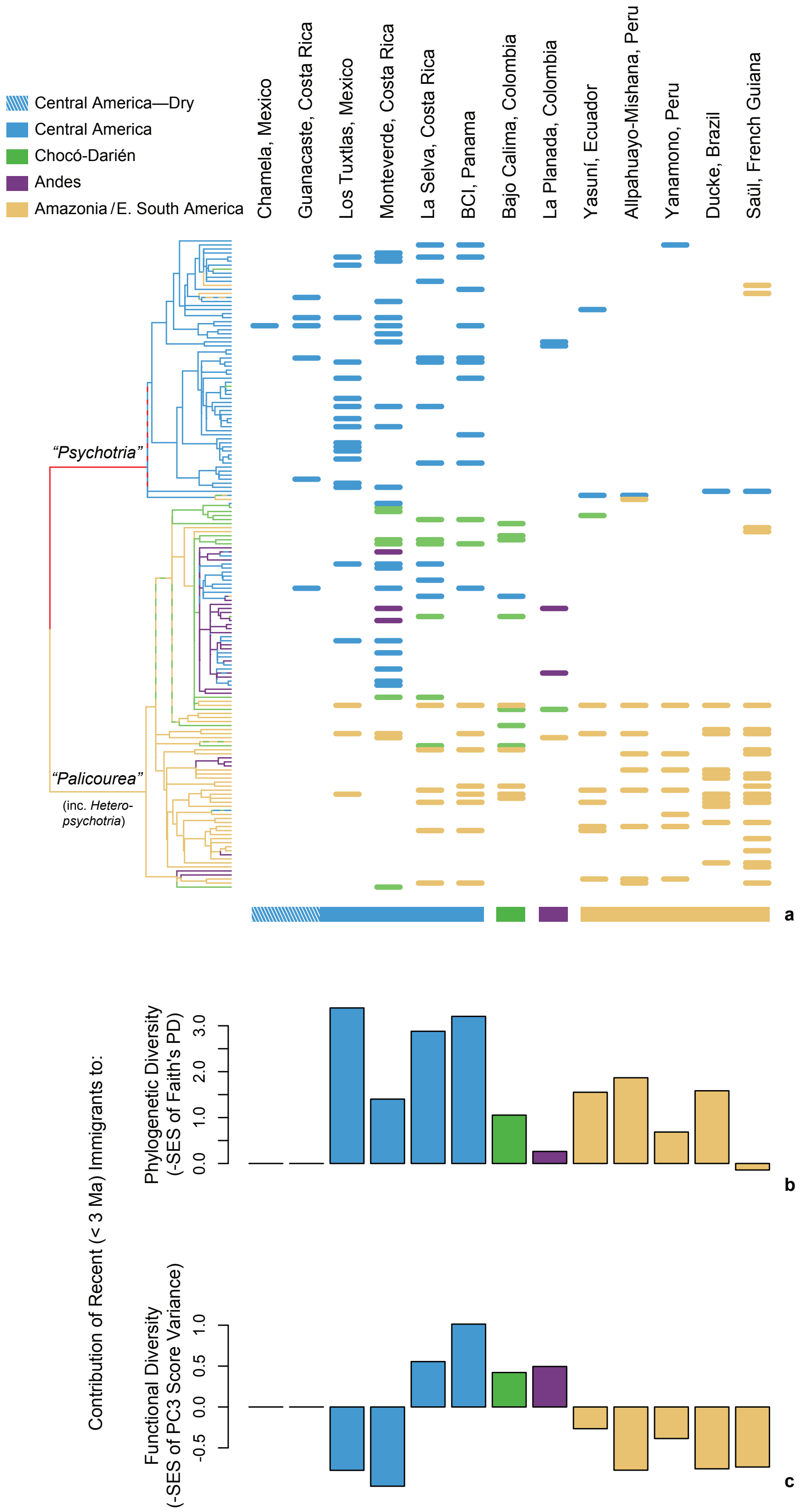

Central America—Dry
Central America
Chocó-Darién
Andes
Amazonia / E. South America
Chamela, Mexico
Guanacaste, Costa Rica
Los Tuxtlas, Mexico
Monteverde, Costa Rica
La Selva, Costa Rica
BCI, Panama
Bajo Calima, Colombia
La Planada, Colombia
Yasuní, Ecuador
Allpahuayo-Mishana, Peru
Yanamono, Peru
Ducke, Brazil
Saül, French Guiana
"Psychotria"
"Palicourea"
(inc. Hetero-psychotria)
a
Contribution of Recent (< 3 Ma) Immigrants to:
Phylogenetic Diversity (-SES of Faith's PD)
0.0
1.0
2.0
3.0
b
Functional Diversity (-SES of PC3 Score Variance)
-0.5
0.0
0.5
1.0
c

Figure 3.3. Biogeographic history of *Psychotria* and *Palicourea* at thirteen Neotropical sites. Geographic history and species composition are shown in (a) where the colors indicate ancestral areas of origin. The upper clade is “Psychotria”. The contribution of immigrants within the last 3 Ma to the phylogenetic (b) and functional (c) diversity of each site reveals phylogenetic and functional asymmetry in the Great American Biotic Interchange. Floristic inventories of each site can be found in Table 3.3. The region in which each site is located is indicated at the bottom of (a).

Variable	PC1	PC2	PC3	PC4
Elevation	-0.30	0.18	-0.06	0.04
Bio1	0.30	-0.16	0.08	-0.06
Bio2	-0.17	-0.07	0.40	0.22
Bio3	0.02	0.36	0.14	-0.27
Bio4	-0.06	-0.33	0.10	0.37
Bio5	0.26	-0.24	0.15	0.04
Bio6	0.32	-0.05	-0.03	-0.17
Bio7	-0.15	-0.25	0.26	0.34
Bio8	0.28	-0.21	0.12	0.00
Bio9	0.31	-0.12	0.03	-0.10
Bio10	0.29	-0.20	0.08	-0.01
Bio11	0.31	-0.10	0.06	-0.13
Bio12	0.21	0.26	-0.15	0.27
Bio13	0.19	0.11	-0.38	0.33
Bio14	0.14	0.29	0.35	0.15
Bio15	-0.09	-0.29	-0.37	0.16
Bio16	0.20	0.14	-0.32	0.35
Bio17	0.16	0.30	0.33	0.13
Bio18	0.18	0.21	0.17	0.45
Bio19	0.23	0.25	-0.12	0.01
Eigenvalue	9.10	5.41	2.50	1.81
% Variance	45.48	27.03	12.49	9.06
Pagel's λ	0.00	0.67	0.06	0.10

Table 3.1. Principal components of climatic niche variation. Factor loadings and squared standard deviations (eigenvalues) are shown for the four principal components of climatic niche variation among 152 Neotropical *Psychotria* and *Palicourea*. Bioclimatic variables: Bio1 = annual mean temperature; Bio2 = mean diurnal temperature range; Bio3 = temperature isothermality; Bio4 = temperature seasonality; Bio5 = maximum temperature of warmest month; Bio6 = minimum temperature of coldest month; Bio7 = temperature annual range; Bio8 = mean temperature of wettest quarter; Bio9 = mean temperature of driest quarter; Bio10 = mean temperature of warmest quarter; Bio11 = mean temperature of coldest quarter; Bio12 = annual precipitation; Bio13 = precipitation of wettest month; Bio14 = precipitation of driest month; Bio15 = precipitation seasonality; Bio16 = precipitation of wettest quarter; Bio17 = precipitation of driest quarter; Bio18 = precipitation of warmest quarter; Bio19 = precipitation of coldest quarter. Pagel's (1999) λ can be interpreted as the degree of phylogenetic signal, with λ = 0 indicating no phylogenetic signal and λ = 1 a Brownian motion model. PC2 and PC4 exhibit weak phylogenetic signal (95% CI PC2 λ 0.242 to 0.877; PC4 λ 0.002 to 0.614). PC1 and PC3 do not depart significantly from λ = 0 (95% CI PC1 λ ranges to 0.556; PC3 λ ranges to 0.133).

Climatic Niche Axis		BM	OU1	White Noise	Clade History OU2	Clade History OU3	Clade History OU4	Taxon History OU4	Andes History OU2
	dof	2	3	2	4	5	6	6	4
PC1	lnL	-417.22	-372.34	-372.34	-372.34	-372.15	-370.53	-369.00	-370.61
	AICc	838.51	750.85	748.76	752.95	754.73	753.66	750.59	749.49
	AICw	0.0000	0.3529	1.0000	0.1231	0.0508	0.0863	0.4010	0.6946
PC2	lnL	-346.54	-327.07	-330.64	-324.09	-322.59	-322.41	-323.44	-327.04
	AICc	697.17	660.30	665.37	656.46	655.60	657.41	659.47	662.37
	AICw	0.0000	0.0951	0.0076	0.6509	1.0000	0.4043	0.1444	0.0339
PC3	lnL	-309.16	-274.74	-275.01	-270.91	-270.36	-269.41	**-266.40**	-274.50
	AICc	622.40	555.65	554.10	550.09	551.13	551.42	**545.40**	557.29
	AICw	0.0000	0.0059	0.0129	0.0957	0.0569	0.0494	**1.0000**	0.0026
PC4	lnL	-306.32	-265.30	-265.30	-260.38	-259.12	-257.96	**-253.91**	-264.80
	AICc	616.72	536.77	534.69	529.04	528.67	528.51	**520.42**	537.88
	AICw	0.0000	0.0003	0.0006	0.0134	0.0161	0.0175	**1.0000**	0.0002

Table 3.2. Alternative models of niche evolution with respect to four climatic axes. Log-likelihoods, size-corrected AIC scores (AICc), and relative Akaike weights (AICw) for eight alternative models of climatic niche evolution. Best-fit models that are significantly better than the second-best model by a likelihood ratio test are shown in boldface. Trait evolution model abbreviations are Brownian Motion (BM), and Ornstein-Uhlenbeck (OU).

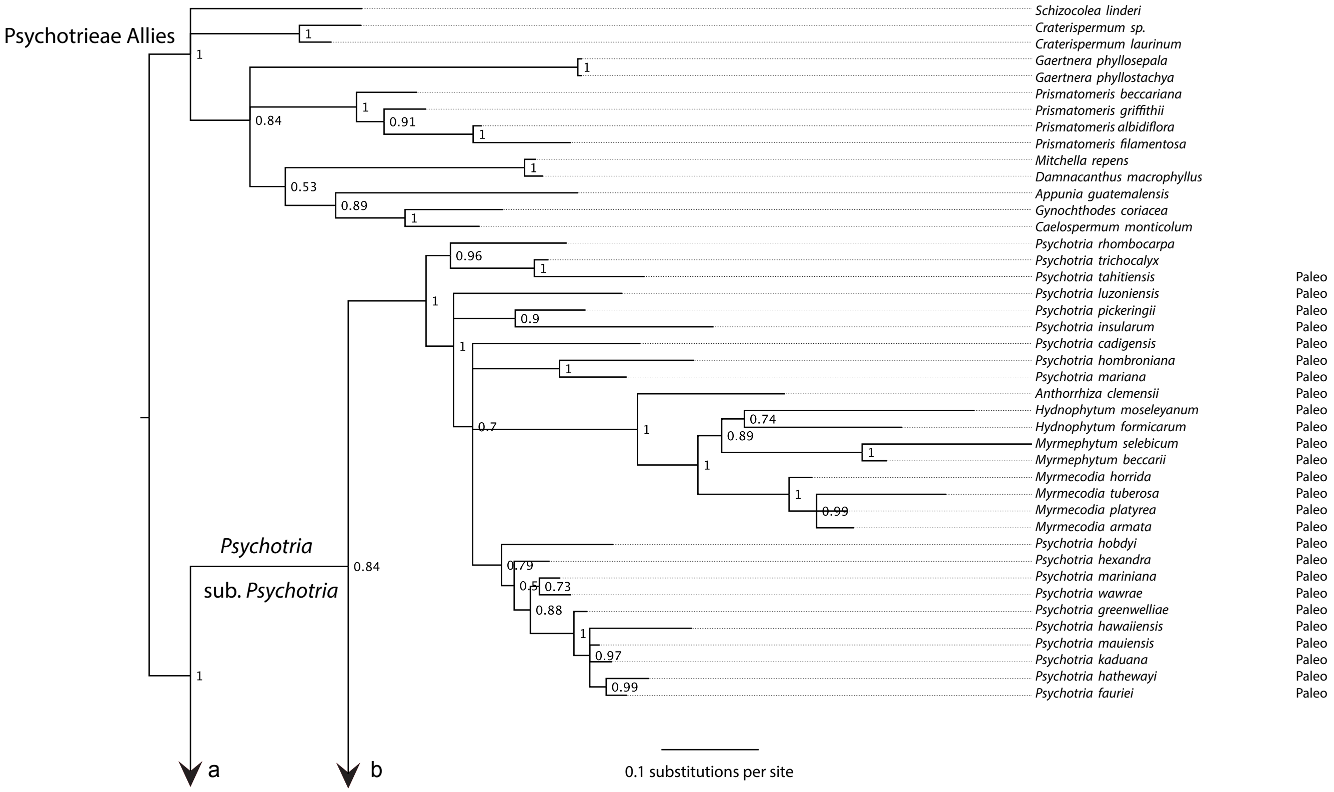


Figure 3.4. 50% majority rule Bayesian consensus tree. Geographic distribution is indicated for species included in the ancestral area reconstruction, wherein the following abbreviations are used: Cent Am—Central America and the Caribbean; Chocó—the Chocó region of western Colombia and Ecuador, the Darién region of eastern Panama, and Magdalena Valley, Colombia; Andes—Andean montane forests above 1500 m in elevation; Amazon—the bulk of northern South America east of the Andes, including the Amazon Basin, the Guiana Shield, and eastern Brazil; Paleo—Asia, Africa, and Pacific islands.

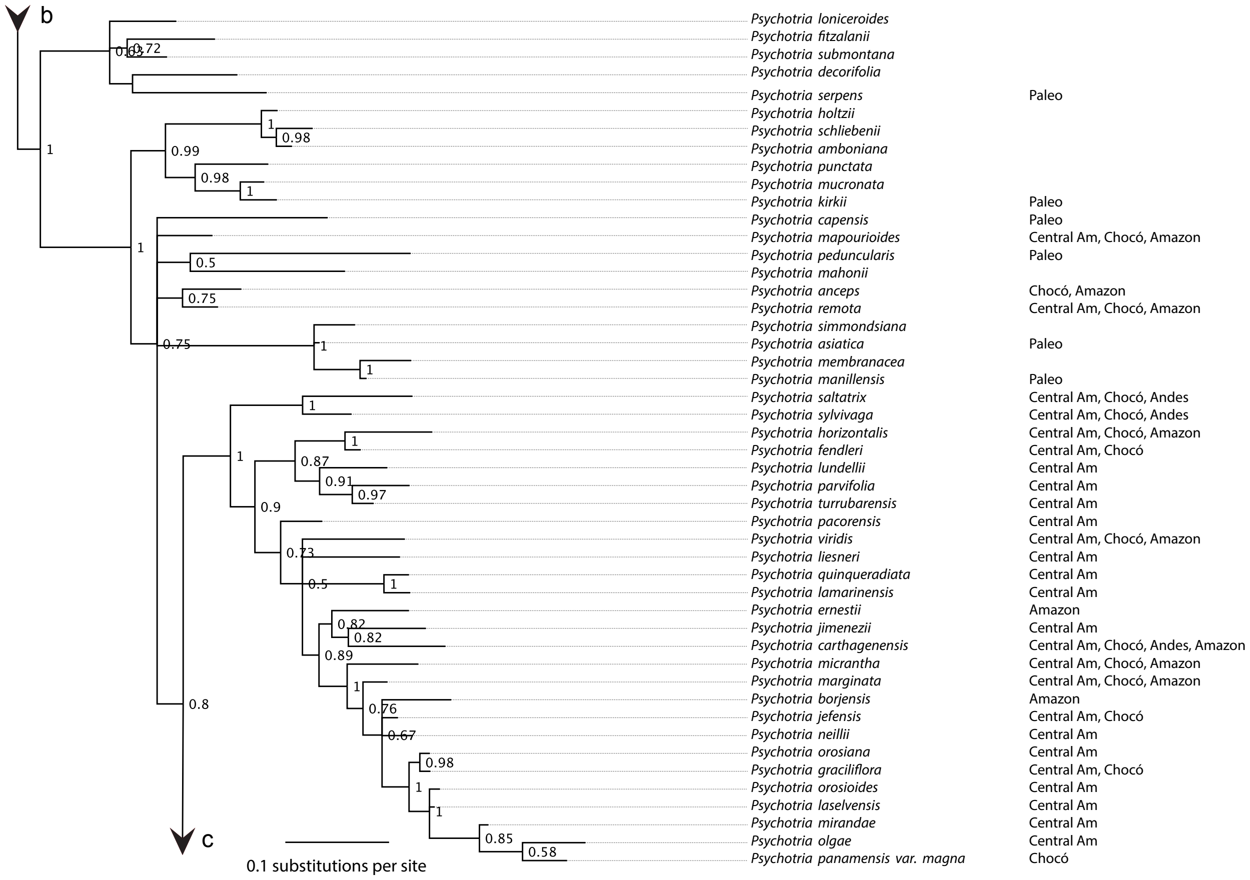
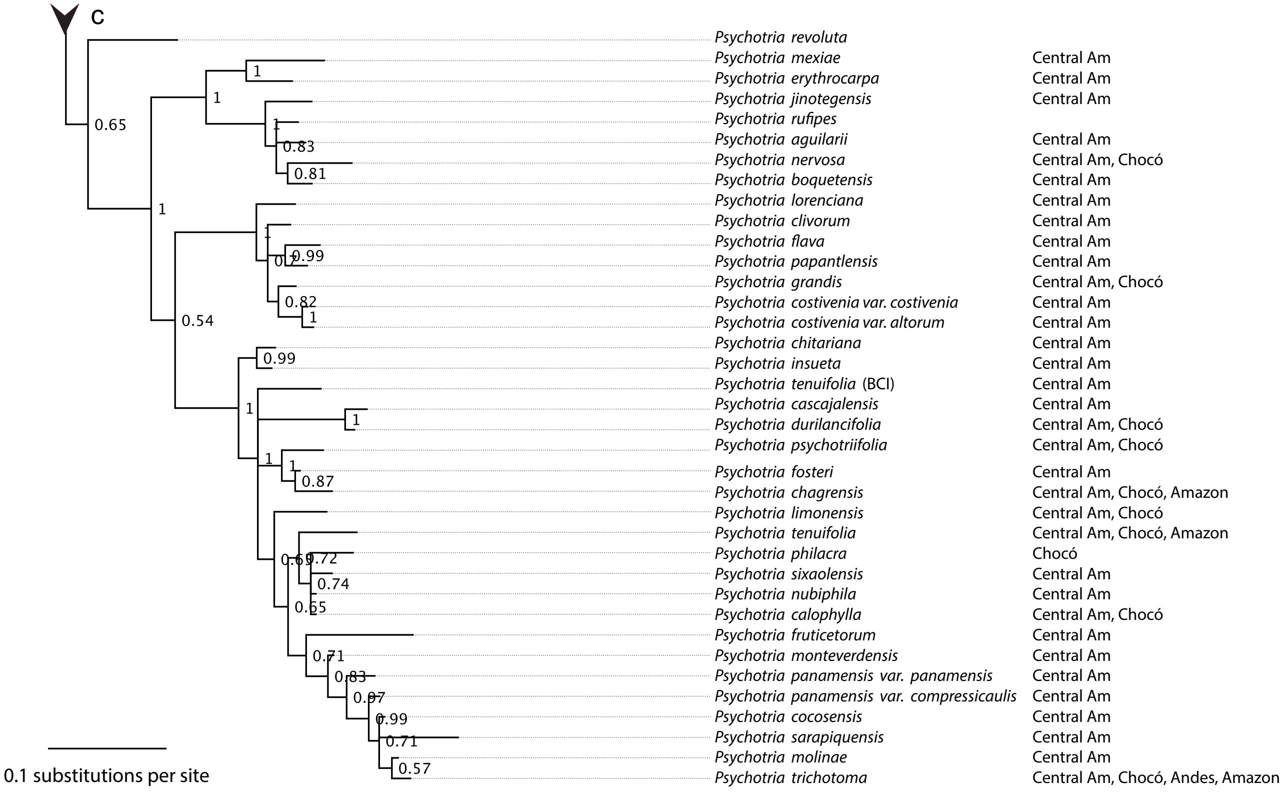


Figure 3.4, *continued*.



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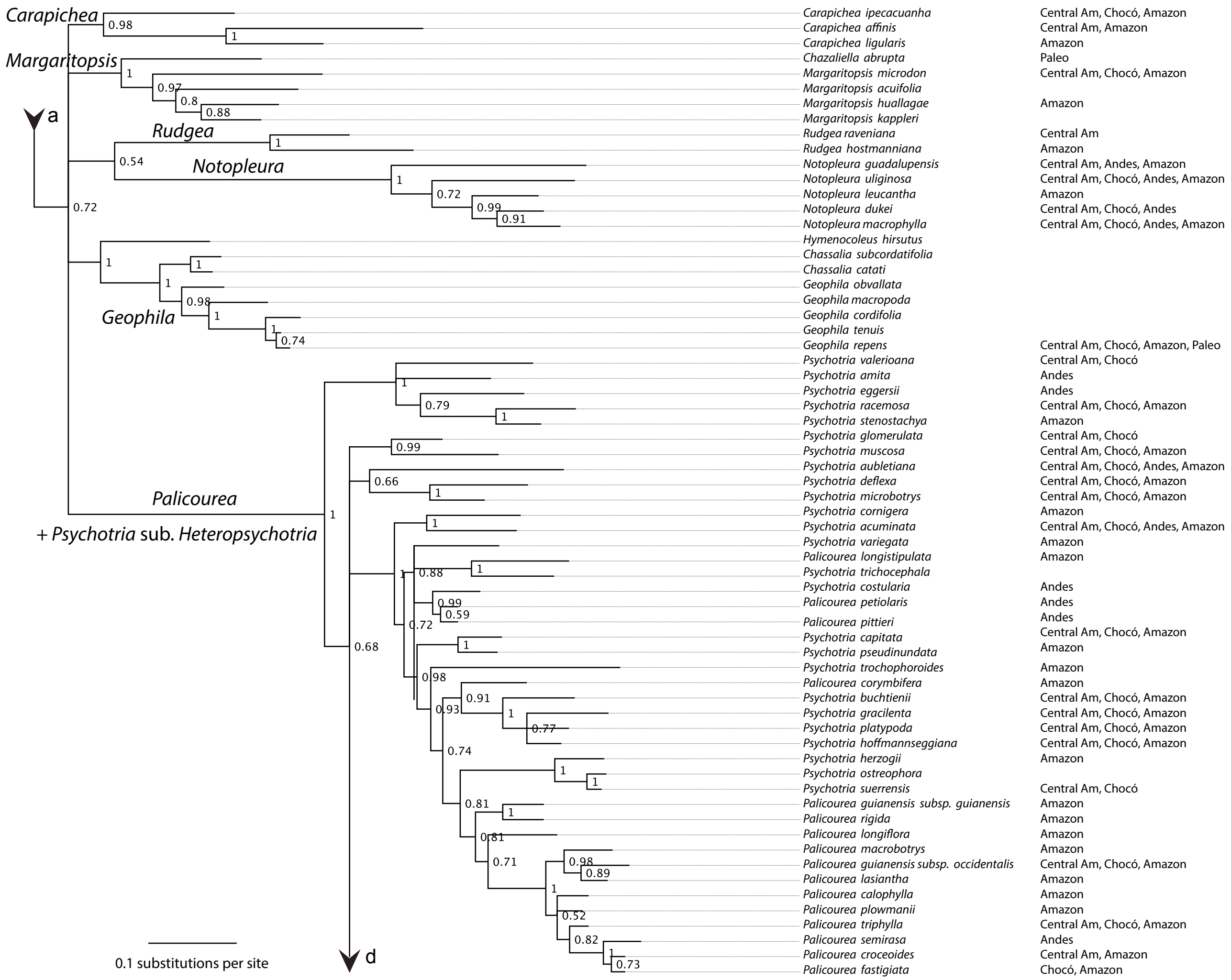

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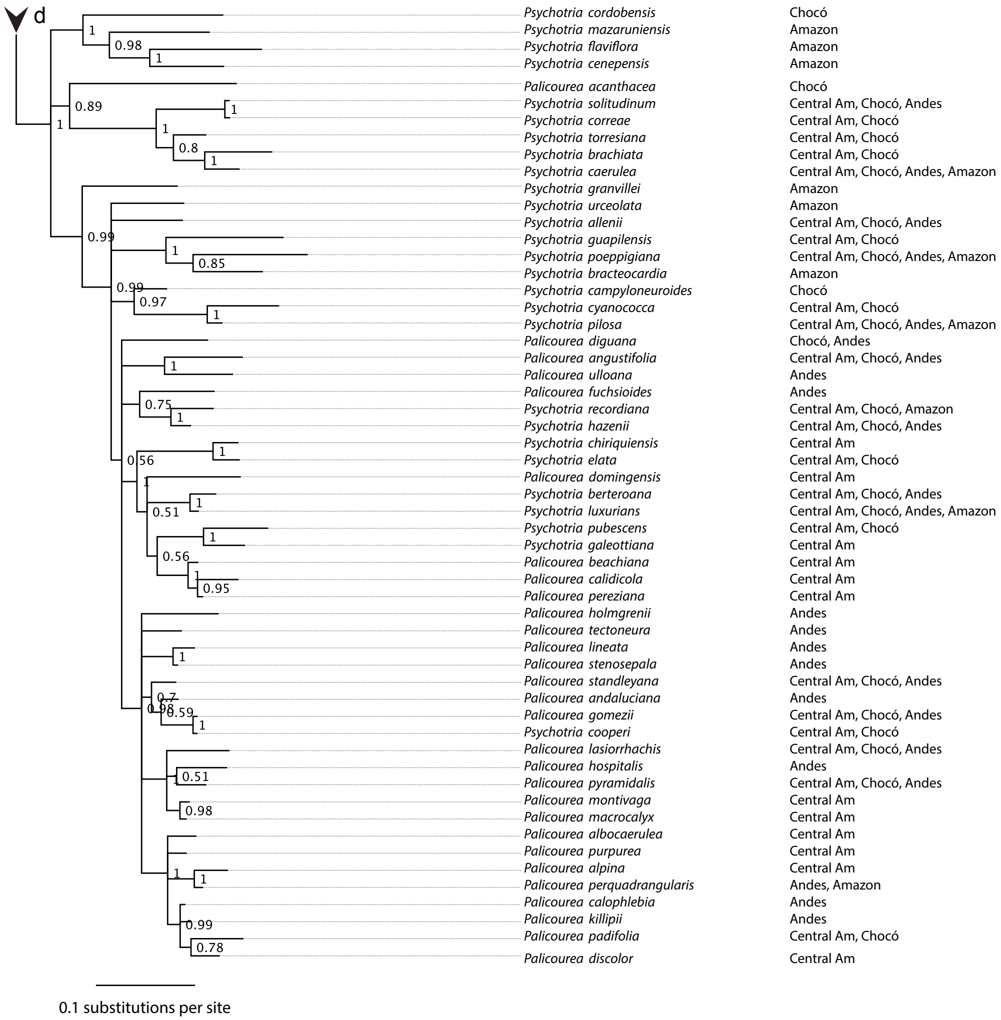

Figure 3.4, *continued*.

	Central America—Dry		Central America—Moist			
Site	Chamela, Mexico	Guanacaste, Costa Rica	Los Tuxtlas, Mexico	Monteverde, Costa Rica	La Selva, Costa Rica	BCI, Panama
Area (ha)	13,142	34,000	155,122	10,500	1,600	1,500
Psychotria s. str. [*Psychotria subg. Psychotria*]	*Ps. chamelaensis* ***Ps. horizontalis***	***Ps. carthagensensis*** ***Ps. horizontalis*** ***Ps. nervosa*** ***Ps. quinqueradiata*** ***Ps. psychotriifolia***	***Ps. clivorum*** ***Ps. costivenia var. costivenia*** ***Ps. chagrensis*** ***Ps. erythrocarpa*** ***Ps. flava*** ***Ps. graciliflora*** ***Ps. limonensis*** ***Ps. mexiae*** ***Ps. mirandae*** ***Ps. panamensis (var. panamensis)*** ***Ps. papatlensis*** ***Ps. quinqueradiata*** ***Ps. sarapiquiensis*** ***Ps. tenuifolia*** ***Ps. trichotoma***	*Ps. alfaroana* ***Ps. graciliflora*** ***Ps. grandis*** ***Ps. horizontalis*** ***Ps. jimenezii*** ***Ps. monteverdensis*** ***Ps. neillii*** ***Ps. mexiae*** ***Ps. orosiana*** ***Ps. panamensis*** ***Ps. parvifolia*** ***Ps. quinqueradiata*** ***Ps. sarapiquiensis*** ***Ps. sylvivaga***	*Ps. alfaroana* ***Ps. chagrensis*** ***Ps. graciliflora*** ***Ps. grandis*** ***Ps. laselvensis*** ***Ps. marginata*** ***Ps. panamensis (var. panamensis)*** ***Ps. psychotriifolia***	***Ps. chagrensis*** ***Ps. graciliflora*** ***Ps. grandis*** ***Ps. horizontalis*** ***Ps. limonensis*** ***Ps. marginata*** ***Ps. micrantha*** ***Ps. psychotriifolia*** ***Ps. tenuifolia***
Palicourea s. lat. [*Palicourea + Psychotria subg. Heteropsychotria*]		***Ps. pubescens***	***Ps. deflexa*** ***Ps. elata*** *Pa. faxlucens* *Pa. gardenioides* ***Ps. hoffmannseggiana*** *Pa. macrantha* ***Pa. padifolia*** *Ps. phanerandra* ***Ps. poepiggiana*** *Ps. simiarum* *Pa. sousae* *Pa. tetragona* *Ps. veracruzensis*	*Pa. adusta* ***Pa. albocaerulea*** *Ps. angustiflora* ***Ps. aubletiana*** ***Ps. chiriquiensis*** ***Ps. correae*** ***Ps. cyanococca*** ***Ps. deflexa*** ***Ps. elata*** *Pa. eurycarpa* *Pa. garciae* ***Pa. gomezii*** ***Ps. guapilensis*** ***Ps. hazenii***	***Ps. acuminata*** *Ps. angustiflora* ***Ps. brachiata*** ***Ps. buchtienii*** ***Pa. calidicola*** ***Ps. cooperi*** *Pa. crocea* ***Ps. cyanococca*** ***Ps. elata*** ***Ps. glomerulata*** ***Ps. gracilenta*** ***Ps. guapilensis*** ***Pa. guianensis*** *Ps. hebeclada*	***Ps. acuminata*** ***Ps. brachiata*** ***Ps. capitata*** ***Ps. cyanococca*** ***Ps. deflexa*** ***Ps. gracilenta*** ***Pa. guianensis*** ***Ps. hoffmannseggiana*** ***Ps. poeppigiana*** ***Ps. pubescens*** ***Ps. racemosa***

				Pa. lasiorrhachis ***Pa. macrocalyx*** ***Pa. montivaga*** ***Pa. padifolia*** ***Ps. pilosa*** ***Ps. pubescens*** ***Ps. solitudinum*** ***Pa. standleyana*** *Ps. steyermarkii* *Pa. tilaranensis* ***Ps. torresiana*** ***Ps. valerioana***	***Ps. luxurians*** ***Ps. pilosa*** ***Ps. poeppigiana*** ***Ps. racemosa*** *Pa. tetragona* *Ps. tsakiana*	

Table 3.3. *Psychotria* and *Palicourea* species composition of thirteen Neotropical sites. Species included in the present study are indicated in boldface. References are: Chamela, Mexico (Dominguez-Licona 2005), Guanacaste, Costa Rica (Janzen & Liesner 1980), Los Tuxtlas, Mexico (Lorence & Ibarra-Mantríquez 1990), Monteverde, Costa Rica (Haber 2000), La Selva, Costa Rica (Taylor *et al.* 2011), Barro Colorado Island, Panama (Sedio *et al.* 2010).

Chocó-Darién		Amazonia				Guiana
Bajo Calima, Colombia	La Planada, Colombia	Yasuní, Ecuador	Allpahuayo-Mishana, Peru	Yanamono, Peru	Ducke, Brazil	Saül, French Guiana
80,000	25	50	57,600	200	10,000	134,000
Ps. megistophylla *Ps. monsalveae*	*Ps. rufiramea* ***Ps. saltatrix*** ***Ps. sylvivaga***	***Ps. remota*** *Ps. romolerouxiana* *Ps. sacciformis* ***Ps. viridis***	*Ps. alba* ***Ps. anceps*** *Ps. marcgraviella* ***Ps. remota***	*Ps. alba* *Ps. marcgraviella* ***Ps. marginata*** *Ps. romolerouxiana* *Ps. sacciformis* *Ps. trivialis*	***Ps. mapourioides***	***Ps. borjensis*** *Ps. cupularis* ***Ps. ernestii*** *Ps. ficigemma* ***Ps. mapourioides*** *Ps. perferruginea*
Pa. acanthacea ***Ps. acuminata*** ***Ps. allenii*** *Pa. amplissima* *Ps. aviculoides* ***Ps. campyloneuroides*** ***Ps. capitata*** *Ps. cincta* ***Ps. cooperi*** *Pa. conferta* ***Ps. cordobensis*** *Pa. grandistipula* ***Ps. glomerulata*** ***Pa. guianensis*** ***Ps. hoffmannseggiana*** ***Ps. luxurians*** ***Ps. pilosa*** ***Ps. platypoda*** ***Ps. poeppigiana***	***Ps. allenii*** ***Ps. aubletiana*** *Pa. demissa* *Pa. garciae* ***Pa. pyramidalis*** ***Pa. standleyana*** *Pa. stipularis* *Pa. tamaensis*	***Ps. buchtienii*** ***Ps. caerulea*** ***Ps. deflexa*** ***Ps. gracilenta*** *Pa. grandiflora* ***Pa. guianensis*** *Ps. huampamiensis* ***Pa. lasiantha*** *Pa. nigricans* *Ps. ostreophora* ***Ps. poeppigiana*** ***Ps. stenostachya***	*Pa. affinis* ***Ps. buchtienii*** ***Ps. cornigera*** *Pa. crocea* ***Ps. deflexa*** *Ps. japurensis* ***Pa. lasiantha*** *Ps. limitanea* *Ps. longicuspis* *Ps. lupulina* *Pa. mansoana* *Pa. nigricans* *Ps. peruviana* ***Ps. poeppigiana*** ***Ps. racemosa*** *Ps. rhodothamna* *Ps. spiciflora* ***Ps. stenostachya*** *Ps. stipulosa* ***Ps. trichocephala*** *Ps. williamsii*	*Pa. affinis* ***Ps. buchtienii*** ***Ps. cornigera*** *Pa. crocea* ***Ps. herzogii*** *Ps. iodotricha* ***Pa. lasiantha*** *Ps. limitanea* *Ps. lupulina* *Pa. mansoana* *Pa. nigricans* *Ps. ostreophora* ***Ps. poeppigiana*** *Ps. rhodothamna* *Ps. spiciflora* ***Ps. stenostachya*** *Pa. subspicata* ***Ps. trichocephala***	*Pa. amapaensis* *Pa. anisoloba* *Ps. apoda* *Ps. bremekampiana* *Ps. colorata* ***Pa. corymbifera*** ***Ps. deflexa*** *Ps. egensis* ***Ps. gracilenta*** ***Pa. guianensis*** ***Ps. hoffmannseggiana*** *Ps. humboldtiana* *Ps. iodotricha* *Ps. longicuspis* ***Pa. longiflora*** ***Pa. longistipulata*** *Ps. lupulina* *Ps. manausensis* ***Ps. microbotrys*** *Ps. paniculata*	***Ps. acuminata*** *Ps. alloantha* *Ps. apoda* *Pa. brachyloba* *Ps. bremekampiana* *Ps. callithrix* ***Pa. calophylla*** ***Ps. capitata*** *Ps. colorata* ***Ps. cornigera*** ***Pa. croceoides*** ***Ps. deflexa*** ***Ps. gracilenta*** ***Ps. granvillei*** ***Pa. guianensis*** ***Ps. hoffmannseggiana*** *Ps. iodotricha* ***Pa. longiflora*** ***Pa. longistipulata*** *Ps. medusula* ***Ps. microbotrys***

Ps. schunkei *Pa. seemannii* *Ps. timbiquensis*					***Ps. platypoda*** ***Ps. poeppigiana*** *Ps. polycephala* *Ps. prancei* *Ps. rhodothamna* *Ps. rhombibractea* *Pa. nitidella* *Ps. sphaerocephala* *Ps. stipulosa* *Ps. subundulata* ***Ps. trichocephala*** *Ps. turbinella* ***Ps. variegata*** *Pa. virens*	*Ps. oblonga* *Ps. officinalis* *Ps. paniculata* ***Ps. platypoda*** ***Ps. poeppigiana*** *Ps. pullei* ***Ps. racemosa*** ***Ps. trichophoroides*** *Ps. ulviformis* ***Ps. urceolata*** ***Ps. variegata*** *Ps. viridibractea*

Table 3.3 ***continued.*** *Psychotria* and *Palicourea* species composition of thirteen Neotropical sites. References are: Bajo Calima, Colombia (Taylor 2011), La Planada, Colombia, and Yasuní, Ecuador (Center for Tropical Forest Science 2011), Allpahuayo-Mishana and Yanamono, Perú (Taylor 1997), Reserva Ducke, Brazil (Taylor *et al.* 2007), Saül, French Guiana (Boom & Delprete 2002).

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Chapter 4: The role of natural enemies in the diversification and coexistence of a hyperdiverse genus of tropical trees.

Abstract

Plant-enemy interactions are hypothesized to play a significant role in both the origin and maintenance of woody plant diversity in species-rich tropical forests. Specialist herbivores and pathogens are thought to facilitate coexistence by preventing their preferred host from becoming locally abundant. Yet many tropical forests are characterized by a small number of locally species-rich tree genera. Moreover, a large proportion of plant enemies exploit multiple, closely related host species even in the tropics. Hence, it is unclear whether host use by natural enemies is constrained sufficiently by local variation in the defensive traits of tropical tree genera to facilitate the partitioning of niche space with respect to enemies. Here, we employ DNA barcodes to reconstruct the host associations of insect herbivores that attack the tree genus *Psychotria* (Rubiaceae) on Barro Colorado Island (BCI), Panama. We examine these associations relative to *Psychotria* secondary chemistry and tree community structure. We ask whether tree species traits are associated with enemy host use and exhibit phylogenetic and community patterns that would indicate a role for defense in the diversification and coexistence of congeneric tropical trees. A hierarchical Bayesian model identified several major axes of secondary chemical variation among *Psychotria* as important

predictors of herbivore host use. Furthermore, whereas species of *Psychotria* that co-occur at a scale of 3 m on BCI are phylogenetically clustered, they are less similar than expected by chance with respect to the secondary chemical axes most strongly associated with herbivore host use. Finally, linear mixed effects models indicate an association between rates of herbivore leaf damage and the neighborhood leaf density with respect to these same principal components of secondary chemical variation. The broad congruence we observed between the traits associated with herbivore host use, community composition of *Psychotria*, and herbivore damage as a function of the trait neighborhood suggest that defense evolution may contribute to the diversification of species-rich tropical tree genera by permitting close relatives to partition niche space locally even while sharing other aspects of their ancestral niche.

Introduction

The quest to identify the mechanisms responsible for the generation and maintenance of species richness in the world's most diverse tropical forests has long been a major driver of scientific inquiry, and has stimulated a multitude of hypotheses (reviewed by Wright 2002, Leigh *et al.* 2004). Forest plots of only a fraction of a km^2 in Ecuador and Borneo contain ca. 1200 tree species, or as many as all of the temperate forests of North America, Europe, and northern Asia combined (Wright 2002). Understanding how such large numbers of tree species coexist despite intense competition for resources remains a basic challenge in ecology (Silvertown 2004). Classical theory maintains that in order to coexist stably, competing species must exploit distinct niches, defined by the way they interact with abiotic resources and other organisms, both mutualist and antagonist (Gause 1934; Hutchinson 1959; MacArthur & Levins 1967; Chesson & Kuang 2008). All plants require CO_2, light, water, and a small number of common nutrients, which seemingly limits the available resource dimensions along which competing species might differentiate (Wright 2002, Silvertown 2004). Among tropical forest trees, niche differences with respect to light intensity, soil moisture, reproduction, and regeneration have been identified, but they rarely explain the coexistence of more than a fraction of the species in the most diverse tropical forests (Wright 2002). Furthermore, habitat filters may often have the effect of increasing the similarity of co-occurring species (Baraloto *et al.* 2012, Sedio *et al.* 2012), further reducing opportunities for differentiation within local assemblages.

The challenge of understanding coexistence appears to be exacerbated by the observation that species richness in many of the world's most diverse tropical forests is disproportionately comprised of a small number of species-rich genera (Gentry 1982). For example, the five largest of ca. 500 tree genera comprise 22% of the tree species recorded on Barro Colorado Island, Panama (Croat 1979). Likewise, 22 species of *Pouteria* (Sapotaceae) and *Inga* (Mimosoideae) have been recorded in census plots of only 1 ha and 0.16 ha, respectively, in Amazonian Ecuador (Valencia *et al.* 1994). Gentry (1982, 1989) referred to similar assemblages of *Miconia* (Melastomataceae), *Piper* (Piperaceae), and *Psychotria* (Rubiaceae) as “species swarms” in tribute to their extreme local diversity and apparent ecological similarity.

Plant genera that exhibit high local diversity pose a challenge to our understanding of coexistence, but also present an opportunity to examine the evolutionary drivers of diversification. Phylogenetically related species share recent common ancestry and thus a recent ancestral niche. As a result, identifying the niche dimensions along which congeneric species differ has the potential to reveal the means of their coexistence, as well as the axes that played a role in lineage diversification.

Examinations of the phylogenetic structure of congeneric assemblages of tree species have revealed that, in some temperate systems, congeneric species tend to diverge over broad environmental gradients. For example, closely related *Quercus* trees were found to exploit distinct habitats defined by fire frequency and soil moisture availability (Cavender-Bares *et al.* 2004), and recent lineage diversification of *Ceanothus* shrubs was associated with evolutionary divergence over temperature and elevation gradients (Ackerly *et al.* 2006). As a result of these evolutionary patterns, local assemblages of *Quercus* and *Ceanothus* tend to be comprised of distantly related congeners, derived from distinct early-diverging clades within each genus. Similarly, adaptation to nutrient-poor white sand soils has occurred independently in several lineages of the tropical genus *Protium*, resulting in assemblages of distantly related congeners (Fine *et al.* 2004, 2005). On the other hand, soil moisture habitat preference appears to be phylogenetically conserved within the tropical genera *Inga* (Kursar *et al.* 2009) and *Psychotria* (Sedio *et al.* 2012), such that habitat filtering assembles local communities of close relatives compared to each genus as a whole. This suggests that habitat-based niches may not only be few relative to the number of tree species in tropical forests (e.g. Wright 2002), but also that ecological divergence associated with speciation in some tropical forest tree genera may be driven by evolution along niche dimensions other than obvious abiotic gradients (or tradeoffs defined by abiotic-biotic interaction as in *Protium*; Fine *et al.* 2004, 2005).

In contrast with the limited number of resource-based niche dimensions available to plants, plant interactions with natural enemies might provide a high-dimensionality niche space within which competing plants might differentiate. Janzen (1970) and Connell (1971) both proposed that plants fail to recruit in the neighborhood of conspecific adults as a consequence of attack by specialized natural enemies, such as insect herbivores or pathogens, that respond either to the presence of adult plants or to the density of seeds or seedlings of their host species. Such negative density-dependent recruitment has the effect of limiting the abundance of any one species and thereby facilitating coexistence among competing species of plants.

A large body of evidence supports the predictions of Janzen and Connell that tree seedlings fail to survive at high densities or in the vicinity of conspecific adults (e.g. Wills *et al.* 1997; Webb & Peart 1999; Harms *et al.* 2000; HilleRisLambers *et al.* 2002;

Carson *et al.* 2008; Comita et al. 2010; Terborgh 2012). Yet whereas the classic Janzen-Connell Hypothesis assumes that plant enemies are highly specialized, surveys of plant-enemy host use in tropical forests suggest that insect herbivores (Basset 1992; Odegaard *et al.* 2000; Novotny *et al.* 2002, 2010; Odegaard *et al.* 2005) and fungal pathogens (Gilbert 2005; Gilbert & Webb 2007; Liu *et al.* 2012) seldom specialize on a single host, but rather exhibit polyphagous host ranges strongly associated with plant phylogeny. Phylogenetic signal in plant-enemy associations at the whole-community scale (i.e. considering multiple plant genera) is most likely a result of conservatism at deep scales of phylogeny in the traits that determine host use (Wink 2003; Barrett & Heil 2012). As a consequence of deep phylogenetic signal in defense traits and enemy host use, competition mediated by shared enemies (Holt 1977) may be partially responsible for the exclusion of closely related heterospecific individuals observed at various stages of recruitment in tropical trees (Webb et al. 2006; Bagchi et al. 2010; Metz et al. 2010).

However, plant defenses can be quite evolutionarily labile within genera. Studies of *Asclepias* milkweeds (Agrawal & Fishbein 2006) and the tropical tree genera *Bursera* (Becerra 1997) and *Inga* (Kursar et al. 2009) reveal convergent evolution of important defense traits resulting in an absence of phylogenetic signal within those genera. Furthermore, assemblages of co-occurring *Bursera* (Becerra 2007) and *Inga* (Kursar et al. 2009) appear to consist of combinations of congeneric species that differ more in defense than expected by chance. These findings suggest that competition mediated by insect herbivores, and perhaps pathogens, works to limit the similarity of co-occurring species with respect to defensive traits. Shared density-responsive natural enemies may thus select for divergence in defensive traits amongst closely related species, and ultimately promote lineage diversification as posited by Ehrlich & Raven (1964).

The handful of studies of the distribution of defense traits over phylogeny and within communities of species-rich plant genera such as Gentry's (1982,1989) "species swarms" suggest a role for enemy-mediated competition as an agent of natural selection for the phenotypic divergence of related plant species that otherwise share important aspects of the niche. In contrast, community-wide studies of herbivore and pathogen host use indicate broad enemy overlap among congeneric plant species (e.g. Novotny *et al.* 2002, 2010; Odegaard *et al.* 2005; Gilbert & Webb 2007). Hence, it remains unclear whether natural enemy host use is influenced by the variation in defensive traits exhibited within tropical tree genera, and whether the traits that are associated with enemy host use exhibit phylogenetic and community patterns that would indicate a role for defense in the diversification and coexistence of congeneric tropical trees.

Here, we characterized host-use among the insect herbivores of 21 sympatric understory shrubs of the genus *Psychotria* (Rubiaceae) on Barro Colorado Island (BCI), Panama through the use of DNA barcodes (Jurado-Rivera *et al.* 2008). We then employed three distinct analytical approaches: a hierarchical Bayesian model to relate plant traits to patterns of herbivore host use, trait-based metrics of community structure to measure the dispersion of traits in communities, and mixed-effects linear models to examine the relationship between herbivore damage and neighborhood trait density. We drew on evidence from each of these approaches to address three principal questions: i) how specialized are the herbivores of a single, diverse plant genus within a community? ii) is host-use within the genus *Psychotria* predicted by species differences in leaf defense traits or host-plant phylogeny? and iii) are *Psychotria* communities assembled in such a way as to minimize species interactions with shared herbivores? Finally, we asked how the *Psychotria* of BCI inform our understanding of the role of defense evolution in the diversification of the genus and coexistence in species-dense local communities.

Materials and Methods

Study System: The Psychotria *of Barro Colorado Island*

BCI (9° 9' N, 79° 51' W) is a 15.9 km^2 island in Gatun Lake, which forms part of the Panama Canal. The area has a tropical monsoon climate under the Köppen system of climatic classification and supports semi-deciduous tropical forest with a mature canopy height of 35 m. Croat (1978), Windsor et al. (1990) and Leigh (1999) provide further descriptions of BCI.

One of Gentry's (1982) "species swarms," *Psychotria* is also one of the largest plant genera, with approximately 1,650 species of shrubs and small trees throughout the world tropics and subtropics (Taylor 1996). The *Psychotria* of central Panama are indeed similar with regard to pollinators (Sakai & Wright 2008), seed dispersers (Poulin et al. 1999) and growth form. Sedio *et al.* (2012) showed that the species of *Psychotria* that co-occur at a 3-m scale on BCI are more closely related than expected by chance (i.e. phylogenetically clustered) as a result of habitat filtering mediated by phylogenetically conserved hydraulic traits, and hence dry season drought tolerance. That pattern is likely caused by the relatively recent colonization of central Panama by *Psychotria* sometime during the Great American Biotic Interchange ~3 Ma. The BCI community is comprised of recent colonists from Central America, the Amazon basin, and the Chocó region of western Colombia and Ecuador, and the BCI colonists continue to exploit microhabitats indicative of the broader climatic conditions of the region from which they dispersed (Sedio *et al.* 2013).

The *Psychotria* of BCI consist of 20 species in two well-defined subgenera, *Psychotria* subgenus *Psychotria* (9 species on BCI) and *Psychotria* subgenus *Heteropsychotria* (10 species on BCI), as well as *Carapichea ipecacuanha* (formerly *Psychotria ipecacuanha*). The *Palicourea* are a polyphyletic collection of species included amongst the *Heteropsychotria* (Nepokroeff *et al.* 1999). The two subgenera diverged approximately 35 Ma (Bremer & Erickson 2009; Sedio *et al.* 2013). For the remainder of the text, we use "*Psychotria*" to refer to the 18 species of *Psychotria* as well as *Carapichea ipecacuanha* and *Palicourea guianensis*.

The closest relative to the *Psychotria* on BCI is *Morinda seibertii*, followed by *Coussarea curvigemmia*, and *Faramea occidentalis* (all Rubiaceae; e.g. Kress *et al.* 2009). *M. seibertii* is extremely rare, hence we utilized *C. curvigemmia* and *F. occidentalis* as a phylogenetic outgroup to the *Psychotria.*

Molecular Methods: DNA Barcoding of Insects and Gut Contents

The species of plant associated with insect specimens collected in the field were identified using the chloroplast *trnH-psbA* intergenic spacer (see Kress *et al*. 2009). Over 2,000 insect specimens were collected from May-October 2010 on *Psychotria*, as well as *C. curvigemmia* and *F. occidentalis*, in 26 collection sites in which species composition was recorded. Specimens were collected on ice and stored at -60 °C at BCI. DNA was extracted using Qiagen Blood & Tissue Extraction Kits (Qiagen, Venlo, Netherlands) and the *trnH-psbA* locus amplified using the *trnH*f and *psbA*3f primers (Sang *et al.* 1997) tagged with 96 unique 10 bp oligonucleotide "Levenshtein" tags (Faircloth & Glenn 2012). Amplicons were cleaned and normalized using SequalPrep (Invitrogen, Carlsbad, CA), combined, and sequenced on a Roche 454 sequencer (F. Hoffman-La Roche, Basel, Switzerland) at the Georgia Genomics Facility. Plant DNA barcode sequence reads derived from insect samples were then compared to a *trnH-psbA* library established previously for the BCI *Psychotria* (Kress *et al.* 2009, Sedio *et al.* 2012)

The mitochondrial DNA barcode CO1 was used to group insect specimens into putative species. The ca. 830 bp portion of the CO1 gene was amplified using the C1-J-2183 and TL2-N-3014 primers (Simon *et al.* 1994) tagged with 96 unique Levenshtein tags (Faircloth & Glenn 2012), cleaned and normalized with SequalPrep, and sequenced using the circular consensus method on a Pacific Biosciences RS II sequencer (Pacific Biosciences, Menlo Park, CA) at the University of Michigan Sequencing Core. CO1 sequences were clustered based on 98% sequence similarity using UCLUST (Edgar 2010), resulting in 125 OTUs including from 1 to 109 individuals per OTU (46 OTUs were represented by at least 2 individuals). OTUs were treated as herbivore species in subsequent analyses.

Analysis of Secondary Chemistry and Physical Defense Traits

We performed a full fiber analysis for fully mature leaves following Westbrook *et al.* (2011). Mature leaf samples were collected on BCI, immediately dried at 60°C and dried again at 105°C for 24 h immediately before analysis. Approximately 500 mg of ground material was washed with neutral detergent using an ANKOM 200 fiber analyzer

(ANKOM Technology, Macedon, NY) to separate leaf tissue from nonpolar and soluble extracts, followed by the isolation of hemicellulose and structural proteins using an acid detergent (Westbrook *et al.* 2011). Cellulose was isolated using 70% H_2SO_4 and remaining material was heated in a muffle furnace at 500°C to determine insoluble mineral ash contents. Dry masses were measured after each round of extraction in order to estimate nonpolar, hemicellulose, cellulose, lignin, and mineral ash mass fractions (g g^{-1}). Fiber data were averaged over two individuals per species for all species but *P. psychotriifolia*; all conspecific replicates differed by < 5% for all fiber components with the exception of *C. ipecacuanha*. Cellulose content is closely related to leaf fractal toughness (Westbrook *at al.* 2011; Kitajima *et al.* 2012). We refer to these five traits as "physical" defense traits, in contrast to the secondary chemistry discussed below. Physical defense traits can be found in Table 4.5.

In order to examine secondary chemical variation, young, expanding leaves were collected from *Psychotria* adults and saplings in the understory between May and September 2010. Between 0.5 g and 2 g of dried leaf tissue was homogenized in a ball mill (Retsch MM200, Haan, Germany) and extracted with 20 mL 90% methanol at pH 5 for 10 min. This was then centrifuged, the supernatant isolated, and the remaining sample re-extracted with 90% methanol. The supernatants were combined and evaporated using a Rotovapor to form a slurry, to which was added water at pH 12. This basic solution was then extracted using dichloromethane (CH_2Cl_2), evaporated, and resuspended in 150 μL methanol. Samples were analyzed using reverse phase high-performance liquid chromatography at high system pressures (UPLC, Waters Inc., Milford, MA) with a solvent gradient from water to 75% acetonitrile and an Acquity

UPLC HSS T3 column. The isoquinoline alkaloid emetine (Sigma-Aldrich, Milwaukee, WI) occurs naturally in one of our study species, *Carapichea ipecacuanha*, and was used as an external standard. Absorbance spectra were collected from 200 to 400 nm on a PDA detector, and chromatograms were quantified at 244 nm (Fig. 4.5).

The chromatograms from each species of *Psychotria* were scaled by the leaf mass analyzed and averaged to produce a mean chromatogram representative of the methanolic extract of secondary chemistry of each of the 22 study species. At least two individuals were represented for all species except *P. graciliflora* and *F. occidentalis*. Principal components analysis (PCA) was conducted using singular-value decomposition on uncentered chromatogram data to estimate the major axes of secondary chemical variation among the 22 species. The first 23 PC axes explained 97.24 % of the interspecific variance in secondary chemistry (Table 4.6). Species factor scores along the first 23 PC axes treated as species mean trait values for secondary chemistry in subsequent analyses. Species PCA factor scores can be found in Table 4.6.

Phylogenetic Signal of Traits and Herbivore Assemblages

Phylogenetic signal in the distribution of traits among species of *Psychotria* was calculated using both maximum likelihood and phylogenetic independent contrasts. Pagel's (1999) λ was estimated using maximum likelihood in the R package 'caper' (Orme *et al.* 2012). A λ value of 1 reflects a Brownian motion model of random-walk trait evolution about a phylogeny, whereas a λ value of 0 indicates no relationship between phylogeny and trait differences among species (Pagel 1999). To corroborate results measured in terms of λ, we also measured Blomberg's (2003) K, which also approaches a Brownian motion expectation when $K = 1$. Significance of phylogenetic signal was tested by comparing observed variance of phylogenetic independent contrasts (PIC) with a distribution of PIC variance measured over 999 phylogenies in which the species' trait values were shuffled using 'picante' (Kembel *et al.* 2008).

The degree to which plant species share insect herbivores and pathogens is strongly related to plant phylogeny within tropical forest plant communities (Novotny *et al.* 2002, 2010; Odegaard *et al.* 2005, Gilbert & Webb 2007). We measured the relationship between the Bray-Curtis similarity of insect herbivore assemblages and the phylogenetic distance between species of *Psychotria* using linear regression.

Bayesian Modeling of Plant-Herbivore Interactions

In order to detect traits relevant to plant-herbivore interactions, we developed a hierarchical Bayesian model relating plant traits to the frequency of appearance in the gut of each insect herbivore species. Under the model, the number of times each *Psychotria* species is found in the stomach of each insect is modeled as a multinomial distribution, and the probability vector governing the multinomial distribution is a function of plant defense traits. The probability p_{ij} that a plant found in the stomach of insect i belongs to *Psychotria* species j is

$$p_{ij} = \frac{e^{q_{ij}}}{\sum_{j'=1}^{n} e^{q_{ij'}}} \ ,$$

where n is the number of *Psychotria* species and

$$q_{ij} = \sum_{k=1}^{K} \beta_{ik} x_{jk}$$

is a linear combination of plant traits x_{jk} weighted by coefficients β_{ik} that reflect the response of insect *i* to trait *k*, with *K* the number of traits. Specifically, β_{ik} represents the log-odds ratio, for insect herbivore *i*, between feeding on two *Psychotria* species with a difference of 1 in trait *k*. Traits were normalized across *Psychotria* species to have mean 0 and standard deviation 1 so that coefficients were directly comparable across traits.

Bayesian hierarchical priors were used to reduce the effective dimensionality of the parameter space. The coefficient β_{ik} for a particular insect species *i* responding to a particular plant trait *k* was drawn from a normal prior for that trait:

$$\beta_{ik} \sim \mathrm{Normal}(\mu_k, \tau_k)\ .$$

The mean μ_k and precision τ_k for each trait's prior distribution were in turn drawn from top-level normal and exponential hyperpriors, respectively:

$$\mu_k \sim \mathrm{Normal}(0, \tau_\mu)$$

$$\tau_k \sim \mathrm{Exponential}(\lambda_\tau)\ ,$$

where the top-level hyperparameters τ_μ and λ_τ are fixed via maximum marginal likelihood (empirical Bayes). We inferred posterior distributions and marginal likelihoods via Markov-chain Monte Carlo using the mc3kit software package (Baskerville 2013a,b).

Top-level hyperparameters τ_μ and λ_τ were fixed at values estimated to maximize the marginal likelihood, identified using pilot MCMC runs with uniform priors assigned to τ_μ and λ_τ. Because all models had the same number of fixed top-level parameters, marginal likelihoods conditional on fixed τ_μ and λ_τ were used as the basis for model selection. Bayes factors (marginal likelihood ratios; Kass and Raftery 1995) were computed relative to the best model. Marginal likelihoods were computed using thermodynamic integration (Lartillot and Philippe 2005).

In order to gauge the impact of each trait on the model post-hoc, we calculated the mean, across posterior samples, of the change in log-likelihood caused by removing each trait from the model, keeping other parameters fixed. Removing a single trait removes its contribution to the relative probabilities of feeding on different plant species, which are still normalized to sum to 1. In addition, we calculated two metrics to measure the variability and strength of herbivore response to each trait, respectively. The posterior standard deviation of β_{ik} over insects i reflects variation in the response of herbivores to each trait k (Table 4.1). The posterior mean of each β_{ik}, normalized by its posterior standard deviation, measures confidence in the sign of a coefficient. The sum of all such positive values for a particular trait k represents a confidence-weighted count of the number of insect herbivores with positive values; likewise for negative values. Traits with large positive and zero negative or large negative and zero positive sums thus have a similar effect across species. Traits with moderate positive and negative sums indicate sign divergence across species.

Community Structure

Psychotria were located and identified in a network of 134 plots of 3 m radius in an earlier study of habitat distributions (Sedio *et al.* 2012). Here, community structure with respect to traits was analyzed by calculating mean distance between every pair of *Psychotria* species occurring within a census plot with respect to phylogeny, insect herbivore assemblages, and each leaf trait. For leaf traits, trait distance was measured as the difference in trait values or chemical PC factor scores between species. Distance with respect to species herbivore assemblages was measured as the Euclidean distance between *Psychotria* species in the quantitative, bitrophic network of plant-herbivore associations. A standardized effect size was then calculated by comparing this observed mean pairwise distance (MPD; Webb 2000) for a particular *Psychotria* census plot to the distribution of MPD's for 999 null communities of equivalent species richness, assembled by an abundance-weighted random draw from the BCI *Psychotria* species pool. An MPD effect size less than zero indicates that observed trait or phylogenetic distances are less than expected by a null assembly mechanism, and hence that co-occurring *Psychotria* are significantly more similar than expected from the BCI species pool. Conversely, an effect size significantly greater than zero indicates that trait or phylogenetic distances of co-occurring species are greater than those generated by random assembly, and thus that local assemblages are more even than expected by chance (Webb 2000). Community structure analyses were calculated using the R package 'picante' (Kembel *et al.* 2008).

Mixed-Effects Models of Herbivore Leaf Damage and the Trait Neighborhood

Between September and November 2010, we recensused 57 arbitrarily chosen plots out of the BCI network of 134 plots (Sedio *et al.* 2012) within a radius of 5 m to record the immediate neighborhood of all individuals within the inner 3 m. For each plant within 3 m of the center of the plot, the youngest pair of fully mature leaves on each stem were temporarily pressed between two plastic sheets, one of which was printed with a 1 X 4 cm scale bar, and photographed. Leaf photographs were then processed electronically to measure the area of each leaf and to estimate the proportion and area of each leaf lost to insect herbivore damage.

Herbivore damage was then modeled as a function of the trait neighborhood of the leaf. This was defined as the mean trait distance of the species of the focal leaf to the traits of the species represented by each other leaf in the plot. Mean neighborhood distance was measured in this way with respect to *Psychotria* phylogeny, herbivore assemblages, and each of the 13 physical and chemical leaf traits.

Light is an important determinant of understory insect abundance and herbivory rates in tropical forests. In the absence of light data contemporary to the period in which the leaves were susceptible to herbivory (i.e. during expansion; Coley & Aide 1991) and in order to account for other unmeasured factors such as edaphic variation and proximity to ant nests, we treated plot as a random effect. Furthermore, in these neighborhood analyses, we treated trait data only in terms of neighborhood trait distance and did not include the species' trait values themselves as predictors of herbivory rates. We therefore treated species as a random effect within the mixed-effects model of herbivory to account for differences in herbivory rates among species resulting from interspecific variation in quantitative defenses or unmeasured factors. Models were developed using the R package 'lme4' (Bates *et al.* 2011).

Results

Network Structure and Host Specificity

Out of a total of 2,018 insects collected on *Psychotria* and its relatives, we recovered *Psychotria* DNA from 525 specimens and unrelated plant species from an additional number of specimens. These 525 individuals were clustered into 127 insect species based on 98% divergence at the CO1 barcode gene, each of which was represented by 1 to 109 individuals (mean = 4 individuals per species). Coleopteran herbivores were most abundant (267 individuals in 69 species), and these were dominated by leaf-beetles in the Eumolpinae subfamily of Chrysomelidae, as well as weevils of the Curculionidae and a smaller number of Buprestidae. Also important were Lepidoptera larvae (134 individuals in 22 species), particularly in the families Crambidae and Sphingidae, and Orthopterans in the family Eumastacidae (103 individuals in 21 species in Orthoptera).

Host specificity of *Psychotria* herbivores was low. Herbivore species represented by more than a single individual were found to feed on an average of 3.46 of the 20 species of *Psychotria* and their two closest, reasonably abundant relatives on BCI (Fig. 4.1). Hemiptera appeared more specialized than other herbivores (Fig. 4.2), though conclusions with respect to this order must be tempered by the small sample size (N = 5 with > 1 individual) of Hemiptera from which *Psychotria* DNA was successfully isolated and sequenced. Orthopteran herbivores of *Psychotria* were the least host-specific, followed by Lepidopteran caterpillars and Coleopteran adult leaf-chewing beetles (Fig. 4.2).

Phylogenetic Signal of Leaf Traits and Herbivore Host Use

A single axis of secondary chemical variation, PC3, exhibited clear phylogenetic signal. In contrast, nonpolar compounds, hemicellulose, and 11 of the 23 axes of secondary chemical variation were significantly less conserved than expected under a Brownian motion, random walk model of trait evolution (Table 4.1). Other traits could not be clearly distinguished from either model, though the maximum likelihood estimate of λ with respect to PC's 2, 5, 12, and 16 approached the Brownian motion expectation of $\lambda = 1$, indicating weak evidence of phylogenetic signal (Table 4.1).

Herbivore similarity increased very weakly, though significantly, with phylogenetic distance between *Psychotria* species ($p = 0.01$, Mantel test; Fig. 4.3). If related species *Coussarea curvigemmia* and *Faramea occidentalis* were also considered (which diverged from *Psychotria* ca. 65 Ma; Bremer & Erickson 2009), no relationship was found between herbivore similarity and the phylogenetic distance between host plants.

Model Selection and Herbivore Associations

The best model of herbivore host use was one that incorporated leaf and stem abundance, the five physical defense traits, and 23 principal components of secondary chemical variation. This model substantially outperformed alternative models that incorporated information pertaining to fewer chemical axes or none at all (Table 4.2). A model that included leaf and stem abundance data but excluded species traits performed worst of all. These results indicate that the physical and chemical leaf traits measured are associated with the host use of herbivores.

The trait coefficient β_{ik} indicates the degree to which each trait k in the host-use model affects the host associations of each herbivore species i. Physical leaf traits were strongly associated with the host-use patterns of herbivores (Table 4.3). Among physical traits, mineral ash, hemicellulose, and lignin had the largest impact on the host-use model, followed by nonpolar compounds and cellulose. *Psychotria* with high mineral ash and hemicellulose content were strongly disfavored in the diets of herbivores (Table 4.3).

The large standard deviation of $\beta_{i,lignin}$ and $\beta_{i,nonpolar}$, in contrast, indicate that a high concentration of lignin or nonpolar compounds was associated with host use among some herbivores and with avoidance by others (Table 4.3).

The 23 principal components of variation in young leaf secondary chemistry varied substantially in their association with herbivore host-use patterns (Table 4.3). The PC axes with the greatest impact on the host-use model were PC4, PC8, PC20, PC3, and PC19, in that order. Axes PC9, PC6, PC2, PC12, PC5, and PC21 were also among the chemical trait axes with the largest impact on the likelihood of the model (Table 4.3). Of these chemical traits, PC4, PC19, PC20, PC5, PC12, and PC3 exhibited the greatest standard deviation in β_{ik}, indicating variation in the response to those traits among herbivore species. In addition, of PC axes with large $\Delta \log L$, PC20, PC3, PC12, and PC21 were strongly negative in their association with herbivore diets. This result means that *Psychotria* species that feature strongly on the positive side of those axes are underutilized by herbivores or, conversely, that *Psychotria* that characterize the negative end of those axes are favored hosts of a large proportion of the herbivore species. PC8 and PC5 were strongly positively associated with herbivore host use. In contrast to the aforementioned axes, PC4, PC20, PC19, PC9, PC6, and PC2 were associated with both positive and negative β_{ik}, indicating that the *Psychotria* that make up the opposite poles of those particular trait axes differ substantially in the herbivores by which they are exploited.

Community Structure

Sedio *et al.* (2012) demonstrated that species of *Psychotria* that co-occur at fine spatial scales on BCI are phylogenetically clustered, or more closely related than expected by random community assembly (mean MPD = -0.3779, $p < 0.0005$, Fig. 4.4). Despite that background of phylogenetic clustering, the *Psychotria* that co-occur within 3 m are less similar than expected with respect to nonpolar compounds (mean MPD effect size = 0.530, $p < 0.0005$) and with respect to the axes of interspecific variation in secondary chemistry represented by PC2, PC3, PC8, PC9, and PC23 (PC2 mean MPD effect size = 0.85, $p < 0.0005$; PC3 mean = 0.75, $p < 0.0005$; PC8 mean = 1.80, $p < 0.0005$; PC9 mean = 0.59, $p = 0.001$; PC23 mean = 0.54, $p < 0.0005$; Fig. 4.4B). The remaining components of secondary chemistry variation exhibit a clustered community distribution, whereas the community structure of *Psychotria* with respect to cellulose, hemicellulose, lignin, and mineral ash do not differ from null assembly models (Fig. 4.4). Co-occurring *Psychotria* are significantly less similar than expected by chance with respect to their insect herbivore assemblages (mean MPD effect size = 1.7085, all p-values Bonferroni-corrected with N = 30; Fig. 4.4B).

Figure 6A indicates that community structure with respect to mean pairwise trait distance between leaves differs little from results pertaining to individual plants (i.e. Fig. 4.4B). As with co-occurring individuals, leaves within each 3-m radius plot were significantly phylogenetically clustered, but more distant than random with respect to nonpolar compounds, secondary chemical axes PC2, PC3, PC8, PC9, PC12, PC13, PC19, PC21 and insect herbivore assemblages.

Herbivore Damage and the Neighborhood

Mixed effects models indicated a relationship between the trait neighborhood in which a leaf occurred and the rate of herbivore leaf damage recorded in census plots (Table 4.3). The best model of all those considered was a model that included nonpolar compounds, hemicellulose, lignin, mineral ash, and all 23 PC axes of secondary chemistry (AIC = 11376.93, likelihood ratio test compared to null: LR = 98.19, p = 5.1 X 10^{-10}). In addition, three smaller models predicted herbivore damage better than the null, which included only *Psychotria* species and plot as random factors (AIC = 11421.12). A model that included the mean distance along chemistry axes PC2 and PC8 within the local neighborhood was significantly associated with herbivore damage (AIC = 11419.32, LR = 5.80, p = 0.02) as were models featuring only axes PC 20 (AIC = 141783, LR = 5.29, p = 0.02) and PC21 (AIC = 11418.64, LR = 4.480, p = 0.03). A model that included only differences in herbivore assemblages among species as a fixed effect exhibited a marginally significant association with leaf damage in the plots (AIC = 11420.43, LR = 2.69, p = 0.10). In contrast to models that incorporated information pertaining to leaf traits or herbivore similarities, herbivory models including *Psychotria* phylogenetic distance (AIC = 11422.69, LR = 0.43, p = 0.51) or strictly conspecific leaf density (AIC = 11421.66, LR = 1.46, p = 0.23) as a fixed effect did not improve on the null model.

Discussion

The *Psychotria* of BCI demonstrated variation in physical and chemical defensive traits sufficient to differentiate themselves from sympatric species with respect to insect herbivores. DNA barcoding of insects and their diets revealed a community of *Psychotria* herbivores that do not specialize narrowly within the genus. Nevertheless, most *Psychotria* herbivores tended to avoid host species with high hemicellulose and mineral content in their leaves, and to exploit *Psychotria* characterized by shared secondary chemistry. Furthermore, *Psychotria* demonstrated nonrandom patterns of co-occurrence that reduced the similarity of species at a neighborhood spatial scale with respect to several key defensive traits. Specifically, *Psychotria* that shared elements of their secondary chemistry represented by axes PC2, PC3, PC8, PC9, and PC21 were more likely to share insect herbivores, (Table 4.3), but were less likely co-occur within 3 m in the forest understory (Fig 4.4). In addition, *Psychotria* experienced greater rates of herbivore damage when occurring in 5-m neighborhoods characterized by a high density of leaves with chemical similarities along axes PC20, PC21, PC2, and PC8 (Table 4.4), all of which were important predictors of shared herbivores in our host-use model (Table 4.3). These results suggest that defense-trait similarities with neighboring species of *Psychotria* have fitness consequences that may favor community assembly that reduces interactions with shared insect herbivores. In addition, the breakdown of phylogenetic signal in secondary chemistry (Table 4.1, Fig. 4.3) compared to physiological traits that determine habitat preferences (Sedio *et al.* 2012) suggests that defense evolution has contributed to the diversification of the genus and may facilitate coexistence within species-dense local communities.

Herbivore Host Use and Leaf Traits

DNA barcoding of insects and their diets revealed a community of congeneric plants with broad overlap in their herbivores. The breadth of the host ranges observed among the herbivores of *Psychotria* is consistent with the specialization exhibited by leaf-chewers in other surveys of tropical forest herbivores (e.g. Novotny *et al.* 2002, 2010). On the other hand, the relative host specificity of Lepidopteran and Coleopteran herbivores of *Psychotria* on BCI differs from whole-community comparisons (e.g. Novotny *et al.* 2002, 2010), in which Coleoptera were substantially less specialized than Lepidoptera. However, Novotny *et al.* (2002) found little difference in herbivore assemblages as a function of phylogenetic distance within the genera *Psychotria* and *Ficus* in Papua New Guinea. Indeed, the weak relationship between herbivore assemblages and intrageneric phylogenetic distance exhibited by the BCI *Psychotria* is largely congruent with previous observations of high herbivore similarity within tropical tree genera (Basset 1992, Novotny *et al.* 2002, Lewinsohn *et al.* 2005, Novotny & Basset 2005, Dyer *et al.* 2010, but see Becerra 1997, 2007).

Despite broad similarity in the herbivore assemblages associated with the BCI *Psychotria*, our results indicated substantial variation in the degree to which pairs of *Psychotria* species differed in the herbivores they supported (Fig. 4.3). Furthermore, whereas interspecific variation in physiological traits important in determining habitat distributions of *Psychotria* on BCI exhibit phylogenetic signal (Sedio *et al.* 2012), only a single principal component of *Psychotria* secondary chemical variation is clearly phylogenetically conserved (Table 4.1). The evolutionary divergence of closely related *Psychotria* with respect to key defensive traits may explain the absence of a phylogenetically-conserved pattern among herbivore assemblages. In fact, the degree to which pairs of *Psychotria* species shared herbivores showed a weakly positive relationship with phylogenetic distance (Fig. 4.3).

Tropical forests have been celebrated as pharmacopeias of plant secondary chemistry and many species-rich genera demonstrate chemical diversity thought to be associated with defense (Becerra 1997, 2007, Wink 2003, Fine *et al.* 2004, 2005, Agrawal 2007, Kursar et al. 2009). Indeed, the very name *Psychotria* invokes the hallucinogenic, or psychotropic, compounds found in some species. If selection for divergence in chemical and other defense traits has contributed to the diversification of species rich plant genera and plays a prominent role in facilitating their coexistence in species-dense local communities, we would expect natural enemy host ranges to be circumscribed at least in part by the interspecific trait variation found within the plant genera.

Despite relatively low host specificity, *Psychotria* herbivores demonstrated nonrandom patterns in their host associations with respect to the trait variation exhibited within the species pool of *Psychotria* on BCI. A host-use model that incorporated species differences in five physical defense traits and 23 axes of secondary chemical variation substantially outperformed alternative models that considered fewer axes of chemical variation or did not account for plant traits at all (Table 4.2). The use of constrained Bayesian priors reduced the number of effective parameters in the model, so although we hypothesize that not all chemical axes reflect significant anti-herbivore defenses, the method permitted us to incorporate weak signals from all available traits. Furthermore, even some chemical axes that explain little of the interspecific variation among *Psychotria* (Table 4.6) were important predictors of shared insect herbivores in our host-use model (Table 4.3). Axes PC19, PC20, and PC21, for example, each represent less than 1% of the chemical variation among species (Table 4.6). However, the amount of variation explained by each PCA axis is strongly associated with the concentration in leaf tissue of the compounds it reflects. In contrast, the potency of secondary compounds as agents of anti-herbivore defense may be only weakly correlated with their concentration in leaf tissue.

The physical traits of mature leaves were strongly associated with the subset of potential *Psychotria* hosts utilized by herbivores (Table 4.3). Furthermore, of the secondary chemical axes that strongly influence herbivore host-use (Table 4.3), the chemical traits that comprise PC4, PC20, PC19, PC9, PC6, and PC2 were notable in that they were positively associated with host use among some insect species but were negatively associated with the host-use patterns of other herbivores, indicating that the *Psychotria* that make up the opposite poles of those trait axes differ substantially in their herbivores. These results suggest that the trait variation found among sympatric species of *Psychotria* does indeed influence the host associations of herbivores. If the *Psychotria*-herbivore interactions mediated by the above traits were of consequence in the assembly of local communities, we would expect *Psychotria* to occur in assemblages that reduce the trait similarity in the immediate neighborhood.

Community Structure

Sedio *et al.* (2012) demonstrated that *Psychotria* co-occurring within 134 census plots of 3 m in radius are more closely related than expected by chance (i.e. are phylogenetically clustered) and that this pattern is likely driven by habitat filtering mediated by phylogenetically-conserved hydraulic traits that influence species differences in drought tolerance. In congruence with this underlying phylogenetic structure of the BCI *Psychotria* community, several leaf traits exhibited a more clustered distribution than expected by chance (Fig. 4.4). In contrast, four axes of secondary chemical variation, PC axes 2, 3, 8, and 9, were substantially more evenly distributed than expected by a null assembly model (i.e. were overdispersed) with respect to both leaves and individuals (Fig. 4.4A and B). Furthermore, co-occurring *Psychotria* individuals were less similar with respect to PC23 (Fig. 4.4B), and leaves less similar with respect to PC12, PC13, PC19, and PC21, than by chance (Fig. 4.4A). Two of these chemical axes were among those most strongly associated with herbivore host use patterns (Table 4.3). The even distribution of defense traits exhibited by *Psychotria* occurring within 3 m in the forest understory therefore suggests that the BCI *Psychotria* partition niche space with respect to herbivores whose diet is constrained by these traits.

The axis PC4 represented a chemical similarity between *Palicourea guianensis* and *Faramea occidentalis*, at one pole, and *Carapichea ipecacuanha* at the other (Table 4.6). *F. occidentalis* was not included in *Psychotria* census plots, and both *F. occidentalis* and *P. guianensis* were excluded from herbivore damage measurements because of their height. These two species did, however, share several herbivores in the form of Buprestid beetles and Sphingid hawkmoth caterpillars with each other and with *P. acuminata*, which also loaded toward the positive end of PC4. Anecdotally, *P.*

guianensis is common in high-light environments, whereas *F. occidentalis* is more persistent in the understory (BES, pers. obs.). Thus, though PC4 was strongly associated with herbivore host use, its contribution to community structure and herbivory rates may have been more notable if *F. occidentalis* were considered among the *Psychotria*.

Other chemical axes that were strongly associated with herbivore host use in our model represented trait axes along which co-occurring *Psychotria* were less similar than expected by random assembly, with the exception of PC20 and PC6 (Fig. 4.4). *F. occidentalis* comprised one extreme of the chemical variation represented by PC20 (Table 4.6). However, the five or so species that shared secondary compounds represented by the opposite pole of PC20 did co-occur with some frequency, despite the fact that high leaf density of species that were similar with respect to PC20 was associated with increased rates of herbivory (Table 4.4). Neighborhood density of PC6, however, was associated with increased rates of herbivory only as part of a model that included all chemical axes (Table 4.4). Some discrepancy between the three major analyses may result from disparities in the superficial damage or fitness consequence of host use among herbivores. For example, not all herbivorous insects included in our host-use model are equal in their propensity to visibly damage leaves. On the other hand, some herbivores, including Hemipterans and weevils, may inflict harm disproportionate to the amount of superficial damage they cause if they act as vectors of plant pathogens.

Sagers & Coley (1995) demonstrated that individuals of *P. horizontalis* exposed to herbivore damage experienced reduced growth and fecundity compared to those that were protected from insect visitation on BCI. Here, we have demonstrated that the incidence of herbivore damage is related to the trait neighborhood defined not only by the density of conspecific individuals, but also by the presence of heterospecific *Psychotria* (Table 4.3). Density-dependent plant-herbivore interactions may, in theory, result in the competitive exclusion of species with shared herbivores ("apparent competition" sensu Holt 1977) or facilitate the coexistence of species with distinct herbivores (Janzen 1970, Connell 1971). Are the *Psychotria*-herbivore interactions identified here likely to limit or to enhance the species richness of the BCI *Psychotria* community relative to what would persist in their absence?

Herbivores that respond to spatial variation in the local density of favorable plant traits should discourage the co-occurrence of their preferred hosts, and hence of phenotypically similar species. Yet whether such community overdispersion of defense traits enhances the species richness of the plant community may depend on the host specificity of plant enemies (Sedio & Ostling 2013). The simulation model of Sedio & Ostling (2013) suggests that the propensity for density-dependent enemies to promote plant coexistence is sensitive to variation in host specificity, whereas the impact enemies have on the trait structure and diversity of the plant community is much more robust to variation in enemy host range. The spatial dispersion of important defense traits in the BCI *Psychotria* community is therefore congruent with theoretical predictions concerning the influence of non-specialist enemies on community trait diversity. In contrast, Sedio & Ostling (2013) suggest some caution in the interpretation of community trait dispersion, as *Psychotria*-herbivore interactions may strongly influence the structure of the community with respect to traits without necessarily stabilizing the coexistence of a greater number of species than would persist in the absence of those enemies.

Character Displacement of Anti-herbivore Defenses and the Diversification of Plant Genera.

Sympatric, congeneric plants that are characterized by phylogenetically conserved habitat preferences nevertheless display a notable absence of phylogenetic signal in traits associated with herbivore host use. Is this evidence of character displacement of anti-herbivore defense traits? Character displacement is an evolutionary divergence in traits caused by interspecific competition (Brown & Wilson 1956). The concept has been applied primarily to traits that mediate resource competition (e.g. Grant 1972, Grant & Grant 2006), but extends to defense traits that affect competition mediated by shared enemies (Chesson & Kuang 2008). The high species richness, low population sizes, and spatial variation in species composition characteristic of tropical forests are thought to weaken the strength of natural selection exerted by pairwise species interactions (Hubbell 2006). Perhaps it is no accident that the classical examples of character displacement pertain to low-diversity localities such as oceanic islands where the strength of pairwise competitive interactions are amplified (e.g. Grant 1972).

Barro Colorado Island is not an oceanic island, but a former hilltop isolated a mere 200 m from mainland Panama by the flooding of Gatun Lake reservoir, part of the Panama Canal. A consideration of the biogeographic history of the *Psychotria* of Panama is therefore instructive. The Isthmus of Panama connected Central and South America only 3 Ma, permitting the Great American Biotic Interchange of terrestrial plants and animals (Coates & Obando 1996). Though some intercontinental dispersal of plants may have been facilitated by an island chain prior to the closure of the Isthmus

(Cody *et al.* 2010) there is evidence that regional floristic composition of Rubiaceae in general (Antonelli *et al.* 2009), and of *Psychotria* in particular (Sedio *et al.* 2013), were strongly influenced by the uplift of the Andes and the closure of Panama. The BCI *Psychotria* community is comprised of several phylogenetic lineages with evolutionary histories in Central America, the Amazon basin, and the Chocó region of western Colombia and Ecuador, each of which dispersed to central Panama relatively recently (Sedio *et al.* 2013). Furthermore, the *Psychotria* that co-occur at 3 m spatial scales on BCI are likely to have originated in the same geographic region. Much of the physiological trait variation exhibited by the BCI *Psychotria* is therefore not likely a result of *in situ* trait evolution in response to local species interactions, but rather reflects the climatic diversity of the regions in which *Psychotria* diversified prior to assembling in central Panama. Defense traits, on the other hand, do distinguish closely related species with shared biogeographic histories (Table 4.1). Such differences, however, may have their origins in interactions that took place prior to the dispersal of close relatives to Panama. Indeed, if divergence in defense was associated with speciation *per se* (e.g. Fine *et al.* 2013), then the interactions responsible for the early divergence of *Psychotria* defenses from those of their common ancestors may have taken place elsewhere for a majority of the species found on BCI.

In sum, there is little evidence that the differences in secondary chemistry exhibited by closely related *Psychotria* on BCI originated through *in situ* trait evolution. In fact, only two of the *Psychotria* species in this study (*P. acuminata* and *P. racemosa*) exhibited intraspecific variation between populations on BCI and in Costa Rica (A. Berger, unpublished data). Yet the absence of phylogenetic signal observed with respect to putative defense traits and to herbivore assemblages contrasts notably with the phylogenetic conservatism exhibited by physiological traits and habitat preferences (Sedio *et al.* 2012). This suggests that chemical leaf traits of close relatives tend to diverge from shared ancestral states at a greater pace than physiological traits important in defining the abiotic niche. Therefore, our results do not necessarily suggest character displacement on BCI itself, yet the contrasting evolutionary history of chemical and physiological traits in the genus implicates herbivore-driven selection for chemical divergence as an important source of evolutionary change in *Psychotria* as a whole.

To understand whether the niche evolution of *Psychotria* is characteristic of other woody plant genera, it is useful to reexamine the patterns of trait evolution and community structure of other species-rich genera, both tropical and temperate. In North America, several major subgeneric lineages of oaks (*Quercus*) appear to have diversified with respect to major soil moisture gradients and fire regimes (Cavender-Bares *et al.* 2004). Similarly, two major clades within the genus *Ceanothus* each appear to have diversified with respect to elevational and precipitation gradients in the American West (Ackerly *et al.* 2006). In both cases, local communities consist of members of distinct early-diverging clades within the respective genus.

In the case of the tropical genus *Protium*, adaptation to alternative edaphic regimes of the Amazon basin is associated with a growth-defense tradeoff, and adaptation to nutrient poor white sand soils has occurred independently in many lineages in the genus (Fine *et al.* 2004, 2005). Assemblages of *Inga*, however, are phylogenetically clustered at broad spatial scales commensurate with soil moisture and edaphic gradients in the Peruvian Amazon, but phylogenetically evenly distributed at a finer spatial scale on

BCI (Kursar *et al.* 2009). *Inga* and *Bursera*, like *Psychotria* (Fig. 4.4), occur in assemblages that are less similar with respect to secondary chemistry and other defense traits than by chance (Becerra 2007, Kursar *et al.* 2009). It remains to be seen if other species-rich tropical genera are also characterized by rapid defense evolution relative to habitat-related niche axes, and assembly patterns indicative of enemy-mediated niche partitioning. A consistent tendency for otherwise ecologically similar “species swarms” to exhibit defense trait patterns like those of *Inga* and *Psychotria* may suggest that enemy-mediated competition is a more important ecological driver of diversification in tropical woody plant genera than in genera that diversified in temperate regions.

Conclusion

We have demonstrated that insect herbivores of *Psychotria* shrubs and treelets on BCI, Panama, exhibit polyphagous host ranges within the plant genus, but nevertheless are likely to exploit a subset of the BCI species pool circumscribed by species differences in physical and chemical defense traits. Furthermore, the results presented here indicate broad congruence among the plant traits implicated in herbivore host-use patterns, *Psychotria* community structure, and herbivore leaf damage relative to the trait neighborhood of the plant. These results suggest that enemy-mediated competitive interactions have played an important role in the divergence of related species with respect to secondary chemistry and other traits that influence host use of herbivores. The association between herbivore leaf damage and the neighborhood similarity of plants with respect to key defensive traits suggests that shared, density-responsive herbivores exert a fitness consequence on *Psychotria* that is likely to select for trait divergence among species, particularly those that exploit the same habitats. The defense trait differences examined here did not likely evolve *in situ* on BCI, but nevertheless influence community assembly at fine spatial scales on the island and may contribute to the stabilization of coexistence of species that differ sufficiently along this important niche axis. Trait evolution within *Psychotria* presents an intriguing contrast between traits that influence microhabitat distributions and those that mediate interactions with herbivores. Physiological traits that mediate species distributions with respect to understory soil moisture gradients are characterized by phylogenetic conservatism (Sedio *et al.* 2012). On the other hand, the secondary chemistry and associated herbivore assemblages examined here revealed evidence of evolutionary differentiation among close relatives. These findings suggest that trait evolution with respect to defense may contribute to the diversification of species rich tropical tree genera such as Gentry's (1982,1989) species swarms by allowing ecologically similar close relatives to partition niche space locally even while sharing other aspects of their ancestral niche.

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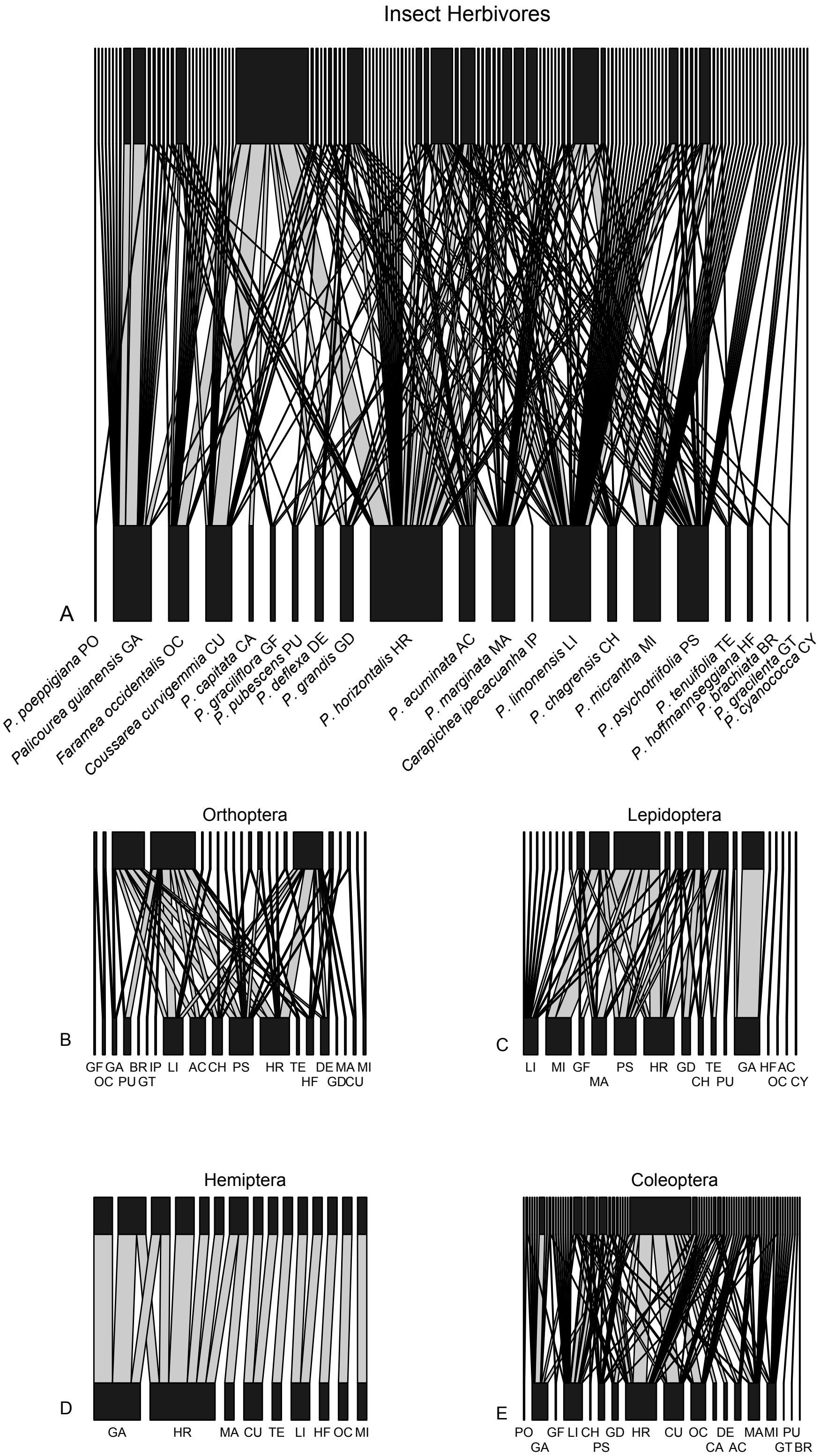

Figure 4.1. Bipartite trophic network of insect herbivores of *Psychotria*.

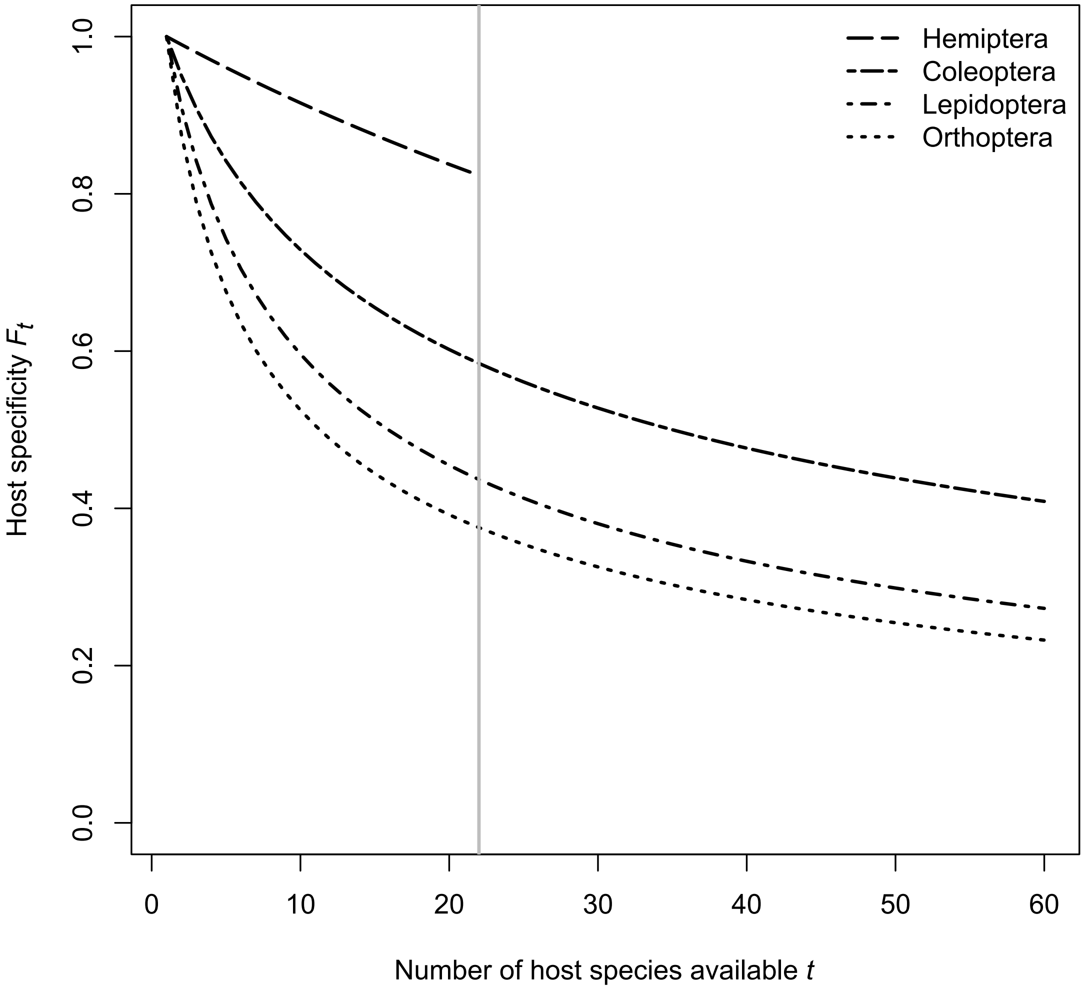

Figure 4.2. Effective specialization F_t of *Psychotria* insect herbivores. Host specificity as a function of available *Psychotria* host species is projected for up to 60 co-occurring plant hosts. The number of host species considered here is indicated by the gray vertical line.

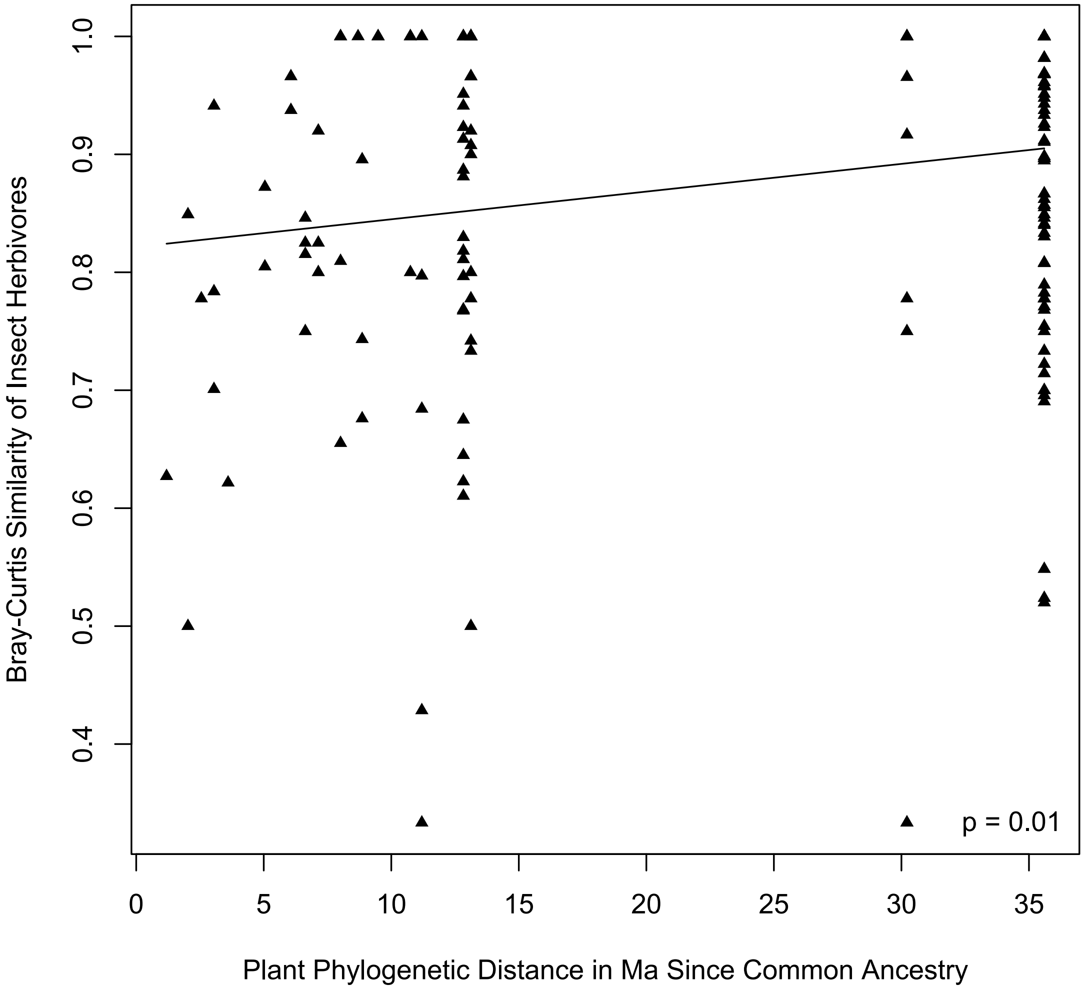

Figure 4.3. Similarity of *Psychotria* herbivore assemblages and phylogenetic distance. All pairwise comparisons of 20 species of *Psychotria* are indicated. Significance was measured using a two-sided Mantel test of the correlation between distance matrices.

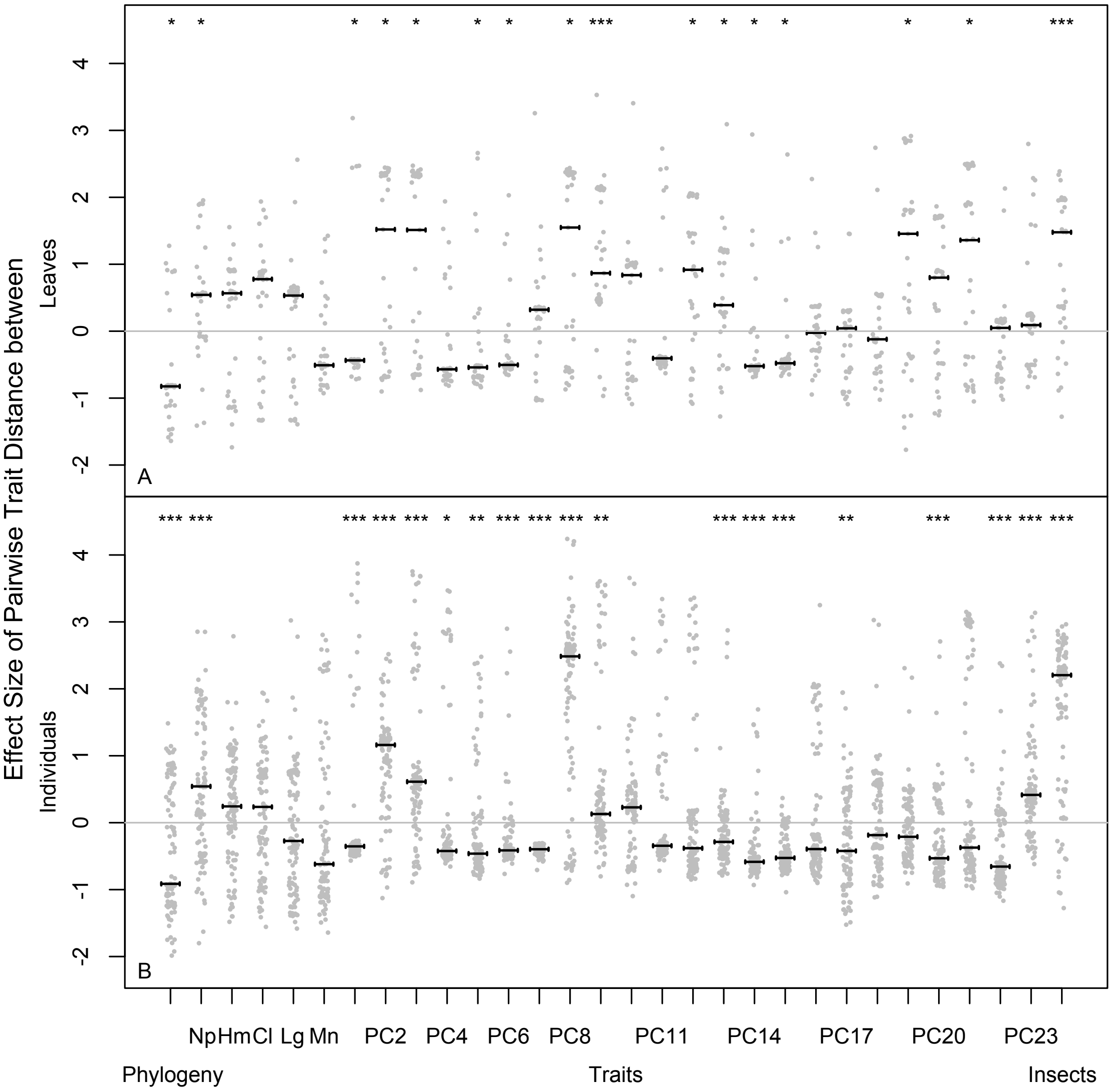

Figure 4.4. Community structure of *Psychotria* with respect to phylogeny, traits, and herbivores. Each point represents the mean pairwise distance (MPD) of the *Psychotria* species occurring in one 3-m radius census plot compared to the null expectation for a plot with an equivalent number of species. Horizontal bars indicate the median effect size for all plots in which leaf herbivory was measured (A; N = 57) or all census plots of *Psychotria* individuals (B; $n = 134$). Significance of Bonferroni-corrected two-sided Wilcoxon rank sum tests for greater evenness (effect size > 0) or clustering (effect size < 0) is indicated as follows: * $p < 0.05$; ** $p < 0.005$; *** $p < 5 \times 10^{-4}$.

Trait	Pagel's λ	p ($\lambda > 0$)	p ($\lambda < 1$)	Blomberg's *K*	p (PIC)
Nonpolar Comp.	0.085	1.000	0.017*	0.2848	1.000
Hemicellulose	0.527	0.278	0.009**	0.3442	1.000
Cellulose	0.221	1.000	0.139	0.3303	1.000
Lignin	0.447	0.400	0.613	0.4404	0.224
Mineral Ash	0.000	1.000	0.520	0.4038	1.000
PC1	0.000	1.000	1.000	0.4378	1.000
PC2	0.993	1.000	1.000	0.7180	0.924
PC3	1.000	0.035*	1.000	0.9162	0.112
PC4	0.000	1.000	1.000	0.4308	1.000
PC5	1.000	1.000	1.000	0.4584	1.000
PC6	0.000	1.000	0.517	0.3332	1.000
PC7	0.000	1.000	0.000***	0.1643	1.000
PC8	0.000	1.000	0.444	0.3244	1.000
PC9	0.000	1.000	0.291	0.3175	1.000
PC10	0.038	1.000	0.030*	0.2561	1.000
PC11	0.000	1.000	0.000***	0.1540	1.000
PC12	1.000	1.000	1.000	0.6084	1.000
PC13	0.000	1.000	1.000	0.4741	1.000
PC14	0.000	1.000	1.000	0.3558	1.000
PC15	0.000	1.000	0.000***	0.1930	1.000
PC16	1.000	0.160	1.000	0.8127	0.140
PC17	0.000	1.000	0.020*	0.2503	1.000
PC18	0.000	1.000	0.000***	0.1897	1.000
PC19	0.000	1.000	0.021*	0.2523	1.000
PC20	0.000	1.000	0.001***	0.1906	1.000
PC21	0.042	1.000	0.011*	0.2366	1.000
PC22	0.000	1.000	0.000***	0.0893	1.000
PC23	0.000	1.000	0.000***	0.1654	1.000

Table 4.1. Phylogenetic signal of 28 leaf traits. Pagel's λ and Blomberg's $K = 1$ under a Brownian Motion, random drift model of trait evolution; $\lambda = 0$ when traits are unrelated to phylogeny. Phylogenetic signal is significantly greater than a random model when $\lambda > 0$ or PIC variance < random; λ significantly < 1 indicates a departure from Brownian motion, random walk evolution, such as that caused by selection. P-values are Bonferroni-corrected (N = 28).

Model	log Marginal Likelihood	Δ Marginal Likelihood	Evidence Ratio
Phys + (PC1-PC23)	-723.03	0	1
Phys + (PC1-PC16)	-731.94	-8.92	0.000134
Phys + (PC1-PC8)	-735.93	-12.91	$2.49 \text{ X } 10^{-06}$
Phys	-860.63	-137.60	$1.75 \text{ X } 10^{-60}$
Abundance	-1031.73	-308.70	$8.58 \text{ X } 10^{-135}$

Table 4.2. Model selection of five alternative hierarchical Bayesian models of herbivore host use. Model "Abundance" included only leaf and stem abundances for each *Psychotria* species. Model "Phys" included leaf and stem abundances, as well as five physical leaf traits. Additional models included leaf and stem abundances, five physical leaf traits, and the first eight, 16, or 23 principal components of secondary chemistry, respectively.

Predictor Variable	Mean β_i	Δ log Likelihood	Mean Standard Deviation β_{ik}	Negative Posterior Mean β_{ik}	Positive Posterior Mean β_{ik}
Individual abund.	0.29	-28.48	0.1892	0.00	126.19
Leaf abund.	-0.06	-9.28	0.1890	-32.58	1.37
Nonpolar comp.	0.25	-44.17	0.2752	-0.25	84.09
Hemicellulose	-0.60	-77.40	0.2180	-228.12	0.00
Cellulose	0.34	-41.17	0.2323	0.00	139.00
Lignin	0.34	-62.84	0.4746	-2.16	85.92
Mineral ash	-0.69	-111.94	0.1751	-328.83	0.00
PC1	-0.32	-4.08	0.2327	-110.07	0.00
PC2	-0.05	-28.66	0.2157	-23.01	2.60
PC3	-0.41	-62.74	0.2265	-148.10	0.00
PC4	-0.16	-129.89	0.7259	-49.60	24.36
PC5	0.32	-27.58	0.2942	-0.07	94.80
PC6	0.01	-30.67	0.2132	-2.91	4.97
PC7	0.29	-9.64	0.2142	0.00	93.83
PC8	0.35	-102.24	0.1425	0.00	178.78
PC9	0.03	-37.22	0.2292	-3.01	14.06
PC10	0.06	-24.58	0.1728	-1.45	26.52
PC11	-0.04	-10.67	0.2101	-16.08	1.84
PC12	-0.07	-28.60	0.2586	-20.66	0.53
PC13	0.33	-19.34	0.2897	-0.13	100.09
PC14	-0.14	-15.10	0.1867	-51.25	0.02
PC15	-0.14	-8.34	0.1791	-48.90	0.04
PC16	-0.10	-7.88	0.1804	-35.93	0.00
PC17	0.06	-9.14	0.2511	-1.48	24.97
PC18	-0.18	-15.85	0.1749	-76.75	0.00
PC19	-0.04	-61.38	0.5333	-25.83	21.45
PC20	-0.36	-87.49	0.3573	-114.16	0.13
PC21	-0.12	-27.48	0.2056	-61.62	0.47
PC22	0.11	-8.74	0.1412	0.00	69.80
PC23	-0.22	-22.06	0.1840	-92.62	0.00

Table 4.3. Contribution of physical and chemical leaf traits to herbivore host use. The coefficient β_{ik} reflects the affinity of herbivor *i* for trait *k*. The Δ(log-likelihood) associated with removing a trait from the model indicates that trait's contribution to the fit of the complete model. The mean standard deviation of β_{ik} over the posterior sample for all insects indicates the variation in response by insect species; greater standard deviation indicates that herbivore species responded differently to that trait. Negative and positive posterior mean β_{ik} reflect confidence in the sign of β_{ik}; coefficients with both negative and positive posterior means indicate differences in response by different species of herbivore.

Model, AIC	Likelihood Ratio Test	Variable	Estimate	Standard Error	t-value
SPEC + PLOT (null)	-	intercept	-2.0108	0.06	-35.40
AIC = 11421.12	-				
CONS + SPEC + PLOT	1.460	intercept	-2.0911	0.0528	-39.63
11421.66	p = 0.23	CONS	0.0012	0.0003	4.20
PHYLO + SPEC + PLOT	0.430	intercept	-1.9938	0.0560	-35.64
11422.69	p = 0.51	PHYLO	-0.1912	0.1093	-1.75
INSC + SPEC + PLOT	2.694	intercept	-1.9842	0.0582	-34.10
11420.43	p = 0.10	INSC	-0.1910	0.0782	-2.44
PC20 + SPEC + PLOT	5.288	intercept	-1.9839	0.0562	-35.33
11417.83	p = 0.02	PC20	-0.3022	0.1072	-2.82
PC21 + SPEC + PLOT	4.480	intercept	-1.9884	0.0578	-34.40
11418.64	p = 0.03	PC21	-0.1962	0.0693	-2.83
PC2 + PC8 + SPEC + PLOT	5.802	intercept	-1.9770	0.0555	-35.64
11419.32	p = 0.02	PC2	-1.9089	1.1330	-1.68
		PC8	1.5499	1.0130	1.53
LIGN + HEMI + NPLR +	98.188	intercept	-2.0013	0.0639	-31.31
MINR + PC1 + … + PC23					
11376.93	p = 5.1 X 10^{-10}	LIGN	1.6977	2.0835	-0.688
		HEMI	-1.1762	1.6222	-0.725
		NONP	0.0330	1.0176	0.032
		MINR	-1.4336	1.3215	1.285
		PC1	12.5463	19.5133	0.643
		PC2	9.1402	17.8673	0.512
		PC3	-1.3894	7.0193	-0.198
		PC4	-79.0370	60.9098	-1.298
		PC5	20.6867	18.8154	1.099
		PC6	55.2716	54.2576	1.019
		PC7	-2.0412	3.0351	-0.673
		PC8	-6.7960	15.6348	-0.435
		PC9	2.9482	3.1533	0.935
		PC10	-3.1563	7.9026	-0.399
		PC11	1.0041	3.1980	0.314
		PC12	1.3145	1.8862	0.697
		PC13	1.4346	1.2168	1.179
		PC14	-19.5048	22.8995	-0.852
		PC15	2.9212	9.7852	0.299
		PC16	2.8152	6.1958	0.454
		PC17	-4.1040	2.9665	-1.383
		PC18	3.3176	5.4566	0.608
		PC19	0.2839	2.0580	0.138
		PC20	6.0078	3.9285	1.529
		PC21	-5.7637	3.3773	-1.707
		PC22	-1.1555	4.5999	-0.251
		PC23	0.9121	2.3339	0.391

Table 4.4. Mixed effects models of herbivore leaf damage. The logarithm of the proportion of leaf damaged was modeled as a function of the mean trait distance between the focal leaf and all other leaves in a plot for 5,389 leaves in 57 census plots, treating species (SPEC) and plot (PLOT) as random effects. Variables are conspecific leaf density (CONS), phylogenetic distance among *Psychotria* (PHYLO), similarity of insect herbivore assemblages measured as network distance (INSC), and the physical leaf traits lignin (LIGN), hemicellulose (HEMI), nonpolar compounds (NPLR), mineral ash (MINR) and principal components of secondary chemistry PC1-PC23. Likelihood ratio tests were performed relative to the null model (SPEC + PLOT)

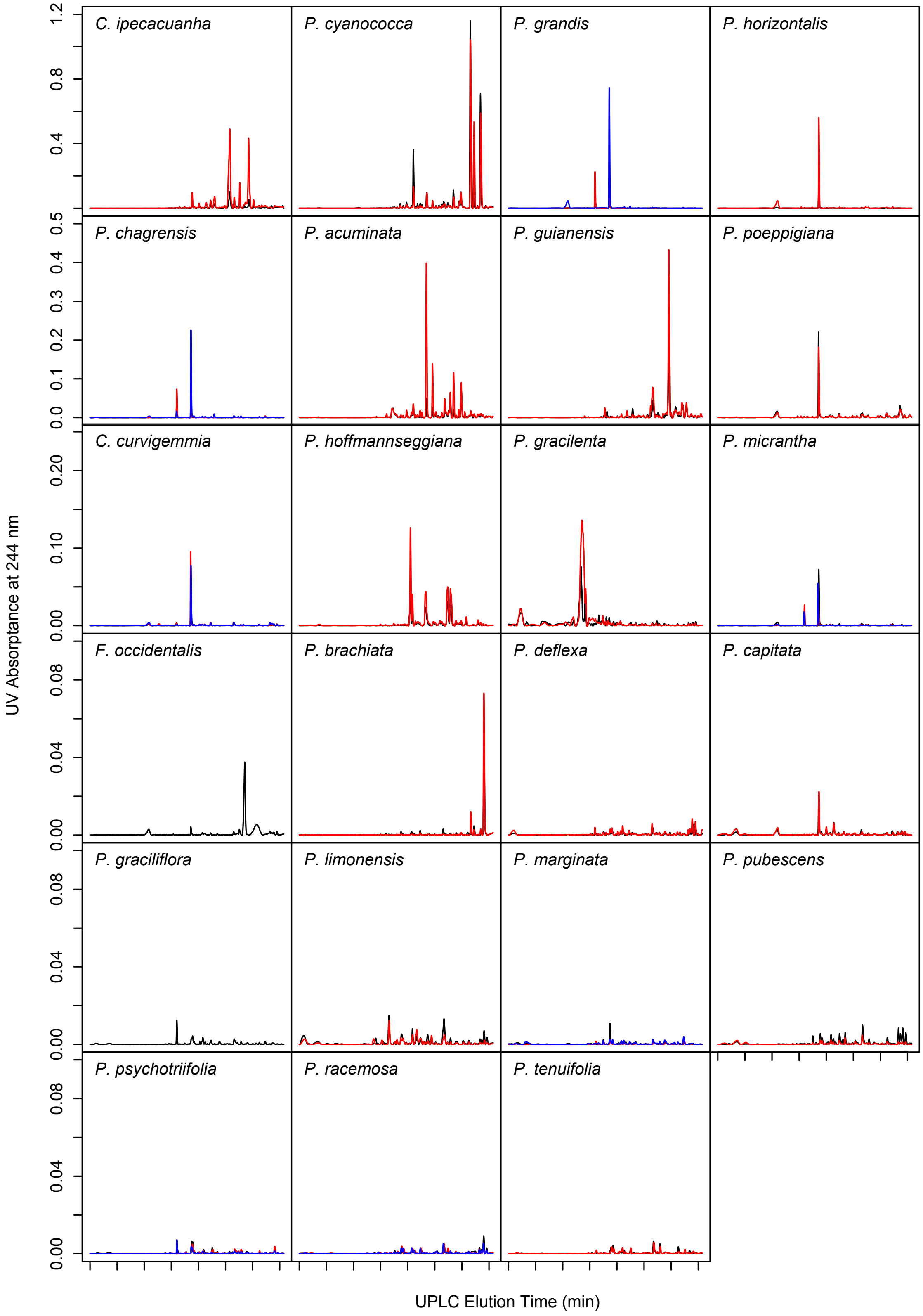
C. ipecacuanha
P. cyanococca
P. grandis
P. horizontalis
P. chagrensis
P. acuminata
P. guianensis
P. poeppigiana
C. curvigemmia
P. hoffmannseggiana
P. gracilenta
P. micrantha
F. occidentalis
P. brachiata
P. deflexa
P. capitata
P. graciliflora
P. limonensis
P. marginata
P. pubescens
P. psychotriifolia
P. racemosa
P. tenuifolia
1.2
0.8
0.4
0.5
0.4
0.3
0.2
0.1
0.0
0.20
0.10
0.00
0.08
0.04
0.00
0.08
0.04
0.00
0.08
0.04
0.00
UV Absorptance at 244 nm
UPLC Elution Time (min)

Figure 4.5. UPLC chromatograms of the BCI *Psychotria* and their relatives in the Rubiaceae. UV absorbance at 244 nm wavelength is shown. Each individual per species is shown in black, red, and blue, respectively. Note that the y-axis, indicating the strength of UV absorbance, varies by row.

Species	Cellulose	Hemicellulose	Lignin	Mineral Ash	Nonpolar
P. chagrensis	0.1949	0.1841	0.1344	0.0008285	0.4857
P. psychotriifolia	0.1957	0.1005	0.1484	0.0003669	0.5549
P. tenuifolia	0.1474	0.0899	0.1226	0.0021763	0.6380
P. limonensis	0.1402	0.0700	0.2070	0.0010769	0.5817
P. grandis	0.1940	0.0987	0.1712	0.0031699	0.5330
P. graciliflora	0.1295	0.1081	0.1204	0.0015918	0.6404
P. micrantha	0.1536	0.0881	0.1681	0.0056961	0.5844
P. marginata	0.1415	0.0837	0.1381	0.0002077	0.6365
P. horizontalis	0.1866	0.1770	0.1365	0.0004213	0.4995
P. brachiata	0.1992	0.1664	0.0910	0.0058669	0.5375
P. cyanococca	0.2060	0.2050	0.0711	0.0011091	0.5169
P. pubescens	0.1671	0.1929	0.1018	0.0002781	0.5380
P. poeppigiana	0.2076	0.1634	0.1812	0.0034435	0.4443
P. capitata	0.1826	0.1690	0.0723	0.0026860	0.5733
Pal. guianensis	0.1410	0.1381	0.1141	0.0006257	0.6063
P. gracilenta	0.1968	0.1522	0.0950	0.0016632	0.5544
P. hoffmannseggiana	0.1935	0.1790	0.0728	0.0024842	0.5523
P. acuminata	0.1784	0.1489	0.1094	0.0052720	0.5580
P. racemosa	0.1910	0.2457	0.0666	0.0004147	0.4964
P. deflexa	0.2081	0.1818	0.0685	0.0008358	0.5407
Car. ipecacuanha	0.2123	0.2189	0.1002	0.0072808	0.4613
Cou. curvigemmia	0.1313	0.1050	0.1008	0.0000000	0.6629
Far. occidentalis	0.1726	0.0994	0.1201	0.0006277	0.6072

Table 4.5. Mass fraction in g g^{-1} of five physical leaf traits measured for fully mature leaves.

Species	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
P. chagrensis	0.001922	0.065117	-0.050004	0.000011	-0.002900	0.001764	-0.008273	0.101763
P. psychotriifolia	0.000445	0.003428	0.000154	-0.000027	0.000278	0.003052	-0.002042	0.009882
P. tenuifolia	0.000924	0.003292	0.001604	0.000848	0.001482	0.003941	-0.002294	0.004953
P. limonensis	0.001314	0.004629	0.001908	0.001846	0.002047	0.007349	-0.011211	0.008030
P. grandis	0.004240	0.258228	-0.206392	-0.000895	0.000091	-0.004633	-0.007213	-0.160596
P. graciliflora	0.000694	0.003861	-0.000295	0.000339	-0.001061	0.002794	-0.004240	0.010630
P. micrantha	0.000897	0.031161	-0.023517	0.000091	-0.000048	0.000798	-0.003415	-0.014395
P. marginata	0.000731	0.003936	-0.000960	0.000476	-0.001028	0.003365	-0.001634	0.009769
P. horizontalis	0.005517	0.234303	-0.184060	-0.000824	-0.003723	0.000255	-0.001912	0.357630
P. brachiata	0.004234	0.002142	0.001755	-0.000338	-0.007690	0.001676	-0.003598	0.003687
P. cyanococca	0.704041	-0.031746	-0.019265	-0.020202	-0.042480	-0.024831	0.000259	-0.001287
P. pubescens	0.001573	0.002978	0.002358	0.000945	0.003289	0.009842	-0.002512	0.001907
P. poeppigiana	0.009949	0.149788	-0.107291	0.004694	-0.010724	0.006586	-0.000629	-0.005325
P. capitata	0.000981	0.015801	-0.010383	0.000180	-0.000226	0.001943	-0.001897	0.018943
Pal. guianensis	0.025677	0.118171	0.149205	0.565130	0.008230	-0.054515	-0.005094	0.011451
P. gracilenta	0.001550	0.011493	-0.003077	0.002373	0.002221	0.021662	-0.643239	-0.022002
P. hoffmannseggiana	0.004335	0.012400	0.010351	0.009406	0.011803	0.092020	-0.014323	0.013081
P. acuminata	0.027767	0.025529	0.022894	0.038015	0.033066	0.571988	0.014613	-0.005308
P. racemosa	0.001006	0.002215	0.001534	0.000809	-0.000143	0.003255	-0.002771	0.001791
P. deflexa	0.001719	0.003312	0.001068	0.001091	0.001252	0.004013	-0.003924	0.004720
Car. ipecacuanha	0.020190	0.328941	0.413850	-0.230286	-0.003399	-0.011763	-0.002095	0.011538
Cou. curvigemmia	0.001747	0.055760	-0.043156	0.000138	-0.000006	-0.000030	0.000739	-0.006380
Far. occidentalis	0.013079	0.098303	0.121434	0.223961	0.004200	0.001440	0.009251	-0.014043
% Variance	26.01	12.04	11.84	8.71	6.84	6.21	3.98	3.44

Table 4.6. Species factor scores with respect to 23 principal components of interspecific variation in secondary chemistry. 79.07 % of chromatographic variation in secondary chemistry.

Species	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16
P. chagrensis	-0.000818	-0.131081	-0.000056	0.001275	-0.007645	0.007208	-0.005498	0.025996
P. psychotriifolia	-0.003851	-0.006020	0.009821	-0.001490	-0.040700	0.000154	-0.004996	0.025028
P. tenuifolia	-0.008149	-0.002674	0.019578	0.008753	-0.042603	0.021181	-0.005499	0.024620
P. limonensis	-0.018385	0.001167	0.017963	0.024620	-0.089630	0.010451	0.009503	0.046251
P. grandis	-0.009956	-0.335164	-0.005516	-0.004561	0.021009	-0.011861	0.003070	-0.018168
P. graciliflora	-0.006472	-0.039856	0.011343	0.002368	-0.027840	0.016886	-0.004994	0.042590
P. micrantha	-0.004590	-0.053421	0.004729	0.000205	-0.007908	0.001785	0.009465	0.010467
P. marginata	0.000140	0.000653	0.007323	0.003775	-0.015760	0.007704	0.002218	0.019951
P. horizontalis	0.019041	0.098376	-0.004348	-0.000471	0.022858	0.000611	0.004747	-0.018830
P. brachiata	-0.011132	-0.002169	0.011937	-0.026341	-0.513231	-0.351322	-0.004985	-0.258566
P. cyanococca	0.003737	-0.000505	-0.000284	-0.000422	0.007883	-0.002557	0.001430	0.000823
P. pubescens	-0.013159	-0.000716	0.011357	0.006918	-0.072878	-0.004934	0.003752	0.026218
P. poeppigiana	-0.031124	0.030204	0.024762	0.028624	-0.151749	0.071243	-0.031095	0.116940
P. capitata	-0.002867	0.006471	0.008733	0.003878	-0.022018	0.000005	0.009495	0.014661
Pal. guianensis	-0.357382	0.010863	-0.027690	-0.084576	0.040938	-0.003484	0.007219	-0.052772
P. gracilenta	0.013192	0.034871	-0.010829	-0.002182	-0.038468	0.060609	0.278072	0.007136
P. hoffmannseggiana	-0.035243	-0.001537	0.642767	0.012508	0.064692	-0.067915	0.010207	0.013766
P. acuminata	-0.008648	0.001875	-0.027146	0.021690	-0.134180	0.328504	-0.105175	-0.156033
P. racemosa	-0.006975	-0.001237	0.012836	0.003647	-0.069019	-0.008695	0.000372	0.003471
P. deflexa	-0.005992	-0.011369	0.012839	0.001282	-0.065497	-0.001191	-0.001186	0.017939
Car. ipecacuanha	-0.122738	-0.001811	0.000918	-0.097118	-0.143743	0.032068	-0.052529	0.306620
Cou. curvigemmia	-0.003938	0.002118	0.003834	0.006053	-0.016089	0.001341	-0.002876	0.009585
Far. occidentalis	0.422998	-0.011648	0.016287	0.038136	-0.057683	0.006518	-0.012026	0.104170
% Variance	3.05	2.87	2.44	1.43	1.35	1.17	1.07	1.01

Table 4.6, *continued*. Species factor scores with respect to 23 principal components of interspecific variation in secondary chemistry. 79.07 % of chromatographic variation in secondary chemistry.

Species	PC17	PC18	PC19	PC20	PC21	PC22	PC23
P. chagrensis	0.221374	0.308286	-0.027418	0.057735	-0.005690	0.234010	-0.007473
P. psychotriifolia	0.015671	0.041615	-0.007427	-0.033190	-0.065483	-0.019922	0.004516
P. tenuifolia	-0.028608	0.048500	-0.010898	-0.041611	-0.147531	-0.001609	0.012475
P. limonensis	-0.098568	0.109975	-0.038789	-0.117548	-0.502437	-0.102774	-0.009642
P. grandis	0.069819	-0.025708	-0.017232	0.044712	-0.029921	-0.035137	-0.003553
P. graciliflora	0.046458	0.120462	0.002514	-0.028435	-0.070340	-0.002606	-0.005554
P. micrantha	0.025448	0.004715	0.143535	-0.283683	0.021654	0.132413	-0.002308
P. marginata	-0.001471	0.022941	0.003386	-0.034503	-0.076603	-0.016434	0.003657
P. horizontalis	0.016501	-0.038547	0.016975	-0.004203	0.008126	-0.021122	0.066200
P. brachiata	0.063198	-0.042085	0.014802	0.023819	0.053423	-0.000568	0.002633
P. cyanococca	0.003730	-0.002779	-0.000648	0.000006	-0.002255	0.000190	0.000046
P. pubescens	-0.074778	0.073173	-0.032370	-0.057711	-0.165492	-0.025778	0.006211
P. poeppigiana	-0.480568	0.282979	-0.012437	-0.008181	0.264013	-0.004240	0.028853
P. capitata	-0.031386	0.031114	0.017796	-0.036625	-0.061419	-0.098752	-0.000151
Pal. guianensis	-0.000157	0.005634	0.012312	0.005667	-0.002859	-0.001307	0.002135
P. gracilenta	0.010994	-0.015597	-0.006998	0.016885	0.016347	0.008611	-0.003896
P. hoffmannseggiana	0.023553	-0.025166	-0.008249	0.008198	0.012351	-0.001111	0.251016
P. acuminata	0.052929	-0.040982	-0.010272	0.001033	0.013240	-0.002110	-0.022154
P. racemosa	-0.050228	0.050502	-0.005598	-0.031688	-0.074351	0.007487	0.008474
P. deflexa	-0.072792	0.059159	0.004914	-0.036663	-0.060664	0.000295	0.012177
Car. ipecacuanha	0.088496	-0.111396	-0.032070	0.007441	0.030879	0.002455	-0.027762
Cou. curvigemmia	-0.043045	0.027281	-0.008386	0.005957	-0.023084	0.142394	-0.020934
Far. occidentalis	-0.033829	-0.011830	0.420723	0.210452	-0.074963	0.018976	0.019842
% Variance	0.74	0.73	0.60	0.58	0.49	0.39	0.35

Table 4.6, *continued.* Species factor scores with respect to 23 principal components of interspecific variation in secondary chemistry. 79.07 % of chromatographic variation in secondary chemistry.

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Chapter 5: How specialised must natural enemies be to facilitate coexistence among plants?

Abstract

The Janzen-Connell hypothesis proposes that plant interactions with host-specific antagonists can impair the fitness of locally abundant species and thereby facilitate coexistence. However, insects and pathogens that associate with multiple hosts may mediate exclusion rather than coexistence. We employ a simulation model to examine the effect of enemy host breadth on plant species richness and defense community structure, and to assess expected diversity maintenance in example systems. Only models in which plant enemy similarity declines rapidly with defense similarity support greater species richness than models of neutral drift. In contrast, a wide range of enemy host breadths result in spatial dispersion of defense traits, at both landscape and local scales, indicating that enemy-mediated competition may increase defense-trait diversity without enhancing species richness. Nevertheless, insect and pathogen host associations in Panama and Papua New Guinea demonstrate the potential to enhance plant species richness and defense-trait diversity comparable to strictly specialized enemies.

Introduction

How large numbers of plant species manage to coexist in the face of intense competition for light, water, and other shared resources remains a fundamental challenge to community ecology (Wright 2002; Silvertown 2004). A now-classical hypothesis put forth independently by Janzen (1970) and Connell (1971) proposes that plants fail to recruit in the neighborhood of conspecific adults as a result of attack by specialized natural enemies, such as insects or pathogens, that respond either to the presence of adult plants or to the density of offspring of their host species. This mechanism is thought to limit the abundance of any given species, and thereby facilitate coexistence among plants.

A considerable amount of evidence has accrued in the last 40 years in support of the predictions of Janzen and Connell, namely that offspring fail to survive at high densities or in the vicinity of conspecific adults in tropical forests (e.g. Wills et al. 1997; Webb & Peart 1999; Harms et al. 2000; HilleRisLambers et al. 2002; Comita et al. 2010; Terborgh 2012). Yet recent large-scale community surveys suggest that within at least some communities of both insect herbivores (Basset 1992; Odegaard *et al.* 2000; Novotny *et al.* 2002; Odegaard *et al.* 2005; Novotny *et al.* 2010) and fungal pathogens (Gilbert 2005; Gilbert & Webb 2007; Liu *et al.* 2012), enemies seldom specialize on a single host, but rather associate with a range of often closely related species. Additionally, recruitment patterns consistent with the expectations of Janzen and Connell are not restricted to the tropics (Johnson *et al.* 2012), despite some suggestions that host-specificity declines with latitude (Dyer *et al.* 2007). More broadly, knowledge of how specialized enemies are, and how large of an effect specialized enemies have on host fitness as compared with their more generalist counterparts, is rather limited. Shared natural enemies may result in coexistence or competitive exclusion depending on the degree to which competitors partition niche space defined by antagonists (Bever 2003; Chesson & Kuang 2008). Hence, it remains unclear whether plants differ in their interactions with enemies sufficiently to facilitate the maintenance of diversity in tropical forests (Freckleton & Lewis 2006) or elsewhere. Are plant antagonists specialized enough to facilitate coexistence? How specialized must they be?

Even if natural enemies are not specialized enough to foster coexistence of competing plant species, they may still influence the phylogenetic or defense trait composition in plant communities. The degree to which plant species share herbivores (Novotny *et al.* 2002; Odegaard *et al.* 2005; Novotny *et al.* 2010) or pathogens (Gilbert & Webb 2007; Liu *et al.* 2012) is broadly associated with plant phylogeny, most likely as a result of conservatism of host use-determining traits at deep scales of phylogeny (Wink 2003; Barrett & Heil 2012). As a result, the exclusion of related heterospecific individuals through competition mediated by shared enemies (Holt 1977) may be partially responsible for observations of phylogenetically even ("overdispersed" sensu Cavender-Bares *et al.* 2004) species assemblages at small spatial scales (Webb *et al.* 2006; Bagchi *et al.* 2010; Metz *et al.* 2010). On the other hand, detailed investigations of *Asclepias* milkweeds (Agrawal & Fishbein 2006) and the tropical tree genera *Bursera* (Becerra 1997) and *Inga* (Kursar *et al.* 2009) suggest that plant defenses can be quite evolutionarily labile at finer scales of phylogeny, such as within a genus. Furthermore, communities of co-occurring *Bursera* (Becerra 2007) and *Inga* (Kursar *et al.* 2009) appear to exhibit a more even distribution of defenses than by chance. These findings suggest that density-responsive insects and pathogens increase the diversity of the plant community with respect to defense traits, and potentially increase plant species richness in the process. Furthermore, by allowing phenotypically distinct plants to escape the enemy load of neighboring heterospecifics, nonspecialist enemies may promote divergence in defense among closely related species, thus linking the Janzen-Connell (J-C) mechanism of diversity maintenance with the role of enemies in promoting plant lineage diversification posited by Ehrlich & Raven (1964).

Here we consider quantitatively a modified version of the strict J-C hypothesis, in which natural enemies of intermediate host-specificity shape the spatial organization of plants with respect to defense and thereby contribute to diversification and the maintenance of species richness. If offspring mortality is dependent on distance to conspecific adults, as well as adults belonging to species with which the offspring is likely to share enemies, subsequent generations ought to exhibit an even distribution of adults with respect to plant defense similarity. The tendency for such trait dispersion should be stronger if enemies are not strict specialists. Substantial maintenance of species diversity may accompany this pattern of spatial dispersion in traits, despite the lack of strict specialization allowing for a simple division of limiting or regulating factors (Levin 1970; Meszena *et al.* 2006) that would enable the possibility of stable coexistence. Webb *et al.* (2006), and later other authors (Bagchi *et al.* 2010; Metz *et al.* 2010; Dyer *et al.* 2010) discussed a similar generalization of the J-C.

We explore this modified J-C hypothesis using a simulation model. We begin with Adler and Muller-Landau's (2005) model of the J-C mechanism and relax its assumption that plant antagonists specialize on a single host species. We assume that the proportion of shared enemies between two plant species is a function of the plants' defense trait similarity. We then manipulate the shape of this function to reflect alternative models of enemy host-breadth, ranging from a model that approaches the J-C model, to one in which antagonists are highly generalist (Fig. 5.1a). We examine not only the number of plant species maintained in the community at equilibrium as a function of this relationship, but also its influence on the spatial structure of defense traits in the community. We also use three case studies of insects and pathogens in Panama and Papua New Guinea to assess expected community structure and diversity maintenance from the modified J-C hypothesis in those systems. Some prior theoretical exploration of coexistence implications of non-specialist enemies exists (Bever 2003; Chesson & Kuang 2008). Here, we specifically consider the J-C mechanism in a stochastic, spatially-explicit framework that permits an assessment of dynamic diversity maintenance compared with a neutral model and of potential diversity maintenance in example communities.

We show that only scenarios in which plant enemy similarity declines rapidly as a function of pairwise defensive trait distance support greater species richness than models of neutral drift. Nonetheless, a wide range of variation in enemy host-specificity results in the dispersion of defensively similar plant species, both at the scale of the landscape and the local neighborhood, indicating that enemy-mediated competition may increase defense trait diversity without necessarily enhancing species richness. Indeed, the strongest trait dispersion seems to occur at intermediate host specificities, where diversity is no higher than in a neutral model. Finally, in exploring predictions of the model using observed host ranges, we found that studies of herbivorous beetles (Odegaard *et al.* 2005) and pathogenic fungi (Gilbert & Webb 2007) in Panama, as well as a large-scale survey of caterpillars in Papua New Guinea (Novotny *et al.* 2010), demonstrate sufficiently narrow host-ranges to substantially enhance both plant species richness and the diversity of plant defense traits in those communities.

The Simulation Model

Each species in a regional species pool of K species is assigned a random position in two-dimensional space used here to represent secondary compounds and other traits that may influence enemy host-use. We refer to this trait space as "defense" space and the Euclidean distance between species pairs, ρ, as "defense" distance. These trait distances exhibit a normal distribution and are scaled so that $\rho_{max} = 1$.

The model follows the fates of a fixed number N of adult plants in a two-dimensional landscape of L by L meters. We employ a torus to eliminate edge effects. The initial community is assembled by selecting N individuals' starting positions randomly and uniformly. Species identities are assigned by selecting at random from a regional pool of species with equal probability with replacement. The J-C recruitment process is then reflected in a series of death and replacement events, in which a randomly chosen adult dies, and potential offspring undergo recruitment trials until one reaches adulthood and is added to the community to replace the dying individual (not necessarily at the same location). These dynamics keep the total number of adults in the community fixed.

In each death and replacement event, there is a probability μ that the replacing individual is an immigrant offspring. In this case, the location of the offspring is chosen randomly and uniformly, and it's species identity chosen with equal probability from the regional species pool. With probability 1-μ, an adult within the community is chosen at random to produce a single offspring, which disperses in a uniformly random direction a distance r chosen from the exponential probability density function $f(r)$, with mean seed dispersal distance σ:

$$f(r) = (1/\sigma)e^{-r/\sigma} \tag{5.1}$$

The probability that any offspring survives after dispersal depends on the local density of natural enemies. In the model of Adler & Muller-Landau (2005), enemy density is a function of distance to conspecific adults. In our model, enemy density is a function of distance to each adult in the landscape, weighted by the probability that the offspring shares an enemy with the species of that adult. The proportion of enemies shared between an offspring of plant species i and an adult of species j is a function of their defense distance ρ:

$$g(\rho) = 1 - \rho_{ij}^{s} \tag{5.2}$$

where s is the shape parameter describing the relationship between defense distance and shared enemies (Fig. 5.1a). Furthermore, the probability of an enemy dispersing to the site x of the offspring is a function of the distance between the offspring and each adult k in the landscape, $r_k(x) = d(x_k,x)$:

$$h(r_k(x)) = [1/(2\pi\alpha)] \exp(-r_k(x)/\alpha) \tag{5.3}$$

where α is the mean enemy dispersal distance. Thus, the number of enemies of species i at site x, $H_i(x)$, is:

$$H_i(x) = \sum_k h(r_k(x))g(\rho_{ij(k)}) \tag{5.4}$$

where $\rho_{ij(k)}$ is the defense distance between species i and the species j to which individual k belongs. The probability that an offspring of species i survives at location x is:

$$\mathrm{P}_i(x) = 1/(1+\beta N H_i(x)) \tag{5.5}$$

where β is a scaling parameter that describes the strength of enemy attack and N is the number of adults. If an offspring does not survive, a new adult is chosen to produce an offspring and this process is repeated until an offpsring survives. If an offspring survives, it immediately becomes an adult and another death and replacement event commences, beginning with the mortality of a new randomly chosen adult.

We consider a range of values for the defense shape parameter, s, that extend from a rapidly declining proportion of shared enemies as a function of plant trait distance (when $s = 1/20 = 0.05$), to a linear decline (when $s = 1$), to a convex relationship in which most plant species pairs share a high proportion of natural enemies ($s = 20$; Fig. 5.1a). In addition to these model variants that account for the influence of plant traits, we consider three simplified models in which traits are ignored. In the strict J-C model, the number of enemies, and ultimately offspring survival, depends only on distance to conspecific adults. In the Generalist model, offspring survival depends on distance to all adults, species identity notwithstanding. Finally, we consider a neutral model (Hubbell 2001) in which recruitment is not influenced by enemies, leaving species richness to be governed by the opposing forces of demographic drift and immigration from the regional species pool.

Asymmetrical resource competition in plants can make offspring establishment directly below an adult impossible. In order to incorporate the influence of adult shading or other resource competition on seedling establishment and potential diversity-maintenance through the J-C mechanism, we incorporated a threshold distance R below which establishment cannot occur:

$$f(r) = 0 \qquad \text{if } r \leq R \tag{5.6}$$

$$f(r) = (1/\sigma)e^{-r/\sigma} \qquad \text{if } r > R \tag{5.7}$$

The threshold parameter has the effect of imposing spatial structure on the distribution of adults in the community, ranging from strongly clustered (at $R = 0$ m) to evenly dispersed (at $R \geq 3$ m).

To allow us to maximally differentiate diversity maintained by different values of s, we used the combination of offspring and enemy dispersal distances ($\alpha = \sigma = 5$ m) that resulted in substantially more species than the neutral case in the strict J-C simulation according to Adler and Muller-Landau (2005) and our own explorations. We explored a range of threshold distance (R) values, and, because the mechanism is mediated by survival, three different functional forms relating offspring survival to enemy density (see Table 5.1). Figs 5.2-5.5 show results for $R = 3$ and survival as in Eq. 5.4, but key results are robust over *R* values and survival functions considered (Figs 5.5-5.8 in Supporting Information).

We employed pairwise distance metrics to examine the influence of *s* on defense trait community structure as well as the relationship between defense trait diversity and species richness. We calculated the mean pairwise defense distance of all species found on the landscape compared to that expected from a random sample of an equal number of species from the regional species pool (Webb 2000) as well as the mean pairwise defense distance within the neighborhood <5m of each individual on the landscape. In addition, we calculated the mean distance to the center of our two-dimensional defense space for all individuals in the landscape to understand the degree to which species on the landscape represent the outer boundaries of trait variation in the regional species pool and calculated the probability of immigration success as a function of trait distance to the most abundant resident.

Three studies have examined the relationship between the proportion of enemies shared between species of plants and the plants' phylogenetic relationships. Odegaard *et al.* (2005) investigated host-use among 1174 species of Coleopteran herbivores of 50 and 52 tree species in a wet and dry tropical forest, respectively, in central Panama. Also working in Panama, Gilbert & Webb (2007) analyzed the probability that a fungal pathogen would infect a host plant as a function of the phylogenetic distance between the host and the plant species from which the pathogen was isolated. These data included 53 species of tissue-killing fungi and 36 plant species (Gilbert & Webb 2007). Finally, in a large-scale, community-wide study of 1490 insect herbivore species in 11 feeding guilds, Novotny *et al.* (2010) examined the phylogenetic relationships of the 88 host plant species utilized by 572 species of leaf-feeding caterpillars in Papua New Guinea. In order to examine the potential for the natural enemies in each of these studies to facilitate coexistence within their respective tree communities via the J-C mechanism, we parameterized our simulation model with the curves in each study reflecting the proportion of enemies shared as a function of phylogenetic distance, a pattern shaped by species differences in secondary chemistry and other defensive traits represented in our theoretical model by the parameter ρ (Fig. 5.1b,c; Table 5.1). We used these studies to explore the implications of the shape parameter s for diversity maintenance and defensive trait similarity in these systems, but did not incorporate other parameters (e.g. enemy dispersal, seed dispersal distances) from these communities. Nevertheless, we were able to compare the potential for diversity maintenance in these three communities to that maintained by strict J-C, generalist, and neutral models under conditions in which the strict J-C mechanism is an effective mechanism of coexistence.

Simulation code was written in R (R Development Core Team 2011). We simulated 400 adults in a 4 ha local community with a regional species pool of $K = 1000$ species. Plotted results are based on 20 different communities, each the state achieved after stochastic simulation of 400,000 adult deaths, or 1000 generations, beginning from a community randomly generated from the regional species pool.

Results

Offspring of rare species encounter a more favorable landscape upon dispersal when s is low, and host specificity high, than do offspring of abundant species (Fig 5.2). This advantage erodes as s increases from 0.10 to 1.00, as the offspring even of a rare species are likely to disperse to a neighborhood teeming with enemies they share with heterospecific adults (Fig 5.2h).

As shown in Fig. 5.3a, increasing the shape parameter, and thereby increasing the proportion of shared enemies over greater defense trait distances (Fig 5.1a), results in reduced species richness relative to the strict J-C model. Models in which s is small ($0.05 \leq s \leq 1.00$) enhance species richness relative to the neutral case, whereas models in which $1.00 \leq s \leq 5.00$, suppress species richness relative to that maintained under neutral drift with immigration. A model in which all enemies are generalists supports no more than the number of species maintained by neutral drift and immigration. Models in which $s \geq 6.67$ behave similarly to the generalist and neutral models. The proportion of enemies shared by most plant species pairs must be low ($s < 1$) in order to confer a rare species advantage (see Fig. 5.9) and thereby slow the loss of diversity through ecological drift. Relative diversity maintenance is robust to the threshold distance R (Fig. 5.5) as well as the function relating offspring survival to enemy density (Figs 5.6,5.7) with the exception that when the threshold distance $R = 0$, the resulting spatial clustering of adult plants allows even generalist enemies to maintain 20% more plant species than neutral drift, and does not suppress species richness in communities with $1.00 \leq s \leq 5.00$.

Nearly all models we considered resulted in highly nonrandom trait structure in the plant community, whether measured at the scale of the landscape (Fig. 5.3b) or the immediate neighborhood surrounding individual adults (Fig. 5.3c). For most values of s, the mean defense distance between species in the landscape differed greatly from that in null communities (Fig. 5.3b). Only the true J-C model and models with exceedingly generalist enemies ($s > 6.67$) exhibited random species composition with respect to defense. The strength of exclusion of defensively similar individuals within the immediate neighborhood (5m) of adults mirrored the degree of landscape-wide trait structure with the exception of the true J-C model. The J-C model results in greater mean neighborhood trait distances than models in which enemies are highly generalist ($3.00 \leq s \leq 20$) simply because conspecifics are excluded from the vicinity of one another. These results are robust to variation in threshold distance and survival functional form considered (Figs 5.5-5.8).

A species on the perimeter of our two-dimensional trait space is 0.35-0.50 units from the center. It therefore appears in Fig. 5.3d that in models in which species richness is suppressed relative to neutral, enemy-mediated competition effectively excludes most species from the community, excepting those that exploit enemy-free space on the periphery of defense space. The relationship between immigration success and trait distance can be found in Fig. 5.10.

The Papuan Lepidoptera studied by Novotny *et al.* (2010) reveal a strong phylogenetic signal to host-use, such that caterpillar similarity declines rapidly as a function of phylogenetic distance between plants and displays a long tail of very low herbivore similarity between distant relatives. In fact, the shape parameter s fitted to these data is 0.0336, or less than the smallest theoretical value we considered (Fig. 5.1a,b; Table 5.1). Papuan caterpillars are specialized to a degree that their interaction with host-plants closely approximates the strict J-C simulation, supporting 1.95 times as many species as a neutral model, compared to 2.05 times more species in the case of the strict J-C model (Fig. 5.4).

The Coleoptera in two Panamanian forests exhibit a similar relationship between host-use and plant phylogeny to the herbivores of Papua New Guinea (Fig. 5.1b,c). As a result, the simulations pertaining to the wet forest of Odegaard *et al.* (2005) maintain an average of 1.90 times as many tree species as a neutral model, and the dry forest 1.94 times more species (Fig. 5.4). As with the Lepidtoperan caterpillars studied by Novotny *et al*. (2010), the Panamanian Coleoptera closely approach the strict J-C model in our simulation. Note also that Figs 5.5-5.8 suggest robustness of these results to variation in *R* and the form of Eq. 5.5.

The primary difference between the pathogenic fungi studied by Gilbert & Webb (2007) and the insects studied by Novotny *et al.* (2010) and Odegaard *et al.* (2005) was not the shape of the relationship between enemy overlap and plant phylogenetic distance, but rather the percent overlap at which the relationship reached an asymptote (Fig. 5.1c), suggesting that distantly related tree species are more likely to share foliar fungal pathogens than insect herbivores. In our simulations, these pathogens maintain an average of 1.40 times more species than extreme generalists, but only 0.67 as many as the strict J-C model (Fig 5.4).

Discussion

The predictions of Janzen (1970) and Connell (1971) concerning the influence of density-dependent recruitment on the community structure of tropical forests have been largely supported by empirical findings (Wright 2002; Leigh *et al.* 2004; Terborgh 2012). Yet studies indicate that insects and pathogens are often less specialized than the classical J-C model assumes (Novotny *et al.* 2002; Odegaard *et al.* 2005; Gilbert & Webb 2007), raising the possibility that the diversity and structure of plant communities is also shaped by generalist enemies (Webb *et al.* 2006; Bagchi *et al.* 2010; Metz *et al.* 2010). Our results suggest that the ability of the J-C mechanism to facilitate the coexistence of plant species is sensitive to the host specificity of plant enemies. However, we also find that strict host specialization is not a prerequisite for coexistence, as models with narrow host ranges (e.g. $0.05 \leq s \leq 0.67$) support greater species richness than does neutral drift (Fig. 5.3a). Yet even a shape parameter of $s = 1$, which results in a perfectly proportional decline in enemy similarity with trait distance, fails to enhance plant species richness beyond that maintained in a neutral model.

Furthermore, our results suggest that host specificity can determine whether enemy-mediated competition serves to enrich or suppress diversity relative to a neutral model. When $1 \leq s \leq 6.67$, enemy-mediated competition effectively excludes most species from the community, except for those that exploit enemy-free space (Jeffries & Lawton 1984) on the periphery of the two dimensions we use to represent variation in defensive traits. Yet, when s is small—and enemies more specialized—it appears that trait space can be more finely partitioned, and potential immigrants need be only a short trait distance from an abundant resident to successfully invade the community (Fig. 5.10).

On the other hand, the propensity of enemy-mediated competition to increase the trait diversity of plant communities appears quite robust to changes in enemy host-specificity. A wide range of models exhibits a substantial signature of nonrandom community structure with respect to defense, whether considered at the scale of the landscape (Fig. 5.3b) or of the immediate neighborhood of each individual plant (Fig. 5.3c). One important implication is that models that enhance species richness ($0.05 \leq s \leq$

0.67), as well as those that suppress it ($1 \leq s \leq 6.67$), promote trait diversity by favoring dissimilar species within the available dimensions of trait variation. Apparent competition may thus select for novel defensive traits independently of its role as a mechanism of coexistence.

Ehrlich and Raven (1964) postulated that lineage diversification often follows defensive innovation in plants, helping to explain patterns of diversity in plants and their herbivores. Our results suggest that the J-C mechanism is consistent with this idea over a broad range of host-specificities. Specifically, trait diversity at neighborhood and community scales are enhanced over a wide range of host specificity ($0.05 \leq s \leq 6.67$; Fig. 5.3b,c). Were novel defenses to arise through character evolution, rather than immigration, our model suggests that these traits would be quite strongly favored demographically (Fig. 5.10). We are left to postulate that demographic success associated with enemy release may translate to increased lineage diversification in taxa in which defenses are particularly evolutionarily labile. The absence of phylogenetic signal with respect to defense traits in a number of species-rich genera (Becerra 1997; Agrawal & Fishbein 2006; Kursar *et al.* 2009) and the chemically-even spatial structure exhibited by those genera (Becerra 2007; Kursar *et al.* 2009) are consistent with this view.

Are natural enemies specialized enough to facilitate coexistence among plants?

Investigations of phytophagous insects (Novotny *et al.* 2002; Novotny *et al.* 2010) and pathogenic fungi (Gilbert 2005; Gilbert & Webb 2007; Liu *et al.* 2012) in the last decade have generally found less host-specialization than earlier estimates (Lewinsohn & Roslin 2008). There are exceptions to these findings, including studies of insect seed predators, such as beetles in the family Bruchidae (Janzen 1980), and some pathogenic Oomycota (Augspurger 1990). Less specialized enemies have been thought to be unlikely agents of coexistence (Freckleton & Lewis 2006). Nevertheless, the plant diversity maintained by the seemingly generalist insect herbivores examined by Odegaard *et al.* (2005) and Novotny *et al.* (2010) closely approximates that maintained by strictly specialist enemies under conditions in which the strict J-C mechanism is an effective mechanism of coexistence.

Our results suggest that the fungal pathogens studied by Gilbert & Webb (2007) are also sufficiently specialized to foster diversity, but to a lesser extent than in the other two case studies (Fig. 5.4). However, the strength of diversity maintenance my be understated by fitting *s* to phylogenetic distance rather than the actual traits that determine host-use among pathogenic fungi. Some plant species pairs in their study exhibited no symptoms when cross-infected (Gilbert & Webb 2007). This suggests that the relationship between shared pathogens and plant similarity with respect to relevant defense traits may exhibit a more negative slope than the enemy-phylogeny curve, and asymptote at zero rather than nearly 30% enemy similarity (Fig. 5.1b i and ii), and hence maintain greater diversity.

Hanski (1981) has shown that density-responsive predation by generalist enemies can maintain coexistence as long as there is a positive relationship between species abundance and spatial aggregation. Adler and Muller-Landau (2005) found the highest species richness at short enemy and offspring dispersal distances that produced highly clustered adult distributions. Our results confirm Hanski's (1981) conclusion that even generalists can maintain higher species richness than a neutral model when plants are allowed to cluster spatially (Fig. 5.5). While density-responsive generalists may not contribute much to coexistence in closed-canopy forests, this mechanism may enhance species richness in understory, xeric, and other spatially aggregated plant communities.

Our model may underrepresent the diversity enhancing effect of shared enemies if such enemies vary in their host preferences, virulence, or damage relative to the species that they feed on or infect. Such variation is a key ingredient in the conditions for stable coexistence of species that share enemies, which follow by analogy from resource competition theory (Tilman 1982), and may explain the ability of generalist herbivores to enhance species richness in some instances (Dyer *et al.* 2010). Given *n* enemies, *n* plant species may stably coexist if each is unique in the enemy which most limits it, which arises through enemy variation in preferences or effects. Indeed, the deterministic models of Chesson & Kuang (2008) and Bever (2003) show that stable coexistence can result when conspecific negative feedback is more severe than heterospecific feedback due to differences in enemy preferences or effects. Our model accounts for such variation indirectly, by being formulated in terms of the emergent pattern of enemy effects as a function of trait distance, but does not for the empirical data sets, which provide potential rather than actual enemy effects. However, the stochastic, spatial modeling approach we take here permits our model to also account for effects on diversity of increased persistence of species resulting from their differences in their enemy communities, even when those differences do not confer stable coexistence

On the other hand, the ability of natural enemies to enhance diversity depends on the details of enemy community structure and enemy and seed dispersal. If seed dispersal distances are long relative to enemy dispersal distances, the J-C mechanism maintains fewer plant species than if both enemies and offspring disperse short distances (Adler & Muller-Landau 2005). Furthermore, a negative association between host-specificity and enemy virulence or abundance could undermine the diversity-enhancing effect of enemies by allowing dynamics to be dominated by destructive or abundant generalists. Janzen (1988), however, found a positive relationship between host-specificity and damage rates in Lepidoptera, and the caterpillars studied by Novotny *et al.* (2010; Fig. 5.1b) were not dominated by generalists. A greater understanding of the fitness effects and host breadths of plant enemies, and the relationship between the two, would improve models of plant-enemy interactions and coexistence.

Conclusions

There is broad consensus that the spatially dependent action of specialist enemies described by Janzen and Connell is a widespread and important mechanism that maintains species richness in tropical forests and other plant communities (Wright 2002; Leigh *et al.* 2004; Terborgh 2012). It remains a challenge to reconcile such patterns with the effects of enemies that associate with a range of often closely related species (Novotny *et al.* 2002; Odegaard *et al.* 2005; Webb *et al.* 2006; Gilbert & Webb 2007; Bagchi *et al.* 2010; Metz *et al.* 2010; but see Janzen 1980). Here, we have shown that the J-C mechanism is indeed sensitive to the host-specificity of natural enemies. Within the range of models extending from the J-C model to one in which plant species are indistinguishable in the eyes of enemies, only those that approach the classic J-C model retain the diversity-enhancing effect of the mechanism. Yet our results also suggest that the case studies of somewhat generalist plant pathogens in Panama and insect herbivores in Panama and Papua New Guinea are sufficiently specialized within their respective plant communities to approach the effectiveness of the classic J-C model at maintaining plant species richness. Future study of the relationship between enemy host use and plant defensive traits in a wider range of systems (both tropical and temperate), and incorporation of that relationship into models like the one we examine here, could provide a more general perspective on the potential influence of enemies on coexistence and spatial organization in plant communities.

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Parameters	Values
L, length/width of the landscape	200 m
N, total population size	400
K, number of species in regional species pool	1000
μ, probability of immigration	$0.001(N)$
β, strength of frequency dependence	25, 2.5
α, mean enemy dispersal distance	5 m
σ, mean offspring dispersal distance	5 m
R, threshold distance for offspring survival	0-5 m
ρ, chemical distance between species	0 - 1
s, shape of the proportion of enemies shared as a function of defense trait distance	0.05, 0.10, 0.15, 0.20, 0.25, 0.33, 0.50, 0.67, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.67 10.00, or 20.00

Observed Plant-Enemy Relationship	Function
P_{wet}, Coleoptera in Panama wet forest (Odegaard et al. 2005)	$1 - \rho^{0.0446}$
P_{dry}, Coleoptera in Panama dry forest (Odegaard et al. 2005)	$1 - \rho^{0.0622}$
P_{Lep}, Lepidoptera in Papua New Guinea (Novotny et al. 2010)	$1 - \rho^{0.0336}$
P_{log}, Lepidoptera in Papua New Guinea, treating log(plant phylogeny) (Novotny et al. 2010)	$0.3966 - \rho(0.4407)$
$P_{nursery}$, Fungal pathogens in Panama (Gilbert & Webb 2007)	$\text{logit}(S) = 3.4096 – 1.7562(\log_{10}(1+\rho*300))$
P_{forest}, Fungal pathogens in Panama (Gilbert & Webb 2007)	$\text{logit}(S) = 2.2327 – 1.3428(\log_{10}(1+\rho*300))$

Functional Forms	
Survival function $P(H)$	$1/(1+\beta NH)$, $1/(1+\beta N\log(H))$, $1/(1+(\beta NH)^2)$

Note: Gilbert and Webb (2007) defined shared pathogens with the logit function: $P = \exp(\text{logit}(S))/[1 + \exp(\text{logit}(S))]$.

Table 5.1. Variables, parameters and functions in the simulation.

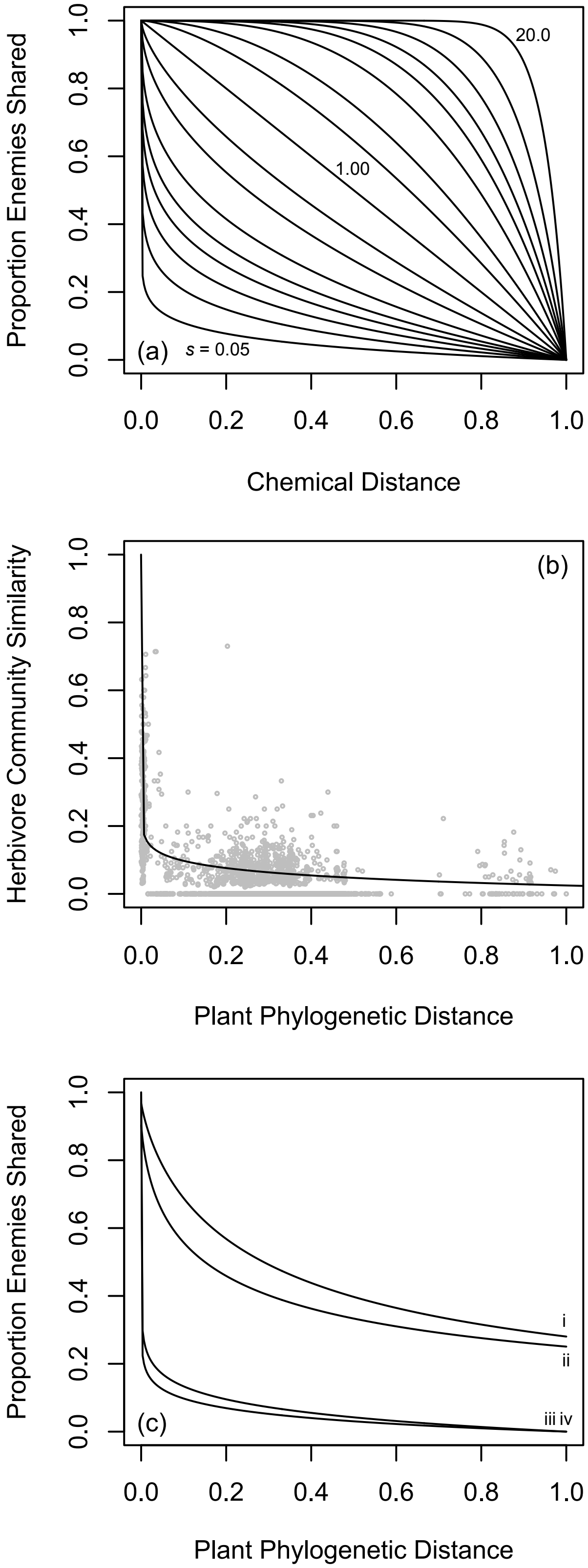
Proportion Enemies Shared
Chemical Distance
20.0
1.00
(a)
$s = 0.05$
Herbivore Community Similarity
Plant Phylogenetic Distance
(b)
Proportion Enemies Shared
Plant Phylogenetic Distance
i
ii
iii
iv
(c)
0.0
0.2
0.4
0.6
0.8
1.0

Figure 5.1. Theoretical and empirical relationships between plant enemy similarity and defense trait or phylogenetic distance. (a) Proportion of enemies shared vs. simulated trait distance for 17 values of the defense shape parameter *s* of the function in Eq. 1. (b) Jaccard index of Lepidopteran herbivore similarity vs. plant phylogenetic distance in Papua New Guinea (Novotny *et al.* 2010). (c) Empirical curves pertaining to i) pathogenic fungi, nursery and ii) moist forest, Panama (Gilbert & Webb 2007), iii) Coleopteran herbivores, wet forest and iv) dry forest, Panama (Odegaard *et al.* 2005).

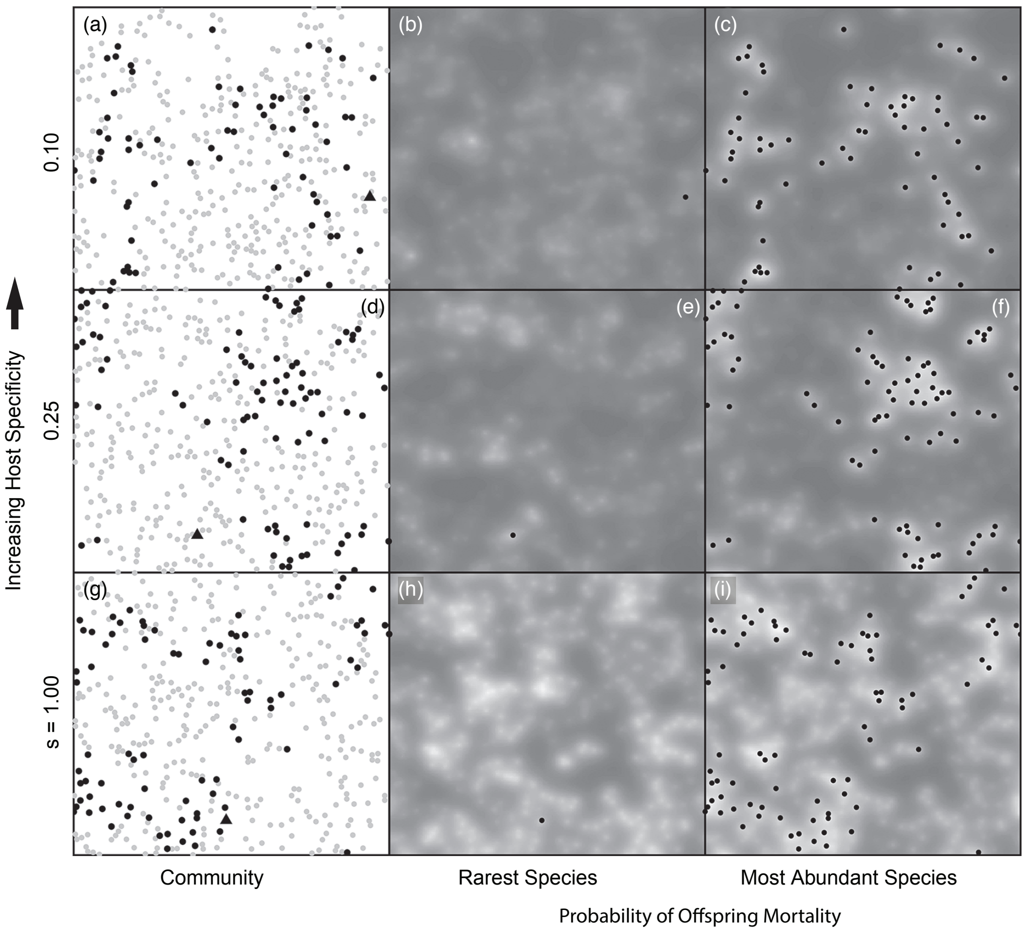

Figure 5.2. Community structure and the probability of offspring mortality of rare and abundant species. Community structure is shown after 50,000 adult deaths for simulations in which $s = 0.1$, 0.25, and 1, respectively. The left panel shows the adult community in gray, with the individuals of the rarest species represented by triangles and those of the most abundant by black circles. In the center and right panels, prevalence of enemies of the rarest and most abundant species are shown respectively as a heat map (low prevalence = dark, high = light) with individuals of the species in question indicated with circles. Differences in enemy prevalence in areas inhabited by neither species are a result of differences in defense traits and the composition of the rest of the community; this is most visually apparent between panels (e) and (f).

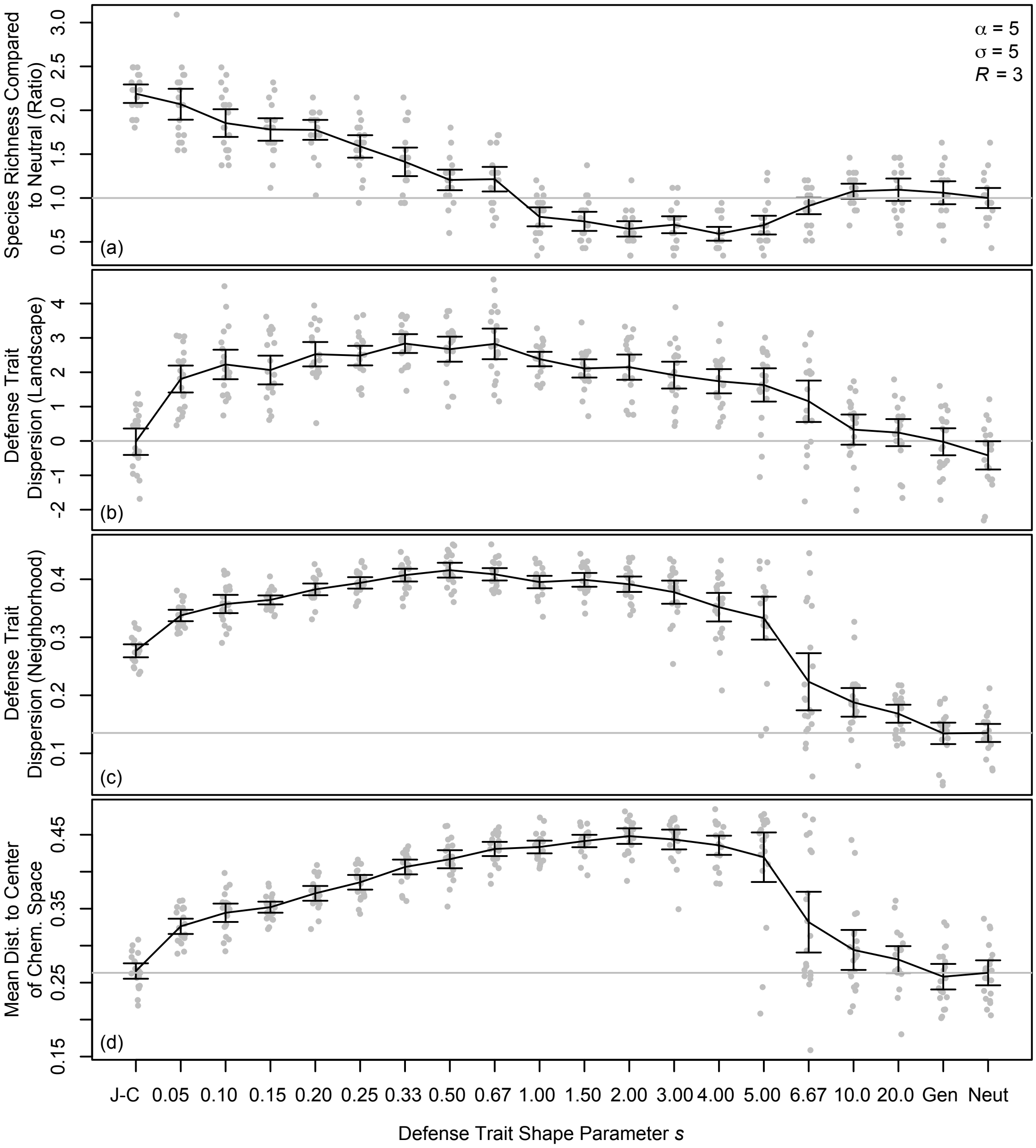

Figure 5.3. Species richness (a), landscape defense trait structure compared to random species assembly (b), neighborhood mean trait distance (MTD; c), and mean distance to the center of two-dimensional defense trait space (d) as a function of the shape parameter relating enemy-overlap to defense trait distance between plant species. MTD was measured between focal individuals and all individuals within 5 m of each focal individual in the simulation landscape. Results shown pertain to an offspring (σ) and enemy (α) dispersal distance of 5 m, an establishment threshold distance (*R*) of 3 m, and β = 25. Each point represents the result of one simulation of 400,000 adult deaths. Means and two standard errors of 20 simulations of each model or value of *s* are indicated.

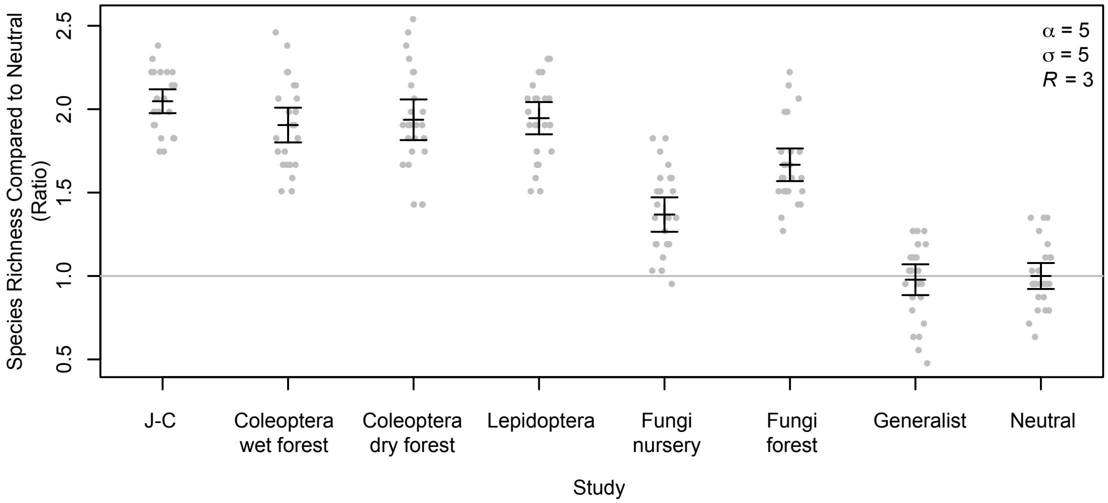

Figure 5.4. Species richness resulting from simulations parameterized with observed enemy overlap as a function of host-plant phylogenetic distance in three studies. Observed datasets are wet and dry forest Coleoptera in Panama (Odegaard *et al.* 2005), Lepidoptera in Papua New Guinea (Novotny *et al.* 2002), and fungal pathogens studied in a nursery and forest in Panama (Gilbert & Webb 2007). Simulation results from the J-C, Generalist, and Neutral models are included for comparison. Results shown pertain to an offspring (σ) and enemy (α) dispersal distance of 5 m and an establishment threshold distance (*R*) of 3 m. Each point represents the result of one simulation of 400,000 adult deaths. Means and two standard errors of 25 simulations of each model are indicated.

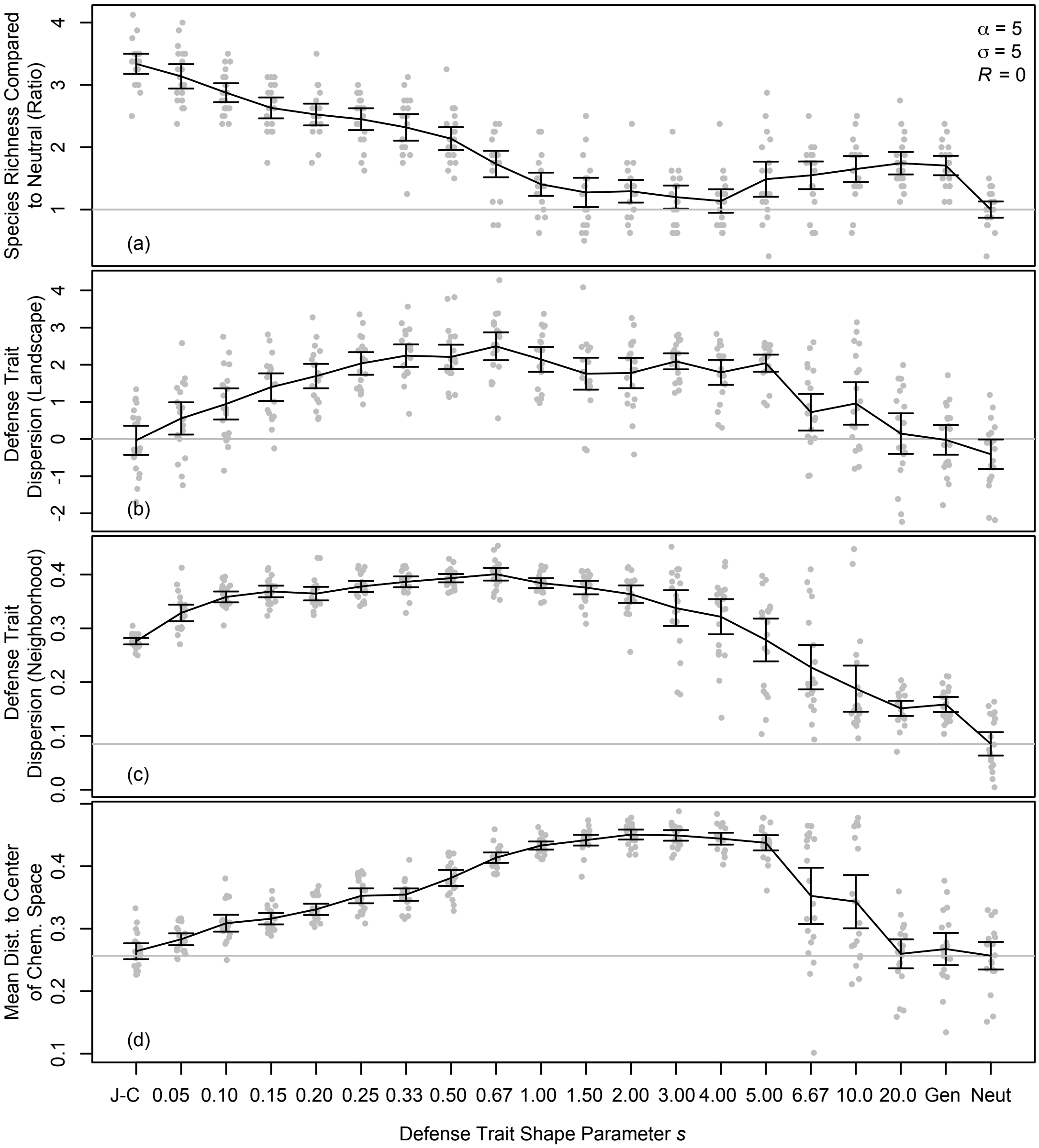

Figure 5.5. Model results when the probability that an offspring of species *i* survives at location *x* is: $P_i(H) = 1/(1+\beta H)$ and the establishment threshold distance $R = 0$. Species richness (a), landscape defense trait structure compared to random species assembly (b), neighborhood mean trait distance (MTD; c), and mean distance to the center of two-dimensional defense trait space (d) is shown as a function of the shape parameter relating enemy-overlap to trait distance between plant species. MTD was measured between focal individuals and all individuals within 5 m of each focal individual in the simulation landscape. Results shown pertain to an offspring (σ) and enemy (α) dispersal distance of 5 m and β = 25. Each point represents the result of one simulation of 400,000 adult deaths.

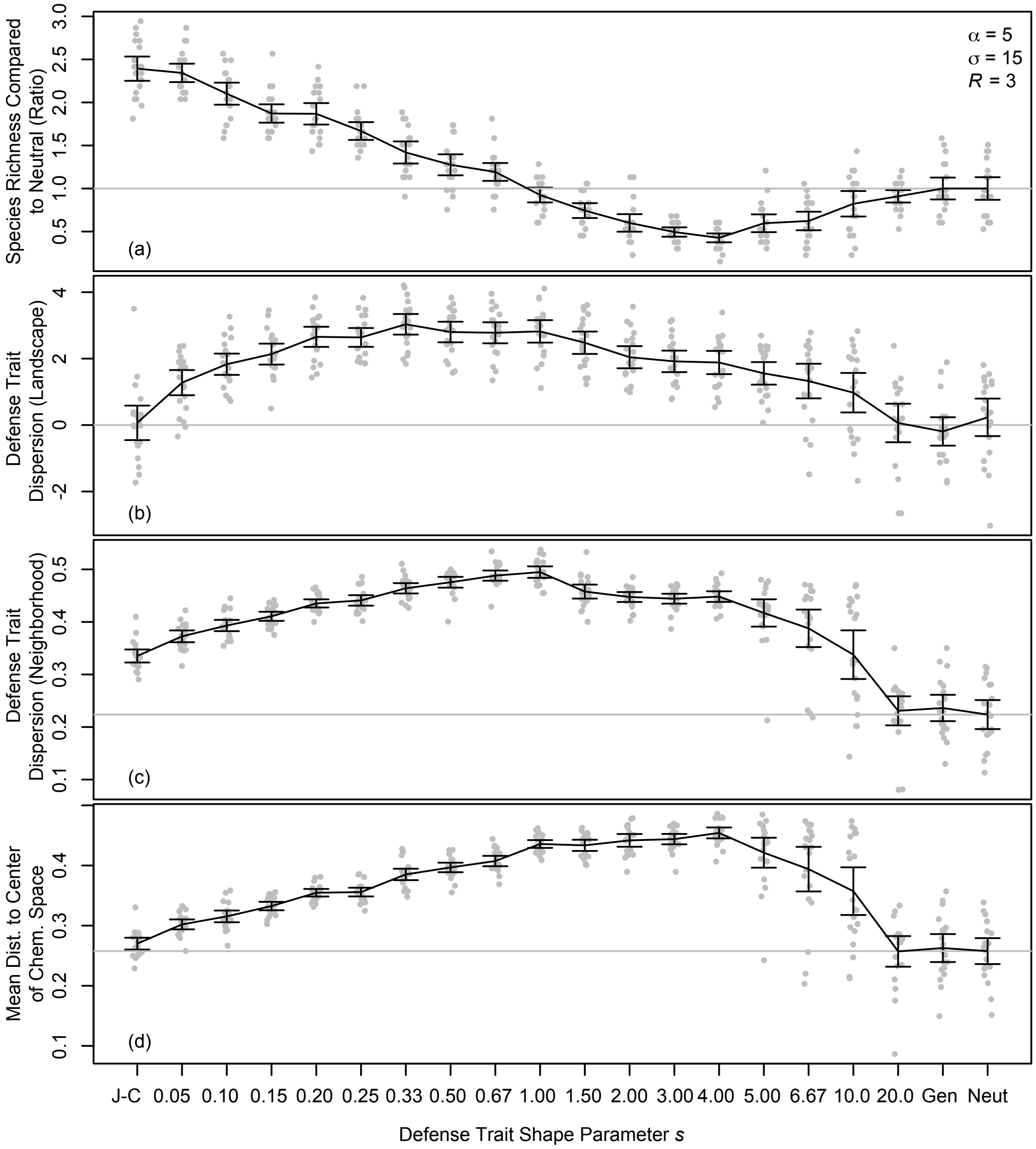

Figure 5.6. Model results when the probability that an offspring of species *i* survives at location *x* is: $P_i(H) = 1/(1+\beta H)$ the establishment threshold distance $R = 3$, and enemy dispersal distance is long relative to seed dispersal distance ($\alpha = 15$, $\sigma = 5$). Species richness (a), landscape defense trait structure compared to random species assembly (b), neighborhood mean trait distance (MTD; c), and mean distance to the center of two-dimensional defense trait space (d) is shown as a function of the shape parameter relating enemy-overlap to trait distance between plant species. MTD was measured between focal individuals and all individuals within 5 m of each focal individual in the simulation landscape. Results shown pertain to $\beta = 25$. Each point represents the result of one simulation of 400,000 adult deaths.

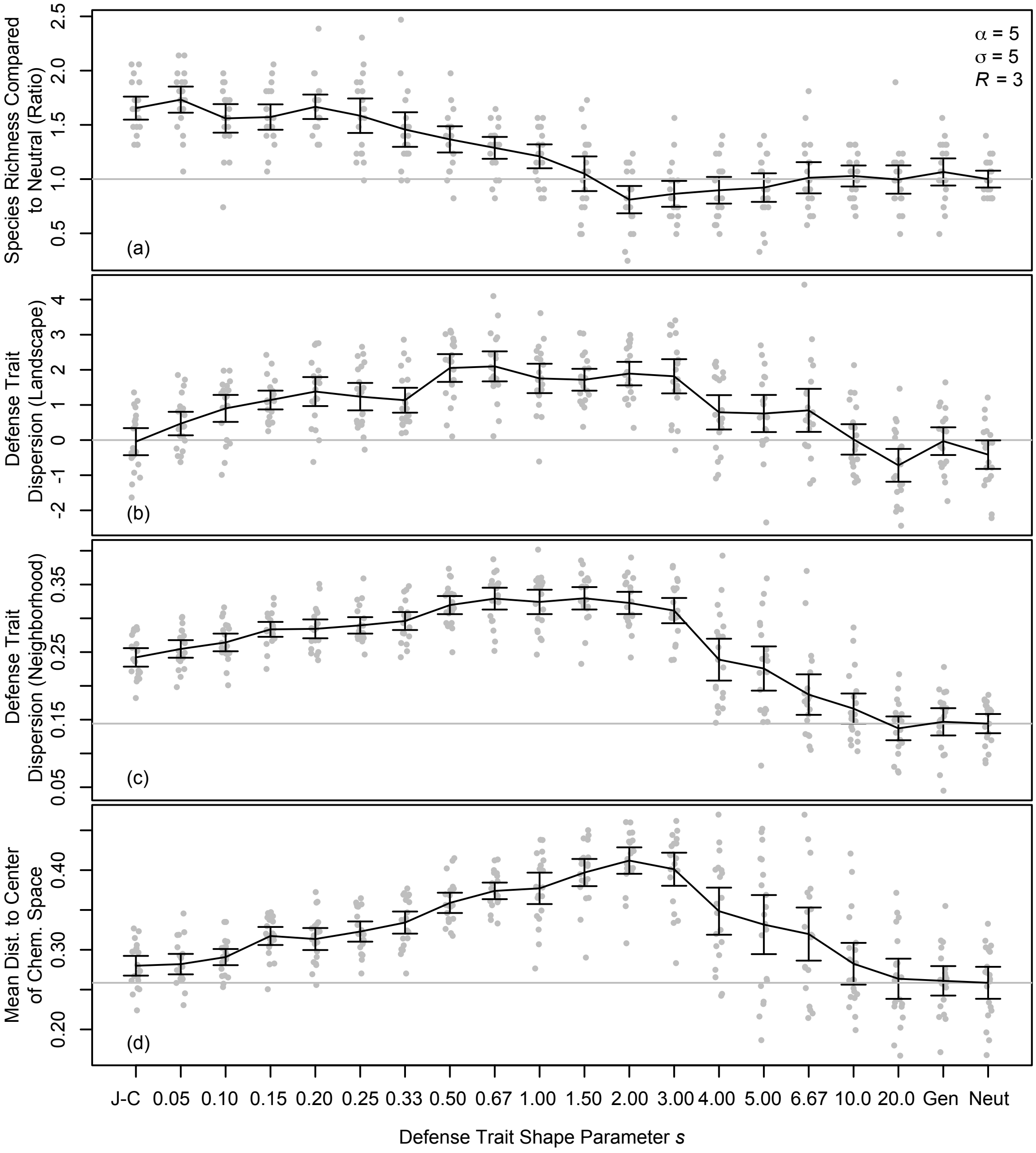

Figure 5.7. Model results when the probability that an offspring of species *i* survives at location *x* is: $P_i(H) = 1/(\beta * \log(1+H))$. Species richness (a), landscape defense trait structure compared to random species assembly (b), neighborhood mean trait distance (MTD; c), and mean distance to the center of two-dimensional defense trait space (d) is shown as a function of the shape parameter relating enemy-overlap to trait distance between plant species. MTD was measured between focal individuals and all individuals within 5 m of each focal individual in the simulation landscape. Results shown pertain to an offspring (σ) and enemy (α) dispersal distance of 5 m, an establishment threshold distance (*R*) of 3 m, and β = 2.5. Each point represents the result of one simulation of 400,000 adult deaths.

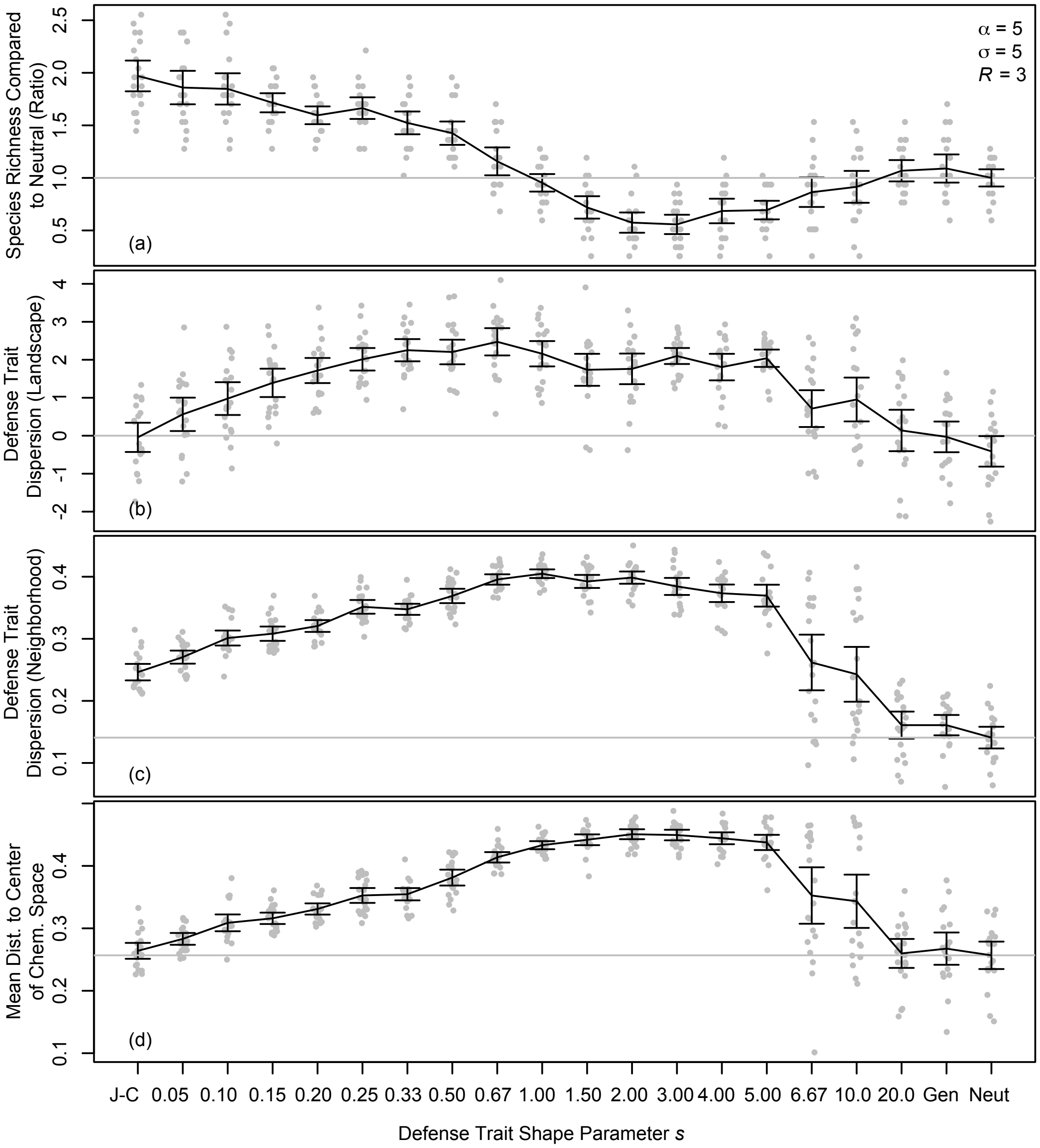

Figure 5.8. Model results when the probability that an offspring of species *i* survives at location *x* is: $P_i(H) = 1/(1+\beta H^2)$). Species richness (a), landscape defense trait structure compared to random species assembly (b), neighborhood mean trait distance (MTD; c), and mean distance to the center of two-dimensional defense trait space (d) is shown as a function of the shape parameter relating enemy-overlap to trait distance between plant species. MTD was measured between focal individuals and all individuals within 5 m of each focal individual in the simulation landscape. Results shown pertain to an offspring (σ) and enemy (α) dispersal distance of 5 m, an establishment threshold distance (*R*) of 3 m, and β = 2.5. Each point represents the result of one simulation of 400,000 adult deaths.

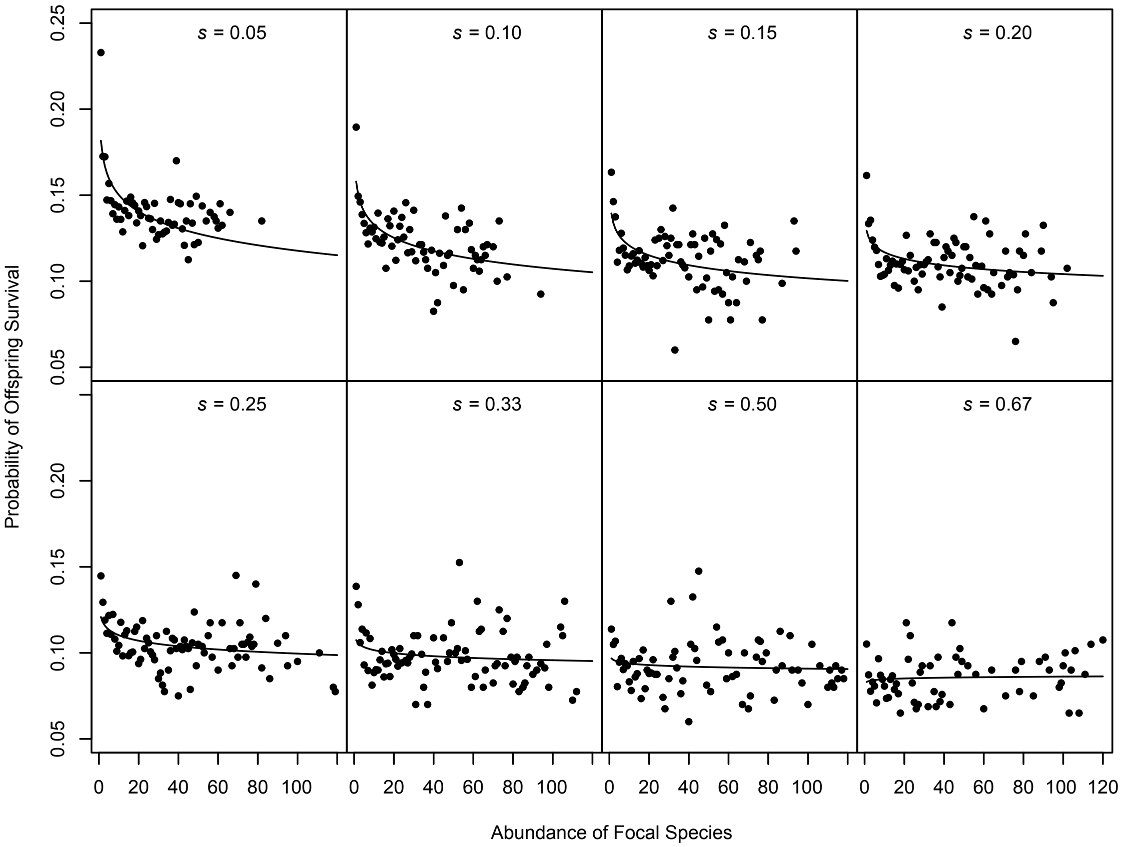

Figure 5.9. Probability of offspring recruitment as a function of abundance of the focal species for eight values of *s*. The rare-species advantage conferred by the J-C mechanism is sensitive to the value of *s*. Lines described by the equation $y = b - x^a$ were fit to simulated offspring recruitment trials using non-linear least squares.

An examination of probability of offspring survival as a function of the abundance of adults of the focal species indicates a strong rare-species advantage in models in which *s* is small (Fig. 5.9). In fact, the strength of this advantage, as indicated by the value of *m* in the curve $y = b - x^m$, is strongly associated with the species richness maintained in the model.

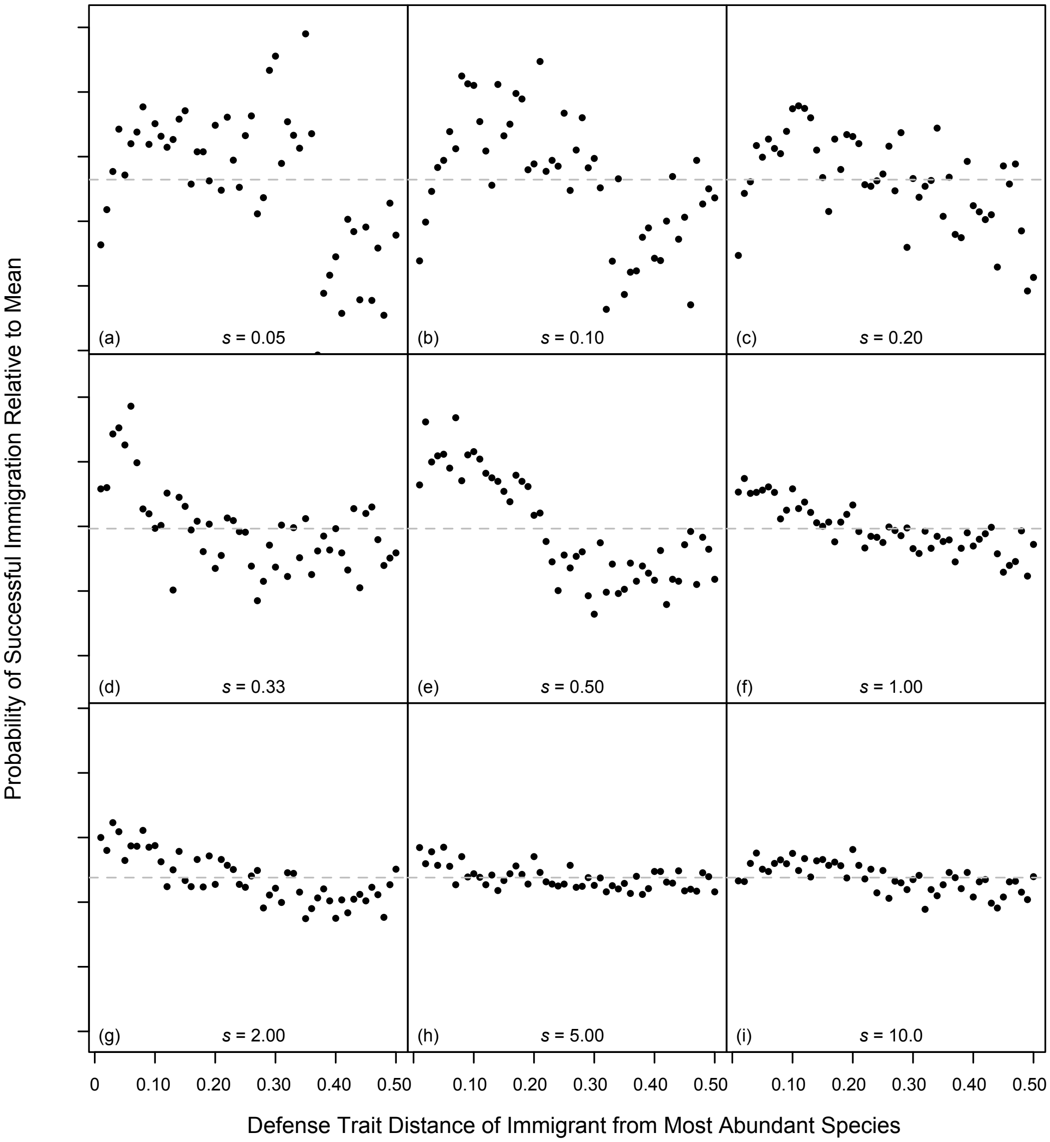

Figure 5.10. Probability of immigration success as a function of defense trait distance to the most abundant species on the landscape for nine values of the shape parameter *s*. All y-axes are on the same scale in which one tick equals 2 units, but each plot is centered about the mean. The mean immigration success for each value of *s* is indicated by a dashed line. Maximum immigration success is attained by immigrant species with intermediate trait distances from the most locally abundant species in panels a-e.

In order to better understand the demographic influence of the shape parameter *s* on species richness and trait community structure, we measured both the probability of offspring recruitment as a function of abundance of each focal species in the landscape as well as the probability of successful immigration as a function of the defense trait distance between the potential immigrant species and the most abundant species in the landscape. The later was conducted by selecting 1000 random points on each of 50 arcs described by radii from 0.01 to 0.50 units of defense trait distance from the location of the most abundant species in defense space. For each point, 1000 uniformly random locations were chosen on the community landscape and the success of the immigration attempt recorded, thus providing a probability of successful immigration.

In models in which *s* is small and species richness is enhanced relative to the neutral case, the probability of immigration is low for potential immigrant species that are defensively similar to the most abundant resident, but increases sharply only a small trait distance from the abundant resident (Fig. 5.10a-e). The probability of successful immigration then declines as potential immigrants approach other abundant residents in trait space. In models in which species richness is suppressed relative to neutral ($1.00 \leq s \leq 5.00$), it is advantageous for a potential immigrant to be defensively similar to the most abundant resident (Fig. 5.10f-h).

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Chapter 6: Conclusions

This dissertation has examined the role of trait evolution in the community assembly of sympatric species of *Psychotria*—one of the most species-rich plant genera in the world (Taylor 1996). A small number of species-rich tree genera contribute disproportionately to the species richness of tropical forests. Genera such as *Psychotria* therefore pose a particularly strong challenge to classical coexistence theory, which maintains that species must differ in important aspects of their niche in order to stably coexist over time. Species differences with respect to soil moisture and shade tolerance, edaphic requirements, and reproduction can facilitate coexistence among species by ensuring that resource competition occurs primarily and most intensely among individuals of the same species (Wright 2002, Silvertown 2004). Species interactions with mutualists, such as pollinators, seed dispersers, and mycorrhizal fungi, as well as with antagonists, such as the herbivore and pathogens that exert a strong pressure on tropical woody plants, can also promote coexistence by favoring a species when it is rare (Wright 2002).

Most sympatric *Psychotria* in central Panama have nearly synchronous fruiting phenology and share a small number of seed dispersers dominated by three native manakins and three migratory thrushes (Poulin *et al.* 1999). The *Psychotria* of BCI also appear to share their principal pollinators, though there is some evidence of asynchronous flowering phenology among these sympatric species (Sakai & Wright 2008). Seed dispersal and pollination do not therefore appear to be ecological services that are partitioned in such a way as to reduce interspecific competition for mutualists, as these reproductive niche axes are in some species-rich genera, such as *Miconia* (Poulin *et al.* 1999) and *Shorea* (Ashton 1969). Furthermore, nearly all *Psychotria* attain a maximum stature well below canopy height and only a few species that occur in Panama are true gap specialists that do not survive in deep shade. In light of these observations, I sought to examine the contribution of species differences with respect to understory soil moisture habitats (Ch. 2) and anti-herbivore defenses (Ch. 4) to the evolutionary divergence of closely related *Psychotria* from the traits of their common ancestors and to the coexistence of sympatric species on Barro Colorado Island (BCI). In addition, I examined the influence of biogeographic history on the evolution of hydraulic traits and hence community assembly with respect to soil moisture (Ch. 3). And finally, I considered the relationship between the degree of specialization of plant enemies with respect to plant defenses and the diversity such enemies maintain via density-dependent interaction with their hosts (Ch. 5). Understanding the biogeographic history of *Psychotria* and the theoretical relationship between enemy specialization and species coexistence allows this dissertation to consider the broader implications of observations made with respect to the *Psychotria* of BCI.

In Chapter II, I examined the relationship between phylogenetic history and the hydraulic and photosynthetic traits and habitat distributions of the *Psychotria* of BCI. The *Psychotria* of BCI do indeed differ with respect to understory habitat gradients defined by soil moisture and light availability. Yet, hydraulic traits exhibit a phylogenetic signal indicating that closely related species have diverged little from the niches of their common ancestors. This is contrary to what one would expect if differentiation with respect to such habitat gradients played a strong role in the evolutionary diversification of *Psychotria* or if similarities in soil moisture preference precluded the coexistence of close relatives. As a result, though the *Psychotria* of BCI exploit a range of soil moisture environments, species tend to share preferred soil moisture habitats with other close relatives within the genus, resulting in phylogenetically clustered assemblages at a scale of 3 m in radius compared to the species pool of the island as a whole.

In Chapter 2, I explored the implications of these patterns for our understanding of the role of species coexistence in the tropical forest understory. I concluded that survival in understory environments is dictated primarily by tolerance of low resource levels. Understory resources are dictated by asymmetric competition with overstory trees. Saplings and other understory plants, including mature *Psychotria*, fail to reach densities great enough for intraguild resource competition to influence resource availability. Therefore, closely related *Psychotria* are unlikely to exclude one another from shared habitats through resource competition because their influence on moisture, light, and other resource levels experienced by their understory neighbors is essentially negligible compared to the impact exerted by the overstory.

The overwhelming importance of asymmetric resource competition with the overstory relative to resource competition among understory plants in closed-canopy forests likely influences the life histories of many species viewed as “canopy” trees because they pass through critical recruitment filters in the understory. Furthermore, nearly 70% of the tree species in central Panama reach a maximum height at maturity below that of the canopy (King *et al.* 2006). A majority of tropical rain forest trees may therefore be influenced by factors that weaken competitive exclusion in the understory. This may contribute to the differences in patterns of niche evolution observed in temperate woody genera (e.g. *Ceanothus* and *Quercus*) and tropical forest genera such as *Inga*, *Bursera*, and *Psychotria*, a point to which we will return below.

The tendency of close relatives to exploit similar, inherited niches, known as phylogenetic niche conservatism, is most apparent when a group of organisms fails to exploit an alternative set of habitats or resources that are instead utilized by other clades (Wiens *et al.* 2010). At first glance, the phylogenetically-conserved hydraulic niches and phylogenetically clustered assemblages that characterize the *Psychotria* of BCI may appear to indicate a failure to adapt to alternative hydraulic habitats within the two major clades of the genus. Yet an examination of the biogeographic history of trait evolution alters this view in favor of a more nuanced understanding of the selection experienced by clades of *Psychotria* over the course of their evolutionary histories.

In Chapter 3, I concluded that the hydraulic traits and habitat affinities of the *Psychotria* of BCI are associated with the geographic region within which the species originated prior to dispersing to central Panama during the Great American Biotic Interchange. The hydraulic niches that characterize species of *Psychotria* with a shared biogeographic region of origin differentiate species on a spatial scale of mere meters in the forest understory. The biogeographic origins of species therefore influence community assembly at a spatial and temporal scale comparable to that of ecological processes including competition and coexistence.

An important implication of the results reported in Chapter 3 is that trait differences among locally sympatric species may not evolve locally (Ricklefs 2004, 2008). Obvious character displacement may be a special case most likely to be observed where populations are isolated from metapopulation dynamics and extraregional geneflow, such as oceanic islands (e.g. Grant & Grant 2006). More frequently, detectable differences in the pace at which lineages evolve with respect to alternative niche axes may provide some insight regarding the ecological dimensions that have played an important role in the diversification of taxa and that may stabilize their coexistence.

In Chapter 4, I investigated the role of differentiation with respect to secondary chemistry and other leaf traits that circumscribe the host-use patterns of insect herbivores of *Psychotria* in the evolutionary diversification of the genus and community assembly of species. My results indicate that insect herbivores of *Psychotria* on BCI exhibit polyphagous host ranges within the plant genus, but nevertheless are likely to exploit a subset of the BCI species pool of *Psychotria* circumscribed by species differences in physical and chemical defense traits. Furthermore, my results indicate broad congruence among the particular plant traits implicated in herbivore host-use patterns, *Psychotria* community structure, and herbivore damage relative to the trait neighborhood of the plant. These results suggest that enemy-mediated competitive interactions have played an important role in the divergence of related species with respect to secondary chemistry and other traits important in determining the hosts exploited by herbivores. The association between herbivore leaf damage and the neighborhood similarity of plants with respect to key defensive traits suggests that shared, density-responsive herbivores exert a fitness consequence on *Psychotria* that is likely to select for trait divergence among species over time, particularly those that exploit the same habitats. The absence of phylogenetic signal observed with respect to defense traits and to herbivore assemblages contrasts notably with the phylogenetic conservatism exhibited by hydraulic traits and soil moisture habitat preferences (Ch. 2). This contrast suggests that chemical defense traits tend to diverge among close relatives at a greater rate than physiological traits important in defining the abiotic niche and implicates selection for divergence in defense traits driven by *Psychotria*-herbivore interactions as an important source of evolutionary change in the genus.

Can the polyphagous natural enemies of *Psychotria* and other species-rich tropical tree genera facilitate coexistence within local communities? In Chapter 5, I addressed this question from a theoretical perspective using a stochastic, spatially-explicit simulation model. There is broad consensus that the spatially dependent action of specialist enemies described by Janzen and Connell is a widespread and important mechanism that maintains species richness in tropical forests and other plant communities (Wright 2002; Leigh *et al.* 2004; Terborgh 2012). However, it remains a challenge to reconcile such negative density-dependent recruitment patterns with the effects of enemies that appear to exploit clades of closely related species (Novotny *et al.* 2002; Odegaard *et al.* 2005; Webb *et al.* 2006; Gilbert & Webb 2007; Bagchi *et al.* 2010; Metz *et al.* 2010).

My theoretical model suggests that the J-C mechanism is indeed sensitive to the host specificity of natural enemies. Within the range of models extending from the J-C model to one in which plant species are indistinguishable in the eyes of enemies, only those that approach the classic J-C model retain the diversity-enhancing effect of the mechanism. Yet, our results also suggest that the case studies of somewhat generalist plant pathogens in Panama and insect herbivores in Panama and Papua New Guinea are sufficiently specialized within their respective plant communities to approach the effectiveness of the classic J-C model at maintaining plant species richness.

The models examined in Chapter 5 suggest some caution in the interpretation of the results pertaining to the dispersion of defense traits presented in Chapter 4. Nonspecialist enemies may play a role in the chemical diversification of *Psychotria* and contribute to the even distribution of *Psychotria* with respect to defense on BCI without stabilizing the coexistence of more species that would persist in the absence of such enemies. On the other hand, the interaction between the hydraulic niche and shared herbivores may contribute to coexistence within the BCI community of *Psychotria*, as species are likely to co-occur within habitats with species with which they are least likely to share herbivores. Evolutionary divergence from defense traits inherited from common ancestors may thus contribute to the ability of closely related species to exploit shared hydraulic habitats on the island.

Future Directions

The work presented in this dissertation provides evidence that secondary chemistry associated with herbivore host use patterns represents both a major ecological dimension along which *Psychotria* have diversified over the evolutionary history of the genus, as well as a trait axis or axes along which species partition niche space in local assemblages. Similar findings with respect to *Bursera* (Becerra 1997, 2007) and *Inga* (Kursar *et al.* 2009) suggest that such patterns may be common among species-rich tropical tree genera. Even recent studies of *Protium* suggest that defense evolution can be quite labile within that genus, though quantitative as well as qualitative differences in defense may often evolve as part of a growth-defense tradeoff associated with adaptation to nutrient-poor edaphic environments (Fine *et al.* 2004, 2005). Despite these intriguing results pertaining to four genera in three families, identification of plant-enemy interactions as the critical axis of evolutionary diversification and medium of diversity maintenance in tropical plant communities would require i) identification of the fitness effects of various guilds of natural enemies, including microbiota, ii) a better understanding of the phenotypic scale of defense variation that circumscribes the host ranges of enemies, and iii) an understanding of the differences in genetic or metabolic architecture that distinguish species-rich, defense-mediated adaptive radiations—in the vein of Ehrlich & Raven (1964)—from their species-poor sister clades with hypothetically less propensity to differentiate with respect to defenses against natural enemies.

Which plant enemies are responsible for the maintenance of tree diversity in forests? Terborgh (2012) suggests that only host-restricted, distance-dependent enemies are likely to prevent plant reproductive success within the vicinity of adult plants, a requirement for enemies to promote species coexistence. This is because density-responsive herbivores exploit a seedling carpet only until its density matches the background density of suitable host or food species in the landscape. Density-dependent plant enemies can therefore reduce per capita propagule survival at high densities, but cannot prevent replacement of a parent tree with a conspecific seedling. Plant enemies with more specialized host ranges are in any case likely to act in a distance-, rather than density-, dependent manner as they associate with large, adult members of their host plant species in the landscape. The spatial association of such enemies and adult host trees can result in near-complete reproductive failure of the parent tree within some distance (Terborgh 2012). In an herbivore exclusion experiment carried out in Cocha Cashu Biological Station, Amazonian Peru, Swamy & Terborgh (2010) concluded that the organisms responsible for distance-dependent seedling mortality were either soil organisms or arthropods small enough to pass through 2 mm mesh. Comita *et al.* (2010) and Mangan *et al.* (2010) further implicate soil microbiota, perhaps fungal pathogens, in seedling mortality associated with proximity to conspecific adults. On the other hand, much remains unknown concerning the host specificity of fungal pathogens. Webb *et al.* (2006) and Gilbert & Webb (2007) suggest fungal pathogens of leaves and wood tissue display quite low host specificity and are likely to exploit multiple closely-related species.

Another intriguing possibility is that insect herbivores which do not themselves cause great mechanical damage, such as Curculionid weevils, may act as vectors of disease caused by fungal, bacterial, and viral plant pathogens. Such tritrophic interactions may couple the spatial distribution and host specificity of the vector (e.g. a weevil) with the greater virulence of a microbial pathogen that would exhibit a greater fundamental host range than that realized by the more circumscribed diet of the insect vector. Hence, despite over 50 years of investigation into, and pervasive evidence of, the negative density dependent recruitment predicted by Janzen (1970) and Connell (1971), much remains to be discovered concerning the mechanisms by which natural enemies influence host abundance and community composition and the identity of the agents responsible for the recruitment patterns we observe.

The difficulty of isolating, identifying, and comparing the kaleidoscope of secondary compounds has long limited our ability to understand plant-enemy interactions in species rich plant communities. The host ranges of herbivores and pathogens, and the community structure of the forest have been investigated with respect to plant phylogeny (e.g. Kembel & Hubbell 2005, Kress *et al.* 2009). Yet, as discussed in Chapter 5, the usefulness of phylogeny as a proxy for plant defense is strongly scale dependent. Ultimately, the traits themselves are necessary to understand the role of enemies in the origin and coexistence of woody plant diversity. More specifically, it remains unclear if the number of niches defined by the secondary compounds and other defenses of trees can approach the number of species in the most species-rich tropical forests, or if much of the chemical variation is redundant in the eyes of natural enemies. Fortunately, recent advances in analytical and computational chemistry may make it feasible to investigate the chemical differences between species in a tropical forest in a comparable way. These advances consist of two critical developments. Firstly, methods that permit the desorption of the chemical components of a sample and their separation and unique determination as part of the analytical process circumvent the need to fraction a plant's secondary compounds based on structural class prior to analysis (sensu Kursar *et al.* 2009). For example, a technique termed "nanospray desorption electrospray ionization tandem mass spectrometry" (nanoDESI MS) has been utilized in the analysis of unextracted, mixed samples, such as a bacterial colony on a plate of agar, and the generation of mass spectra for each compound in the sample (Watrous *et al.* 2012). Secondly, novel computational methods facilitate the organization of vast amounts of spectral data into "molecular networks," the topology of which indicates the structural similarity between compounds that differ by as little as a single atom or between those as different as a lipid and an alkaloid (Watrous *et al.* 2012). Molecular networking of mass spectra produced using nanoDESI MS has not yet been applied to the study of plant secondary chemistry, though it has the potential to provide both a standardizeable extraction protocol and a framework for quantifying secondary chemical differences among species over a wide range of phylogenetic relationships. The pairing of a large-scale secondary chemistry network with a plant-herbivore host association network in a forest plot in which all individuals ≥ 1 cm dbh are measured and identified, such as those managed by the Center for Tropical Forest Science, could provide a potent combination of datasets. The field application of new analytical and DNA barcoding methods might therefore provide the opportunity to identify the plant traits that determine enemy host use at a whole-community scale, and to understand how the enemy neighborhood influences recruitment, growth, survival, and the community composition of plants even in species-rich tropical forests.

Gentry (1982,1989) coined the term "species swarms" in response to his observations of high local species richness and apparent ecological similarity of *Miconia*, *Piper*, and *Psychotria*. Assemblages of *Pouteria, Inga* and *Eugenia* also exhibit high local species richness in Neotropical forests, with some examples of 22 species of *Pouteria* and *Inga* in plots of only 1 ha and 0.16 ha, respectively (Valencia *et al.* 1994). Likewise, Southeast Asian forests can be dominated by *Shorea*, or members of the Dipterocarpaceae more broadly (Ashton 1969; LaFrankie *et al.* 2006). Is there something about the genetic or metabolic architecture of these lineages that distinguish them from their sister taxa in ways that make them more evolutionarily labile with respect to secondary chemical defenses? New developments in genomics may make it possible to answer such questions. The exceedingly long generation times of trees make traditional approaches to genetics, or even genomics, simply unfeasible. However, the decreasing costs of genome sequencing have made feasible a practice known as genome “resequencing,” the sequencing of whole genomes of many individuals in a population so as to facilitate the comparison of genome-level variation responsible for population differences. For example, Friesen *et al.* (2010) were able to identify candidate genes associated with saline adaptation in wild populations of *Medicago trunculata* by employing genome-wide scans for population-level polymorphism. If Gentry’s species swarms represent adaptive radiations driven by elevated rates of evolution driven by selection for divergence of defensive traits, the genes involved in the metabolic pathways responsible for defensive secondary compounds may exhibit genomic evidence of selection that would stand out against a background of otherwise more conserved genomic features. Indeed, there is evidence for divergent selection in the monoterpene synthase gene responsible for expressing the small-molecular building blocks of the secondary chemical defenses of *Protium* and other Burseraceae (Zapata & Fine 2013). The divergence times of some tree genera may complicate any attempt to apply population-level genomic tools to the study of interspecific trait evolution in tropical trees. The two subgenera of *Psychotria*, for example, last shared a common ancestor ca. 35 Ma, making the genomic trail of selection for chemical divergence perhaps difficult to pick out against a background that has been influenced by many factors over past millennia. *Inga*, on the other hand, represents ca. 300 species that all radiated from a single common ancestor within the last ca. 2 Ma and displays some of the lowest amounts of genetic variation among congeneric plant species known (Richardson *et al.* 2001, Kursar *et al.* 2009, Kress *et al.* 2009). The species of the genus *Inga*, which differ notably and exhibit little phylogenetic signal with respect to their secondary chemistry, may provide an excellent system in which to examine the genetic and metabolic factors that may couple chemical evolution and lineage diversification in trees. In other instances, identifying the trait evolution associated with cladogenesis in woody plants may require the genomic comparison of sister species or populations.

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