Traits, Habitats, and Clades: Identifying Traits of Potential Importance to Environmental Filtering.

Author(s): Margaret M. Mayfield, Maciej F. Boni, and David D. Ackerly

Source: *The American Naturalist*, Vol. 174, No. 1 (July 2009), pp. E1-E22
Published by: The University of Chicago Press for The American Society of Naturalists
Stable URL: http://www.jstor.org/stable/10.1086/599293
Accessed: 06/10/2015 21:15

E-Article

Traits, Habitats, and Clades: Identifying Traits of Potential Importance to Environmental Filtering

Margaret M. Mayfield,[1,*] Maciej F. Boni,[2,3] and David D. Ackerly[4]

1. The University of Queensland, School of Biological Sciences, St. Lucia campus, Brisbane, Queensland 4072, Australia; 2. Resources for the Future, Washington, DC 20036; 3. Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey 08544; 4. Department of Integrative Biology, University of California, Berkeley, California 94720

Submitted January 14, 2008; Accepted January 6, 2009; Electronically published May 22, 2009

Online enhancement: computer code.

abstract: Environmental filtering is a fundamental process in the ecological assembly of communities. Recently developed phylogenetic tools identify patterns associated with environmental filtering across whole communities. Here we introduce a novel method that allows the detection of traits involved in the environmental filtering of species from specific clades in specific habitat types. Our approach identifies nonindependent trait/habitat/clade (THC) associations and also provides a framework for detecting clearly defined two-way trait/clade, trait/habitat, and clade/habitat associations. The THC method relies on exact binomial tests and differentiates THC associations resulting from a three-way interaction from those that are generated by one or more underlying significant two-way interactions. It can also detect THC associations for which there are no significant two-way associations (trait/habitat, trait/clade, clade/habitat). To illustrate the THC method, we examine plant pollination and dispersal traits from six habitat types in a fragmented Costa Rican landscape. Results suggest that these traits are not widely important for the environmental filtering of most clades in this landscape, but animal dispersal and insect pollination are involved in the filtering of monocots and the Piperaceae in rain forest understory.

Keywords: community assembly, functional traits, exact binomial test, environmental filtering, pollination mechanisms, dispersal mechanisms.

Introduction

Identifying the mechanisms that determine the species composition of natural communities remains a focal topic in community ecology. Recently developed phylogenetic tools designed to detect nonrandom patterns in the relatedness of co-occurring species provide new insights into this topic (Belyea and Lancaster 1999; Webb 2000). From these patterns we can infer the importance of ecological processes, such as environmental filtering, to community assembly (Webb 2000; Kraft et al. 2007; Swenson et al. 2007). Interpretation of community-level phylogenetic patterns depends on the distribution of ecologically relevant traits among clades represented in communities. This approach does not, however, allow for the identification of individual traits involved in maintaining particular species groups in specific environments. To make such an inference, a significant three-way association among a trait, a clade, and an environment (or habitat), must be shown. It has been suggested that for a given trait, clade, and habitat, three significant two-way associations—between the trait and habitat, habitat and clade, and clade and trait—reflect a significant three-way trait/habitat/clade association (Chazdon et al. 2003). As we illustrate below, this is not necessarily the case.

Environmental filtering is the process by which certain physiologically and ecologically compatible species survive and persist in a community while others do not. Results from phylogenetic-based analyses have resulted in a growing appreciation for the role this process plays in the assembly of many communities (Webb et al. 2002; Mayfield et al. 2005; Horner-Devine et al. 2007). Environmental filtering is commonly detected by comparing species similarity in real communities to "null" communities (a theoretical community assembling under neutral conditions; Weiher and Keddy 1995; Gotelli and Graves 1996). When species within a community are found to be more closely related to one another or to have significantly more similar trait values (for assembly-relevant traits; e.g., Cornwell et al. 2006) than a "null" community, then environmental filtering is theorized to be a prominent process involved in that community's assembly. To date, most studies of environmental filtering have aimed to detect this process across entire communities, with only a few studies considering the distinct evolutionary history and ecology of species groups within communities (Harvey 1996; Chazdon et al. 2003; Cavender-Bares et al. 2004).

In this article, we present a novel method for detecting

* Corresponding author; e-mail: m.mayfield@uq.edu.au.

Am. Nat. 2009. Vol. 174, pp. E1–E22.
DOI: 10.1086/599293

nonindependent associations among categorical functional traits, phylogenetic clades (or taxonomic groups), and habitat types (different environments) within a landscape that shares a regional species pool. A theoretically correct interpretation of a trait-habitat-clade (THC) association identified as significant with this method is that the trait is involved in the environmental filtering of species in the clade into the habitat. We refer to our approach as the THC method. To explain this method, we examine the association of pollination and dispersal mechanisms (traits) with plant clades represented in six common habitat types in a forest-pasture mosaic landscape in southern Costa Rica (Mayfield and Daily 2005). This landscape is ideal for testing which functional traits are important to the environmental filtering of plants living in adjacent but distinct habitats because it is composed of small habitat patches and is old enough to reflect assembly rather than just extinction processes associated with initial deforestation (Mayfield and Daily 2005).

The THC Method

The THC method identifies statistically significant associations among traits, habitats, and clades (THC associations), which can be interpreted as reflecting the importance of the trait (T) to the environmental filtering of species from the clade (C) into the habitat (H). The approach we describe can be used to find both positive and negative associations, but we focus on positive associations for simplicity. The THC approach requires: (1) a list of species from multiple communities within a region (a regional species pool) linked by a common species pool, (2) habitat categorizations (or environmental distinctions) for the sampled communities, (3) a phylogeny or taxonomic classification for the species in the regional species pool (i.e., clades), and (4) categorical trait data for the sampled species.

The THC method can be used to assess different types of associations among any trait, habitat, and clade. To describe these associations, we use a simple conceptual contingency cube (a "THC cube"; fig. 1). The THC cube separates all the species in the regional species pool according to whether they are from the clade and the habitat of interest and have the trait of interest (fig. 1). Using the THC cube to examine associations among these factors, it becomes clear that at least three statistically distinct questions can be asked about each pair of THC factors and all three factors. To describe these distinct question forms, we first consider two factors: a hypothetical habitat (understory) and clade (monocots). We can ask these questions about the association between monocots and understory: (i) If a plant is found in the understory (H), is it more likely to be a monocot (C) relative to a plant selected at random from any habitat? (ii) If a plant is a monocot (C), is it more likely to be found in the understory (H), relative to a plant selected at random from the phylogeny of all species in the landscape? (iii) Are monocots (C) assembling independently of whether the habitat is understory (H)?

Questions i, ii, and iii can be written as statistical statements 1, 2, and 3, respectively:

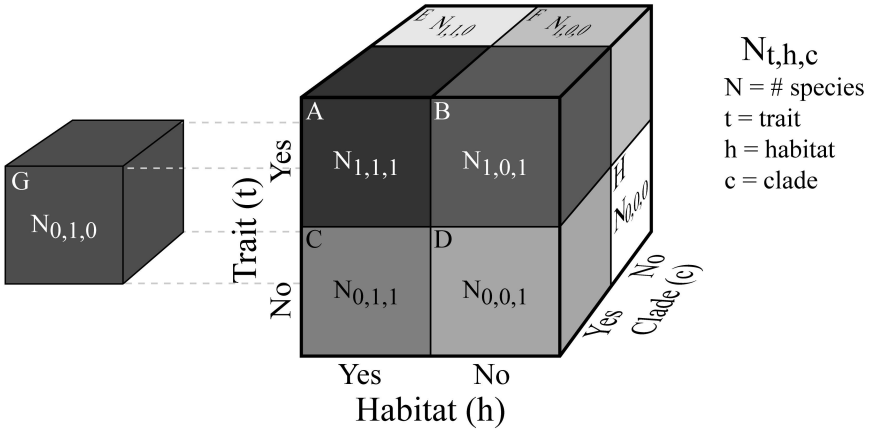


Figure 1: Trait-habitat-clade (THC) contingency cube. For any group of communities linked by a common regional species pool, there is a conceptual THC cube for each trio of clades, traits, and habitat types. Each subcube represents the number of species with each combination of the three factors. N = total number of species in the regional species pool. Subscripts of the N in each subcube are coded as "yes" (1) and "no" (0). Thus, each subcube is the portion of N species with and without the indicated T, H, and C. For example, subcube B is the number of species with the trait (1), not in the habitat (0), and in the clade (1).

$$\frac{A+C}{A+C+E+G} \gg \frac{A+B+C+D}{N}, \quad (1)$$

$$\frac{A+C}{A+B+C+D} \gg \frac{A+C+E+G}{N}, \quad (2)$$

$$\frac{A+C}{N} \gg \frac{A+B+C+D}{N} \times \frac{A+C+E+G}{N}. \quad (3)$$

In these statements, letters refer to the subcubes in the THC cube (fig. 1) and the operator means "is significantly greater than." Using exact binomial tests, we can compare the observed (left side of statements) and expected (right side of equation) proportion (f) of species in each statement. Unlike categorical analytical tools, such as G-tests, exact binomial tests can be used effectively for small sample sizes and in cases when there are no species in some subcubes (fig. 1; app. A). Binomial tests are also computationally much easier and faster than statistically comparable randomization tests.

From the statements above it is evident that for any trait, habitat, and clade, there are nine types of two-factor associations. While there are many reasons we may be interested in any or all nine of these two-factor associations (Chazdon et al. 2003; Box 1: "A Guide to Interpreting THC Results"), they cannot be used to reliably identify significant three-factor associations of environmental filtering importance. Even if all nine of the two-factor questions are answered yes, a significant three-factor association may not exist. This is because a trait T can be conserved in clade C and overrepresented in habitat H and species from clade C can be phylogenetically clustered in habitat H without the same species being involved in all three associations. For this reason, a direct three-factor test must be used to identify whether a biologically meaningful three-way THC association exists (app. A).

As with two-factor associations, the THC test can be used to ask conditional questions about all three factors in the format of questions i, ii, and iii. The most direct approach for identifying THC associations of importance to environmental filtering is question form iii, which can be written as

$$\frac{A}{N} \gg \left(\frac{A+B+E+F}{N}\right)\left(\frac{A+C+E+G}{N}\right)\left(\frac{A+B+C+D}{N}\right). \quad (4)$$

The null expectation for the binomial test of equation (4) is that each trait, habitat, and clade assort independently, in other words, that the expected frequency of the trio is the product of the individual trait, habitat, and clade frequencies ($f_T f_H f_C$; eq. [4]). Thus, a particular THC trio does not assort independently if its observed frequency ($A/N = f_{THC}$; eq. [4]) is significantly greater (or smaller) than the expected frequency $f_T f_H f_C$ (examples of calculations in app. A).

One issue to be mindful of with this approach is that a statistically strong two-way association may, in some cases, be responsible for the significance of a three-way association. To identify these cases, we use a post hoc test to correct for the contribution of each significant two-factor association. We do this by redefining the expectation frequency ($f_T f_H f_C$) to incorporate the significant two-factor frequency (e.g., $f_H f_{TC}$). To explain, consider a case where $T_1H_1C_1$ and T_1C_1 are found to be significant associations. If T_1C_1 is generating a spurious three-way association, then the number of species with the T, H, and C will be close to the total number of species in the region times the frequency of the habitat and the combined TC frequency ($N \times f_H \times f_{TC}$). If, however, f_{THC} is significantly greater than $N \times f_H \times f_{TC}$, then T_1, H_1, and C_1 are significantly associated over and above any underlying significant T_1C_1 association.

One of the benefits of the binomial THC method is that it allows large numbers of traits, habitats, and clades to be assessed at once; however, this also results in a large number of statistical tests being performed. There is no specific multiple comparisons correction method tied to

Box 1: A Guide to Interpreting THC Results

1. *Significant TH Associations.* In basic assembly theory, if a trait T significantly affects assembly, then there will be a significant TH association regardless of clade. The THC method can further identify whether the observed TH association is the result of filtering acting on trait T of species from one clade, C_1. If the THC_1 and TH associations are significant, this is robust evidence of environmental filtering for THC_1. If a THC with a significant TH association is found for all or many clades (THC_1, THC_2, THC_3, …), there is even stronger evidence of the importance of the trait to the environmental filtering of the greater community.

2. *THC Results from a Significant TH Association.* If a post hoc test shows that a two-factor association (e.g., TH) is causing the THC association, then trait T is generally important for filtering in habitat H, and there may be some additional characteristic of species from clade C that facilitates the filtering of trait T into habitat H. We may not, however, have sufficient statistical evidence to show that clade C plays any significant role in the filtering of trait T into habitat H.

3. *Significant THC Associations with No Two-Way Associations.* This pattern reflects the importance of trait T (or an unmeasured trait correlated with T) for the environmental filtering of species only from clade C in habitat H but not for the entire community. Note that such cases cannot be detected with two-factor analyses alone.

the THC method, but results should always be interpreted with such corrections in mind (Garcia 2004). For conservative correction methods, such as the Dunn-Sidák method used in our case study, some biologically meaningful comparisons may be mistakenly disregarded. Fortunately, *P* values from binomial tests tend to be quite small even for moderately significant associations, helping keep false negatives to a minimum (table 1). We recommend that any user of the THC method consider which correction procedure is best for their study and examine "close cases" to ensure that important associations are not overlooked.

While the THC method utilizes phylogenetically organized species groups (clades), the binomial test compares species in each clade to all species in the regional species pool, functionally ignoring the phylogenetic structure both above and below the node defining the clade of interest. In this respect, it is similar to taxonomic approaches that focus on traditionally recognized genera or families as units of analysis, with the important advantage that analyses can be conducted across the entire tree, rather than on an arbitrarily defined taxonomic rank. On the other hand, results pertaining to closely nested nodes may reflect nonrandom patterns that propagate up the tree, and care must be exercised to avoid overinterpretation in such cases. One way to incorporate phylogenetic structure into the method would be to compare species in each clade to species in a sister clade rather than to all other species in the regional phylogeny. THC Binomial Analysis software was written to compare species in each clade to the whole regional species pool, but it can easily be modified for this alternative approach.[1]

Costa Rica Example

To illustrate the THC method, we test for the importance of dispersal and pollination mechanisms to the environmental filtering of herbaceous and shrubby plant species in six common habitat types in a southern Costa Rica landscape. In addition to illustrating one application of the THC method, results from this example advance our understanding of the role that these traits play in the environmental filtering of plant communities in human-altered tropical landscapes. We ask a specific question about this data set: Which THC trios are assorting non-independently in this landscape?

In addition to results from the THC method, we present, as an appendix, results from a two-factor analysis in which we infer three-factor associations from this data set in cases where all three two-factor associations (trait/habitat, trait/clade, and habitat/clade) are found to be significant. This two-factor approach uses randomization tests for identifying trait/clade and habitat/clade associations (question form ii; Webb et al. 2008) and ANOVA for identifying trait/habitat associations (Mayfield et al. 2006; app. B). We compare results obtained from these two approaches to highlight the benefits of the THC approach.

Complete details of the data set used for this case study are available in Mayfield and Daily (2005) and Mayfield et al. (2006). Brief methods are provided in appendix B. The complete regional phylogeny is presented in figure 2.

The full data set used for the THC analysis consisted of 488 plant species from 159 clades (including genera, families, and deeper multifamily clades; fig. 2), with each species coded for 25 traits (15 pollination mechanisms and 10 dispersal mechanisms) and recorded living in one or more of 58 plant communities, divided among six habitat types (app. B). To test whether a particular THC combination was present with more species than expected, we use the exact binomial test as described above (eq. [4]) with the expectation equal to $488 \times f_T \times f_H \times f_C$ (representing independent assortment in this landscape). In the full THC analysis, we performed all 28,929 possible tests (including all two-way tests) and corrected each *P* value for multiple comparisons with a Dunn-Sidák correction (corrected *P* value cutoff of 1.78E−6). Corrected and uncorrected *P* values are presented in table 2 and appendix B. For those significant THC combinations that also have significant two-factor associations, we used our above-described post hoc binomial test to examine the contribution of each two-factor association to the significant three-way association. The THC code is available in the online edition of the *American Naturalist.*

Results

Binomial THC

Out of 23,850 three-way combinations, exact binomial tests revealed 100 significant THC associations after corrections for multiple comparisons: 43 for dispersal traits and 57 for pollination traits (table 1; app. C). Eighty-seven of the 100 significant THC combinations had one significant two-way interaction, two combinations had two significant two-way interactions, and the remaining 11 had no significant two-way interactions (app. C). There were no significant THC associations for which all three two-factor associations were significant. Trait-clade was the significant two-way interaction for 86 of the THC combinations with a single significant two-way interaction; the other was between a habitat and clade (HC in app. C).

[1] This C++ code will run the THC test as used in this article. If you would like assistance in modifying the code for another use, please feel free to contact M. M. Mayfield. Code that appears in the *American Naturalist* has not been peer-reviewed, nor does the journal provide support.

Table 1: Trait-habitat-clade (THC) combinations found to be statistically significant using the THC binomial test and the two-factor binomial post hoc correction

Habitat	Clade	Trait *f*	Habitat *f*	Clade *f*	Expected	Observed	THC *P*	Two-factor *P*	Status
Dispersal traits:									
Endozoochory:									
Gap	Alismatales: Philodendron	.23 (111)	.43 (212)	.03 (15)	1.48	12	5.41E−08	None	Yes
Understory[a]	Monocots	.23 (111)	.22 (109)	.26 (127)	6.45	33	5.70E−14	2.89E−11 (TC); 5.96E−07 (HC)	Yes
Understory	Alismatales: Philodendron	.23 (111)	.22 (109)	.03 (15)	.76	8	1.37E−06	None	Yes
Understory[a]	Monocots-Alismatales	.23 (111)	.22 (109)	.15 (71)	3.61	22	3.69E−11	3.89E−10	Probable
Understory[a]	Asparagales + Commelinids	.23 (111)	.22 (109)	.13 (61)	3.10	19	7.35E−10	1.12E−07	Probable
Understory	Commelinids	.23 (111)	.22 (109)	.12 (56)	2.85	19	1.83E−10	4.13E−08	Probable
Understory	Arecales	.23 (111)	.22 (109)	.03 (13)	.66	10	2.21E−09	None	Yes
Exozoochory:									
Pasture	Malvaceae: Sida	.06 (30)	.26 (125)	.01 (5)	.08	4	1.49E−06	None	Yes
Bird/bat:									
Understory[a]	Magnoliids	.10 (49)	.22 (109)	.08 (38)	.85	18	2.94E−18	1.95E−21	Probable
Understory	Piperales	.10 (49)	.22 (109)	.08 (37)	.83	18	1.86E−18	8.64E−22	Probable
Understory	Piperales: Piper	.10 (49)	.22 (109)	.07 (33)	.74	18	2.57E−19	2.79E−22	Probable
Gravity:									
Verge	Rubiaceae: Spermacoce	.035 (17)	.43 (212)	.008 (4)	.06	4	5.27E−07	None	Yes
General:									
Forest riverbank	Urticaceae: Urera	.04 (20)	.25 (122)	.01 (4)	.04	4	1.12E−07	None	Yes
Pasture riverbank	Poales	.04 (20)	.18 (86)	.01 (7)	.05	4	2.58E−07	None	Yes
Pollination traits:									
Bird:									
Understory	Monocots-Alismatales	.05 (23)	.22 (109)	.15 (71)	.75	8	1.19E−06	None	Yes
Understory	Asparagales + Commelinids	.05 (23)	.22 (109)	.13 (61)	.64	8	3.87E−07	None	Yes
Wind:									
Forest riverbank	Urticaceae: Urera	.05 (26)	.25 (122)	.01 (4)	.05	4	3.18E−07	None	Yes
General (abiotic and entomophilous):									
Understory	Magnoliids	.11 (51)	.22 (109)	.08 (38)	.89	19	2.63E−19	7.21E−24	Probable
Understory	Piperales	.11 (51)	.22 (109)	.08 (37)	.86	19	1.62E−19	2.95E−24	Probable
Understory	Piperales: Piper	.11 (51)	.22 (109)	.07 (33)	.77	18	5.14E−19	9.23E−22	Probable
Entomophilous:									
Understory	Arecales	.26 (127)	.22 (109)	.03 (13)	.76	10	7.79E−09	None	Yes

Note: The table shows dispersal traits and pollination traits, and the trait for each THC trio is indicated at the top of each section. Column headings: Trait *f*, Habitat *f*, and Clade *f* = frequencies of the trait, habitat, and clade, respectively (total number of species with each factor); Expected = expected number of species ($N \times f_T f_H f_C$); Observed = observed number of species with the THC trio (*A*/*N*; fig. 1); THC *P* = *P* value for the three-way THC exact binomial test (uncorrected); Two-factor *P* = *P* value for the significant two-factor association(s) for a given THC trio, which is TC unless indicated otherwise in the cell.

[a] The THC association was also identified using two-factor analyses presented in appendixes B and D.

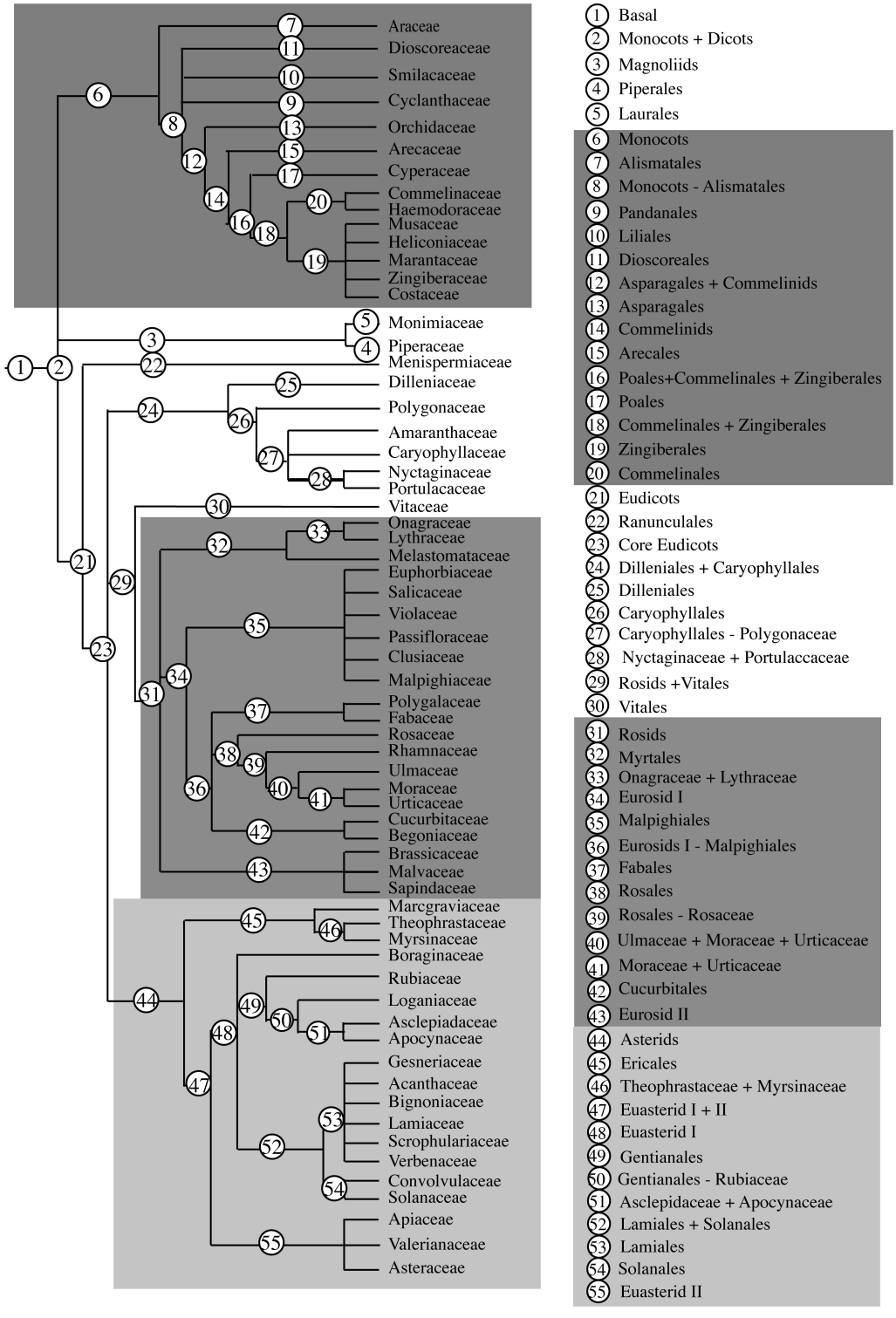


Figure 2: Regional phylogeny for the Costa Rica case study. Numbers correspond to codes used in tables 1 and 2 and the appendixes. The phylogeny was constructed using Phylomatic's conservative backbone tree (Webb and Donoghue 2005), derived from Peter Steven's world angiosperm tree (app. B). Numbered nodes leading to single families indicate instances where an order is represented by a single family. Genera are not included here but were part of the trait-habitat-clade analysis. Shading indicates key clades: *dark gray* = monocots (6–20), *medium gray* = Rosids (31–43), and *pale gray* = Asterid (44–55).

Table 2: Example results from our Costa Rica case study using the trait-habitat-clade (THC) method and the two-factor method (app. B)

Trait	Habitat	Clade	No. species Obs. THC (exp. no.)	TH	TC	HC	THC *P* (corrected)	Sig. THC	Sig. two-fact.
Endozoochory (111)	Understory (109)	6 (127)	33 (6.45)	41	69	57	5.70E−14 (1.65E−9)	Yes	Yes
Bird/bat dispersal (49)	Understory (109)	3 (38)	18 (.85)	22	35	19	1.95E−21 (5.65E−17)	Probable	Yes
Beetle pollination (27)	Understory (109)	6 (127)	14 (1.57)	14	27	57	1.26E−9 (3.65E−5)	Weak	Yes
Bird pollination (23)	Understory (109)	12 (61)	8 (.64)	9	14	26	3.87E−7 (1.1E−2)	Yes	No
Endozoochory (111)	Understory (109)	15 (13)	10 (.66)	41	13	10	2.21E−9 (6.40E−5)	Yes	No
Bird/bat dispersal (49)	Understory (109)	4 (37)	18 (.83)	22	34	19	1.86E−18 (5.39E−14)	Probable	No
Bird dispersal (122)	Gap (212)	Sap. (8)	5 (.87)	64	8	5	1.99E−3 (1.0)	No	Yes
Endozoochory (111)	Understory (109)	31 (142)	3 (7.21)	41	26	14	9.76E−1 (1.0)	No	Yes
Gravity dispersal (17)	Pasture (125)	44 (155)	6 (1.38)	8	9	47	2.97E−3 (1.0)	No	Yes
Exozoochory (30)	Pasture (125)	21 (321)	14 (5.05)	14	30	90	7.16E−4 (1.0)	No	Yes

Note: Total numbers of species with each trait, habitat, or clade are listed in parentheses in columns 1–3. The numbers and "Sap." (Sapindaceae) in the "Clade" column refer to figure 2. Column headings: Obs. THC = number of observed species that have the trait, are in the habitat, and are in the clade (subcube *A*, fig. 1), with the expected number of species with that THC in parentheses; TH, TC, and HC = numbers of species with both the indicated trait and habitat, trait and clade, and habitat and clade, respectively; THC *P* value = uncorrected *P* value obtained for the three-way THC association, with the corrected value in parentheses; Sig. THC = strength of significance for each THC association (table 1); and "Sig. two-fact." indicates whether the two-factor approach (app. B) detected that the THC association was significant. The table shows THC combinations that were significant at $P < .05$ (status: yes) and $P < .1$ (status: probable) and those significant THC trios with no significant two-factor associations (status: yes).

The significant THC combinations with two significant two-way interactions were: endozoochorous dispersal/monocots/understory and beetle pollination/monocots/understory, both of which had trait-clade and habitat-clade as two-factor significant associations (table 1).

The strongest evidence for nonindependent assortment of the trait, habitat, and clade lies in the 11 THC combinations without any two-way interactions (table 1; Box 1: "A Guide to Interpreting THC Results"). Post hoc binomial tests revealed that 45 of the remaining 89 significant THC combinations are most likely caused by a strongly significant two-factor association ("no" status in app. C), and only one was found to be a strongly significant THC association after correction for multiple comparisons: endozoochory/monocots/understory ("yes" status in table 1). Of the remaining 44 THC associations, nine had *P* values <.1 after post hoc tests ("probable" status in table 1; app. C), and 35 had uncorrected *P* values <.05 that were no longer significant after correction ("weak" status in app. C).

Comparison of Three-Factor THC and Two-Factor Multistatistic Results

There were major differences in the THC associations identified using the THC method and those selected based on three significant two-factor associations (table 2; apps. B–D). The two-factor analysis identified 157 trait, habitat, and clade combinations (out of a more restricted 1,404 combinations tested; app. B) for which all three two-factor associations (trait-clade, clade-habitat, habitat-trait) were significant (112 with a dispersal mechanism and 45 with a pollination mechanism; app. D). Of the 157 identified associations, only 16 were also identified as three-way relationships using the THC test (asterisk in app. C; table 1). Some of these 157 associations were identified as significant using the THC method before correction for multiple comparisons, suggesting that our conservative correction may falsely eliminate some weaker associations. Other associations, however, were not significant at all using the THC approach, indicating that the two-factor approach does lead to inferences regarding three-way relationships that are not supported by direct analysis of three-way associations (examples provided in table 2). Only one association inferred from the underlying two-way relationships, endozoochory/understory/monocots, was also found to be a strong independent association using the THC method after all corrections ("yes" status in table 1; app. C; table 2).

Discussion

The THC method is a simple tool for studying environmental filtering, a key process in ecological community assembly. In particular, it determines whether functional traits significantly associated with particular habitat types are overrepresented because of their role in the environmental filtering of particular species groups also associated with those habitats. The THC approach also provides a statistically precise and versatile method for detecting trait/clade, trait/habitat, and habitat/clade associations, which

are important for understanding the role of environmental filtering across entire communities.

Statistically significant THC associations detected with the THC method from data collected from fine spatial scales (as in our case study) for multiple habitats can be interpreted to mean the trait is involved in the environmental filtering of species from the clade within that habitat. As with any correlative statistic, however, care should be taken with the interpretation of results (Cohen 1971). For example, a positive THC association may alternatively reflect that the trait is linked to a different unexamined trait in clade C that is the actual biologically relevant trait (Box 1: "A Guide to Interpreting THC Results"). The association may also reflect local evolutionary radiation of the clade within a habitat type. Although this is an unlikely interpretation when data describe communities at a fine spatial scale, it should be seriously considered for studies of large areas, like states or countries. Care should also be taken when using a species pool that contains only species known to be compatible with the habitat of interest. In such cases, significant associations may reflect competitive processes rather than environmental filtering.

Significant THC correlations do not indicate that the trait is the only trait important for the persistence of species in clade C, just that it is one important trait in the complex process of environmental filtering. Correlations also cannot be used to identify the proportional contribution of a given THC association to the complete assembly of a community, nor can they provide details about which aspects of the habitat contribute to the importance of the trait to filtering of species in the clade (unless environmental factors are used instead of broad habitat groupings). Such biological details can be determined only through direct study of associations and even then may be difficult to determine.

The comparison of results obtained from the THC method and those inferred from two-factor associations (table 2; apps. B–D) illustrates the benefits of the THC method by showing the types of associations that can be falsely identified and those that cannot be detected at all using a two-factor inferential approach (table 2; apps. B, D). In our Costa Rican case study, we found that pollination and dispersal traits are important for only a few clades in forest communities (table 1; app. C). Had we used only results inferred from significant two-factor analyses (apps. B, D), we would have concluded that pollination and dispersal traits are considerably more important to the environmental filtering of communities in these landscapes than is likely in reality. This seems likely even if our correction for multiple comparisons was too conservative (tables 1, 2). Importantly, the two-factor approach also missed 11 of the 21 most significant THC associations because they did not have any significant two-factor associations.

Alternative Approaches

In addition to the binomial tests proposed here, there are several alternative approaches for detecting significant two-factor associations between categorical traits and clades or habitats (Pagel and Meade 2006; Webb et al. 2008). Conventional approaches to such problems utilize either χ^2 or *G*-tests, which cannot handle small sample sizes or zero values. For tests of correlated evolution with categorical traits, Pagel and Meade (2006) have developed a likelihood ratio method that accounts for phylogenetic branch lengths and compares models in which habitats and traits vary independently and dependently on a phylogeny. This method is best for taxonomically restricted studies and studies using very well resolved and dated phylogenies. Randomization methods, such as those provided in Phylocom (app. B) result in the same two-factor results (using question form ii) as in the THC test, but these tests become computationally cumbersome with very large data sets. It is for this reason that we examined only 1,404 out the 23,850 possible THC combinations for our Costa Rica data set with this approach (app. B).

A limitation of the THC method is that it can handle only categorical trait data. Many important functional traits are continuous variables. Continuous traits can be placed in categories for analysis with the THC method, or other methods, such as those available in Phylocom (Webb et al. 2008), can be used to detect associations between continuous traits and clades.

Case Study: Costa Rican Countryside Plant Communities

In Mayfield et al. (2006), we found numerous significant associations between pollination and dispersal traits (T) and the same six habitat types (H) examined in this study. Results from that study provided evidence for the importance of these traits to the assembly of these communities (Box 1: "A Guide to Interpreting THC Results"). Results from our THC study, however, indicate that very few pollination and dispersal traits are important for maintaining individual clades in these habitats. Only 54 THC associations were found to be even weakly significant using the THC method, out of 23,850 tested combinations. Of these, only 12 were clearly significant "yes," and nine were "probable" (table 1). Due to this large number of multiple comparisons, there is some concern that we "overcorrected" and missed some meaningful associations. In table 2 we provide examples of THC combinations that become nonsignificant following correction as well as associations that remain significant following correction to illustrate the

range of *P* values influenced by our correction method. Even if our conservative correction resulted in the exclusion of some biologically meaningful THC results, it seems clear that few associations are significant for pollination and dispersal mechanisms in this system (table 1). This suggests that many of the TH associations identified by Mayfield et al. 2006 are probably due to correlated unmeasured traits (Box 1: "A Guide to Interpreting THC Results").

Of our 18 most significant THC associations, all but four were in forest and involved either monocots or the Piperaceae. None were further supported by a significant TH association ("Box 1: A Guide to Interpreting THC Results"). Biologically, it is not surprising that these traits are not of major environmental filtering importance in this system. It is interesting, however, that insect pollination and animal dispersal do play a significant role in maintaining monocots and the Piperaceae in forest habitats and that wind dispersal is important for some Asterids in deforested habitats (table 1; app. C). These findings also give within-community details to the whole-community patterns found by Mayfield et al. (2005), which showed that dispersal is more important for the environmental filtering of forest communities than deforested communities in this landscape.

Conservation Applications

The type of information obtainable using the THC method has important implications for advancing a more ecological and less count-based approach to biodiversity conservation. There is a growing interest in understanding how human activities alter the ecology of natural communities (Holt and Gomulkiewicz 2004; Mayfield et al. 2005; Edwards et al. 2007). The THC method may be of particular use in identifying subtle but important ecological differences between remnants of pristine ecosystems and those under a range of human pressures. The THC associations may also be useful indicators of success in restoration projects or for identifying communities in human-modified landscapes of value for the protection of target species or for the provisioning of ecosystem services.

Acknowledgments

We are grateful to J. HilleRisLambers, C. Webb, and M. Westoby for providing valuable suggestions during the development of this work. A special thanks to J. Dwyer for graphical assistance and to numerous great field assistants and the staffs of the Las Cruces Biological Field Station of the Organization for Tropical Studies and the Rio Tigre Lodge for help with data collection. We are deeply indebted to R. Liesner (Missouri Botanical Garden) and many others at the Missouri Botanical Garden and InBio for plant identification. For financial support, we are extremely grateful to H. Bing and P. Bing, the Teresa Heinz Foundation, the National Science Foundation, the Winslow Foundation, Stanford University, Resources for the Future, and National Institutes of Health/National Institute of General Medical Sciences grant P50GM071508.

APPENDIX A

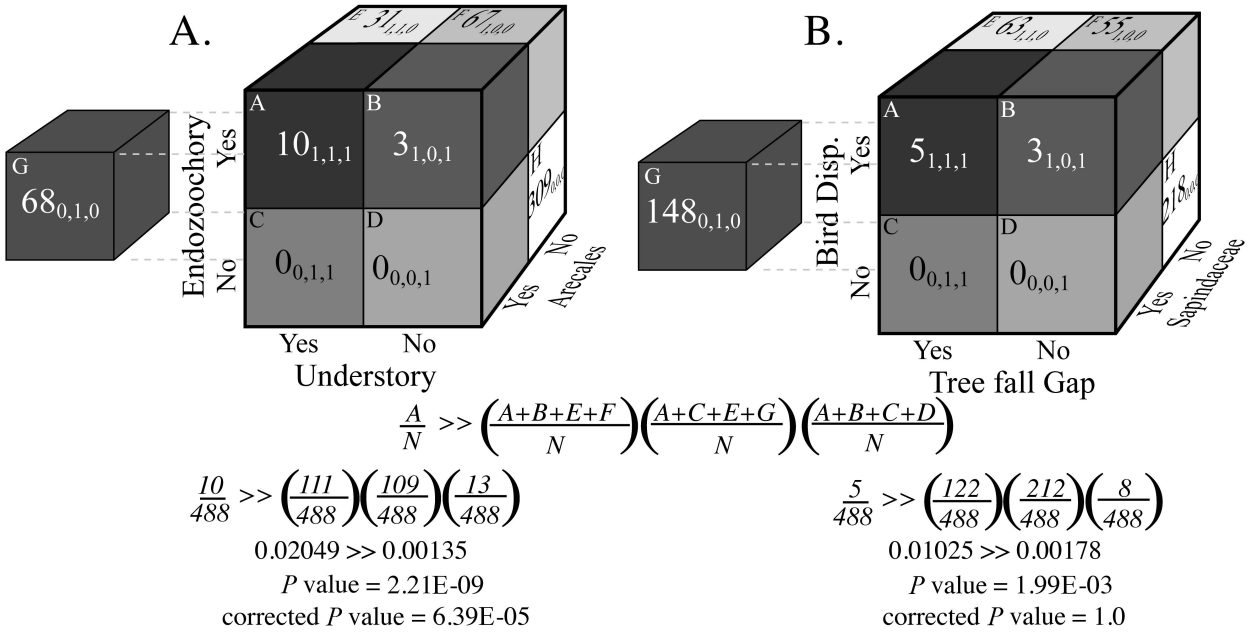


Figure A1: Two-example trait-habitat-clade (THC) analyses from our Costa Rica case study. *A* is a case where the THC approach revealed a significant THC association but for which there were no significant two-way associations. In *B*, the THC combination was identified using the two-factor approach presented in appendixes B and D but not by the THC approach. Letters in the equations refer to those in the THC cubes above them, which are the same as those explained in figure 1. The *P* values were calculated using the THC binomial test described in the main text.

APPENDIX B

Methods for the Costa Rica Case Study and Results of Two-Factor Analysis of the Costa Rica Data Set

Here we present the site and data collection details and phylogeny construction methods used for the THC analysis presented in the main text. We also present all methods and results for the two-factor analysis for inferring three-factor THC associations used for comparative purposes in the main text. As with the study presented in the main text, the aim of this analysis is to determine traits important for the environmental filtering of species in specific clades in specific habitats. To do this, we start with trait/habitat associations detected using conventional statistics (ANOVA; Mayfield et al. 2006) and then use phylogenetic analyses to identify trait/clade and habitat/clade associations. Using these significant associations, we look for sets of traits, habitats, and clades for which all three two-factor associations are significant. Tests of trait/clade and habitat/clade associations presented in this appendix all take the form of question (ii) in "The THC Method." By comparing the results from the two-factor analyses presented here with those obtained using the THC approach (in the main text), we can more clearly illustrate the importance of using a direct three-factor THC method for identifying THC combinations of environmental filtering importance.

Costa Rican Study Methods

Location and Plot Selection for THC and Two-Factor Analyses. Data used for this study are more thoroughly described by Mayfield and Daily (2005) and Mayfield et al. (2006). This study examines 58 plant communities from three forest and three deforested habitats on the Osa Peninsula of southern Costa Rica. Plant communities were surveyed from June to August 2001 and in February 2003. Sampled sites were divided among three common forest habitat types (forest understory, 1–2-year-old tree-fall gaps, and riverbanks through primary forest) and three common deforested habitat types (actively grazed cattle pasture, ungrazed road verges, and riverbanks through grazed pasture riverbanks) in a human-dominated heterogeneous landscape. The 58 communities include 12 sites each in understory, tree-fall gaps, pasture, and road verges; four sites in forest riverbanks; and six sites in pas-

ture riverbanks. All plots were 80-m^2 rectangular areas, divided into 20 noncontiguous 1 × 1-m quadrats spaced as uniformly as possible. Within each plot, we recorded the number and abundance of herbs, vines, and shrubs (woody species up to 5 m high) in each quadrat (see Mayfield and Daily 2005 for details). Forest riverbanks and pasture riverbanks were not included in this set of analyses because of the different sampling effort of these habitat types. Data from these habitats are included in the THC analysis presented in the main text.

Pollination and Dispersal Mechanisms Used in THC and Two-Factor Tests. Pollination and dispersal mechanisms were recorded for species identified to the genus or species level (of 551 total species, 365 were identified to species and an additional 123 were identified to genus; 488 total) based on information provided in appropriate floras (Croat 1978; Stevens et al. 2001; Weber et al. 2001). Pollination and dispersal information was not available for all species and genera. When specific pollination and dispersal information was not available, we coded as many species as possible as most likely to have the mechanisms, given their respective flower or fruit structures. Generally entomophilous pollination and endozoochory were given to those species with unknown but very general flower structures or fleshy fruit, respectively, as well as those known to be pollinated or dispersed by a wide range of species (for more details, see Mayfield et al. 2006). For all analyses in this study and the THC analyses in the main text, each species was limited to one pollination and one dispersal mechanism, as the THC method does not easily accommodate polymorphism.

Phylogeny Construction for THC and Two-Factor Analyses. The regional phylogeny we use for all analyses includes all 488 species (resolved to the family level) found in our 58 study communities and is based on Phylomatic's conservative backbone tree (Webb 2005; Webb and Donoghue 2005). Phylomatic's conservative tree is derived from the world angiosperm tree generated by Peter Stevens (Apweb tree; Missouri Botanical Garden; http://www.mobot.org/MOBOT/research/APweb/). This tree is resolved to the family level and contains all currently recognized angiosperm families. Phylomatic's conservative tree uses only branches from the Apweb tree with bootstrap *P* values greater than 80%. The Apweb angiosperm tree is a dynamic tree updated as phylogenetic relationships become available. Our results are based on the tree available in March 2005 (Phylomatic tree C20040402).

Two-Factor Associations for Comparison to THC Results. Associations between individual pollination and dispersal mechanisms and each habitat type (TH) were determined in a previous publication (Mayfield et al. 2006). We use a subset of results from that study in combination with new phylogenetic analyses aimed at identifying HC and TC associations. Based on these three analyses, we compile a list of all trait, habitat, and clade combinations for which there are significant TH, TC, and HC associations (app. D). The full methods used to identify significant TH associations are provided in Mayfield et al. (2006). Briefly, two-way ANOVAs were run with habitat type as the fixed factor and the proportion of species with each mechanism per plot as the dependent factor. Tukey HSD tests were used to identify differences between the habitat types for each dispersal and pollination mechanism (SAS Institute 2003). From all significant results reported in Mayfield et al. (2006), we selected five pollination mechanisms and eight dispersal mechanisms that were significantly more common in one or two of the habitat types than in any of the others to test for trait-clade associations. For trait-clade analyses, we selected pollination mechanisms (and associated habitat type[s]) mediated by Diptera (pasture), general entomophily (pasture), wasps (road verges), beetles (understory), and weevils (understory). Dispersal traits were birds (understory, tree-fall gaps), exozoochory (pasture), general endozoochory (understory), gravity (pasture, road verges), and propulsion (pasture, road verges).

To determine which clades in our regional phylogeny were clustered (significantly more related than expected and thus "associated") in communities from one of the six habitat types (HC), we used the "nodesig" module of Phylocom (Webb et al. 2008). This program tests for overabundance of terminal taxa distal to each node in each community compared with 999 random communities drawn from the regional phylogenetic tree (fig. 2). This program provides a list of nodes that are significantly (*P* value <.05) over- and underdispersed in each community. Using data generated by Phylocom "nodesig," we mapped the number of communities for each habitat type (except riverbanks) that were significantly clustered or overdispersed for each clade (all species terminal to a given node) in our regional phylogeny.

To determine whether the 13 selected traits are associated with specific clades in the regional phylogeny (TC), we used the Phylocom "AOT" module (Webb et al. 2008). "AOT" examines the presence and absence of each trait within each clade of the regional phylogeny across all habitat types combined. The real distribution of traits is then compared with the average of 999 null distributions of a null trait across the phylogeny.

We mapped results for a selection of our 13 traits onto regional phylogenies already marked for species clustering by habitat type (fig. B1). Full results for these analyses are presented in table B1 and figure B1.

Table B1: Pollination mechanism results of Phylocom analysis of trait-clade and clade-habitat associations

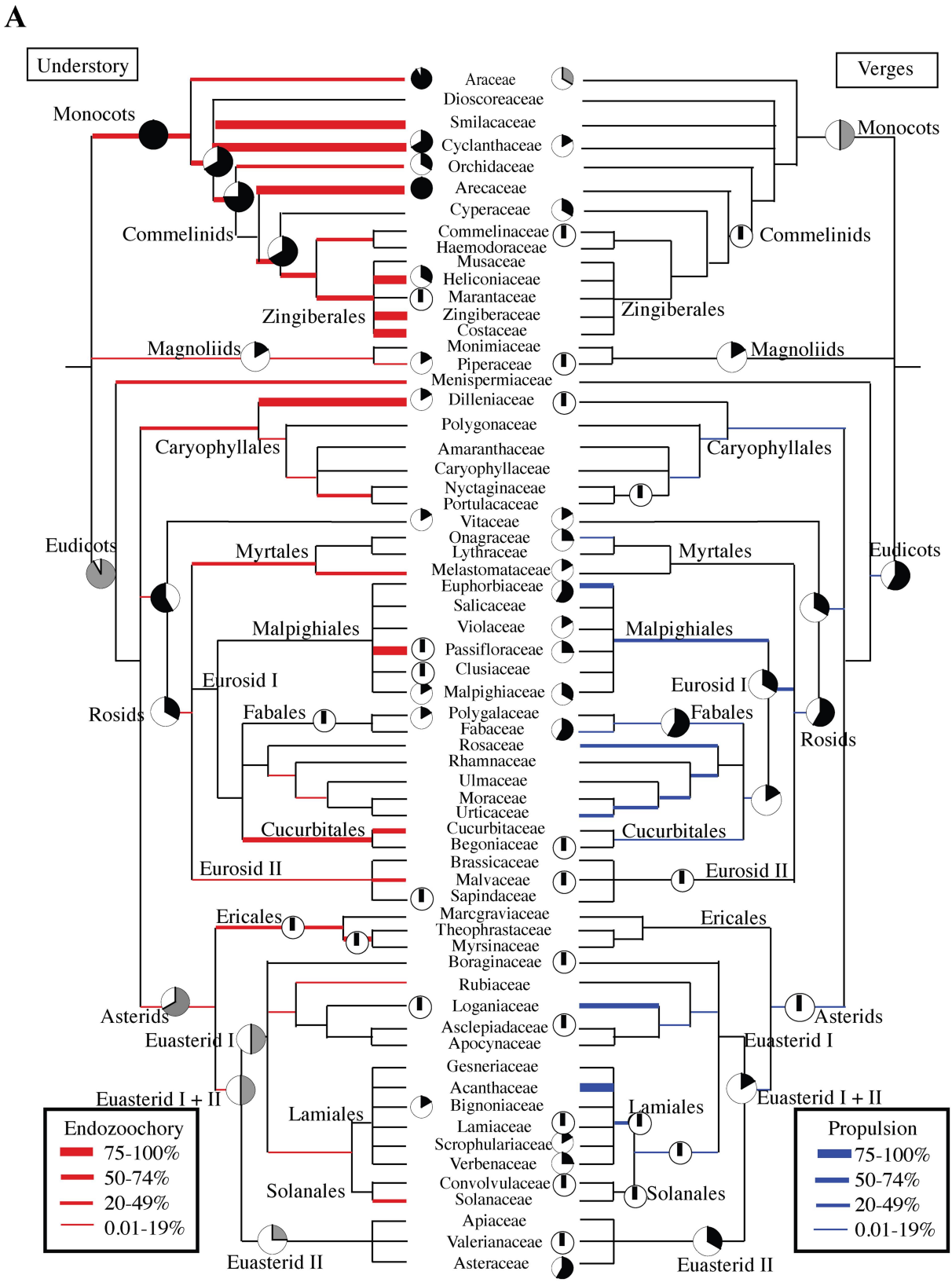
		Pasture			Verge		Understory		
Clade	Total spp.	% spp.	Diptera (%)	Entomo. (%)	% spp.	Wasps (%)	% spp.	Beetle (%)	Weevil (%)
Percent in common			25	100		14		29	43
Monocots	127						10.2	21.3	3.9
Monocot-Araceae	71						6.2		7.0
Araceae	56						12.2	48.2	
Cyclanthaceae	5				8.3		16.7		100
Magnoliids	38	.90		2.6	2.9		2.2		
Eudicots	321	2.4	2.5	23.4	5.1	1.6			
Menispermiaceae	5	1.7		100					
Portulacaceae + Nyctaginaceae	3	2.8		100					
Ameranthaceae + Caryophyllaceae + Portullaccaceae + Nyctaginaceae	11	1.5		36.4	4.2				
Rosids + Vitales	147	1.1	5.4	19.0	3.9	3.4			
Vitaceae	5				6.7	100	3.3		
Rosids	142	1.1	5.6	19.7	5.1				
Eurosid I	82	1.6	9.8	15.9	4.8				
Malpighiales	32	.70	25.0	9.4	6.0				
Euphorbiaceae	15	6.6	53.3	13.3	16.1				
Fabales + Rosales + Cucurbitales	50	.83		20.0	2.5				
Cucurbitaceae	8	1.0		75.0					
Fabales	26	3.5		11.5	8.3		.96		
Fabaceae	24	3.8		8.3	9.0				
Eurosid II	27	2.5		55.6	1.5				
Malvaceae	18	4.6		83.3	2.3				
Asterids	155	1.6		22.6	2.3				
Euasterid 1 + 2	151	1.6		21.9	1.5				
Rubiaceae	35	1.7		51.4					
Lamiales + Solanales	53	.94		22.6	1.1				
Lamiales	37	1.4		32.4	.90				
Verbenaceae	6	6.9		100	8.3				
Euasterids II	40	2.9		5.0	6.7				
Asteraceae	37	.90		2.7	10.6				

Note: Clustering of pollination mechanisms that are most common in pasture, verge, or understory. Percent in common = percent of clades that are both clustered in the indicated habitat type and were found to have the indicated trait significantly clustered in them. Total spp. = number of species in the clade listed to the left; % spp. = the mean percentage of species from the listed clade that are clustered for the given habitat type. The numbers listed in the column for each pollination mechanism are the mean percent of species (averaged across replicate sites) from the listed clade with that pollination mechanism (Phylocom "AOT" results). The clades listed are only those found to have significant clusters of one or more of these pollination mechanisms. Entomo. = generally entomophilic. The low percentages of species clustered in each habitat are due to averaging across all 12 plots from each habitat regardless of whether plots were clustered at that clade.

Table B2: Dispersal mechanism results of Phylocom analysis of trait-clade and clade-habitat associations

		Pasture				Verge			Understory			Gaps	
Clades	Total spp.	% spp.	Exoz. (%)	Propul. (%)	Gravity (%)	% spp.	Propul. (%)	Gravity (%)	% spp.	Bird (%)	Endoz. (%)	% spp.	Bird (%)
Percent in common			67	56	52		48	52		61	71 (1)		78 (11)
Monocots	127								10.2	27.2	54.3	10.4	26.0
Monocots-Araceae	71				4.2				6.2	14.1	64.8	4.0	14.1
Araceae	56								12.2	41.1	41.1	10.3	41.1
Cyclanthaceae	5					8.3			16.7		100		
Asperigales, Commelinids	61								6.7	16.4	60.7	4.2	16.4
Orchidaceae	5								6.7		2.0	3.3	
Commelinids	56								5.8	17.9	64.3	4.17	17.9
Arecaceae	13								24.3		100	7.1	
Poales + Commelinales + Zingiberales	43					1.7		7.0					23.3
Zingiberales + Commelinales	36												27.8
Commelinaceae	3					2.8		100					
Zingiberales	32												31.2
Heliconiaceae	12	3.5							5.6		100		
Marantaceae	9								1.9	100		1.9	100
Magnoliids	38					2.9			2.2	2.6	7.9	2.6	2.6
Piperaceae	37	10.8				1.6			2.25			2.7	8.1
Eudicots	321	2.4	9.3	10.3	4.4	5.2	10.3	4.4					27.4
Menispermiaceae	5	1.7											80.0
Dilleniaceae	2					4.2					100	4.2	
Amaranthaceae + Caryophyllaceae + Portullacacae + Nyctaginaceae	11	1.5	9.1	9.1					8.3				
Portulacaceae + Nyctaginaceae	3	2.8	33.3			2.8							
Rosids + Vitales	147	1.1	12.9	12.9	3.4	3.9	12.9	3.4	3.9			.74	28.6
Vitaceae	5					6.7			3.3	100		1.7	100
Rosids	142	1.1	13.4	13.4	3.5	5.1	13.4	3.5				.7	26.1
Myrtales	33												45.5
Onagraceae	6	2.8		16.7		11.1	16.7						
Melastomataceae	27												55.6
Eurosid I	82	1.6	8.5	22.0	6.1	4.8	22.0	6.1		17.1			17.1
Malpighiales	32	.78		31.2	6.2	6.0	31.2	6.2					21.9
Euphorbiaceae	15	6.7		66.7	6.7	16.1	66.7	6.7					
Passifloraceae	5	1.7				10.0			1.7		100	10.0	
Clusiaceae	5								1.7	100		3.3	100
Fabales + Rosales + Cucurbitales	50	.83	14.0	16.0	6.0	2.5	16.0	6.0	.96				
Fabales	26	3.5	26.9	15.4		8.3	15.4			19.2		1.0	19.2
Fabaceae	24	3.8	29.2	16.7		9.0	16.7						20.8
Eurosid II	27	2.5	44.4		2.5	1.5		2.5					
Malvaceae	18	4.6	66.7			2.3							
Sapindaceae	7											7.14	100
Asterid	155	1.6	6.5	8.4	5.8	2.3	8.4	5.8					
Ericales	4								2.1	25	25.0	6.25	25.0
Theophrasaceae, Myrsinaceae	2								4.1	50	50.0	8.3	50.0
Euasterid 1 + 2	151	1.6	6.6	8.6	6.0	1.5	8.6	6.0				.64	
Gentianales	52												46.2
Rubiaceae	35	1.7			17.1								
Loganiaceae	6								1.4		50.0		
Lamiales + Solanales	53	.94	11.3	18.9	3.8	1.1	18.9	3.8					
Lamiales	37	1.3	16.2	27.0	5.4	.90	27.0	5.4					
Gesneriaceae	7											2.4	85.7
Lamiaceae	6	11.1	100			2.8							
Schrophulariaceae	2	4.2			100	8.3		100					
Euasterids II	40	2.0	10.0			6.7							
Asteraceae	37	.90	10.8			10.6							

Note: Clustering of dispersal mechanisms that are most common in pasture, verge, understory, and tree-fall gaps, respectively. All table details are as described for table B1, except for dispersal rather than for pollination traits. Exoz. = exozoochory, Propul. = propulsion, and Endoz. = endozoochory.



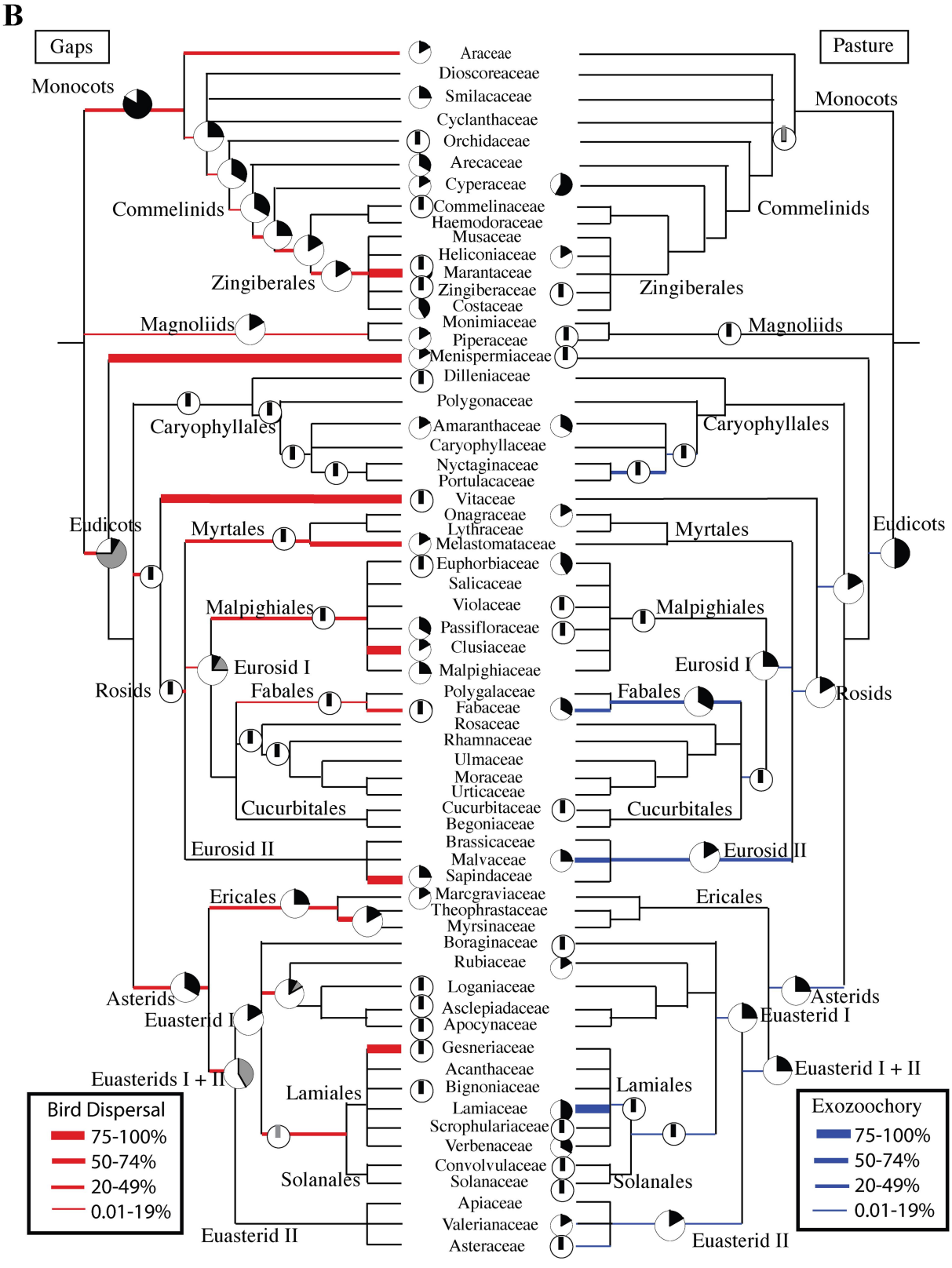


Figure B1: Selected results from Phylocom analyses of clade/habitat associations and trait/clade associations. *A*, Distribution of clades significantly clustered in understory and verge sites and for which clades endozoochory and propulsion are significantly clustered in regardless of habitat. *B* shows which clades are significantly clustered in tree-fall gaps (Gap) and pastures and in which clades bird dispersal and exozoochory are clustered across all habitat types. Pie charts are divided into 12 pieces, one for each community (site) sampled for the indicated habitat type. The number of blackened pie pieces indicates the number of sites for which the clade-habitat association was significant (e.g., the pie charts next to Araceae for understory indicate that 11 of 12 understory sites are significantly clustered for that node). Gray portions of each pie chart are the number of plots phylogenetically underrepresented for the given node (a negative "nodesig" result). Colored lines indicate nodes with trait clustering across all habitats. Line thickness indicates the mean percent of species in the clade with the indicated trait.

Results

"Nodesig" (HC). Understory communities were found to have significant clustering at deep and terminal nodes of the monocots, indicating significant associations in this branch of the regional phylogeny (fig. B1*A*). Tree-fall gap communities also showed strong significant clustering in the monocots but less consistently (fewer plots clustered at the same clades) than in understory communities (fig. B1*B*). There were multiple deep clades in the Eudicots that were significantly clustered in road verge communities, with particularly strong associations in the Rosids and Asterids (fig. B1*A*). A few road verge communities were found to have significant clusters of monocot clades; the extent and frequency of monocot clustering was much reduced from that seen in the forest communities (fig. B1*A*). Pasture communities showed similar clustering patterns to those observed in road verge communities, but patterns were weaker and terminal clustering was more disperse. Significant clustering was most prevalent in the deep clades of the Eudicots, most prominently in the Asterids. There was almost no clustering in the monocots for pasture communities with the exception of strong and frequent clustering in the Cyperaceae (sedges; fig. B1*B*).

"AOT" (TC). Phylogenetic clustering patterns varied substantially by trait (fig. B1; table B1). Significant phylogenetic associations with endozoochory, propulsion, bird dispersal, and exozoochory are shown in figure B1. Others are summarized in table B1. As with clade-habitat associations identified with the "nodesig" analyses, there were similarities between which traits and clades clustered in road verges and pastures and which clustered in understory and gaps (fig. B1; table B1).

APPENDIX C

One Hundred Significant THC Associations

Table C1: The 100 significant THC associations identified using the THC method

Habitat	Clade	Trait *f*	Habitat *f*	Clade *f*	Exp.	Obs.	THC *P*	Two-factor *P*	Status
Dispersal traits									
Endozoochory:									
Gap	6	.227 (111)	.434 (212)	.260 (127)	12.55	46	9.26E−14	2.89E−11	Weak
Gap	Araceae: Philodendron	.227 (111)	.434 (212)	.031 (15)	1.48	12	5.41E−08	None	Yes
Gap	8	.227 (111)	.434 (212)	.145 (71)	7.02	28	1.21E−09	2.89E−11	Weak
Gap	12	.227 (111)	.434 (212)	.125 (61)	6.03	21	1.25E−06	1.12E−07	No
Gap	14	.227 (111)	.434 (212)	.115 (56)	5.53	20	1.29E−06	4.13E−08	No
Understory[a]	6	.227 (111)	.223 (109)	.260 (127)	6.45	33	5.70E−14	2.89E−11[(TC)] 5.96E−07[(HC)]	Yes
Understory	Araceae: Philodendron	.227 (111)	.223 (109)	.031 (15)	.76	8	1.37E−06	None	Yes
Understory[a]	8	.227 (111)	.223 (109)	.145 (71)	3.61	22	3.69E−11	3.89E−10	Probable
Understory[a]	12	.227 (111)	.223 (109)	.125 (61)	3.1	19	7.35E−10	1.12E−07	Probable
Understory	14	.227 (111)	.223 (109)	.115 (56)	2.85	19	1.83E−10	4.13E−08	Probable
Understory	15	.227 (111)	.223 (109)	.027 (13)	.66	10	2.21E−09	None	Yes
Exozoochory:									
Pasture[a]	Malvaceae	.061 (30)	.256 (125)	.037 (18)	.28	6	5.50E−07	2.27E−09	No
Pasture	Malvaceae: Sida	.061 (30)	.256 (125)	.010 (5)	.08	4	1.49E−06	None	Yes
Verge	Malvaceae	.061 (30)	.434 (212)	.037 (18)	.48	7	7.46E−07	2.27E−09	No

Table C1 (*Continued*)

Habitat	Clade	Trait *f*	Habitat *f*	Clade *f*	Exp.	Obs.	THC *P*	Two-factor *P*	Status
Birds and bats:									
FR	3	.100 (49)	.250 (122)	.078 (38)	.95	13	3.13E−11	1.95E−21	No
FR	4	.100 (49)	.250 (122)	.076 (37)	.93	13	2.26E−11	8.64E−22	No
FR	Piperaceae: Piper	.100 (49)	.250 (122)	.068 (33)	.83	12	9.03E−11	2.79E−22	No
Gap	Araceae: Anthurium	.100 (49)	.434 (212)	.020 (10)	.44	8	2.10E−08	1.07E−07	No
Gap[a]	3	.100 (49)	.434 (212)	.078 (38)	1.66	15	2.68E−10	1.95E−21	No
Gap	4	.100 (49)	.434 (212)	.076 (37)	1.61	15	1.87E−10	8.64E−22	No
Gap	Piperaceae: Piper	.100 (49)	.434 (212)	.068 (33)	1.44	14	4.24E−10	2.79E−22	No
Pasture	3	.100 (49)	.256 (125)	.078 (38)	.98	9	8.81E−07	1.95E−21	No
Pasture	4	.100 (49)	.256 (125)	.076 (37)	.95	9	7.09E−07	8.64E−22	No
Understory[a]	3	.100 (49)	.223 (109)	.078 (38)	.85	18	2.94E−18	1.95E−21	Probable
Understory	4	.100 (49)	.223 (109)	.076 (37)	.83	18	1.86E−18	8.64E−22	Probable
Understory	Piperaceae: Piper	.100 (49)	.223 (109)	.068 (33)	.74	18	2.57E−19	2.79E−22	Probable
Verge	3	.100 (49)	.434 (212)	.078 (38)	1.66	17	2.52E−12	1.95E−21	No
Verge	4	.100 (49)	.434 (212)	.076 (37)	1.61	17	1.67E−12	8.64E−22	No
Verge	Piperaceae: Piper	.100 (49)	.434 (212)	.068 (33)	1.44	16	3.42E−12	2.79E−22	No
Gravity:									
Verge	Rubiaceae: Spermacoce	.035 (17)	.43 (212)	.008 (4)	.06	4	5.27E−07	None	Yes
Propulsion:									
FR	Acanthaceae	.068 (33)	.250 (122)	.020 (10)	.17	5	9.81E−07	2.75E−09	No
Pasture[a]	Euphorbiaceae	.068 (33)	.256 (125)	.031 (15)	.26	6	3.33E−07	1.18E−07	Weak
Verge[a]	Euphorbiaceae	.068 (33)	.434 (212)	.031 (15)	.44	8	2.27E−08	1.18E−07	No
Wind:									
Pasture	44	.162 (79)	.256 (125)	.318 (155)	6.43	22	9.30E−07	3.20E−11	No
Pasture	47	.162 (79)	.256 (125)	.309 (151)	6.26	22	6.09E−07	1.10E−11	No
Pasture	55	.162 (79)	.256 (125)	.082 (40)	1.66	16	2.71E−11	1.95E−15	Weak
Pasture	Asteraceae	.162 (79)	.256 (125)	.076 (37)	1.53	15	9.40E−11	7.47E−15	Weak
Verge	44	.162 (79)	.434 (212)	.318 (155)	10.9	36	8.11E−10	3.20E−11	No
Verge	47	.162 (79)	.434 (212)	.309 (151)	10.62	36	4.09E−10	1.10E−11	No
Verge	55	.162 (79)	.434 (212)	.082 (40)	2.81	25	4.49E−16	1.95E−15	Weak
Verge	Asteraceae	.162 (79)	.434 (212)	.076 (37)	2.6	24	7.82E−16	7.47E−15	Weak
General:									
FR	Urticaceae-Urera	.041 (20)	.250 (122)	.008 (4)	.04	4	1.12E−07	None	Yes
PR	17	.041 (20)	.176 (86)	.014 (7)	.05	4	2.58E−07	None	Yes
Pollination method									
Birds:									
Pasture	Heliconiaceae	.047 (23)	.256 (125)	.025 (12)	.14	5	4.62E−07	1.17E−12	No
Understory	8	.047 (23)	.223 (109)	.145 (71)	.75	8	1.19E−06	None	Yes
Understory	12	.047 (23)	.223 (109)	.125 (61)	.64	8	3.87E−07	None	Yes
Understory	14	.047 (23)	.223 (109)	.115 (56)	.59	8	2.04E−07	6.97E−07	Weak
Understory	16	.047 (23)	.223 (109)	.088 (43)	.45	8	2.78E−08	3.00E−08	Weak
Understory	18	.047 (23)	.223 (109)	.074 (36)	.38	8	7.16E−09	3.35E−09	Weak
Understory	19	.047 (23)	.223 (109)	.066 (32)	.34	8	2.89E−09	7.65E−10	Weak
Understory	Heliconiaceae	.047 (23)	.223 (109)	.025 (12)	.13	7	8.75E−11	1.17E−12	Weak
Beetles:									
FR	7	.055 (27)	.250 (122)	.115 (56)	.77	8	1.55E−06	4.77E−17	No
Gap[a]	7	.055 (27)	.434 (212)	.115 (56)	1.35	19	4.78E−16	4.77E−17	Weak

Table C1 (*Continued*)

Habitat	Clade	Trait f	Habitat f	Clade f	Exp.	Obs.	THC P	Two-factor P	Status
Gap[a]	6	.055 (27)	.434 (212)	.260 (127)	3.05	19	5.75E−10	5.26E−09	Weak
Gap	Araceae: Philodendron	.055 (27)	.434 (212)	.031 (15)	.36	12	6.35E−15	1.77E−14	Weak
Understory[a]	7	.055 (27)	.223 (109)	.115 (56)	.69	14	2.93E−14	4.77E−17	Weak
Understory[a]	6	.055 (27)	.223 (109)	.260 (127)	1.57	14	1.26E−09	5.26E−09[(TC)] 5.96E−07[(HC)]	Weak
Understory	Araceae: Philodendron	.055 (27)	.223 (109)	.031 (15)	.19	8	2.78E−11	1.77E−14	Weak
Weevils:									
FR	9	.010 (5)	.250 (122)	.010 (5)	.01	3	3.45E−07	2.76E−09	No
Gap	9	.010 (5)	.434 (212)	.010 (5)	.02	4	9.92E−09	2.76E−09	No
Understory[a]	9	.010 (5)	.223 (109)	.010 (5)	.01	3	2.46E−07	2.76E−09	No
Wasps:									
Verge	Vitaceae: Cissus	.010 (5)	.434 (212)	.008 (4)	.02	3	9.22E−07	1.12E−07	No
Wind:									
FR	38	.053 (26)	.250 (122)	.029 (14)	.19	6	4.84E−08	6.90E−09	Weak
FR	39	.053 (26)	.250 (122)	.027 (13)	.17	6	3.14E−08	3.45E−09	Weak
FR	40	.053 (26)	.250 (122)	.025 (12)	.16	6	1.96E−08	1.62E−09	Weak
FR	41	.053 (26)	.250 (122)	.023 (11)	.15	6	1.18E−08	1.25E−08	Weak
FR	Urticaceae	.053 (26)	.250 (122)	.020 (10)	.13	6	6.72E−09	5.53E−09	Weak
FR	Urticaceae: Urera	.053 (26)	.250 (122)	.008 (4)	.05	4	3.18E−07	None	Yes
PR	17	.053 (26)	.176 (86)	.014 (7)	.07	4	7.29E−07	1.38E−07	Weak
Verge	17	.053 (26)	.434 (212)	.014 (7)	.16	5	7.98E−07	1.38E−07	Weak
General (abiotic and entomophilous):									
FR	3	.105 (51)	.250 (122)	.078 (38)	.99	13	5.08E−11	7.21E−24	No
FR	4	.105 (51)	.250 (122)	.076 (37)	.97	13	3.68E−11	2.95E−24	No
FR	Piper	.105 (51)	.250 (122)	.068 (33)	.86	12	1.42E−10	9.23E−22	No
Gap	3	.105 (51)	.434 (212)	.078 (38)	1.73	16	4.78E−11	7.21E−24	No
Gap	4	.105 (51)	.434 (212)	.076 (37)	1.68	16	3.25E−11	2.95E−24	No
Gap	Piper	.105 (51)	.434 (212)	.068 (33)	1.5	14	7.03E−10	9.23E−22	No
Pasture	3	.105 (51)	.256 (125)	.078 (38)	1.02	10	1.21E−07	7.21E−24	No
Pasture	4	.105 (51)	.256 (125)	.076 (37)	.99	10	9.47E−08	2.95E−24	No
Understory	3	.105 (51)	.223 (109)	.078 (38)	.89	19	2.63E−19	7.21E−24	Probable
Understory	4	.105 (51)	.223 (109)	.076 (37)	.86	19	1.62E−19	2.95E−24	Probable
Understory	Piper	.105 (51)	.223 (109)	.068 (33)	.77	18	5.14E−19	9.23E−22	Probable
Verge	3	.105 (51)	.434 (212)	.078 (38)	1.73	18	4.32E−13	7.21E−24	No
Verge	4	.105 (51)	.434 (212)	.076 (37)	1.68	18	2.79E−13	2.95E−24	No
Verge	Piper	.105 (51)	.434 (212)	.068 (33)	1.5	16	6.15E−12	9.23E−22	No
Diptera:									
Pasture[a]	Euphorbiaceae	.016 (8)	.256 (125)	.031 (15)	.06	5	7.68E−09	2.53E−10	No
Pasture[a]	35	.016 (8)	.256 (125)	.066 (32)	.13	5	3.20E−20	8.50E−08	No
Verge	Euphorbiaceae	.016 (8)	.434 (212)	.031 (15)	.11	6	1.83E−09	2.53E−10	No
Verge	35	.016 (8)	.434 (212)	.066 (32)	.23	6	1.56E−07	8.50E−08	No
Entomophilous:									
Understory	Arecaceae	.260 (127)	.223 (109)	.027 (13)	.76	10	7.79E−09	None	Yes
Understory[a]	6	.260 (127)	.223 (109)	.260 (127)	7.38	25	2.06E−07	5.96E−07[(HC)]	Weak
Self:									
FR	55	.088 (43)	.250 (122)	.082 (40)	.88	10	3.24E−08	1.12E−26	No
FR	Asteraceae	.088 (43)	.250 (122)	.076 (37)	.82	9	1.98E−07	1.13E−25	No
Pasture	44	.088 (43)	.256 (125)	.318 (155)	3.5	18	2.89E−08	2.56E−08	Weak
Pasture	47	.088 (43)	.256 (125)	.309 (151)	3.41	18	1.96E−08	1.31E−08	Weak

Table C1 (*Continued*)

Habitat	Clade	Trait *f*	Habitat *f*	Clade *f*	Exp.	Obs.	THC *P*	Two-factor *P*	Status
Pasture	55	.088 (43)	.256 (125)	.082 (40)	.9	18	7.93E−18	1.12E−26	Weak
Pasture	Asteraceae	.088 (43)	.256 (125)	.076 (37)	.84	17	4.63E−17	1.13E−25	Weak
Verge	44	.088 (43)	.434 (212)	.318 (155)	5.93	28	2.98E−11	2.56E−08	Weak
Verge	47	.088 (43)	.434 (212)	.309 (151)	5.78	28	1.65E−11	1.31E−08	Weak
Verge	55	.088 (43)	.434 (212)	.082 (40)	1.53	28	5.60E−26	1.12E−26	Weak
Verge	Asteraceae	.088 (43)	.434 (212)	.076 (37)	1.42	27	1.46E−25	1.13E−25	Weak

Note: The trait associated with each habitat and clade is indicated at the top of each section. Headings: Habitat = the habitat in question; Clade = the clade code number corresponding to figure 2 or the name of the terminal clade. Trait *f*, habitat *f*, and clade *f* = frequencies of the trait, habitat, and clade, respectively, in the regional species pool, followed in parentheses by the total number of species with the trait, habitat, or clade, respectively. Exp. = expected number of species ($N \times f_T f_H f_C$); Obs. = observed number of species with the indicate THC trio (A/N; fig. 1); THC *P* = *P* value calculated using the exact binomial test (not corrected for multiple comparisons); Two-factor *P* = *P* value for two-factor associations that were also significant based on the THC-binomial test. All significant two-factor associations were between the trait and clade (TC) unless otherwise indicated in the cell. In the Status column, "Yes" indicates that the association had a $P < .05$ after binomial post hoc tests or had no significant two-factor associations, "Probable" had a $P < .1$ after post hoc tests, and "Weak" indicates that the association was significant before post hoc tests but had a $P > .1$ following this test.

[a] The THC association was also identified using the non-THC two-factor analyses (app. B).

APPENDIX D

Predicted THC Combinations

Table D1: Predicted THC combinations based on trait/habitat, trait/clade, and clade/habitat two-factor analyses presented in appendix B

Dispersal mechanisms			Pollination mechanisms		
Trait	Habitat	Clade	Trait	Habitat	Clade
Endozoochory	Understory	3	Beetles	Understory	6
Endozoochory	Understory	4	Beetles	Understory	7
Endozoochory	Understory	6	Beetles	Gaps	6
Endozoochory	Understory	7	Beetles	Gaps	7
Endozoochory	Understory	8	Diptera	Verges	21
Endozoochory	Understory	9	Diptera	Verges	23
Endozoochory	Understory	12	Diptera	Verges	29
Endozoochory	Understory	13	Diptera	Verges	31
Endozoochory	Understory	Heliconiaceae	Diptera	Verges	34
Endozoochory	Understory	25	Diptera	Verges	35
Endozoochory	Understory	29	Diptera	Verges	Euphorbiaceae
Endozoochory	Understory	31	Entomophilous	Understory	6
Exozoochory	Pasture	21	Entomophilous	Understory	7
Exozoochory	Pasture	29	Entomophilous	Understory	8
Exozoochory	Pasture	31	Entomophilous	Understory	12
Exozoochory	Pasture	37	Entomophilous	Understory	16
Exozoochory	Pasture	Fabaceae	Entomophilous	Pasture	3
Exozoochory	Pasture	34	Entomophilous	Pasture	21
Exozoochory	Pasture	43	Entomophilous	Pasture	22
Exozoochory	Pasture	Malvaceae	Entomophilous	Pasture	27
Exozoochory	Pasture	44	Entomophilous	Pasture	28
Exozoochory	Pasture	47	Entomophilous	Pasture	29
Exozoochory	Pasture	48	Entomophilous	Pasture	31
Exozoochory	Pasture	Lamiaceae	Entomophilous	Pasture	34
Exozoochory	Pasture	55	Entomophilous	Pasture	35
Birds and bats	Understory	3	Entomophilous	Pasture	Euphorbiaceae
Birds and bats	Understory	6	Entomophilous	Pasture	36
Birds and bats	Understory	7	Entomophilous	Pasture	37

Table D1 (*Continued*)

Dispersal mechanisms			Pollination mechanisms		
Trait	Habitat	Clade	Trait	Habitat	Clade
Birds and bats	Understory	8	Entomophilous	Pasture	Rosid
Birds and bats	Understory	12	Entomophilous	Pasture	Rosid
Birds and bats	Understory	14	Entomophilous	Pasture	43
Birds and bats	Understory	Marantaceae	Entomophilous	Pasture	Rosid
Birds and bats	Understory	30	Entomophilous	Pasture	44
Birds and bats	Understory	34	Entomophilous	Pasture	47
Birds and bats	Understory	Clusiaceae	Entomophilous	Pasture	Asterid
Birds and bats	Understory	37	Entomophilous	Pasture	52
Birds and bats	Understory	45	Entomophilous	Pasture	53
Birds and bats	Understory	46	Entomophilous	Pasture	Asterid
Birds and bats	Gaps	3	Entomophilous	Pasture	55
Birds and bats	Gaps	6	Entomophilous	Pasture	Asterid
Birds and bats	Gaps	7	Weevil	Understory	6
Birds and bats	Gaps	8	Weevil	Understory	8
Birds and bats	Gaps	12	Weevil	Understory	Mono
Birds and bats	Gaps	14	Weevil	Gaps	6
Birds and bats	Gaps	16	Weevil	Gaps	8
Birds and bats	Gaps	18			
Birds and bats	Gaps	19			
Birds and bats	Gaps	22			
Birds and bats	Gaps	30			
Birds and bats	Gaps	Melastomataceae			
Birds and bats	Gaps	Clusiaceae			
Birds and bats	Gaps	Sapindaceae			
Birds and bats	Gaps	44			
Birds and bats	Gaps	45			
Birds and bats	Gaps	46			
Birds and bats	Gaps	48			
Gravity	Pasture	8			
Gravity	Pasture	21			
Gravity	Pasture	29			
Gravity	Pasture	31			
Gravity	Pasture	34			
Gravity	Pasture	35			
Gravity	Pasture	Euphorbiaceae			
Gravity	Pasture	36			
Gravity	Pasture	43			
Gravity	Pasture	44			
Gravity	Pasture	47			
Gravity	Pasture	Rubiaceae			
Gravity	Pasture	52			
Gravity	Pasture	Scrophulariaceae			
Gravity	Verges	16			
Gravity	Verges	Commelinaceae			
Gravity	Verges	21			
Gravity	Verges	29			
Gravity	Verges	31			
Gravity	Verges	34			
Gravity	Verges	35			
Gravity	Verges	Euphorbiaceae			
Gravity	Verges	36			
Gravity	Verges	43			
Gravity	Verges	44			
Gravity	Verges	47			

Table D1 (*Continued*)

Dispersal mechanisms			Pollination mechanisms		
Trait	Habitat	Clade	Trait	Habitat	Clade
Gravity	Verges	52			
Gravity	Verges	53			
Gravity	Verges	Scrophulariaceae			
Propulsion	Pasture	21			
Propulsion	Pasture	23			
Propulsion	Pasture	31			
Propulsion	Pasture	Euphorbiaceae			
Propulsion	Pasture	34			
Propulsion	Pasture	35			
Propulsion	Pasture	Onagraceae			
Propulsion	Pasture	36			
Propulsion	Pasture	37			
Propulsion	Pasture	Fabaceae			
Propulsion	Pasture	44			
Propulsion	Pasture	47			
Propulsion	Pasture	52			
Propulsion	Pasture	53			
Propulsion	Verges	21			
Propulsion	Verges	29			
Propulsion	Verges	31			
Propulsion	Verges	34			
Propulsion	Verges	Euphorbiaceae			
Propulsion	Verges	Onagraceae			
Propulsion	Verges	36			
Propulsion	Verges	37			
Propulsion	Verges	Fabaceae			
Propulsion	Pasture	27			

Note: Numbers and abbreviations listed under "Clade" correspond to those in figure 2.

Literature Cited

Belyea, L. R., and J. Lancaster. 1999. Assembly rules within a contingent ecology. Oikos 86:402–416.

Cavender-Bares, J., K. Kitajima, and F. A. Bazzaz. 2004. Multiple trait associations in relation to habitat differentiation among 17 oak species in north central Florida. Ecological Monographs 74:635–662.

Chazdon, R. L., S. Careaga, C. O. Webb, and O. Vargas. 2003. Community and phylogenetic structure of reproductive traits of woody species in wet tropical forests. Ecological Monographs 73:331–348.

Cohen, J. E. 1971. Estimation and interaction in a censored 2 × 2 × 2 contingency table. Biometrics 27:379–386.

Cornwell, W. K., D. W. Schwilk, and D. D. Ackerly. 2006. A trait-based test for habitat filtering: convex hull volume. Ecology 87: 1465–1471.

Croat, T. B. 1978. Flora of Barro Colorado Island. Stanford University Press, Stanford, CA.

Edwards, E. J., C. J. Still, and M. J. Donoghue. 2007. The relevance of phylogeny to studies of global change. Trends in Ecology & Evolution 22:243–249.

Garcia, L. V. 2004. Escaping the Bonferroni iron claw in ecological studies. Oikos 105:657–663.

Gotelli, N. J., and G. R. Graves. 1996. Null models in ecology. Smithsonian Books, Washington, DC.

Harvey, P. H. 1996. The eleventh Tansley Lecture: phylogenies for ecologists. Journal of Animal Ecology 65:255–263.

Holt, R. D., and R. Gomulkiewicz. 2004. Conservation implications of niche conservatism and evolution in heterogeneous environments. Pages 244–264 *in* R. Ferriere, U. Dieckmann, and D. Couvet, eds. Evolutionary conservation biology. Cambridge University Press, Cambridge.

Horner-Devine, M. C., J. M. Silver, M. A. Leibold, B. J. M. Bohannan, R. K. Colwell, J. A. Furhman, J. L. Green, et al. 2007. A comparison of taxon co-occurrence patterns for macro- and microorganisms. Ecology 88:1345–1353.

Kraft, N. J. B., W. K. Cornwell, C. O. Webb, and D. D. Ackerly. 2007. Trait evolution, community assembly, and the phylogenetic structure of ecological communities. American Naturalist 170:271–283.

Mayfield, M. M., and G. C. Daily. 2005. Countryside biogeography of Neotropical herbaceous and shrubby plants. Ecological Applications 15:423–439.

Mayfield, M. M., M. F. Boni, G. C. Daily, and D. Ackerly. 2005. Species and functional diversity of native and human-dominated plant communities. Ecology 86:2365–2372.

Mayfield, M. M., D. Ackerly, and G. C. Daily. 2006. The diversity and conservation of plant reproductive and dispersal functional traits in human-dominated tropical landscapes. Journal of Ecology 94:522–536.

Pagel, M., and A. Meade. 2006. Bayesian analysis of correlated evo-

lution of discrete characters by reversible-jump Markov chain Monte Carlo. American Naturalist 167:808–825

SAS Institute. 2003. JMP: the discovery software. Version 5.0.1.2. SAS Institute, Cary, NC.

Stevens, W. D., C. Ulloa Ulloa, A. Pool, and O. M. Montiel. 2001. Flora de Nicaragua. Monographs in Systematic Botany from the Missouri Botanical Garden. Vol. 85. Missouri Botanical Garden Press, St. Louis.

Swenson, N. G., B. J. Enquist, J. N. Thompson, and J. K. Zimmermann. 2007. The influence of spatial and size scale on phylogenetic relatedness in tropical forest communities. Ecology 88:1770–1780.

Webb, C. O. 2000. Exploring the phylogenetic structure of ecological communities: an example for rainforest trees. American Naturalist 156:145–155.

———. 2005. Phylomatic: tree assembly for applied phylogenetics. Molecular Ecology Notes 5:181–183.

Webb, C. O., and M. J. Donoghue. 2005. Phylomatic. Freeware: http://www.phylodiversity.net/phylomatic/phylomatic.html.

Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics 33:475–505.

Webb, C. O., D. D. Ackerly, and S. W. Kembel. 2008. Phylocom: software for the analysis of phylogenetic community structure and trait evolution. Bioinformatics 24:2098–2100.

Weber, A., W. Huber, A. Weissenhofer, N. Zamora, and G. Zimmermann. 2001. An introductory field guide to the flowering plants of Golfo Dulce rain forests of Costa Rica. U. of V. Institute of Botany, INBIO, and S. J. Museo Nacional de Costa Rica, eds. Biologiezentrum des Oberösterreichisches Landesmuseums, Linz, Austria.

Weiher, E., and P. Keddy. 1995. Assembly rules, null models, and trait dispersion: new questions from old patterns. Oikos 74:159–164.

Associate Editor: Susan Kalisz
Editor: Monica A. Geber