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Prevalence of phylogenetic clustering at multiple scales in an African rain forest tree community

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Summary

1. In highly diverse ecosystems, such as tropical forests, the relative importance of mechanisms underlying species coexistence (e.g. habitat filtering, competitive exclusion, neutral dynamics) is still poorly known and probably varies depending on spatial and phylogenetic scales.

2. Here, we develop new approaches for dissecting simultaneously the phylogenetic structure of communities at different phylogenetic depths and spatial scales. We tested with simulations that our method is able to disentangle overdispersion and clustering effects occurring at contrasted phylogenetic depths.

3. We applied our approaches to a 50 ha Forest Dynamic Plot located in Korup National Park (Cameroon) where 329,000 tree stems ≥ 1 cm in diameter were identified and mapped, and using a newly generated dated molecular phylogenetic tree based on 2 plastid loci (*rbcL* and *matK*), including 272 species from Korup (97% of the individuals).

4. Significant patterns of phylogenetic turnover were detected across 20 × 20 m^2 quadrats at most spatial scales, with higher turnover between topographic habitats than within habitats, indicating the prevalence of habitat filtering processes. Spatial phylogenetic clustering was detected over the entire range of phylogenetic depths indicating that competitive exclusion does not generate a pattern of phylogenetic overdispersion at this scale, even at a shallow phylogenetic depth.

5. Using an individual-based approach, we also show that closely related species tended to aggregate spatially until a scale of 1 m. However, the signal vanishes at smaller distance, suggesting that competitive exclusion can balance the impact of environmental filtering at a very fine spatial scale.

6. *Synthesis.* Using new methods to characterize the structure of communities across spatial and phylogenetic scales, we inferred the relative importance of the mechanisms underlying species coexistence in tropical forests. Our analysis confirms that environmental filtering processes are key in the structuring of natural communities at most spatial scales. Although negative-density tends to limit coexistence of closely related species at very short distance (<1 m), its influence is largely veiled by environmental filtering at larger distances.

Key-words: African rain forest, clustering, competition, determinants of plant community diversity and structure, overdispersion, phylogenetic depth, phylogenetic structure, rain forest, spatial scale, trees

Introduction

Identifying causal mechanisms in the assembly of ecological communities is a challenging task, especially in species-rich communities. This should help improve our understanding of the origin of biodiversity and our ability to predict the response of ecosystems to ongoing global change. However, many processes have been identified as potential drivers of species coexistence (Hubbell 2001; Wright 2002; Ricklefs 2004) and their relative importance and the spatial and temporal scales at which they act are still hotly debated.

At the scale of co-occurring individuals, three processes are expected to play a role in the spatial distribution of plant species. First, limited dispersal should cause a spatial aggregation of conspecifics (Hubbell 2001). Second, according to the Janzen-Connell hypothesis (Janzen 1970; Connell 1971), negative density dependence leads to lower growth and survival of individuals if surrounded by conspecifics, or related

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species, due to shared natural enemies. This phenomenon has been evidenced at the species level (Wright 2002; Bagchi *et al.* 2010), but also beyond the species level (Webb, Gilbert & Donoghue 2006; Gonzalez *et al.* 2010; Liu *et al.* 2012; Paine *et al.* 2012). Third, environmental filtering should promote the coexistence of species that display similar ecological strategies (due to phylogenetic inertia or convergent evolution). However, interspecific competition has also been seen as a major driver of species diversification, such that the coexistence of species that diverge in ecological strategy (limiting similarity) should be promoted. These two niche-based processes might simultaneously contribute to the maintenance of the high tree species diversity in rain forests (Kraft, Valencia & Ackerly 2008; Swenson & Enquist 2009).

Functional traits are now commonly used as a proxy of the ecological niche of species (Kraft, Valencia & Ackerly 2008). Yet, this strategy is challenging in diverse communities given the high costs associated with trait measurement (Swenson 2013), and the difficulties to select and measure the most relevant traits (Baraloto *et al.* 2010, 2012). At broad taxonomic scales, many ecological functions are expected to be more similar in closely allied species because of phylogenetic inertia. The relative positions of species in a phylogeny should thus convey indirect information about trait similarity as recently shown by Baraloto *et al.* (2012) and Lebrija-Trejos *et al.* (2013) in tropical tree communities (see also Burns & Strauss 2011). Hence, phylogenetic distance has been usually used as a surrogate for niche overlap (e.g. Webb *et al.* 2002) and over the past decade inferring assembly processes from the phylogenetic structure of communities has been the topic of an active research program (Webb *et al.* 2002; Cavender-Bares *et al.* 2009; Mouquet *et al.* 2012; Swenson 2013).

While this research programme sounds appealing and has indeed motivated a great deal of recent research, phylogenetic patterns may be difficult to interpret. One problem is that opposite processes may cancel each other out: if species traits are phylogenetically conserved, environmental filtering favours phenotypic and phylogenetic clustering within habitat patches, leading to phylogenetic turnover between patches subject to different environmental filters (Graham & Fine 2008; Parmentier & Hardy 2009; Hardy *et al.* 2012). Spatial phylogenetic clustering is also expected if either mutualism or facilitation occur in closely related species (Vamosi *et al.* 2009), although the pattern does not necessarily depends on habitat heterogeneity in this case. On the other hand, negative density dependence may result in spatial phylogenetic overdispersion (Webb *et al.* 2002; Gilbert & Webb 2007; Hardy & Senterre 2007; Paine *et al.* 2012). Lastly, under neutral species assembly, the expected pattern is a random phylogenetic structure, at least at scales where biogeographic effects are negligible (Hardy 2008). If such processes occur simultaneously but at contrasting phylogenetic depths or at different spatial scales, we expect that their effect could be distinguished by decomposing community patterns at different spatial and phylogenetic scales. This is the key objective of the present study.

Previous studies have already been able to detect an increase in phylogenetic overdispersion as the spatial scale decrease, confirming that biotic interactions increase at smaller spatial scales in tree communities (Cavender-Bares, Keen & Miles 2006; Kembel & Hubbell 2006; Swenson *et al.* 2006). However, scaling the phylogenetic structure and dynamics of tree communities from its locally biotic environment to the whole community level (i.e. plot level) is far more challenging (Uriarte *et al.* 2010). Another important point is that the influence of phylogenetic depth on the phylogenetic structure of communities has also been seldom addressed. Swenson *et al.* (2006) found that phylogenetic overdispersion increased from the Euasterid I clade to the Rubiaceae clade (see also Cavender-Bares, Keen & Miles 2006 for a similar approach). Even if based on only few lineages (a single family in Swenson *et al.*'s 2006 work), such results are of considerable interest as they suggest stronger biotic interactions between closely related species. Hence, ecological processes may simultaneously lead to the spatial phylogenetic clustering of species at large spatial and taxonomic scales and to an overdispersion at small spatial scale and shallow phylogenetic depth, a regime called the 'Darwin-Hutchinson' zone by Vamosi *et al.* (2009).

In this study, new methods to detect patterns at different spatial and phylogenetic scales were developed. To test these methods, we analyse the simultaneous influence of the spatial and phylogenetic scales on the phylogenetic structure of a tropical tree community. We used a Forest Dynamic Plot (FDP) of 50 ha where all trees $\geq$ 1 cm in diameter were mapped at Korup, Cameroon (Chuyong *et al.* 2004a). We generated a resolved phylogeny constructed from two coding plastid DNA sequences (*rbcL* and *matK*) from a subset of species representing 97% of the 329,000 individuals. Here we address the following questions: (i) Do ecological processes leave a detectable signature in the phylogenetic structure of communities? (ii) How does phylogenetic turnover depend on the phylogenetic depth considered (recent versus ancient diversifications)? (iii) Is a spatial phylogenetical overdispersion of communities observed at small spatial scales and at shallow phylogenetic depth? Finally, Kress *et al.* (2009) and Pei *et al.* (2011) have recently argued that phylogenetic community inference depends critically on a good resolution of the phylogenetic tree, while many previous plant community phylogenetic studies relied on phylogenetic supertrees poorly resolved below the family level. Hence, we ask (iv) does the use of a family-level supertree affect our conclusions?

Materials and methods

STUDY SITES AND HABITAT CLASSIFICATION

The study site is a 50 ha FDP located in a lowland evergreen forest of the Korup National Park (05° 04′ N - 08° 51′ E) in southwestern Cameroon and lies within the Guineo-Congolian forest of tropical Africa (White 1983; Chuyong *et al.* 2011). The FDP is managed by the Korup Forest Dynamics Plot Programme (KFDP), affiliated with the Centre for Tropical Forest Science of the Smithsonian Tropical

Research Institute. This lowland tropical rain forest stands on highly weathered syenite and other igneous rocks; because of the high rainfall they are skeletal and sandy at the surface, highly leached, and poor in nutrients (Newbery, Songwe & Chuyong 1998; Chuyong, Newbery & Songwe 2002). Climate at Korup is equatorial, with mean annual rainfall around 5000 mm and mean annual temperature of 30.6 °C (Chuyong, Newbery & Songwe 2004b). Elevation varies between 150 m and 240 m asl. Five topographic habitats, mapped at a 20-m resolution, were considered within the FDP following the classification of Chuyong *et al.* (2011): valley (V; elevation < 165 m, slope < 15° and convexity < 0), low-slope (LS; elevation < 165 m, slope < 15° and convexity ≥ 0), high-gully (HG; elevation ≥ 165 m, slope ≥ 15° and convexity < 0), ridge-top (RT; elevation ≥ 165 m, slope < 15°) and high-slope (HS; elevation ≥ 165 m, slope ≥ 15° and convexity ≥ 0). About 330,000 tree stems ≥1 cm diameter at 1.3 m height have been identified and mapped within the 50 ha FDP (Thomas *et al.* 2003). Because habitat data were available for each 20 × 20 m^2 quadrat, community data analyses were partly performed using the 1250 quadrats as local community units.

TISSUE SAMPLING, DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Tissue samples were collected within the Korup FDP plot. Material for DNA extraction consisted of 5–50 cm^2 of leaf tissue immediately dried in silica-gel. These samples were included in the African rain forest tree DNA samples collection of the Université Libre de Bruxelles. Around 494 tree species occur in the FDP, including many rare species which are likely to be inaccurately identified (Kenfack *et al.* 2007). We sampled all species represented by more than 50 trees with a diameter at breast height >1 cm according to a survey done in 1996. These 272 species represented 97% of the trees in the plot and belonged to 159 genera and 51 families. We collected leaf material from 3 to 4 individuals per species, along with voucher specimens deposited at the Missouri Botanical Garden (MO), the Herbarium of the Université Libre de Bruxelles (BRLU) and at the KFDP base camp in Mundemba (Cameroon). A total of 726 individual trees were sampled.

DNA barcodes (*rbcL* and *matK*) were generated for 1–4 individuals of 262 species (see Parmentier *et al.* 2013). All sequences have been deposited in the Barcode of Life Data Systems (BOLD; Ratnasingham & Hebert 2007) and are available on GenBank (Table S1). If several sequences were obtained for the same barcode and the same species, the consensus sequence of all individuals was used in the phylogenetic reconstruction. Degenerate base codes were used for sites showing intra-species polymorphism; this concerned only 2% and 5% of the species for *rbcL* and *matK*, respectively (Parmentier *et al.* 2013).

PHYLOGENETIC RECONSTRUCTION

A species-resolved phylogeny was constructed from the *rbcL* and *matK* sequences. Additional sequences were retrieved from GenBank and added to the DNA matrices to improve the fossil calibration step (Table S2). Sequences were aligned independently for the two markers, using MUSCLE (Edgar 2004) as implemented in the MEGA 5.05 software (Tamura *et al.* 2011). Alignments were checked and manually edited. The final matrix contained 346 sequences and 1441 bp (*rbcL*: 727 bp and *matK*: 709 bp) (see Table S1).

A preliminary tree was constructed using RAxML v7.2.8 (Stamatakis, Hoover & Rougemont 2008) and it was dated using r8s v1.71 (Sanderson 2003). This dated tree was then used as an input in a Bayesian phylogenetic reconstruction by BEAST v1.7.2 (Drummond & Rambaut 2007). Full details on the phylogenetic reconstruction are provided in Appendix S1. This tree was subsequently completed with polytomies for 10 species with minimum 50 trees in the FDP for which we had been unable to obtain DNA sequences, but which had congeneric species. This tree is available on request from the corresponding author.

Additionally, to evaluate the gain of power provided by our newly produced tree, a tree resolved to the family level was constructed by pruning a previously published phylogenetic tree including all angiosperm families (Davies *et al.* 2004) using the program Phylomatic (Webb & Donoghue 2005). Within families, genera and species were positioned as polytomies with node ages equal to 2/3 and 1/3 the age of the family, respectively.

PHYLOGENETIC TURNOVER AT THE SCALE OF 20 × 20 M^2 QUADRATS

We used the 1996 census data of the Korup FDP to quantify the phylogenetic structure of tree assemblages within and among habitats. Phylogenetic patterns in the assemblage were tested using the Π_{ST} metric of Hardy & Senterre (2007), as implemented in the software SPACODI (Hardy 2010). Π_{ST} measures phylogenetic turnover independently of taxonomic turnover by comparing the mean phylogenetic distance between species found in different sites compared with species found within sites. Thus it is a measure of relative β-phylodiversity. Its calculation requires a species per quadrat presence/absence matrix (Hardy & Senterre 2007). Positive Π_{ST} values indicate spatial phylogenetic clustering (or positive phylogenetic turnover) and negative values spatial phylogenetic overdispersion. Thus, $\Pi_{ST} = 0$ indicates no phylogenetic structure in the assemblage. Π_{ST} is essentially unbiased by phylogenetic signals originating from the distribution of the species abundances in the plot (Hardy 2008), a clear advantage over previously proposed metrics (e.g. mean phylogenetic distance, Webb *et al.* 2002; Webb, Ackerly & Kembel 2008).

ΠST was computed for each pair (q, r) of 1250 quadrats (20 × 20 m) as:

$$\Pi_{ST}(q,r) = 1 - \frac{1}{2}\left[\frac{I(q,q)}{S_q(S_q-1)} + \frac{I(r,r)}{S_r(S_r-1)}\right] \times \left[\frac{I(q,r)}{S_qS_r - S_{qr}}\right]^{-1} \quad \text{eqn1}$$

where $I(q,r) = \sum_{s=1}^{S}\sum_{t=1}^{S}\delta_{st}p_{sq}p_{tr}$. Here S is the total number of species, S_q and S_r are the numbers of species in quadrat q and r, respectively, S_{qr} is the number of species shared by quadrats q and r, $p_{sq} = 1$ if species s is present in quadrat q, otherwise $p_{sq} = 0$, and δ_{st} is the divergence time between species s and t ($\delta_{st} = 0$ for $s = t$). Averaged values were then calculated for pairs of quadrats belonging to the same habitat (within habitats) or to different habitats (between habitats), and for different spatial distance classes between quadrats. The significance of the Π_{ST} values was tested by a phylogenetic tree randomization, permuting species among the tips of the tree (n = 200 permutations; Hardy 2008). To test the influence of the resolution of the phylogenetic tree on the phylogenetic structure results, the same analysis was run with the family-resolved tree (Davies phylogeny). The same analyses were also performed using an abundance-weighted version of Π_{ST}, the B_{ST} metric (Baraloto *et al.* 2012). As both metrics gave the same general patterns but higher power and less stochasticity were observed using the Π_{ST} metric, we only report the latter hereafter.

TESTING THE PHYLOGENETIC STRUCTURE AT DIFFERENT PHYLOGENETIC DEPTHS

To get insight on the phylogenetic structure at different phylogenetic depths, we investigated how the phylogenetic signal (Π_{ST}) varies if the information on older diversifications is altered. To this end, we transformed the original phylogenetic tree (Fig. 1a) to create artificial polytomies beyond defined time thresholds: 0, 1, 2, 5, 10, 20, 40, 60, 80, 100, 120 and 140 Ma (Fig. 1b). These truncated trees having lost the information on old speciation events but not on recent events, any phylogenetic signal due to diversification events before the time threshold is eliminated and the remaining signal corresponds to that produced by shallower phylogenetic depths.

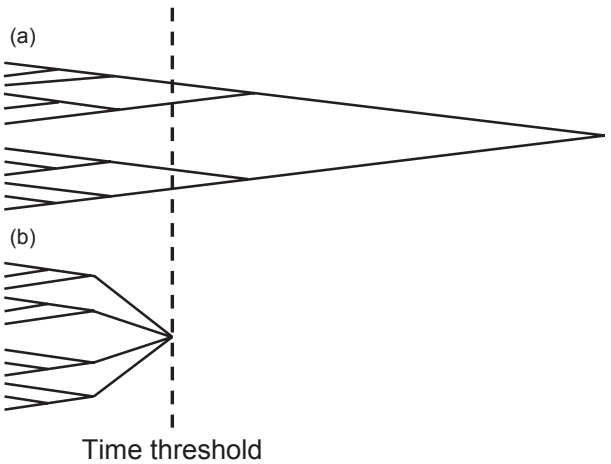

Fig. 1. Illustration of a tree transformation approach to study the phylogenetic structure at different phylogenetic depths. (a) Untransformed phylogenetic tree. (b) Phylogenetic tree turned into a star-shaped phylogeny beyond a time threshold. The transformed tree hides the influence of ancient diversification events.

To evaluate this approach, we compared the observed pattern with what could be expected in a simulated community subject to competitive exclusion between closely related species by artificially transforming the data set. Our data set contained 175 species pairs that diverged < 10 My ago according to the resolved phylogeny. We randomly selected 25%, 50%, 75% or 100% of these species pairs, and for each selected species pair considered in turn, we suppressed from the data set all individuals of the most abundant species located < 20 m from an individual of the less abundant species. Hence for the 100% case, no closely related species co-occurs at < 20 m in the whole 50-ha plot.

TESTING THE PHYLOGENETIC STRUCTURE AT SHORT SPATIAL DISTANCE

In order to detect phylogenetic structure at smaller spatial scales than 20×20 m^2 quadrats, we developed a novel method at the individual level, which analyses the spatial attraction or repulsion between species pairs (*A* and *B*; Fig. 2). For each individual of a focal species *A*, we computed the relative frequency of species *B* within a given spatial distance class (after excluding species A). We then calculated a measure of co-occurrence of *B* at a distance *c* surrounding *A* ($f_{A,B,c}$) as the difference between the frequency of species *B* at distance class *c* (first term of eq. 2) and the frequency of *B* in the plot (second term of eq. 2: number of individuals of species *B* divided by the total number of individuals in the plot except those belonging to species *A*):

$$f_{A,B,c} = \frac{\sum_{i,j} \varepsilon_{A,i}\varepsilon_{B,j}\omega_{i,j,c}}{\sum_{i,j} \varepsilon_{A,i}\omega_{i,j,c}(1-\varepsilon_{A,j})} - \frac{\sum_i \varepsilon_{B,i}}{\sum_i (1-\varepsilon_{A,i})} \qquad \text{eqn 2}$$

This formula has the following meaning: for individuals *i* and *j*, $\varepsilon_{A,i} = 1$ if individual *i* belongs to species *A* and $\varepsilon_{A,i} = 0$ otherwise; *c* is

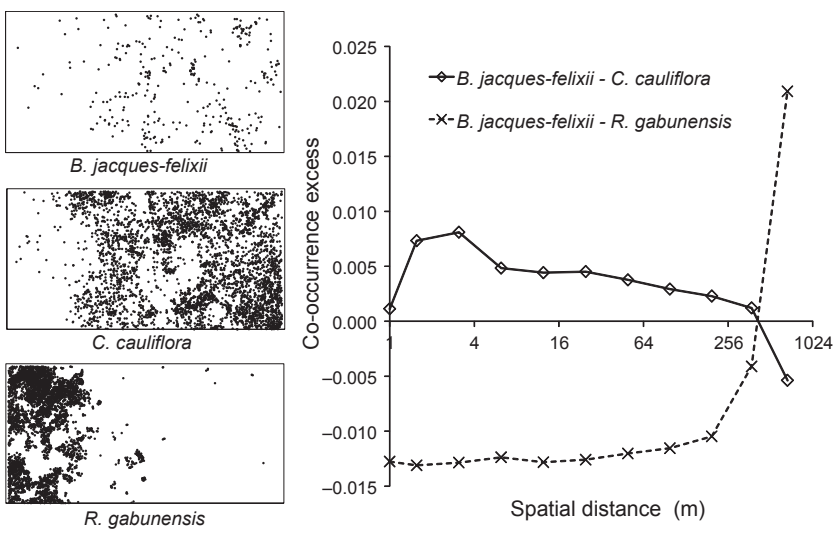

Fig. 2. Illustration of an individual-based approach to infer the relative spatial attraction or repulsion between two species pairs according to spatial distance. In this example, *Beilschmiedia jacques-felixii* and *Cola cauliflora* have similar broad-scale spatial distributions in the Korup 50 ha plot, contrasting with that of *Rinorea gabunensis* (left panels). The co-occurrence curves (right panel) display the excess (positive value) or deficit (negative value) of the relative frequency of *B. jacques-felixii* individuals among the individuals situated at a given distance interval from *C. cauliflora* or *R. gabunensis* individuals. The sign of the slope detects the presence of an attraction (−) or repulsion (+) between species. The distance of the steepest slope informs on the scale at which attraction or repulsion occurs the most (here at relatively large distances). Averaging these co-occurrence curves over pairs of species by classes of phylogenetic relatedness yields a community-wide spatial phylogenetic structure at all spatial scales (Fig. 5).

a distance interval; $\omega_{i,j,c} = 1$ if the distance between i and j is included in c, otherwise $\omega_{i,j,c} = 0$. The sums are taken over all individuals i and/or j from the plot. When the co-occurrence measure $f_{A,B,c} > 0$, species B tends to be more frequent among the individuals surrounding individuals of species A at a distance class c than expected at random. Conversely, $f_{A,B,c} < 0$ indicates that species B tend to be less frequent among the individuals surrounding individuals of species A. Thus, a curve of $f_{A,B,c}$ decaying with spatial distance indicates spatial attraction between A and B, while a curve increasing with spatial distance indicates repulsion. Note that the relative frequencies of species B are computed after excluding species A to ensure that the $f_{A,B,c}$ curves remain unaffected by the degree of spatial aggregation of species A. Such curves thus help explore patterns of species co-occurrence across spatial scales (Fig. 2).

Finally, we linked these spatial patterns of species co-occurrence with their phylogenetic relationships. Species pairs were classified according to their phylogenetic divergence time (<2 Ma, <10 Ma, <50 Ma, >100 Ma). The means of the $f_{A,B,c}$ across groups of species pairs were then plotted against spatial distance. The significance of the co-occurrence measures was tested by a phylogenetic tree randomization, permuting species among the tips of the tree ($n = 5000$ permutations).

All analyses were performed using the SPACODI 0.10 program (Hardy 2010) and using R version 3.0.1. (R Core Development Team 2013).

Results

IMPACTS OF SPATIAL DISTANCE AND TOPOGRAPHIC HABITATS ON THE PHYLOGENETIC TURNOVER

We found that Π_{ST} was positive on average (mean pairwise $\Pi_{ST} = 0.0013$; $P < 0.01$) indicating that species within a quadrat are phylogenetically more closely related than between quadrats (spatial phylogenetic clustering), demonstrating a positive phylogenetic turnover among habitats. Spatial phylogenetic clustering between pairs of quadrats increases regularly up to approximately 60 m, both within and among habitats (Fig. 3). At larger distances, Π_{ST} reaches a plateau within habitats but increases further among habitats; hence phylogenetic turnover among quadrats becomes more important between habitats than within habitats. Null model tests show that the phylogenetic clustering signal is statistically significant at all distances, except within habitats at large distances, which can be explained by the low number of pairs of quadrats limiting the testing power.

The same analyses conducted with a low-resolution tree, as often used in previous studies, gave similar results, with clustering within and among habitats at all distances (Fig. S1), and similar average Π_{ST} (0.0011).

PHYLOGENETIC TURNOVER AT SHALLOW PHYLOGENETIC DEPTH

Using truncated phylogenetic trees (Fig. 1), when the time threshold varied from 140 Ma (full phylogenetic signal) to 0.1 Ma (tree reduced to a single polytomy of species and with no phylogenetic signal), mean Π_{ST} consistently decreased to zero but remained significantly positive (phylogenetic clustering, Fig. 4), except when the threshold was ≤1 Ma.

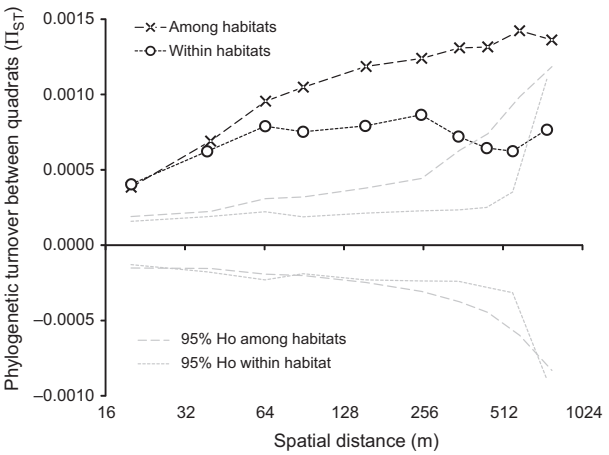

Fig. 3. Impact of spatial distance and topographically defined habitat types on phylogenetic turnover across 20 × 20 m^2 quadrats. The mean pairwise Π_{ST} between quadrats is plotted against the spatial distance within and among habitats. 95% Ho: confidence envelope under the null hypothesis of no phylogenetic structuring constructed by randomizing the species among the tips of the phylogenetic tree. See Fig. S1 for the same analysis with a family-resolved phylogenetic tree.

We also analysed transformed data sets simulating a community subject to competitive exclusion between closely related species at a local scale (20 m). The resulting Π_{ST} were lower and tended to display a minimum when the time threshold reached 10–40 Ma, at least when competitive exclusion was imposed on at least 50% of the pairs of closely related species (Fig. 4). In this scenario, Π_{ST} was negative when the phylogenetic tree was truncated below 60 Ma (null model tests significant for time thresholds ranging between 1 Ma

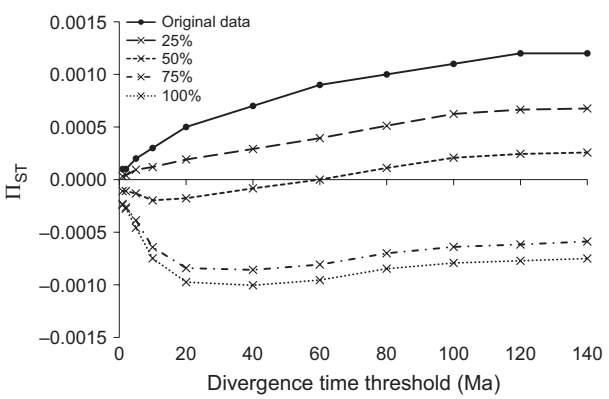

Fig. 4. Overall signal of phylogenetic turnover among quadrats (mean Π_{ST}, solid line) depending on phylogenetic depth (in Ma). To eliminate signal occurring above a given phylogenetic depth, the mean Π_{ST} is computed using truncated phylogenetic trees (see Fig. 1) for a range of divergence time thresholds. Simulated data help compare the observed pattern (i.e. original data) with a situation where competitive exclusion would have occurred between closely related species over a distance range of 20 meters (dashed lines) for 25% to 100% of the species pairs having diverged <10 Ma ago.

and 20 Ma, $P < 0.05$), and non-significantly positive when it was truncated above 60 Ma (Fig. 4).

PHYLOGENETIC STRUCTURE AT FINE SPATIAL SCALES: INDIVIDUAL-BASED ANALYSES

At the shortest distances (<1 m), the neighbouring scale, closely related species displayed a non significant spatial repulsion (Fig. 5). At intermediate distances (2–400 m), the average measure of co-occurrence decreased with spatial distance for closely related species pairs. This trend of attraction between phylogenetically related species was reinforced when divergence time between species was smaller. Conversely, the most unrelated species pairs (divergence time >100 Ma) showed an average trend of repulsion with slight, but significant, negative average measure of co-occurrence at short distances (<50 m).

Discussion

In this study, we found significant patterns of spatial phylogenetic clustering at most spatial scales, confirming that environmental filtering plays a crucial role in the phylogenetic structure of tropical tree communities. This interpretation is supported by the higher phylogenetic turnover found between habitats than within habitats. We also showed that the phylogenetic clustering signal at the quadrat scale (20 × 20 m²) built up progressively with phylogenetic depth. There is thus no evidence of a 'Darwin-Hutchinson' zone (Vamosi *et al.* 2009) where overdispersion would occur at a shallow phylogenetic depth at this spatial scale. Finally, we evidenced a slight but not significant overdispersion pattern at the smallest scale between close neighbours (<1 m). This trend of overdispersion was consistently stronger at shallower phylogenetic depth.

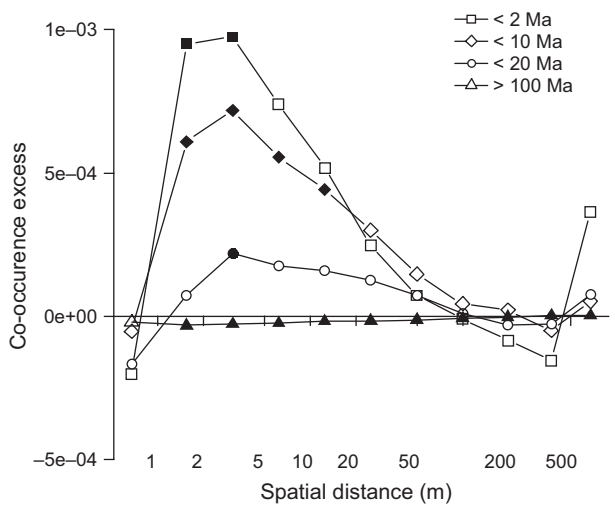

Fig. 5. Trends of spatial attraction (negative slope) or repulsion (positive slope) for species pairs according to their divergence time at different spatial distances. The mean species pair co-occurrence measure, indicating if species pairs are more (positive values) or less (negative values) frequent at a given distance around each other (Fig. 2), is plotted against spatial distance for species pairs that diverged <10, 20 or 50 Ma, or more than 100 Ma ago. Significance ($P < 0.05$) is illustrated by filled symbols and non-significance ($P \geq 0.05$) by open symbols (confidence envelopes for the four group of species are illustrated in Fig. S2).

PHYLOGENETIC TURNOVER WITHIN AND ACROSS HABITATS

If biogeographic effects can be ruled out (which is the case at the local scale of our study), testing the phylogenetic structure of plant assemblages can be interpreted as a neutrality test (Hardy & Senterre 2007). The significant phylogenetic structure observed in the tree assemblages within the 50 ha of the Korup FDP demonstrates that the tree assemblage is not neutrally assembled. We detected almost no phylogenetic overdispersion, except a slight trend at very small spatial scale (<1 m). The observed significant signal of phylogenetic clustering within quadrats was similar when comparing quadrats between habitats and within habitats at spatial distance <60 m, but more intense across habitats than within habitats beyond approximately 60 m. This could be attributed to mass effects (Shmida & Whittaker 1981) expected to dilute the relationship between species and environment at the boundary of habitats. The mass effect vanishes as the spatial distance increases between quadrats, due to dispersal limitation (Réjou-Méchain & Hardy 2011). At larger spatial scales, the higher phylogenetic turnover between habitats than within habitats indicates that habitat filtering is a major driver of the community phylogenetic structure, which is expected if ecological sorting of species is coupled to niche conservatism throughout the evolutionary history of the assemblage. It would be possible to further assess this claim by characterizing the habitat affinities of each species, measuring their functional traits and finally assessing whether these traits show phylogenetic signal (Baraloto *et al.* 2012).

Though phylogenetic turnover between habitats was found to be higher than within habitats at large distance, there was still a positive and significant turnover within habitat. This suggests that the classification of habitats based on topographic categories (Chuyong *et al.* 2011) captures only part of the environmental variability relevant to species filtering. For example, gap dynamics are expected to create strong environmental contrasts and could thus lead to a strong phylogenetic clustering even in similar habitats.

INFLUENCE OF PHYLOGENETIC TREE RESOLUTION FOR QUADRAT-BASED ANALYSES

We did not obtain conflicting results when analysing the phylogenetic structure with a tree inferred from DNA sequences generated for the purpose of this study, versus a family-resolved tree. This finding is in contrast to that of Kress *et al.* (2009) in a tropical FDP in Panama and to Pei *et al.* (2011) in a subtropical FDP in China. These authors based their results on the Net Relatedness Index (NRI) and the Nearest Taxon Index (NTI) rather than on our metric Π_{ST}. Applying

these metrics on our data set we found that the NRI or NTI values per quadrat were well correlated using both phylogenetic trees (Pearson's $r = 0.95$ and $r = 0.76$, respectively, Fig. S3), but the proportion of significant tests was higher using the barcode phylogeny for NTI (18.6% of quadrats versus 12.4% using the family-resolved phylogeny), though not for NRI (6.6% and 8.4% of quadrats displaying significant NRI using the barcode and family-resolved phylogenies, respectively). In yet another study Baraloto *et al.* (2012) used the Π_{ST} metric to analyse the phylogenetic structure of rain forest tree assemblages in French Guiana. They also obtained congruent results and similar Π_{ST} values with a species-resolved tree and with a family-level tree. Hence, the Π_{ST} metric might be more robust to the quality of the phylogenetic tree than the NRI or NTI metrics, an issue that would require further theoretical work.

PHYLOGENETIC STRUCTURE AT DIFFERENT PHYLOGENETIC DEPTHS AND AT SHORT DISTANCE

Using a quadrat-based analysis, we detected spatial phylogenetic clustering patterns irrespective of the spatial scale and the phylogenetic depth investigated. Interestingly, a significant portion of this phylogenetic turnover was contributed by niche differentiation among clades that had diverged long ago (see Fig. 4). When the tree was truncated to account only for diversification events more recent than a given threshold, the clustering signal persisted, and there was no sign of overdispersion between species at the tips of the phylogenetic tree.

Our individual-based analysis refined the spatial scale at which potential spatial repulsion patterns between phylogenetically related species are observable. Consistently with quadrat-based analyses, we observed a pattern of attraction between phylogenetically related individuals at most spatial scales (>1 m). This attraction decreased with an increase of species divergence time, confirming that closely related species contribute significantly to the overall phylogenetic clustering pattern. In a recent study conducted in a Panamanian forest (Barro Colorado Island), Lebrija-Trejos *et al.* (2013) consistently found that seedling survival tended to increase in the presence of closely related neighbors. In our study, the stronger attraction between closely related species was observed at relatively small distances (1–10 m) suggesting that microenvironment variations (e.g. nutrients or light variability), or facilitation processes, strongly shape species coexistence at very small spatial scales. However, this pattern of attraction disappeared at very short distance (<1 m) where we detected a non-significant tendency of spatial repulsion, which was stronger for phylogenetically closer species. Given that the average stem density is 0.66 m^{-2}, it means that an effect of repulsion between closely related species would only occur between neighbouring individuals. This result is consistent with negative density dependence already evidenced at small spatial scales, likely due to more intense competition or more shared natural pests between closely related species (Webb, Gilbert & Donoghue 2006; Gilbert & Webb 2007). Hence, our results suggest that spatial attraction of phylogenetically related species is largely predominant in tropical tree assembly at most distances, even if a slight compensation effect may occur between neighbouring individuals through negative density dependence effects.

NEW METHODS FOR DISSECTING THE PHYLOGENETIC STRUCTURE ACROSS SPATIAL AND PHYLOGENETIC SCALES

It has been often pointed out in the past that phylogenetic community structure analysis suffers from the inability to dissect the intensity of patterns across spatial and phylogenetic scales (Vamosi *et al.* 2009; Baraloto *et al.* 2012; Swenson 2013). We thus designed two novel approaches based on the truncation of the phylogenetic tree (Fig. 1) and on individual tree distribution (Fig. 2). The truncated-tree approach is a way of tracking how the phylogenetic signature is distributed along the phylogenetic tree and thus of detecting a signal related to recent versus ancient diversification events. We validated this approach using transformed data sets that mimicked artificially the spatial repulsion between closely related species at short spatial distance (20 m; i.e. overdispersion at shallow phylogenetic depth, Fig. 4). These simulations showed that our approach does disentangle a pattern of phylogenetic overdispersion at shallow phylogenetic depth due to competitive exclusion between closely related species, overlaid to an overall signal of phylogenetic clustering due to habitat filtering and phylogenetic conservatism. This new approach thus detects the simultaneous effects of habitat filtering causing clustering at a deep phylogenetic depth and of competitive exclusion causing overdispersion at a shallow phylogenetic depth (e.g. when repulsion occurs in 50% the pairs of closely related species, Fig. 4). However, fully resolved phylogenetic trees are required to use this method because family-resolved trees (e.g. Davies *et al.* 2004) ignore the more recent diversification events.

Second we developed an individual-based approach, where multiple species pair comparisons were considered. This proved helpful to understand how phylogenetic structure is influenced by both the spatial scales and phylogenetic relatedness at a fine spatial resolution. In the last decades, several studies have investigated the effect of competition thanks to neighbourhood models of tree demography (Uriarte *et al.* 2004). In most of these studies, tree growth and mortality were analysed as a function of the sizes and distances to neighbouring trees. A recent study even integrates the phylogenetic and functional relatedness in such framework (Uriarte *et al.* 2010). Our new approach investigates the patterns of co-occurrence as a function of both spatial distance and phylogenetic relatedness, instead of considering tree demography. This approach captures any spatial structure, contrary to quadrat-based analyses where fine spatial scales cannot be investigated. It is related to the individual-based phylogenetic mark correlation function developed by Shen *et al.* (2013). The latter describes the ratio of mean phylogenetic distance of two heterospecifics separated by a given spatial distance over the mean phylogenetic distance of two heterospecifics taken randomly. The

main difference is that our approach describes mean spatial patterns of co-occurrence between species separated by a given range of phylogenetic distances, allowing us to explore patterns across both spatial and phylogenetic scales.

A general limitation of phylogenetic approaches to infer ecological processes is that the link is indirect: different combinations of ecological and evolutionary processes might result in similar community phylogenetic patterns (Hardy & Senterre 2007; Cavender-Bares *et al.* 2009). Although we developed methods refining the description of phylogenetic patterns, our study does not escape this limitation. If well chosen, functional traits should provide better proxies to infer niche-related processes. It is worth noting that our methods can easily be extended to analyse trait data by replacing phylogenetic distance, δ_{st} in eq. (1), by a functional distance based on species traits, or by relating our co-occurrence measure for species pairs, eq. (2), with their functional proximity. Interestingly, comparing Π_{ST} and a trait-based equivalent, τ_{ST}, Baraloto *et al.* (2012) showed that the phylogenetic structure of tropical tree communities closely paralleled its functional structure, but with a weaker signal. This was explained by a positive but weak to moderate phylogenetic signal of the functional traits.

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Data accessibility

DNA sequences are deposited on GenBank (Table S1). The Korup FDP data set can be requested from the FDP managers: David Kenfack (KenfackD@si.edu), George Chuyong (chuyong99@yahoo.com), Duncan Thomas (duncanwt@gmail.com).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Details on the Phylogenetic reconstruction.

Figure S1. Impact of the resolution of the phylogenetic tree on the patterns of phylogenetic turnover.

Figure S2. Confidence envelopes of the spatial attraction or repulsion for species pairs according to their divergence time at different spatial distances.

Figure S3. Impact of the resolution of the phylogenetic tree on the Net Relatedness Index (NRI).

Table S1. List of the species used in the phylogenetic reconstruction and their accession number in GenBank.

Table S2. Fossils used in the the phylogenetic reconstruction and the priors used for calibration.