

Spatial associations of tree species in a subtropical evergreen broad-leaved forest

Zheng R. Luo¹, Ming J. Yu¹, De L. Chen², You G. Wu² and
Bing Y. Ding^{3,*}

¹ Key Laboratory of Conservation Biology for Endangered Wildlife of the Ministry of Education, and College of Life Science, Zhejiang University, Hangzhou 310058, China

² Management of Baishanzu, Fengyangshan-Baishanzu National Nature Reserve, Qingyuan 323800, China

³ Department of Biology Science, The School of Life and Environmental Science, Wenzhou University, Wenzhou 325027, China

*Correspondence address. The School of Life and Environmental Science, Wenzhou University, Wenzhou 325027, China. Tel/Fax: 086-577-86689259; E-mail: dby@wzu.edu.cn

Abstract

Aims

The spatial segregation hypothesis and the low-frequency hypothesis are two important proposed mechanisms that delay or prevent competitive exclusion in ecosystems. Because tree species interact with their neighbors, the importance of these potential processes can be investigated by analyzing the spatial structures of tree species.

Methods

The distribution of the adults of 27 common tree species in a fully mapped 5-ha subtropical forest plot in Baishanzu, eastern China, was analyzed to investigate the community-level intra- and interspecific spatial association patterns. We first tested for the overall spatial pattern in the 5- to 40-m neighborhoods and classified first-order bivariate associations with a diametric scheme based on Ripley's *K* and nearest-neighbor statistic (*G*-function). Then heterogeneous Poisson null models were used to distinguish second-order interactions from overall spatial associations (including first-order effects). Finally, we analyzed correlations between the existence of species interactions and some attributes of the species involved.

Important Findings

Partial overlap and segregation increased with scale, whereas mixing decreased. Nearly 70% of the species pairs occurred less than expected at random, and only 3.4% of the species pairs were well mixed; 11.0% of all species pairs showed significant small-scale interactions, which was a greater frequency than expected by chance if species are abundant or prefer the same habitat, but less frequent than expected if species are highly aggregated. This suggests that both spatial segregation and low frequency of species facilitate species coexistence by reducing the opportunity that trees of two species encounter each other. The study also revealed that positive interactions were more prevalent than negative interactions in the forest, which indicates that positive interactions may have important effects on forest species assemblies.

Keywords: Baishanzu • point pattern analysis • spatial segregation • low frequency • interaction opportunity

Received: 1 May 2011 Revised: 30 October 2011 Accepted: 3 December 2011

INTRODUCTION

The relative importance of inter- and intraspecific interactions is one of the central debates in ecology. Although positive interactions between species have recently been reported, especially in stressful habitats (Callaway *et al.* 2002; Martinez *et al.* 2010), competition has been accepted as a prevalent process in nature since the 1930s (Gause 1934). Specifically, competition has important effects on composition and structure of

plant communities (e.g. Chesson 2000; Grime 1977; Tilman 1994). Due to interspecific competition, species in a community will exclude each other until only one species remains (Wright 2002). However, many mechanisms have been proposed that delay or prevent competitive exclusion. Given the fact that plants are sessile organisms, the frequency at which two species directly interact will be influenced by the spatial patterns they (separately and jointly) exhibit and their relative abundances. The Spatial Segregation Hypothesis contends that highly patchy

distributions lead to interspecific segregation and, thus, on average individuals will interact with conspecifics rather than heterospecifics (Pacala 1997). Most tropical and subtropical forests are species rich, which usually means low densities of individual species. This generally weakens direct interspecific interactions among species. If two species occur at low frequencies, they do rarely encounter each other as proximal neighbors (Perry *et al.* 2009). The Low-Frequency Hypothesis states that, due to the low frequencies of most species in forests, pairwise species interactions are rare occurrences. The common ground between these hypotheses is that reducing the probability of encounters between pairs of species avoids competitive exclusion.

Whatever coexistence mechanisms are operating in the forest, they should leave a spatial signature (Hubbell *et al.* 2001). Space can be used as a surrogate for uncovering ecological process through the study and analysis of spatial patterns (McIntire and Fajardo 2009). If spatial mechanisms, such as the spatial segregation hypothesis and the low-frequency hypothesis, are indeed important for species coexistence, one would expect emergence of distinct spatial patterns, not only intraspecific but also interspecific. However, while there have been plenty of studies that investigate intraspecific spatial structure, few attempts have been made to analyze species spatial associations as a subset of species biological interactions (e.g. Kubota *et al.* 2007; Martinez *et al.* 2010; Perry *et al.* 2009; Wang *et al.* 2010; Wiegand *et al.* 2007). Such studies are necessary to reveal potential biological processes that control the assembly, dynamics and functioning of forest ecosystems.

Interspecific spatial repulsion is demonstrated by fewer heterospecific neighbors on average than expected in a random distribution and can be explained by negative interactions (e.g. competition). Interspecific spatial attraction, on the other hand, can be a result of positive interactions (e.g. facilitation) and manifests itself as higher than expected neighborhood densities of heterospecifics. However, other substantially different processes performed at different scales can create similar spatial patterns. For example, spatial repulsion (or attraction) between species may be also explained by different (or similar) microhabitat preferences (i.e. shading, soil moisture or nutrient levels; Wright 2002). Large-scale interspecific distribution patterns that are usually determined by species' habitat preferences will confound small-scale patterns if the analyses are not conducted appropriately (Wang *et al.* 2010). This makes the true underlying ecological processes behind patterns in species distributions difficult to elucidate (i.e. biological limitation). Thus, it is essential to integrate all relevant ecological information when making *a priori* inferences from ecological theory, which will help break up the biological limitation (McIntire and Fajardo 2009). For instance, if the Spatial Segregation Hypothesis is true in the forest, (i) most species should be aggregated and most species pairs should be spatially segregated especially at large spatial scales, (ii) the probabilities of significant interspecific associations should negatively correlate with the degrees of aggregations of species involved and (iii) species pairs with the same habitat preference should as-

sociate more frequently at small spatial scales. Alternatively, given that the Low-Frequency Hypothesis applies to an ecosystem, (i) the probabilities of significant interspecific associations should positively correlate with densities (or abundances) of involved species and (ii) a higher mean relative species density of a forest (i.e. abundance of one species relative to the total abundance of all species or the inverse of species richness) will lead to a lower frequency of interspecific associations in the forest.

Precise application of analytical tools is important to effectively test the above inferences we have made (McIntire and Fajardo 2009). A considerable problem in this study is to separate the effect of the abiotic environment from the effect of direct plant interactions. One effective approach to studying species interactions is to use heterogeneous Poisson processes as null models to account for larger spatial scale patterns when small-scale patterns are of primary interest (Wiegand *et al.* 2007). This approach is in the spirit of the empirically based knowledge that plant-plant interactions (second-order effects) usually work at small scales (e.g. 0–15 m) and species environment interactions (first-order effects) work at large scales (e.g. >15m). Another problem is to precisely describe the overall heterogeneous patterns of interspecific associations. Information of relative neighborhood density or nearest-neighbor distance alone does not unambiguously characterize heterogeneous patterns. Wiegand *et al.* (2007) developed a simple scheme integrating information of relative neighborhood density and nearest-neighbor distance to classify overall associations.

In this article, we use the above-mentioned analytical tools to assess the type and frequency of species distributions and associations in a subtropical forest in eastern China. In order to test the (i) Spatial Segregation Hypothesis and (ii) Low-Frequency Hypothesis for species coexistence, we tested the *priori* inferences drawn from the hypotheses. Specifically, we analyzed (i) how many species pairs were spatially segregated, (ii) how many species pairs associated at small scales, (iii) how the probability of interspecific association was related to the species' properties, such as species density, degree of intraspecific aggregation, and species habitat preference, and (iv) the relationship between community-wide relative density and frequency of association. In order to answer the last two questions, we conducted further analyses: we correlated the *P* values of interspecific associations with the intraspecific $g(r)$ values (see Methods for calculations of these two statistics) and abundances of species involved; we checked the relationship between the frequency of significant interspecific associations and habitat preference of species involved with permutation tests, and finally, we compared the percentages of significant interspecific associations among different forests.

Study site

The study site (Baishanzu Forest Dynamic Plot, hereafter Baishanzu FDP), a 250 × 200 m permanent forest plot, is located in the Baishanzu mountains in southeast China (119°3'53"E,

27°40' 54" N). In summer, the region is influenced by the south-east monsoon, which carries a large amount of water from the Pacific Ocean. The Baishanzu FDP is representative of mid-altitude subtropical mountain evergreen broadleaf forests, which is the typical vegetation in the subtropical monsoon climate. Many rare species exist in the old-growth species-rich community of the Baishanzu FDP, while the abundance of dominant species is much higher than other species (Xu *et al.* 2007). The site is on a north-facing hillside with a slope ranging from 7.27° to 44.12°.

The Baishanzu FDP (1 400–1 600 m elevation) was established in July 2003, when it was divided into 120 20 × 20 m and 10 20 × 10 m grids by using a total station. All trees with ≥ 1 cm diameter at 1.3 m height (DBH) and seedlings (individuals with DBH < 1 cm and/or height < 1.3 m) of non-shrub species were tagged, measured, stem mapped and identified to the species level. We monitored 156 tree species in the census. They show varying degrees of association to topography (Wang *et al.* 2011).

Study species

In this study, all species with >30 adult trees in the census were included. In order to include more shrub and understory species, we developed criteria to define adult trees. Trees were considered adult when DBH ≥ 8 cm for canopy species; DBH ≥ 4 cm for understory species and DBH ≥ 2.5 cm for shrub species. Adult specimens of 27 species (7 canopy species, 13 understory species and 7 shrub species) were analyzed. These 27 species accounted for ~93.8% of the total number of adults in the plot. There were 6 species with >1 000 adults, including the most abundant species, *Rhododendron latoucheae*, with >6 000 adults. All these species showed aggregation at some scale (Luo *et al.* 2009). There were 14 species that showed significant habitat preference (Wang *et al.* 2011). The ecological attributes of these species are shown in Table S1 in the online supplementary material.

Spatial pattern analysis

Summary second-order statistics.

Sophisticated techniques of spatial point pattern analysis were used to quantify species associations. Specifically, we chose the summary statistics of Ripley's (1976) K -function, pair-correlation function (Stoyan and Stoyan 1994) and the distribution function of near neighbor distances $G(y)$ (Diggle 2003) to analyze the data. The bivariate K -function $K_{12}(r)$ was defined as the expected number of Type 2 points within radius r of an arbitrary Type 1 point, divided by intensity λ_2 of Type 2 (i.e. the expected number of points of Type 2 per unit area). The bivariate pair-correlation function $g_{12}(r)$ based on point-to-point distance is related to the derivative of the K_{12} -function, i.e. $g_{12}(r) = K'_{12}(r)/2\pi r$ (Stoyan and Stoyan 1994). $K_{12}(r)$ is a cumulative distribution function where the values of $K_{12}(r)$ at larger scales include the values of $K_{12}(r)$ at smaller scales. In contrast, the pair-correlation func-

tion $g_{12}(r)$ is a non-cumulative distribution function in which $g_{12}(r)$ is the expected density of Type 2 points in a ring (its width Δr is very small) of a given distance r around a Type 1 focal point divided by intensity λ_2 of Type 2 (Wiegand and Moloney 2004). By factoring out the interference among different distances, the pair-correlation function allows for a precise assessment of scales where significant point-to-point interactions occur (Wiegand *et al.* 2007). The univariate K and pair-correlation functions are analogous to the bivariate functions, but the focal point is not counted (Wiegand and Moloney 2004). The statistic $G_{12}(y)$ evaluates the fraction of points of the focal Type 1 that have their nearest Type 2 neighbor within distance y (Diggle 2003; Illian *et al.* 2008). This statistic provides information on the bivariate emptiness probability not provided by $K_{12}(r)$ (Wiegand *et al.* 2007).

Statistical hypotheses and null models

Spatial analysis 1: Overall non-random association.

The null statistical hypothesis of this analysis (i.e. homogeneous process) was that Species 2 is randomly distributed in the plot, irrespective of the distribution of Species 1. In order to distinguish the difference between observed associations and random distribution hypothesis, we implemented complete spatial randomness (CSR) as the null model of this test. In this null model, the locations of the focal species were fixed, but Species 2 were distributed randomly and independently of the locations of Species 1. Additionally, with the scheme developed by Wiegand *et al.* (2007), we categorized all associations of the heterogeneous bivariate patterns. The commonly used statistics bivariate $K_{12}(r)$ and the $G_{12}(r)$ were implemented to construct the two axes of the scheme. The two axes P and M are defined as

$$P(r) = \hat{G}_{12}(r) - G_{12}^{\text{exp}}(r). \quad (1)$$

$$M(r) = \ln(\hat{K}_{12}(r)) - \ln(K_{12}^{\text{exp}}(r)). \quad (2)$$

The theoretical value of the two summary statistics under CSR is known ($G_{12}^{\text{exp}}(r) = 1 - e^{-\lambda_2 \pi r^2}$ and $K_{12}^{\text{exp}}(r) = \pi r^2$), but we used the mean of the Monte Carlo simulations because the edge-corrected estimates of $K_{12}(r)$ are not unbiased (Perry *et al.* 2006). The M axis and P axis evaluate two fundamental aspects of bivariate point patterns like the bivariate K_{12} - and G_{12} -functions. The M axis indicates the extent to which there are more (positive value) or less (negative value) points of Species 2 than expected (K -function), while the P axis indicates the extent to which the probability of having a nearest neighbor of Species 2 within distance r is higher (positive value) or lower (negative value) than expected (emptiness probability). $P(r)$ can distinguish the state in which many Species 1 points have no Species 2 neighbor but few Species 1 points have many Species 2 neighbors (with low values of $P(r)$) from the state where all Species 1 points have a similar number of Species 2 neighbors (with high values of $P(r)$); this

can not be distinguished by $M(r)$. $P(r)$ will be negative if the proportion of nearest neighbors within distance r is smaller than expected and positive if the proportion is larger than expected. Similarly, $M(r)$ will be negative if the average number of neighbors within distance r is smaller than expected and positive if the number is larger than expected. Further discussion about this scheme can be found in Wiegand *et al.* (2007) and Wang *et al.* (2010). Following this scheme, species spatial associations can be roughly classified into five types:

1. 'segregation' ($P(r) < 0$ and $M(r) < 0$) where two species are segregated in space;
2. 'partial overlap' ($P(r) < 0$ and $M(r) > 0$) where many plants of Species 1 have no Species 2 neighbors within distance r , but other plants of Species 1 have plenty of Species 2 neighbors;
3. 'mixing' ($P(r) > 0$ and $M(r) > 0$) where two species are mixed well in space;
4. 'strong interaction' ($P(r) > 0$ and $M(r) < 0$) where most individuals of the two species are segregated, but occasionally plants of Species 2 are the common nearest neighbors of all the focal plants in the strongly aggregated cluster; and
5. 'null association' (or Type 0, $P(r) = 0$ and $M(r) = 0$) proposed by Martinez *et al.* (2010). A null association arises if neither $K_{12}(r)$ nor $G_{12}(r)$ shows significant departures from the CSR.

Spatial analysis 2: small-scale plant-plant interactions

A univariate g -function was used to investigate the spatial patterns of conspecific adults. In order to reveal significant second-order effects in the univariate patterns (i.e. regularity or aggregation), we examined the discrepancies between the observed g -function and the 95% confidence envelope of the simulated null model. Here we used heterogeneous Poisson point processes (HP) as the null model because it is a shortcut for separating the first- (i.e. habitat preference) and second (i.e. direct plant-plant interactions)-order effects (Wiegand *et al.* 2007). Diggle (2003) suggested that small-scale effects are attributed to second-order plant-plant interactions and large-scale effects are attributed to environmental heterogeneity. In HP, the occurrence of any point is independent of that of any other, but the points are distributed in accordance with an intensity function $\lambda(x, y)$ that varies with location (x, y) (Stoyan and Stoyan 1994; Wiegand and Moloney 2004). The Epanechnikov kernel function, a nonparametric method, was used to estimate the intensity function $\lambda(x, y)$ of a given point pattern (Wiegand *et al.* 2007).

In order to reveal significant second-order effects in the bivariate patterns (i.e. repulsion or attraction), we kept the location of the individuals of the first species fixed and randomized the locations of the individuals of the second species under the HP model. Estimation of intensity of Species 2 was conducted as in the univariate patterns analysis. This allowed us to assess, given the intensity of Species 2, whether points of Species 2 were found more or less frequently than expected

around points of Species 1, which would suggest interspecific attraction or repulsion, respectively. We tested all possible pairs of the 27 species. Each species was analyzed as Species 1 and Species 2 because there was no reason to assume that the interactions would be symmetric.

Significant tests comparing patterns with the null model

Significant departures from the underlying null model were tested by overlap with 95% confidence envelope, which was constructed by using the 5th lowest and the 5th highest value of 199 Monte Carlo iterations, or, if the number of trees of Species 2 was < 200 , by using the 10th lowest and the 10th highest value of 399 Monte Carlo iterations. We performed different numbers of iterations for different abundances, because as the number of events in the event set decreases, the test's power falls and the ability to discriminate between different patterns is lost (Perry *et al.* 2006).

In order to minimize type I error, we used a goodness-of-fit (GOF) test to assess significant departures from the null model. The P value of the observed pattern is calculated as follows:

$$\hat{p} = 1 - \frac{\sum_{j=1}^s I(u_0 > u_j)}{s + 1} \quad (3)$$

where $I(u_0 > u_j)$ is an indicator function that equals 1 if $u_0 > u_j$ and equals 0 otherwise, and s represents the number of simulation iterations. u_i is a summary statistic that measures the discrepancy between the empirical measurement and the theoretical pair correlation function over a distance interval of interest, with

$$u_i = \sum_{r_k = r_{\min}}^{r_{\max}} [\hat{g}_i(r_k) - \bar{g}_i(r_k)]^2 \delta r_k, \quad (4)$$

where r_k is distance, r_{\min} and r_{\max} are lower and upper limits of the summation in terms of distance. $\hat{g}_i(r_k)$ is the empirical measurement for pattern i for $g(r)$, $\bar{g}_i(r_k)$ is the mean result computed for all simulated patterns except for i , and $\delta r_k = (t_{k+1} - t_k)$ is the width of the distance interval (Diggle 2003; Loosmore and Ford 2006).

Bandwidth selection for intensity estimation of each species

The Epanechnikov kernel function that was applied to estimate the intensities of species depends on the parameter bandwidth R . It is important to select a suitable bandwidth parameter for separating biological effects, as an unsuitable R may underestimate or overestimate intensity $\lambda(x, y)$. For example, Zhu *et al.* (2010) reported that the HP null model led many randomly distributed species show fake large-scale regularity in terms of statistical test. This may be caused by overestimation of intensity λ . In general, plant-plant interactions are restricted to within a limited spatial separation. An individual-based analysis of plant survival in this forest revealed that direct interactions usually occur among plants within 15 m of each other. This observation was used to select the bandwidth for each species.

We used a procedure to select the best bandwidths for each species (listed in Table S1 in the online supplementary material), as follows:

1. Given $R = R^*$, we estimated the intensity function of a species using the Epanechnikov kernel function.
2. Then, we simulated 199 patterns under the HP null model with the intensity function estimated from the species.
3. We calculated $g(r)$ values of these modeled patterns and the observed pattern.
4. After that, we estimated the P value of the GOF test using Equations (3) and (4) and restricting r_k to 15–35 m (i.e. $t_{\min} = 15$, $t_{\max} = 35$).
5. We repeated Step 1 through 4 using R^* values ranging from 10 to 25 m, with step intervals of 1 m and assessed P values at each step.
6. We chose the bandwidth corresponding to the largest P value as the suitable bandwidth for the species.

Additional tests

In order to assess how the probability of significant interspecific interactions varies with species aggregation and abundance, we correlated the P value of the GOF with the number of stems of species pairs and the univariate $g(r)$ of focal or neighbor species at scales $r = 0, 2, 6, 10, 15$ and 30 m using Spearman's ρ . Significance of these correlations was determined with a t -test. Testing for similar habitat preferences of species pairs between which interactions have been identified is much more difficult. Here we used a permutation test: (i) based on the matrix of interspecific interactions (i.e. Table S2 in the online supplementary material), the observed number of species pairs (note that Species 1 vs. Species 2 and Species 2 vs. Species 1 are considered as different species pairs) which had the same habitat preference and showed a significant interaction (i.e. 'p' or 'n' in Table S2 (see online supplementary material) indicating positive and negative interaction respectively) were counted; (ii) ps and ns were randomly replaced over the matrix (but the diagonal of the matrix should always keep empty); (iii) the number of species pairs with the same habitat preference and a significant interaction were counted again based on the new distribution of ps and ns in the matrix; and (iv) we repeated steps 2 and 3 999 times resulting in 1 000 predictions of number of interacting species pairs with similar habitat preferences if the relationship were random. If the observed number belong to the largest (or smallest) 2.5% of the total numbers, interspecific interaction was concluded to occur more (or less) frequently between species with the same habitat preference than between species with different habitat preferences.

All these analyses were conducted in R statistical software (R Development Core Team 2009). We used the package *spatstat* (Baddeley and Turner 2005) in R to perform spatial point pattern analyses.

RESULTS

Analysis 1: Overall non-random associations

As shown in Fig. 1, the relative frequencies of overall interspecific associations depended on scale. Most changes in the relative frequencies of the different bivariate association types occurred at scales smaller than 15 m (Fig. 1). The frequency of species pairs where neither summary statistic (i.e. K_{12} nor G_{12}) showed significant departure from the CSR model was relatively high in small neighborhoods. Partial overlap and segregation increased and mixing decreased with increasing spatial scale. The analyzed 702 species pairs were not equally distributed within the 2D classification space (Fig. 1, 2). Taking the proportion of cases (i.e. species pairs) at the 20-m spatial scale as an example, segregation and partial overlap were the two most frequent associations, making up 34.2 and 35.6% of all cases, respectively. As expected, type IV associations

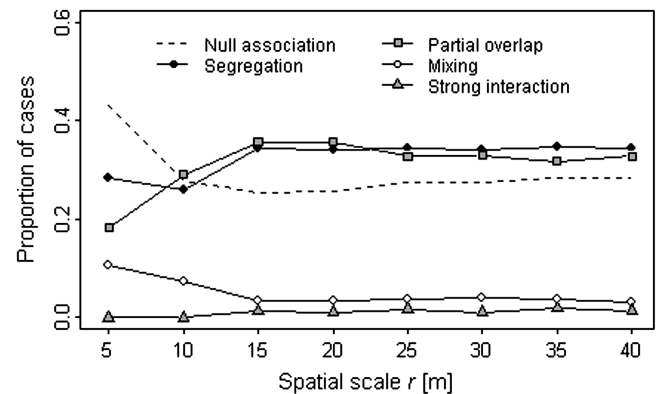


Figure 1: assessment of scale dependence on overall associations. The figure shows the proportion of the 702 species pairs studied categorized into five association types.

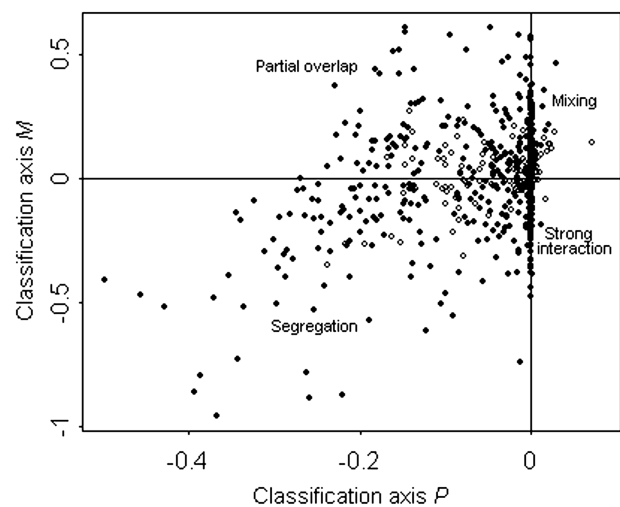


Figure 2: allocation of the overall association of the 702 species pairs involving 27 species in the Baishanzu FDP at 20 m based on the classification scheme described in Analysis 1.

(i.e. strong interaction) occurred in only 1.2% of all cases and only 3.4% species pairs were well mixed. This distribution pattern of classifications suggested that for most species pairs, trees encounter a neighbor from the counterpart species less often than expected by chance [$P(r) < 0$], though there are still 25.6% of species pairs whose association can be predicted by their abundance alone (i.e. not significant under CRS model).

Analysis 2: small-scale plant–plant interactions

Univariate case.

In 27 adult patterns examined, 20 of them showed significant departure from HP null models (i.e. P value from GOF test < 0.05). Nineteen species were fine-scale aggregated. The univariate pair-correlation function showed that none of the species was significantly regular at scale < 5 m and 3 species exhibited regularity at mid-scale (5–15 m). Among these three species, only *Acer olivaceum* did not show aggregation at small scale while the other two species did. The remaining seven species followed the HP null models.

In order to roughly estimate the effect of scale on species spatial patterns, we counted the number of species (with P value < 0.05 by GOF test) for each detail scale r where the observed pattern showed significant aggregation or repulsion (i.e. $g(r)$ value was above or below the envelopes constructed by Monte Carlo simulations). Results showed that the number of species exhibiting aggregation peaked at 3–4 m and decreased with the increasing scale, while the number of species exhibiting regularity fluctuated with the increasing scale from 5 to 15 m (Fig. 3a).

Bivariate case.

A total of 702 bivariate point pattern analyses for all adult pairs of the 27 species were executed. For 77 of these species pairs, the GOF test revealed a significant association (11.0%); in 58 of these cases, the small-scale association was positive (attraction), and in 19, it was negative (repulsion). In order to estimate the magnitude of scale dependence, we also counted for each scale the number of species exhibiting significant attraction or repulsion (using only species pairs where the P value of

the GOF test was < 0.05). We found that attraction occurred more frequently than repulsion, especially at small scales; the count of attraction showed a decreasing trend with increasing scale and stabilized for scales > 15 m at a count of ~ 0 –2 species, while the count of repulsion peaked at 2 m with 13 species pairs and decreased gradually with increasing scale (Fig. 3b).

Among 77 significantly interacting species pairs, 42 of them were symmetric, while the other 35 species pairs were asymmetric (see Table S2 in the online supplementary material). Both positive and negative interactions showed some symmetrical cases (19 and 2 cases, respectively). Symmetric interactions usually involved an abundant species. From the 27 species analyzed, only one species (*Illicium angustisepalum*) did not show any significant small-scale (0–15 m) association to another species. This species showed a relatively high degree of univariate clustering and relatively low abundance. On the other hand, two species (*R. latoucheae* and *Cleyera pachyphylla*) showed significant interaction with > 10 other species (14 and 17 species, respectively). *Rhododendron latoucheae* was the most abundant species in the community and was scattered throughout the plot. Most interactions involving this species were positive and symmetric; only one species (*Sycopsis sinensis*) negatively interacted with *R. latoucheae*. *Cleyera pachyphylla* showed negative association with 9 species. It is interesting to note that this species showed strong clonal reproduction.

The P value of the GOF test was moderately and negatively correlated with the number of stems of neighbor species (Species 2) ($\rho = -0.25$; $P < 0.01$), weakly and negatively correlated with the number of stems of focal species (Species 1) ($\rho = -0.17$; $p < 0.01$) and negatively correlated with the product and sum of the number of stems of two species ($\rho = -0.31$ and $\rho = -0.30$, respectively, both $P < 0.01$, see Table 1). This suggests that the abundant species interacted more frequently with other species. We also found a positive but weak correlation of P value with the clumping of two species. The two correlations both peaked at scale 2 m ($\rho = 0.19$ for Species 1 and $\rho = 0.12$ for Species 2, see Table 1). This suggested that small-scale clumping species were less likely to interact with other species.

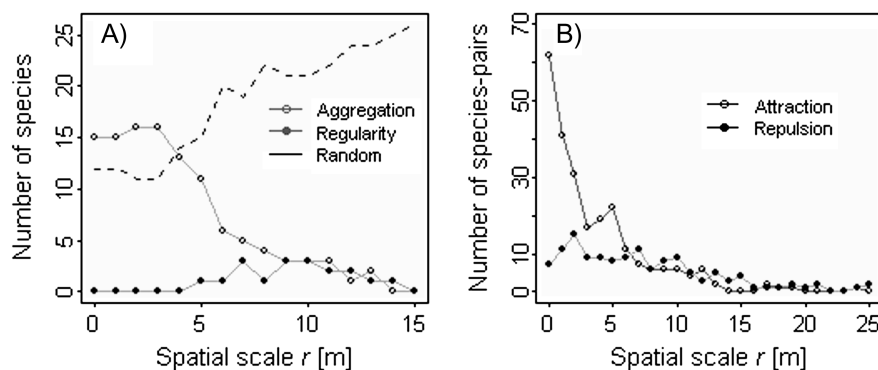


Figure 3: Scale-dependent biotic interactions. The figures exhibit number of species (univariate) and species pairs (bivariate) showing significant positive and negative adult interactions over different scales in the Baishanzu FDP.

Table 1: correlation of the P value of the GOF test of the bivariate analysis 2 with several variables describing species abundance and aggregation

| Variable | Correlation ρ |
|-----------------------------|--------------------|
| $n_{s1} \times n_{s2}$ | -0.31*** |
| $n_{s1} + n_{s2}$ | -0.30*** |
| n_{s1} | -0.17*** |
| n_{s2} | -0.25*** |
| $g_{11}(r = 0.1 \text{ m})$ | 0.05 |
| $g_{11}(r = 2 \text{ m})$ | 0.19*** |
| $g_{11}(r = 6 \text{ m})$ | 0.18*** |
| $g_{11}(r = 10 \text{ m})$ | 0.12*** |
| $g_{11}(r = 15 \text{ m})$ | 0.14*** |
| $g_{11}(r = 30 \text{ m})$ | 0.14*** |
| $g_{22}(r = 0.1 \text{ m})$ | 0.03 |
| $g_{22}(r = 2 \text{ m})$ | 0.12*** |
| $g_{22}(r = 6 \text{ m})$ | 0.06 |
| $g_{22}(r = 10 \text{ m})$ | 0.06* |
| $g_{22}(r = 15 \text{ m})$ | -0.05 |
| $g_{22}(r = 30 \text{ m})$ | -0.06 |

The correlations are not corrected for multiple testing. n_{s1} , the number of individuals of Species 1; n_{s2} , the number of individuals of Species 2; g_{11} and g_{22} are the values of the univariate pair correlation functions of Species 1 and 2, respectively, at the specified spatial scale r .

* $P < 0.1$; ** $P < 0.05$; *** $P < 0.01$.

Additionally, the permutation test showed that significant interactions, especially for positive interactions ($P < 0.01$), occurred somewhat more frequently for those species with the same habitat preference ($P < 0.05$). These results indicate that species with similar habitat preference tended to interact.

DISCUSSION

The comprehensive spatial analyses of species distributions and associations among adult trees of 27 common species in a subtropical evergreen broad-leaved forest in eastern China revealed a variety of strong scale-dependent spatial structures. We found that most species were aggregated at small scales, and segregation and overlap were dominant in overall species associations. More than a quarter of species pairs co-occurred at scales >10 m, by chance alone. Selective analysis of small-scale effects revealed that 89% of species pairs did not exhibit significant interactions between adult plants (note that we did not perform multiple testing here, if we had, the percentage would have been higher). The findings highlight that neutral species–species associations are prevalent in this subtropical forest, which would facilitate species coexistence. Many theories may explain the lack of positive or negative species–species interactions (e.g. Chesson 2000; Hubbell 2001; Wright 2002). Here we mainly discuss the importance of the segregation hypothesis and the low-density hypothesis in interpreting the patterns we observed.

The segregation hypothesis and the low-frequency hypothesis

These two hypotheses are known as mechanisms that violate the condition of the competitive exclusion principle (Chesson 2000; Wright 2002). The common underlying mechanism of these hypotheses is that spatial segregation or low densities among species decreases the probability of interspecific encounters with the effect of weakening interspecific interaction (Palmer 1994; Vazquez *et al.* 2007). All 27 species were significantly aggregated, which separate different species in space (Luo *et al.* 2009). Our study showed that spatial segregation and partial overlap were the most dominant (near 70%) overall species association types, which suggests that trees encounter a neighbor from the counterpart species less often than expected by chance [$P(r) < 0$]. More than a third of species pairs were segregated completely; these pairs of species rarely occupy the same areas, allowing plants of different species to ‘avoid’ each other. This observation is compatible with the segregation hypothesis and explains why 86.6% of all species pairs did not exhibit significant small-scale associations. The finding that small-scale clumping species are less likely to interact with other species (i.e. the P value of the GOF test positively correlated with the clumping of two species) also strongly supports the segregation hypothesis. This hypothesis is also supported by the finding that species with similar habitat preferences tended to interact (especially attract) significantly. Habitat heterogeneity attributed to differences in topography had profound effects on species associations. On one hand, species with the same association usually inhabit the same area, which gives them more opportunity to interact; on the other hand, adults usually modify their local environment, benefiting heterospecifics with similar preferences and allowing them to take up the same area.

Low frequency of most species is also a reason that results in a low percentage of significant species interactions. If most species were present at low abundances relative to the number of species, chance alone would make it unlikely that they encounter each other as neighbors (Lieberman and Lieberman 2007; Perry *et al.* 2009). Our results show that significant small-scale interspecific interactions are more likely if one or both species are more abundant. A good example is the most abundant species, *R. latoucheae*, which shows significant interactions with 14 other species, while the rare canopy species, *I. angustisepalum*, is independent of all other species. An important difference between species-rich and species-poor communities is that the mean relative species density of species-rich communities is often low, while that of species-poor communities is often high. Under the low-frequency hypothesis, the high relative species density in species-poor communities would lead to increased frequencies of species–species interactions. A comparison of results from Changbaishan (Wang *et al.* 2010), Sinharaja (Wiegand *et al.* 2007) and Baishanzu (this paper) supported our expectation that lower mean relative species densities (i.e. higher species richness) in a forest lead to

a lower frequency of interspecific association (see Table S3 in the online supplementary material). These results suggest that the low-frequency hypothesis elucidates important mechanisms influencing the frequency of small-scale interspecies interactions within forests. However, further comparisons with statistical test across sites are still necessary to confirm the effect of low species density across communities because the frequency of significant interactions and mean relative species density are reciprocally dependent.

Furthermore, the low probability of interspecific encounters has profound influence on species trait evolution that is related to a species' ability to compete. Since the set of neighbors encountered by individuals of a given species within the immediate neighborhood is quite variable and unpredictable for the individual (i.e. high biotic uncertainty), Hubbell and Foster (1986) argued that natural selection may be diffuse. Under these conditions, species are unlikely to develop specific interactions with other species. In this scenario, interspecific interaction would be weak even if individuals of the two species sometimes encountered each other.

Our study supports the idea that reducing the probability of interspecific encounters may strikingly weaken species interactions. Naturally, if species live in different habitats and have no direct or indirect interactions with each other, they should have no difficulty coexisting in a region (Chesson 2000). However, species do not have to be strictly segregated in space for regional coexistence. There are still a third of species pairs that overlapped and some species pairs mixed well. Spatial patterns are usually controlled by multiple processes, which work at different scales (He *et al.* 1997; Luo *et al.* 2009). In a broad sense, species differing in the resources they exploit (i.e. niche complementarity) would weaken interspecific interactions (Chesson 2000; Wills *et al.* 2006).

Interpretations of intraspecific aggregation

Similar to results from other tropical, subtropical and temperate forests (e.g. Condit *et al.* 2000; Li *et al.* 2009; Wang *et al.* 2010), we found that intraspecific aggregation is prevalent in this subtropical forest. The high degree of individual species aggregation has important effects on species spatial separation and their interactions (DeBoeck *et al.* 2006; Seidler and Plotkin 2006). Species habitat preference is an important process leading to the aggregation pattern here (Luo *et al.* 2009). More than half of these species exhibited habitat preference (see Table S1 in the online supplementary material). This suggests that the benefit of growing in favorable habitats may overwhelm the negative effects of sharing that habitat with conspecifics (Getzin *et al.* 2008). Our study showed that the observed patterns of seven species were undistinguishable from predictions of a heterogeneous Poisson model, suggesting that patterns of these species can be explained by habitat heterogeneity alone. Preceding studies also indicate that dispersal limitation also contributes to aggregation patterns (Grubb 1977; He *et al.* 1997). Indeed, we found that even after accounting for the effects of habitat preference, there were 19 species that were

aggregated at a small scale. This suggests that the effects of dispersal limitation are very strong. Fruit type and seed size of these species show that most species in the community are not dispersed far from their parent trees, which disperse their seed by gravity or wind. Habitat heterogeneity and dispersal limitation are the two most important effects that determined species distribution patterns in forests (Shen *et al.* 2009). Moreover, the lack of evidence of spatial regularity of individual species at small scales suggests Janzen–Connell effects and intraspecific competition is absent or weak at least. The Janzen–Connell hypothesis posits that mother trees impair survival of offspring where natural enemies are aggregated (Connell 1971; Janzen 1970). The effect will lead to a regular spatial distribution of adults. However, further studies are needed to confirm the absence of Janzen–Connell effects and intraspecific competition because strong effects of dispersal limitation and habitat preference may mask them.

The importance of positive interactions in forests

Positive associations have been documented in stressed environments like alpine, arid and Mediterranean-type plant communities (Armas and Pungnaire 2005; Callaway *et al.* 2002; Riginos *et al.* 2005), but are rare in forest communities (but see Kubota *et al.* 2007 and Martinez *et al.* 2010). Our selective analysis of small-scale effects revealed that positive associations are more prevalent than negative associations in Baishanzu FDP, especially between species pairs involving non-canopy species with similar habitat preferences. The finding is not surprising when species have similar requirements for establishment; canopy trees modify the local environment in their vicinity, which facilitates small conspecifics and heterospecifics with similar preferences (Dovciak *et al.* 2001; Kubota *et al.* 2007); and/or species' niches differ (Chesson 2000).

Such small-scale attractions, which enable species to exploit a greater portion of available resources, profoundly influence community structure. We find that the most abundant species, *R. latoucheae*, associates with 14 other species and only one of these associations is negative. Most species can recruit under adults of *R. latoucheae*. This has interesting implications for community assembly. By providing habitats for numerous species, the net effect of foundation species on species diversity can actually be positive. This phenomenon may need to be integrated into forest ecological theory (Bruno *et al.* 2003). For example, Shen *et al.* (2009) used the joint effects of dispersal limitation and habitat heterogeneity to explain species-area curves in Gutianshan plot (25 ha, in subtropics) and BCI (Barro Colorado Island) plot (50 ha, in tropics) and showed that the joint effects of these two processes did not fit the observed species-area curves well at intermediate spatial scales (they usually predicted lower than the observed species area). The influence of positive interactions between species may explain this departure. Positive facilitative interactions between species occurring at some life stages can compensate for negative competitive interactions at other stages (Callaway *et al.* 2002; Illian *et al.* 2009; Martinez *et al.* 2010). In this case, the patterns that finally emerged would

be predominantly neutral as those found here (Wiegand *et al.* 2007). It would be interesting to study species interactions at different life stages and habitats.

SUPPLEMENTARY MATERIAL

Supplementary Tables S1–S3 are available at *Journal of Plant Ecology* online.

FUNDING

Appropriative Foundation of Ecology and Environment Protection of Zhejiang Province (ZCJ200317); China National Program for R & D Infrastructure and Facility Development (2008BAC39B02).

ACKNOWLEDGEMENTS

We are grateful to Chaozong Zheng Xiaofeng Jin, Jianguo Ai, and Xu Yang for their technical support for species identification. The authors thank the many students from Zhejiang University and many colleagues from Management of Baishanzu for their contributions to the establishment of this 5 ha permanent forest plot. We also thank Guochun Shen, Helge Bruelheide and three anonymous reviewers for critical comments of this manuscript. Last but not least, we appreciate Christine Verhille at the University of British Columbia and Lauren Barry for their assistance with English language and grammatical editing of the manuscript.

Conflict of interest statement. None declared.

REFERENCES

- Armas C, Pungnaire FI (2005) Plant interactions govern population dynamics in a semi-arid plant community. *J Ecol* **93**:978–89.
- Baddeley A, Turner R (2005) Spatstat: an R package for analyzing spatial point patterns. *J Stat Softw* **12**:1–42.
- Bruno JF, Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. *Trends Ecol Evol* **18**:119–25.
- Callaway RM, Brooker RW, Choler P, *et al.* (2002) Positive interactions among alpine plants increase with stress. *Nature* **417**:844–8.
- Chesson P (2000) Mechanisms of maintenance of species diversity. *Annu Rev Ecol Syst* **31**:343–66.
- Condit R, Ashton PS, Baker P, *et al.* (2000) Spatial patterns in the distribution of tropical tree species. *Science* **288**:1414–8.
- Connell JH (1971) On the role of natural enemies in preventing competitive exclusion in some marine animals and in rain forest trees. In denBoer PJ, Gradwell GR (eds). *Dynamics of Populations*. Wageningen, The Netherlands: Centre for agricultural publication and documentation, 298–312.
- DeBoeck HJ, Nijs I, Lemmens CMHM, *et al.* (2006) Underlying effects of spatial aggregation (clumping) in relationships between plant diversity and resource uptake. *Oikos* **113**:269–78.
- Diggle P (2003) *Statistical Analysis of Spatial Point Patterns*. London, UK: Arnold.
- Dovciak M, Frelich LE, Reich PB (2001) Discordance in spatial patterns of white pine (*Pinus strobus*) size-classes in a patchy near-boreal forest. *J Ecol* **89**:280–91.
- Gause GF (1934) *The Struggle for Existence*. Baltimore, MD: Williams & Wilkins.
- Getzin S, Wiegand T, Wiegand K, *et al.* (2008) Heterogeneity influences spatial patterns and demographics in forest stands. *J Ecol* **96**:807–20.
- Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am Nat* **111**:1169–94.
- Grubb PJ (1977) The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biol Rev* **53**:107–45.
- He FL, Legendre P, LaFrankie JV (1997) Distribution patterns of tree species in a Malaysian tropical rain forest. *J Veg Sci* **8**:105–14.
- Hubbell SP (2001) *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton, NJ: Princeton University Press.
- Hubbell SP, Ahumada JA, Condit R, *et al.* (2001) Local neighborhood effects on long-term survival of individual trees in a neotropical forest. *Ecol Res* **16**:859–75.
- Hubbell SP, Foster RB (1986) Biology, chance and history and the structure of tropical rain forest tree communities. In Diamond JM, Case TJ (eds). *Community Ecology*. New York: Harper and Row, 314–29.
- Illian JB, Penttinen A, Stoyan H, *et al.* (2008) *Statistical Analysis and Modeling of Spatial Point Patterns*. Chichester, UK: John Wiley & Sons.
- Illian JB, Moller J, Waagepetersen RP (2009) Hierarchical spatial point process analysis for a plant community with high biodiversity. *Environ Ecol Stat* **16**:389–405.
- Janzen DH (1970) herbivores and the number of tree species in tropical forests. *Am Nat* **104**:501–28.
- Kubota Y, Kubo H, Shimatani K (2007) Spatial pattern dynamics over 10 years in a conifer/broadleaved forest, northern Japan. *Plant Ecol* **190**:143–57.
- Li L, Huang ZL, Ye WH, *et al.* (2009) Spatial distributions of tree species in a subtropical forest of China. *Oikos* **118**:495–502.
- Lieberman M, Lieberman D (2007) Nearest-neighbor tree species combinations in tropical forest: the role of chance, and some consequences of high diversity. *Oikos* **116**:377–86.
- Loosmore NB, Ford ED (2006) Statistical inference using the G or K point pattern. *Ecology* **87**:1925–31.
- Luo ZR, Ding BY, Mi XC, *et al.* (2009) Distribution patterns of tree species in an evergreen broadleaved forest in eastern China. *Front Biol China* **4**:531–8.
- Martinez I, Wiegand T, Gonzalez-Taboada F, *et al.* (2010) Spatial associations among tree species in a temperate forest community in north-western Spain. *For Ecol Manag* **260**:456–65.
- McIntire EJB, Fajardo A (2009) Beyond description: the active and effective way to infer processes from spatial patterns. *Ecology* **90**:46–56.
- Pacala SW (1997) Dynamics of plant communities. In Crawley MJ (ed). *Plant Ecology*. Oxford: Blackwell Science, 532–55.
- Palmer MW (1994) Variation in species richness: towards a unification of hypotheses. *Folia Geobot* **29**:511–30.
- Perry GLW, Enright NJ, Miller BP (2009) Nearest-neighbour interactions in species-rich shrublands: the roles of abundance, spatial patterns and resources. *Oikos* **118**:161–74.
- Perry GLW, Miller BP, Enright NJ (2006) A comparison of methods for the statistical analysis of spatial point patterns in plant ecology. *Plant Ecol* **187**:59–82.

- R Development Core Team (2009) R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.
- Riginos C, Milton SJ, Wiegand T (2005) Context-dependent interactions between adult shrubs and seedlings in a semi-arid shrubland. *J Veg Sci* **16**:331–40.
- Ripley BD (1976) The second-order analysis of stationary point processes. *J Appl Probab* **13**:255–66.
- Seidler TG, Plotkin JB (2006) Seed dispersal and spatial pattern in tropical trees. *PLoS Biol* **4**:2132–7.
- Shen GC, Yu MJ, Hu XS, *et al.* (2009) Species-area relationships explained by the joint effects of dispersal limitation and habitat heterogeneity. *Ecology* **90**:3033–41.
- Stoyan D, Stoyan H (1994) Fractals, Random Shapes and Point Fields: Methods of Geometrical Statistics. Chichester, UK: John Wiley & Sons.
- Tilman D (1994) Competition and biodiversity in spatially structured habitat. *Ecology* **75**:2–16.
- Vazquez DP, Melian CJ, Williams NM, *et al.* (2007) Species abundance and asymmetric interaction strength in ecological networks. *Oikos* **116**:1120–7.
- Wang XG, Wiegand T, Hao ZQ, *et al.* (2010) Species associations in an old-growth temperate forest in north-eastern China. *J Ecol* **98**:674–86.
- Wang W, Luo ZR, Zhou RF, *et al.* (2011) Habitat associations of woody plant species in Baishanzu subtropical broad-leaved evergreen forest. *Biodiv Sci* **19**:134–42.
- Wiegand T, Gunatilleke S, Gunatilleke N (2007) Species associations in a heterogeneous Sri Lankan dipterocarp forest. *Am Nat* **170**:77–95.
- Wiegand T, Moloney KA (2004) Rings, circles, and null-models for point pattern analysis in ecology. *Ecography* **104**:109–29.
- Wills C, Harms KE, Condit R, *et al.* (2006) Nonrandom processes maintain diversity in tropical forest. *Science* **311**:527–31.
- Wright J (2002) Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* **130**:1–14.
- Xu M, Luo ZR, Yu MJ, *et al.* (2007) Floristic composition and community structure of mid-montane evergreen broad-leaved forest in north slope of Baishanzu mountain. *J Zhejiang Uni (Agric & Life Sci)* **33**:450–7.
- Zhu Y, Mi XC, Ren HB, *et al.* (2010) Density dependence is prevalent in a heterogeneous subtropical forest. *Oikos* **119**:109–19.