

## LETTER

# Intra-specific relatedness, spatial clustering and reduced demographic performance in tropical rainforest trees

Xiaona Shao,<sup>1,2</sup> Calum Brown,<sup>3</sup>  
Samantha J. Worthy,<sup>4</sup> Lu Liu,<sup>1,2</sup>  
Min Cao,<sup>1</sup> Qiaoming Li,<sup>1\*</sup>  
Luxiang Lin<sup>1,5\*</sup> and  
Nathan G. Swenson<sup>1,4</sup>

### Abstract

Intra-specific negative density dependence promotes species coexistence by regulating population sizes. Patterns consistent with such density dependence are frequently reported in diverse tropical tree communities. Empirical evidence demonstrating whether intra-specific variation is related to these patterns, however, is lacking. The present study addresses this important knowledge gap by genotyping all individuals of a tropical tree in a long-term forest dynamics plot in tropical China. We show that related individuals are often spatially clustered, but having closely related neighbours reduces the growth performance of focal trees. We infer from the evidence that dispersal limitation and negative density dependence are operating simultaneously to impact the spatial distributions of genotypes in a natural population. Furthermore, dispersal limitation decreases local intra-specific genetic diversity and increases negative density dependence thereby promoting niche differences and species coexistence as predicted by theory.

### Keywords

Density dependence, forest dynamics, forest ecology, rainforest, relatedness.

*Ecology Letters* (2018) 21: 1174–1181

## INTRODUCTION

Theory has shown that the relative strength of negative intra-specific interactions (i.e. niche differences) plays a fundamental role in determining whether two species can coexist (Lotka 1920; Volterra 1926; MacArthur & Levins 1967; Levin 1970; Chesson 2000). Specifically, if the negative impact of adding an additional conspecific outweighs the negative impact of adding an additional heterospecific, species coexistence is promoted. That is, intra-specific negative density dependence prevents a single species from forming a monoculture and increases the probability of stable coexistence. Though, it should be noted that recent theoretical advances have demonstrated that coexistence is possible with little-to-no niche differences so long as there are little-to-no differences in performance between the two species (Chesson 2000).

The accumulation of long-term forest dynamics datasets over the past several decades is now permitting forest ecologists to quantify the strength of intra-specific negative density dependence and whether neighbouring conspecific and heterospecific individuals have negative impacts on focal individual demographic rates. A general outcome of this work is that intra-specific negative density dependence is widespread in tree communities from the temperate zone to the tropics (Harms *et al.* 2000; Chen *et al.* 2010; Lin *et al.* 2012; Comita *et al.* 2014). Further, the strength of this density dependence has been shown to vary with latitude and tree diversity (Lamanna *et al.* 2017) and potentially local relative

abundance (Comita *et al.* 2010; but see Zhu *et al.* 2015). Thus, a key regulating mechanism of tree populations is routinely evident and should promote coexistence so long as the negative impact of neighbouring heterospecific individuals is relatively weak. Neighbourhood analyses of tree demographic rates, where the demographic rate of a focal individual is modeled partly as a response to the conspecific and heterospecific individual densities within a given radius, have generally found that conspecific densities have a far more negative impact on focal tree growth and survival rates than heterospecific densities (e.g. Chen *et al.* 2017). This result is even found when functional traits or phylogenetic relationships are used to scale the similarity of heterospecific individuals to the focal individual (e.g. Uriarte *et al.* 2010; Wu *et al.* 2016). Indeed, in perhaps the most extensive study to date scaling heterospecific neighbourhood similarity by functional trait data (Kunstler *et al.* 2016), conspecific densities were strongly related to demographic success whereas the role of heterospecific trait similarity was far weaker or non-existent. This leads to the obvious questions of whether ecologists are simply measuring the wrong traits (Swenson 2012, 2013) and even more importantly why so little focus has been placed in neighbourhood studies on intra-specific variation (Bolnick *et al.* 2011; Levine 2016). In sum, strong niche differences resulting from strong intra-specific negative density dependence are generally detected in tree community datasets worldwide, which combined with apparently weak heterospecific interactions should promote coexistence.

<sup>1</sup>Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Kunming, Yunnan 650201, China

<sup>2</sup>University of Chinese Academy of Sciences, Beijing 100049, China

<sup>3</sup>Institute of Meteorology and Climate Research, Atmospheric Environmental Research, Karlsruhe Institute of Technology, Kreuzackbahnstraße 19, 82467 Garmisch-Partenkirchen, Germany

<sup>4</sup>Department of Biology, University of Maryland, College Park, MD 20742, USA

<sup>5</sup>Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China

\*Correspondence

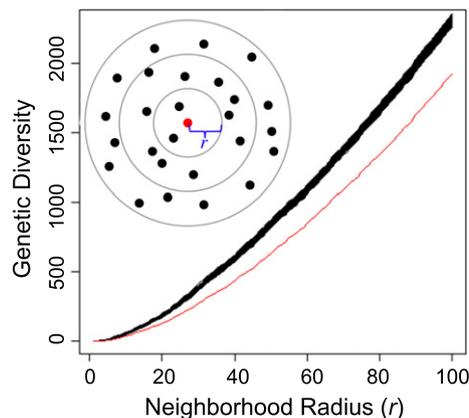
E-mails: lqm@xtbg.ac.cn and linluxa@xtbg.ac.cn

A present challenge in tree community ecology is to elucidate how intra-specific functional trait or genetic variation is naturally distributed in populations and how it is related to intra-specific negative density dependence. This is particularly true in highly diverse tropical rainforests where negative density dependence is likely stronger and species are potentially more dispersal limited. Analyses of the genetic relatedness of conspecific individuals in natural populations would be greatly useful in resolving these outstanding issues. Specifically, the spatial distribution of phenotypes would elucidate whether dispersal limitation is occurring and presumably the traits underlying negative interactions have some genetic component. Previous work in tropical trees examining relatedness has largely focused on the spatial distribution of genotypes as it relates to gene flow, dispersal limitation and habitat fragmentation (e.g. Aldrich *et al.* 1998; Nason *et al.* 1998; Dayanandan *et al.* 1999; Hamilton 1999; Jones & Hubbell 2006). However, very few studies exist linking genetic diversity to demographic rates of individuals across life stages (e.g. Browne & Karubian 2016). There are multiple ways in which intra-specific genetic diversity will influence coexistence and vice versa. First, strong negative conspecific interactions that are dependent upon relatedness should promote an increase in local genetic diversity. This outcome may be detected at any point in time, but can also be captured by analysing genetic diversity across size classes. For example, Jones & Hubbell (2006) report an increase in genetic diversity across size classes in a Panamanian tree species presumably due to a thinning of related conspecifics through time. Second, dispersal limitation and/or genotype specific habitat preferences or competitive dominance may reduce local genetic diversity. Both of these mechanisms can be related to niche differences as the first would indicate strong niche differences while the second would restrain intra-specific variation that could weaken niche differences (Hart *et al.* 2016).

One approach to elucidating whether neighbourhood intra-specific genetic diversity influences the distribution and demographic rates of species may be to simply quantify these patterns across many individuals in a locality. A potential problem with such an approach is that the observed levels of spatial clustering in genotypes may be dependent on the scale used for the study and the diversity of genotypes may change across ontogeny (e.g. Jones & Hubbell 2006). Thus, traditional neighbourhood analyses that do not consider multiple spatial scales or size classes may fail to uncover results. We propose that these two obstacles may be overcome using individual species area relationships (ISARs). The first ISARs were formulated by Wiegand *et al.* (2007), where the species richness of neighbours around a focal tree was quantified and recalculated as neighbourhood size (i.e. a radius length around the focal tree) increased. These observed individual species area relationships were then compared to a null distribution of individual species area relationships to determine whether species tend to be surrounded by more, less or no different from the expected number of species in a neighbourhood. Wiegand *et al.* (2007) then considered the impact of size class on the individual species area relationships they quantified to determine whether neutral or deterministic mechanisms were more prevalent in particular size classes. This

original approach has subsequently been modified by employing more sophisticated spatial null models (e.g. Wang *et al.* 2015) and incorporating other dimensions of biodiversity (e.g. Yang 2013; Wiegand *et al.* 2017). Here, we propose a reinterpretation of this approach that focuses on intra-specific diversity across spatial scales and size classes. Specifically, in a genotyped population, the genetic diversity of neighbouring individuals is summed across increasingly large spatial scales and compared to a null expectation to form what we call an individual genetic diversity area relationship (IGDAR) (Fig. 1). If the observed genetic diversity is smaller than expected, this indicates that related individuals are spatially aggregated potentially due to dispersal limitation or genotype-specific superior performance in a given habitat. Conversely, if the observed genetic diversity of neighbours is higher than expected, then this may indicate a repulsion of similar genotypes potentially due to competitive interactions and/or shared enemies. Importantly, these mechanisms are not mutually exclusive. For example, individuals may be dispersal limited such that there is spatial aggregation of genotypes, but intra-specific negative density dependence would reduce the demographic performance (e.g. growth) of these individuals and may ultimately lead to a reduction in the spatial aggregation of genotypes as time proceeds. Thus, integrating the spatially continuous IGDAR with demographic rates across size classes may be a powerful way to elucidate these processes that are likely occurring simultaneously in natural populations that cannot be experimentally manipulated.

Here, we employ an IGDAR and ISAR frameworks (Fig. 1) to study the spatial aggregation and demographic



**Figure 1** A graphical explanation of the individual genetic diversity area relationship (IGDAR) utilised in this study. For a given individual focal tree (red point in insert) the genetic diversity of neighbouring conspecifics (black points in insert) is summed as the neighbourhood radius ( $r$ ) increases. This observed cumulative sum (red line) is plotted against  $r$ . The observed is then compared to a null distribution. Here the 95% confidence interval range of the null distribution is plotted in black. Thus, in this example the observed data have lower than expected neighbourhood conspecific genetic diversity at larger scales (i.e. individuals surrounded by closer relatives) and no different from expected at smaller scales (i.e. neutral individuals). If the red line was higher than the 95% confidence interval it would be an individual surrounded by more distant relatives (i.e. neighbouring conspecifics that are more distantly related than expected).

performance of a population of trees in a hyper-diverse rain-forest in southwest China. The work developed microsatellites to genotype individuals of *Beilschmiedia roxburghiana* (Lauraceae) in a long-term forest dynamics plot where growth and survival has been monitored for 10 years. The specific research questions we ask in this study are: (1) Are genotypes non-randomly distributed in the forest plot and does the degree of spatial aggregation decrease across size classes?; (2) At what spatial scales is the aggregation of similar genotypes most apparent?; (3) Does individual growth and survival differ between individuals that are surrounded by conspecifics that are more or less related than expected by chance and how do these demographic relate to the diversity of heterospecifics in the neighbourhood?; and (4) How does intra-specific genetic diversity relate, if at all, to mechanisms that would promote the coexistence of large numbers of species in hyper-diverse tropical tree communities?

## METHODS

### Study species and site

*Beilschmiedia roxburghiana* is an evergreen, small- to medium-sized tree that can reach 20 m in height. This species is widespread, mainly distributed in tropical evergreen broadleaf forests in the southeast of the Tibet autonomous region, in Yunnan province, in northeastern Myanmar, and in India. As a typically forest-dwelling species, *B. roxburghiana* generally occupies the second and third layers of the canopy. It has hermaphroditic flowers that are pollinated by insects and its elliptic berry dispersed by gravity and vertebrates, such as birds and small mammals (Li 1982).

This study was conducted in the 20-ha Xishuangbanna tropical seasonal rainforest dynamics plot (XSBN plot) in Xishuangbanna, Southwest China. Elevation in XSBN plot ranges from 708.2 to 869.1 m. The monsoon climate in Xishuangbanna is strongly seasonal with distinct alterations between the wet season (May–October) and the dry season (November–April). The mean annual temperature is 21.8°C and mean annual precipitation is 1493 mm (Cao *et al.* 2008). More than 400 individuals of *B. roxburghiana* are found in XSBN plot, making it one of the common tree species.

### Microsatellite genotyping of individuals

We genotyped all the individuals of *B. roxburghiana* in the XSBN plot using nine pairs of microsatellite DNA primers. The details of the protocols of the microsatellite genotyping and the primer sequences were described in Liu *et al.* (2013). Briefly, the total genomic DNAs were extracted from dried leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle 1991). Following PCRs, all the PCR products were separated in an ABI 3730 sequencer (Applied Biosystems, Carlsbad, CA, USA), and the fragment lengths were analysed using ABI GeneMapper software version 3.7 (Applied Biosystems). Finally, we removed individuals with missing data for one or more microsatellite loci, which meant that 363 individuals of *B. roxburghiana* were applied for further analyses.

### Quantifying individual genetic diversity area relationships (IGDARs)

A genetic distances matrix among microsatellite phenotypes was generated in MSAnalyser (Dieringer & Schlötterer 2003). To measure scale-dependent local genetic diversity structures around individuals of a population, we propose the framework of individual genetic diversity area relationships (IGDARs). We sum the genetic distances between focal individuals and all the conspecific neighbours in a certain circular area and also produce the null values for the sum of the above genetic distance by shuffling the focal individuals' positions on the genetic distances matrix by 999 times. If the observed value for a focal individual was higher than 97.5% of null values, it would be an individual surrounded by more distant relatives (i.e. neighbouring conspecifics that are more distantly related than expected). If the observed value for a focal individual was less than 97.5% of null values, it would be an individual surrounded by closer relatives (i.e. neighbouring conspecifics that are more highly related than expected). If the observed value of a focal individual was within the simulation envelopes, it would be a neutral individual. Lastly, we also calculated standardised effect sizes (SES) for each individual at each scale using the null distribution. Specifically, the mean of the null distribution of neighbourhood genetic diversities was subtracted from the observed genetic diversity in the neighbourhood and this value was divided by the standard deviation of the null distribution. Positive SES values therefore indicate higher than expected neighbourhood genetic diversity and negative SES values indicate a lower than expected neighbourhood genetic diversity. The null model utilised in this study was chosen because it does not randomize the spatial location of individuals in the forest. Thus, the natural spatial clustering, or lack thereof, in the population is maintained and only the variable of interest (i.e. genotypes) was randomised (Gotelli & Graves 1996).

In the next step, the proportion of individuals surrounded by closer relatives, individuals surrounded by more distant relatives and neutral individuals were counted at each scale  $r$ , respectively, for assessing the scale-dependent effects. We also ran the above analysis for two diameter size classes ( $> 26$  mm DBH and  $\leq 26$  mm DBH). These two size classes were selected as 26 mm is the median DBH for all focal individuals. Taking into account the effect of dispersal limitation, the maximum scale was  $r_{\max} = 50$  m with steps of 1 m. To make sure that all focal individuals were calculated in all scales without edge effects, we selected the individuals with distance at least 50 m from the plot edge as focal individuals. In total, there are 191 focal individuals in the following analysis.

### Quantifying Individual Species Area Relationships (ISARs)

The next part of the study aimed to quantify the richness of neighbouring heterospecific species in the neighbourhoods of the 191 focal *B. roxburghiana* individuals. To accomplish this, we quantified individual species area relationships (ISARs) as described by Wiegand *et al.* (2007). Briefly, we quantified the number of species in neighbourhood radii ranging from 1–50 m for each focal individual. We then used a complete

spatial randomness null model which randomised focal individual locations in the forest. The randomization was repeated 999 times with the heterospecific species richness recorded at each neighbourhood radius to generate a null distribution. A SES value was then calculated for each *B. roxburghiana* focal individual at each neighbourhood radius. Thus, positive SES values indicate more than expected heterospecific neighbours and negative SES values indicate fewer than expected heterospecific neighbours.

#### Relationship between IGDARs and ISARs and individual growth and death

The IGDAR analyses described above classified each individual tree as an individual surrounded by closer relatives, an individual surrounded by more distant relatives or a neutral individual at each of the 50 scales analysed. Similarly, the ISAR analyses asked whether focal individuals were surrounded by more, less or no different than expected heterospecific species richness across the 50 scales. Both analyses resulted in SES values that we used for subsequent analyses.

We asked whether the average relative growth rates (RGRs) differed between these three groups for two diameter size classes ( $> 26$  mm DBH and  $\leq 26$  mm DBH). The RGRs were calculated using the DBH measurements of the focal trees from the 2007 and 2017 censuses of the XSNB plot as  $(DBH_{2017} - DBH_{2007})/DBH_{2007}$ . We used Pearson correlation tests to quantify whether focal individual RGR was correlated with the IGDAR and ISAR SES values. With respect to IGDAR, a positive correlation indicates that RGR was elevated when the individual was surrounded by distant relatives (i.e. positive SES values) and a negative correlation indicates that RGR was elevated when the individual was surrounded by close relatives (i.e. negative SES values). With respect to the ISAR, a positive correlation indicates that RGR was elevated when surrounded by a richer heterospecific neighbourhood (i.e. positive SES values) and a negative correlation indicates that RGR was elevated when the individual was surrounded by a poor heterospecific neighbourhood (i.e. negative SES values). The correlations were calculated across all neighbourhood radius scales.

Similar to the RGR analyses, we wanted to quantify whether different groups had different proportions of individuals that died over the 10-year interval. This was accomplished using logistic regression analyses where death (1) and survival (0) over the 10-year interval was the response and the SES values from the IGDAR and ISAR analyses were used as the independent variables in separate analyses.

## RESULTS

### Individual genetic diversity area relationships (IGDARs)

The proportion of individuals surrounded by closer relatives, individuals surrounded by more distant relatives and neutral individuals were similar for both all individuals and individuals with DBH less than or equal to 26 mm at all scales (Fig. 2). As the scale increased, the proportion of individuals

surrounded by closer relatives increased. About 40% of focal individuals were individuals surrounded by closer relatives at the scale of 50 m, while few individuals surrounded by more distant relatives were found at scales  $r$  from 1–50 m (Fig. 2). In contrast, the proportion of individuals surrounded by closer relatives for neighbourhood individuals with DBH greater than 26 mm increased relatively slow and with about 20% of focal individuals as individuals surrounded by closer relatives at the scale of 50 m.

### Relationship between IGDARs, ISARs and individual growth

The results from the Pearson correlation between RGR and IGDAR SES values generally had positive values (Fig. 3). Positive correlations indicate that RGR is higher in individuals surrounded by more distant relatives. However, when considering all individuals, this correlation was significant at one spatial scale. When considering individual  $> 26$  mm DBH, the correlations were not significant. When considering individuals  $\leq 26$  mm DBH, significant positive correlations were found at many scales (Fig. 3). We do note that the  $P$ -values (Table S1) from these analyses would not pass a strict Bonferroni  $P$ -value correction for multiple tests. We also note that the lack of a correlation at some spatial scales, particularly for large individuals, may be due to a lack of statistical power due to having few neighbouring conspecific individuals.

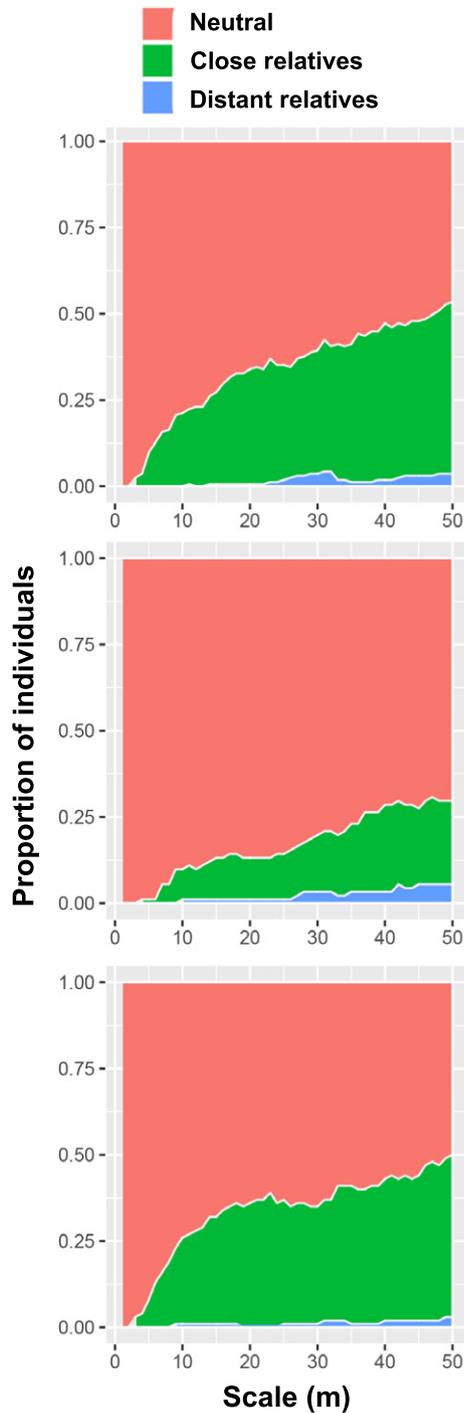
The Pearson correlation results for RGR and ISARs SES values generally found no significant correlations (Fig. 4). Specifically, the correlation values were slightly negative and the only significant value was at the second smaller spatial scale for focal individuals  $\leq 26$  mm DBH (Fig. 4). A negative correlation indicates higher RGR when surrounded by fewer heterospecific species. The  $P$ -values from these analyses are available in Table S2.

### Relationship between IGDARs, ISARs and individual death

Logistic regression was used to relate death (1) and survival (0) over the 10-year interval to the SES values from the IGDAR and ISAR analyses. In both cases, we found no significant relationships (Tables S3 and S4). In other words, the death of an individual was not related to whether it was surrounded by closely or distantly related conspecifics or whether it was surrounded by a rich or poor assemblage of heterospecifics.

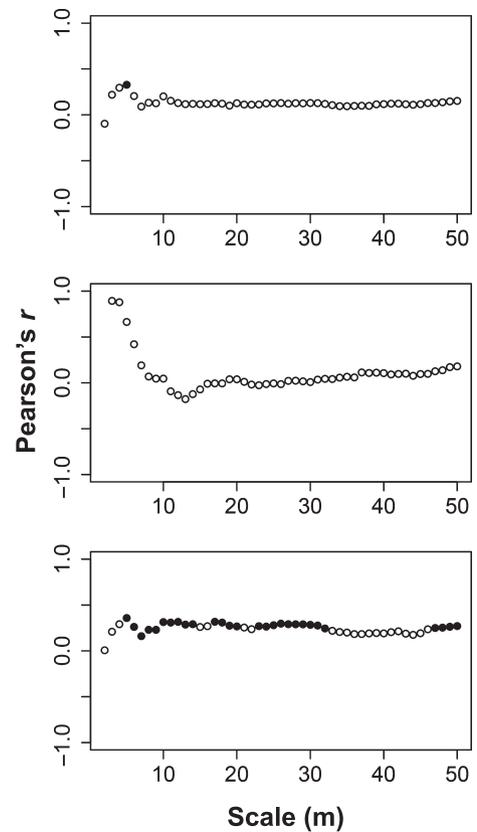
## DISCUSSION

Intra-specific negative density dependence promotes species coexistence by preventing a single species from forming a monoculture (Wright 2002). The pervasiveness of intra-specific negative density dependence in tree communities from the temperate zone to the tropics is now clear (Harms *et al.* 2000; Comita *et al.* 2014; Lamanna *et al.* 2017). Less clear is how intra-specific variation is empirically related to this important mechanism. The present work has focused on the spatial distribution of intra-specific genetic diversity in a natural population of a tropical tree and the demographic implications of related neighbours. We find that spatial clustering of



**Figure 2** The results from the individual genetic diversity area relationship (IGDAR) analyses for all stems (top), only considering stems > 26 mm diameter at breast height (DBH; middle), or only considering stems  $\leq$  26 mm DBH (bottom). The y-axis describes the proportion of individual trees that were identified as neutral individuals (red), individuals surrounded by close relatives (green) or individuals surrounded by more distant relatives (blue). The x-axis is the spatial scale of the neighbourhood.

genotypes is evident across spatial scales, particularly in smaller individuals, and that individuals that are surrounded by closely related conspecifics have lower relative growth rates

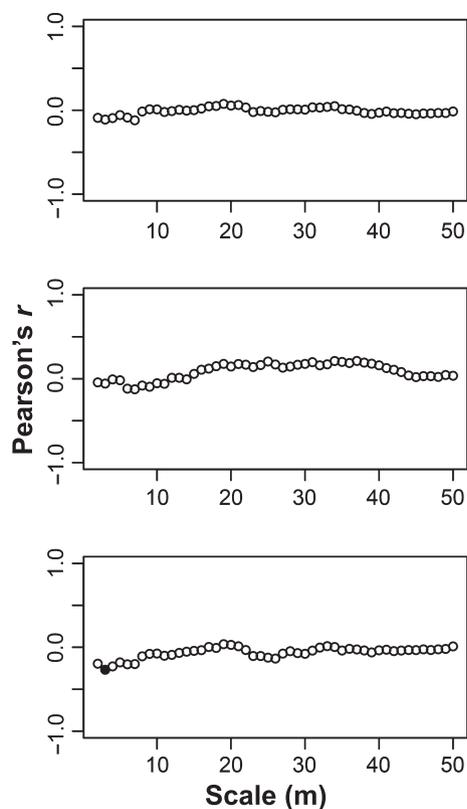


**Figure 3** Pearson correlations (y-axis) between the individual genetic diversity area relationship standardised effect sizes (SES) and *B. roxburghiana* relative growth rates across neighbourhood scales (x-axis). The top panel includes all individuals of *B. roxburghiana*, the middle panel is only individuals > 26 mm and the bottom panel is all individual  $\leq$  26 mm. Positive correlations indicate growth rates are elevated when individuals are surrounded by more distantly related than expected conspecifics (i.e. positive SES values). Negative correlations indicate growth rates are elevated when individuals are surrounded by more closely related than expected conspecifics (i.e. negative SES values). Significant correlations (i.e.  $P < 0.05$ ) are represented by black points and non-significant correlations are represented by white points.

than individuals surrounded by random or more diverse than expected conspecific neighbours. Furthermore, the species richness of neighbouring heterospecifics had no impact on the demographic rates of focal individuals. In sum, the work demonstrates that spatial aggregation of genotypes likely due to dispersal limitation comes with a demographic cost that should ultimately promote species coexistence. In the following, we discuss our results in more detail.

### Spatial aggregation of related individuals

We reinterpreted the method of calculating individual species area relationships (ISARs) (Wiegand *et al.* 2007, 2017) to quantify the accumulation of genetic diversity with distance from focal individuals and compared the levels of diversity to a null expectation (Fig. 1). The results of these analyses show that in spatial neighbourhoods with a radius 20 m or less the majority of individuals are surrounded by a genotypically random assemblage of conspecific individuals. Roughly 20–30%



**Figure 4** Pearson correlations ( $y$ -axis) between the individual species area relationship standardised effect sizes (SES) and *B. roxburghiana* relative growth rates across neighbourhood scales ( $x$ -axis). The top panel includes all individuals of *B. roxburghiana*, the middle panel is only individuals  $> 26$  mm and the bottom panel is all individual  $\leq 26$  mm. Positive correlations indicate growth rates are elevated when individuals are surrounded by more than expected heterospecific species (i.e. positive SES values). Negative correlations indicate growth rates are elevated when individuals are surrounded by less than expected heterospecific species (i.e. negative SES values). Significant correlations (i.e.  $P < 0.05$ ) are represented by black points and non-significant correlations are represented by white points.

of the individuals are surrounded by conspecific individuals that are more related than expected and  $< 10\%$  of the individuals were surrounded by a more diverse genotypic assemblage of conspecifics than expected (Fig. 2). The degree of genotype aggregation is stronger in trees  $\leq 26$  mm in diameter whereas non-random spatial distributions are far rarer for large trees ( $> 26$  mm in diameter) (Fig. 2). The degree of dispersal limitation and gene flow in tropical trees varies substantially across species (e.g. Nason *et al.* 1998; Hamilton 1999). The natural history of our focal species provides insights into why the presented patterns arose. Specifically, the species is pollinated by animals and dispersed by gravity and animals. Thus, it is expected that genotypes may cluster spatially, but animal vectors leave open the possibility of long-distance dispersal (Nason *et al.* 1998).

The decrease in spatial aggregation in genotypes in larger individuals is similar to that found for a tropical tree species in a Panamanian forest dynamics plot (Jones & Hubbell 2006). This static pattern of changes in genotypic diversity

across size classes may be tentatively used to infer population dynamics through time. Specifically, the results may indicate that genotypes are clustered early during ontogeny, likely due to dispersal limitation, and are non-randomly thinned through time. In other words, the static pattern could be evidence that intra-specific negative density dependence based upon relatedness is an important process governing population dynamics in this forest. In the next aspect of the study, we sought to directly quantify the relationship between neighbour relatedness and focal tree demographic performance to further elucidate this possibility.

#### Conspecific neighbours and individual demographic rates

Growth rates were correlated with the standardised effect size (SES) values from the individual genetic diversity area relationships (IGDAR; Fig. 1). We found that individuals surrounded by close relatives had significantly lower growth rates for trees  $\leq 26$  mm in diameter across most spatial scales (Fig. 3). Thus, trees surrounded by closely related conspecific individuals were at a demographic disadvantage. The results are consistent with expectations from intra-specific competition increasing with relatedness or depressed growth due to shared enemies between close relatives. We do not have evidence directly supporting one of these possibilities over the other. When considering all individuals together or only larger individuals ( $> 26$  cm in diameter), we found little-to-no evidence of a relationship between growth rates and the SES values from the IGDAR results (Fig. 3). This lack of a significant relationship may stem simply from having few large conspecific neighbouring individuals, particularly in small spatial neighbourhoods, which would reduce statistical power and any density dependent effects. Reduced growth and survival rates in tropical trees due to neighbouring conspecific individuals have been widely documented (e.g. Chen *et al.* 2017). However, there is little prior evidence showing that the degree of relatedness between a focal individual and its conspecific neighbours has an impact on growth rates.

A recent study from Ecuador focusing on a species in the palm genus *Oenocarpus* found that increased levels of relatedness between neighbouring seedlings negatively impacted survival rates (Browne & Karubian 2016). We examined whether an individual's survival during the 10-year study period was associated with the levels of conspecific relatedness in its neighbourhood. We found no correlation (Table S3). The apparent inconsistency between the results of our study and that of Browne & Karubian (2016) could be attributed to the different size classes studied. Negative density dependent mortality is typically strong in seedlings likely due to the importance of shared enemies at this fragile ontogenetic stage. Large individuals suffer lower rates of mortality due to neighbouring conspecifics, while negative effects on growth are still evident. However, we are unable to assign a mechanism to these observations in the present study. To determine the drivers of negative density dependence across the life cycle, future work should aim to examine the effects of relatedness across all ontogenetic stages and across multiple species in the same forest.

### Heterospecific neighbours and individual demographic rates

Niche differences are one of the key promoters of species coexistence (Chesson 2000). Specifically, if negative intra-specific interactions outweigh the negative interactions between heterospecific individuals, then coexistence will be promoted. Thus, we were interested in gauging the impact of neighbouring heterospecifics. We approximated these impacts by quantifying ISARs around each individual of *B. roxburghiana* in our system where we asked if an individual was surrounded by more, less or no different than expected numbers of species in neighbourhoods ranging from 1–50 m in radius. We found that the species richness of heterospecific neighbours around individuals of *B. roxburghiana* had no impact on their growth or death rates (Fig. 4; Table S4). We do note that heterospecific neighbour richness does not directly address whether one particular heterospecific species has a greater impact than a conspecific and weighting heterospecifics by their similarity may help elucidate such interactions. However, our previous work in this forest using neighbourhood modeling has found that heterospecifics weighted by their similarity have weak effects on focal tree performance and if they do have an effect it is in the opposite direction. That is, having similar heterospecific neighbours results in higher survival rates for the focal individual (Wu *et al.* 2016), which may be more important for performance differences and competitive exclusion than niche differences. In sum, present and past analyses indicate that heterospecific negative interactions are far weaker in this forest and that the strong negative intra-specific interactions affiliated with genetic relatedness likely play a dominant role in regulating populations and, therefore, promoting coexistence.

### CONCLUSIONS

Species coexistence is more likely when the negative demographic impact of conspecifics exceeds that of heterospecifics (Chesson 2000). Furthermore, theory predicts that low levels of intra-specific functional trait or genetic diversity will promote coexistence by reducing the strength of inter-specific interactions and increasing the strength of intra-specific interactions (Hart *et al.* 2016). If the traits that dictate negative interactions have a strong genetic component, then it is expected that the degree of relatedness between neighbouring conspecifics will have demographic impacts influencing species coexistence. Here, by genotyping a natural population of a tropical tree and monitoring demographic rates over a decade we have shown that genotypes are often clustered spatially and those trees surrounded by very closely related conspecifics grow more slowly in smaller size classes. Thus, we have uncovered three pieces of evidence that should promote species coexistence in a hyper-diverse tropical tree community—dispersal limitation, low intra-specific diversity in local neighbourhoods and negative demographic consequences of closely related neighbours. Future work should seek to examine multiple species across ontogeny in the same community to determine the generality of these findings and the degree to which intra-specific diversity is indeed a major driver of species coexistence. Additionally, while we believe the work

demonstrates the power of examining conspecific relatedness in tropical tree communities and such studies will become more common as sequencing costs and logistical barriers erode, a critical next goal is to determine why relatedness matters. That is, linking extraordinarily popular functional trait approaches, that often ignore intra-specific variation, to community genetics approaches should be a priority (Crutsinger 2016).

### ACKNOWLEDGEMENTS

This study was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDPB0203), the National Key Basic Research Program of China (Grant No. 2014CB954100), the National Natural Science Foundation of China (31370445, 31570430, 31370267), the Southeast Asia Biodiversity Research Institute of Chinese Academy of Sciences (Grant No. 2015CASE-ABRI004) and the CAS 135 program (No. 2017XTBG-T01). We are grateful to the people who have contributed to the establishment of the 20-ha Xishuangbanna tropical seasonal rainforest dynamics plot, and Ms. Jingjing Li who contributed to the measurement of DBH for all focal individuals in 2017. Logistical support was provided by Xishuangbanna Station of Tropical Rainforest Ecosystem Studies (National Forest Ecosystem Research Station at Xishuangbanna), Chinese Academy of Sciences. We thank the two anonymous reviewers for providing insightful comments to the manuscript. NGS was funded by NSF US-China Dimensions of Biodiversity grants (DEB-1241136, DEB-1046113).

### AUTHORSHIP

LL and NGS designed the study, XS, CB, LL and NGS performed spatial and demographic analysis, LL and QL collected genetic data and analyzed genetic diversity, MC provided the plot census data, XS, SJW, LL and NGS wrote the manuscript, and XS, SJW, LL and NGS contributed to revisions.

### DATA ACCESSIBILITY STATEMENT

The data supporting the results are archived at Dryad <https://doi.org/10.5061/dryad.hp77gb5>

### REFERENCES

- Aldrich, P.R., Hamrick, J.L., Chavarriaga, P. & Kochert, G. (1998). Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. *Molec. Ecol.*, *7*, 933–944.
- Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M. *et al.* (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecol. Evol.*, *26*, 183–192.
- Browne, L. & Karubian, J. (2016). Frequency-dependent selection for rare genotypes promotes genetic diversity of a tropical palm. *Ecol. Lett.*, *19*, 1439–1447.
- Cao, M., Zhu, H., Wang, H., Lan, G., Hu, Y., Zhou, S. *et al.* (2008). *Xishuangbanna Tropical Seasonal Rainforest Dynamics Plot: Tree Distribution Maps, Diameter Tables and Species Documentation*. Yunnan Science and Technology Press, Kunming.

- Chen, L., Mi, X., Comita, L.S., Zhang, L., Ren, H. & Ma, K. (2010). Community-level consequences of density dependence and habitat association in a subtropical broad-leaved forest. *Ecol. Lett.*, 13, 695–704.
- Chen, L., Comita, L.S., Wright, S.J., Swenson, N.G., Zimmerman, J.K., Mi, X. *et al.* (2017). Forest tree neighborhoods are structured more by negative conspecific density dependence than by interactions among closely related species. *Ecography*. <https://doi.org/10.1111/ecog.03389>.
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Ann. Rev. Ecol. Syst.*, 31, 343–366.
- Comita, L.S., Muller-Landau, H.C., Aguilar, S. & Hubbell, S.P. (2010). Asymmetric density dependence shapes species abundance in a tropical tree community. *Science*, 329, 330–332.
- Comita, L.S., Queenborough, S.A., Murphy, S.J., Eck, J.L., Xu, K., Krishnadas, M. *et al.* (2014). Testing predictions of the Janzen-Connell hypothesis: a meta-analysis of experimental evidence for distance and density-dependent seed and seedling survival. *J. Ecol.*, 102, 845–856.
- Crutsinger, G.M. (2016). A community genetics perspectives: opportunities for the coming decade. *New Phytol.*, 210, 65–70.
- Dayanandan, S., Dole, J., Bawa, K. & Kesseli, R. (1999). Population structure delineated with microsatellite markers in fragmented populations of a tropical tree, *Carapa guianensis* (Meliaceae). *Molec. Ecol.*, 8, 1585–1592.
- Dieringer, D. & Schlötterer, C. (2003). Microsatellite analyser (msa): a platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Res.*, 3, 167–169.
- Doyle, J.J. (1991). DNA protocols for plants—CTAB total DNA isolation. In: *Molecular Techniques in Taxonomy*. (eds Hewitt, G.M., Johnston, A.). Springer-Verlag, Berlin, Germany, pp. 283–293.
- Gotelli, N.J. & Graves, G.R. (1996) *Null Models in Ecology*. Smithsonian Institution, Washington, D.C., USA.
- Hamilton, M.B. (1999). Tropical tree gene flow and seed dispersal. *Nature*, 401, 129–130.
- Harms, K.E., Wright, S.J., Calderon, O., Hernandez, A. & Herre, E.A. (2000). Pervasive density-dependent recruitment enhances seedling diversity in a tropical forest. *Nature*, 404, 493–495.
- Hart, S.P., Schreiber, S.J. & Levine, J.M. (2016). How variation between individuals affects species coexistence. *Ecol. Lett.*, 19, 825–838.
- Jones, F.A. & Hubbell, S.P. (2006). Demographic spatial genetic structure of the Neotropical tree, *Jacaranda copaia*. *Molec. Ecol.*, 15, 3205–3217.
- Kunstler, G., Falster, D., Coomes, D.A., Hui, F., Kooyman, R.M., Laughlin, D.C. *et al.* (2016). Plant functional traits have globally consistent effects on competition. *Nature*, 529, 204–207.
- Lamanna, J.A., Mangan, S.A., Alonso, A., Bourg, N.A., Brockelman, W.Y., Bunyavejchewin, S. *et al.* (2017). Plant diversity increases with the strength of negative density dependence at the global scale. *Science*, 356, 1389–1392.
- Levin, S.A. (1970). Community equilibria and stability, and an extension of the competitive exclusion principle. *Amer. Nat.*, 104, 413–423.
- Levine, J.M. (2016). Ecology: a trail map for trait-based studies. *Nature*, 529, 163–164.
- Li, H. (1982). *Flora Reipublicae Popularis Sinicae*. (in Chinese) Science Press, Beijing, China, vol. 31, 133–134.
- Lin, L., Comita, L.S., Zheng, Z. & Cao, M. (2012). Seasonal differentiation in density-dependent seedling survival in a tropical rainforest. *J. Ecol.*, 100, 905–914.
- Liu, L., Wang, Z., Li, Q., Ye, W., Cao, H. & Wang, Z. (2013). Isolation and characterization of microsatellite markers in *Beilschmiedia roxburghiana* (Lauraceae). *App. Plant Sci.*, 1, 81–92.
- Lotka, A.J. (1920). Analytical note on certain rhythmic relations in organic systems. *Proc. Nat. Acad. Sci. U.S.A.*, 6, 410–415.
- MacArthur, R.H. & Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *Amer. Nat.*, 101, 377–385.
- Nason, J.D., Herre, E.A. & Hamrick, J.L. (1998). The breeding structure of a tropical keystone plant resource. *Nature*, 391, 685–687.
- Swenson, N.G. (2012). The functional ecology and diversity of tropical tree assemblages through space and time: from local to regional and from traits to transcriptomes. *ISRN Forestry*, 2012, 743617.
- Swenson, N.G. (2013). The assembly of tropical tree communities - the advances and shortcomings of phylogenetic and functional trait analyses. *Ecography*, 36, 264–276.
- Uriarte, M., Swenson, N.G., Chazdon, R.L., Comita, L.S., John, K.W., Erickson, D. *et al.* (2010). Trait similarity, shared ancestry, and the structure of neighborhood interactions in a subtropical wet forest: implications for community assembly. *Ecol. Lett.*, 13, 1503–1514.
- Volterra, V. (1926). Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. *Mem. Acad. Lincei*, 2, 31–113.
- Wang, X., Wiegand, T., Swenson, N.G., Wolf, A.T. & Howe, R.W. (2015). Mechanisms underlying local functional and phylogenetic beta diversity in two temperate forests. *Ecology*, 96, 1062–1073.
- Wiegand, T., Gunatilleke, C.V.S., Gunatilleke, N. & Huth, A. (2007). How individual species structure diversity in tropical forests. *Proc. Nat. Acad. Sci. U.S.A.*, 104, 19029–19033.
- Wiegand, T., Uriarte, M., Kraft, N.J.B., Shen, G., Wang, X. & He, F. (2017). Spatially explicit metrics of species diversity, functional diversity, and phylogenetic diversity: insights into plant community assembly processes. *Ann. Rev. Ecol. Evol. Syst.*, 48, 329–351.
- Wright, S.J. (2002). Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia*, 130, 1–14.
- Wu, J., Swenson, N.G., Brown, C., Zhang, C., Yang, J., Ci, X. *et al.* (2016). How does habitat filtering affect the detection of conspecific and phylogenetic negative density dependence? *Ecology*, 97, 1182–1193.
- Yang, J. (2013). A phylogenetic perspective on the individual species-area relationship in temperate and tropical tree communities. *PLoS ONE*, 8, e63192.
- Zhu, K., Woodall, C.W., Monteiro, J.V.D. & Clark, J.S. (2015). Prevalence and strength of density-dependent tree recruitment. *Ecology*, 96, 2319–2327.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information section at the end of the article.

Editor, Jonathan Chase

Manuscript received 19 December 2017

First decision made 7 February 2018

Second decision made 12 April 2018

Manuscript accepted 16 April 2018