Differential impacts of adult trees on offspring and non-offspring recruits in a subtropical forest

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An important mechanism promoting species coexistence is conspecific negative density dependence (CNDD), which inhibits conspecific neighbors by accumulating host-specific enemies near adult trees. Natural enemies may be genotype-specific and regulate offspring dynamics more strongly than non-offspring, which is often neglected due to the difficulty in ascertaining genetic relatedness. Here, we investigated whether offspring and non-offspring of a dominant species, *Castanopsis eyrei*, suffered from different strength of CNDD based on parentage assignment in a subtropical forest. We found decreased recruitment efficiency (proxy of survival probability) of offspring compared with non-offspring near adult trees during the seedling-sapling transition, suggesting genotype-dependent interactions drive tree demographic dynamics. Furthermore, the genetic similarity between individuals of same cohort decreased in late life history stages, indicating genetic-relatedness-dependent tree mortality throughout ontogeny. Our results demonstrate that within-species genetic relatedness significantly affects the strength of CNDD, implying genotype-specific natural enemies may contribute to population dynamics in natural forests.

genetic relatedness, conspecific negative density dependence, Janzen-Connell hypothesis, species coexistence, forest dynamics plot, subtropical forest

INTRODUCTION

As one of the key theories explaining species coexistence in community ecology, the Janzen-Connell hypothesis (JCH) has been addressed in various communities over half a century (Connell, 1971; Janzen, 1970; Petermann et al., 2008; Terborgh, 2020; Wright, 2002). The JCH explains plant species coexistence by the suppression of conspecific

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recruits near adults due to attacks of specialist natural enemies that are attracted by those adults and thus by freeing up space for heterospecific individuals (Connell, 1971; Janzen, 1970). Empirical evidence shows that high densities of conspecific adult neighbors can reduce seedling survival (Chen et al., 2019; Eisenhauer, 2022; Johnson et al., 2014; Liu and Zhang, 2020; Qin et al., 2020; Zhu et al., 2015), which is also known as conspecific negative density dependence (CNDD; Bagchi et al., 2014; Harms et al., 2000; Johnson et al., 2012). Since pathogens and pests may be genotype-specific during the co-evolutionary process with host plants (e.g., Bergelson et al., 2001; Price et al., 2004), conspecific neighbors from the same or different genotypes may have different strength of CNDD on the target seedling. While it has also been suggested that parent trees may exert stronger negative effects on their offspring than non-offspring (Eck et al., 2019), this issue remains largely unexplored in natural forests due to the difficulty in assessing the parent-offspring relationship between individuals within natural populations.

In contrast to the implicit assumption in most previous studies that all recruits are equally impacted by their conspecific neighboring adults (Johnson et al., 2014; Murphy et al., 2017; Qin et al., 2020; Zhu et al., 2015), recent studies indicate that the strength of CNDD does depend on the genetic relatedness between focal seedlings and nearby conspecific trees (Eck et al., 2019; Liu et al., 2015). For example, shade-house experiments suggested that seedlings growing in soils from putative mother trees or closely related conspecifics had reduced performance and this negative effect disappeared after soil sterilization (Eck et al., 2019; Liu et al., 2015), suggesting that genotype-specific soil pathogens mediate within-species negative effect from parent trees. Similarly, Shao et al. (2018) also found that the growth rate of focal trees in an early life stage was reduced when surrounded by closely related conspecific individuals in a tropical forest. However, although the influence of genetic relatedness on CNDD has been documented in some studies, the potential role of genetic relatedness in regulating the recruitment pattern of natural populations remains unclear.

The ratio of offspring vs. non-offspring in the vicinity of adult trees may be vital for the strength of CNDD due to the presence of genotype-specific natural enemies. Compared with non-offspring, offspring may share much more genotype-specific natural enemies with their parent trees and suffer stronger negative parent-tree effects. Thus, incorporating parentage analysis and genetic relatedness among individuals with a fully mapped tree census dataset will provide new insights into the role of genotype-specific natural enemies in promoting species diversity. However, few studies have directly identified the parent-offspring relationship for tree species in natural forests to test the recruitment pattern of offspring as Janzen-Connell hypothesis

suggested (Steinitz et al., 2011).

Here, we investigated whether the offspring- and non-offspring recruits suffered different effects from conspecific adults in a subtropical forest. To attain this goal, we used 12 microsatellite markers to quantify the genetic relatedness (i. e., parent-offspring relationship) for seedlings, saplings, juveniles and adults of the most dominant tree species *Castanopsis eyrei* (Fagaceae; Figure S1 in Supporting Information) in a 24-ha subtropical evergreen broad-leaved forest dynamics plot (FDP). We hypothesize that (1) the distance of the peak recruitment of offspring will shift away from parent trees with ontogeny due to high mortality of offspring near the parent tree during the seedling-sapling and sapling-juvenile transitions (Hypothesis 1, Figure 1A), (2) offspring recruits will suffer from a stronger negative impact near their parent trees than non-offspring recruits due to the genetic-relatedness-dependent CNDD (Hypothesis 2, Figure 1B), and (3) genetic similarity between individuals of same cohort will decrease across life stages because of genetic-relatedness-dependent tree mortality (Hypothesis 3).

RESULTS

Shifts in the offspring recruitment distance with life stages

Overall, 400 mother trees were assigned to 1,204 offspring (268 seedlings, 388 saplings, 548 juveniles, Table S1 in Supporting Information) of which 864 were assigned to a single parent and 340 offspring to parent pairs with the nearest parent considered as the mother tree while with the other parent as the father tree. The offspring recruitment patterns within 80 m from mother trees significantly shifted from seedling to sapling rather than from sapling to juvenile (Figure 2). These patterns were evident as sampling area showed no significant effects on recruitment patterns across five sampling scales for both sapling offspring ($F_{4,773}$=1.04, P=0.400) and juveniles ($F_{4,1047}$=0.451, P=0.772).

The mean recruitment distances of seedlings, saplings and juveniles were 40.6±2.6 m (SE) (range 1.4–253.4 m), 60.6± 2.7 m (2.3–312.3 m) and 59.1±2.1 m (1.4–264.8 m), respectively (Figure S2 in Supporting Information). The recruitment distance significantly increased from seedling to sapling stage (Tukey multiple-comparisons test, P<0.001), but did not differ between saplings and juveniles (Tukey multiple-comparisons test, P=0.881). The distances of peak recruitment increased from 11.0 m for seedlings to 21.4 and 23.9 m for saplings and juveniles (Figure S2 in Supporting Information).

For the offspring recruitment distance from father tree, 340 offspring were assigned to 188 fathers with a mean distance of 77.8 m (Figure S3 in Supporting Information). The father-offspring distance did not differ between seedlings and
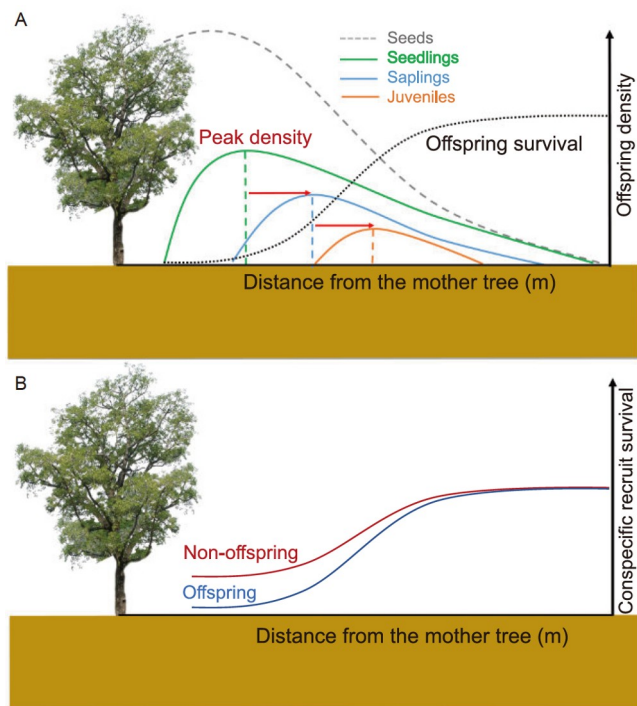
Figure 1 The expected consequences of negative effect from parent trees on offspring and non-offspring mediated by genotype-specific natural enemies. A, The unimodal recruitment curves of offspring seedlings (green), saplings (blue) and juveniles (orange) as a function of distance from the parent trees. The unimodal recruitment curves are results of the probability of effective seed dispersal (dashed line) and the probability of survival (dotted line) of offspring. B, The different survival probability of offspring (red) and non-offspring (blue) as a function of distance from the parent trees.

saplings (Tukey multiple-comparisons test, P=0.053), saplings and juveniles (Tukey multiple-comparisons test, P=0.167). The effective pollen dispersal distance from father tree to mother tree of 340 offspring ranged from 4.7–335.8 m with a mean of 47.2 m (Figure S4 in Supporting Information).

Adult effects on offspring and non-offspring

In the three core areas, 854 offspring (201 seedlings, 270 saplings, 383 juveniles) were assigned to 212 mother trees, including 206 offspring assigned to paired parents. The offspring/non-offspring ratio was higher for seedlings as compared with saplings and juveniles within 20 m to the mother tree (Figure S5A in Supporting Information). The offspring/non-offspring ratio was also higher for seedlings as compared with saplings and juveniles within 20 m to the father tree (Figure S5B in Supporting Information), but the differences were quite small (only from 0.011 to 0.013). Considering there were only 26 offspring (18 seedlings, 4 saplings, and 4 juveniles) within 20 m to the father tree and the effects from the mother tree (the mean distances of the mother and father tree to 26 offspring were 7.6 m and

13.0 m, respectively) could not be excluded, only the effect of mother trees on offspring and non-offspring was evaluated in further analyses.

Although the patterns of recruitment efficiency (RE, the recruit ratio of early life stage to the later) varied with Core Plots (Figure S6 in Supporting Information), genetic relatedness (offspring and non-offspring) and life stages were important in determining the overall REs (Figure 3). For the seedling-sapling transition, the REs of both offspring and non-offspring increased with the distance from the focal trees as predicted by the JCH and the REs of offspring were significantly lower than that of non-offspring within 40 m from the focal tree Figure 3A and Figure S7A in Supporting Information, P<0.01 for all distance groups except P<0.05 for the 0–5 m group when removing effects from non-offspring's own mother trees and the 20–40 m group when all non-offspring were included). For the sapling-juvenile transition, there were no significant difference in REs between offspring and non-offspring along the distance from mother trees except for the 10–20 m and 40–80 m distance classes (Figure 3B and Figure S7B in Supporting Information).

Changes in genetic structure across life stages

Spatial genetic structures with significantly positive autocorrelations were observed across life stages at distance classes up to 40 m (P=0.001, Figure 4) except for seedlings in the 5–10 m distance class. The strength of spatial genetic autocorrelation between recruits decreased with distance for all life stages. Seedlings presented a significantly stronger spatial genetic structure than that of saplings (t^2=23.115, P=0.001) and juveniles (t^2=39.710, P=0.001) in the 0–5 m distance class (Table S2 in Supporting Information).

DISCUSSION

Our results of the present study on the dominant tree species *C. eyrei* support all the three hypotheses, that is, (1) peak recruitment distances of offspring shift away from mother trees over life stages, (2) offspring have significantly lower recruitment efficiencies than non-offspring in the vicinity of mother trees, and (3) genetic similarity between recruits within 5 m distance significantly decreases from seedlings to saplings and juveniles. These results provide clear evidence that genetic relatedness can influence the strength of CNDD.

Seed dispersal is an important determinant in seedling establishment, affecting the genetic structure and diversity of plant populations (Berens et al., 2013; Dow and Ashley, 1996; Hardy et al., 2019; Harms et al., 2000). Escaping away from parent trees and other conspecific adults may increase the probability of per capita seedling recruitment and survival when strong CNDD is present (Harms et al., 2000). In
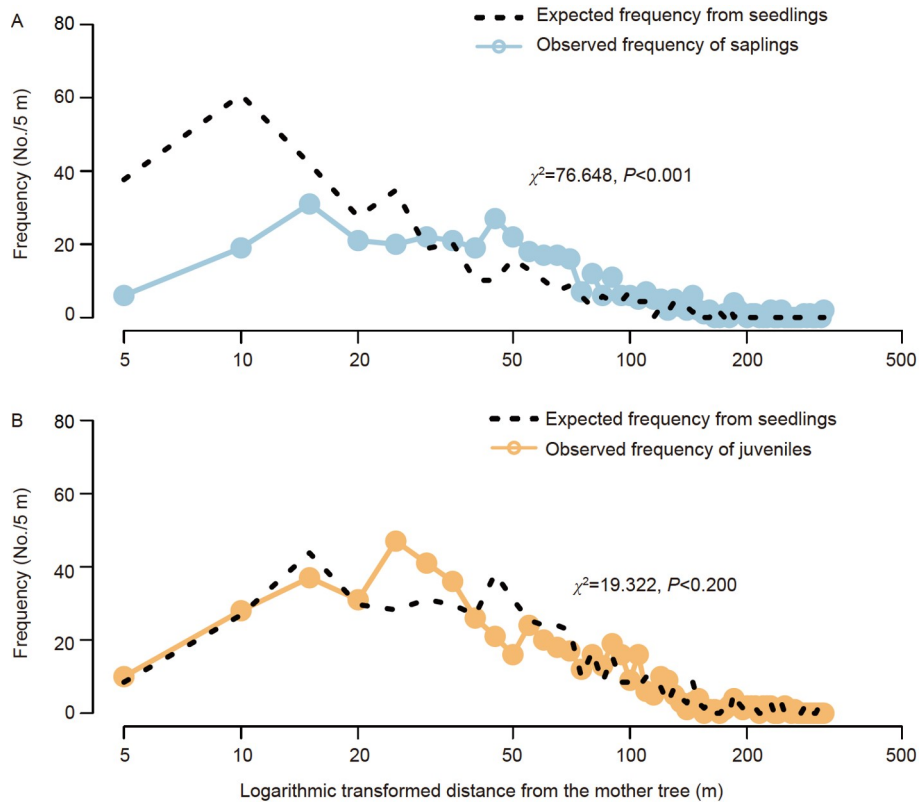
Figure 2 Recruitment pattern shifts across life stages for offspring of *Castanopsis eyrei*. A, Observed (blue) vs. expected (black) sapling distribution estimated from the seedling distribution. B, Observed (orange) vs. expected (black) juvenile recruitment distribution estimated from the sapling distribution.

our study, the recruitment distance of *C. eyrei* to the parent tree in the forest plot increased from seedlings to late life stages. The peak recruitment distances of offspring were much larger than the mean crown diameter of adult *C. eyrei* (~7 m, pers. obs.) (Figure S2 in Supporting Information). Given that 25.4%, 3.4%, and 3.3% of seedling, sapling, and juvenile offspring distributed within 10 m to the mother tree, it seems that seedlings recruiting near their mother trees have a high mortality rate.

Genotype-specific natural enemies (e.g., pathogens) together with dispersal limitation have been recently suggested as potential key drivers in promoting coexistence of plant species (Comita and Stump, 2020; Eck et al., 2019; Liu et al., 2015; Stump and Comita, 2020). Assuming dispersal limitation of genotype-specific natural enemies and that they cannot hitchhike on the dispersal units of plants, it is likely that they find offspring close to parent trees more easily than further away. Furthermore, because enemies are genotype-specific at least to some degree, non-offspring recruits close to conspecific adults should be less attacked. In our study, we found a stronger CNDD on offspring near the parent tree relative to non-offspring for *C. eyrei* during the seedling-sapling transition (Figure 3A, Figure S5A and Figure S7A in Supporting Information), implying genotype-specific natural enemies may have been responsible for the observed spatial pattern of tree recruitment in natural forests. Our findings are

in line with results from previous studies which showed that soil-borne pathogens from closely related conspecifics may reduce seedling performances (Eck et al., 2019; Liu et al., 2015). Our study elucidated the parent-offspring relationship for individuals at multiple spatial scales and life stages with explicit spatial information, which allowed us to detect an obvious reduction of recruitment efficiencies of offspring across life stages. The calculation of REs in the present study is based on static data from only one survey and further long-term monitoring on offspring and non-offspring cohorts will be helpful to find the real recruitment efficiencies of those cohorts and their responses to CNDD.

There are two possible mechanisms for the observed within-species genetic-relatedness-dependent CNDD. First, trees of different genotypes can host specific pathogens (Cordier et al., 2012; Purahong et al., 2016) and have different pathogen susceptibilities (Bruns et al., 2012), and the shared pathogens and similar pathogen susceptibility between parents and offspring will account for genetic-relatedness-dependent CNDD. Second, it may be that the genetically related recruits tended to utilize similar resources and offspring may face stronger competition from parent trees and siblings than non-offspring (Cheplick, 1992). Similar to competitive exclusion of ecologically similar species in local habitats (Webb et al., 2002), the stronger competition between genetically related offspring and parent
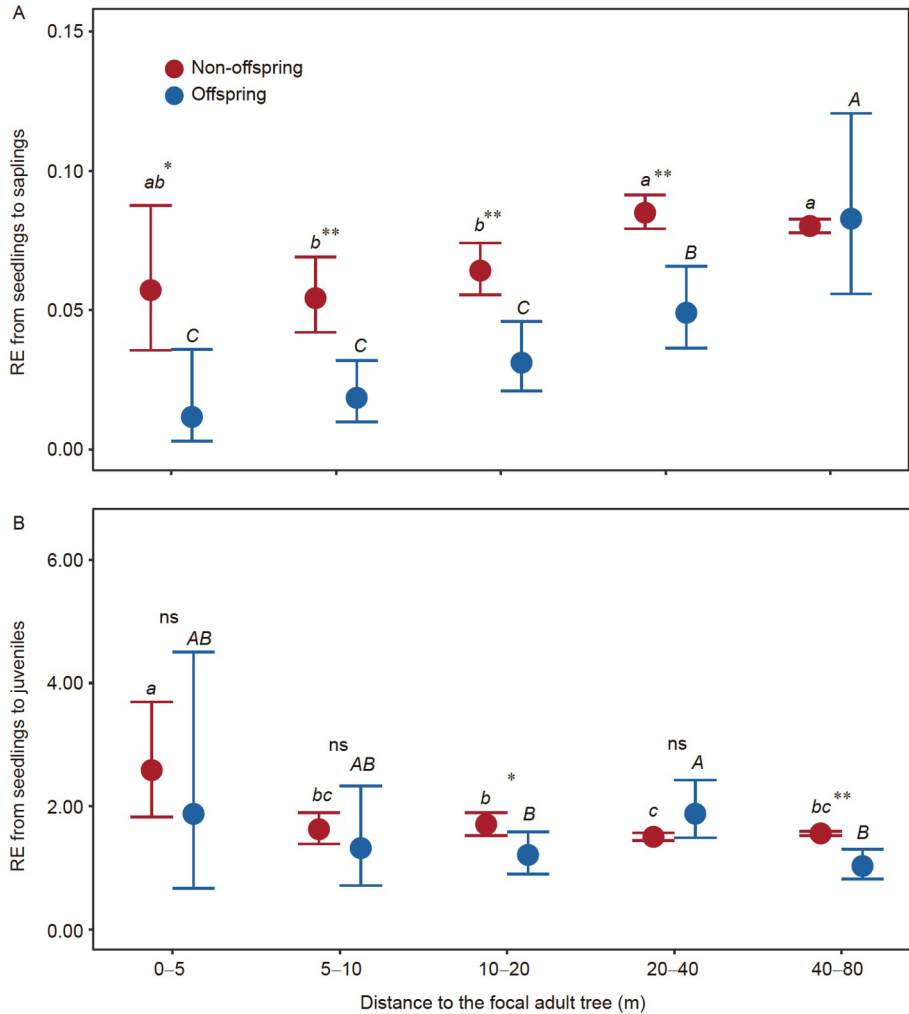
Figure 3   Recruitment efficiency (RE, the recruit ratio of early life stage to the later) of seedling-sapling (A) and sapling-juvenile (B) transitions along the distance from the focal trees for *Castanopsis eyrei* (removing effects from non-offspring's own mother trees). The blue and red solid circles are mean REs of offspring and non-offspring, respectively, in each distance class generated by 10,000 bootstrap replicates. Only non-offspring whose own mother trees located at ≥80 m away were considered. The error bars are 95.0% bootstrap confidence intervals. Difference in REs between offspring and non-offspring is significant if their 95.0%, 99.0% or 99.9% confidence intervals do not overlap the other's mean separately. The asterisks above error bar represents significant differences between offspring and non-offspring at α=0.05 (*), 0.01 (**) or 0.001 (***) based on 95.0%, 99.0% or 99.9% confidence intervals, respectively. Different upper and lower-case letters indicate significant differences between distance groups for offspring and non-offspring at α=0.05, respectively.

trees will lead to a lower RE near parent trees. Although both mechanisms may contribute, the first interpretation is supported by shade-house experiments with tropical and subtropical trees in previous studies (Eck et al., 2019; Liu et al., 2015) and it is also suggested that competition for resources among plant individuals are quite weak during the seedling stage (Forrister et al., 2019; Paine et al., 2008).

The changes of spatial genetic structure in a plant population across life stages may provide insights into the roles of gene dispersal, micro-environmental selection, and mating patterns in shaping spatial genetic pattern and help to understand the mechanisms driving population dynamics (Jones and Hubbell, 2006; Michalski and Durka, 2007). In our study, *C. eyrei* displays a significant decrease in genetic similarity for the seedling-sapling transition (Figure 4). This

result is consistent with changes of spatial genetic structure of a tropical tree species *Prunus africana*, with decreased spatial genetic structure from seed and seedling to older juvenile stages (Berens et al., 2014). The low RE of offspring and decreased genetic similarity for the seedling-sapling transition near the mother trees will, in turn, enable seedlings of genotype distinct to the adult tree have higher survival rates and maintain a higher genetic diversity in the overall tree population. Considered that high genetic diversity in a population is helpful for plant adaptation to various abiotic environments and could also dilute and inhibit genotype specific pathogens (Ehlers et al., 2016; Leimu et al., 2006), the genetic-relatedness-dependent CNDD found in our study may enhance plant adaptation to both abiotic and biotic environments through the enhanced population genetic di-
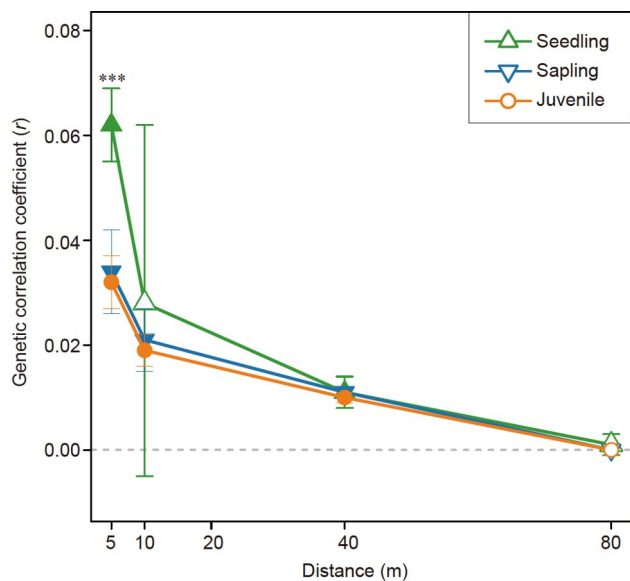
Figure 4 Correlogram of spatial genetic structure for recruits of *Castanopsis eyrei* in three life stages, seedlings (green line), saplings (blue line) and juveniles (orange line), in a subtropical forest. Data points represent the observed genetic correlation coefficient (*r*) between paired individuals within each distance class. Error bars indicate the 95% confidence intervals estimated from bootstrapping 1,000 times. Solid symbols represent significant spatial genetic structure in the distance class for each life stage (*P*=0.001). The asterisks above error bar represents significant differences between seedling- and two later life stages in 0–5 m distance class

versity.

Overall, our findings provide field evidence for the relationship between genetic relatedness and neighborhood interactions, namely, that the effects of CNDD between plants have a strong "genetic signal". Our results indicate that CNDD depends on both genetic relatedness and spatial distance to conspecific adults, and the strength of that dependence and the consequent negative effects decreased from seedling to later life stages. These results coupled with other studies focused on seedling performance with different soil sources (Eck et al., 2019; Liu et al., 2015) imply that the genetic relatedness of plant hosts, genotype-specific enemies, and seed dispersal ability may jointly regulate the strength of CNDD. Further research on the interaction between genotype-specific enemies and their host plants is essential to fully understand the underlying mechanisms of CNDD.

MATERIALS AND METHODS

Study site

The study was conducted in a 24-ha subtropical evergreen broad-leaved forest dynamics plot (FDP; 29°15′6″–29°15′21″ N, 118°07′1″–118°07′24″ E; 446.3–714.9 m altitude) at Gutianshan National Nature Reserve in Zhejiang province, China (Legendre et al., 2009). This FDP is part of the Chinese Forest Biodiversity Monitoring Network (CForBio) and

the Forest Global Earth Observatory (ForestGEO; Davies et al., 2021). The FDP is 400 m×600 m in area and divided into 600 grids of 20 m×20 m. All tree individuals with DBH (diameter at breast height) ≥1 cm were tagged, measured, spatially mapped and identified to species since 2005 and censused every 5 years (Legendre et al., 2009). Seedling plots (5 m×5 m each) in every second 20 m×20 m grid were set in 2012, where all seedlings with DBH<1 cm and height ≥10 cm were tagged, measured, and mapped.

Sampling and DNA extraction

Castanopsis eyrei (Champ. ex Benth.) Tutch. (Fagaceae) is an evergreen, monoecious and anemophilous dominant tree species (Huang et al., 1999; Mao et al., 2016) that grows up to 35 m and has a maximum recorded DBH of 95 cm in the FDP. *C. eyrei* usually begins to produce seeds at DBH >14 cm (pers. obs.) and more than 10,700 individuals with DBH ≥1 cm were recorded in the FDP in 2010. Seeds of *C. eyrei* are dispersed by gravity and animals (Du et al., 2009). In June and July of 2014, we collected leaf tissue from all mapped individuals within three core plots (core 1: 100 m× 100 m, core 2 and 3: 120 m×120 m; Figure S1 in Supporting Information), which represent three main habitat types (i.e., low valley, low ridge and high ridge; Chen et al., 2010). In order to include as much parents as possible, additional adults and seedlings in extended area were also collected (Figure S1 in Supporting Information). See Appendix S1 in Supporting Information for additional context. In total, we sampled 3,054 individuals, including 1,224 adults (DBH >14 cm), 871 juveniles (2 cm<DBH ≤14 cm), 585 saplings (1 cm≤DBH≤2 cm), and 374 seedlings. All leaves were dried with silica gel before DNA extraction.

We extracted DNA from dry leaf tissue using the Tiangen Plant Genomic DNA kit (TIANGEN Biotech Co., Ltd, Beijing, China) and performed PCRs according to a modified protocol described by Ueno et al., (2009), using 12 nuclear microsatellite markers (Table S3 in Supporting Information). All PCR products were analyzed using an ABI 3730 sequencer (Applied Biosystems, USA) and the alleles were scored using GeneMapper Version 4.0 (Applied Biosystems, Beijing, China).

Genetic analyses

As *C. eyrei* has a strong sprouting capability (Ye et al., 2017), we identified root sprouts (clonal ramets) using the R package RClone (Arnaud-haond and Belkhir, 2007) while considering the identity analysis results conducted in CERVUS 3.0.7 (Kalinowski et al., 2007; Appendix S1 in Supporting Information). Fifty-two putative root sprouts were removed and a total of 3,002 individuals (366 seedlings, 576 saplings, 857 juveniles and 1,203 adults) were included in

the downstream analyses. The number of alleles ranged from 12 to 43 across the 12 microsatellite markers, giving a total of 285 alleles and low null allele frequencies (0 to 0.0969, Table S3 in Supporting Information) by using CERVUS 3.0.7 (Kalinowski et al., 2007). The combined exclusion probabilities across the 12 microsatellite loci for the first parent and parent pair were both 0.9999, which validates the effectiveness of markers used in the parentage analyses. The parentage analyses were performed using CERVUS 3.0.7 (Kalinowski et al., 2007). The parent-pairs and single parents were assigned based on critical values of LOD (the logarithm of the likelihood-odds ratio; Kalinowski et al., 2007) obtained through simulations at a 99% confidence level. The simulation parameters were as follows: 100,000 runs, 60% sampled candidate parents, and a 5% genotyping error rate. All 1,203 adult trees were used as candidate parents and all other parameters of simulations were set as default values. A single identified parent and the closer parent within an identified parent pair was considered as the mother tree, since seed dispersal in general is strongly declining with distance and offspring are more likely to grow around their mother trees (Millerón et al., 2013).

Statistical analyses

To test if the offspring recruitment patterns shifts away from their mother trees across ontogeny (seedlings, saplings, and juveniles) as the Janzen-Connell hypothesis predicts, we first calculated the frequency distribution of offspring from the mother tree with 5 m distance intervals for each life stage. The proportion of seedlings in each distance group was then multiplied by the total number of saplings to obtain an "expected" number of saplings in each distance group based on the simple assumption that if all seedlings are equal, sapling density should be directly proportional to seedling density (Swamy et al., 2011). Similarly, the "expected" number of juveniles in each distance group were obtained by multiplying the total number of juveniles by the proportion of saplings in each distance group. The expected frequency distribution of offspring within 80 m was compared with the observed distribution using a chi-square test. We then fitted recruitment kernels for the three life stages with Gaussian kernel using the density function in the R package stats, separately. Tukey multiple-comparisons were performed to compare the differences of recruitment distances (mother-offspring distances) and father-offspring distances among life stages using the TukeyHSD function in the R package stats. To test the influence of sampling scales on the recruitment pattern of offspring, we selected 47 20 m×20 m grids with seedling plots in three core areas (Figure S1 in Supporting Information) and resampled saplings (and juveniles) with known mother trees in those grids at four scales (5 m×5 m, 10 m×10 m, 15 m×15 m, and 20 m×20 m). The

recruitment patterns of sapling (and juvenile) offspring across four scales and all core areas were compared by using analysis of variance.

To explore the negative effects of focal adult trees on their offspring and non-offspring along the distance from focal trees, we used recruitment efficiency (RE) as a proxy variable of survival probability, which was calculated as the ratio of recruit number at later to earlier stage. Recruits of three life stages around the focal trees were grouped separately into offspring and non-offspring relative to the focal trees based on the parentage analysis. The non-offspring whose mother trees located within 80 m from them were removed from the calculation of RE to eliminate effects from non-offspring's own mother trees. Then, the ratios of sapling/ seedling and juvenile/sapling in specific distance intervals (circular or annular area) from the focal trees were calculated for offspring and non-offspring separately to obtain the RE at that distance interval (Swamy et al., 2011). All mother trees in core areas were used as focal trees in this analysis. We chose distance classes in geometric sequence (0–5 m, 5–10 m, 10–20 m, 20–40 m and 40–80 m), and calculated RE of the recruits within each distance class as follows:

$$RE_{ij} = W_A \times N_j / N_i,$$

where RE_{ij} stands for the recruitment efficiency of recruits during the transition from life stage i to j in each distance class, N_i and N_j are the total numbers of recruits in life stage i and j in each distance classes respectively, and W_A is a coefficient for area correction which is the ratio of sampling area of life stage i to j. We calculated 95%, 99%, and 99.9% confidence intervals of RE using the percentile method by bootstrapping 10,000 times of focal trees using boot package in R (Canty and Ripley, 2021; Davison and Hinkley, 1997). Difference in REs between offspring and non-offspring and distance classes is significant (α=0.05, 0.01, and 0.001) if their confidence intervals do not overlap the other's mean (Searle and Chen, 2020).

To explore the changes of fine scale spatial genetic structure across ontogeny, we performed spatial autocorrelation analysis for the recruits of three life stages (seedlings, saplings and juveniles) and analyzed the differences in spatial genetic structure between life stages using the nonparametric heterogeneity tests (Smouse et al., 2008) implemented in the program GenAlEx 6.51b2 (Peakall and Smouse, 2006; Peakall and Smouse, 2012). The genetic autocorrelation coefficient, r, was calculated for all paired samples falling within specific distance class and 95% confidence intervals for r were estimated by 999 bootstraps. The squared paired-sample t-test statistic "t^2" between life-stages for each distance class were calculated using 999 permutations to estimate the significance of genetic heterogeneity across ontogeny (Smouse et al., 2008). Distance classes were defined to ensure a minimum of 50 sample pairs of each life

stage at each distance class (0–5 m, 5–10 m, 10–40 m and 40–80 m).

The sample sizes of adults, seedlings, saplings, and juveniles in different analyses were provided in Tabel S1 in Supporting Information.

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SUPPORTING INFORMATION

The supporting information is available online at http://doi.org/10.1007/s11427-021-2148-7. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.