

# Landscapes drive the dispersal of *Monochamus alternatus*, vector of the pinewood nematode, revealed by Whole-genome resequencing

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## Abstract

Pine wilt disease (PWD), *Bursaphelenchus xylophilus*, is an extremely threatening invasion forest disease throughout the world, especially in Asia. *B. xylophilus* is spread in Asia by vector beetles of *Monochamus alternatus*, which has long no effective control method. Understanding of landscape effects on the dispersal and outbreaks of forest pests is crucial to establishing effective ecological control strategies. Here, we analyzed the samples of *M. alternatus* collected at landscapes in order to estimate the effects of landscape types on the genetic structure and dispersal of *M. alternatus*. The landscapes included the geographical scales, forest types and land uses. The individuals of *M. alternatus* were genotyped by using whole-genome resequencing. Population genetic structures were clearly differentiated at the intermediate scale, suggesting the intermediate scale is an effective barrier against natural dispersal of *M. alternatus*. We used the least-cost distances, least-cost transect analysis, and distance-based redundancy analysis to estimate the effects of forest types and land uses within the fine scales. The results showed that the gene flow and genetic diversity were positively correlated with host and mixed forests, whereas negatively with non-host forests. Among land-use landscapes, the roads had the positive effect on gene flow and genetic diversity but farmland and urban uses had negative effects. This highlights that human-mediated transport via roads was likely to be the main factor leading to the long-distance invasion of *M. alternatus*, whereas non-host landscapes could suppress the spread of this species. These findings may be useful to control the PWD dispersed by *M. alternatus*.

## KEYWORDS

*Monochamus alternatus*, pine wilt disease, whole-genome resequencing, least-cost transect analysis, distance-based redundancy analysis, landscape types

## 1 INTRODUCTION

Biotic invaders are a major threat, causing great destruction to the environment, economies, and biological safety, and with the development of transportation and commerce under globalization, few, if any, areas have escaped these invasions (Mack et al., 2000; Sun, Yuan, & Ou, 2002; Walker & Steffen, 1996). Pine wilt disease (PWD) is a major disease that invades forests and is highly destructive to its host pines. The disease spreads quickly, causes disease over a wide range, and is difficult to control and cure; thereby, PWD is also known as the “cancer” of pines (Naves, Camacho, Sousa, & Quartau, 2007; Wu et al., 2013). The disease, native to North America, was introduced into Japan in 1905, and then outbreaks occurred successively in

China, South Korea, Mexico, Portugal, and Spain (Mota & Vieira, 2008; Zhao, Mota, Vieira, Butcher, & Sun, 2013). To date, PWD has caused serious negative effects on the economy and the environment of many countries. From 2001 to 2012, the European Union spent thirty million euros on the surveillance and control of the disease, with projected losses of up to twenty billion euros in Europe by 2030 (Soliman et al., 2012). In Japan, at least 700,000 m<sup>3</sup> of pine trees are lost every year (Mamiya & Shoji, 2009). In China, the direct and indirect economic losses due to PWD have reached 100 billion yuan, and the area of *Pinus massoniana*, its main host, decreased by 8.07 million ha from 1994 to 2013, with 60 million ha of pine forests that remain greatly threatened by this disease (Ye, 2019). In addition, according to the hypothesis of an insect–fungus complex (Lu, Wingfield, Gillette, & Sun, 2011), the native ecosystems are at risk of reinvasion because pine wilt disease acquired greater adaptability in the invasion of new habitats (Maehara & Futai, 1997; Zhao et al., 2013).

The causal agent of PWD is the pinewood nematode (PWN, *Bursaphelenchus xylophilus*), which is spread by vector beetles of the genus *Monochamus* (Mota & Vieira, 2008). The main vector beetles in North America include *M. carolinensis*, *M. scutellatus*, and *M. mutator* (Akbulut & Stamps, 2011). *M. galloprovincialis* is the only insect vector in Portugal and other European countries (Sousa et al., 2001); whereas in several Asian countries, including China, the main vector is *M. alternatus*. Therefore, the effective control of *M. alternatus* plays an important role in controlling the continued spread of PWD in Asia (Zhao et al., 2008). In addition to self-migration, human-mediated and environmental factors are also important in the spread of *M. alternatus*. Moreover, because of the host dominance and ecosystem dominance of forest pests (Lian & Zhang, 2005), it is necessary to better understand the influence of host and nonhost landscapes on the dispersal behavior of this species.

The landscape plays an important role in the ecological processes (Turner, 1989). Landscape diversity can have major effects on the dispersal of forest pests (Thibaud et al., 2014; Foley et al., 2005), by either promoting or inhibiting the gene flow of dispersing insects and their movement between different landscape types (Fontaine, Bergerot, Le Viol, & Julliard, 2016; Yadav, Stow, & Dudaniec, 2019). The abundance of insects generally increases with the density of host plants, and insects have a tendency to migrate to patches of host plants (Underwood, Inouye, & Hambäck, 2014). The evidence is also increasing that with high tree diversity associational resistance can form against pests and reduce their damage (Damien et al., 2016; Harri, Koricheva, & Kai, 2007; Jactel, Goulard, Menassieu, & Goujon, 2002; Jactel & Brockerhoff, 2007; Jacte et al., 2015). Especially in mixed stands with hosts and nonhosts, the nonhosts can reduce the proportion of hosts available to the pest, and the natural enemies of pests are also typically more abundant, both of which increase the resistance to forest pests (Barbosa et al., 2009; Quayle, Regniere, Cappuccino, & Dupont, 2003). In addition to the effects of tree species in the landscape, other landscape types (e.g., urban areas, farmlands, and roads) can also have different effects on the gene flow and dispersal of insects (Keller, Strien, & Holderegger, 2012; Ortego, Bonal, & Muñoz, 2010; Yadav et al., 2019). Therefore, in a heterogeneous landscape, it is particularly important to understand the relationships between landscape types and pest dispersal behavior, population genetic structure, and gene flow. With such an understanding, the mechanisms to explain pest occurrence can be revealed, and scientific and effective ecological control methods can be established.

Although the relationship between landscape and some longhorn beetles has been described, most studies used microsatellite or mitochondrial DNA markers to study the invasion history and the influence of environmental factors such as climate and altitude (David, Giffard, Piou, Roques, & Jactel, 2016; Haran, Roques, Barnard, Robinet, & Roux, 2015; Haran et al., 2017; Javal et al., 2017; Javal et al., 2019; Koutroumpa, Rougon, Bertheau, Lieutier, & Roux, 2013; Tsykun et al., 2019). However, because of the limited number of polymorphic sites and the lack of detailed information on tree species, the dispersal behavior and the genetic model of *M. alternatus* at a fine scale has not been studied. In particular, a comprehensive description of the relationships between this species and finely classified tree species and nonhost landscapes such as urban areas, farmlands, and roads is lacking. This information is particularly important to clarify the dispersal behavior of *M. alternatus* in a heterogeneous landscape, as well as to efficiently manage and control PWD. With the emergence of next-generation sequencing, millions of single nucleotide polymorphisms (SNPs) can

be detected and compared with microsatellite markers. In addition, fewer samples are needed to obtain more accurate and better information on population structure. Thus, next-generation sequencing not only facilitates the development of landscape genetics but also is expected to become the standard in environmental association analysis in coming years (Rellstab et al., 2015; Jeffries et al., 2016). Currently, SNP analysis is becoming more widely accepted and applied in landscape research (Bay et al., 2018; Nancy et al., 2020; Zhen et al., 2017). Moreover, with the large numbers of SNPs, the analysis is more powerful, and the differences between populations can be detected at a fine scale (Liu et al., 2019; Rašić, Filipović, Weeks, & Hoffmann, 2014).

In this study, the whole genome of *M. alternatus* was resequenced at different landscape scales (intermediate and fine scales), and the tree species in the forested landscape were classified in detail based on a forest inventory of China. To analyze the influence of multiple scales, first, the population structure at intermediate and fine scales was compared, and then, the relations between gene flow and genetic diversity and forests with different tree species, urban areas, farmlands, roads, and water sources were studied at a fine scale. At the fine scale, the isolation-by-distance (IBD) and the least-cost distance (LCP) models (Adriaensen et al., 2003) were used, and the distances calculated by the models were correlated with genetic distance. To define the resistance surface more objectively and further study the effects of different landscape types on gene flow and dispersal of *M. alternatus*, the least-cost transect analysis (LCTA) developed by Strien, Keller, & Holderegger. (2012) and maximum-likelihood population effects (MLPE) developed by Clarke, Rothery, & Raybould. (2002) were adopted. The distance-based redundancy analysis (db-RDA) (Legendre & Anderson, 1999) and the generalized additive model (GAM) (Lengyel & Podani, 2015) were also used to test the influence of the landscape composition around each sample point on genetic diversity.

## 2 MATERIALS AND METHODS

### 2.1 Study area and sampling

*M. alternatus* is the main vector of PWN in Asia and is especially widely distributed in southern China. This study was mainly conducted in Shunchang (26°39' to 27°12'N, 117°29' to 118°14'E) and Xiapu (26°25' to 27°16'N, 119°46' to 120°28'E) in Fujian Province of southern China, with a geographical distance of approximately 250 km separating the two areas. Both sites have a subtropical monsoon climate. In Shunchang, the average annual temperature is 18.5 degC, and the average annual rainfall is 1,756 mm. In Xiapu, the average annual temperature is 18.8 degC, and the average annual rainfall is 1,800 mm.

*P. massoniana* and *P. elliotii* are natural hosts of *M. alternatus*, and they are widely distributed in the two areas. Moreover, urban areas, farmlands, roads, and water sources are embedded in the forested landscape. The landscape types were divided into ten categories: *P. massoniana*, *C. lanceolata*, *P. elliotii*, mixed forest (including some hosts), broad-leaved forest, urban, farmland, road, water, and ruderal (Figure 1). Samples were collected when adults are active from May to October in 2018 from 22 sites (12 sites in Shunchang (SC) and 10 sites in Xiapu (XP); Figure 1; Table 1) using a trapping method. The sample size ranged from seven to fourteen individuals per site (mean = 9.77 ± 1.93(SE); Table 1). The maximum geographic distance between fine-scale sampling points was 10 km and a the minimum of was 400 m. All adult beetles were preserved in absolute ethanol and stored at -80 degC. A total of 215 adults *M. alternatus* were collected (115 in SC and 100 in XP).

### 2.2 DNA extraction, library preparation, and sequencing

Total genomic DNA was extracted from mesothorax muscle tissues of each beetle for library preparation and sequencing using the TruSeq DNA Sample Preparation kit (Illumina, USA) according to the manufacturer's instructions. Sequencing libraries were generated using the TruSeq Nano DNA HT Sample Preparation kit (Illumina, USA) following the manufacturer's recommendations, and index codes were added to label sequences from each sample. For each individual, the DNA sample was sheared into fragments of 350 bp fragments by using a Covaris S2 (Covaris). The DNA fragments were end-polished, A-tailed, and ligated

with a full-length adapter for Illumina sequencing with further PCR amplification. The PCR products were purified (AMPure XP system), and the libraries were analyzed for size distribution using an Agilent 2100 Bioanalyzer and quantified using real-time PCR. The DNA fragments were then processed and sequenced using the Illumina HiSeq PE150 platform. Quality control was achieved by removing low costs and low-quality paired reads (reads with  $\geq 10\%$  unidentified nucleotides;  $>10$  nt aligned to the adaptor, allowing  $\geq 10\%$  mismatches;  $>50\%$  of bases having phred quality  $<5$ ; and putative PCR duplicates generated in the library construction process), which mainly resulted from base-calling duplicates and adaptor contamination.

## 2.3 Reads mapping and SNP calling

Raw sequence reads were mapped to the *M. alternatus* reference genome using the Burrows–Wheeler Aligner (BWA) with the command ‘mem -t 4 -k 32 -M’ (Li & Durbin, 2009). To reduce mismatches generated by PCR amplification before sequencing, duplicated reads were removed with SAMtools (Li et al., 2009). After alignment, SNP calling was performed on a population scale using a Bayesian approach as implemented in the package SAMtools, and then genotype likelihoods from reads for each individual at each genomic location and the allele frequencies in the sample were calculated with a Bayesian approach. The ‘mpileup’ command was used to identify SNPs with the parameters as ‘-q 1 -C 50 -t SP -t DP -m 2 -F 0.002’. Specifically, a SNP was retained when it met the following criteria: coverage depth  $\geq 2$  and  $\geq 30$ , minor allele frequency (MAF)  $\geq 0.05$ , MISS  $\geq 0.1$ .

## 2.4 Population genetics analyses

Within-population diversity was assessed by expected heterozygosity ( $H_E$ ), average self-coancestry ( $s$ ), fixation index ( $F_{IS}$ ), allelic diversity ( $A$ ), and mean number of alleles per locus ( $K$ ) using Metapop2 (Lopez-Cortegano, Perez-Figueroa, & Caballero, 2019). To analyze the population structure at the intermediate scale, a phylogenetic tree between Shunchang and Xiapu was constructed through 100 calculations of bootstrap values based on the neighbor-joining method and a principal component analysis was conducted, using MEGA 7.0 and genome-wide complex trait analysis (GCTA), respectively (Sudhir, Glen, & Koichiro, 2016; Yang, Lee, Goddard, & Visscher, 2011). ADMIXTURE version 1.3.0 (Alexander, Novembre, & Lange, 2009) was used to analyze population genetic structure at the two scales. The  $k$  genetic clusters were tested, with  $k$  ranging from one to seven, and the best  $k$  was determined according to the smallest cross-validation (CV) error. The vcftools (Auton & Marcketta, 2015) was used to calculate the  $F_{ST}$  between all pairs of sampled populations by averaging the values less than 0 after 5,000 bp windows and filtering (Supporting information Table S3, S4).

## 2.5 Influence of landscape on gene flow and genetic distance

To preliminarily predict the influence of the different landscape types on the genetic differentiation of *M. alternatus*, the distance models IBD and LCP were used at the fine scale (with Shunchang as an example). In the LCP model, four groups of resistance values were set for the ten landscape types base on the opinion method (Supporting information Table S5): *i*) host landscapes and roads were given the lowest resistance value (the resistance value of *P. massoniana*, *P. elliottii*, mixed-forest with hosts, and roads was 1), and nonhost landscapes (except roads) were divided into medium and high resistance (the medium resistance value of broad-leaved forest, *C. lanceolata*, water, and nudation was 8; the high resistance value of farmland was 64 and for urban was 512); *ii*) host landscapes and roads were given the medium resistance value of 8, whereas nonhost landscapes (except roads) were given the lowest resistance value of 1; *iii*) host landscapes and roads were given the medium resistance value of 64, whereas nonhost landscapes (except roads) were given the lowest resistance value of 1; *iiii*) host landscapes and roads were given the high resistance value of 512, whereas nonhost landscapes (except roads) were given the lowest resistance value of 1. The LCP on the four resistance surfaces was calculated using the R package *gdistance* (Vanetten, 2014). Finally, Mantel tests between the two distances and genetic distances ( $F_{ST}(1-F_{ST})$ ) were performed in the R package *ecodist* (Goslee & Urban, 2007).

In addition, to maintain objectivity, the least-cost transect analysis (LCTA) developed by Strien et al. (2012) was used to study the influence of landscape types on dispersal behavior and gene flow of *M. alternatus* at the

fine scale. This analysis provided an effective solution to objectively define the resistance values of different landscape types based on transects. In the analysis, each landscape type in a linear path, with different transect widths, between two sampling points is quantified, and the correlation between those values and the genetic distance is analyzed. The analysis can also determine the probable migration habitats and the landscape types that either inhibit or facilitate the gene flow. However, it is unlikely that species will migrate directly between two points following a straight line. Therefore, the LCTA uses the least-cost path to replace the straight line between two points and then calculates the percentage of each landscape type in the transect. For each transect, the proportions of each of the landscape types and the transect length were used as explanatory variables.

In this study, the first step in the LCTA was to create a series of resistance surfaces, in which each landscape type (*P. massoniana*, *C. lanceolata*, *P. elliotii*, mixed forest (including some hosts), broad-leaved forest, urban, farmland, road, water, and nudation) was successively considered as the optimal dispersal habitat. The optimal dispersal habitat was given the lowest resistance value of 1, whereas the other landscape types were given gradually increasing resistance values ( $2^3$ ,  $2^6$ ,  $2^9$ ) (Supporting information Table S6). A variety of transect widths (200 m, 300 m, 600 m) were evaluated. In each transect, Geospatial Modelling Environment v0.7.4.0 (Beyer, 2015) was used to calculate the proportions of each of the landscape types and the transect length. A total of 90 LCP data sets were obtained (10 land cover types x 3 resistance values x 3 transect widths). The correlations between those variables (proportions of each of the landscape types and the transect length) and the  $F_{ST}$  were tested through maximum-likelihood population effects (MLPE) (Clarke et al., 2002). In each MLPE, the model was estimated with restricted estimation maximum likelihood (REML), and the individual deme was used as a random effect. To explain the relationships between the different landscape types and the gene flow, Akaike's Information Criteria (AIC) (Gurka, 2006), which is considered to be effective in choosing an REML hybrid model, was used to select the optimal model from the 90 data sets.

## 2.6 Influence of landscape on genetic diversity

To reveal the influence of the composition of the landscape types around each sampling point on the genetic diversity, Arcgis10.2 (ESRI) was used to create circular buffers with radii of 300 m, 800 m, and 1,000 m and with each sample point as the center. Then, the proportions of *P. massoniana*, *C. lanceolata*, *P. elliotii*, mixed forest (including some hosts), broad-leaved forest, urban, farmland, road, water, and nudation landscape types in each circular buffer were calculated. The db-RDA model (Legendre & Anderson, 1999) was used to analyze whether the ten landscape types at the three scales could explain the genetic diversity. In addition, to explain the nonlinear relationship in the db-RDA analysis and explore whether those variables inhibited or facilitated the genetic diversity of *M. alternatus*, a flexible fitting method (GAM) (Lengyel & Podani, 2015) was used to map the explanatory variables over the first two principal coordinates analysis (PCoA) axes.

## 3 RESULTS

### 3.1 Whole-genome resequencing, mapping, and SNP detection

The genome size was 836,201,995 bp, and individual genomes were sequenced to an average of 7x coverage, with the average coverage of 90% (Supporting information Table S1, S2). A total of 31,759,600 SNP markers were initially obtained from Shunchang, whereas 29,918,163 SNPs were obtained from Xiapu. After stringent filtering, 4,152,751 SNPs were identified from Shunchang and 3,298,993 from Xiapu. The filtered SNPs were used for subsequent analysis.

### 3.2 Within-population diversity at a fine scale

In Shunchang, the expected heterozygosity ( $H_E$ ) ranged from 0.2208 to 0.2383, the average self-coancestry ( $s$ ) ranged from 0.875 to 0.8799, the allele richness ( $A$ ) ranged from 0.7613 to 0.7752, and the mean number of alleles per locus ( $K$ ) was close to 2 (Table 2). In Xiapu, the  $H_E$  ranged from 0.2015 to 0.2127, the  $s$  ranged from 0.8861 to 0.8895, the  $A$  ranged from 0.7754 to 0.7674, and the  $K$  was also close to 2 (Table 2).

According to these five parameters, although the genetic diversity of populations at the fine scale was weak, there were certain differences.

### 3.3 Population structure at different scales

*M. alternatus* population structure was compared at intermediate and fine scales. As shown in the phylogenetic tree, the population in the two regions was divided into two branches at the intermediate scale (Figure 2A). Principal component analysis confirmed this result, having the populations from the two regions clearly separated (Figure 2B). ADMIXTURE was also used to analyze the population genetic structure between the two regions. In the ADMIXTURE figure (Figure 2C), each column represents an individual, with the length of the different colored segments representing the proportion of an ancestor in the individual genome. Because the CV error was the smallest at  $K = 2$  (CV error = 0.42066), the population genetic structure revealed two main differentiated gene pools (Figure 2C). Thus, the population in Shunchang was originated from one ancestor and the one from Xiapu was originated from a different ancestor, which is a conclusion consistent with the results of the phylogenetic tree and the PCA. In conclusion, the populations were clearly differentiated at the intermediate scale ( $>250$  km), which clearly indicated the importance of geographic barriers against natural dispersal and gene flow of *M. alternatus* over long distances.

To reveal whether there were differences in population genetic structure at the fine scale, ADMIXTURE was also used to analyze the population genetic structures within Shunchang and Xiapu. According to the smallest CV error in Shunchang and Xiapu at  $K = 2$  (CV error = 0.49264 and 0.47180, respectively; Supporting information Figure S1), the populations at the fine scale formed two clusters. Thus, the populations in SC included two clusters, and those XP included two other clusters (Figure 3). Although the fine-scale genetic structure detected by ADMIXTURE was weak, with an increase in the  $K$  value, seven meaningful clusters were obtained (Supporting information Figure S2).

### 3.3 Influence of landscape on gene flow and genetic distance

According to the IBD model and the Mantel test in the Shunchang County, there was no significant ( $r(F_{ST}, IBD) = 0.38$ ,  $P = 0.075$ ) (Figure 4A), but different landscape types had influence (Supporting information Figure S3). Among the four groups of resistance values, only the resistance surface created by the first group (the resistance value of *P. massoniana*, *P. elliotii*, mixed-forest with hosts, and road was 1; the medium resistance value of broad-leaved forest, *C. lanceolata*, water, and nudation was 8; the high resistance value of farmland was 64 and that of urban 512) (Figure 4C) was significantly correlated ( $r(F_{ST}, LCP) = 0.44$ ,  $P = 0.045$ ) (Figure 4B). There was no correlation with the other three resistance surfaces, in which the resistance values of host and road landscapes were higher than those of nonhost landscapes (except roads). This result suggested that at the fine scale ( $<10$  km), the genetic differentiation of *M. alternatus* could not be determined by distance alone, although landscape types could have an effect. Among the landscapes, the host landscapes and roads had low resistance to local dispersal; whereas nonhost landscapes were more likely to inhibit movement in this species.

The top ten models in the LCTA of the two areas all demonstrated that *P. massoniana*, mixed forests with hosts, *P. elliotii*, and roads could facilitate the dispersal of *M. alternatus* (Table 3), which is also consistent with the LCP model in which the host and road landscapes had a lower resistance value. In Shunchang, the top model showed that *P. massoniana* was the most suitable dispersal habitat for this species, with the transect width of 600 m and the landscape variable of *P. elliotii*, which could facilitate gene flow (Table 3). However, in the fifth to tenth models, except for the eighth model in which roads had a positive effect, the other models all showed that both farmland and urban landscapes had a negative effect on gene flow (Table 3). In Xiapu, the top model suggested that mixed forest with hosts was the most suitable dispersal habitat for this species, with the transect width of 600 m and the landscape variable of urban, which had a negative effect on gene flow with the highest resistance value of  $2^9$  (Table 3). The second and third models also showed that urban landscapes inhibited gene flow (Table 3). In the fifth to eighth models, *P. elliotii* was also a suitable habitat for this species to disperse, whereas *C. lanceolata* had a negative effect on gene flow (Table 3). Overall, in the two regions, the host and road landscapes were likely conducive to the dispersal

of *M. alternatus* at a fine scale, whereas the nonhost landscapes had significant negative effects on dispersal and gene flow in this species.

### 3.4 Influence of landscape on genetic diversity

Analysis of the db-RDA model in Shunchang County showed that different landscape types had significant effects on the genetic diversity at three scales (Table 4). At the three scales of 300 m, 800 m, and 1,000 m, the percentage of *P. massoniana* was significantly related to the genetic diversity of this species ( $P$  (300 m) = 0.031;  $P$  (800 m) = 0.022;  $P$  (1,000 m) = 0.006), explaining 47.478%, 45.730%, and 43.342% of the variation, respectively (Table 4). Urban landscape was significantly correlated with the genetic diversity only at 800 m ( $P$  = 0.046), whereas mixed forest with hosts had significant effects at scales of 800 m and 1,000 m ( $P$  (800 m) = 0.038;  $P$  (1,000 m) = 0.031). At the 1,000 m scale, additional types of landscapes had significant correlations, including *P. elliotii* ( $P$  = 0.04), farmland ( $P$  = 0.018), and roads ( $P$  = 0.046) (Table 4). In Xiapu, in addition to the influence of *P. massoniana* at the three scales, other landscape types at different scales also showed significance (Table 5). At the 800 m scale, *P. elliotii* was significantly correlated with the genetic diversity ( $P$  = 0.019), explaining 23.237% of the variation (Table 5). At the 1,000 m scale, similar to Shunchang, there were more relevant landscape variables than at the two smaller scales, primarily including mixed forest with hosts ( $P$  = 0.032), roads ( $P$  = 0.032), farmland ( $P$  = 0.007), and urban ( $P$  = 0.041) (Table 5). In both areas, the different landscape types could affect the genetic diversity of *M. alternatus*, and the number of landscape types with effects increased with the increase in scale.

GAM analysis was performed to explain the nonlinear relationship in the db-RDA analysis. According to the results, the landscape variables fit well on the first two dimensions of the PCoA ordination (Figures 5 (Shunchang) and 6 (Xiapu)). *Pinus massoniana* increased gradually with the first PCoA axis or the second axis in both areas, indicating that *P. massoniana* was positively correlated with genetic diversity (Figure 5A, B, E; Figure 6A, B, E). The landscape types that increased along the first PCoA axis also included roads in Shunchang (Figure 5I), whereas mixed forest with hosts and *P. elliotii* mainly increased with the second PCoA axis (Figure 5C, F, G). In Xiapu, mixed forest with hosts at the 800 m scale and *P. elliotii* and mixed forest with hosts at the 1,000 m scale also increased with the first PCoA axis (Figure 6C, D, F, H). Urban decreased with the second PCoA axis in both areas (Figure 5D; Figure 6G), whereas farmland decreased with the first PCoA axis or the second axis (Figure 5H or Figure 6I), which indicated that the genetic diversity was negatively correlated with urban and farmland landscapes.

## 4 DISCUSSION

*M. alternatus* is the primary beetle vector of PWD in Asia, a major disease invading the forests. This study describes the first of the population structure of *M. alternatus* at intermediate and fine scales using whole-genome resequencing. In addition, the relationships between host and nonhost landscapes and this species were analyzed at a fine scale, which clarified the influence of multiple scales and different landscape types on dispersal behavior, gene flow, and genetic diversity. The results suggested that host forests could facilitate gene flow and dispersal of this species in a heterogeneous landscape and that human-mediated actions via roads were also conducive to dispersal. However, nonhost forest, urban, and farmland landscapes had negative effects on gene flow and dispersal. The different responses of *M. alternatus* to the heterogeneous landscapes indicate the importance of fully considering tree species and human-mediated landscapes when designing and managing forests and also suggest that PWD can be predicted and controlled in advance by determining the distribution of landscape types.

### 4.1 Method of data analysis and model selection of landscape genetics with whole-genome resequencing

The common markers in landscape genetics are microsatellites, mitochondrial DNA, amplified fragment length polymorphisms, and the Y chromosome (Manel, Schwartz, Luikart, & Taberlet, 2003). In recent years, single nucleotide polymorphisms (SNPs) have become another major and widely used marking method. The greatest advantage and characteristic of SNPs is that the number of polymorphic sites increases greatly compared with that of other molecular markers. However, the increase in number of markers also increases

the difficulty and the time needed for data analysis. Therefore, by selecting the correct analysis tool, the genetic model in whole-genome resequencing can be accurately obtained and the analysis time effectively shortened. Pairwise estimation of  $F_{ST}$  is an important parameter in population genetic analysis that can conveniently summarize the population structure (Weir & Cockerham, 1984). Pairwise  $F_{ST}$  is generally calculated using GENEPOP (Rousset, 2008), the R package *adegenet*, or GenAlEx 6.5 (Peakall & Smouse, 2012). In this study, after filtering, 4,152,751 SNPs were obtained in Shunchang and 3,298,993 SNPs were obtained in Xiapu. Then, the R package *adegenet* was used to calculate pairwise  $F_{ST}$ ; however, the calculation required a long time, approximately one month. Therefore, the same data were used to calculate  $F_{ST}$  through 5,000-bp windowing in vcftools software (Auton & Marcketta, 2015), and the calculation required only 7 h, which showed that using this method to calculate pairwise  $F_{ST}$  could effectively shorten the calculation time.

Observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity, fixation index ( $F_{IS}$ ), allele diversity ( $A$ ), and mean number of alleles per locus ( $K$ ) are also important parameters in population genetic analysis, which can be calculated by software such as Arlequin 3.11 (Excoffier, Laval, & Schneider, 2005), GENETIX 4.05 (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 2004), or ADZE 1.0 (Szpiech, Jakobsson, & Rosenberg, 2008). The commonly used software for calculating these parameters in Restriction-site associated DNA sequencing (RAD-seq) or Genotyping by sequencing (GBS) is GenAlEx 6.5, but because the number of SNPs in whole-genome resequencing is usually a hundred times that of RAD-seq or GBS, GenAlEx 6.5 did not appear to be able to support such a large data set. Therefore, in this case, Metapop2 software (Lopez-Cortegano et al., 2019) was selected, which can not only calculate the complete genetic diversity but can also effectively analyze a large number of SNPs (e.g., >100,000 SNPs) in the latest optimized version. The software calculated the population genetic parameters in a total of 60 h, which indicated that Metapop2 can effectively analyze millions or even tens of millions of SNPs in a short time.

To quantify the landscape structure and determine its effect on population genetics we used method of least-cost path based on resistance surface or straight-line transects (Spear et al., 2010). To eliminate the shortcomings of those two methods, Strien et al. (2012) developed the least-cost transect analysis (LCTA) by combining the two methods. While realizing objectivity, this analysis can also use buffers to form transect widths to quantify the proportions of each of the landscape types. In this study, LCTA was performed at a fine scale (<10 km) based on whole-genome resequencing, which clearly revealed the relationships between different tree types, urban areas, roads, and farmland and the dispersal and gene flow of *M. alternatus*. Thus, this analysis was effective with SNP markers and could identify the effects of landscape types at a fine scale. Cleary et al. (2017) used this analysis to describe the landscape genetics of two frugivorous bats under agricultural intensification and also obtained a better interpretation of effects. The db-RDA model also effectively described landscape genetics at a fine scale. This model breaks through the limitation that the existing method of RDA can only be performed by using Euclidian distance and allows the use of Bray-Curtis or other ecologically meaningful measures (Legendre & Anderson, 1999). The LCTA and db-RDA well explained the effects of different landscape types on *M. alternatus* at a fine scale, and because the results obtained by the two models were relatively consistent, both are applicable to landscape genetics at a fine scale under whole-genome resequencing.

#### 4.2 Population structure of *M. alternatus* at different scales

The analysis of population structure at different scales revealed that there was obvious differentiation among populations at the intermediate scale (>250 km); whereas the difference at the fine-scale (<10 km) was weak but still had certain influence (Figure 2, 3). The phylogenetic tree, PCA, and ADMIXTURE all showed that the two areas had different populations of *M. alternatus*, indicating that natural dispersal and gene flow at the intermediate scale were difficult. In the study of other species, the geographical distance and the genetic distance also are strongly correlated, and the geographical distance can also affect the genome-wide variation (Bay et al., 2018). This result demonstrates that the natural spread of *M. alternatus* can be isolated at a distance of more than 250 km, which also implies that the PWD can not be transmitted naturally at this distance. At the same time, further study are required to collect some populations at the scale of 10-250 km, so as to analyze the influence of this range on the spread of *M. alternatus*.



In the analysis of genetic structure and diversity at the fine-scale, the results indicated that the populations in Shunchang and Xiapu were composed of two subpopulations, although the difference level within each subpopulation was not high (Figure 3; Table 2). According to the Mantel test, there was no significant correlation between genetic distance and IBD at the fine scale (Figure 4A). In *Monochamus* species, the flight distance is up to 22 km in the field, and based on a combination of flight mill and mark–release–recapture experiments, the cumulative flying distance averages 63 km by the end of the adult stage in these species (Takasu et al., 2000; David, Giffard, Piou, & Jactel, 2014; Robinet et al., 2019). As a result, strong inbreeding occurs in populations, leading to weak local genetic structure (Kawai et al., 2006; Shoda-Kagaya, 2007; Haran et al., 2015). Indeed, in this study, the genetic structure of the populations was weak at the fine-scale. The distribution of the host may also lead to the weakening of the population genetic structure. According to other studies, as long as the landscape type contains sufficient hosts for a species to inhabit and forage, the genetic structure may be insignificant at a distance of more than 120 km (McCulloch et al., 2013). In Shunchang and Xiapu, the landscape type was primarily forest, and there were many *P. massoniana* and *P. elliotii* distributed in the forest landscape. Because these tree species are important hosts of *M. alternatus*, the two species likely facilitate the dispersal of the beetle, and as a result, the genetic structure was poorly defined at the fine-scale. Although the geographic distance can have an important effect on the genetic structure of a species at large scales, differences in the genetic structure and obstruction of gene flow at a fine scale may be more affected by landscape types than by the geographic distance alone (Aurelie et al., 2017). Thus, with the increase in landscape scale, the influence of the geographical distance on the natural dispersal of *M. alternatus* was also increased, whereas at a fine scale, the influence of landscape types was greater than that of distance.

#### 4.3 Different effects of nonforest landscapes on gene flow and dispersal

Urban landscapes are generally considered to be barriers to gene flow, and an increase in farmland landscapes can also directly or indirectly negatively affect the ecological processes and dispersal of many species (Butler, Vickery, & Norris, 2007; Johnson & Munshi-South, 2017; Lane-Degraaf, Fuentes, & Hollocher, 2014; Ruell et al., 2012; ). According to the Mantel test of the correlation between the LCP of multiple resistance surfaces and the genetic distance, they were significantly correlated only when the urban and farmland landscapes were given high resistance values (Figures 4A, B). By contrast, no significant correlations were obtained with the other three resistance surfaces in which low resistance was given to urban and farmland landscapes (Supporting information Figure S3). Thus, by acting as barriers, urban and farmland landscapes played an important role in the dispersal and the gene flow of *M. alternatus*. This effect is possibly due to the limited food sources of the species in cities and farmland. Moreover, the use of pesticides in farmlands can also affect insects (Sonoda, Izumi, Kohara, Koshiyama, & Yoshida, 2011), likely affecting the identification of hosts by *M. alternatus* and inhibiting dispersal.

Notably, the LCTA, in which the resistance value was objectively defined, also indicated that urban and farmland landscapes had a negative effect on gene flow (Table 3). Especially in Xiapu, the best three models in the LCTA showed that urban landscapes inhibited gene flow. In addition, the GAM model also suggested that urban and farmland landscapes were negatively correlated with genetic diversity at the 800 to 1,000-m scale (Figure 6). Urbanization is one of the important factors that cause the loss of species habitat as well as forest fragmentation and the gradual but eventual disappearance of small areas of forest (Caspersen & Olafsson, 2010), and the distribution of farmland destroys the continuity of host distribution. Because of the decline in forest coverage and the disappearance of host continuity, the population of *M. alternatus* may be separated to form isolated populations, thus reducing the gene flow. Indeed, farmland can directly affect the distribution and spread of insects and even hinder insect migration, which has important effects on population dynamics (Ge, Ou, & Men, 2016). In a study on other species of *Monochamus*, the flying time of this species was short, sometimes only 1 km (David, 2014), so the farmland and urban landscapes at the scale of 800 to 1,000 m would most likely be barriers due to the unwillingness to fly. Thus, at a fine-scale, urban and farmland landscapes distributed are important barriers to dispersal and migration in this species.

Corridors in landscapes can promote the dispersal of species among different landscape types (Lapolla, 1993;

Nicholls, Parrella, & Altieri, 2001). Among nonforest landscapes, roads are very important corridors, and especially in areas with high landscape diversity, road patches can often play a role in connectivity. The fourth model of the LCTA in Shunchang showed that roads were the most suitable dispersal habitat for this species (Table 3). The LCP model showed that roads had the same low resistance value as other host landscapes (Figure 4C). The db-RDA and GAM models also indicated that roads were positively correlated with genetic diversity (Figure 5, 6; Table 4, 5). These results all suggest that roads are important and promote the dispersal of *M. alternatus* in a heterogeneous landscape. Although the existence of road networks can inhibit the dispersal and gene flow in some other species (Garcia-Gonzalez, Campo, Pola, & Garcia-Vazquez, 2012; Hartmann, Steyer, Kraus, Segelbacher, & Nowak, 2013), *M. alternatus* has the characteristic of the human-mediated movement, such as in the transport of infested wood from one area to another, including lumber and wood packaging material (Haack, 2006; Ye, 2019). This type of movement may cause the dispersal of this species in heterogeneous landscapes and may even lead to long-distance transmission. In a previous study on the genetic structure of *M. alternatus*, the road transportation system was also found to promote the dispersal of this species in mainland China (Hu, Ning, Fu, & Haack, 2013). Therefore, roads can facilitate dispersal and gene flow of *M. alternatus* in heterogeneous landscapes by promoting long-distance, human-mediated transport.

#### 4.4 Relationships between detailed forest landscapes and population structure of *M. alternatus*

This study showed that *P. massoniana*, *P. elliotii*, and mixed forest with hosts had the lowest resistance values and were the most suitable habitats for dispersal, whereas *C. lanceolata* had a negative effect on the gene flow (Table 3, 4, 5). The db-RDA and GAM models also indicated that *P. massoniana*, *P. elliotii*, and mixed forest with hosts could promote genetic diversity and that *P. massoniana* had significant influence at each scale (i.e., 300 m, 800 m, 1,000 m). *Pinus massoniana* and *P. elliotii* are important host tree species of *M. alternatus* (Hao, Zhang, Dai, & Wan, 2005; Li, Xu, & Zhang, 2003), and therefore, the three forest landscapes with hosts showed a promoting effect. By contrast, *C. lanceolata* was not a food source and was also the least preferred species of the tree species (Tu et al., 2019), indicating that the distribution of *C. lanceolata* could greatly hinder the dispersal of *M. alternatus*.

The promoting effects of hosts observed in this study are not supported by results in previous studies based on microsatellites in *M. galloprovincialis* (Haran et al., 2017; Koutroumpa, Rougon, Bertheau, Lieutier, & Roux, 2013). However, another study found that the distribution of hosts could increase the gene flow of *M. galloprovincialis* on both sides of a mountain range (Haran, Roques, Barnard, Robine, & Roux, 2015), and in China, the occurrence area of PWD is also significantly positively correlated with that of the host (Bai et al., 2015). The distribution of hosts most likely has different effects on *Monochamus* because of several reasons. First, there are different species of beetles in the same genus Allison, Strom, Sweeney, & Mayo, 2019), and adult *M. alternatus* in forests also behave differently, depending on the stage (e.g., spawning period and mating period). Second, even when all forests have hosts, the tree age, height, canopy density, tree potential, and other factors may determine whether hosts inhibit or facilitate the dispersal of this species (Ding, Lv, Han, Pu, & Wu, 2001; Jiang, Wan, Yao, Xu, & Li, 2020). Last, the role of hosts is also closely related to the landscape scale. Therefore, more in-depth and detailed study and discussion on the influence of hosts are necessary.

Mixed forests are generally considered better able to resist and hinder forest pests than single stands. However, not all mixed forests have this effect, and the specific tree species, growth rates, and their relative proportions within and between stands in mixed forests must be fully considered (Damien et al., 2016). In this study, the mixed forest with hosts did not act as barriers to *M. alternatus* but had a certain promoting effect on its dispersal (Table 3, 4, 5; Figure 5, 6), which was probably related to the partial distribution of *P. massoniana* in the mixed forest. Indeed, in some studies, PWD was more likely to occur in stands mixed with other tree species (Nakamura, Togashi, & Takahashi, 1997; Togashi, Aida, Nakamura, Horikoshi, & Takahashi, 1997). Haran et al. (2017) also suggest that a low density of pine trees is not a barrier to the dispersal of a species in the same genus, *M. galloprovincialis*. Thus, even when there are other tree species in a mixed forest or when the proportion of host trees is reduced because of other tree species, the dispersal

of this species can be facilitated as long as there are host trees.

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## DATA ACCESSIBILITY STATEMENT

Whole-genome resequencing data is planned to be publicly accessed on NCBI in the form of VCF files (<https://www.ncbi.nlm.nih.gov/>). Genebank accession numbers will also provide.

## AUTHOR CONTRIBUTION

YCL, SQW and FPZ designed the whole experiment. YCL, XHH and XQW were survey study area and collect samples. WLH performs data analysis of Whole-genome resequencing. YCL, XHH and XQW construction and analysis model. RW, XH, GHL, SGH, SQW and FPZ procured funding. CLL and RCL have modified the article. YCL finished writing with the participation of all authors, and all authors approved the manuscript.

## TABLES

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## FIGURE LEGENDS

Figure 1.-Landscape types and populations sampled across the Shunchang County and Xiapu County in China.

A. Shunchang County; B. Xiapu County

The intermediate scale with the two counties shaded is shown in the map below the fine scale maps of the two study areas, each with ten landscape types.

Figure 2.-Population structure analysis of *M. alternatus* at an intermediate scale.

Neighbor-joining phylogenetic tree of all individuals. (B) Principal component analysis of all individuals. (C) ADMIXTURE analysis, with only the K with the smallest CV error shown ( $K = 2$ ). SC, Shunchang; XP, Xiapu.

Figure 3.-Smallest cross-validation error of genetic structure detected by ADMIXTURE at a fine scale ( $K = 2$ ).

The two genetic clusters in Shunchang County (blue and green); (B) The two genetic clusters in Xiapu County (mint green and red). The size of each chart is proportional to the sample size. The gray area represents all forest habitats, and the white area represents other habitats.

Figure 4.-Mantel correlation tests between genetic distance and (A) Euclidian or (B) least-cost distance between pairs of populations.

Least-cost distances were calculated using the opinion method resistance surface relying on the land cover surface. Only the resistance surface 1 (B) and its resistance values (C) are shown, because the other three groups of resistance values were not significant.

Figure 5.-Principal coordinates analysis of predictor variables and fitted as trend surfaces a posteriori by generalized additive model in Shunchang County.

300 m, *P. massoniana* ; (B) 800 m, *P. massoniana* ; (C) 800 m, Mixed forest; (D) 800 m, Urban; (E) 1,000 m, *P. massoniana* ; (F) 1,000 m, Mixed forest; (G) 1,000 m, *P. elliottii* ; (H) 1,000 m, Farmland; (I) 1,000 m, Road.

Figure 6.-Principal coordinates analysis of predictor variables and fitted as trend surfaces aposteriori by generalized additive model in Xiapu County.

(A) 300 m, *P. massoniana* ; (B) 800 m, *P. massoniana* ; (C) 800 m, Mixed forest; (D) 800 m, *P. elliotii* ; (E) 1,000 m, *P. massoniana* ; (F) 1,000 m, Mixed forest; (G) 1,000 m, Farmland; (H) 1,000 m, Road: (I) 1,000 m, Urban.

Table 1. -Sampling point information

Study location	Site	Sample no	Latitude (N)	Longitude (E)
SC	SC1	7	26.78810278	117.7934222
	SC2	9	26.79221944	117.8110306
	SC3	12	26.810075	117.8108444
	SC4	7	26.80866667	117.7933306
	SC5	8	26.80754444	117.78365
	SC6	10	26.81075556	117.77665
	SC7	14	26.81578889	117.7851528
	SC8	14	26.81764444	117.7789222
	SC9	10	26.82101111	117.7741056
	SC10	7	26.77182778	117.7358667
	SC11	10	26.85954444	117.8003167
	SC12	7	26.87125278	117.7454056
XP	XP1	10	26.989183	120.222639
	XP2	10	26.990892	120.213067
	XP3	10	26.998219	120.194725
	XP4	9	26.980986	120.171342
	XP5	10	26.986389	120.169403
	XP6	10	26.990178	120.162661
	XP7	9	27.001125	120.163372
	XP8	10	26.953933	120.176708
	XP9	10	26.963456	120.165219
	XP10	12	27.041250	120.142739

Table 2.-Estimates of population diversity

Location	Site	H <sub>E</sub>	s	A	K
SC	SC1	0.2228	0.8784	0.7637	1.794
	SC2	0.2248	0.8799	0.7613	1.8529
	SC3	0.2325	0.8772	0.766	1.9149
	SC4	0.2208	0.8798	0.7629	1.7901
	SC5	0.2328	0.875	0.7752	1.8448
	SC6	0.2338	0.876	0.7709	1.8874
	SC7	0.2383	0.8758	0.7726	1.9441
	SC8	0.2351	0.8792	0.7666	1.9397
	SC9	0.2304	0.8773	0.7638	1.8785
	SC10	0.2246	0.8777	0.7677	1.7994
	SC11	0.2293	0.8785	0.7652	1.8808
	SC12	0.2242	0.8779	0.7655	1.798
XP	XP1	0.2127	0.8861	0.7674	1.8696
	XP2	0.2082	0.8885	0.7596	1.8601
	XP3	0.2097	0.8869	0.7639	1.84
	XP4	0.2038	0.8886	0.7626	1.7693
	XP5	0.2055	0.8893	0.756	1.8538
	XP6	0.2043	0.8892	0.7575	1.8293

XP7	0.2076	0.8879	0.7616	1.8362
XP8	0.2074	0.889	0.7602	1.8596
XP9	0.2015	0.8895	0.7554	1.7924
XP10	0.2083	0.8889	0.7586	1.8973

*Note:* Expected heterozygosity ( $H_E$ ), self-coancestry ( $s$ ), allelic diversity ( $A$ ), and mean number of alleles per locus ( $K$ ).

Table 3.-Maximum-likelihood population effects models with genetic distance ( $F_{ST}$ ) as the response variable and results from the least-cost transect analyses as the explanatory variables

Location	Genetic differentiation	Dispersal habitat	Resistance level	Transect width (m)	Variables
SC	$F_{ST}$	<i>P. massoniana</i>	$2^9$	600	<i>P. elliotii</i> (-)
		<i>P. massoniana</i>	$2^3$	600	<i>P. elliotii</i> (-)
		<i>P. massoniana</i>	$2^6$	600	<i>P. elliotii</i> (-)
		Road	$2^9$	600	<i>P. elliotii</i> (-)
		Mixed forest	$2^9$	200	Farmland (-)
		Mixed forest	$2^6$	200	Farmland (-)
		<i>P. massoniana</i>	$2^3$	600	Urban (-)
		<i>P. massoniana</i>	$2^6$	400	Road (+)
		Mixed forest	$2^9$	600	Farmland (-)
		<i>P. massoniana</i>	$2^3$	400	Urban (-)
XP	$F_{ST}$	Mixed forest	$2^9$	600	Urban (-)
		Mixed forest	$2^9$	400	Urban (-)
		Mixed forest	$2^9$	200	Urban (-)
		<i>P. massoniana</i>	$2^9$	600	Farmland (-)
		<i>P. elliotii</i>	$2^6$	400	<i>C. lanceolata</i> (-)
		<i>P. elliotii</i>	$2^9$	600	<i>C. lanceolata</i> (-)
		<i>P. elliotii</i>	$2^9$	400	<i>C. lanceolata</i> (-)
		<i>P. elliotii</i>	$2^6$	600	<i>C. lanceolata</i> (-)
		<i>P. massoniana</i>	$2^3$	200	Farmland (-)
		<i>P. massoniana</i>	$2^6$	600	Urban (-)

*Note:* This table shows the top ten models of SC and XP based on AIC respectively. The variables in each model are positively correlated with "+" and negatively correlated with "-".

Table 4.-Summary statistics for the db-RDA model of genetic diversity in Shunchang, influenced by the percentage of different landscapes in circular buffers with radii of 300 m, 800 m, and 1,000 m

Circular buffer	Landscape	Explained variation %	F	P
300 m	% <i>P. massoniana</i>	47.478	9.6003	0.031*
800 m	% <i>P. massoniana</i>	45.730	37.230	0.022*
	% Mixed forest	22.811	18.571	0.038*
	% Urban	13.595	11.068	0.046*
1,000 m	% <i>P. massoniana</i>	43.342	132.812	0.006**
	% Mixed forest	13.450	40.448	0.031*
	% <i>P. elliotii</i>	10.500	32.174	0.04*
	% Farmland	18.292	56.054	0.018*
	% Road	9.350	28.648	0.046*

Note: significant ( $*P < 0.05$ ) , the same bleow.

Table 5. Summary statistics for the db-RDA model of genetic diversity in Xiapu, influenced by the percentage of different landscapes in circular buffers with radii of 300 m, 800 m, and 1,000 m

Circular buffer	Landscape	Explained variation %	F	P
300 m	% <i>P. massoniana</i>	35.271	8.4176	0.047*
800 m	% <i>P. massoniana</i>	15.563	22.9195	0.031*
	% Mixed forest	30.792	45.3488	0.01*
	% <i>P. elliotii</i>	23.237	34.2221	0.019*
1,000 m	% <i>P. massoniana</i>	39.474	114.3156	0.003**
	% Mixed forest	8.214	23.7867	0.032*
	% Road	8.185	23.7045	0.032*
	% Urban	24.596	71.2305	0.007**
	% Farmland	6.557	18.9874	0.041*

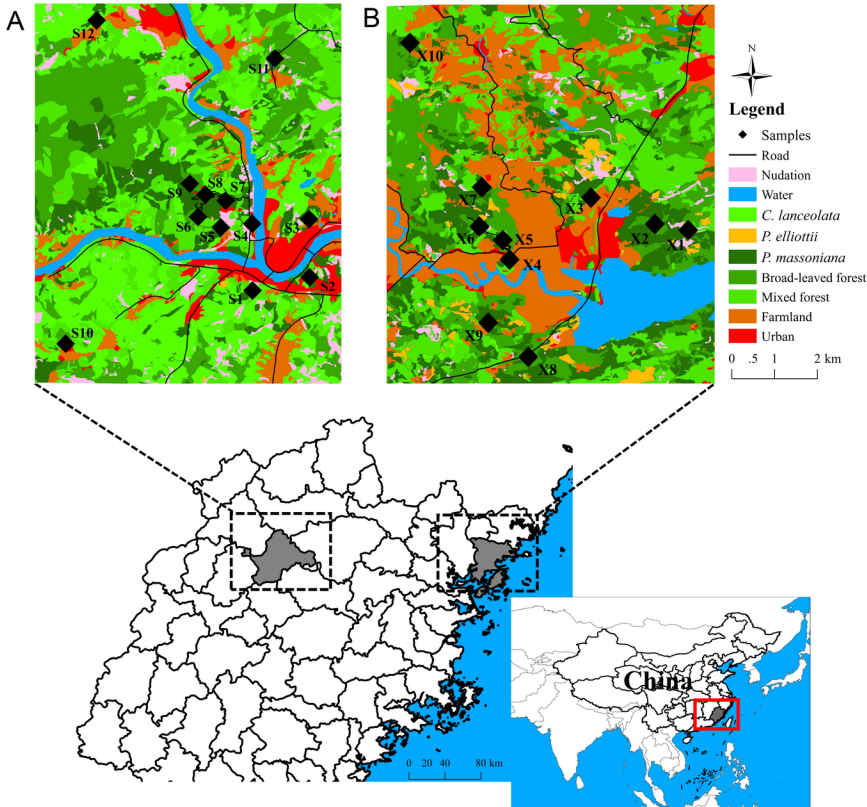


Figure 1.-Landscape types and populations sampled across the Shunchang County and Xiapu County in China.

A. Shunchang County; B. Xiapu County

The intermediate scale with the two counties shaded is shown in the map below the fine scale maps of the two study areas, each with ten landscape types.

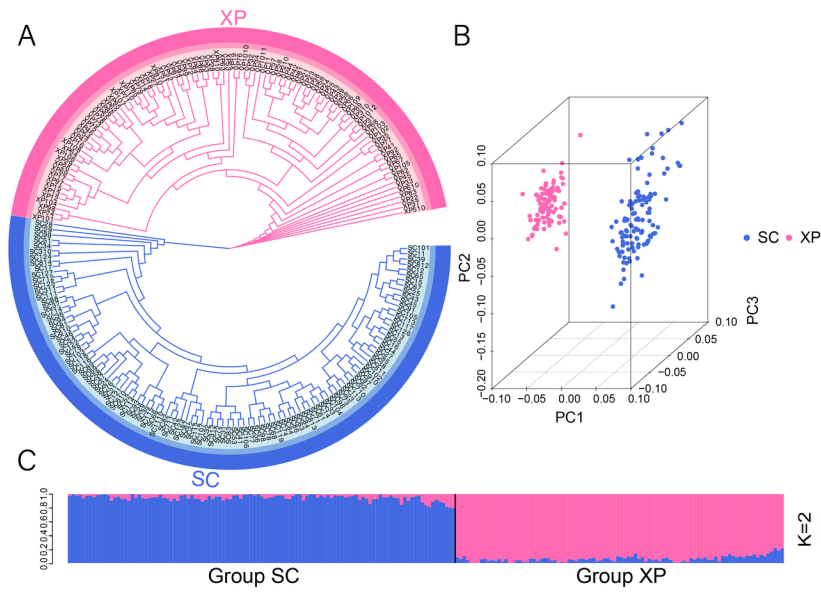


Figure 2.-Population structure analysis of *M. alternatus* at an intermediate scale.

Neighbor-joining phylogenetic tree of all individuals. (B) Principal component analysis of all individuals. (C) ADMIXTURE analysis, with only the K with the smallest CV error shown ( $K = 2$ ). SC, Shunchang; XP, Xiapu.

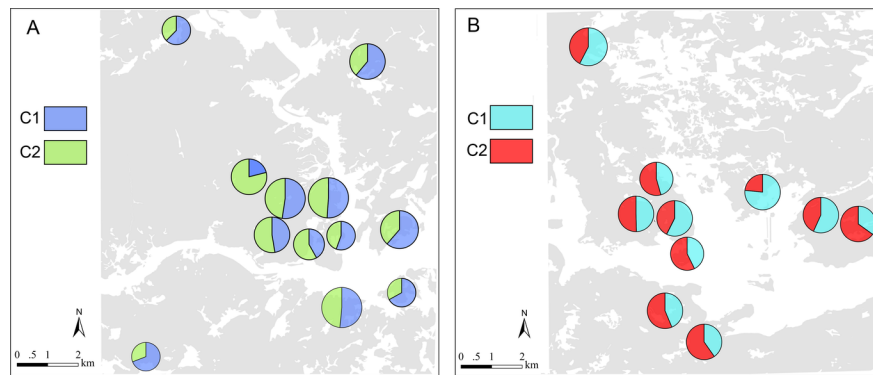


Figure 3.-Smallest cross-validation error of genetic structure detected by ADMIXTURE at a fine scale ( $K = 2$ ).

The two genetic clusters in Shunchang County (blue and green); (B) The two genetic clusters in Xiapu County (mint green and red). The size of each chart is proportional to the sample size. The gray area represents all forest habitats, and the white area represents other habitats.

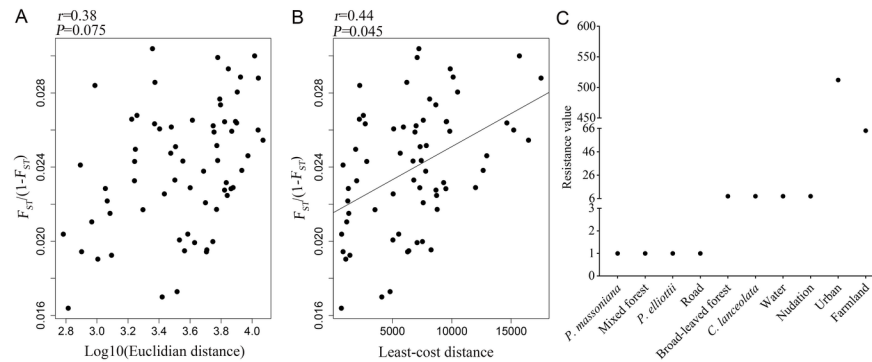


Figure 4.-Mantel correlation tests between genetic distance and (A) Euclidian or (B) least-cost distance between pairs of populations.

Least-cost distances were calculated using the opinion method resistance surface relying on the land cover surface. Only the resistance surface 1 (B) and its resistance values (C) are shown, because the other three groups of resistance values were not significant.

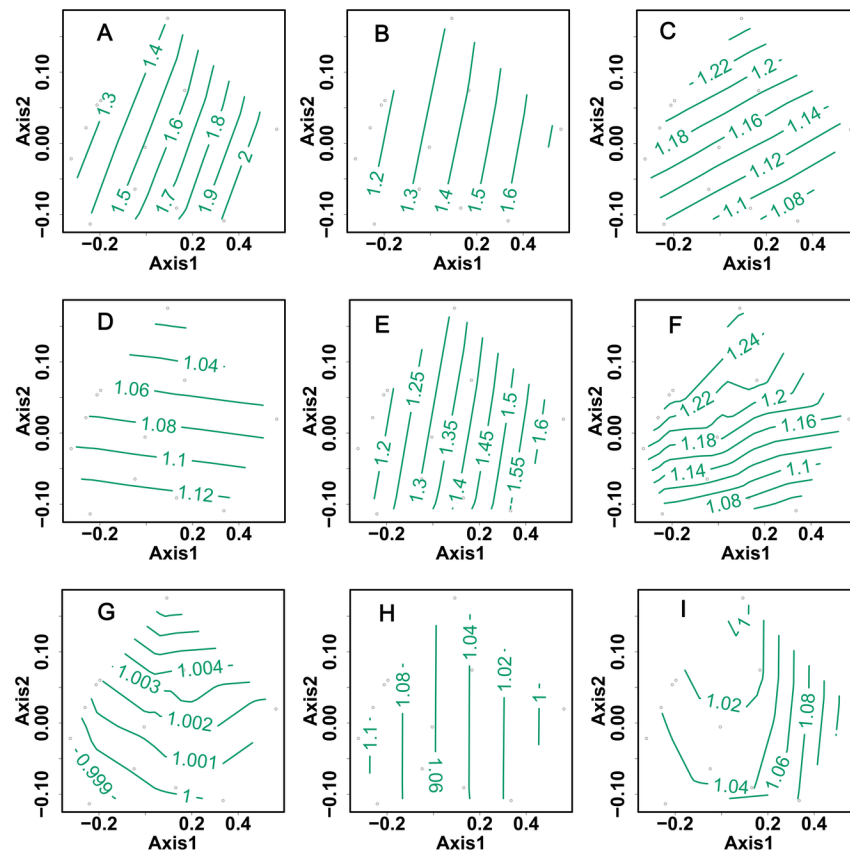


Figure 5.-Principal coordinates analysis of predictor variables and fitted as trend surfaces a posteriori by generalized additive model in Shunchang County.

300 m, *P. massoniana* ; (B) 800 m, *P. massoniana* ; (C) 800 m, Mixed forest; (D) 800 m, Urban; (E) 1,000 m, *P. massoniana* ; (F) 1,000 m, Mixed forest; (G) 1,000 m, *P. elliotii* ; (H) 1,000 m, Farmland; (I) 1,000 m, Road.

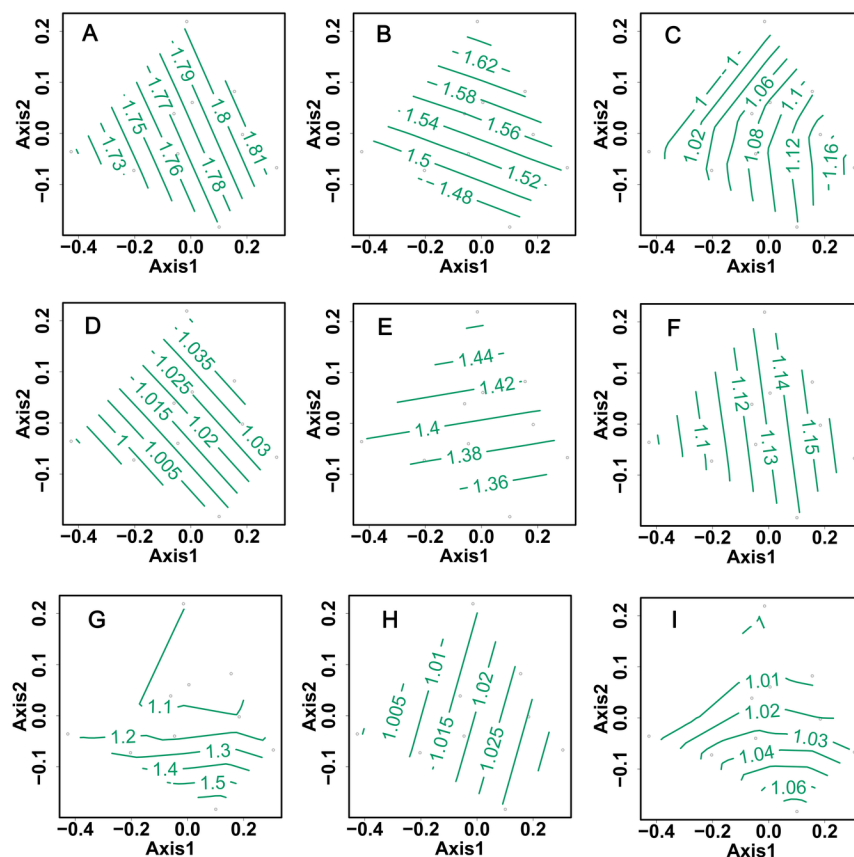


Figure 6.-Principal coordinates analysis of predictor variables and fitted as trend surfaces aposteriori by generalized additive model in Xiapu County.

(A) 300 m, *P. massoniana* ; (B) 800 m, *P. massoniana* ; (C) 800 m, Mixed forest; (D) 800 m, *P. elliotii* ; (E) 1,000 m, *P. massoniana* ; (F) 1,000 m, Mixed forest; (G) 1,000 m, Farmland; (H) 1,000 m, Road; (I) 1,000 m, Urban.