Temporal genetic variation and dispersal patterns of Aedes albopictus (Diptera: Culicidae) among three different temperature regions of China

Jian Gao  
Beijing Institute of Microbiology and Epidemiology

Hengduan Zhang  
Beijing Institute of Microbiology and Epidemiology

Xiaoxia Guo  
Beijing Institute of Microbiology and Epidemiology

Dan Xing  
Beijing Institute of Microbiology and Epidemiology

YanDe Dong  
Beijing Institute of Microbiology and Epidemiology

CeJie Lan  
Beijing Institute of Microbiology and Epidemiology

Ge Wang  
Beijing Institute of Microbiology and Epidemiology

ChaoJie Li  
Beijing Institute of Microbiology and Epidemiology

ChunXiao Li  
Beijing Institute of Microbiology and Epidemiology

TongYan Zhao (✉ tongyanzhao@126.com)

Research

Keywords: Aedes albopictus, Genetic variation, Haplotype, Dispersion pattern, Temperature regions, Microsatellite loci, Environmental factors.

DOI: https://doi.org/10.21203/rs.3.rs-38761/v1

License: 🍀 This work is licensed under a Creative Commons Attribution 4.0 International License.  
Read Full License
Abstract

Background *Aedes albopictus* is an indigenous and primary vector for Dengue and Zika viruses in China. Compare with its insecticide resistance, biology, and vector competence; little was known about its genetic variation, corresponding to environmental variations. Thus, the present study aims to discuss how *Ae. albopictus* population varies among different temperatures regions of China and decipher its potential dispersal patterns.

Methods The genetic variation and population structure of all 17 *Ae. albopictus* populations, collected from three temperature regions of China, were investigated with 11 microsatellite loci and mitochondrial COI gene.

Results 11 pairs out of 44 isolated microsatellite markers were chosen for genotyping analysis with the average PIC value of 0.713, which was high polymorphism. The number of alleles was high for each population, with the $n_e$ value increased from the Temperate region (3.876) to the Tropical region (4.144). 25 COI Haplotypes were detected, and the highest diversity was observed among the Tropical region. The mean $Ho$ value (ca. 0.557) of all temperature regions, was significantly lower than the mean $He$ values (ca. 0.684), with nearly all populations significantly departed from the HWE test and displayed significant population expansion (p-value < 0.05). Two genetically isolated groups and three Haplotype clades were evaluated via STRUCTURE and Haplotype phylogenetic analyses, with Tropical populations isolated from other regions, significantly. Meanwhile, the majority genetic variation of *Ae. albopictus* populations were detected within populations and individuals at 31.40% and 63.04%, respectively, via AMOVA test, and a relatively significant positive correlation was merely observed among populations from the temperate region via Isolation by distance (IBD) analysis ($R^2 = 0.6614, p = 0.048$). Recent dispersions were observed among different *Ae. albopictus* populations and a total of four major migration trends were rebuilt between the Tropical and the other two regions with the high genetic flows (Nm>0.5). Environmental factors, especially temperature and rainfall, may be the leading cause of genetic diversity differences of different temperature regions.

Conclusions Continuous dispersion contributes to the similarity of *Ae. albopictus* populations among different temperature regions, and environmental factors, especially temperature and rainfall, may be the leading cause of genetic variation.

Background

*Aedes (Stegomyia) albopictus*, also known as Asian tiger, is an epidemiologically critical vector for its transmission of several Arboviruses and Filarial nematodes, including Dengue virus, Zika virus, Chikungunya virus, and *Dirofilaria immitis*[1, 2, 3, 4]. Compared with *Aedes aegypti*, the primary vector for DENV and ZIKV, *Ae. albopictus* is generally considered to be an inefficient vector for its less anthropophilic and adaption to urban domestic environments[5]. However, its broad temperature adaptability and high competence for several Arboviruses make it a comprehensive important vector as
Ae. aegypti. Meanwhile, its strong ecological plasticity and global trades such as the movement of used tires and “lucky bamboo” also accelerate the global spread of this species from the original South-eastern Asian to nearly every continent except Antarctica[6]. Now, it is among the list of the top 100 worst invasive species, threatening the health of people all around the world[7].

With the development of urbanization in China, the natural environment was altered tremendously, more people have moved from rural areas to the cities, and trades increased between cities and areas, all these processes create suitable habitats for Ae. albopictus and facilitate its breeding and diffusion across the whole country[8, 9, 10, 11]. Meanwhile, the thriving international traveling and trades, providing the essential routines for the continual importing of arboviruses from other countries, especially from Southeast Asian, where Ae. albopictus originated[12], putting all people at risk. During the past ten years, at least 22 ZIKV imported cased were confirmed by the China CDC, and the persistent emergence of Dengue in Southern China, especially in Guangdong and Yunnan province, is positively related to the widespread of Aedes mosquitoes among these regions[13]. In contrast to Ae. aegypti (an invasion mosquito, only distributed in the Southern area of China), Ae. albopictus is an indigenous mosquito of China, ranging from Dalian in the north to Hainan in the south[14, 15]. Moreover, it also has been considered the sole voter responsible for numerous recent dengue fever outbreaks in China. Its wider distribution and high competence for numerous arboviruses and nematode parasites emphasize the need to study more extensively the biology, distribution, and dispersion patterns of this species.

Natural environmental variation is responsible for the fluctuation of insects’ population dynamics, distribution, and biology, including the abundance of population, intensity, and feeding behavior[16]. As a powerful and rapid adaptive insect with a high fecundity rate and short life cycle, the population dynamics and vector competence of Ae. albopictus are also greatly influenced by environmental conditions[17, 18]. Numerous previous studies discussed the influence of environmental variations on the Ae. albopictus population, mainly based on their influence on mosquito’s abundance, survival, size-fecundity, and competence for certain arbovirus[19, 20, 21, 22, 23]. However, less focused on monitoring the changes in genetic diversity and population structure of the Aedes albopictus population, corresponding to the various environmental conditions, which are essential for rebuilding the dispersion patterns of Ae. albopictus among certain regions and providing necessary information for the subsequent mosquito control.

Genetic variation is ubiquitous in the vector population, especially for invasive vectors, such as Ae. aegypti and Ae. albopictus, in helping them to occupy diverse niches and respond quickly to evolutionary challenges[24]. Microsatellites are the preferred markers in studying the genetic variation of vectors for its co-dominant, highly informative, and vast abundance throughout vector genomics[25]. Up to the present, multiplies microsatellite loci associated with Ae. albopictus were successfully isolated and employed in Ae. albopictus population study on a global scale and continue to be a popular choice of genetic marker[22, 26, 27, 28]. Simultaneously, independently from the Ae. albopictus genome, mitochondrial COI gene was also used for the mosquito barcoding and monitoring invasion species, frequently, for its
conservation and accuracy of distinguishing sequence variation more sufficiently at the inter-species [29, 30, 31, 32].

To evaluate how *Ae. albopictus* populations vary with the environmental variations among different temperatures regions of China genetically and decipher the potential dispersal patterns of *Ae.albopictus* among these regions, It was examined 17 *Ae. albopictus* population based on 11 microsatellite loci and COI gene. The results can provide some basic guidelines for the future vector control of *Ae. albopictus* in China.

**Materials And Methods**

**Mosquitoes sampling and DNA isolation**

In the present study, *ca.* 600 *Ae. albopictus* larvae were sampled from seventeen geographically sites across three environmentally distinct regions of China from June to August 2018, and all the sampling sites information as described in Fig. 1 and Additional file 1: Table S1. For each of the samples, larvae were reared and emerged, independently, at 25°C ± 1°C at 75% ± 5% relative humidity (RH) under a 14 h-light/10 h-dark (LD) photoperiod and all the Female adult mosquitoes were identified under the microscope before DNA isolation. In order to avoid the inbreeding interference, each pooled female mosquito for a given locality was picked up from at least five wild breeding places within 500 meters. The following DNA isolation work was conducted with Qiagen DNA isolate Kit (No. 69504) under the manufacturer's protocol, and all the DNA samples were stored under −80°C.

**Microsatellite isolation, processing, and COI gene amplification**

Referring to CHAMBERS E.W. et al.[33], microsatellite markers were isolated from the whole genome of *Ae. albopictus* by magnetic-bead enrichment and PCR screening method, and all markers were tested as high polymorphism via Denatured Polyacrylamide Gel Electrophoresis (D-PAGE). A set of 11 microsatellite loci were employed for genotyping 17 *Ae. albopictus* populations. Detailed microsatellite primers information was list in Table 1. All PCR reactions were performed on a T100 Thermal Cycler (Bio-Rad) under a 50 µl reaction system containing ten ng of DNA, 0.25 U of PrimeStar HS DNA Polymerase (TaKaRa), six µM of dNTPs and ca.5 µM of each primer, with the program set as 35 cycles of 95 °C for 30 sec, 57 °C for 30 sec and 72 °C for 1 min and final elongation at 72 °C for 10 min. All products were then checked with 2% agarose gel electrophoresis under UV light and run on a 3730XL DNA Genetic Analyzer (Applied Biosystems, California, USA).
| No. | Primer name | Forward sequence (5'-3') | Reverse sequence (5'-3') | Repeat Motifs | Size range (bp) | Dye     | GenBank No. |
|-----|-------------|--------------------------|--------------------------|---------------|----------------|---------|-------------|
| 1   | BW-P1       | TTAGCA TCCATCT ATTCTG GC | AAACATT CCTACG CATTTCAC | (GT)6         | 230–260       | 5'-HEX  | MT64244 6   |
| 2   | BW-P3       | GAAAATA TGTTCT ATCAAAT G | AAGTCA GTAAAA CAGGAG TCT | (GT)3GC(GT)3TTT | 132–178     | 5'-FAM  | MT64244 7   |
| 3   | BW-P6       | GAATTG GGAGCT TGTTAA AAC | CGCCTA CTGGAG AAACAC TGA | (TG)5         | 124–200      | 5'-HEX  | MT64244 8   |
| 4   | BW-P18      | CACTGG TTCTCTA TCCTAA GC | GTGTTA TCAGTT GGAAGC GTT | (GT)24        | 158–203      | 5'-HEX  | MT64244 9   |
| 5   | BW-P22      | GGGGTC CCCCCA ACATAACTC | CGGCTC CGTCCT CCTCTT CCC | (GT)5(GC GT)2(GT)3 | 187–244 | 5'-HEX  | MT64245 0   |
| 6   | BW-P23      | GGATAA GAATGA CACAGG CAC | CAAAGA GGAACA CCATAG GAA | (GAC)7        | 133–177      | 5'-FAM  | MT64245 1   |
| 7   | BW-P24      | ACGAAA CATACTT ACAATT GCA | AACCTA GAGTCC GAGAGA GAAC | (AC)8         | 145–239      | 5'-FAM  | MT64245 2   |
| 8   | BW-P26      | CGTGGT GTTCTAG GTCCAT GTT | TCGCTT TCGGCT CTAGTC AAT | (GT)5         | 107–233      | 5'-HEX  | MT64245 3   |
| 9   | BW-P27      | TTATACA AAAGC GAACAT CC | CACACA CATAGAA AAAAGC AA | (ACG)6        | 249–281      | 5'-FAM  | MT64245 4   |
| 10  | BW-P35      | TATTTG CACATC CATTTC GTCT | TTCAAA ACCTGA TTTCCG ACTG | (CA)7TTT     | 83–120       | 5'-HEX  | MT64245 5   |
As Kamgang et al. described[29], COI sequence polymorphism of each locality was investigated among at least 20 individuals. Briefly, DNA amplification of a 550-bp fragment of COI was performed on a T100 Thermal Cycler (Bio-Rad) with the following two sets of primers: 5′-GGAGGATTGG-AAATTGATTAGTTCC-3′ (F-COI) and 5′-CCCGGTAAAATTAAAATATAA-CTTC-3′(R-COI) in a 50 ul reaction mix containing 10 ul PCR reaction Buffer (TaKaRa), 4 ul of dNTPs (TaKaRa), 1 ul of Primers (10 pmol/ul), 0.5 ul PrimeStar HS DNA Polymerase (TaKaRa), respectively. The PCR amplification program was set as Pre-denaturation at 94℃ for 3 min, followed by 35 cycles of denaturation at 94℃ for 30 sec, annealing at 54℃ for 45 sec, and elongation at 72℃ for 1 min, with the final elongation at 72℃ for 10 min. All the PCR products were detected and separated by 2% agarose gel electrophoresis. The target fragments for COI were then cut from the gel under the UV light, and purified with GenElute™ PCR Clean-Up Kit (NA1020, Sigma-Aldrich). Each purified PCR product was then cloned to pCR™2.1 Vector with TA Cloning™ Kit (K202040, Invitrogen), and selected by Bacteria Liquid PCR with the T7 promoter primers. Finally, at least 20 positive clones for each PCR product were sequenced on both strands using ABI 3730XL automatic sequencer (Applied Biosystems).

**Population structure analyses, Phylogenetic genotyping and migration analyses**

Microsatellite dates were processed with GeneMapper v.4.0 (Applied Biosystems). All markers were tested polymorphism with PIC values via PIC-Calc 0.6, and the Null allele frequency of each locus was assessed with Microchecker version2.2.3. Genetic diversity and population structure of all localities were evaluated via multiple genetic indices with different software. Allele frequency indices including \( n_a \) and \( n_e \), were assessed with Cervus version 3.0.7. \( Ho \), \( He \), and \( F_{IS} \) values of all populations were calculated with Arlequin version 3.5.2.2. Departure from Hardy-Weinberg and heterozygosity deficient were assessed via Bottleneck version1.2.02. Additionally, combined with Evanno et al.’s \( \Delta K \) methods, STRUCTURE version 2.3.4 was employed to calculate the optimal K value; the parameters set as follows: (1) K ranged from 1 to 20 with ten interactions for each K value. (2) an admixture model was chosen with the Markov chain Monte Carlo algorithm of 100,000 iterations and 1,000,000 repetitions. The optimal K value was calculated via Structure Harvester: http://taylor0.biology.ucla.edu/structureHarvester/ and depicted with Distribut version1.1. R packages, “Adegenet 2.1.3” & “Genpop 4.7.5”, were used to illustrate population structure and its relationship with genetic distance, respectively; meanwhile, genetic variation and \( F_{ST} \) value among each population were evaluated with AMOVA test via Arlequin version 3.5.2.2.
Haplotypes of all populations were screened by Dnasp version 6.0, and the genetic relationship of all haplotypes was displayed with TCS network, constructed by Network 10.0.0.0. Moreover, BEast version 1.8.4 and R package “pheatmap” were used to build the phylogenetic tree of all haplotypes and discuss the distribution of all haplotypes among different temperature regions, with the best model selected by JModelTest 2.1.10. The mismatch distribution and Bayesian Skyline plot analyses were conducted with Arlequin version 3.5.2.2 and BEast version 1.8.4, respectively, to investigate the current dispersal incidences of each population; Concurrently, the possible migration routines were rebuilt via the R package “divMigrate”. Finally, the relationship between genetic indices and environmental factors was described via Principal Component Analysis (PCA) and Multiple Factor Analysis (MFA) with the R package “FactoMineR”.

Results

Microsatellite maker isolation and assessment

In the present study, a total of 44 pairs of microsatellite markers were isolated from the whole genome of *Ae. albopictus*, 11 pairs of which were tested as high polymorphism and chosen for the microsatellite genotyping analysis (Table 1). The allele number of each locus ranged from 10 to 33, with a mean of 17.545 per locus. The polymorphism information content (PIC) values of each locus ranged from 0.334 to 0.925, with a mean value of 0.713. According to the definition of Allah *et al.* (2018) about PIC value, nearly all the markers selected were highly informative (PIC value > 0.5) except BW-P18, which was 0.357 and considered as an informative marker. The Micro-checker results suggest that null alleles were present in all loci, with the frequency ranged from 0.064 to 0.157 (Additional file 8 Table S5). Linkage disequilibrium (LD) test showed that a total of 302 pairs of loci out of 1870 (16.15%) across all locations were tested significantly after Bonferroni correction, while no consistency was found among them (Additional File 5: Figure S1).

Genetic diversity and variation

The observed number of alleles (n<sub>a</sub>) of each *Ae. albopictus* population was very high, and the Mean n<sub>a</sub> value of each temperature region ranged from 6.909 to 8.091 without significant difference. In contrast, the effective number of alleles (n<sub>e</sub>) ranged from 3.501 to 4.525, and the n<sub>e</sub> value increased from the Temperate region (3.876) to the Tropical region (4.144). The mean value of observed heterozygosity (H<sub>o</sub>) for all temperature regions was ca. 0.557, which was significantly lower than that of expected heterozygosity (H<sub>e</sub>) (ca. 0.684). The F<sub>IS</sub> value of each temperature region ranged from 0.266 to 0.359, and nearly all *Ae. albopictus* populations significantly departed from the Hardy-Weinberg equilibrium test (HWE) except two locations SHJD and KZXZ (Table 2). Based on the SMM model, Heterozygosity tests of all 17 *Ae. albopictus* populations revealed that nearly all populations from Temperate and Subtropical regions displayed significant population expansion with p-value < 0.05, while no significance was observed among all these tests of *Ae. albopictus* populations from tropical regions after Bonferroni correction (Additional file 2: Table S2).
| Regions          | SC  | SS | n<sub>a</sub> | n<sub>e</sub> | F<sub>IS</sub> | Ho   | He   | HWE  |
|------------------|-----|-----|-------------|-------------|----------|------|------|------|
| Tropical Region  |     |     |             |             |          |      |      |      |
| HKWN 30          | 8.455 | 4.525 | 0.388       | 0.561       | 0.733    | 0.442*** |
| JYJB 30          | 7.636 | 4.355 | 0.385       | 0.584       | 0.699    | 0.535*** |
| JKCH 25          | 7.000 | 3.553 | 0.292       | 0.532       | 0.671    | 0.246*** |
| Mean 29          | 7.697 | 4.144 | 0.355       | 0.559       | 0.701    | 0.408*** |
| South-Subtropical Region |     |     |             |             |          |      |      |      |
| NNXD 30          | 7.909 | 4.000 | 0.336       | 0.533       | 0.700    | 0.347**  |
| NNXZ 30          | 7.909 | 4.190 | 0.473       | 0.627       | 0.725    | 0.480*** |
| GZTH 30          | 8.455 | 4.292 | 0.172       | 0.467       | 0.714    | 0.192*   |
| Mean 30          | 8.091 | 4.161 | 0.327       | 0.542       | 0.713    | 0.340**  |
| North-Subtropical Region |     |     |             |             |          |      |      |      |
| NJTH 30          | 7.455 | 3.836 | 0.392       | 0.590       | 0.682    | 0.473*** |
| NJDX 30          | 8.455 | 4.119 | 0.465       | 0.637       | 0.706    | 0.551*** |
| KZXZ 30          | 6.182 | 3.681 | 0.189       | 0.474       | 0.677    | 0.331    |
| SHJD 30          | 5.455 | 3.106 | 0.035       | 0.455       | 0.606    | 0.123    |
| Mean 30          | 6.909 | 3.677 | 0.266       | 0.542       | 0.660    | 0.369*   |
| Temperate Region |     |     |             |             |          |      |      |      |
| HNDX 30          | 7.000 | 3.642 | 0.247       | 0.553       | 0.627    | 0.365**  |
| QDDX 30          | 7.909 | 3.921 | 0.361       | 0.570       | 0.664    | 0.380**  |
| BHBG 22          | 8.091 | 3.838 | 0.433       | 0.641       | 0.637    | 0.447*** |
| BJLG 30          | 8.636 | 4.341 | 0.408       | 0.574       | 0.728    | 0.456*** |
| ZGND 30          | 6.909 | 3.728 | 0.425       | 0.631       | 0.636    | 0.419*** |
| SXJW 30          | 6.455 | 3.501 | 0.270       | 0.572       | 0.622    | 0.421**  |
| HBSD 30          | 7.546 | 3.926 | 0.256       | 0.508       | 0.687    | 0.341**  |

SC: Sample Code; SS: Sample Size; n<sub>a</sub>: Observed number of alleles; n<sub>e</sub>: Effective number of alleles (Kimura and Crow, 1964); HWE: Hardy-Weinberg disequilibrium; Ho: Observed Heterozygosity; He: Expected Heterozygosity (Nei's, 1973)

***: p < 0.001; **: p < 0.01; *: p < 0.05
### Population structure and differentiation

In the present study, all *Ae. albopictus* populations were adequately allocated to two groups with significant genetic differences, and the best K value, assessed via Evanno et al.’s ΔK methods, was also equal to two (Fig. 2a). Combined with STRUCTURE bar plots analysis, the Bayesian clustering analysis displayed that *Ae. albopictus* populations from the Tropical region were genetically isolated with the subtropical and temperature regions (Fig. 2b&c). Moreover, a total of 86.4% of variation was explained by 50 PCs in the DAPC analysis and the results revealed that two genetically isolated groups were obtained, and there was no clear relationship between *Ae. albopictus* population structure and their distribution temperature areas (Fig. 2d).

AMOVA test revealed that the majority genetic variation of *Ae. albopictus* populations were detected within populations and individuals at 31.40% and 63.04%, respectively, with the significant Fixation indices ($F_{IS}$=0.33253, $F_{IT}$=0.36962, and all $p=0.0000$; Table 3). Meanwhile, Pairwise $F_{ST}$ values between each population ranged from 0.008 (ZGND and NNXD) to 0.141 (SHJD and JKCH). Nearly all genetic differentiation was significant between all sampled populations after Bonferroni corrections ($p<0.05$), except five pairs of $F_{ST}$ values among the population NNXC, MJTH, ZGND, NJDX, BHBG, QDDX, HBSD and KZXZ (Additional file 4: Table S4). In contrast to the individual differentiation, a relatively significant positive correlation was merely observed among populations from the temperate region via Isolation by distance (IBD) analysis ($R^2 = 0.6614$, $p = 0.048$), while no such evidence was observed among the other two regions (Additional file 6: Figure S2).
Table 3
Analysis of molecular variance (AMOVA) test of 17 Ae. albopictus populations sampled from three temperature regions.

| Source of Variation | d. f. | Sum of Squares | Variance Component | Percentage of Variation | P-value   | Fixation indices |
|---------------------|-------|----------------|--------------------|------------------------|-----------|-----------------|
| Among groups        | 5     | 142.425        | 0.12789Va          | 3.62                   | P = 0.0000 | F<sub>CT</sub> = 0.03620 |
| Among population s within groups | 11   | 93.157         | 0.06845Vb          | 1.94                   | P = 0.0000 | F<sub>SC</sub> = 0.02010 |
| Among individuals within population s | 485  | 2156.367       | 1.10951Vc          | 31.40                  | P = 0.0000 | F<sub>IS</sub> = 0.33253 |
| Within individuals | 502   | 1118.000       | 2.22709Vd          | 63.04                  | P = 0.0000 | F<sub>IT</sub> = 0.36962 |
| Total               | 1003  | 3509.949       |                    | 3.53294                |           |                 |

**Haplotype network and phylogenetic analysis**

Three major Haplotype clades, distributed across three central temperature regions and closely related to each other, were rebuilt via TCS network. Haplotype (H1) of Clade was the most frequent Haplotype of all and distributed from tropical to temperate areas with an increasing trend. Nearly all other Haplotypes derived from H1 with one or two mutants, with Clade mainly distributed at the tropical region and Clade mainly distributed at the South subtropical region (Fig. 3b).

A phylogenetic tree, combined with heatmap analysis, was well-established with sequences of all 25 Haplotypes, and it also demonstrated that all 25 haplotypes were divided into three major well-supported clades. As expected, Clade was separated from Clade and Clade with a 100% bootstrap support rate, including Haplotype H9, H10, H11, H12, H19, and H20, which was only observed at the tropical region. In comparison, Clade was isolated from Clade with a relatively lower bootstrap support rate (70.74%), including five Haplotypes (H2-H3, and H5-H7, respectively) that distributed merely at South subtropical region. Nevertheless, Clade is an admixture group containing all the rest 14 Haplotypes, whereas only two of them were observed in the tropical region and four at the temperature region (Fig. 3a).

**Migration and the correlation analyses between genetic indices and environmental factors**

Migration patterns were rebuilt using divMigrate networks among all 17 Ae. albopictus populations. As expected, Ae. albopictus was observed migrating back and forth between the Tropical and Temperate areas frequently (Fig. 1). A total of four major migration trends were observed among different
temperature regions with the high genetic flows ($Nm > 0.5$). Two routines were observed originated from the Tropical and subtropical areas and destined to Hebei and Beijing of the Temperate area, while another two destined to Guangdong and Guangxi of the Southern subtropical area. Compared with the south to north routines, the latter migration routines were substantially higher. Meanwhile, nearly all Tajima's D and Fu’s $Fs$ values were tested negatively with no statistically significant $p$ values except population JKCH (Additional file 3: Table S3). Based on the COI sequences, mismatch analysis results showed that Harpending's Raggedenes indexes for all three Haplotype clades were relatively low (ranged from 0.1054 to 0.1812, $p > 0.05$) and unimodal mismatch distributions were observed among different *Ae. albopictus* populations (Additional file 7: Figure S3).

As illustrated in Fig. 4, all five genetic indices and two environmental factors (*i.e.*, Temperature and Rainfall) contributed equivalently to the first axis of the PCA up to 95.2% except the environmental factor Latitude, which contributes more to the second axis with a proportion of 17.5%. Combined with Hierarchical clustering, performed via multiple factor analysis (MFA), all 17 *Ae. albopictus* populations were then clustered into three groups, with cluster II and cluster III closely related to each other. When environmental factors were regarded as the major influencing factors, molecular diversity indices of the Tropical populations were significantly higher than that of other regions. It also demonstrated that environmental factors, especially temperature and rainfall, were the leading cause of genetic diversity differences of different temperature regions.

**Discussion**

For their high mutation rate, co-dominant expression, and universal distribution throughout the eukaryotic genome, microsatellite loci have been widely used to evaluate the genetic variation and population structure of vectors, especially for those without fully annotated genome[26, 33]. Some studies mentioned that the vast existence of the null allele might have effects on classical estimates of population differentiation, especially for the assessment of heterozygosity deficient[34, 35, 36]. In the present study, even though nearly all loci were tested high informative (PIC > 0.5), the null allele was still observed at all loci ranged from 0.02 to 0.158, with an average of 0.078. In order to improve the accuracy of the findings, the mitochondrial COI gene was also employed to investigate the genetic variation of all individuals with microsatellite loci together.

Consistent with Zhong et al.[37], *Ae. albopictus* population of the Tropical region showed higher diversity than the other two regions. Continuous dispersion and a better survival environment may be the best explanation for this phenomenon. Geographically, Southern china directly borders with many countries of Southeast Asian where *Ae. albopictus* originates, including Laos, Philippines, and Myanmar et al.[12]. Frequent border trades and personnel exchanges among these areas result in the continuous dispersion of *Ae. albopictus*, in turn, enrich the diversity of the local population, which is also confirmed by the results of bottleneck analysis that nearly all populations from Temperate and Subtropical regions experienced bottleneck effect except the populations of the Tropical region. Moreover, the hot and humid climate of Southern China is more suitable for the breeding and development of *Ae. albopictus*[38, 39,
Stimulously, all the mean $Ho$ values of the Temperate and Subtropical regions were observed significantly lower than the mean $He$ values, and nearly all populations significantly depart from the HWE test. It may be closely related to the application of mosquito control programs in these areas, and the continuous dispersion of *Ae. albopictus* from Southeast Asian countries to the Tropical region helps the recovery of the local mosquito population.

The population differentiation analyses showed that nearly all pairwise $F_{ST}$ among populations were significant but not high, which indicates the potential communications between populations across different temperature regions, especially among populations ZGND, NJTH, NJDX, and QDDX. Correspondingly, two genetically separated groups were observed among these populations, and molecular variations within populations and individuals contribute to the differentiation between populations. As a global invasion species, *Ae. albopictus* has developed several strategies to cope with a broader range of temperatures and adapt to local thermal conditions[17, 40], which helps this mosquito disposal and colonize at different locations successfully. Migration analysis results displayed four major migration trends that were observed among different temperature regions with the high genetic flows ($Nm > 0.5$). All these results revealed that human-aided dispersion might be the main reason for the similarity of populations among different regions. This hypothesis is also verified via IBD analysis that long-distance is not significantly associated with the genetic variation of individuals.

Among all the 25 haplotypes detected in the present study, nearly 44% (11 out of 25) of these haplotypes were only observed at the Tropical and Subtropical regions, the $p$-value of the mantle test for the Tropical region was significant. It means continuous dispersal from the neighborhood Southeast Asia countries maybe the reason for the higher diversity for the *Ae. albopictus* populations of the Tropical and Subtropical regions and the origination for the *Ae. albopictus* populations of these two regions may be different. Furthermore, the mismatch analysis results also revealed that recent population dispersal among different temperature regions, which is also the main reason for the universal distribution of the rest haplotypes. As a powerful and rapid adaptive insect, the biology of *Ae. albopictus* is greatly influenced by environmental conditions[17, 18]. In the present study, The PCA analysis among genetic indices and environmental factors revealed that temperature and rainfall were the leading cause of genetic diversity differences of *Ae. albopictus* populations among different temperature regions.

Overall, energetic dispersal patterns are not only observed only in the southern part of China, but frequent diffusions also exist from the Tropical and Subtropical regions to the Temperate region. Since Dengue fever continues to break out in southern China every year, the potential diffusion routines between the south and north regions may put the rest areas of China in danger.

**Conclusion**

The present study systematically evaluated the genetic variation, population structure, and haplotype phylogenetic relationships of *Ae. albopictus* populations across different temperature regions of China. In accordance with the locality or region, all 17 *Ae. albopictus* populations were genetically structured, and
three major Haplotype clusters were observed via COI phylogenetic analysis, which suggests different evolutionary histories under changing environments, especially temperature and rainfall. Meanwhile, four major migration trends were observed among different temperature regions with high genetic flows, which contributes to the similarity of all *Ae. albopictus* populations. Overall, the results of the present study suggest that more effective control strategies should be employed to prevent the multiple dispersion patterns of *Ae. albopictus* in China.

**Abbreviations**

COI: Mitochondrial cytochrome c oxidase subunit 1;

PIC: Polymorphism Information Content;

Ho: Observed heterozygosity;

He: Expected heterozygosity;

WE test: Hardy–Weinberg equilibrium test;

AMOVA test: Analysis of molecular variation test;

IBD: Isolation by distance;

WHO: World Health Organization;

CDC: Centers for Disease Control;

na: Observed number of alleles;

ne: Effective number of alleles;

FIS: Inbreeding coefficient;

FST: Genetic differences among populations;

FIT: Genetic differences within individuals.

PCA: Principal Component Analysis;

MFA: Multiple Factor Analysis;

LD: Linkage disequilibrium;

Hd: Haplotype diversity;

DAPC: Discriminant analysis of principal components;
Nm: Number of migrants.

**Declarations**

**Acknowledgment**

Not applicable.

**Founding**

This work was funded by grants from the Infective Diseases Prevention and Cure Project of China (No.2017ZX1000303404)

**Availability of data and materials**

Data are available on request to the corresponding author.

**Authors’ contributions**

JG, HDZ, CXL and TYZ jointly designed and coordinated the study, with contributions from XXG, DX and YDD. JG drafted the article with contributions from HDZ. HDZ, JG, CJL, and GW collected samples from different temperature regions of China. JG, HDZ, GW, and CJL carried out the laboratory work and performed the statistical analysis. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Author Details**

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, Beijing, Fengtai District, China.

**References**

1. Cancrini G, Frangipane di Regalbono A, Ricci I, Tessarin C, Gabrielli S, Pietrobelli M. Aedes albopictus is a natural vector of Dirofilaria immitis in Italy. Vet Parasitol. 2003;118 3-4:195-202; doi:
2. Grard G, Caron M, Mombo IM, Nkoghe D, Mboui Ondo S, Jiolle D, et al. Zika virus in Gabon (Central Africa)–2007: a new threat from Aedes albopictus? PLoS Negl Trop Dis. 2014;8 2:e2681; doi: 10.1371/journal.pntd.0002681. https://www.ncbi.nlm.nih.gov/pubmed/24516683.

3. Kress A, Kuch U, Oehlmann J, Muller R. Effects of diapause and cold acclimation on egg ultrastructure: new insights into the cold hardness mechanisms of the Asian tiger mosquito Aedes (Stegomyia) albopictus. J Vector Ecol. 2016;41 1:142-50; doi: 10.1111/jvec.12206. https://www.ncbi.nlm.nih.gov/pubmed/27232137.

4. Wong PS, Li MZ, Chong CS, Ng LC, Tan CH. Aedes (Stegomyia) albopictus (Skuse): a potential vector of Zika virus in Singapore. PLoS Negl Trop Dis. 2013;7 8:e2348; doi: 10.1371/journal.pntd.0002348. https://www.ncbi.nlm.nih.gov/pubmed/23936579.

5. Rezza G. Aedes albopictus and the reemergence of Dengue. BMC Public Health. 2012;12:72; doi: 10.1186/1471-2458-12-72. https://www.ncbi.nlm.nih.gov/pubmed/22272602.

6. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, et al. The global distribution of the arbovirus vectors Aedes aegypti and Ae. albopictus. Elife. 2015;4:e08347; doi: 10.7554/eLife.08347. https://www.ncbi.nlm.nih.gov/pubmed/26126267.

7. Bonizzoni M, Gasperi G, Chen X, James AA. The invasive mosquito species Aedes albopictus: current knowledge and future perspectives. Trends Parasitol. 2013;29 9:460-8; doi: 10.1016/j.pt.2013.07.003. https://www.ncbi.nlm.nih.gov/pubmed/23916878.

8. Chadee DD, Martinez R. Aedes aegypti (L.) in Latin American and Caribbean region: With growing evidence for vector adaptation to climate change? Acta Trop. 2016;156:137-43; doi: 10.1016/j.actatropica.2015.12.022. https://www.ncbi.nlm.nih.gov/pubmed/26796862.

9. Jia P, Chen X, Chen J, Lu L, Liu Q, Tan X. How does the dengue vector mosquito Aedes albopictus respond to global warming? Parasit Vectors. 2017;10 1:140; doi: 10.1186/s13071-017-2071-2. https://www.ncbi.nlm.nih.gov/pubmed/28284225.

10. Johnson MTJ, Munshi-South J. Evolution of life in urban environments. Science. 2017;358 6363; doi: 10.1126/science.aam8327. https://www.ncbi.nlm.nih.gov/pubmed/29097520.

11. Reba M, Reitsma F, Seto KC. Spatializing 6,000 years of global urbanization from 3700 BC to AD 2000. Sci Data. 2016;3:160034; doi: 10.1038/sdata.2016.34. https://www.ncbi.nlm.nih.gov/pubmed/27271481.

12. Lai S, Johansson MA, Yin W, Wardrop NA, van Panhuis WG, Wesolowski A, et al. Seasonal and interannual risks of dengue introduction from South-East Asia into China, 2005-2015. PLoS Negl Trop Dis. 2018;12 11:e0006743; doi: 10.1371/journal.pntd.0006743. https://www.ncbi.nlm.nih.gov/pubmed/30412575.

13. Xiang B, Gao P, Kang Y, Ren T. Importation of Zika Virus in China: A significant risk in southern China. J Infect. 2017;74 3:328-30; doi: 10.1016/j.jinf.2017.01.004. https://www.ncbi.nlm.nih.gov/pubmed/28109676.
14. Wu F, Liu Q, Lu L, Wang J, Song X, Ren D. Distribution of Aedes albopictus (Diptera: Culicidae) in northwestern China. Vector Borne Zoonotic Dis. 2011;11 8:1181-6; doi: 10.1089/vbz.2010.0032. https://www.ncbi.nlm.nih.gov/pubmed/21254912.

15. Zheng X, Zhong D, He Y, Zhou G. Seasonality modeling of the distribution of Aedes albopictus in China based on climatic and environmental suitability. Infect Dis Poverty. 2019;8 1:98; doi: 10.1186/s40249-019-0612-y. https://www.ncbi.nlm.nih.gov/pubmed/31791409.

16. Ayres JS, Schneider DS. The role of anorexia in resistance and tolerance to infections in Drosophila. PLoS Biol. 2009;7 7:e1000150; doi: 10.1371/journal.pbio.1000150. https://www.ncbi.nlm.nih.gov/pubmed/19597539.

17. Culbert NJ, Gilles JRL, Bouyer J. Investigating the impact of chilling temperature on male Aedes aegypti and Aedes albopictus survival. PLoS One. 2019;14 8:e0221822; doi: 10.1371/journal.pone.0221822. https://www.ncbi.nlm.nih.gov/pubmed/31454400.

18. Kweka E, Baraka V, Mathias L, Mwang’onde B, Baraka G, Lyaruu L, et al. Ecology of Aedes Mosquitoes, the Major Vectors of Arboviruses in Human Population. Dengue Fever - a Resilient Threat in the Face of Innovation; 2019.

19. Catherine J. Westbrook MHR, Kendra N. Pesko,Krystle E. Greene, and L. Philip Lounibos. Larval Environmental Temperature and the Susceptibility of Aedes albopictus Skuse (Diptera: Culicidae) to Chikungunya Virus. VECTOR-BORNE AND ZOONOTIC DISEASES. 2010;10:241-7.

20. Costanzo KS, Westby KM, Medley KA. Genetic and environmental influences on the size-fecundity relationship in Aedes albopictus (Diptera: Culicidae): Impacts on population growth estimates? PLoS One. 2018;13 8:e0201465; doi: 10.1371/journal.pone.0201465. https://www.ncbi.nlm.nih.gov/pubmed/30071049.

21. Ephantus J. Muturi MBJ, and Allison Montgomery. Temperature and density-dependent effects of larval environment on Aedes aegypti competence for an alphavirus. Journal of Vector Ecology. 2012;37 1:154-61.

22. Lubinda J, Trevino CJ, Walsh MR, Moore AJ, Hanafi-Bojd AA, Akgun S, et al. Environmental suitability for Aedes aegypti and Aedes albopictus and the spatial distribution of major arboviral infections in Mexico. Parasite Epidemiol Control. 2019;6:e00116; doi: 10.1016/j.parepi.2019.e00116. https://www.ncbi.nlm.nih.gov/pubmed/31528740.

23. Murdock CC, Evans MV, McClanahan TD, Miazgowicz KL, Tesla B. Fine-scale variation in microclimate across an urban landscape shapes variation in mosquito population dynamics and the potential of Aedes albopictus to transmit arboviral disease. PLoS Negl Trop Dis. 2017;11 5:e0005640; doi: 10.1371/journal.pntd.0005640. https://www.ncbi.nlm.nih.gov/pubmed/28558030.

24. Powell JR. Genetic Variation in Insect Vectors: Death of Typology? Insects. 2018;9 4; doi: 10.3390/insects9040139. https://www.ncbi.nlm.nih.gov/pubmed/30314367.

25. Vieira ML, Santini L, Diniz AL, Munhoz Cde F. Microsatellite markers: what they mean and why they are so useful. Genet Mol Biol. 2016;39 3:312-28; doi: 10.1590/1678-4685-GMB-2016-0027. https://www.ncbi.nlm.nih.gov/pubmed/27561112.
26. Manni M, Gomulski LM, Aketarawong N, Tait G, Scolari F, Somboon P, et al. Molecular markers for analyses of intraspecific genetic diversity in the Asian Tiger mosquito, Aedes albopictus. Parasit Vectors. 2015;8:188; doi: 10.1186/s13071-015-0794-5. https://www.ncbi.nlm.nih.gov/pubmed/25890257.

27. Multini LC, Marrelli MT, Wilke AB. Microsatellite loci cross-species transferability in Aedes fluviatilis (Diptera:Culicidae): a cost-effective approach for population genetics studies. Parasit Vectors. 2015;8:635; doi: 10.1186/s13071-015-1256-9. https://www.ncbi.nlm.nih.gov/pubmed/26667177.

28. Porretta D, Gargani M, Bellini R, Calvitti M, Urbanelli S. Isolation of microsatellite markers in the tiger mosquito Aedes albopictus (Skuse). Molecular Ecology Notes. 2006;6 3:880-1; doi: 10.1111/j.1471-8286.2006.01384.x.

29. Kamgang B, Brengues C, Fontenille D, Njiokou F, Simard F, Paupy C. Genetic structure of the tiger mosquito, Aedes albopictus, in Cameroon (Central Africa). PLoS One. 2011;6 5:e20257; doi: 10.1371/journal.pone.0020257; doi: https://www.ncbi.nlm.nih.gov/pubmed/21629655.

30. L MH-T, V AB, N IN, Ignacio R-A, Barrero E, Thorne L, et al. DNA barcoding of British mosquitoes (Diptera, Culicidae) to support species identification, discovery of cryptic genetic diversity and monitoring invasive species. Zookeys. 2019;832:57-76; doi: 10.3897/zookeys.832.32257. https://www.ncbi.nlm.nih.gov/pubmed/30930645.

31. Park DS, Suh SJ, Hebert PD, Oh HW, Hong KJ. DNA barcodes for two scale insect families, mealybugs (Hemiptera: Pseudococcidae) and armored scales (Hemiptera: Diaspididae). Bull Entomol Res. 2011;101 4:429-34; doi: 10.1017/S0007485310000714. https://www.ncbi.nlm.nih.gov/pubmed/21272395.

32. Shaikevich E, Talbalaghi A. Molecular Characterization of the Asian Tiger MosquitoAedes albopictus(Skuse) (Diptera: Culicidae) in Northern Italy. ISRN Entomology. 2013;2013:1-6; doi: 10.1155/2013/157426.

33. Chambers EW, Meece JK, McGowan JA, Lovin DD, Hemme RR, Chadee DD, et al. Microsatellite isolation and linkage group identification in the yellow fever mosquito Aedes aegypti. J Hered. 2007;98 3:202-10; doi: 10.1093/jhered/esm015. https://www.ncbi.nlm.nih.gov/pubmed/17420178.

34. Chapuis MP, Estoup A. Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol. 2007;24 3:621-31; doi: 10.1093/molbev/msl191. https://www.ncbi.nlm.nih.gov/pubmed/17150975.

35. Girard P. A robust statistical method to detect null alleles in microsatellite and SNP datasets in both panmictic and inbred populations. Stat Appl Genet Mol Biol. 2011;10:Article 9; doi: 10.2202/1544-6115.1620. https://www.ncbi.nlm.nih.gov/pubmed/21381434.

36. Rico C, Cuesta JA, Drake P, Macpherson E, Bernatchez L, Marie AD. Null alleles are ubiquitous at microsatellite loci in the Wedge Clam (Donax trunculus). PeerJ. 2017;5:e3188; doi: 10.7717/peerj.3188. https://www.ncbi.nlm.nih.gov/pubmed/28439464.

37. Zhong D, Lo E, Hu R, Metzger ME, Cummings R, Bonizzoni M, et al. Genetic analysis of invasive Aedes albopictus populations in Los Angeles County, California and its potential public health
impact. PLoS One. 2013;8 7:e68586; doi: 10.1371/journal.pone.0068586. 
https://www.ncbi.nlm.nih.gov/pubmed/23861921.

38. Jia P, Liang L, Tan X, Chen J, Chen X. Potential effects of heat waves on the population dynamics of the dengue mosquito Aedes albopictus. PLoS Negl Trop Dis. 2019;13 7:e0007528; doi: 10.1371/journal.pntd.0007528. https://www.ncbi.nlm.nih.gov/pubmed/31276467.

39. Mogi M, Armbruster PA, Tuno N, Aranda C, Yong HS. The Climate Range Expansion of Aedes albopictus (Diptera: Culicidae) in Asia Inferred From the Distribution of Albopictus Subgroup Species of Aedes (Stegomyia). J Med Entomol. 2017;54 6:1615-25; doi: 10.1093/jme/tjx156. 
https://www.ncbi.nlm.nih.gov/pubmed/28968769.

40. Rafael A. Marinho EBB, Maria A. Bezerra-Gusmão, Valbia de S. Porto, Ricardo A. Olinda, and Carlos A. C. dos Santos. Effects of temperature on the life cycle, expansion, and dispersion of Aedes aegypti (Diptera: Culicidae) in three cities in Paraiba, Brazil. Journal of Vector Ecology. 2016;41 1:10.

Additional Files

Additional file 1: Table S1 Sampling information of 17 Ae. albopictus populations collected from three different temperature zones of China.

Additional file 2: Table S2 Heterozygosity tests of all 17 Ae. albopictus populations based on SMM model.

Additional file 3: Table S3 Haplotype diversity of 17 Ae. albopictus populations based on COI collected from three different climatic regions of China.

Additional file 4: Table S4 Population differentiation estimation of the Fst value (below the diagonal) and Geographic distance (above the diagonal) between all 17 Ae. albopictus populations.

Additional file 5: Figure S1 Linkage disequilibrium analysis at each pair of Loci across all 17 Ae. albopictus populations.

Additional file 6: Figure S2 Isolation by distance analysis of all 17 Ae. albopictus populations.

Additional file 7: Figure S3 Historical demography analysis of Ae. albopictus inferred from mtDNA COI sequences.

Additional file 8 Table S5 Genetic diversity of 11 microsatellite loci developed for Ae. albopictus based on samples(n=502) collected from three different climatic regions of China.

Figures
Figure 1

Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.
Figure 2

The population structure analysis of all 17 Ae. albopictus populations based on 11 microsatellite loci. (a) K values assessed via Evanno et al.’s ΔK methods; (b) STRUCTURE bar plots (k=2); (c) Bayesian clustering analysis of all Ae. albopictus populations; (d) DAPC analysis of all Ae. albopictus populations, and 86.4% of variation was explained by 50 PCs.
Figure 3

The Bayesian and Haplotype network analysis of the representing 25 haplotypes among all 17 Ae. albopictus populations based on COI sequences. (a) Bayesian tree of the representing 25 haplotypes was constructed via BEast version1.8.4 with the best model “HKY+G” selected by JModelTest 2.1.10; (b) TCS haplotype network for the COI gene of all Ae. albopictus individuals (n=502) from different temperature regions of China. The sizes of circles are proportional to haplotype frequency, and each line segment represents a single mutation.
Figure 4

Multiple factor analysis (MFA) and Hierarchical clustering performed on 17 Ae. albopictus populations sampled from three different temperature regions. (a) Multiple factor analysis between genetic indices of all individuals and three environmental variables (temperature, rainfall and altitude) of 17 Ae. albopictus populations, populations of different clusters were marked with different colors; (b) Hierarchical clustering of all 17 Ae. albopictus populations on the factor map.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- AdditionalFile1TableS1.doc
- AdditionalFile2TableS2.doc
- AdditionalFile3TableS3.doc
- AdditionalFile4TableS4.doc
- AdditionalFile5FigureS1.tif
- AdditionalFile6FigureS2.tif
- AdditionalFile7FigureS3.tif
- AdditionalFile8TableS5.doc
- Graphicalabstract.tif