Genetic structure and vector competence for DENV-2 of *Aedes albopictus* (Diptera: Culicidae) populations in China

Yong Wei

Southern Medical University

Jiatian Wang

Southern Medical University

Shengqun Deng

Southern Medical University

Peiyang Fan

Southern Medical University

Yulan He

Southern Medical University

Ke Hu

Southern Medical University

Yulan Chen

Southern Medical University

Xinyi Yu

Southern Medical University

Yiwei Wang

Southern Medical University

Guofa Zhou

University of California Irvine

Daibin Zhong

University of California Irvine

Xueli Zheng (✉ zhengxueli2001@126.com)

Research

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Abstract

Background: As a vector of over 20 arboviruses, *Aedes albopictus* has spread throughout the world mostly since the second half of the twentieth century, and it is now found on every continent except Antarctica. Approximately 50-100 million people are infected with dengue virus (DENV) transmitted by *Aedes* mosquitoes each year, leading to a heavy economic burden on both governments and individuals. Understanding the population genetics and vector competence of vector species is critical to effectively preventing and controlling vector-borne diseases. The aim of this study was to examine the genetic structure and vector competence for dengue virus type 2 (DENV-2) of *Ae. albopictus* populations across China and their relationship.

Methods: From July to September in 2019, *Ae. albopictus* eggs were collected by using ovitraps in 8 localities across China and reared to adults in laboratory. The mitochondrial gene cytochrome *c* oxidase subunit 1 (*cox1*) were used to examine the patterns of genetic diversity and population structure among native *Ae. albopictus* populations. The vector competence was detected by quantitative analysis of DENV-2 virus titer in mosquito tissues (midguts, heads and salivary glands) through qRT-PCR. The correlation between population genetic indices and DENV-2 loads in the mosquitoes' tissues was also examined.

Results: A total of 20 haplotypes of mtDNA cytochrome *c* oxidase subunit 1 (*cox1*) were identified in the 120 specimens from the eight tested populations. The dominate haplotype H01 was detected in seven geographic populations of mainland China. Genetic parameters such as haplotype diversity (H_d), nucleotide diversity (π), and fixation index (F_{ST}) revealed the population diversity decreasing from south to north, and low population genetic differentiation. STRUCTURE analysis indicated that *Ae. albopictus* populations in southern China were clustered. In addition, The Mantel test indicated a positive correlation between genetic distance and geographical distance ($R^2 = 0.364$, $P = 0.003$). We observed no correlation between population genetic indices of *cox1* in *Ae. albopictus* populations and DENV-2 virus loads. However, the southern populations had the low DENV-2 virus loads generally.

Conclusion: Conventional genetic markers such as *cox1* may not reflect genetic differences in mosquitoes' vector competence in different regions. The fact of lower DENV-2 loads in southern populations may be associated with the stable immunity system established in mosquitoes due to the long-term prevalence of dengue disease in these areas. The genetic structure and vector competence of *Ae. albopictus* populations in this study may have implications for understanding the epidemiology, prevention and control of vector-borne diseases.

Background

Aedes (Stegomyia) albopictus (Skuse, 1894) is an anthropophilic mosquito which invaded from Southeast Asia into many countries across all continents except Antarctica [1]. This species is the vector of more than 20 arboviruses, and of which some are strongly pathogenic and transmissible viruses, such as dengue virus (DENV), Chikungunya virus (CHIKV), and yellow fever viruses [2, 3]. Global warming, strong adaptability of *Ae. albopictus*, and human-aided transportation have contributed to the reemergence and wide spread of mosquito-borne diseases with the expansion of mosquito habitats [4–6].

Population genetic studies of *Ae. albopictus* can provide information about the transmission dynamics of specific pathogens, which aids in designing strategies for controlling vector-borne diseases [7, 8]. There are approximately 50 million new cases of dengue each year, among an estimated 2.5 to 4 billion people living in over 100 countries where DENV transmission occurs [9, 10]. In China, dengue fever and dengue hemorrhagic fever epidemics frequently occurred in the southern regions. Prior to 2000 this mainly included Guangdong, Hainan, Guangxi, and Fujian Provinces [11]. However, dengue fever epidemics have headed northward to Zhejiang province (in 2005 and 2011) in southeastern China and to Henan province (in 2013) in temperate central China, which is far north of any previous epidemics [12]. The recent waves of dengue outbreak in China highlight the need to improve our knowledge of *Ae. albopictus* population distribution and dynamics. In this study, we performed genetic analysis of *Ae. albopictus* based on the mitochondrial gene cytochrome *c* oxidase subunit 1 (*cox1*), which is also widely used for barcoding, and numerous published sequences from different geographical origins are available [13–15].

The vector competence for DENV of *Aedes* mosquitoes is influenced not only by the ecological structure, climate, virus titer, the serotype and virulence of DENV, but also by population genetic characteristics of mosquitoes [16–20]. The environmental

factors and the mosquito density were used to predict the risk of dengue outbreaks. The susceptibility to dengue virus of different mosquito species are significantly different, so are those of different geographic strains of the same mosquito species. Significant variation in susceptibility to four dengue serotypes was observed among 13 geographic strains of *Ae. albopictus* [21] and among 13 geographic strains of *Ae. aegypti* [22]. Boromisa et al. [23] reported the eight geographic strains of *Ae. albopictus* from Asia and North America showed marked variation in susceptibility to oral infection with DENV-1. Paupy et al. [24] found that the populations of *Ae. albopictus* from the east coast were more susceptible to DENV-2 than those from the west coast on Réunion. In our previous study, we have divided the 34 populations of *Ae. albopictus* across northern and southern China into 5 different genetic clustering groups according to the genetic distance. We expected to select 8 populations of *Ae. albopictus* from different genetic clusters to carry out virus-infection experiments for exploring the impact of genetic factors of *Ae. albopictus* on dengue susceptibility.

The present study continued with our previous study to address the following questions: (i) What is the genetic structure of different geographic strains of *Ae. albopictus* populations in China?; (ii) What are the vector competence for DENV-2 of different geographic strains of *Ae. albopictus* populations in China?; (iii) Is there any association between genetic diversity indices and vector competence to DENV-2 of *Ae. albopictus* populations? To address these questions, 8 populations of *Ae. albopictus* from different genetic clusters and DENV-2 were used in the study. The data gained from this study could provide useful information for understanding the epidemiology, prevention, and control of vector-borne diseases.

Materials And Methods

Mosquito sampling

From July 20th to September 25th, 2019, we collected *Ae. albopictus* eggs from the 8 locations in different genetic clusters from our previous study [25] in China (Table 1). The abbreviations of the 8 locations are as followed: LS, Lingshui; ZJ, Zhanjiang; GZ, Guangzhou; MS, Meishan; WH, Wuhan; HZ, Hangzhou; SJZ, Shijiazhuang; BJ, Beijing. In each location, 8–12 oviposition sites at 400–3000 m apart were selected randomly for eggs. And the ovitraps placed under the bushes were used for eggs collection.

Table 1
Population information, genetic polymorphism and neutrality tests of *Ae. albopictus* in China

Populations	Abbreviation	Latitude	longitude	n	S	h	H _d	k	π	Tajima's D	Fu's F _s
Beijing	BJ	39°51'36"N	116°11'45"E	15	0	1	0.000	0.000	0.000	0.000	–
Shijiazhuang	SJZ	37°54'55"N	114°27'49"E	15	1	2	0.133	0.133	0.017	-1.159	-0.649
Hangzhou	HZ	30°18'42"N	120°07'09"E	15	4	5	0.638	0.762	0.096	-1.220	-2.233*
Wuhan	WH	30°30'30"N	114°22'39"E	15	2	3	0.457	0.495	0.062	-0.513	-0.447
Meishan	MS	30°11'55"N	103°52'01"E	15	6	6	0.848	1.771	0.223	-0.140	-1.149
Guangzhou	GZ	23°11'15"N	113°19'42"E	15	5	5	0.752	1.162	0.146	-0.824	-1.146
Zhanjiang	ZJ	21°05'37"N	109°42'60"E	15	7	7	0.886	2.076	0.261	-0.128	-1.768
Lingshui	LS	18°30'27"N	110°01'59"E	15	6	6	0.829	1.924	0.242	0.149	-0.926

Eggs were returned to the laboratory and reared to adults. The larvae were fed daily with turtle food (Shenzhen INCH-GOLD Fish Food, LTD, Shenzhen, CHA). *Ae. albopictus* adults were identified [26] and selected for further breeding at (28 ± 1) °C, 16 h:8 h light:dark diurnal cycle at (80 ± 5) % relative humidity in 20 cm × 20 cm × 35 cm cages covered with nylon mesh. Mosquito adults were provided with 10% glucose solution. Every third day, over a two-week period, sugar was removed from the cages 24 h prior to feeding on defibrinated sheep blood (Solarbio, Beijing, CHA). Bloodfed females were then given constant access to wet paper towels as an oviposition substrate.

PCR amplification and sequencing of mitochondrial DNA (mtDNA)

Fifteen adult female *Ae. albopictus* mosquitoes from different oviposition sites in each location were used for DNA extraction. Total genomic DNA was individually extracted using the Insect DNA Kit (Omega Bio-tek, GA, USA), following the manufacturer's protocol. The mitochondrial gene cytochrome c oxidase subunit 1 (*cox1*) was used to examine sequence polymorphism among mosquito samples. PCR was performed to amplify a 796 bp fragment of the 5' *cox1* region of mtDNA using the DNA primer pairs 2027F (5'-CCCGTATTAGCCGGAGCTAT-3') and 2886R (5'-ATGGGGAAAGAAGGAGTTCG-3') [13]. The PCR reaction mixture consisted of 40 ng genomic DNA, 12.5 µl 2 × PCR Master Mix (Promega, Madison, WI, USA), 1 µl each of the forward and reverse primers at 10 µmol/l, and sufficient nuclease-free water to make 25 µl. PCR conditions were as follows: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min; and a final extension at 72 °C for 10 min. The amplified fragments were run on a 1% agarose gel to check integrity, stained with ethidium bromide and analyzed under UV light. PCR products were purified using a gel extraction kit (Omega Bio-tek, GA, USA) and sequenced with PCR primer 2027F using the ABI 3730XL automatic sequencer (Applied Biosystems, Foster City, CA, USA). The sequences of *cox1* unique haplotypes were deposited to the GenBank database under the accession numbers MT188111-MT188130.

Mosquito oral infections

DENV-2 virus (New Guinea C strain, GenBank: AF038403.1) was provided by the Key Laboratory of Tropical Disease Control of Sun Yat-sen University (Guangzhou, China). The virus stock was obtained after 5–7 days incubation in C6/36 cells at 28 °C, and stored at -80 °C. The frozen virus stock was passaged once through C6/36 cells before the mosquitoes were infected. The fresh virus suspension was used to prepare the blood meal for mosquito oral infections.

The 5- to 7-day-old and non-blood-fed female mosquitoes were starved for 24 h before infection. The females were fed with an infectious blood meal (the fresh DENV-2 virus suspension was diluted in defibrinated sheep blood to $5.77 \times 10^5 \log_{10}$ copies/µl). After 30 min of exposure to the infectious blood meal, fully engorged mosquitoes were transferred into the 1500-ml plastic bucket covered with nylon mesh. They were provided with 10% glucose and maintained for 14 days in an HP400GS incubator (Ruihua, Wuhai, China) at 28 °C, 80% relative humidity, and a light:dark cycle of 16 h:8 h.

RNA extraction and qPCR

At 14 days post-infection (dpi), 30 mosquitoes were randomly collected and cold-anesthetized. The legs and wings of the mosquitoes were removed, then the heads, salivary glands and midguts were dissected, washed three times in cold phosphate-buffered saline and transferred to 50 µl TRIzol (Ambion, Life Technologies, Carlsbad, CA, USA). The total RNA of each tissue was extracted with TRIzol reagent following the manufacturer's protocol and reverse-transcribed into cDNA using a 5 × All-In-One MasterMix with AccuRT Genomic DNA Removal Kit (Abm, Richmond, Canada). For DENV-2 quantification, a standard plasmid was constructed. A 127-bp fragment of the untranslated region (UTR) of DENV-2 was amplified using specific primers (forward: 5' – TCCCTTACAAATCGCAGCAAC– 3'; and reverse: 5' – TGGTCTTTCCAGCGTCAAT– 3') [27] and cloned into a pMD18-T vector. After sequencing, the recombinant plasmid was linearized by *EcoR* I. The concentration of the plasmid was detected by NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The 127-bp fragment of DENV-2 UTR was the detecting target for qRT-PCR, and a standard curve was generated from a range of serial 10-fold dilutions of the plasmid. The viral RNA copies in all of these tissues were quantified by detecting the cDNA of DENV-2 using Hieff® qPCR SYBR® Green Master Mix (YEASEN, Shanghai, China). The qRT-PCR reaction mixture per well contained 10 µl SYBR® green master mix, 1 µl of each primer (10 µM), 2 µl cDNA or the plasmid standard, and 6 µl RNase-free water. The reaction was performed in a QuantStudio™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) under the following conditions: 95 °C for 5 min; 40 cycles of 95 °C for 10 s, 60 °C for 30 s. DENV-2 RNA copies from each sample were quantified by comparing cycle threshold value with the standard curve.

Data analysis

The *cox1* gene sequences from 120 mosquitoes were aligned using Clustal W multiple alignment in BioEdit version 7.2.5 [28]. The number of segregating sites (S), haplotype diversity (Hd), average number of nucleotide differences (k), and nucleotide diversity (π) within each population were determined using DnaSP version 5.10.1 [29]. Deviations from selective neutrality were

tested using Fu's F_s statistic [30] and Tajima's D [31]. Neutrality test was performed for each population to examine population expansion. To determine the genealogical relationships among haplotypes, a haplotype network was constructed using a statistical parsimony algorithm implemented in TCS version 1.21 [32]. The minimum number of mutational steps between sequences was calculated with > 95% confidence. Genetic differentiation among populations was estimated, and analysis of molecular variance (AMOVA) was conducted using Arlequin version 3.5.2.2 [33]. Bayesian clustering analysis was used to infer population structure in STRUCTURE V2.3 [34]. The burn-in was set to 100,000 steps and was followed by 2,000,000 Markov Chain Monte Carlo replications. All runs were repeated 20 times for each number of possible clusters from 1 to 8 (the number of populations). The optimal number of clusters (K) was determined using the Delta K method of Evanno et al. [35] in the online version of Structure Harvester v.0.6.94 [36]. Individuals were partitioned into multiple clusters according to the membership coefficient (Q) that ranges from 0 (lowest affinity to a cluster) to 1 (highest affinity to a cluster) across the K clusters. Finally, the programs CLUMPP [37] and DISTRUCT [38] were used to average replicate runs and to generate bar graphs of structure results, respectively.

The vector competence of the *Ae. albopictus* mosquitoes was evaluated by calculating the infection rate (IR; no. infected midguts/no. tested midguts), dissemination rate (DR; no. infected heads/no. infected midguts), potential transmission rate (TR; no. infected salivary glands/no. infected midguts) and potential population transmission rate (PTR; no. infected salivary glands/no. tested mosquitoes) [39]. Pearson's Chi-square tests or Fisher's exact tests were applied to compare IR, DR, TR and PTR among different geographical strains. Fisher's exact test was used when the minimum expected count was < 5. The DENV-2 RNA copy levels were log-transformed and then compared among different populations by using one-way analysis of variance (ANOVA) post hoc Tukey's honestly significant difference (HSD) tests. Pearson's correlation coefficient was used to analyze the correlation between the average dengue virus load in each tissue and genetic indices, including haplotype diversity (H_d), average number of nucleotide differences (k), nucleotide diversity (π), Tajima's D and Fu's F_s . All analyses were performed using SPSS 20.0 statistical software (IBM, Chicago, IL, USA). A significance level at $P < 0.05$ was set for all statistical tests, and the sequential Bonferroni correction [40] was used when significant correlations were detected between the paired data.

Results

Sequence variation and haplotype network

PCR amplification and sequencing of the mitochondrial *cox1* gene resulted in a 796 bp fragment for each individual study subject, with no insertions or deletions. All of the 120 sequences were identical or possessed > 99% similarity with *Ae. albopictus* (GenBank: KR068634). 18 variable sites were observed and 14 of them were parsimony informative (Table 1). Among eight populations, the Zhanjiang (ZJ) population had the highest values of polymorphism sites ($S = 7$), haplotype diversity ($H_d = 0.886$), nucleotide diversity ($\pi = 0.261$) and the highest average number of nucleotide differences ($k = 2.076$). However, the Beijing (BJ) population had the lowest values of these genetic indices for it had only one haplotype. Tajima's D tests for all populations were not statistically significant (Table 1), indicating that the populations are in genetic equilibrium, consistent with the neutral mutation hypothesis. Likewise, Fu's F_s tests were not statistically significant and rejected the population expansion/bottleneck model, with the exception of one Hanzhou population (HZ, $F_s = -2.233$, $P = 0.015$) (Table 1).

A total of 20 haplotypes of mtDNA *cox1* were detected in the 120 specimens (GenBank: MT188111-MT188130, Additional file 1: Table S1). To determine the relationships among the samples or haplotypes, we constructed a median-joining network using haplotypes based on sequence variation (Fig. 1). The most samples were identified as H01 (49.2%) in *Ae. albopictus* populations, but no haplotype H01 in Lingshui population (LS) (Fig. 1). Other haplotypes were either unique to a specific population (such as H03 in HZ; H06, H07, H08, H10 in MS; H11 in GZ; H12, H14, H15 in ZJ; H17, H18, H19, H20 in LS), or had a limited geographical distribution (such as H13 and H16 in southern China (ZJ and LS), H09 in southern-western China (MS, GZ and ZJ)) (Fig. 1).

Genetic clustering and differentiation

Based on Bayesian clustering analysis and the Delta K method, the optimal partitioning of all samples was obtained for $K = 4$ with structure analysis by the Delta K method (Fig. 2). The proportional membership coefficient of individuals was showed in the pie charts from the eight *Ae. albopictus* populations studied (Fig. 3, Additional file 2: Table S2). The largest membership coefficient value (Q), or the proportion of individuals assigned to a cluster, were high for Lingshui ($Q = 0.630$), suggesting a strong affinity to be included in a single cluster (cluster 3). This cluster was shared with some of the individuals from Zhanjiang and Guangzhou, consistent with the results obtained in the network analysis (Fig. 1). Individuals from the rest populations mainly constituted three genetic clusters (clusters 1, 2, and 4; Fig. 3), suggesting a strong gene flow among these populations. Based on these results and previous study [25], we divided the eight populations into two groups: southern (GZ, ZJ and LS) and other populations (BJ, SJZ, HZ, WH, MS). AMOVA results (Table 2) indicate that the majority of the variation in *Ae. albopictus* was within populations, accounting for 54.19% of the variation, while variations among groups and populations within groups accounted for 35.10% and 10.72% of the total variation, respectively. Fisher's exact test showed that there was significant genetic variation at these three levels.

Table 2
Analysis of molecular variance (AMOVA) of two groups of populations of *Ae. albopictus* in China

Source of variation	df	SS	Variance components	Percentage of variation	P-value	Fixation index
Among groups	1	21.018	0.337	35.10	$P = 0.012$	$F_{CT} = 0.351$
Among populations within groups	6	12.382	0.103	10.72	$P < 0.001$	$F_{SC} = 0.165$
Within populations	112	58.267	0.520	54.19	$P < 0.001$	$F_{ST} = 0.458$
Total	119	91.667	0.960			
Abbreviations: df, degrees of freedom; SS, sum of squares						

Twenty-one of 28 pairwise tests were significant at $P < 0.05$ after Bonferroni correction, and pairwise F_{ST} values ranged from 0.117 (between HZ and GZ) to 0.620 (between BJ and LS), with an average of 0.333 (Table 3). The Mantel test showed statistically significant correlation ($y = 0.52x - 3.18$, $R^2 = 0.364$, $P = 0.003$) between the genetic distance (y , estimated as $F_{ST} / (1 - F_{ST})$) and geographical distance (x , estimated as $\ln(\text{km})$) between populations.

Table 3
Pairwise genetic differentiation and geographical distance between *Ae. albopictus* populations from China

	BJ	SJZ	HZ	WH	MS	GZ	ZJ	LS
BJ	-	5.573	7.021	6.959	7.347	7.536	7.685	7.803
SJZ	0.000	-	6.900	6.714	7.169	7.403	7.563	7.696
HZ	0.048	0.003	-	6.312	7.352	6.947	7.286	7.415
WH	0.071	0.004	-0.025	-	6.916	6.710	7.044	7.247
MS	0.170*	0.160*	0.136*	0.150*	-	7.107	7.064	7.273
GZ	0.208*	0.181*	0.117*	0.141*	0.160*	-	6.084	6.434
ZJ	0.513*	0.498*	0.432*	0.448*	0.346*	0.243*	-	5.668
LS	0.620*	0.602*	0.526*	0.542*	0.441*	0.354*	0.022	-
Pairwise genetic differentiation (F_{ST}) between all populations displayed below the diagonal; geographical distance [ln (km)] displayed above the diagonal								
*Asterisks indicate significant values after Bonferroni correction ($P < 0.05$)								

Vector competence of *Ae. albopictus* for DENV-2

At 14 days post-infection (dpi), The IR, DR, TR and PTR were all 100.00% (30/30) in BJ and SJZ populations ; 96.67% (29/30), 96.55% (28/29), 93.10% (27/29) and 90.00% (27/30), respectively, in HZ population; 96.67% (29/30), 96.55% (28/29), 89.66% (26/29) and 86.67% (26/30), respectively, in WH population; 93.33% (28/30), 96.43% (27/28), 92.86% (26/28) and 86.67% (26/30), respectively, in MS population; 93.33% (28/30), 96.43% (27/28), 89.29% (25/28) and 83.33% (25/30), respectively, in GZ population; 93.33% (28/30), 89.29% (25/28), 85.71% (24/28) and 80.00% (24/30), respectively, in ZJ population; 96.67% (29/30), 93.10% (27/29), 89.66% (26/29) and 86.67% (26/30), respectively, in LS population (Table 4). The IR, DR and TR of DENV-2 in mosquitoes were not significantly different among the eight population of *Ae. albopictus* (IR: $\chi^2 = 4.52$, df = 7, $P = 0.838$; DR: $\chi^2 = 5.72$, df = 7, $P = 0.456$; TR: $\chi^2 = 8.56$, df = 7, $P = 0.221$). However, there was significant difference in the PTR among them ($\chi^2 = 13.18$, df = 7, $P = 0.043$). The PTR between populations was further compared by using ANOVA post hoc Tukey's HSD tests. The PTR in ZJ population showed significantly different with that in BJ population ($\chi^2 = 6.67$, df = 1, $P = 0.024$) and SJZ population ($\chi^2 = 6.67$, df = 1, $P = 0.024$), respectively.

Table 4

Rates of dengue virus infection, dissemination, potential transmission and population potential transmission by *Ae. albopictus* females from eight different populations

Populations	IR	DR	TR	PTR
BJ	100.00% (30/30)	100.00% (30/30)	100.00% (30/30)	100.00% (30/30)
SJZ	100.00% (30/30)	100.00% (30/30)	100.00% (30/30)	100.00% (30/30)
HZ	96.67% (29/30)	96.55% (28/29)	93.10% (27/29)	90.00% (27/30)
WH	96.67% (29/30)	96.55% (28/29)	89.66% (26/29)	86.67% (26/30)
MS	93.33% (28/30)	96.43% (27/28)	92.86% (26/28)	86.67% (26/30)
GZ	93.33% (28/30)	96.43% (27/28)	89.29% (25/28)	83.33% (25/30)
ZJ	93.33% (28/30)	89.29% (25/28)	85.71% (24/28)	80.00% (24/30)
LS	96.67% (29/30)	93.10% (27/29)	89.66% (26/29)	86.67% (26/30)
Abbreviations: IR: infection rate = no. infected midguts/no. tested midguts (%); DR: dissemination rate = no. infected heads/no. infected midguts (%); TR: potential transmission rate = no. infected salivary glands/no. infected midguts (%); PTR: potential population transmission rate = no. infected salivary glands/no. infected mosquitoes (%).				

The amount of dengue virus in the mosquito midguts, heads and salivary glands was measured by qRT-PCR (Fig. 4). At 14 days post-infection, the average DENV RNA copies ($\log_{10}$) in midguts for each population were 4.82 (BJ), 4.55 (SJZ), 4.38 (HZ), 5.11 (WH), 5.21 (MS), 3.24 (GZ), 4.18 (ZJ), 4.51 (LS), respectively. There was significant difference ($P < 0.05$) in midgut between GZ and each one of BJ, SJZ, HZ, WH, MS, LS; and between MS and ZJ. The average DENV RNA copies ($\log_{10}$) in heads for each population were 4.25 (BJ), 3.93 (SJZ), 3.79 (HZ), 4.69 (WH), 3.99 (MS), 3.23 (GZ), 3.51 (ZJ), 3.68 (LS), respectively. There was significant difference ($P < 0.05$) in heads between WH and each one of SJZ, HZ, MS, GZ, ZJ, LS; between GZ and each one of BJ, SJZ, MS; and between BJ and ZJ. The average DENV RNA copies ($\log_{10}$) in salivary glands for each population were 3.72 (BJ), 3.63 (SJZ), 3.35 (HZ), 3.85 (WH), 3.45 (MS), 3.13 (GZ), 2.94 (ZJ), 3.13 (LS), respectively. There was significant difference ($P < 0.05$) in salivary glands between GZ and each one of BJ, SJZ, WH; between ZJ and each one of BJ, SJZ, WH, MS; between LS and each one of BJ, SJZ, WH; and between WH and HZ.

Correlation between population genetic indices and vector competence

In order to explore the association between population genetic structure and vector competence, we conducted Pearson's correlation analysis of five genetic indices and the average dengue virus load in each tissue in different populations (Additional file 3: Table S3). The average dengue virus load in midguts and heads showed no statistically significant correlation to the five genetic indices. The average dengue virus load in salivary glands showed a negative correlation with H_d ($r = -0.772$, $P = 0.025$), k ($r = -0.810$, $P = 0.015$), and π ($r = -0.810$, $P = 0.015$). However, the significance was lost after the Bonferroni correction for multiple testing (adjusted P -value < 0.00179).

Discussion

At present, there have been many studies on the application of *cox1* in mosquito population genetics. But it has not been discussed whether the genetic factors from *cox1* analysis could affect mosquito vector competence. As we know, the outbreak of mosquito borne diseases would be affected by climate, mosquito density, vector competence and so on [41–44]. Would mosquito populations of different geographical strains affect their vector competence due to genetic factors, leading to differences in incidence rate of mosquito borne diseases in different areas? From this aspect, we would carry out experiments and data analysis to explore the genetic characteristics of mosquito populations in different regions and the relationship between the genetic characteristics and mosquito vector competence in these regions.

Population genetic of *Ae. albopictus* would be helpful for understanding the degree of population spread and establishment, which facilitate dengue prevention and vector control. In this study, the dominate haplotype H01 was detected in 7 geographic populations of mainland China, suggesting it was relatively conservative and primordial. The haplotype diversity decreased from south to north, which was similar to our previous study [25]. The suitable climate in the south may be conducive to the growth, development and reproduction of each genotype of *Ae. albopictus*, while the cold and dry climate in the north may play a role in mosquito genotype selection [45, 46]. Results of Tajima's D and Fu's F_s tests (Table 2) indicated no sign of population bottleneck/expansion but in demographic equilibrium. Thus, these populations could have existed stably rather than recently invaded as a new founding population [47]. Our results showed that F_{ST} -values were low and not significantly different among BJ, SJZ, HZ and WH, and they couldn't be separated by clustering analysis, whereas they were significantly different and separated in our previous study using microsatellites. These results also indicated the strong gene flow among these populations. Through comparing the results of clustering and population differentiation between this study and our previous study [25], we could infer that weaker separation between populations was obtained with compared to microsatellites, which was associated with that microsatellites evolved more rapidly than mitochondrial sequence data [48]. The F_{ST} -value was not significantly different between ZJ and LS, but these two populations had differentiation with any other population, which might be related to the fact that the two populations had gene flow with *Ae. albopictus* from other countries through ships for they were close to ocean. Divergence of specific haplotypes in ZJ and LS was observed from network structures, such as H12, H14, H15 and H19. Reasons for this phenomenon needed to explore based on more samples, in order to reveal this differentiation was caused by immigrant samples or local population divergences. Mantel test suggested that population differentiation of *Ae. albopictus* had positive relation with geographic distance, which was similar to the previous study [25].

There are several factors influencing vector competence of *Aedes* mosquitoes, such as mosquitoes' midgut microbiota, innate immune system, climate, the virulence of the virus strain and the genetic background of mosquito populations tested [21, 27, 49–52]. In this study, the eight different geographical strains of *Ae. albopictus* were fed with a blood meal containing the same DENV-2 strain at the same concentration and kept in the same condition for 14 days. Therefore, the vector's genetic characteristics are probably an important parameter in the observed difference in vector competence. An advantage of the current study is that we were able to quantify the amount of DENV-2 virus in the midgut and disseminated infections through real-time quantitative PCR while other studies have only compared infection, dissemination or transmission rates [53, 54]. Our results showed that there was not significant difference in IR, DR, TR and PTR among the eight population of *Ae. albopictus*, with the exception of the significant difference in PTR between ZJ and BJ/SJZ population. That indicated ZJ population of *Ae. albopictus* had lower potential population transmission rate than BJ and SJZ populations. The amount of DENV-2 virus of midguts, heads and salivary glands in GZ population of *Ae. albopictus* were significantly lower than that in BJ, SJZ and WH populations. The amount of DENV-2 virus of salivary glands in ZJ and LS populations of *Ae. albopictus* were significantly lower than that in BJ, SJZ and WH populations. We could find that the amount of DENV-2 virus in southern populations (GZ, ZJ and LS) was generally lower than other populations. This seems to be similar to the division of population genetic structure. So we also did the correlation analysis between the genetic indices of each population and dengue loads in the tissues of *Ae. albopictus*. However, there were no statistically significant correlation between them. One possible reason might be that the mosquito vector competence of the same species is not affected by the genetic factors of different geographical strains, but mainly affected by the climate and the microbial communities *in vivo* [27, 49–51]. Another one might be that the genetic level of the samples we collected is not enough to represent the overall level of the region, which need more collecting sites and more samples. And another one might be that other genetic markers or loci associated with immunity should have been used to analyze the population genetic structure of mosquitoes in different regions in order to establish a link with vector competence [55–58]. For example, Lambrechts et al. [59] found that Dicer-2 genotype was associated with resistance to dengue virus in *Ae. aegypti*. The fact of lower DENV-2 loads in southern populations might be associated with the stable immunity system established in mosquitoes due to the long-term prevalence of dengue disease in these areas [11, 12]. Viral infection triggers the activation of innate immunity pathways and leads to the transcription of genes responsible for antiviral responses in mosquitoes [60–62]. Innate immunity is a functional response against foreign organisms, formed in the long-term evolution process of mosquitoes after frequent viral infection, including RNA interference pathways, JAK-STAT, Toll and Imd pathways [63, 64]. We will further focus on studying the relationship between mosquitoes' innate immune system or immune-related genetic loci and vector competence.

Conclusions

This study reported not only the genetic structure and vector competence of *Ae. albopictus* from eight locations across China but also the correlation between them. Our results showed the population differentiation among *Ae. albopictus* was low and positively related with geographic distance, which was similar to our previous study. There was no significant correlation between vector competence and the genetic indices of each population from *cox1* analysis. However, the southern populations had the low DENV-2 loads generally, which may be associated with innate immunity and worth deep research. The genetic structure and vector competence of *Ae. albopictus* populations may have implications for understanding the epidemiology, prevention and control of vector-borne diseases. Further researches are needed to focus on mosquitoes' innate immune system or immune-related genetic loci to reduce vector competence for prevention and control of vector-borne diseases.

Abbreviations

AMOVA

analysis of molecular variance; *cox1*:cytochrome c oxidase subunit 1; DENV-2:dengue virus type 2; IR:infection rate; DR:dissemination rate; TR:potential transmission rate; PTR:potential population transmission rate.

Declarations

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Ethics approval and consent to participate

No specific permits were required for the field studies. After explanation of the purposes and activities of the study, oral consent was obtained from the local participating residents prior to mosquito collection. No sites were protected by law and this study did not involve endangered or protected species.

Consent for publication

Not applicable.

Availability of data and materials

The data sets supporting the results are included within the article and its additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YW, XLZ and DBZ conceived and designed the experiments. YW, JTW, SQD, PYF, YLH, KH, YLC, XYY and YWW performed the experiments. YW and DBZ analyzed the data. YW, XLZ, DBZ and GFZ wrote and revised the manuscript. All authors read and approved the final manuscript.

Author details

¹Department of Pathogen Biology, School of Public Health, Southern Medical University, Guangzhou, China. ²Program in Public Health, College of Health Sciences, University of California, Irvine, USA.

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Figures

Figure 1

Phylogenetic network of 20 mitochondrial haplotypes of *cox1* gene in *Aedes albopictus* from 8 localities. Localities are indicated by different color (bottom-right). Sizes of circles are approximately proportional to the number of individuals with the given haplotype. Abbreviations: LS, Lingshui; ZJ, Zhanjiang; GZ, Guangzhou; MS, Meishan; WH, Wuhan; HZ, Hangzhou; SJZ, Shijiazhuang; BJ, Beijing

Figure 2

Scatter plots of Log probability of the data (a) and Delta K (b) for *Ae. albopictus* populations. Delta K plots are based on the rate of change in the log probability of the data between successive K values

Figure 3

Genetic structure of *Ae. albopictus* within 8 locations. Each vertical bar in the bar plots represents an individual sample and each color represents a cluster, where the color of the bar indicates the probability of assignment to each of 4 clusters (different colors) determined using Evanno et al.'s ΔK methods. Pie charts showing the composition of proportional membership coefficient of *Ae. albopictus* populations within the 4 clusters

Figure 4

Dengue virus RNA copies in infected midguts (a), heads (b), and salivary glands (c) of *Ae. albopictus* in China. The results are expressed as means $\pm$ standard error (SE). Different letters with the same color above bars represent significant differences in relative expression levels at the $P < 0.05$ level

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