Spatial distribution of mutations in voltage-gated sodium channel genes of Culex pipiens pallens/Culex pipiens quinquefasciatus in China

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Research Article

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Abstract

Background

*Culex pipiens pallens* and *Culex pipiens quinquefasciatus* are the main species of bloodsucking mosquitoes in China. The aim of this study is to understand the current situation of VGSC genes of *Cx. pipiens pallens* and *Cx. pipiens quinquefasciatus* in China and the impact of pyrethroid insecticides on the long-term evolution of mosquito populations, providing a basis for scientific prevention and control.

Methods

Study 28 geographic populations in 22 provinces. Partial fragments of the voltage-gated sodium channel (VGSC) gene on the nerve cell membrane were amplified by PCR. The mutation, mutation frequency and phylogenetic were analyzed after sequencing.

Results

There were 6 alleles and 6 genotypes at the L1014 locus, which were wild-type TTA / L and CTA / L, mutant TTT / F, TTC / F, TCT / S and TCA / S. The proportion of homozygous L/L and mutant homozygous F/F in genotype is similar, which are more than 30.00% The geographic populations with frequency less than 20.00% are mainly concentrated in the north of 38 ° N, and the geographic populations with frequency more than 80.00% in the south of 30 ° N. frequency increases with the decrease of latitude, and frequency has urban tendency. mutation is correlated with introns. The mutant allele TCA / S has only one intron, TTT / F has three introns, and the wild allele TTA / L has 17 introns.

Conclusions

The allelic genotype of *Culex pipiens pallens/Culex pipiens quinquefasciatus* in China is diversified, and the resistance of *Culex pipiens pallens/Culex pipiens quinquefasciatus* in most areas has been developed, and the degree of resistance is regional. gene has a polymorphism in its adjacent downstream intron and is related to mutation.

Background

*Culex pipiens pallens* and *Culex pipiens quinquefasciatus* are both geographic subspecies of *Culex pipiens*, which are widely distributed in China, with *Culex pipiens pallens* mainly distributed in northern China and *Cx. pipiens quinquefasciatus* dominating in southern China[1]. The larvae of *Cx. pipiens pallens* often breed in polluted water bodies, and the adult mosquitoes are the main domestic nuisance
mosquito species in China, with biting and blood-sucking, which is an important target for urban mosquito control. They can transmit Bancroftian filariasis\(^2\).

Insecticides are widely used to control mosquito vectors because of their high efficiency, rapidity, broad-spectrum, convenience, etc. Since the first discovery of resistance in 1908, resistance has been found in more and more insect populations. One of the more widely used for pyrethroid insecticides, pyrethroids can act on the sodium channel, so that the sodium channel mutation, produces resistance to insecticides, that is, knockdown resistance (\(kdr\)). The current study found that a mutation at site 1014 of the SI\(\text{II6}\) fragment of the para-type sodium channel caused the mutation in mosquitoes\(^3\text{–}^5\). Mutations at locus 1014 were found in both \(C\text{x.\, p. pallens}\) and \(C\text{x.\, p. quinquefasciarus}\)\(^6,^7\). The emergence of drug resistance in wild populations of mosquitoes is a complex process, which is subjected to insecticide selection pressure during the evolutionary process from a wild purebred SS mutated to a wild/mutant heterozygote RS, which then evolved into a mutant purebred RR\(^8\). Mosquito resistance is not only due to insecticide selection pressure, but individual migration, genetic drift, and neutral evolution during the long-term evolution of mosquitoes may affect the emergence and development of resistance. Mutations at the locus induced by selective pressure from pyrethroid insecticides may lead to reduced nucleotide diversity in haplotypes\(^9,^{10}\). Variation in the neighboring intronic region of the gene can provide information about the long-term effects of pyrethroid insecticides on the evolution of mosquito populations\(^11\). Genetic diversity analysis of the locus, including the intronic region, can help to understand population genetic characteristics and predict the risk of localized emergence of resistance. However, at present, there are large time intervals and regional limitations in the monitoring of resistance to \(C\text{x.\, p. pallens/\, C\text{x.\, p. quinquefasciarus}\). For example, in Anhui province, after the survey of \(C\text{x.\, p. quinquefasciarus}\) resistance in some areas in the 1990s, the survey of \(C\text{x.\, p. quinquefasciarus}\) resistance in north-central Anhui province was conducted only after a lapse of 20 years\(^12\). To understand the current situation of \(C\text{x.\, p. pallens/\, C\text{x.\, p. quinquefasciarus}\) resistance in China, this paper examined the mutation of \(C\text{x.\, p. pallens/\, C\text{x.\, p. quinquefasciarus}\) in 22 provinces. We analyzed the composition of downstream introns adjacent to their genes to understand the effects of pyrethroid insecticides on the long-term evolution of mosquito populations. Provide a basis for research on resistance mechanisms and monitoring priority directions.

**Methods**

**Mosquito collection and species identification**

Sampling was conducted from July to September 2021 in representative habitats in 28 municipalities of 22 provinces in China. The spoon fishing method was used to collect larvae, and the mosquito trapping lamp method was used to collect adult mosquitoes. A total of 8000 mosquitoes from 28 populations were collected (Fig. 1A). Combining morphology and molecular biology for mosquito species identification. Primer COI-F: 5’-GGC CAA CAA ATC ATA AAG ATA TTG G-3’, COI-R: 5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’.
Nucleic acid extraction and amplification

The DNA of a single adult *Culex mosquito* was extracted using a magnetic bead-based microtissue genomic DNA extraction kit. The whole genomic DNA was used as a template to amplify the structural domain IIS6 of the gene of voltage-gated sodium channel (VGSC) using primer CD. CD1-F: 5’-GAC CTG CCA CGG TGG AAC T-3', CD2-R: 5’-TTG GAC AAA AGC AAG GCT AAG-3’. The PCR reaction system contained 2 µL of whole genomic DNA, 12.5 µL of 2×Taq PCR premix, 1 µL of each of the 10 µmol/L positive (negative) primers, and ddH2O was added to 25 µL. The PCR reaction conditions were 94 ℃ for 3 min. 94 ℃ for 30 s, 60 ℃ for 30 s, and 72 ℃ for 30 s, with a total of 35 cycles. And 72 ℃ for 8 min, and stored at 4 ℃. PCR amplification products were detected using 1% agarose gel electrophoresis, and samples with clear bands and no trailing were selected for forward sequencing.

Phylogenetic analysis

Seqman software was applied to compare and analyze the peak maps of the measured partial sequences of the VGSC gene. Muscle comparison of sequences using MEGA 11. MEGA 11 and ChiPlot to construct Neighbor-Joining (NJ) trees. GeneDoc to plot sequence comparisons. The TCS network was built using PopART and the haplotype network was plotted.

Statistical analyses

Excel 2021 (Microsoft, Washington, USA) was used to conduct statistical analysis. Use Seqman and MEGA X software to compare and analyze the peak plots of the measured *kdr* gene sequences, and calculate the knock down resistance mutation frequency (*kdr* frequency).

\[
    kdr\text{ frequency} = \frac{RR + RS}{N^2} \quad (RR \text{ is resistant pure or resistant heterozygote. RS is heterozygote genotype of wild and resistant genes).}
\]

Results

Mutation types at locus 1014 of the gene

A total of 1308 sequences from structural domain II were obtained in this study. The codon 1014 site of structural domain II was analyzed, and six allelic genotypes and six genotypes were identified (Table 1). One category is non-synonymous mutations. Non-synonymous mutations can be categorized into two types, one is the mutation of leucine to phenylalanine (L1014F) and the other is the mutation of leucine to serine (L1014S). The second type is the base double mutation of L1014F /L1014S, which means that leucine is mutated to both phenylalanine and serine at the same time (F1014S). And the other type is the base synonymous mutation (L1014L). Alleles TTA/L and CTA/L encoding leucine, TTT/F and TTC/F encoding phenylalanine, and TCT/S and TCA/S encoding serine were obtained. The frequencies of TTT/F and TTA/L were higher, with frequencies of 41.17% and 43.58%. Genotypes included wild-type
purebred L/L, wild/mutant heterozygotes F/L and S/L, mutant purebred F/F and S/S, and mutant heterozygotes F/S, with frequencies ranging from 37.11% (F/F) to 1.72% (S/L).

<table>
<thead>
<tr>
<th>Table 1 Type of mutation at 1014 locus of <em>Cx. pipiens pallens</em> / <em>Cx. pipiens quinquefasciatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of mutation at 1014 locus (n = 1308)</td>
</tr>
<tr>
<td>Allelic genotype</td>
</tr>
<tr>
<td>TTA/L</td>
</tr>
<tr>
<td>CTA/L</td>
</tr>
<tr>
<td>TTT/F</td>
</tr>
<tr>
<td>TTC/F</td>
</tr>
<tr>
<td>TCA/S</td>
</tr>
<tr>
<td>TCT/S</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>L/L</td>
</tr>
<tr>
<td>F/L</td>
</tr>
<tr>
<td>S/L</td>
</tr>
<tr>
<td>F/F</td>
</tr>
<tr>
<td>S/S</td>
</tr>
<tr>
<td>F/S</td>
</tr>
</tbody>
</table>

Note: TTA, CTA, TTT, TTC, TCA, and TCT are codons. L, F, and S are concatenated bases representing leucine, phenylalanine, and serine. TTA and CTA encode leucine. TTT and TTC encode phenylalanine. TCA and TCT encode serine. n represents the total number of test worms.

**Spatial distribution of the gene**

The mutant allele TTT/F was detected in all 28 populations. TTT/F frequency from 98.9% (YNJH) to 2.08% (JLTH). TCT/S was detected in the JXNC and SDLC populations at a frequency of 1.20%. CTA/L was found only in the SDLC population at a frequency of 2.38%. Mutant heterozygotes F/S were detected in 14 populations of ZJNB, HNSY, and SDZB with frequencies ranging from 26.67% (ZJNB) to 1.19% (HNLY). The S/L frequencies were all less than 10.00% and were present in nine populations. In addition, F/F was detected in all 28 populations, with frequencies ranging from 97.87% (YNJH) to 2.08% (JLTH). Overall, TTT/F was the predominant mutant allele type.

Geographic populations within the same latitudinal range are similar in their allelic and genotypic composition. frequency increases with decreasing latitude (Fig. 1B-C). Geographic populations north of
38°N are predominantly TTA/L, L/L, with the allelic mutation being TTT/F, and they have fewer mutant purebreds and a single mutation type. The frequency of mutant alleles rises in populations at 30–38°N latitude compared to high latitude populations, with the emergence of TCA/S, TCT/S mutant allele types, and mutant heterozygotes F/S. Alleles and genotypes tend to be enriched in mid-latitude mid-populations. The percentage of mutant alleles in some populations south of 30°N latitude was more than 90.00%, dominated by TTT/F. Lower latitude populations have increased mutant purities, mainly F/F. Alleles and genotypes are more homogeneous. Geographic populations with frequencies less than 20.00% were concentrated north of 38°N. Frequencies of 20.00%-80.00% were mostly in 30–38°N. And geographic populations south of 30°N latitude have frequencies greater than 80.00%. (Fig. 1D) Within the same latitudinal range, the frequency was significantly higher in the big city population than in the other populations. The frequencies of BJCP, HBWH, SHSJ, CQSPB, and GDGZ were 36.52%, 93.24%, 86.84%, 90.48%, and 84.15, which were higher than those of other populations at the same latitude (Table 2).
Table 2
frequency of Cx. pipiens pallens/ Cx. pipiens quinquefasciatus in different geographical populations

<table>
<thead>
<tr>
<th>District</th>
<th>Code</th>
<th>N</th>
<th>SS (%)</th>
<th>RS (%)</th>
<th>RR1 (%)</th>
<th>RR2 (%)</th>
<th>frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jilin Tonghua</td>
<td>JLTH</td>
<td>48</td>
<td>47(97.92)</td>
<td>0(0.00)</td>
<td>1(2.08)</td>
<td>0(0.00)</td>
<td>2.08</td>
</tr>
<tr>
<td>Liaoning Shenyang</td>
<td>LNSY</td>
<td>42</td>
<td>35(83.33)</td>
<td>5(11.91)</td>
<td>2(4.76)</td>
<td>0(0.00)</td>
<td>10.71</td>
</tr>
<tr>
<td>Hebei Chengde</td>
<td>HBCD</td>
<td>41</td>
<td>35(85.37)</td>
<td>3(7.32)</td>
<td>3(7.32)</td>
<td>0(0.00)</td>
<td>10.98</td>
</tr>
<tr>
<td>Beijing Changping</td>
<td>BJCP</td>
<td>89</td>
<td>35(39.33)</td>
<td>43(48.31)</td>
<td>11(12.36)</td>
<td>0(0.00)</td>
<td>36.52</td>
</tr>
<tr>
<td>Tianjin Xiqing</td>
<td>TJXQ</td>
<td>47</td>
<td>37(78.72)</td>
<td>8(17.02)</td>
<td>2(4.26)</td>
<td>0(0.00)</td>
<td>12.77</td>
</tr>
<tr>
<td>Tianjin Hedong</td>
<td>TJHD</td>
<td>47</td>
<td>33(70.21)</td>
<td>10(21.28)</td>
<td>4(8.51)</td>
<td>0(0.00)</td>
<td>19.15</td>
</tr>
<tr>
<td>Ningxia Yinchuan</td>
<td>NXYC</td>
<td>40</td>
<td>33(82.50)</td>
<td>6(15.00)</td>
<td>1(2.50)</td>
<td>0(0.00)</td>
<td>10.00</td>
</tr>
<tr>
<td>Shandong Zibo</td>
<td>SDZB</td>
<td>45</td>
<td>19(42.22)</td>
<td>2(4.44)</td>
<td>14(31.11)</td>
<td>10(22.22)</td>
<td>55.56</td>
</tr>
<tr>
<td>Shandong Liaocheng</td>
<td>SDLC</td>
<td>42</td>
<td>9(21.42)</td>
<td>9(21.43)</td>
<td>17(40.48)</td>
<td>7(16.67)</td>
<td>67.86</td>
</tr>
<tr>
<td>Shandong Qingdao</td>
<td>SDQD</td>
<td>47</td>
<td>30(63.83)</td>
<td>12(25.53)</td>
<td>4(8.51)</td>
<td>1(2.13)</td>
<td>23.40</td>
</tr>
<tr>
<td>Gansu Pingliang</td>
<td>GSPL</td>
<td>34</td>
<td>23(67.65)</td>
<td>5(14.71)</td>
<td>6(17.65)</td>
<td>0(0.00)</td>
<td>25.00</td>
</tr>
<tr>
<td>Shanxi Jincheng</td>
<td>SXJC</td>
<td>42</td>
<td>14(33.34)</td>
<td>12(28.57)</td>
<td>14(33.33)</td>
<td>2(4.76)</td>
<td>52.38</td>
</tr>
<tr>
<td>Henan Luoyang</td>
<td>HNLY</td>
<td>84</td>
<td>16(19.05)</td>
<td>18(21.43)</td>
<td>49(58.33)</td>
<td>1(1.19)</td>
<td>70.24</td>
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<tr>
<td>Shanxi Ankang</td>
<td>SXAK</td>
<td>46</td>
<td>18(39.13)</td>
<td>17(36.96)</td>
<td>11(23.91)</td>
<td>0(0.00)</td>
<td>42.39</td>
</tr>
<tr>
<td>Jiangsu Taizhou</td>
<td>JSTZ</td>
<td>48</td>
<td>28(58.33)</td>
<td>10(20.83)</td>
<td>9(18.75)</td>
<td>1(2.08)</td>
<td>32.29</td>
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<tr>
<td>Hubei Xiangyang</td>
<td>HBXY</td>
<td>39</td>
<td>10(25.64)</td>
<td>10(25.64)</td>
<td>15(38.46)</td>
<td>4(10.26)</td>
<td>61.54</td>
</tr>
<tr>
<td>Shanghai Songjiang</td>
<td>SHSJ</td>
<td>38</td>
<td>3(7.89)</td>
<td>4(10.53)</td>
<td>29(76.32)</td>
<td>2(5.26)</td>
<td>86.84</td>
</tr>
<tr>
<td>Hubei Wuhan</td>
<td>HBWH</td>
<td>37</td>
<td>1(2.70)</td>
<td>3(8.11)</td>
<td>28(75.68)</td>
<td>5(13.51)</td>
<td>93.24</td>
</tr>
<tr>
<td>Zhejiang Ningbo</td>
<td>ZJNB</td>
<td>45</td>
<td>1(2.22)</td>
<td>12(26.67)</td>
<td>20(44.44)</td>
<td>12(26.67)</td>
<td>84.44</td>
</tr>
<tr>
<td>Chongqing Shapingba</td>
<td>CQSPB</td>
<td>42</td>
<td>4(9.52)</td>
<td>0(0.00)</td>
<td>35(83.33)</td>
<td>3(7.14)</td>
<td>90.48</td>
</tr>
<tr>
<td>Jiangxi Nanchang</td>
<td>JXNC</td>
<td>41</td>
<td>4(9.76)</td>
<td>15(36.59)</td>
<td>14(34.15)</td>
<td>8(19.51)</td>
<td>71.95</td>
</tr>
<tr>
<td>Hunan Changsha</td>
<td>HNCS</td>
<td>40</td>
<td>4(10.00)</td>
<td>33(82.50)</td>
<td>1(2.50)</td>
<td>2(5.00)</td>
<td>88.75</td>
</tr>
<tr>
<td>Hunan Loudi</td>
<td>HNLG</td>
<td>39</td>
<td>3(7.69)</td>
<td>5(12.82)</td>
<td>31(79.49)</td>
<td>0(0.00)</td>
<td>85.90</td>
</tr>
<tr>
<td>Guizhou Guiyang</td>
<td>GZGY</td>
<td>22</td>
<td>5(22.73)</td>
<td>0(0.00)</td>
<td>17(77.27)</td>
<td>0(0.00)</td>
<td>77.27</td>
</tr>
</tbody>
</table>
Gene neighboring downstream introns characterization

Twenty-one haplotypes were obtained from 614 D2 sequences. Sequence analysis showed that the gene of *Cx. pipiens pallens / Cx. pipiens quinquefasciatus* was adjacent to the downstream intron, and the 5'end of GT,3' was AG (Fig. 2), which accorded with the characteristics of intron\(^{[16]}\). Introns consist of four bases, adenine (A), thymine (T), cytosine (C), and guanine (G). Overall, the total AT content was more than the total GC content in all cases. The difference between AT and GC was most pronounced at the first base site, and A was most predominant at all but the second base site (Table 3). It suggests that AT values are used more in the downstream intron adjacent to the gene of *Cx. pipiens pallens / Cx. pipiens quinquefasciatus*, and that adenine A is preferred at the codon site.

### Table 3

Sequence characteristics of introns adjacent to downstream of gene

<table>
<thead>
<tr>
<th>Base site</th>
<th>A (%)</th>
<th>T (%)</th>
<th>C (%)</th>
<th>G (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First base site (108bp*)</td>
<td>30.2</td>
<td>28.8</td>
<td>23.8</td>
<td>17.2</td>
</tr>
<tr>
<td>Second base site (110bp*)</td>
<td>26.7</td>
<td>28.4</td>
<td>22.8</td>
<td>22.2</td>
</tr>
<tr>
<td>Third base site (109bp*)</td>
<td>29.9</td>
<td>25.8</td>
<td>23.9</td>
<td>20.4</td>
</tr>
<tr>
<td>Total Fragment (326bp*)</td>
<td>28.9</td>
<td>27.7</td>
<td>23.5</td>
<td>19.9</td>
</tr>
</tbody>
</table>

Note: T represents thymine. A represents adenine. G represents guanine. C represents cytosine. % Indicates the proportion of the nucleic acid in the fragment. * represents the fragment length (bp), which is the average fragment length of 28 geographical populations.

Haplotype analysis of neighboring downstream introns of the gene
The phylogenetic evolutionary tree of intronic haplotypes adjacent to the gene shows that most of the intronic haplotypes are clustered in one large lineage, with H6 and H16 as independent branches. The intronic haplotype of TTA/L is widely distributed. Mutant allele-linked intronic haplotypes are mainly distributed in the same major lineage, in which TTT/F intronic haplotypes H8 and H10 originate from the same branch and are sister groups to each other. Neighboring intronic haplogroups H1 and H5 of TTT/F and TTA/L are sister groups to each other. Neighboring intronic haplotypes H11 and H14 of TCA/S and TTA/L are sister groups to each other (Fig. 3). The haplotype network diagram shows that H11 is the dominant haplotype, H11 may be the ancestral sequence, and other haplotypes may have evolved for H11. The mutant alleles TTT/F and TCA/S are derived from different TTA/Ls, with at least one sensitive haplotype on each branch. The mutant allele TCA/S has only one haplotype H11 in the neighboring downstream intron and TTT/F has three haplotypes H1, H8, and H10 in the neighboring downstream intron, whereas the wild allele TTA/L has 17 haplotypes in the neighboring downstream intron (Fig. 4). It can be inferred that the neighboring downstream introns of the mutant allele are derived from the introns of the wild-type allele and are homologous to some of the wild-type introns. The introns of the mutant allele tend to be stabilized, suggesting that the mutation is correlated with the introns.

**Discussion**

A total of three mutations were detected in this study, one was a non-synonymous mutation, leucine L1014 was mutated to phenylalanine L1014F (TTA-TTT) or serine L1014S (TTA-TCA). The second is a base double mutation of L1014F/L1014S, leucine L1014 is mutated to both phenylalanine and serine F1014S. The other is a base synonymous mutation of L1014L (TTA-CTA). Consistent with the results of existing studies\(^{18-19}\), the L1014F mutation is not conserved. The existence of multiple mutation types at locus 1014 suggests that the mutations in *Cx. pipiens pallens / Cx. pipiens quinquefasciatus* in China tend to be diversified, and the genotype is polymorphic. Increased mutational orientation of the 1014 locus under insecticide selective pressure and enhanced adaptation to the environment. The present study revealed significant geographic variation in the type, number and frequency of mutations. The allelic and genotypic composition of *Cx. pipiens pallens / Cx. pipiens quinquefasciatus* varied with latitude, from single to diverse, before shifting to single. Genotypes are more monotypic north of 38°N latitude. 30–38°N genotype composition is diversified. Populations south of 30°N latitude are again homogeneous in terms of allelic and genotypic composition. Populations north of 38°N have a high percentage of L/L, while most populations south of 30°N have mutant genotypes, with no wild pureblood genotypes detected in YNJH and HNSY, and less than 5% of wild types in HBWH and ZJNB. Populations south of 30°N latitude have a high mutation frequency and a small percentage of sensitive genotypes. Mutant phenotypes of *Cx. pipiens pallens / Cx. pipiens quinquefasciatus* are increasing in adaptability under the selective pressure of insecticides. Mutant genes can be inherited stably in populations, suggesting that we should conserve genes in the wild.
In this study, the synonymous mutation CTA/L was detected only in the Liaocheng population in Shandong, China. Most of the 1014 loci of *Aedes albopictus* from Anhui and Hunan Zhuzhou populations were detected as CTA/L. It is possible that CTA/L is non-mutated for *Aedes albopictus* because the 1014 locus is a CTA/L background. In contrast, the wild type of *Cx. pipiens pallens / Cx. pipiens quinquefasciatus* is TTA/L, and although CTA and TTA encode leucine at the same time, CTA may be mutated in *Cx. pipiens pallens / Cx. pipiens quinquefasciatus*. In the Zibo population, CTA and TTC allele genotypes were not detected, in contrast to the results of Zhang Wei’s study[20]. Possibly because: 1. Related to different sampling habitats and sampling times. 2. Convenient transportation, frequent transportation of goods, and well-developed tourism increase the chances of mosquitoes’ passive movement, which increases the possibility of gene exchange between populations and changes the frequency of allele genotypes of the populations. 3. The populations have a low content of mutant alleles.

Resistance has developed in *Cx. pipiens pallens / Cx. pipiens quinquefasciatus* in most areas. The laboratory strain of *Cx. pipiens pallens*(BJ strain) had a frequency of 15.96% and a mortality rate of 50.00% at exposure to a diagnostic dose of deltamethrin of 0.025%. The BJ strain was resistant to deltamethrin[21]. Combining the results of BJ bioassay and frequency, it can be seen that field populations with frequency < 20.00% have developed resistance to pyrethroid insecticides. The larger the frequency, the lower the sensitivity to pyrethroid insecticides. It indicates that the light-colored *Culex mosquitoes* in most parts of the country have become resistant to the drug. Even populations with low frequencies may have developed resistance. A combination of biological and molecular methods should be used to determine the level of resistance in populations, especially those with low frequencies, in a comprehensive manner.

Wild population resistance is regional in nature. The frequency increases as latitude decreases. This study reveals that the mutation in *Cx. pipiens pallens / Cx. pipiens quinquefasciatus* in China exhibits significant spatial heterogeneity. The number and frequency of mutations showed significant geographic differences. Geographic populations with frequencies < 20.00% were mainly concentrated north of 38°N latitude. Whereas, geographic populations south of 30°N latitude have frequencies > 80.00%. The populations south of 30°N latitude, such as HNSY, YNJH, CQSPB and HBWH populations have frequency > 90.00%. No wild-type allele genotypes were detected in the HNSY population, with a frequency of 100.00%. It may be because the lower the latitude, the higher the temperature, and temperature is one of the most important factors affecting the growth cycle of mosquitoes and the amount of breeding. Tire-causing *Cx. pipiens quinquefasciatus* were caught throughout the year in Hainan and Guangdong. In Jilin and Liaoning, the peak occurred in August, and after October, there were basically no catches of *Cx. pipiens pallens*[22]. And mosquitoes overwinter differently, affecting the number of mosquitoes in the second year, which in turn affects the genetics of resistance. The more mosquitoes that overwinter, the more mosquitoes there will be the following year[23]. The greater the percentage of resistant mosquitoes that survive overwintering, the greater the probability of resistance inheritance. Shandong’s *Cx. pipiens pallens* overwinter in high-temperature, high humidity and light sheltered kiln, and the larvae can not
overwinter\textsuperscript{[24]}. \textit{Cx. p. quinquefasciatus} in Jiangsu overwinters in underground garages, stairwells on the ground floor of buildings, and private houses on the outskirts of the city in different forms\textsuperscript{[25]}. Yunnan has a high incidence of dengue fever\textsuperscript{[26]}. Chongqing had a dengue outbreak in 2019\textsuperscript{[27–28]}. The frequency, dose and duration of insecticide use are higher in these areas than in others. Insecticide residues are increased in mosquito breeding sites. The duration of exposure of mosquitoes to insecticides increases, leading to increased mosquito resistance. The frequency also has urban convergence. The frequency is related to natural factors such as latitude and temperature, but also to social factors such as economic, demographic, and pesticide use please-conditions. BJCP, SHSJ, and HBWH are large cities in terms of population, economy, and transportation. These cities are densely populated and serve as important transportation hubs with high logistics and people ow. Developed regions have a long history of insecticide use, and mosquitoes are exposed to insecticides for long periods of time, increasing cumulative toxicity. The frequency of is higher in large cities within the same latitude.

**Conclusions**

This study described the spatial distribution of mutations in 28 populations of \textit{Cx. p. pallens / Cx. p. quinquefasciatus} in China. In most areas had developed drug resistance, and the degree of resistance was regional. The adjacent downstream intron of gene is polymorphic and is associated with mutation. These results highlight the importance of monitoring the level of pyrethroid resistance in field mosquitoes, and limiting the spread of alleles will help to prevent the development of insecticide resistance. To establish a long-term monitoring mechanism of drug resistance and understand the development trend of drug resistance is helpful to implement integrated mosquito control measures according to local conditions.

**Declarations**

**Supplementary Information**

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**Authors’ contributions**

Data acquisition, WYL, DLM, QZM, XXZ, DDH, CCZ, JW, QYL and FXM. Data sorting and analysis, WYL. Funding acquisition, FXM. Methodology, software, WYL. Supervision, FXM. Draft writing, WYL. Writing—review and editing, FXM. WYL and DLM contributed equally to this work. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

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Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

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**Figures**
Figure 1

Status of 1014 loci of *Cx. pipiens pallens / Cx. pipiens quinquefasciatus* from 28 geographical populations in China. A Distribution of sampling points. B Distribution of alleles at 1014 locus. C Genotype distribution of 1014 locus. D Distribution of frequency.
Figure 2

Sequence characteristics

Figure 3

Haploid evolution tree of adjacent introns of gene

Note: All unlabeled are TTA/L. Pie charts indicate percentages at different latitudes.
Figure 4

Haploid network of adjacent introns of gene

Note: Colored circles indicate detected haplotypes. Black dots indicate deletions or not actually observed in the sample. Circle size indicates haplotype frequency. Consecutive vertical lines on the line indicate the presence of mutations between haplotypes. Colored dashed lines indicate different clusters. Red circles indicate mutant alleles. Unlabeled are TTA/L.