CHAPTER III

RESULTS

1. Partial cDNA sequencing of the *Ae. aegypti* voltage-gated sodium channel gene

Partial sodium channel sequencing was carried out on two *Ae. aegypti* strains, PMD-R and PMD, which are laboratory strains of permethrin resistance and susceptible, respectively. Total RNA extracted from the two strains was used to synthesize the first strand cDNA. Three DNA fragments encompassing 29 putative amino acid mutations (Davies *et al.*, 2007) were amplified from cDNA templates with three pairs of specific primers. Single specific fragments were then purified and directly sequenced (Figure 3.1). The nucleotide sequences of these fragments covered a total of 2,410 nucleotides of the voltage-gated sodium channel gene including the IS4 - IS6 (768 bp), IIS1 - IIS6 (812 bp) and IIIS6 - IVS4 (830 bp) domains. The IS4 - IS6 domains sequences of the voltage-gated sodium channel gene were shown in figure 3.2 and 3.3 and deposited in Genbank under accession numbers EU259807 and EU259812. The cDNA sequences of IIS1 - IIS6 (Figures 3.4 and 3.5) and IIIS6 - IVS4 (Figures 3.6 and 3.7) domains were also reported in Genbank under accession numbers EU259808, EU259809, EU259810 and EU259811, respectively.

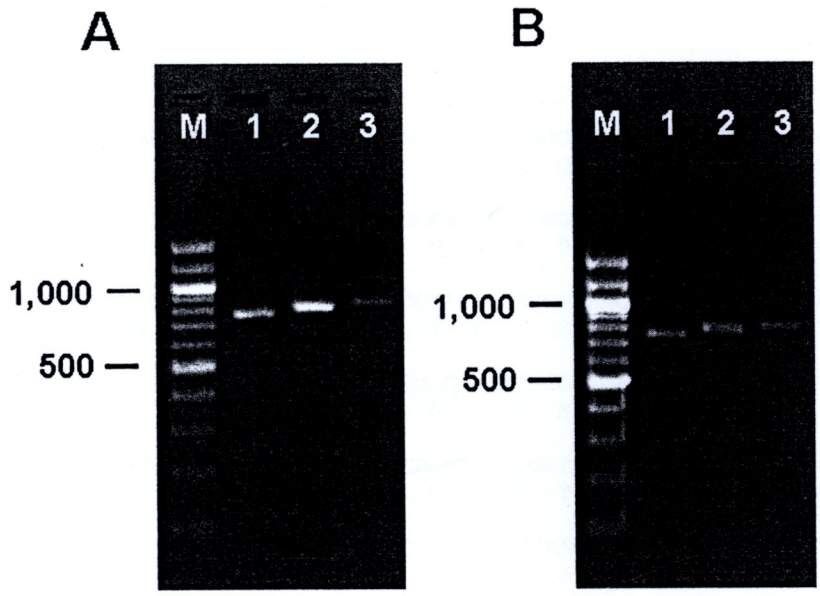


Figure 3.1 Amplification of the *Ae. aegypti* voltage-gated sodium channel gene from PMD-R (A) and PMD (B) strains. PCR products were amplified using IS4-6_F and IS4-6_R primers, IIS1-6_F and IIS6_R primers and IIIS4-6_F and IIIS4-6_R primers were shown in lane 1, 2 and 3 respectively. Markers (lane M) are 100 bp DNA Ladder.

```
ATCTCGCTGCATTGAGAACATTCAGGGTACTACGAGCTCTCAAAACAGTGG     51
  L  A  A  L  R  T  F  R  V  L  R  A  L  K  T  V  A     17

CCATCGTTCCAGGTCTCAAGACCATCGTCGGCGCTGTCATAGAGTCCGTTA    102
  I  V  P  G  L  K  T  I  V  G  A  V  I  E  S  V  K     34

AGAATCTCAGAGATGTGATAATTTTAACAATGTTTTCGTTATCGGTGTTTG    153
  N  L  R  D  V  I  I  L  T  M  F  S  L  S  V  F  A     51

CTTTAATGGGGCTGCAGATCTACATGGGCGTGCTGACGCAGAAGTGCATCC    204
  L  M  G  L  Q  I  Y  M  G  V  L  T  Q  K  C  I  R     68

GGGAGTTCCCGATGGACGGTTCGTGGGGCAACCTGTCGGACGAGAACTGGG    255
  E  F  P  M  D  G  S  W  G  N  L  S  D  E  N  W  E     85

AACGGTTCAACAATAACGACTCCAATTGGTACTTCTCGGAAACTGGAGACA    306
  R  F  N  N  N  D  S  N  W  Y  F  S  E  T  G  D  T    102

CGCCTCTTTGTGGGAACTCGTCGGGTGCTGGCCAATGCGAAGAAGGATATA    357
  P  L  C  G  N  S  S  G  A  G  Q  C  E  E  G  Y  I    119

TTTGTTTACAAGGTTATGGAGATAATCCAAATTACGGGTATACAAGTTTCG    408
  C  L  Q  G  Y  G  D  N  P  N  Y  G  Y  T  S  F  D    136

ATACTTTCGGATGGGCATTCTTATCTGCCTTTCGTCTAATGACCCAAGACT    459
  T  F  G  W  A  F  L  S  A  F  R  L  M  T  Q  D  Y    153

ATTGGGAGAATCTTTATCAACTGGTGTTACGATCAGCTGGACCGTGGCACA    510
  W  E  N  L  Y  Q  L  V  L  R  S  A  G  P  W  H  M    170

TGCTCTTCTTCATTGTGATTATCTTCTTGGGTTCGTTCTACCTTGTAAATT    561
  L  F  F  I  V  I  I  F  L  G  S  F  Y  L  V  N  L    187

TGATCTTGGCCATTGTCGCCATGTCGTACGACGAACTCCAGAAGAGGGCCG    612
  I  L  A  I  V  A  M  S  Y  D  E  L  Q  K  R  A  E    204

AAGAGGAAGAGGCCGCCGAGGAAGAAGCGCTTCGGGAAGCGGAGGAAGCAG    663
  E  E  E  A  A  E  E  E  A  L  R  E  A  E  E  A  A    221

CTGCAGCGAAAGCGGCCAAACTCGAGGCCCAAGCGGCGGCAGCGGCGGCCG    714
  A  A  K  A  A  K  L  E  A  Q  A  A  A  A  A  A  A    238

CAGCCAACCCGGAGATCGCCAAGAGCCCGTCGGACTTTTCCTGCCACAGCT    765
  A  N  P  E  I  A  K  S  P  S  D  F  S  C  H  S  Y

ACG                                                     768
```

Figure 3.2 The cDNA sequences of the IS4 - IS6 domains of the *Ae. aegypti* voltage-gated sodium channel gene from PMD-R and its deduced amino acid sequences.

```
ATCTCGCTGCATTGAGAACATTCAGGGTACTACGAGCTCTCAAAACAGTGG     51
  L  A  A  L  R  T  F  R  V  L  R  A  L  K  T  V  A     17

CCATCGTTCCAGGTCTCAAGACCATCGTCGGCGCTGTCATAGAGTCCGTTA    102
  I  V  P  G  L  K  T  I  V  G  A  V  I  E  S  V  K     34

AGAATCTCAGAGATGTGATAATTTTAACAATGTTTTCGTTATCGGTGTTTG    153
  N  L  R  D  V  I  I  L  T  M  F  S  L  S  V  F  A     51

CTTTAATGGGGCTGCAGATCTACATGGGCGTGCTGACGCAGAAGTGCATCC    204
  L  M  G  L  Q  I  Y  M  G  V  L  T  Q  K  C  I  R     68

GGGAGTTCCCGATGGACGGTTCGTGGGGCAACCTGTCGGACGAGAACTGGG    255
  E  F  P  M  D  G  S  W  G  N  L  S  D  E  N  W  E     85

AACGGTTCAACAATAACGACTCCAATTGGTACTTCTCGGAAACTGGAGACA    306
  R  F  N  N  N  D  S  N  W  Y  F  S  E  T  G  D  T    102

CGCCTCTTTGTGGGAACTCGTCGGGTGCTGGCCAATGCGAAGAAGGATATA    357
  P  L  C  G  N  S  S  G  A  G  Q  C  E  E  G  Y  I    119

TTTGTTTACAAGGTTATGGAGATAATCCAAATTACGGGTATACAAGTTTCG    408
  C  L  Q  G  Y  G  D  N  P  N  Y  G  Y  T  S  F  D    136

ATACTTTCGGATGGGCATTCTTATCTGCCTTTCGTCTAATGACCCAAGATT    459
  T  F  G  W  A  F  L  S  A  F  R  L  M  T  Q  D  Y    153

ATTGGGAGAATCTTTATCAACTGGTGTTACGATCAGCTGGACCGTGGCACA    510
  W  E  N  L  Y  Q  L  V  L  R  S  A  G  P  W  H  M    170

TGCTCTTCTTCATTGTGATTATCTTCTTGGGTTCGTTCTACCTTGTAAATT    561
  L  F  F  I  V  I  I  F  L  G  S  F  Y  L  V  N  L    187

TGATCTTGGCCATTGTCGCCATGTCGTACGACGAACTCCAGAAGAGGGCCG    612
  I  L  A  I  V  A  M  S  Y  D  E  L  Q  K  R  A  E    204

AAGAGGAAGAGGCCGCCGAGGAAGAAGCGCTTCGGGAAGCGGAGGAAGCAG    663
  E  E  E  A  A  E  E  E  A  L  R  E  A  E  E  A  A    221

CTGCAGCGAAAGCGGCCAAACTCGAGGCCCAAGCGGCGGCAGCAGCGGCCG    714
  A  A  K  A  A  K  L  E  A  Q  A  A  A  A  A  A  A    238

CAGCCAACCCGGAGATCGCCAAGAGCCCGTCGGACTTTTCCTGCCACAGCT    765
  A  N  P  E  I  A  K  S  P  S  D  F  S  C  H  S  Y

ACG                                                     768
```

Figure 3.3 The cDNA sequences of the IS4 - IS6 domains of the *Ae. aegypti* voltage-gated sodium channel gene from PMD and its deduced amino acid sequences.

```
GGTCCAACGTTCAAGGACAAGGCCCTGGAGTTCACGATGCGGATGATCGAC     51
G  P  T  F  K  D  K  A  L  E  F  T  M  R  M  I  D      17

GTCTTCTGCGTGTGGGACTGCTGCTGGGTGTGGCTCAAGTTCCAGGAGTGG    102
V  F  C  V  W  D  C  C  W  V  W  L  K  F  Q  E  W      34

GTTGCCTTCATTGTGTTCGACCCGTTCGTCGAGCTGTTCATCACCCTGTGT    153
V  A  F  I  V  F  D  P  F  V  E  L  F  I  T  L  C      51

ATCGTGGTCAACACGCTGTTCATGGCCCTGGATCACCACGATATGGACCCG    204
I  V  V  N  T  L  F  M  A  L  D  H  H  D  M  D  P      68

GACATGGAGCGGGCCCTCAAGAGTGGTAACTATTTTTTCACGGCGACCTTC    255
D  M  E  R  A  L  K  S  G  N  Y  F  F  T  A  T  F      68

GCGATAGAAGCAACGATGAAGCTGATTGCGATGAGTCCCAAGTACTACTTC    306
A  I  E  A  T  M  K  L  I  A  M  S  P  K  Y  Y  F     102

CAAGAGGGCTGGAACATATTCGATTTCATCATCGTGGCGCTGTCGTTGCTC    357
Q  E  G  W  N  I  F  D  F  I  I  V  A  L  S  L  L     119

GAGCTGGGTCTGGAAGGTGTTCAGGGATTGTCAGTATTACGTTCATTCCGT    408
E  L  G  L  E  G  V  Q  G  L  S  V  L  R  S  F  R     136

TTGCTTCGAGTGTTCAAGCTAGCGAAATCGTGGCCGACGTTGAACTTACTC    459
L  L  R  V  F  K  L  A  K  S  W  P  T  L  N  L  L     153

ATTTCCATCATGGGTCGAACGATGGGTGCGTTAGGTAATCTGACGTTTGTG    510
I  S  I  M  G  R  T  M  G  A  L  G  N  L  T  F  V     170

CTCTGCATTATCATCTTCATCTTTGCCGTGATGGGAATGCAGCTGTTCGGC    561
L  C  I  I  I  F  I  F  A  V  M  G  M  Q  L  F  G     187

AAGAACTACATCGACAATGTGGATCGCTTCCCGGACAAGGACCTGCCACGG    612
K  N  Y  I  D  N  V  D  R  F  P  D  K  D  L  P  R     204

TGGAACTTCACCGACTTCATGCACTCATTCATGATCGTGTTCCGGGTATTG    663
W  N  F  T  D  F  M  H  S  F  M  I  V  F  R  V  L     221

TGCGGCGAGTGGATCGAATCCATGTGGGATTGTATGCTTGTGGGTGACGTG    714
C  G  E  W  I  E  S  M  W  D  C  M  L  V  G  D  V     238

TCCTGTATTCCGTTCTTTTTGGCCACCGTAGTGATAGGAAATCTAGTAGTA    765
S  C  I  P  F  F  L  A  T  V  V  I  G  N  L  V  V     255

CTTAACCTTTTCTTAGCCTTGCTTTTGTCCAATTTCGGTTCATCCTC       812
L  N  L  F  L  A  L  L  L  S  N  F  G  S  S           270
```

Figure 3.4 The cDNA sequences of the IIS1-IIS6 domains of the *Ae. aegypti* voltage-gated sodium channel gene from PMD-R and its deduced amino acid sequences.

```
GGTCCAACGTTCAAGGACAAGGCCCTGGAGTTCACGATGCGGATGATCGAC    51
G  P  T  F  K  D  K  A  L  E  F  T  M  R  M  I  D     17

GTCTTCTGCGTGTGGGACTGCTGCTGGGTGTGGCTCAAGTTCCAGGAGTGG   102
V  F  C  V  W  D  C  C  W  V  W  L  K  F  Q  E  W     34

GTTGCCTTCATTGTGTTCGACCCGTTCGTCGAGCTGTTCATCACCCTGTGT   153
V  A  F  I  V  F  D  P  F  V  E  L  F  I  T  L  C     51

ATCGTGGTCAACACGCTGTTCATGGCCCTGGATCACCACGATATGGACCCG   204
I  V  V  N  T  L  F  M  A  L  D  H  H  D  M  D  P     68

GACATGGAGCGGGCCCTCAAGAGTGGTAACTATTTTTTCACGGCGACCTTC   255
D  M  E  R  A  L  K  S  G  N  Y  F  F  T  A  T  F     68

GCGATAGAAGCAACGATGAAGCTGATTGCGATGAGTCCCAAGTACTACTTC   306
A  I  E  A  T  M  K  L  I  A  M  S  P  K  Y  Y  F    102

CAAGAGGGCTGGAACATATTCGATTTCATCATCGTGGCGCTGTCGTTGCTC   357
Q  E  G  W  N  I  F  D  F  I  I  V  A  L  S  L  L    119

GAGCTGGGTCTGGAAGGTGTTCAGGGATTGTCAGTATTACGTTCATTCCGT   408
E  L  G  L  E  G  V  Q  G  L  S  V  L  R  S  F  R    136

TTGCTTCGAGTGTTCAAGCTAGCGAAATCGTGGCCGACGTTGAACTTACTC   459
L  L  R  V  F  K  L  A  K  S  W  P  T  L  N  L  L    153

ATTTCCATCATGGGTCGAACGATGGGTGCGTTAGGTAATCTGACGTTTGTG   510
I  S  I  M  G  R  T  M  G  A  L  G  N  L  T  F  V    170

CTCTGCATTATCATCTTCATCTTTGCCGTGATGGGAATGCAGCTGTTCGGC   561
L  C  I  I  I  F  I  F  A  V  M  G  M  Q  L  F  G    187

AAGAACTACATCGACAATGTGGATCGCTTCCCGGACAAAGACCTGCCACGG   612
K  N  Y  I  D  N  V  D  R  F  P  D  K  D  L  P  R    204

TGGAACTTCACCGACTTCATGCACTCATTCATGATCGTGTTCCGGGTATTA   663
W  N  F  T  D  F  M  H  S  F  M  I  V  F  R  V  L    221

TGCGGCGAGTGGATCGAATCCATGTGGGATTGTATGCTTGTGGGTGACGTG   714
C  G  E  W  I  E  S  M  W  D  C  M  L  V  G  D  V    238

TCCTGTATTCCGTTCTTTTTGGCCACCGTAGTGATAGGAAATCTAGTAGTA   765
S  C  I  P  F  F  L  A  T  V  V  I  G  N  L  V  V    255

CTTAACCTTTTCTTAGCCTTGCTTTTGTCCAATTTCGGTTCATCCTC      812
L  N  L  F  L  A  L  L  L  S  N  F  G  S  S          270
```

Figure 3.5 The cDNA sequences of the IIS1-IIS6 domains of the *Ae. aegypti* voltage-gated sodium channel gene from PMD and its deduced amino acid sequences.

```
TTCAAGCATTCAAAACAATGCGAACTCTTAGAGCACTGAGACCGCTACGTG    51
  Q  A  F  K  T  M  R  T  L  R  A  L  R  P  L  R  A    17

CCATGTCCCGTATGCAGGGTATGAGGGTTGTCGTCAATGCATTGGTACAGG   102
  M  S  R  M  Q  G  M  R  V  V  V  N  A  L  V  Q  A    34

CTATACCGTCCATCTTCAACGTGTTATTGGTGTGTTTGATCTTTTGGTTGA   153
  I  P  S  I  F  N  V  L  L  V  C  L  I  F  W  L  I    51

TTTTCGCTATTATGGGTGTGCAGCTGTTTGCTGGCAAGTATTTTAAGTGCG   204
  F  A  I  M  G  V  Q  L  F  A  G  K  Y  F  K  C  V    68

TCGACAAGAACAAGACGACGCTGTCGCACGAGATCATTCCGGATGTGAACG   255
  D  K  N  K  T  T  L  S  H  E  I  I  P  D  V  N  A    85

CGTGCGTCGCGGAGAACTACACGTGGGAGAACTCGCCGATGAACTTCGACC   306
  C  V  A  E  N  Y  T  W  E  N  S  P  M  N  F  D  H   102

ACGTGGGGAAGGCGTACCTGTGTCTGTTCCAGGTGGCAACGTTCAAGGGCT   357
  V  G  K  A  Y  L  C  L  F  Q  V  A  T  F  K  G  W   119

GGATCCAGATCATGAACGACGCCATCGACTCGCGGGAGGTGGGAAAGCAGC   408
  I  Q  I  M  N  D  A  I  D  S  R  E  V  G  K  Q  P   136

CGATTCGCGAGACCAACATCTACATGTACCTCTACTTTGTGTTCTTCATCA   459
  I  R  E  T  N  I  Y  M  Y  L  Y  F  V  F  F  I  I   153

TCTGCGGGTCGTTCTTCACGCTGAATCTGTTCATCGGTGTCATCATCGACA   510
  C  G  S  F  F  T  L  N  L  F  I  G  V  I  I  D  N   170

ACTTCAACGAGCAGAAGAAGAAAGCCGGTGGCTCACTGGAAATGTTCATGA   561
  F  N  E  Q  K  K  K  A  G  G  S  L  E  M  F  M  T   187

CGGAGGATCAGAAAAAGTACTACAACGCCATGAAAAAGATGGGCTCGAAGA   612
  E  D  Q  K  K  Y  Y  N  A  M  K  K  M  G  S  K  K   204

AGCCGCTGAAAGCTATTCCACGGCCTAGGTGGCGACCACAAGCAATAGTAT   663
  P  L  K  A  I  P  R  P  R  W  R  P  Q  A  I  V  F   221

TCGAAATAGTTACCAATAAGAAGTTCGACATGATCATCATGTTGTTCATCG   714
  E  I  V  T  N  K  K  F  D  M  I  I  M  L  F  I  G   238

GGTTCAACATGTTGACGATGACGCTCGATCACTACAAGCAGACGGACACGT   765
  F  N  M  L  T  M  T  L  D  H  Y  K  Q  T  D  T  F   255

TCAGCGCGGTGCTAGACTATCTGAACATGATCTTCATCTGCATCTTCAGTA   816
  S  A  V  L  D  Y  L  N  M  I  F  I  C  I  F  S  S   272

GCGAGTGTCTGATG                                        830
  E  C  L  M                                          276
```

Figure 3.6 The cDNA sequences of the IIIS4-IVS2 domains of the *Ae. aegypti* voltage-gated sodium channel gene from PMD-R and its deduced amino acid sequences.

```
TTCAAGCATTCAAAACAATGCGAACTCTTAGAGCACTGAGACCGCTACGTG    51
  Q  A  F  K  T  M  R  T  L  R  A  L  R  P  L  R  A    17

CCATGTCCCGTATGCAGGGTATGAGGGTTGTCGTCAATGCATTGGTACAGG   102
  M  S  R  M  Q  G  M  R  V  V  V  N  A  L  V  Q  A    34

CTATACCGTCCATCTTCAACGTGTTATTGGTGTGTTTGATCTTTTGGTTGA   153
  I  P  S  I  F  N  V  L  L  V  C  L  I  F  W  L  I    51

TTTTCGCTATTATGGGTGTGCAGCTGTTTGCTGGCAAGTATTTTAAGTGCG   204
  F  A  I  M  G  V  Q  L  F  A  G  K  Y  F  K  C  V    68

TCGACAAGAACAAGACGACGCTGTCGCACGAGATCATTCCGGATGTGAACG   255
  D  K  N  K  T  T  L  S  H  E  I  I  P  D  V  N  A    85

CGTGCGTCGCGGAGAACTACACGTGGGAGAACTCGCCGATGAACTTCGACC   306
  C  V  A  E  N  Y  T  W  E  N  S  P  M  N  F  D  H   102

ACGTGGGGAAGGCGTACCTGTGTCTGTTCCAGGTGGCAACGTTCAAGGGCT   357
  V  G  K  A  Y  L  C  L  F  Q  V  A  T  F  K  G  W   119

GGATCCAGATCATGAACGACGCCATCGACTCGCGGGAGGTGGGAAAGCAGC   408
  I  Q  I  M  N  D  A  I  D  S  R  E  V  G  K  Q  P   136

CGATTCGCGAGACCAACATCTACATGTACCTCTACTTTGTGTTCTTCATCA   459
  I  R  E  T  N  I  Y  M  Y  L  Y  F  V  F  F  I  I   153

TCTTCGGGTCGTTCTTCACGCTGAATCTGTTCATCGGTGTCATCATCGACA   510
  F  G  S  F  F  T  L  N  L  F  I  G  V  I  I  D  N   170

ACTTCAACGAGCAGAAGAAGAAAGCCGGTGGCTCACTGGAAATGTTCATGA   561
  F  N  E  Q  K  K  K  A  G  G  S  L  E  M  F  M  T   187

CGGAGGATCAGAAAAAGTACTACAACGCCATGAAAAAGATGGGCTCGAAGA   612
  E  D  Q  K  K  Y  Y  N  A  M  K  K  M  G  S  K  K   204

AGCCGCTGAAAGCTATTCCACGGCCTAGGTGGCGACCACAAGCAATAGTAT   663
  P  L  K  A  I  P  R  P  R  W  R  P  Q  A  I  V  F   221

TCGAAATAGTTACCAATAAGAAGTTCGACATGATCATCATGTTGTTCATCG   714
  E  I  V  T  N  K  K  F  D  M  I  I  M  L  F  I  G   238

GGTTCAACATGTTGACGATGACGCTCGATCACTACAAGCAGACGGACACGT   765
  F  N  M  L  T  M  T  L  D  H  Y  K  Q  T  D  T  F   255

TCAGCGCGGTGCTAGACTATCTGAACATGATCTTCATCTGCATCTTCAGTA   816
  S  A  V  L  D  Y  L  N  M  I  F  I  C  I  F  S  S   272

GCGAGTGTCTGATG                                        830
  E  C  L  M                                          276
```

Figure 3.7 The cDNA sequences of the IIIS4-IVS2 domains of the *Ae. aegypti* voltage-gated sodium channel gene from PMD and its deduced amino acid sequences.

2. Sequence analyses of the *Ae. aegypti* voltage-gated sodium channel gene

The cDNA sequences of three *Ae. aegypti* voltage-gated sodium channel regions, IS4 - IS6, IIS1 - IIS6 and IIIS6 - IVS4 domains, from PMD-R and PMD strains were translated to protein consisting of 255, 270 and 276 amino acids, respectively (Figures 3.2 - 3.7). Comparison of the PMD-R amino acid sequence with the susceptible PMD sequence showed 100% sequence identity for the sodium channel regions of IS4 - IS6 and IIS1 - IIS6 domains (Figures 3.8 and 3.9). For the regions of IIIS6 - IVS4 domains, comparison of the amino acid sequences revealed one amino acid substitution; Phenylalanine (F) in PMD strain is substituted by Cysteine (C) in PMD-R strain (Figure 3.10). This substitution was due to a single nucleotide change in the codon TTC to TGC in the IIIS6 coding region of the sodium channel cDNA. The F to C mutation is at the amino acid position 1552 (F1552C) which numbering is based on the sodium channel sequence of the *Ae. aegypti* Liverpool strain (Figure 3.11).

This mutation was referred as F1534C in reference to the equivalent mutation F1534C in the *Musca domestica* house fly *Vssc1* sequences (Genbank accession number: AAB47604) (Ingles *et al.*, 1996). The numbering of the amino acid sequences and other mutations are also based on those of housefly sequences for convenience and used throughout this text. In comparison with other stains, the F1534C mutation is specifically found in PMD-R strain while the amino acid Phenylalanine (F1534) is conserved among all three *Ae. aegypti* permethrin susceptible strains, PMD, Liverpool and China. Figure 3.12 shows sequence comparison between *Ae. aegypti* PMD-R strain from this study and the other

arthropods and non-arthropods. The amino acid F1534 is also found conserved across all the arthropods and non-arthropods.

From the sequence comparison (Figures 3.8 - 3.10), the PMD-R and PMD strains both differ from the Liverpool susceptible strain (Genbank accession numbers: XP_001657358 - 61, inclusive) at amino acid position 427, in having an Arginine (R) rather than a Lysine (K). However a susceptible strain from China (GenBank accession number: AAT69681) also has an Arginine (R) at amino acid position 427. The PMD-R and PMD sodium channel sequences also differ from the China susceptible sequence at amino acid position 292, 295, 318, 377 and 773 but they are identical to the Liverpool sequence. Therefore, these differences do not seem to be related to insecticide resistance. None of the 29 putative amino acid mutations (Davies *et al.*, 2007) were detected in the PMD-R sample.

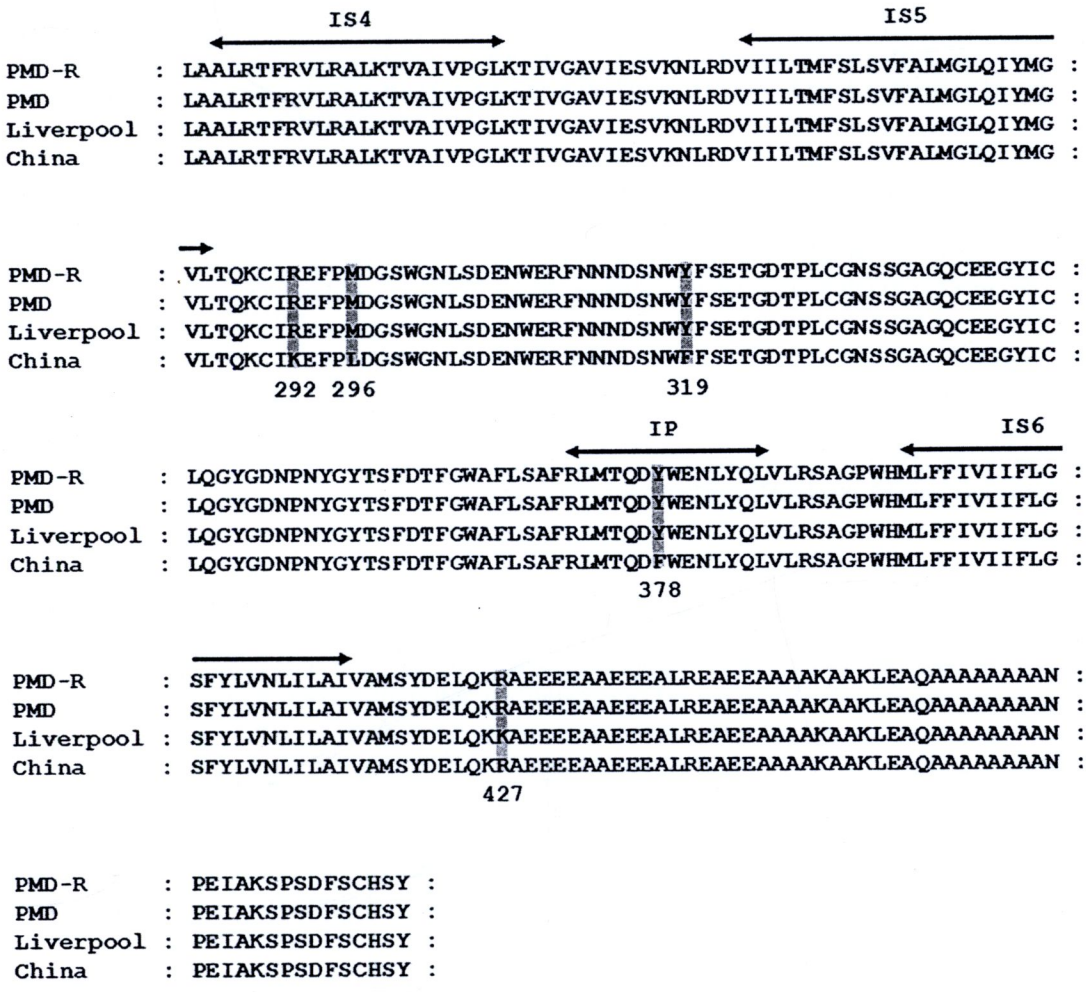


Figure 3.8 Alignment of the amino acid sequences of the *Ae. aegypti* voltage-gated sodium channel protein, IS4 - IS6 domains, of PMD-R strain with that of the susceptible strains, PMD, Liverpool and China strains. The amino acid polymorphisms are shaded in gray and the amino acid positions are labeled according to the house fly *Vssc1* sequences. The locations of putative transmembrane domains (IS4, IS5 and IS6) and putative pore-forming domains (IP) are marked by solid bar above the amino acid sequence.

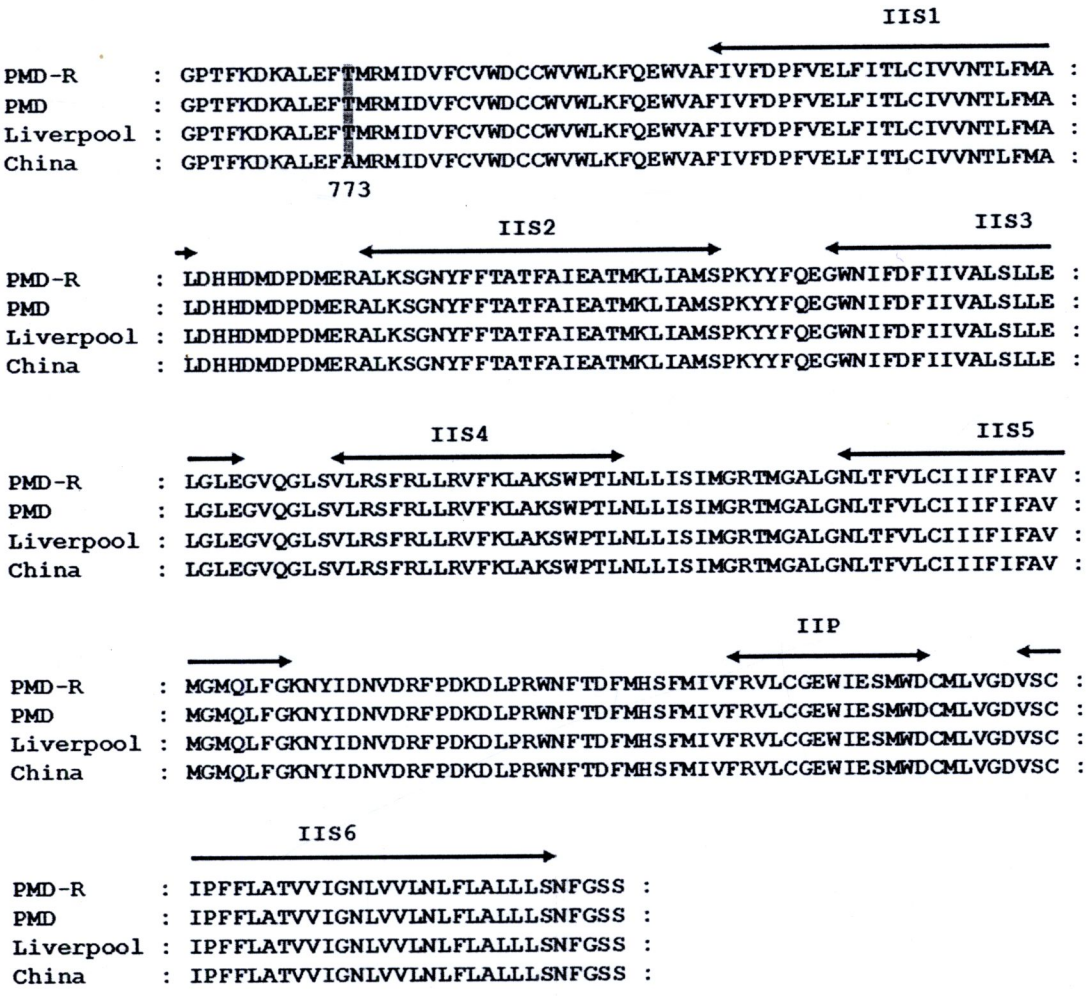


Figure 3.9 Alignment of the amino acid sequences of the *Ae. aegypti* voltage-gated sodium channel protein, IIS1-IIS6 domains, of PMD-R strain with that of the susceptible strains, PMD, Liverpool and China strains. The amino acid polymorphism is shaded in gray and the amino acid position is labeled according to the house fly *Vssc1* sequences. The locations of putative transmembrane domains (IIS1, IIS2, IIS3, IIS4, IIS5 and IIS6) and putative pore-forming domains (IIP) are marked by solid bar above the amino acid sequence.

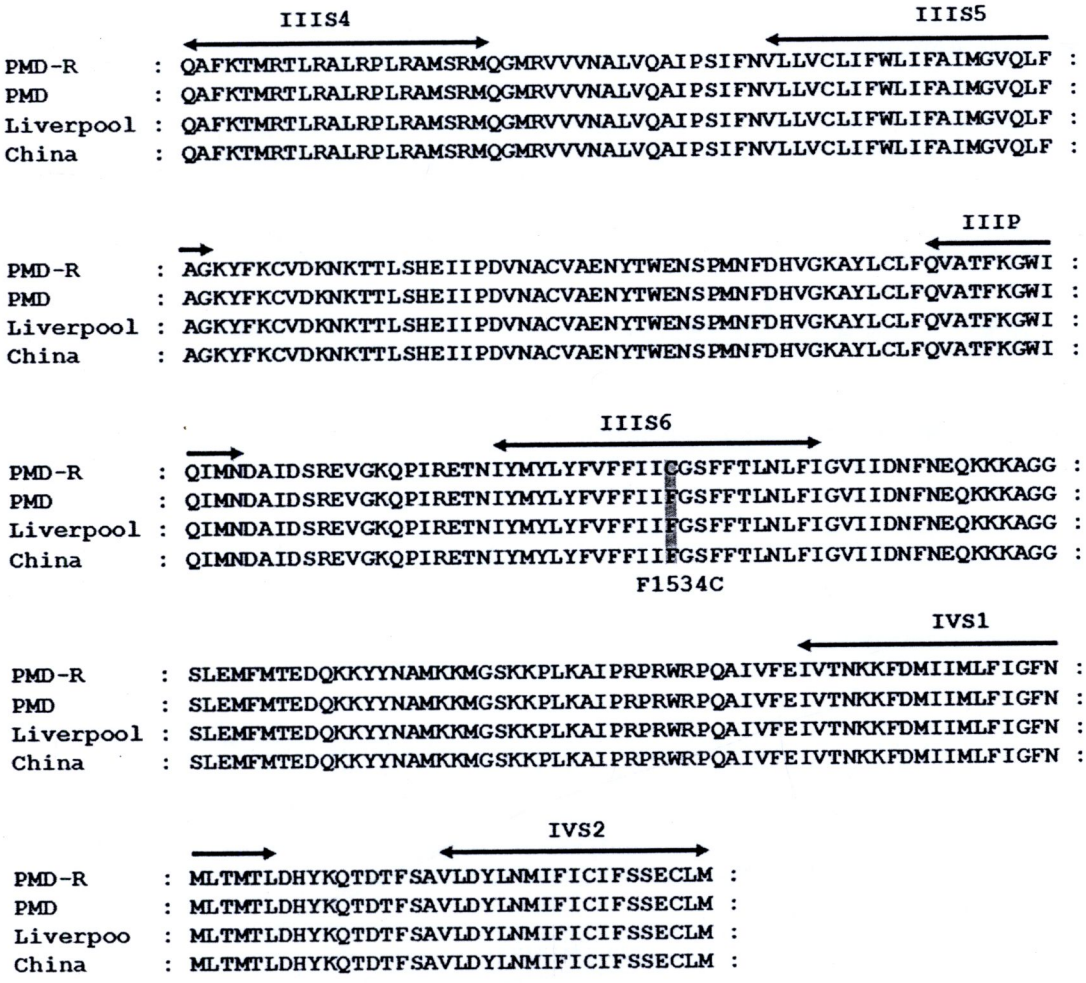


Figure 3.10 Alignment of the amino acid sequences of the *Ae. aegypti* voltage-gated sodium channel protein, IIIS4-IVS2 domains, of PMD-R strain with that of the susceptible strains, PMD, Liverpool and China strains. The F1534C mutation is shaded in gray and the amino acid position is numbered according to the house fly *Vssc1* sequences. The locations of putative transmembrane domains (IIIS4, IIIS5, IIIS6, IVS1 and IVS2) and putative pore-forming domains (IIIP) are marked by solid bar above the amino acid sequence.

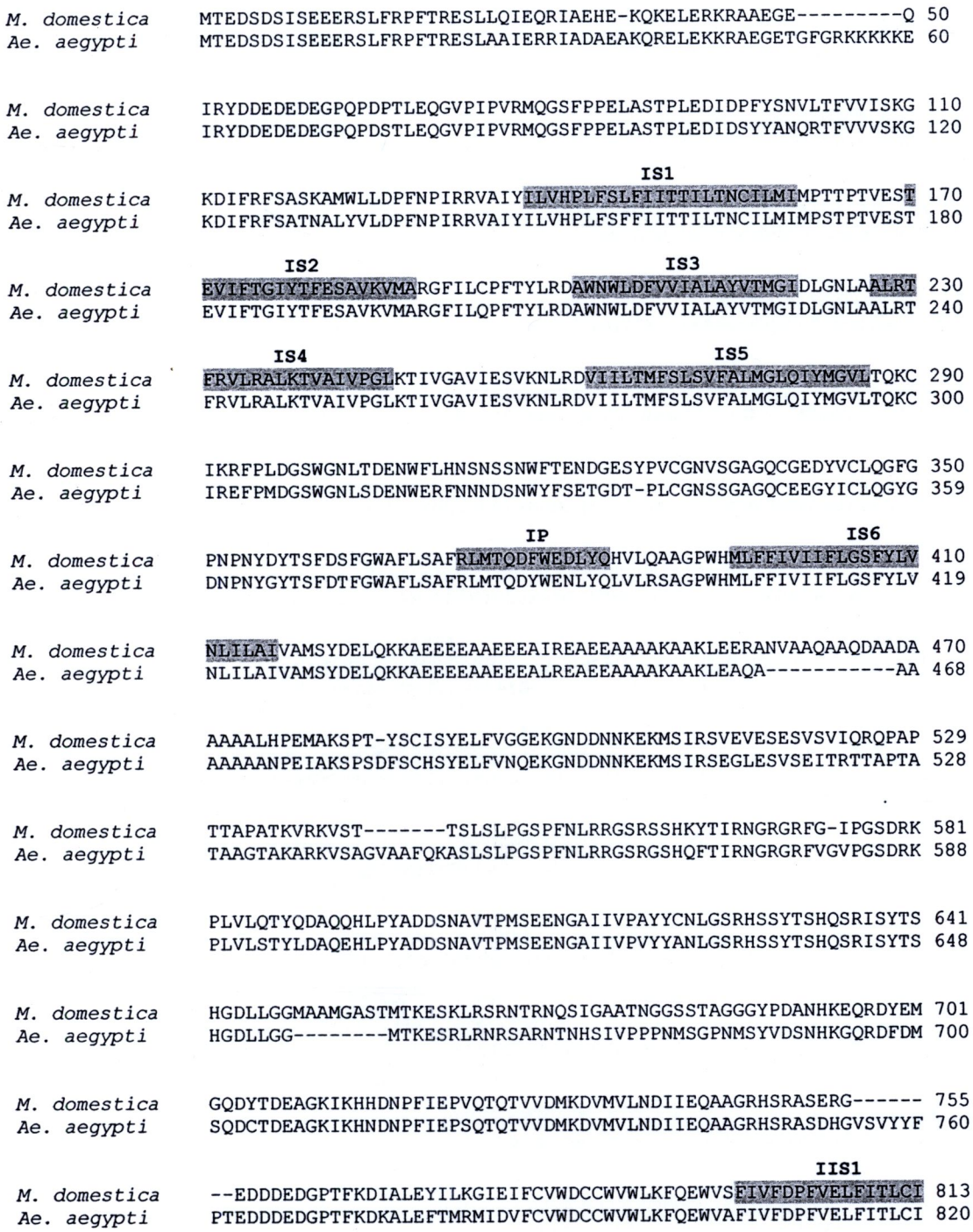


Figure 3.11 Alignment of the sodium channel amino acid sequence of the *Ae. aegypti* Liverpool strain with that of *M. domestica* housefly *Vssc1*.

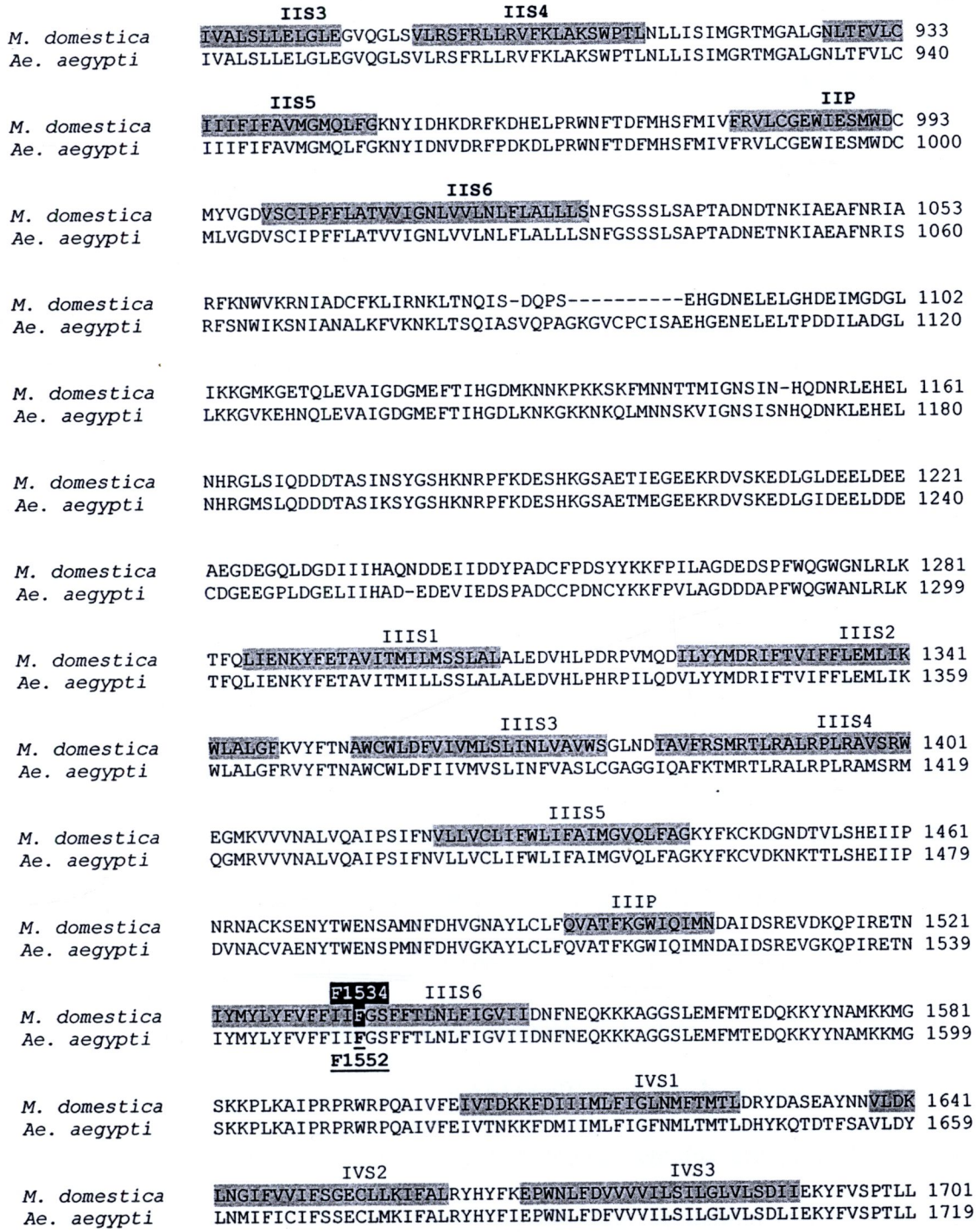


Figure 3.11 Alignment of the sodium channel amino acid sequence of the *Ae. aegypti* Liverpool strain with that of *M. domestica* housefly *Vssc1* (Continued).

```
                                IVS4                                                IVS5
M. domestica    RVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFAIFGMSFFMHV 1761
Ae. aegypti     RVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFAIFGMSFFMHV 1779

                                                    IVP
M. domestica    KEKSGINAVYNFKTFGQSMILLFQMSTSAGWDGVLDAIINEEDCDPPDNDKGYPGNCGSA 1821
Ae. aegypti     KDKSGLDDVYNFKTFGQSMILLFQMSTSAGWDGVLDGIINEDECLPPDNDKGYPGNCGSA 1839

                                IVS6
M. domestica    TVGITFLLSYLVISFLIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPEG 1881
Ae. aegypti     TIGITYLLAYLVISFLIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIWQQFDPDG 1899

M. domestica    TQYIRYDQLSEFLDVLEPPLQIHKPNKYKIISMDMPICRGDMMYCVDILDALTKDFFARK 1941
Ae. aegypti     TQYIRYDQLSDFLDVLEPPLQIHKPNKYKIISMDIPICRGDMMFCVDILDALTKDFFARK 1959

M. domestica    GNPIEETGEIGEIAARPDTEGYDPVSSTLWRQREEYCAKLIQNAWRRYKN----GPPQEG 1997
Ae. aegypti     GNPIEETAELGEVQARPDEVGYEPVSSTLWRQREEYCARVIQHAWRKHKERQAGGGGGDD 2019

M. domestica    DEGEAAGGEDGAEG--GEGEGGSGGGGGDDGGSATGATAAAGATSPSDPDAGEAD--GAS 2053
Ae. aegypti     TDADACDNDDGDDGGGGAGDGGSAGGGGVTSPGVGSGSIVGGGTTPGSGGGGSQANLGIV 2079

M. domestica    VGGPLSPGCVSGGSN---GRQTAVLVESDGFVTKNGHKVVIHSRSPSITSRTADV      2105
Ae. aegypti     VEHNLSPKESPDGNNDPQGRQTAVLVESDGFVTKNGHRVVIHSRSPSITSRSADV      2134
```

Figure 3.11 Alignment of the sodium channel amino acid sequence of the *Ae. aegypti* Liverpool strain with that of *M. domestica* housefly *Vssc1* (Continued).

The deduced amino acid sequences of the *Ae. aegypti* sodium channel gene of the Liverpool strain obtained from manual alignment of 3 VectorBase ID AAEL004612, AAEL008297 and AAEL006019. The locations of putative transmembrane domains and putative pore-forming domains are printed on gray background. The amino acid F1552 numbering based on the sodium channel sequence of the *Ae. aegypti* Liverpool stain is shown with the underlined letters. The equivalent amino acid F1534 in the house fly *Vssc1* sequences is printed on dark background. The gaps (-) are introduced to maximize alignment.

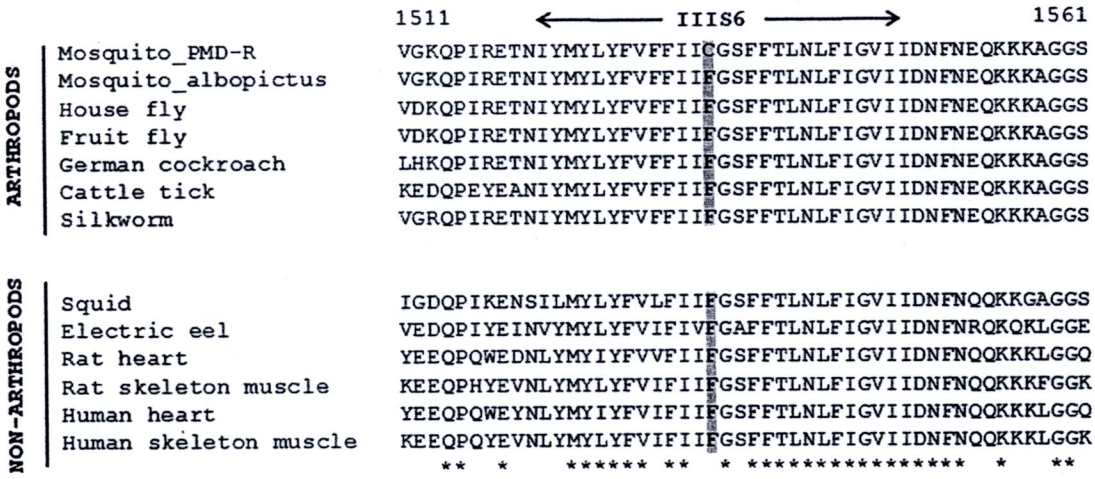


Figure 3.12 Alignment of voltage-gated sodium channel sequences from *Ae. aegypti* PMD-R strain with that from the other arthropods and non-arthropods in the region of IIIS6 domain. The amino acid position 1534 is shaded and the identical amino acids are indicated by asterisk (*). The numbers are shown in relation to the housefly *Vssc1* sequence. Sodium channel sequences, Mosquito_albopictus (*Aedes albopictus*), House fly (*Musca domestica*), Fruit fly (*Drosophila melanogaster*), German cockroach (*Blattella germanica*), Cattle tick (Boophilus *microplus*), Silkworm (*Bombyx mori*), Squid (*Loligo opalescens*), Electric eel (*Electrophorus electricus*), Rat heart (*Rattus norvegicus*) , Rat skeleton muscle (*Rattus norvegicus*), Human heart (*Homo sapiens*), Human skeleton muscle (*Homo sapiens*) have following accession numbers in the Genbank: AAT69680, AAB47605, P35500, AAB82037, AAD23600, NP_001136084, AAA16202, P02719, P15389, P15390, Q14524 and P35499, respectively.

3. Genetic inheritance of the F1534 and C1534 alleles in *Ae. aegypti*

Data from adult permethrin susceptibility test were analysed by a plot of time-mortality response. The log time- probit mortality line of the parental strains, the F1 hybrids and the F2 backcrosses after exposure to 0.75% permethrin paper are shown in Figure 3.13. Statistical data from the probit analysis are presented in table 3.1. The resistance ratio (RR) of the permethrin susceptible (PMD) and resistant (PMD-R) strains was about 54 as determined by LT_{50}.

Data from larval permethrin susceptibility tests of the parental strains, the F1 hybrids and the F2 backcrosses were analyzed by a plot of concentration-mortality response (Figure 3.14). Statistical data from the probit analysis are presented in table 3.2. The resistance ratio (RR) of the permethrin susceptible (PMD) and resistant (PMD-R) strains was about 25 as determined by LC_{50}. The concentration-mortality line of F1 hybrid mosquitoes shifts from intermediate toward, but does not reach, the susceptible line with the RR reduced to about 2.94.

Sequencing of the amplified fragment from adults and larvae of F1 hybrid progeny confirmed that they are heterozygous (F/C1534), suggesting that resistance is highly associated with the homozygous point mutation. In addition, the calculated degree of dominance (Stone, 1968) based on LC_{50} are -0.308 for PMD (F) x PMD-R (M) and -0.347 for PMD (M) x PMD-R (F). All these results clearly suggest that permethrin resistance in PMD-R strain is incompletely recessive. The LC_{50} values of the F1 progeny obtained from both directions of crosses were similar as were the backcrosses (Table 3.2) suggesting that there are no maternal effects or sex linkage, and thus resistance is autosomally inherited.

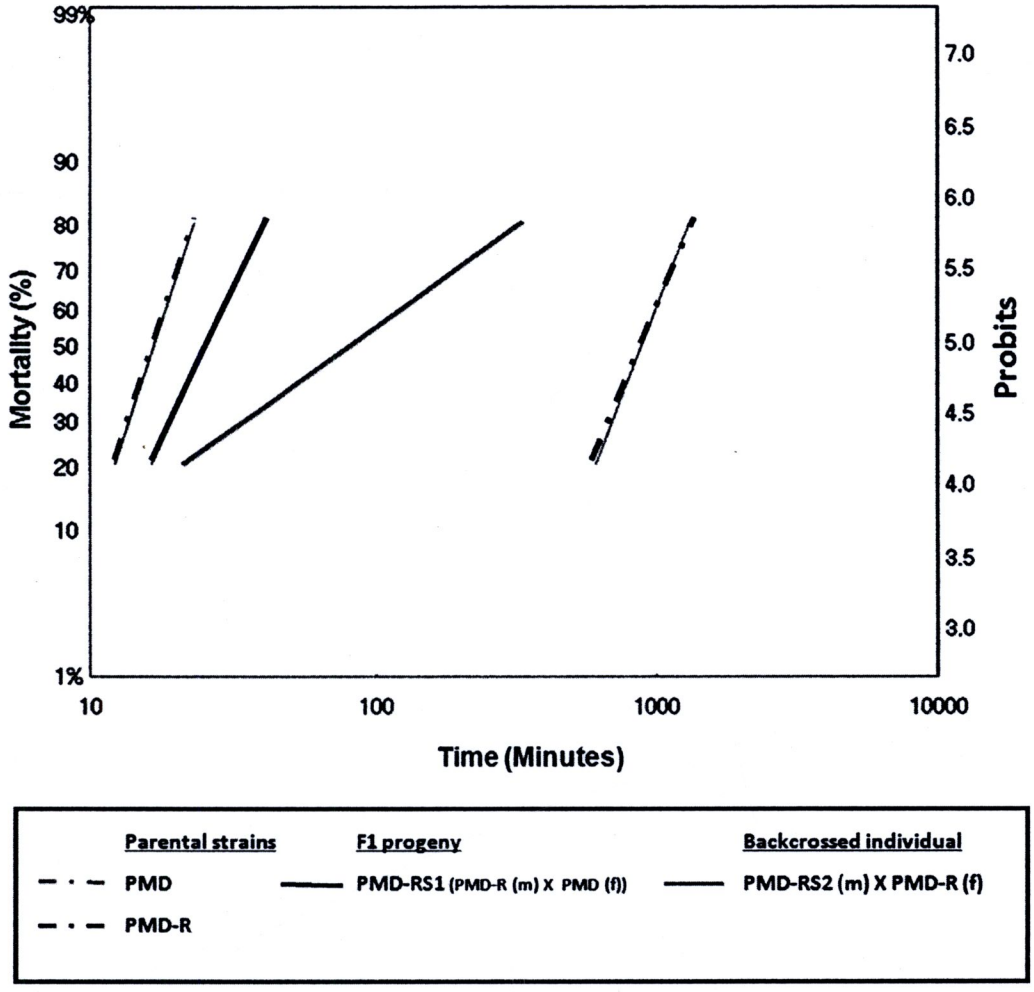


Figure 3.13 The log time- probit mortality line of the parental strains, the F1 hybrids and the F2 backcrosses after exposure to 0.75% permethrin paper.

Table 3.1 Responses of the *Ae. aegypti* adults to 0.75% permethrin for the parental strains, their reciprocal crosses and backcrosses.

Strains	LC_{50} (95% CI) (Minutes)	Slope (±SE)	$\chi 2$	*P*
Parental strains				
- PMD	16.93 (15.99 – 17.88)	6.00 (±0.45)	5.65 (n = 6, df = 5)	0.23
- PMD-R	909.21 (858.32-959.71)	5.0 (±0.32)	5.0 (n = 6, df = 5)	0.31
F1 progeny				
- PMD-RS1 (PMD (f) x PMD-R (m))	25.90 (24.12-27.69)	4.28 (±0.28)	9.04 (n = 6, df = 5)	0.06
Backcross progeny				
- PMD-R (f) x PMD-RS1 (m)	85.63 (71.58- 103.23)	1.40 (±0.09)	4.65 (n = 7, df = 6)	0.46

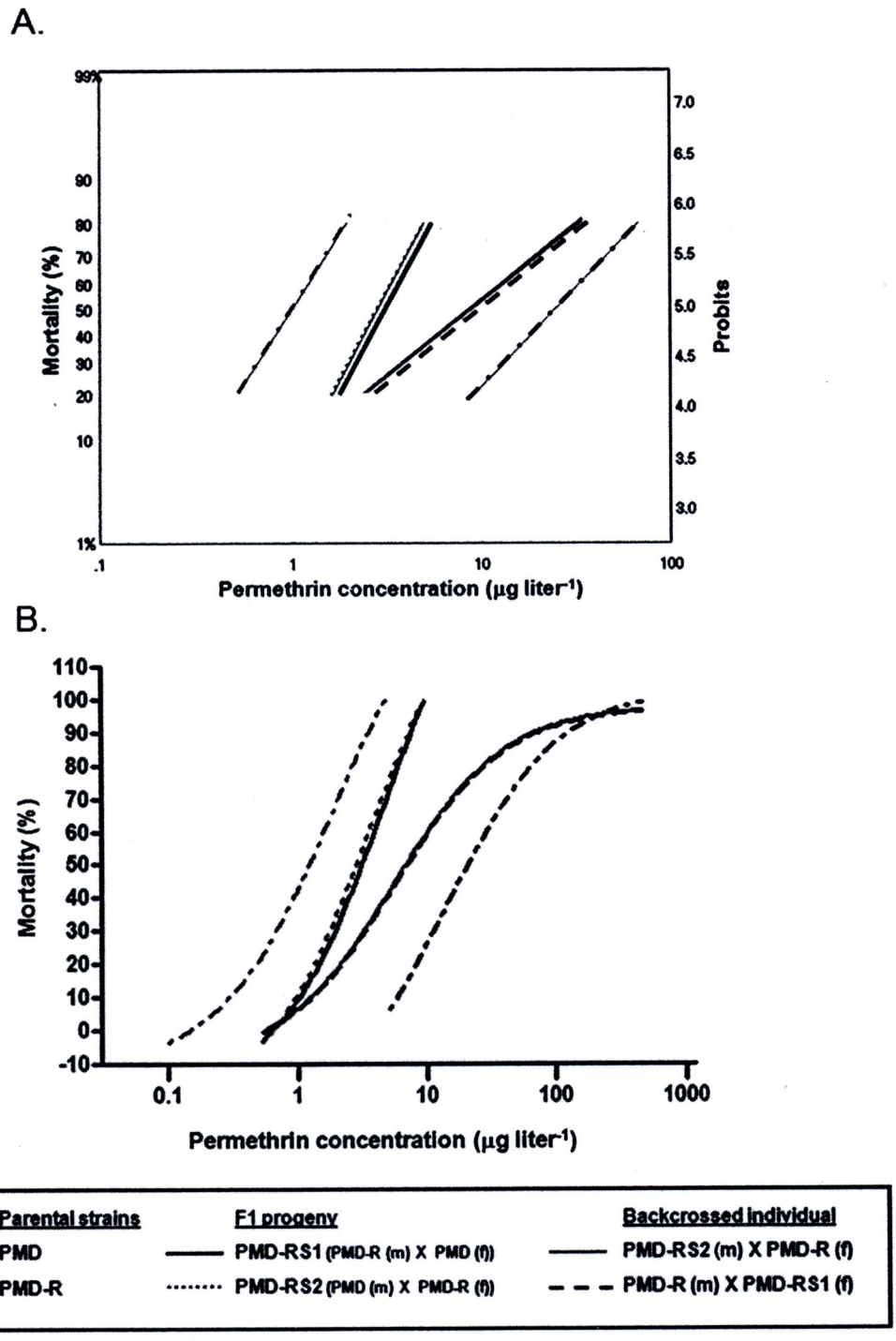


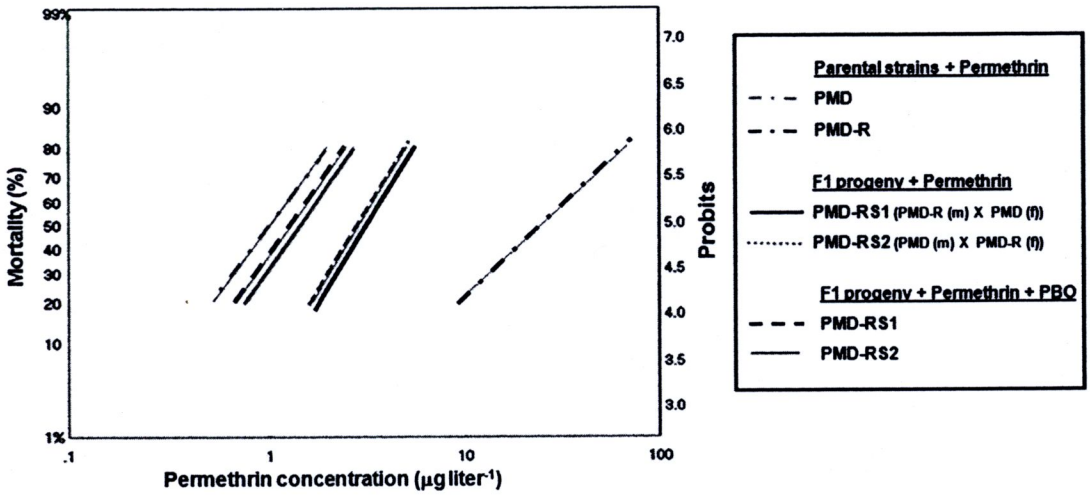
Figure 3.14 The log concentration-probit mortality lines (A) and log concentration-mortality response curves (B) of the parental strains, F1 hybrids and backcrossed individuals after exposure to permethrin.

Table 3.2 Responses of the *Ae. aegypti* larvae to permethrin for the parental strains, their reciprocal crosses and backcrosses.

Strains	LC_{50} (95% CI) ($\mu g\ liter^{-1}$)	Slope (±SE)	$\chi 2$	*P*
Parental strains				
- PMD	1.03 (0.92 – 1.17)	2.91 (±0.25)	7.53 (n = 6, df = 5)	0.11
- PMD-R	25.64 (21.54-30.49)	1.95 (±0.14)	3.11 (n = 5, df = 4)	0.38
F1 progeny				
- PMD-RS1 (PMD (f) x PMD-R (m))	3.13 (2.82-3.45)	3.46 (±0.24)	3.24 (n = 6, df = 5)	0.52
- PMD-RS2 (PMD (m) x PMD-R (f))	2.94 (2.65-3.24)	3.48 (±0.24)	2.08 (n = 6, df = 5)	0.72
Backcross progeny				
- PMD-RS2 (f) x PMD-R (m)	8.96 (7.38-10.84)	1.48 (±0.085)	9.18 (n = 7, df = 6)	0.10
- PMD-R (f) x PMD-RS1 (m)	9.36 (7.69-11.38)	1.48 (±0.088)	10.79 (n = 7, df = 6)	0.06

The offspring obtained from backcrossing the F1 progeny with either the male or female resistant parents do not show a clear plateau curve between the concentration-mortality lines of the resistant and F1 hybrid individuals at the 50% mortality level (Figure 3.14B). In addition, estimated slopes of log concentration-probit mortality plots were lower for backcross progeny than for the parental strains and their F1 hybrid progeny (Table 3.2). These patterns suggest an increased genetic variance in the backcross progeny compared with that of parental populations and F1 progeny. These genotypes possess varying levels of resistance which suggests involvement of other factors or mechanisms (Georghiou, 1969).

In addition, the effect of the oxidase inhibitor, piperonyl butoxide (PBO), on the efficacy of permethrin was evaluated in the F1 progeny with heterozygous F/C1534 (Figure 3.15). The LC_{50} values of F1 progeny when adding the PBO synergist were reduced 2.4-fold and 2.0-fold for PMD-RS1 and PMD-RS2, respectively compare to the original level.



Strains	LC_{50} (95% CI) (µg liter^{-1})	Slope (±SE)	$\chi 2$	*P*
F1 progeny				
Permethrin + PBO				
- PMD-RS1	1.32 (1.20-1.4)	2.99 (±0.22)	10.83 (n = 7, df = 6)	0.05
- PMD-RS2	1.44 (1.31-1.58)	3.00 (±0.21)	9.35 (n = 7, df = 6)	0.09

Figure 3.15 The log concentration –probit mortality lines of the parental strains, F1 progeny after exposure to permethrin compare to those of the F1 hybrids after exposure to permethrin with the PBO synergist. Toxicity of permethrin with the PBO to F1 progeny was also shown.

4. Development of TaqMan SNP genotyping assay

The characteristic cycling graphs for each genotype are presented in Figure 3.16. An exponential increase of either the VIC or FAM fluorescence indicated the homozygous wild type (F/F1534) and the homozygous mutant (C/C1534), respectively, whereas a simultaneous increase in both signals indicated the heterozygous (F/C1534). Scatter plots of relative end point fluorescence intensities of each sample (Figure 3.17) show a clear clustering for each sample group of homozygous (C/C1534) mutant, homozygous (F/F1534) wild type and the heterozygotes (F/C1534). The limit of detection for each genotyping was a 1:100 dilution, equivalent to 1 ng of DNA (Figure 3.18).

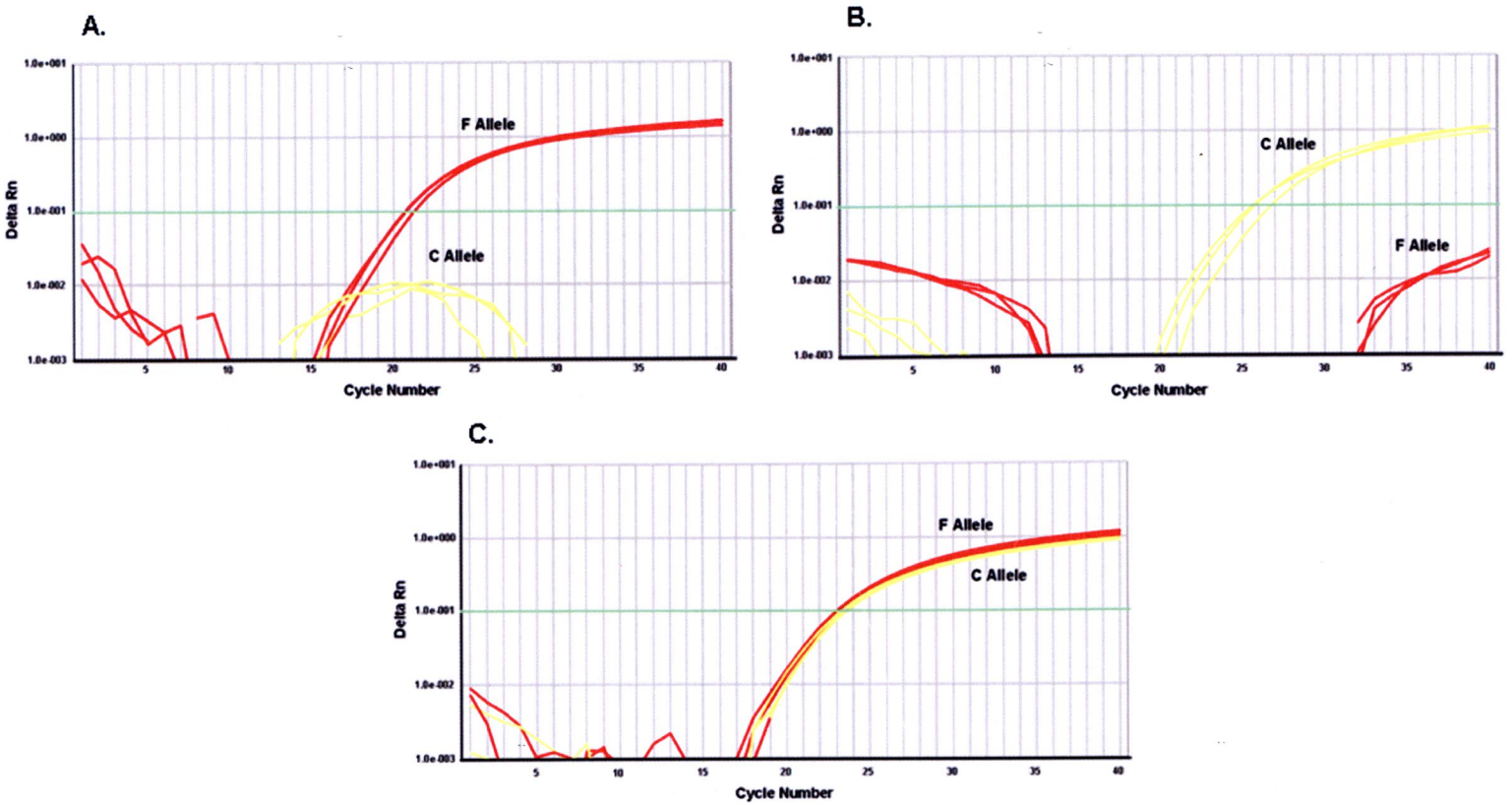


Figure 3.16 Detection of the F1534C genotyping using TaqMan SNP genotyping assay. Amplification plots of results from the TaqMan assay for all genotypes are presented. The patterns are **A.** homozygous F/F1534, **B.** homozygous C/C1534 and **C.** heterozygous F/C1534.

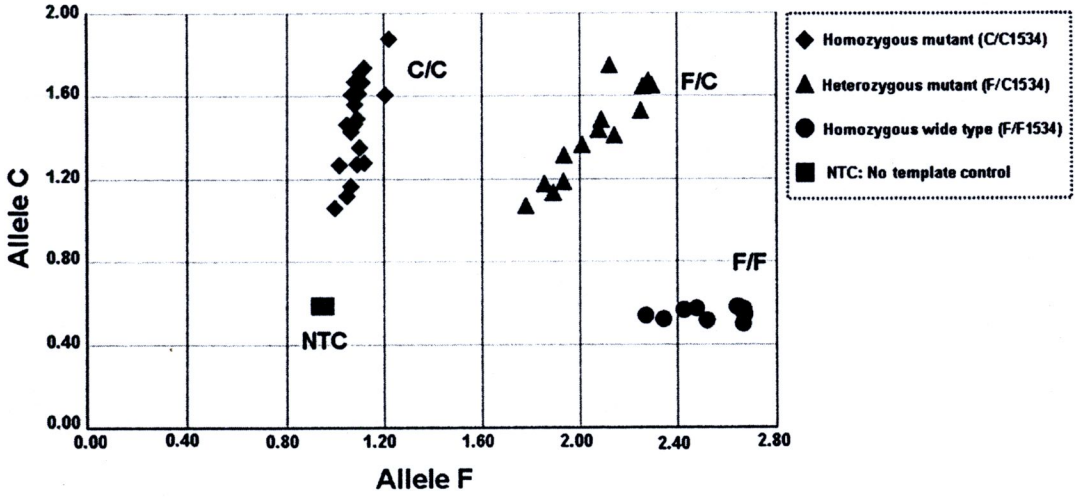


Figure 3.17 Scatter plot of end point fluorescence intensities using the TaqMan SNP genotyping assay. In this example the TaqMan assay was carried out on 43 genomic DNA samples and two no template negative controls. The VIC-labeled probe hybridises with the F allele and is represented on the X-axis; the FAM-labeled probe hybridises with the C allele and is represented on the Y-axis.

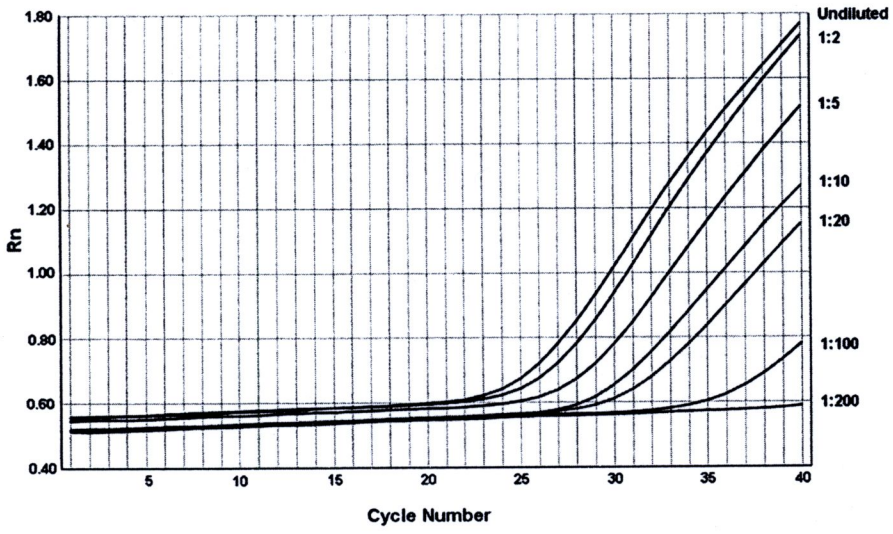


Figure 3.18 Effect of sample dilution on fluorescence intensity. Curves show the increase in fluorescence over time generated for mutant DNA of the following concentrations from left to right: undiluted (original 100 ng DNA template), 1:2, 1:5, 1:10, 1:20, 1: 100 and 1: 200.

5. Development of Allele Specific PCR (AS-PCR)

The wild type homozygous samples (F/F1534) gave a single 93 bp band, the homozygous mutant samples (C/C1534) gave a single 113 bp band and the heterozygous (F/C1534) samples gave both of those bands (Figure 3.19). The sensitivity of this method was evaluated by testing with a set of DNA dilutions of each genotype. The calculated limit of detection was at a 1: 100 dilution, equivalent to 1 ng of genomic DNA.

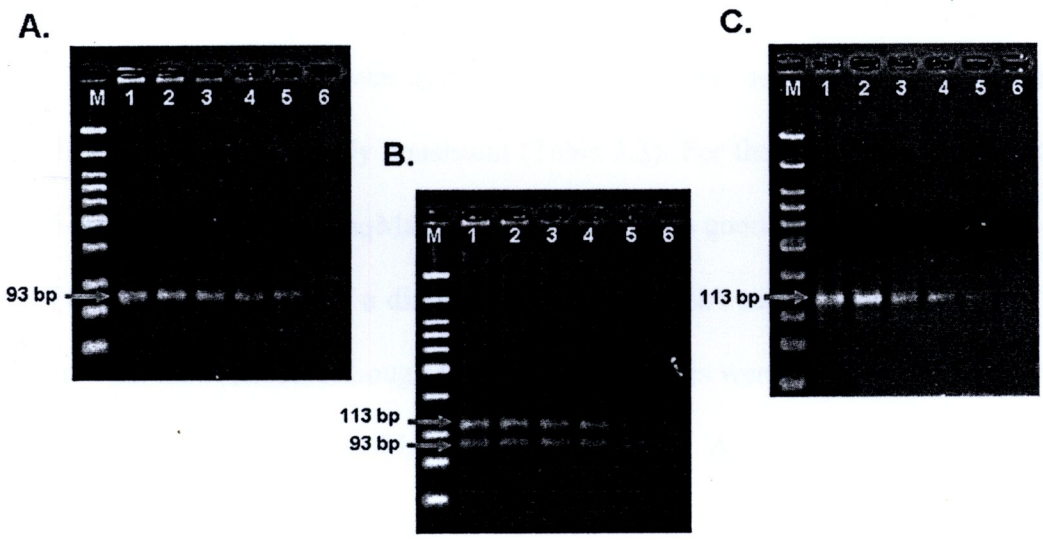


Figure 3.19 Characteristic agarose gel and sensitivity test for the AS-PCR assay for the detection of the F1534C mutation. Lane M: 100 bp DNA ladder; lane 1: 100 ng DNA template; Lane 2-6: dilutions of 1:2, 1:10, 1:20, 1:100 and 1:200, respectively of the original 100 ng DNA template. **A.** Homozygous for F/F1534 allele, **B.** Heterozygous for F/C1534 and **C.** Homozygous for C/C1534.

6. Comparison of DNA sequencing with the TaqMan SNP and AS-PCR assays

In testing 150 known genotyped laboratory *Ae. aegypti* female mosquitoes, the three methods were fully consistent (Table 3.3). For the wild caught mosquitoes, of 85 samples tested, the TaqMan SNP method was as good as DNA sequencing. The AS-PCR, however, showed a discrepancy with the DNA sequencing for 2 out of 103 wild caught mosquitoes, although these two mosquitoes were scored the same by both DNA sequencing and TaqMan SNP. Thus, the AS-PCR apparently slightly overestimated the mutant C1534 allele by 1.8%.

Table 3.3 Comparison of genotype results for the F1534C mutation obtained from the TaqMan SNP and AS-PCR assays with DNA sequencing.

Strains	TaqMan SNP genotyping / DNA sequencing (no. of samples)				AS-PCR / DNA sequencing (no. of samples)			
	F/F1534	F/C1534	C/C1534	Total	F/F1534	F/C1534	C/C1534	Total
Laboratory strains								
PMD	30/30	0/0	0/0	30/30	30/30	0/0	0/0	30/30
PMD-R	0/0	0/0	30/30	30/30	0/0	0/0	30/30	30/30
PMD-RS1	0/0	30/30	0/0	30/30	0/0	30/30	0/0	30/30
PMD-RS2	0/0	30/30	0/0	30/30	0/0	30/30	0/0	30/30
New Orleans	30/30	0/0	0/0	30/30	30/30	0/0	0/0	30/30
Total	60/60	60/60	30/30	150/150	60/60	60/60	30/30	150/150
Wild caught strains								
Chiang Mai city	6/6	12/12	14/14	32/32	13/13	12/12	14/14	39/39
Mae Taeng District	0/0	9/9	18/18	27/27	6/6	9/9	18/18	33/33
Lampang city	2/2	7/7	10/10	19/19	5[a]/6	7/7	11[b]/10	23/23
Mae Sariang District	7/7	0/0	0/0	7/7	8/8	0/0	0/0	8/8
Total	15/15	28/28	42/42	85/85	32/33	28/28	43/42	103/103

[a] One sample was heterozygous F/C1534 by AS-PCR, but homozygous F/F1534 by DNA sequencing.

[b] One sample was homozygous C/C1534 by AS-PCR, but heterozygous F/C1534 by DNA sequencing.

7. Distribution of the F1534C mutation in *Ae. aegypti* populations

In the insecticide bioassay test of 2, 154 *Ae. aegypti* females from 14 localities in four regions of Thailand, only 41 (1.9%) insects died (susceptible) with mortality rates ranging from 0 - 13.3%. Table 3.4 shows the genotyping results determined from the 41 dead mosquitoes and 465 individuals randomly selected from the 2, 113 survivors (resistant). The genotype frequencies of the dead and survivors were significantly different ($\chi^2 = 80.8$, df = 2, $p < 0.0001$). The overall mutant allele frequency was significantly higher in the survivor group (0.84) than the dead group (0.56) ($\chi^2 = 52.1$, df = 1, $p < 0.0001$).

Some of the survivor group possessed a homozygous wild type F/F1534 genotype (n = 19). We predicted that this may be due to a different mutation undetectable by our primers. Hence, we sequenced the samples and found that all were homozygous for mutations V1016G and S989P in domain II. The former, V1016G mutation, has been reported to confer permethrin resistance in *Ae. aegypti* (Brengues *et al.*, 2003). The serine to proline substitution at position 989 was due to a previously unreported mutation in IIP, a nucleotide substitution at the first base in codon 989 (TCC to CCC).

Figure 3.20 summarises the estimated genotype frequencies and distribution of F1534C among 2, 267 mosquitoes of *Ae. aegypti* populations from 20 localities of Thailand. The data were derived partly from the 14 populations (n = 2, 154) in table 3.4 together with 6 other populations that were genotyped but not tested by the permethrin paper. As not all individuals in the survivor group from table 3.4 were genotyped, we prevented any bias in the estimation of the population genotype frequencies (Figure 3.20) by estimating the absolute number of genotypes in the

survivor group by multiplying the determined genotype frequencies with the total number of survivors. In a total of 2, 267 mosquitoes, the homozygous wild type F/F1534 genotype was rarely observed, with estimated frequencies ranging from 0 – 0.31. The F1534C mutation was widely distributed with the heterozygous F/C1534 genotype ranging from 0 - 0.53 and the homozygous mutant C/C1534 genotype ranging from 0.20 - 1.00. The estimated overall genotype frequencies of F/F1534, F/C1534 and C/C1534 were 9.71%, 26.36%, and 63.93%, respectively and the estimated mutant allele frequency was 0.77. Although the frequency of this resistance mutation is, overall, high in Thailand, it appears to be lower in the Provinces of Phetchaburi (sites Nongyapong and Phetchaburi city), Mae Hong Son (Mae Sariang) and Chaing Mai (Mae Taeng and Chiang Mai city), indicating some geographical variation in its distribution.

The F1534C mutation was also detected by AS-PCR in *Ae. aegypti* in the neighboring countries of Myanmar (Yangon city) and Cambodia (Battambang town). Four samples of *Ae. aegypti* from Myanmar were three homozygous mutant C/C1534 and one heterozygous F/C1534. All of ten samples from Cambodia were homozygous mutant C/C1534. The result indicated that this resistance mutation is widespread in Southeast Asia.

Table 3.4 Frequency of the F1534C mutation in the *Ae. aegypti* voltage-gated sodium channel gene within dead and survivor mosquitoes from 14 localities of Thailand determined using the AS-PCR method.

Region	Province	Locality	No dead/Total (% mortality)	Dead				Survivors			
				No mosquitoes			Freq C allele	No mosquitoes			Freq C Allele
				F/F	F/C	C/C		F/F	F/C	C/C	
Northern	Chiang Mai	Chiang Mai City	5/751 (0.7)	5	0	0	0.00	8[a]	20	33	0.70
		Mae Taeng District	10/116 (8.6)	6	4	0	0.20	0	7	48	0.93
	Lampang	Lampang city	0/250 (0.0)	0	0	0	0.00	5[a]	26	18	0.63
		Donchai, Thoen District	0/150 (0.0)	0	0	0	0.00	0	10	22	0.84
	Mae Hong Son	Mae Sariang District	5/67 (7.5)	4	1	0	0.10	6[a]	8	13	0.63
	Chiang Rai	Chiang Sane District	3/28 (10.7)	0	0	3	1.00	0	2	21	0.96
	Uttraradit	Uttraradit city	2/15 (13.3)	0	0	2	1.00	0	0	13	1.00
Central	Phitsanulok	Phitsanulok City	0/70 (0.0)	0	0	0	0.00	0	11	21	0.83
	Phetchabun	Phetchabun City	0/30 (0.0)	0	0	0	0.00	0	0	29	1.00
	Nakhonsawan	Nakhonsawon city	3/134(2.2)	0	0	3	1.00	0	10	19	0.83
Eastern	Trat	Koh Chang Subdistrict	2/40 (5.0)	0	0	2	1.00	0	9	21	0.85
Western	Tak	Tak city	8/241 (3.3)	0	0	8	1.00	0	0	24	1.00
		Mae Kasa, Mae Sot District	2/138 (1.4)	0	1	1	0.75	0	5	25	0.92
		Mae Sot, Mae Sot District	1/124 (0.8)	0	0	1	1.00	0	1	30	0.98
Total			41/2154 (1.9)	15	6	20	0.56	19	109	337	0.84

[a] The V1016G and S989P mutations in domain II of the *Ae. aegypti* voltage-gated sodium channel gene were detected.

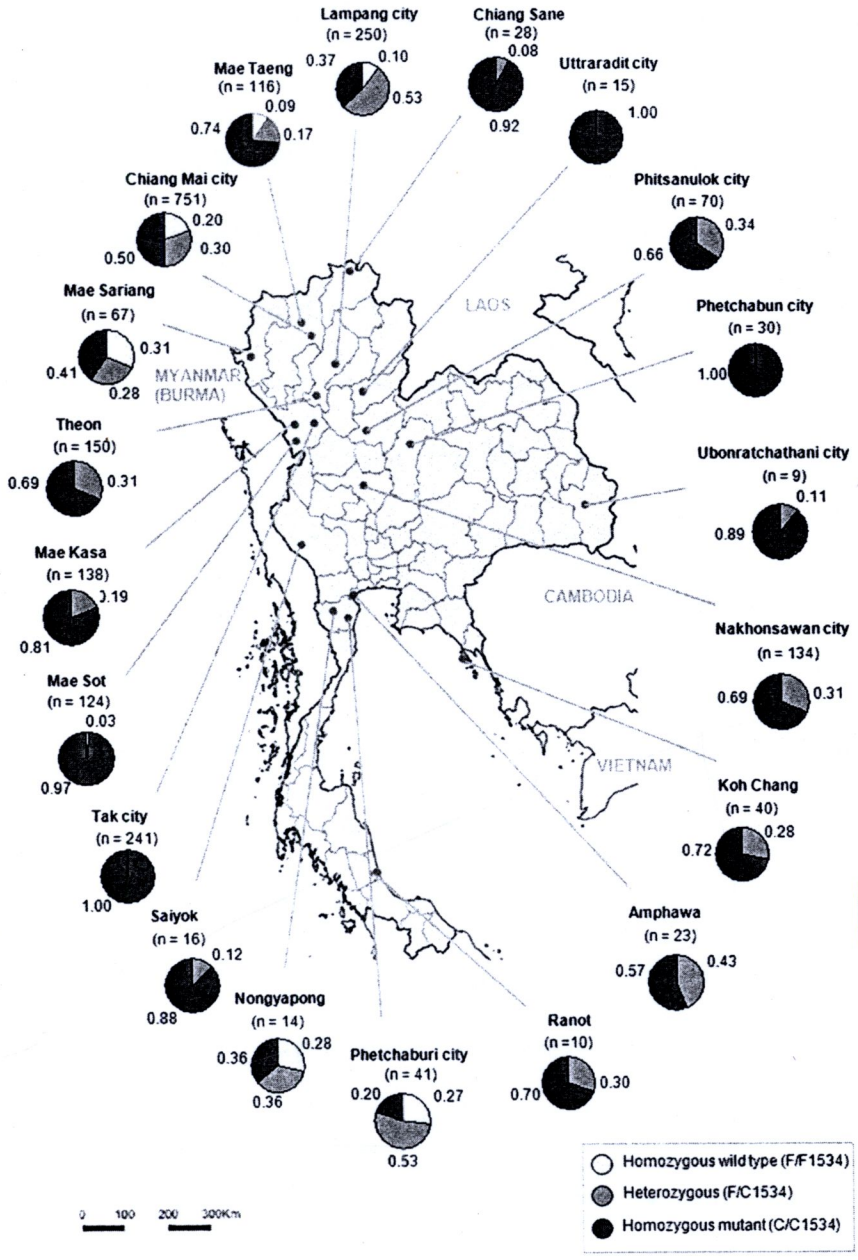


Figure 3.20 Estimated frequency distributions of the homozygous wild type (F/F1534), heterozygous (F/C1534) and homozygous mutant (C/C1534) genotypes in *Ae. aegypti* in Thailand. For the populations from Table 3.4, population genotype frequencies were estimated by combining the total numbers of genotypes from the survivor and dead groups. These numbers were determined directly for the dead group and estimated for the survivor group (see main text).

8. Molecular variation of the IIP-IIS6 region of the *Ae. aegypti* voltage-gated sodium channel gene

As we showed in table 3.4, the V1016G and S989P mutations in domain II were observed in the resistance mosquitoes that were genotyped for the homozygous wild type F/F1534 in domain III. To determine if both mutations in domain II co-existed with F1534C mutation, we sequenced the IIP-IIS6 region in 33 survivors homozygous for C/C1534 from Chiang Mai city (11), Lampang city (10) and Mae Sariang District (12) (Table 3.5). None of them possessed either the V1016G or the S989P mutation. We also sequenced the IIP-IIS6 region in 9 heterozygous F/C1534 individuals from Chiang Mai city (6) and Mae Sariang District (3) and found one of the latter was heterozygous for V/G1016 and S/P989. In addition, we found that the S989P mutation was always linked to the present of the V1016G mutation (Table 3.5)

The nucleotide sequences of the IIP-IIS6 region, including the exon 16, 17 and the intervening intron between exon 16 and 17 (Vectorbase ID AAEL006019), were analyzed and shown in Figure 3.21. Two types of the intervening intron between exon 16 and 17 were detected with the differences in both nucleotide sequences and size. Based on the intron length differences, sequence were classified as haplotype groups A (250 bp) and B (234 bp). Both groups were also separated by the nucleotide sequence with a synonymous polymorphism (A to G) in exon 16 at the position 53. The haplotype groups were previously identified in the sodium channel sequences of *Ae. aegypti* from Brazil (Martins *et al.*, 2009).

In a total of 55 permethin resistant sequences, 34.5% belonged to haplotype group A which was mostly observed the homozygous V1016G and S989P mutations (Table 3.5). The other sequences belonged to haplotype group B with no amino acid

mutation in IIP-IIS6 region of sodium channel gene. However, all of haplotype group B mosquitoes were found the F1534C mutation in IIIS6 region.

Table 3.5 Molecular variation of IIP-IIS6 region of sodium channel gene and the correlation with the F1534C mutation in the *Ae. ageypti* permethin resistance from Thailand

F1534C genotyping sample	Number	Sequence analysis of the IIP-IIS6 region of sodium channel gene					
		V1016G and S989P genotyping[a]				Haplotype group[b]	
		V/V, S/S	V/G, S/S	V/G, P/S	G/G, P/P	A	B
F/F1534	19	0	0	0	19	19	0
C/C1534	33	33	0	0	0	0	33
F/C 1534	9	2	6[c]	1	0	1	2

[a] V/V, S/S indicated no mutation at both the 1016 and 989 codons, V/G, S/S indicated heterozygous at only the 1016 codon, V/G, P/S indicated heterozygous at both the 1016 and 989 codons, G/G, P/P indicated homozygous mutant at both the 1016 and 989 codons.

[b] Haplotype groups were classified from intervening intron sequence between exon 16 and 17 (Vectorbase ID AAEL006019).

[c] Samples are cDNA which could not identified the haplotype groups.

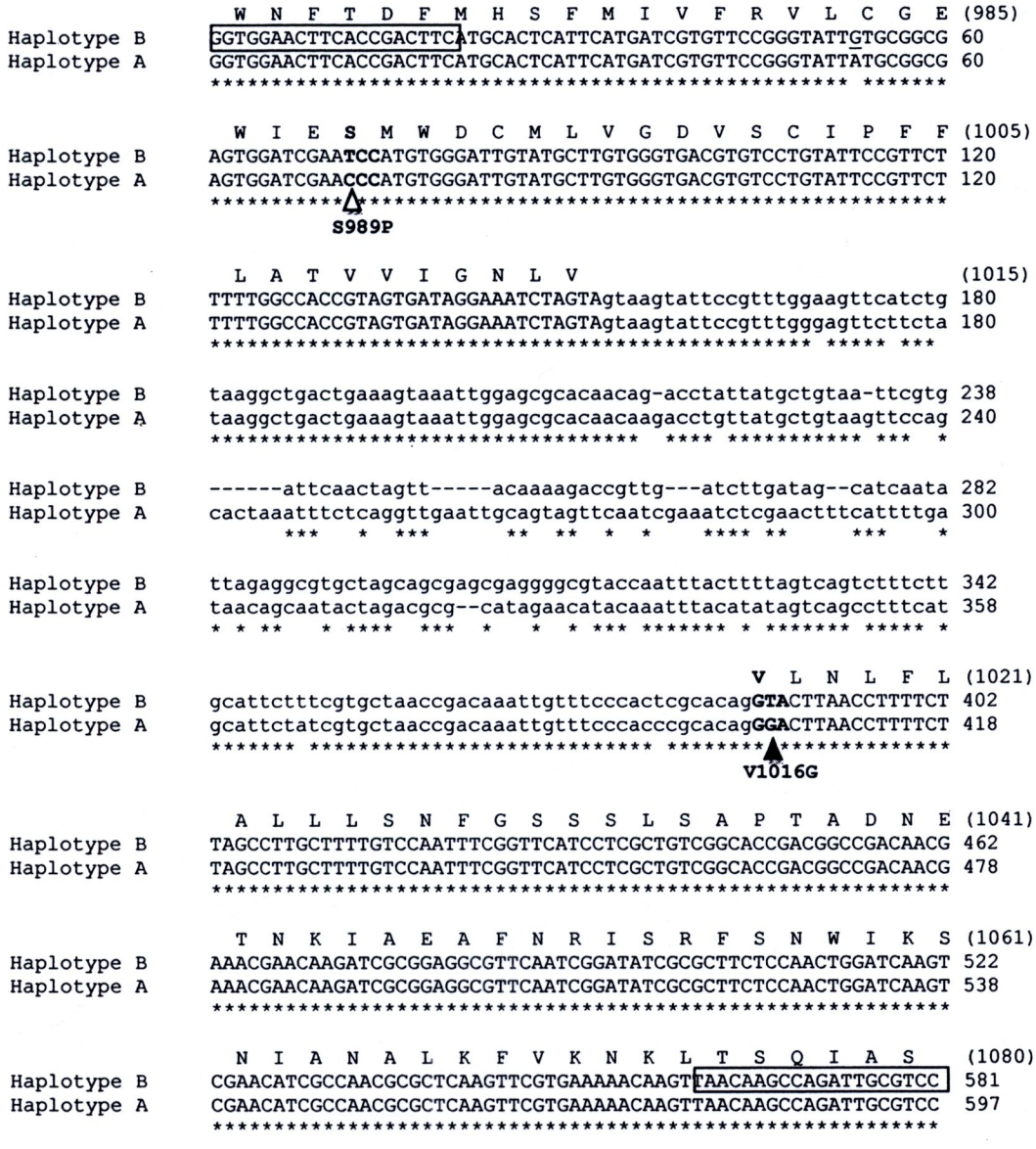


Figure 3.21 (Figure Legend on next page)

Figure 3.21 Sequences alignment of the IIP-IIS6 region of the *Ae. aegypti* sodium channel gene. Nucleotides in upper case letters correspond to the coding region and those in lower case refer to the intron. The gaps (-) are introduced to maximize alignment. Nucleotide numbering at the bottom of each block refers to the sequence showed in the alignment. The identical amino acids are indicated by asterisk (*). Nucleotide at position 53, underlined, is synnonymoous polymorphic sites that differ between haplotype A and B sequences. The numbering of amino acids is in accordance with the house fly *Vssc1* sequence. The S989P and V1016G mutations are marked by open and close arrowheads, respectively. Boxes regions indicate primer positions.