Molecular evidence for historical presence of knock-down resistance in *Anopheles albimanus*, a key malaria vector in Latin America

Juan C Lol¹, María E Castellanos¹, Kelly A Liebman², Audrey Lenhart², Pamela M Pennington¹ and Norma R Padilla¹*

**Abstract**

**Background:** *Anopheles albimanus* is a key malaria vector in the northern neotropics. Current vector control measures in the region are based on mass distributions of long-lasting insecticidal nets (LLINs) and focal indoor residual spraying (IRS) with pyrethroids. Resistance to pyrethroid insecticides can be mediated by increased esterase and/or multi-function oxidase activity and/or mutations in the voltage-gated sodium channel gene. The aim of this work was to characterize the homologous *kdr* region of the voltage-gated sodium channel gene in *An. albimanus* and to conduct a preliminary retrospective analysis of field samples collected in the 1990’s, coinciding with a time of intense pyrethroid application related to agricultural and public health insect control in the region.

**Methods:** Degenerate primers were designed to amplify the homologous *kdr* region in a pyrethroid-susceptible laboratory strain (Sanarate) of *An. albimanus*. Subsequently, a more specific primer pair was used to amplify and sequence the region that contains the 1014 codon associated with pyrethroid resistance in other *Anopheles* spp. (L1014F, L1014S or L1014C).

**Results:** Direct sequencing of the PCR products confirmed the presence of the susceptible *kdr* allele in the Sanarate strain (L1014) and the presence of homozygous-resistant *kdr* alleles in field-collected individuals from Mexico (L1014F), Nicaragua (L1014C) and Costa Rica (L1014C).

**Conclusions:** For the first time, the *kdr* region in *An. albimanus* is described. Furthermore, molecular evidence suggests the presence of *kdr*-type resistance in field-collected *An. albimanus* in Mesoamerica in the 1990s. Further research is needed to conclusively determine an association between the genotypes and resistant phenotypes, and to what extent they may compromise current vector control efforts.

**Keywords:** *Anopheles albimanus*, Pyrethroid resistance, Voltage-gated sodium channel gene, *kdr* mutations

**Background**

*Anopheles albimanus* is one of the key malaria vectors of Latin America and is widely distributed throughout the region [1,2]. In recent years, insecticide resistance has emerged in malaria vectors worldwide as a result of increased intensity of insecticide use, principally via the widespread use of indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) in malaria endemic areas [3-5]. Malaria control in the region currently relies heavily on the use of LLINs, which are treated with pyrethroid insecticides [6]. The widespread use of insecticide treated nets (ITNs) [7-11], LLINs [12-14] and both the historical and ongoing use of DDT and pyrethroid insecticides for IRS [13,15-17] elicit selection pressures on local vector populations. As such, the routine surveillance of insecticide resistance must be implemented in the context of vector control programs to verify that control tools are maintaining their efficacy. The timely detection of insecticide resistance and the characterization of the mechanisms underlying insecticide resistance in a vector population can provide valuable data regarding which...
insecticides should be used to maintain maximum vector control impact.

Resistance to pyrethroid insecticides in malaria vectors can be primarily mediated by either metabolic mechanisms or target site insensitivity, such as mutations on the voltage-gated sodium channel (VGSC) gene [3,18]. Despite reports of pyrethroid resistance throughout the region, none of these mechanisms have been well-described at the molecular level for malaria vectors in Latin America [19]. Previous studies using biochemical assays and bioassays with synergists on pyrethroid resistant *An. albimanus* from Guatemala and Mexico suggest that an increase in the activity levels of esterases and multi-function oxidases are at least partially responsible for the resistance detected in these populations [20-24]. Elevated oxidase activity has been associated with cross-resistance to pyrethroids and DDT in *An. albimanus* [23]. One previous study carried out on *An. albimanus* from Mexico suggested that a target-site mechanism may be involved in cross-resistance between pyrethroids and DDT [25]. Knock-down resistance (kdr) is a target-site mechanism reported in other anopheline species that results in cross-resistance to both pyrethroids and DDT [26,27]. In anophelines, *kdr* is linked to single nucleotide polymorphisms on transmembrane segment 6 of domain II of the VGSC gene. The mutations previously reported for anophelines occur on codon 1014, resulting in an amino acid change of leucine to phenylalanine, serine or cysteine [28-34]. To date, similar mutations have not been described in *An. albimanus*.

The present study describes for the first time the homologous *kdr* region of the VGSC gene in *An. albimanus* where mutations in other anopheline species have been detected that are associated with *kdr*-type resistance. Furthermore, we report molecular evidence of *kdr* resistant-type alleles in field mosquitoes collected in Mexico, Nicaragua and Costa Rica in the 1990s.

**Methods**

**Primer design**

DNA and cDNA sequences of the VGSC gene of different *Anopheles* spp. were retrieved from GenBank (Table 1). Conserved regions were identified from a multiple alignment (MEGA 5.0 [35]) and degenerate primers were designed based on conserved codons using *An. punctipennis* as a basis [GenBank: AY283039-AY283041]. The strategy used to design the primers to amplify the VGSC gene in *An. albimanus* is presented in Figure 1A.

**Mosquito population**

The *An. albimanus* Sanarate laboratory strain, maintained in the insectary of Center for Health Studies (CHS) of Universidad del Valle de Guatemala (Guatemala, Guatemala) was used to validate the designed primers. The Sanarate strain is susceptible to DDT, deltamethrin, permethrin, bendiocarb and malathion (unpublished observations) according to bottle bioassay susceptibility tests [36]. Genomic DNA from individual mosquitoes was isolated following the method described by Collins *et al.* [37].

**Amplification, cloning and sequencing of the VGSC gene**

The amplification of segment 6 of domain II of the VGSC gene with degenerate primers was carried out in a 50 μl reaction mixture containing 1X Colorless GoTaq® Flexi Buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 2.5 μM of each degenerate primer (AAKDRF and AAKDRR), 1 unit

---

**Table 1 DNA sequences of the VGSC gene from different *Anopheles* spp. used in the primer design**

| Specie (subgenus) | Sequence identification |
|-------------------|------------------------|
| *Anopheles aconitus* (Cellia) | GenBank:EU155388 |
| *An. annulans* (Cellia) | GenBank:DQ026443 |
| *An. arabiensis* (Cellia) | GenBank:DQ263749 |
| *An. culicifacies* (Cellia) | GenBank:GQ279245 |
| *An. dirus* (Cellia) | GenBank:DQ279246 |
| *An. funestus* (Cellia) | GenBank:DQ279247 |
| *An. gambiae* (Cellia) | GenBank:EU155384 |
| *An. harrisi* (Cellia) | GenBank:EU155387 |
| *An. jeyporiensis* (Cellia) | GenBank:EU155389 |
| *An. kochi* (Cellia) | GenBank:DQ26446 |
| *An. maculatus* (Cellia) | GenBank:DQ26445 |
| *An. minimus* (Cellia) | GenBank:GU064930 |
| *An. paralae* (Anopheles) | GenBank:GQ225104 |
| *An. peditaeniatus* (Anopheles) | GenBank:GQ225106 |
| *An. punctipennis* (Anopheles) | GenBank:AY283041 |
| *An. stephensi* (Cellia) | GenBank:AY283039 |
| *An. subpictus* (Cellia) | GenBank:AY283040 |
| *An. sinensis* (Anopheles) | GenBank:JN002364 |
| *An. sinensis* (Anopheles) | GenBank:GQ225102 |
| *An. tarsalis* (Cellia) | GenBank:FG04953 |
| *An. subpictus* (Cellia) | GenBank:EU05385 |
| *An. tarsalis* (Cellia) | GenBank:DQ333331 |
| *An. tarsalis* (Cellia) | GenBank:DQ075250 |
| *An. vagus* (Cellia) | GenBank:GQ225100 |
of GoTaq® HotStart Polymerase (Promega, Fitchburg, Wisconsin) and 10 to 30 ng of genomic DNA. The degenerate PCR conditions were 95°C for 3 min, followed by 35 cycles of 95°C for 45 sec, 40.5°C for 45 sec and 72°C for 1 min with a final extension step at 72°C for 5 min in a Px2 Thermal Cycler (Thermo Fisher Scientific, Waltham, Massachusetts).

Non-specific amplification was obtained in An. albimanus from the Sanarate strain using the degenerate primers (Figure 1B). Four different-sized PCR products were isolated for specific amplification using the band-stab PCR technique [38]. These purified PCR products were directly sequenced by Macrogen Inc. (Korea) using AAKDRF and AAKDRR as sequencing primers. BLAST analysis showed that a fragment of approximately 250 bp corresponded to the VGSC gene in An. albimanus. To confirm these findings and to obtain a high-quality DNA sequence of this fragment, PCR products were cloned using a TA Cloning® Kit (Invitrogen, Carlsbad, California) according to the manufacturer's instructions. The plasmids

---

**Figure 1** Strategy to amplify segment 6 of domain II of the VGSC gene in *Anopheles albimanus*. (A) Diagrammatic representation of the design of degenerate and specific primers for *An. albimanus* [GenBank: KF137581] based on *An. gambiae* [GenBank: Y13592] and *An. punctipennis* [GenBank: AY283041]. The identical positions are indicated by an asterisk and mutation site is enclosed by a box. Intron position is indicated by a black line below the sequence. AAKDRF (5′-AGATGGAAYTTYACNGAYTTC-′3); AAKDRF2 (5′-CATTCATTTATGATTGTGTTTCGTG-′3); AAKDRR (5′-GCAANGCTAAGAANAGRTTNAG-′3). (B) PCR products using degenerate and specific primers. The PCR products were separated on a 2% agarose gel containing ethidium bromide. Lane 1: 50 bp DNA ladder (Novagen); Lane 2: degenerate PCR products (using AAKDRF and AAKDRR primers); Lane 3: negative control of degenerate PCR (H2O); Lane 4: specific PCR product (using AAKDRF2 and AAKDRR primers); Lane 5: negative control of specific PCR (H2O).
of the positive clones that contained the fragment of VGSC gene were isolated with the PureLink™ HQ Mini Plasmid Purification Kit (Invitrogen, Carlsbad, California) according to the manufacturer’s instructions. Plasmids were sequenced with M13 universal primers using 3500XL Genetic Analyzer (Applied Biosystems, Foster City, California) with BigDye® Terminator v1.1.

**PCR assay to detect kdr-type resistance**
A second, non-degenerate forward primer (AAKDRF2) was designed based on the sequence of the VGSC gene of *An. albimanus* (GenBank: KF137581) obtained with the degenerate primers (Figure 1A). The amplification with the specific forward (AAKDRF2) and AAKDRR primer was performed using the same reaction specifications as in the degenerate PCR, except that 0.5 μM of each primer were used. The PCR conditions consisted of an initial denaturation at 95°C for 3 min, followed by 40 cycles at 95°C for 45 sec, 51.5°C for 45 sec and 72°C for 1 min, with a final extension step at 72°C for 5 min in an iCycler (BioRad, Hercules, California). The PCR assay with AAKDRF2 and AAKDRR primers amplified a single band of 225 bp in *An. albimanus* from the Sanarate strain (Figure 1B), which corresponds to the VGSC gene of *An. albimanus*. These primers were used to amplify the VGSC gene in DNA samples of *An. albimanus* from Guatemala (collected in 1995), Mexico (collected in 1991), Nicaragua (collected in 1995), Costa Rica (collected in 1995), Ecuador (collected in 1991) and Colombia (collected in 1992) previously used in population genetic studies [39,40]. The PCR products were sequenced by Macrogen Inc. (Korea) using AAKDRF2 and AAKDRR primers.

**Results and discussion**
Sequence analysis showed that segment 6 of domain II of the VGSC gene (excluding the intron sequence) of *An. albimanus* has a sequence identity of 92% with *An. gambiae* and 83% with *An. punctipennis* at the nucleotide level. Variations in the nucleotide sequence of *An. albimanus* did not produce changes in the amino acid sequence (100% identity with *An. gambiae* and *An. punctipennis*, Figure 2). The position of intron II was established through comparison with the VGSC cDNA sequence from *An. gambiae* [GenBank: Y13592]. The size of intron II in *An. albimanus* (71 bp) was greater than in *An. gambiae* (57 bp) and *An. punctipennis* (68 bp). Variation in the size of intron II has been detected in *An. vestitipennis* and *An. pseudopunctipennis* (unpublished observations), and may potentially be used for taxonomic identification of malaria vectors from Latin America, as proposed for other anopheline species [41].

Sequence results from the Sanarate strain of *An. albimanus* showed that the individuals contained the susceptible/wild type *kdr* allele, TTG (L1014), previously reported in *An. sacharovi*, *An. sinensis* and other anopheline species from the Mekong region [34,42,43]. In the field-collected mosquitoes from Latin America, polymorphisms at codon 1014 were detected in several of the samples (Figure 3A). The field samples from Guatemala, Ecuador and Colombia also contained the susceptible TTG (L1014) allele. A non-synonymous homozygous mutation, TGT (cysteine, L1014C), was detected in field samples from Mexico and Nicaragua. This mutation has previously been associated with permethrin, deltamethrin and beta-cypermethrin resistance in *An. sinensis* [34,44,45]. A field sample from Costa Rica contained a homozygous ‘TTC polymorphism (phenylalanine, L1014F), previously reported in populations of *An. gambiae* resistant to permethrin and DDT, *An. sinensis* resistant to deltamethrin and *An. peditaeniatus* resistant to DDT, permethrin, alpha-cypermethrin, lambda-cyhalothrin and etofenprox [28,43,45]. With the exception of certain individuals from Nicaragua and Guatemala, all *kdr* alleles were found to be homozygous (Figure 3B). The heterozygote alleles from Nicaragua were TKY and from Guatemala were TTK. Interestingly, the *kdr* allele reported in *An. gambiae* from East Africa (L1014S) [29] was not detected.

*An. albimanus* populations are panmictic over at least 600 km in Central America, West of Panama [46]. In this region, insecticide resistance in *An. albimanus* has been reported and the main source of its selection has been the extensive use of pesticides in large scale agricultural activities [47-50]. During the nineties, populations in the area in continued exposure to agricultural insecticides plus pressures from the use of insecticides for vector control could have maintained a constant selection pressure on Mesoamerican *An. albimanus* populations, possibly explaining the finding of three homozygous *kdr* variants in

---

**Figure 2** Amino acid sequence comparison of *kdr* region of *Anopheles albimanus* with other anopheline species. The sequence of the segment 6 of domain II of the VGSC gene of *An. albimanus* was compared to *An. gambiae* [GenBank: CAA73920] and *An. punctipennis* [GenBank: AAP60053]. Identical positions are indicated by an asterisk and mutation site (codon 1014) is enclosed by a red box. The amino acid at the mutation site corresponds to the pyrethroid and DDT susceptible (wild-type) genotypes.
Mexico, Nicaragua and Costa Rica with mutations that have been associated with pyrethroid and DDT resistance in other anopheline species. Even though to date the role of \textit{kdr} has not been directly implicated in the insecticide resistance documented in the region, it is highly likely that \textit{kdr} is an important resistance mechanism in Latin American malaria vectors.

**Conclusions**

Our findings describe for the first time the \textit{kdr} region in \textit{An. albimanus}, including the presence of polymorphisms associated with insecticide resistance in other anopheline species. We have documented the presence of homozygous \textit{kdr} alleles associated with resistance in other anopheline species in \textit{An. albimanus} individuals collected across Mesoamerica at a time of intense agricultural and public health insecticide use. This suggests that pyrethroid and DDT resistance in the region could have been mediated in the past by a \textit{kdr} mechanism. Future work will endeavor to link resistant phenotypes with the \textit{kdr} polymorphisms described here, as well as lead to the development of allele-specific diagnostic assays for \textit{An. albimanus} and other malaria vectors across the region.

**Competing interests**

The authors declared that they have no competing interests.

**Authors’ contributions**

JCL carried out the molecular assays, data analysis and drafted the manuscript. MEC conducted molecular assays and contributed to the manuscript. KL conducted molecular assays and sequencing. AL assisted with the analysis and interpretation of results and contributed to the manuscript. PMP designed and guided the study, performed data analysis and contributed to the manuscript. NRP conceived the study and participated in analysis and interpretation of data and contributed to the drafting of the manuscript. All authors have read and approved the final manuscript.

**Acknowledgements**

Partial funding for this work was provided by the US Agency for International Development (USAID) under Amazon Malaria Initiative, Cooperative Agreement Guatemala Public Health/No. 1US1GH000011 and the Center for Health Studies, Universidad del Valle de Guatemala. Ana Maria de Mérida and Alvaro Molina for providing historic mosquito samples. We also thank Ellen Dotson from CDC (MR4) for providing the \textit{kdr} positive controls and primers, and William Brogdon for his helpful comments.

**Author details**

1Centro de Estudios en Salud, Universidad del Valle de Guatemala (CES-UVG), 18 avenida 11-95 zona 15 Vista Hermosa 3, Guatemala, Guatemala. 2Centers for Disease Control and Prevention (CDC), Center for Global Health, Division of Parasitic Disease and Malaria, Entomology Branch, 1600 Clifton Road, Atlanta, GA 30329, USA.

Received: 18 July 2013 Accepted: 12 September 2013 Published: 18 September 2013
References

1. Zimmerman RH: Ecology of malaria vectors in the Americas and future direction. Mem Inst Oswaldo Cruz 1992, 87:371–383.
2. Sinka ME, Rubio-Palis Y, Manguin S, Patil AP, Temperley WH, Gething PW, Van Boeckel T, Kabara CW, Harbach RE, Hay SI: The dominant Anopheles vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic proofs. Parasit Vectors 2010, 3:72.
3. Ranson H, N’Gueyre R, Lines J, Moiroux N, Nikuni Z, Corbel V: Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol 2011, 27:91–98.
4. Czehz C, Labbo R, Arzika I, Duchemin JB: Evidence of increasing Leu-Phe knockdown resistance mutation in Anopheles gambiae from Niger following a nationwide long-lasting insecticide-treated nets implementation. Malar J 2008, 7:189.
5. Balkew M, Ibrahim M, Koekemoer LL, Brooke BD, Engers H, Aseffa A, Gebre-Michael T, Elissen A: Insecticide resistance in Anopheles arabiensis (Diptera: Culicidae) from villages in central, northern and south west Ethiopia and detection of kdr mutation. Parasit Vectors 2010, 3:40.
6. WHO: World malaria report. 2010. Geneve: World Health Organization; 2010.
7. Kroeger A, Gonzalez M, Ordonez-Gonzalez J: Insecticide-treated materials for malaria control in Latin America: to use or not to use? Trans R Soc Trop Med Hyg 1999, 93:565–570.
8. Kroeger A, Manchino M, Alarcon J, Pesse K: Insecticide-impregnated bed nets for malaria control: varying experiences from Ecuador, Colombia, and Peru concerning acceptability and effectiveness. Am J Trop Med Hyg 1995, 53:323–329.
9. Fanello C, Kolaczkinski JH, Conway DJ, Carnevale P, Curtis CF: The kdr pyrethroid resistance gene in Anopheles gambiae: tests of non-pyrethroid insecticides and a new detection method for the gene. Parasitologia 1999, 41:323–326.
10. Kolaczkinski JH, Fanello C, Heve JP, Conway DJ, Carnevale P, Curtis CF: Experimental and molecular genetic analysis of the impact of pyrethroid and non-pyrethroid insecticide impregnated bednets for mosquito control in an area of pyrethroid resistance. Bull Entomol Res 2000, 90:125–132.
11. Valule JM, Beach RF, Atieli FK, Roberts JM, Mount DL, Mwangi RW: Reduced susceptibility of Anopheles gambiae to permethrin associated with the use of permethrin-impregnated bednets and curtains in Kenya. Med Vet Entomol 1995, 9:213–223.
12. Yewhalaw D, Borret WJ, Denis L, Coosemans M, Duchateau L, Speybroeck N: First evidence of high knockdown resistance frequency in Anopheles arabiensis (Diptera: Culicidae) from Ethiopia. Am J Trop Med Hyg 2010, 83:122–125.
13. Protophopan N, Verhaegen K, Van Borre W, Roelants P, Marcotty T, Baza D, D’Alessandro U, Coosemans M: A significant increase in kdr in Anopheles gambiae is associated with an intensive vector control intervention in Burundi highlands. Trop Med Int Health 2008, 13:1479–1487.
14. Ndath MO, Sougoufa S, Gaye A, Mazenc C, Konatte L, Faye O, Sokhna C, Trape JF: Resistance to DDT and pyrethroids and increased kdr mutation frequency in An. gambiae after the implementation of permethrin-impregnated nets in Senegal. PLoS One 2012, 7:e31943.
15. Himeleidy YE, Chen H, Chandre F, Donnelly ML, Yan G: Short report: permethrin and DDT resistance in the malaria vector Anopheles arabiensis from eastern Sudan. Am J Trop Med Hyg 2007, 77:1066–1068.
16. Arevalo-Hereira M, Quinones ML, Guerra C, Cespedes N, Giron S, Ahumada M, Pineros JG, Padilla N, Terrientes Z, Rosas A, et al: Malaria in selected non-Amazonian countries of Latin America. Acta Trop 2012, 121:303–314.
17. Brown AW: Insecticide resistance in mosquitoes: a pragmatic review. J Am Mosq Control Assoc 1986, 2:123–140.
18. Aizouni N, Aikpon R, Padonou GG, Oussou O, Olke-Agbio F, Grangounou V, Osse R, Akogbeto M: Mixed-function oxidases and esterases associated with permethrin, deltamethrin and bendiocarb resistance in Anopheles gambiae s.l. in the south–north transect Benin, West Africa. Parasit Vectors 2013, 6:223.
19. Penilla RP, Rodriguez AD, Hemingway J, Trejo A, Lopez AD, Rodriguez MI: Cytochrome P450-based resistance mechanism and pyrethroid resistance in the field Anopheles albimanus resistance management trial. Pestic Biochem Physiol 2007, 89:111–117.
20. Soderlund DM, Knipple DC: The molecular biology of knockdown resistance to pyrethroid insecticides. Insect Biochem Mol Biol 2003, 33:565–577.
21. Dong K: Insect sodium channels and insecticide resistance. Invert Neurosci 2007, 7:17–30.
22. Martinez-Torres D, Chandre F, Williamson MS, Dariel F, Berge JB, Devonshire AL, Guillett P, Pasteur N, Pauron D: Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector Anopheles gambiae s.s. Infect Genet Evol 2008, 8:79–84.
23. Yewhalaw D, Larsen B, Valule JM, Wang X, Hemingway J, Collins FH: Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Insect Mol Biol 2000, 9:491–497.
24. Syahuddin D, Hidayati AP, Ash PB, Hawley WA, Sukowati S, Lob NF: Detection of 1014F kdr mutation in four major Anopheophilene malaria vectors in Indonesia. Malar J 2010, 9:215.
25. Enayati AA, Vatandoost H, Ladoni H, Townson H, Hemingway J: Molecular evidence for a kdr-like pyrethroid resistance mechanism in the malaria vector Anopheles stephensi. Med Vet Entomol 2003, 17:138–144.
26. Singh OP, Dykes CL, Das MK, Pradhan S, Bhatt RM, Agrawal OP, Adak T: Presence of two alternative kdr-like mutations, L1014F and L1014S, and a novel mutation, V1010L, in the voltage gated N+ channel of Anopheles culicifacies from Orissa, India. Malar J 2010, 9:146.
27. Collins FH, McAllister JC, Convin AM, Cordon-Rosales C, Brogdon WG: Detoxicifying esterases may limit the use of pyrethroids for malaria vector control in the Americas. Parasitol Today 1989, 5:326–327.
28. Brogdon WG, McAllister JC, Convin AM, Cordon-Rosales C: Independent selection of multiple mechanisms for pyrethroid resistance in Guatemalan Anopheles albimanus (Diptera: Culicidae). J Econ Entomol 1999, 92:298–302.
29. Brogdon WG, McAllister JC, Convin AM, Cordon-Rosales C: Oxidase-based DDT-pyrethroid cross-resistance in guatemalan anopheles albimanus. Pestic Biochem Physiol 1999, 64:101–111.
30. Scott JA, Collins FH, Fyereisen R: Diversity of cytochrome P450 genes in the mosquito, Anopheles albimanus. Biochem Biophys Res Commun 1994, 205:1452–1459.
31. Singh OP, Dykes CL, Lather M, Agrawal OP, Adak T: Knockdown resistance (kdr)-like mutations in the voltage-gated sodium channel of a malaria vector Anopheles stephensi and PCR assays for their detection. Malar J 2011, 10:59.
32. Kim H, Baek JH, Lee W, Lee SH: Frequency detection of pyrethroid resistance allele in Anopheles sinensis populations by real-time PCR amplification of specific allele (rtPASA). Pestic Biochem Physiol 2007, 87:54–61.
33. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S: MEGAS: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011, 28:2731–2739.
34. Brogdon WG, McAllister JC: Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. J Am Mosq Control Assoc 1998, 14:159–164.
35. Collins FH, Mendez MA, Rasmussen MO, Mheaefy PC, Besansky NJ, Finnerty V: A ribosomal RNA gene probe differentiates member species of the Anopheles gambiae complex. Am J Trop Med Hyg 1987, 37:37–41.
36. Bjurlin A, Cooper JE: Band-stab PCR: a simple technique for the purification of individual PCR products. Nucleic Acids Res 1992, 20:6675.
37. De Merida AM, De Mata MP, Molina E, Porter CH, Black WC: Variation in ribosomal DNA intergenic spacer regions among populations of Anopheles albimanus in South and Central America. Am J Trop Med Hyg 1995, 53:469–477.
38. De Merida AM, Palmieri Y, Yumita M, Molina A, Molina E, Black WC: Mitochondrial DNA variation among Anopheles albimanus populations. Am J Trop Med Hyg 1999, 61:230–239.
39. Henny-Haldin CN, Nadesakumaran K, Keven JB, Zimmerman AM, Siba P, Mueller L, Hetzel MW, Kazura JW, Thomsen E, Reimer L, Zimmerman PA: Multiplex assay for species identification and monitoring of insecticide resistance in Anopheles punctulatus group populations of Papua New Guinea. Am J Trop Med Hyg 2012, 86:140–151.
42. Luleyap HU, Alptekin D, Kasap H, Kasap M: Detection of knockdown resistance mutations in Anopheles sacharovi (Diptera: Culicidae) and genetic distance with Anopheles gambiae (Diptera: Culicidae) using cDNA sequencing of the voltage-gated sodium channel gene. J Med Entomol 2002, 39:870–874.

43. Verhaeghen K, Van Bortel W, Trung HD, Sochantha T, Keokenchanh K, Coosemans M: Knockdown resistance in Anopheles vagus, An. sinensis, An. paraliae and An. peditaeniatus populations of the Mekong region. Parasit Vectors 2010, 3:59.

44. Tan WL, Wang ZM, Li CX, Chu HL, Xu Y, Dong YD, Wang ZC, Chen DY, Liu H, Liu OP, et al: First report on co-occurrence knockdown resistance mutations and susceptibility to beta-cypermethrin in Anopheles sinensis from Jiangsu Province, China. PLoS One 2012, 7:e20242.

45. Zhong D, Chang X, Zhou G, He Z, Fu F, Yan Z, Zhu G, Xu T, Bonizzoni M, Wang MH, et al: Relationship between Knockdown Resistance, Metabolic Detoxification and Organismal Resistance to Pyrethroids in Anopheles sinensis. PLoS One 2013, 8:e55475.

46. Molina-Cruz A, de Merida AM, Mills K, Rodriguez F, Schoua C, Yurrita MM, Molina E, Palmieri M, Black WC: Gene flow among Anopheles albimanus populations in Central America, South America, and the Caribbean assessed by microsatellites and mitochondrial DNA. Am J Trop Med Hyg 2004, 71:350–359.

47. WHO: Vector resistance to pesticides. Fifteenth Report of the WHO Expert Committee on Vector Biology and Control. World Health Organ Tech Rep Ser 1992, 818:1–62.

48. PAHO: Results of insecticide susceptibility tests carried out in four Central American Countries between 1994–1997. Epidemiol Bull 1997, 18:11–14.

49. Lines JD: Do agricultural insecticides select for insecticide resistance in mosquitoes? A look at the evidence. Parasitology today 1998, 4:517–520.

50. Mouchet J: Agriculture and vector resistance. Insect Science and its Application 1998, 9:297–302.

doi:10.1186/1756-3305-6-268
Cite this article as: Lol et al: Molecular evidence for historical presence of knock-down resistance in Anopheles albimanus, a key malaria vector in Latin America. Parasites & Vectors 2013 6:268.