SSCP Analysis of scnDNA for Genetic Profiling of *Aedes aegypti*

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**Abstract.** We characterized genetic profiling markers for *Aedes aegypti* using single-strand conformation polymorphism (SSCP) analysis of single copy nuclear genes (scnDNA). Nucleotide variations at 18 loci were evaluated in 173 wild *Ae. aegypti* collected from a single population in northwestern Thailand. We identified seven scnDNAs with polymorphisms sufficient to determine a unique genetic profile for each mosquito examined. Six markers were derived from previously mapped cDNA loci. One marker was developed from a non-coding region of a gene. The number of alleles at each scnDNA locus ranged from 3 to 9. The described scnDNAs can be used to quickly fingerprint large numbers of *Ae. aegypti* to track the behavior of individual mosquitoes in the field.

**INTRODUCTION**

Dengue viruses cause more human morbidity and mortality than any other arthropod-borne virus.1 Because no vaccine or antiviral therapy is currently available, dengue prevention relies on suppression of the principal mosquito vector, *Aedes aegypti*.2 Despite a history of detailed studies in the laboratory, much remains unknown about the behavior and ecology of *Ae. aegypti* in nature. The limited efficacy of conventional vector-control methods such as source reduction, larvicide application, and adulticide spraying has led to exploration of genetic control strategies against *Ae. aegypti*. Successful genetic control of mosquitoes, however, will require a more complete understanding of the behavior of target populations.3

Results of previous genetic control trials have highlighted the importance of understanding the mating behavior of natural mosquito populations.3 Laboratory colonization and/or genetic modification of mosquitoes may lead to a mating disadvantage, or assortative mating, such that released individuals mate infrequently or not at all with mosquitoes in the wild population. Field release experiments involving genetically altered and/or sterilized *Culex tritaeniorhynchus,* Anopheles culicifacies,*5,6* and *Culex tarsalis* in the 1970s and 1980s were unsuccessful in part because released males failed to mate competitively with wild females. Similarly, unpredicted polyandry in the target population can present an obstacle to genetic control programs. Introduced transgenes are expected to spread within mosquito populations according to patterns of reproduction. Deviations from monandry may prevent or slow the spread of transgenes,1 particularly if wild females of reproduction. Deviations from monandry may prevent or spread within mosquito populations according to patterns genetic control programs. Introduced transgenes are expected andry in the target population can present an obstacle to competitively with wild females. Similarly, unpredicted polyandry, such that released individuals cannot be identified.13 RAPDs require extraction of large amounts of genomic DNA, which restricts the number of loci that can be examined for a single mosquito specimen.12

Microsatellite markers have been successfully used to study the frequency of polyandry in *Anopheles gambiae* in Mali.14 In studies of *Ae. aegypti*, researchers have used microsatellites to examine population genetics in Vietnam,15 Cambodia,16 Côte d’Ivoire,17 Mexico,18 and Cameroon.19 Microsatellite markers are less abundant, however, in *Ae. aegypti* than other mosquito species.20,21 Because of low frequency and a tendency to be embedded in repetitive regions of the genome,20–22 the number of useful *Ae. aegypti* microsatellites may remain limited,23 leaving room for discovery of other genetic markers for this species.

The purpose of this study was to characterize polymorphic genetic markers for *Ae. aegypti* using single strand conformation polymorphism (SSCP) analysis of single copy nuclear genes (scnDNAs). SSCP is a sensitive electrophoretic technique for detecting nucleotide polymorphisms.25 SSCP analysis has been used to identify numerous polymorphic loci for fingerprinting natural field populations. Compared with RAPDs and RFLPs, markers previously used to study *Ae. aegypti* behavior, automated high-throughput SSCP analysis of scnDNAs is faster and less labor intensive. The scnDNA markers we characterized can be used for a variety of purposes that involve tracking or reconstructing behavior of individual mosquitoes in the field.

**MATERIALS AND METHODS**

*Aedes aegypti* were collected during August 2002 from the villages of Pai Lom (16°45′ N, 98°33′ E) and Lao Bao (16°45′ N, 98°34′ E) in northwestern Thailand. These villages are separated by only 300 m,26 and we expect that the collected...
mosquitoes represent a single population. Adult *Ae. aegypti* were collected from 64 houses with backpack aspirators and preserved in 70% ethanol at ambient temperature until processed for genetic analysis at the University of California, Davis (Davis, CA). Total genomic DNA was purified from 173 adult mosquitoes (42 females and 131 males) by potassium acetate/ethanol precipitation. DNA was re-suspended in 200 μL ddH2O and stored at −80°C until used for polymerase chain reaction (PCR).

We evaluated 18 sequences published in GenBank for nucleotide polymorphisms in our study population from Thailand (Table 1). Fifteen loci represented cDNAs mapped in the *Ae. aegypti* genome by Fulton and others using SSCP analysis. Primer sequences and PCR fragment lengths for these cDNA loci followed Fulton and others. For the remaining three loci, we tried to examine non-coding sequences within the vitelligenin receptor gene (forward: AACCGCTCTCTGCTGTGATCAC, reverse: TGGAGCAGACGGCAATCATC, 302 bp), vacular ATPase B subunit gene (forward: CGAATTCATACCACGAGA, reverse: CTATCGCTTGTGATTGGTAA, 329 bp), and chitinase gene (forward: CAACGGTCTCATGATCAGT, reverse: GGGGTACGCTCCTAATA, 312 bp). Primers for these three loci were designed using Oligo (Molecular Biology Insights, Cascade, CO).

We used 6-FAM-labeled forward primers and HEX-labeled reverse primers (Applied Biosystems, Foster City, CA) during PCR amplification of scnDNA fragments. PCR reactions were performed using puReTaq Ready-To-Go PCR Beads (GE Healthcare, Piscataway, NJ). Each 25-μL reaction contained 1.5 μL of genomic DNA, 0.6 μmol/L of each primer, 10 mmol/L Tris-HCl, 50 mmol/L KCl, 15 mmol/L MgCl2, 200 μmol/L of each dNTP, and 2.5 units puReTaq DNA polymerase. PCR cycling conditions were 95°C for 5 minutes, followed by 30 or 35 cycles of 1) 95°C for 45 seconds, 2) Ta for 30–35 cycles or 1 minute for the remaining cycles, and 3) 72°C for 1 minute, followed by a final extension at 72°C for 7 minutes.

SSCP analysis of scnDNA fragments was carried out on an ABI Prism 3100 Genetic Analyzer, an automated capillary electrophoresis sequencer (Applied Biosystems). To prepare samples for analysis, 1 μL of diluted PCR product (1:20 in ddH2O) was mixed with 10.5 μL HiDi formamide (Applied Biosystems), 0.5 μL NaOH, and 0.5 μL GeneScan ROX size standard (400 or 500 HD) (Applied Biosystems). Samples were denatured at 95°C for 5 minutes, snap-cooled on wet ice for 2–5 minutes, and subjected to capillary electrophoresis. Samples were run for 50 minutes at 25°C using 5% GeneScan polymer (Applied Biosystems) with 10% glycerol in 1× Tris/Boric Acid/EDTA (TBE). Resulting SSCP profiles were analyzed using ABI Prism GeneScan and Genotyper software (Applied Biosystems). Genotyper calculated the position of all forward (6-FAM) and reverse (HEX) peaks relative to ROX size standard peaks included with each sample.

For each locus, we selected at least one individual representative of each distinct SSCP profile for subsequent cloning and sequencing of alleles. Genomic DNA from the selected individuals was PCR amplified using unlabeled primers (Invitrogen, Carlsbad, CA) and Pfu Turbo polymerase (Stratagene, La Jolla, CA). Each 50-μL reaction contained 1.0 μL genomic DNA, 0.3 μmol/L of each primer, 20 mmol/L Tris-HCl, 10 mmol/L KCl, 10 mmol/L (NH4)2SO4, 2 mmol/L Mg SO4, 0.1% Triton X-100, 0.1 mg/mL bovine serum albumin

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* Primer sequences previously published by Fulton and others. Ta = annealing temperature; H O = observed heterozygosity; H E = expected heterozygosity (100,000 Markov chains, χ<sup>2</sup> value cut-off = 0.05).
(BSA), 100 μmol/L each dNTP, and 2.5 units Pfu polymerase. PCR cycling conditions were 95°C for 2 minutes, followed by 35 cycles of 1) 95°C for 30 seconds, 2) Ta for 30 seconds, and 3) 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes. Unlabeled PCR products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and Taq-polished to add the 3' A overhang necessary for cloning.

Each 40-μL Taq-polishing reaction contained 10 mmol/L Tris-HCl, 50 mmol/L KCl, 0.1% Triton-X, 1.5 mmol/L MgCl2, 0.5 units Taq polymerase (Promega, Madison, WI), 100 μmol/L dATP (Enzypol, London, Ontario, Canada), and 27 μL purified PCR product. Polishing was performed at 72°C for 10 minutes. Polished fragments were purified a second time using the QIAquick PCR Purification Kit (Qiagen) to eliminate excess dATPs. PCR products were ligated into vectors and transformed into TOP10 cells using the TOPO TA Cloning Kit (Invitrogen). Transformed bacteria were plated onto imMedia Amp Blue LB agar (Invitrogen) containing ampicillin, X-gal, and isopropyl-β-D-thiogalactopyranoside (IPTG), and incubated at 37°C overnight. For each individual, 16 colonies were randomly selected for a second round of PCR amplification with fluorescent-labeled primers and SSCP analysis. Distinct alleles in the population were identified, and two to four clones representative of each allele were sequenced. For each allele, the sequenced clones were isolated from at least two different individuals to ensure that identical SSCP profiles corresponded to identical nucleotide sequences. Colonies selected for sequencing were grown overnight in 3 mL LB broth with ampicillin at 37°C. Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen) and submitted for sequencing by the Division of Biological Sciences Sequencing Facility (University of California, Davis) with M13R and M13 (−21) primers. Sequences from each clone were aligned with Sequencher (Gene Codes Corp., Ann Arbor, MI) and analyzed using ClustalX.28

Arlequin29 was used to calculate the observed and expected heterozygosity (H0 and He) for each marker locus and to test for significant deviations from Hardy-Weinberg equilibrium (100,000 Markov chains).

RESULTS

Of the 18 loci evaluated, 7 exhibited polymorphisms sufficient to be useful as genetic fingerprinting markers (Table 1). Six markers were derived from cDNA loci mapped by Fulton and others,25 and one marker was located within a non-coding region of the vacuolar ATPase B subunit gene.

During SSCP analysis, we were able to consistently detect nucleotide sequence polymorphisms. In homozygous individuals, all clone SSCP profiles were identical to the original genomic profile. In heterozygous individuals, two distinct patterns were evenly represented in the clone SSCP profiles, each corresponding to a single allele (Figure 1). Sequencing of clones isolated from different individual mosquitoes confirmed that identical SSCP profiles were the result of identical nucleotide sequences.

The degree of polymorphism varied among marker loci, with the number of alleles at each locus ranging from three to nine. Allele frequencies for our study population are presented in Figure 2. There were no significant differences in allele frequencies between males and females for any locus (transferrin precursor, P = 0.10; trypsin-Barillas Mury, P = 0.18; maltase, P = 0.07; carboxypeptidase A, P = 0.69; sali-

![Figure 1](https://via.placeholder.com/150)

**Figure 1.** SSCP analysis of the maltase locus in homozygous (A and B) and heterozygous (C and D) *Ae. aegypti* collected from Thailand. In each panel, red denotes size markers, blue forward sequences, and green reverse sequences. Peaks in panels in the top row are products from individual mosquito genomic DNA. Peaks in panels in the bottom three rows represent individual alleles and are products from clones of material in their respective panel in the top row. Letters above each panel represent different mosquitoes, numbers are allele designations.
vary vasodilatory protein-sialokinin 1, $P = 0.95$; vitelline membrane protein 15a, $P = 0.11$; vacuolar ATPase B subunit, $P = 0.11$; $\chi^2$). GenBank accession numbers for all allele sequences are listed in Table 2. No individuals shared the same genotype across all seven marker loci. Analysis using GenePop showed no significant linkage disequilibrium between any loci. Based on the observed allele frequencies at each locus, we calculated the probability of any two randomly-selected individuals sharing the most common genotype to be $1.4 \times 10^{-7}$.

The observed and expected heterozygosity for each locus is shown in Table 1. In our study population, significant deviations from Hardy-Weinberg proportions were detected by $\chi^2$ test for five of seven loci, even after Bonferroni correction for multiple tests. Only transferrin precursor and carboxypeptidase A conformed to Hardy-Weinberg equilibrium. Heterozygote deficiencies were observed for vitelline membrane protein 15a, maltase, and trypsin-Barillas Mury. For vacuolar ATPase B subunit and salivary vasodilatory protein-sialokinin 1, some genotypes were over-represented, whereas others were under-represented, but there was no overall excess or deficiency of heterozygotes.

Of the 11 loci that could not be used as markers, 4 were not polymorphic in our study population, 2 exhibited skewed al-
were multiple copy genes (Table 1).

lele frequencies (one allele present at > 90%), 2 were not
distinguishable by SSCP, 1 was not amenable to cloning, and
2 were multiple copy genes (Table 1).

**DISCUSSION**

Approximately one third of the loci examined met our
genotyping criteria of being single copy, heritable, polymorphic,
and co-dominant. With the seven scnDNA markers
characterized in this study, we were able to individually
distinguish 173 field-collected *Ae. aegypti*. Because the allele
frequencies described herein are characteristic of a population
at a particular time and location, the use of these
scnDNA fingerprinting markers may not be universal. Before
beginning any fingerprinting study, a sample of the target
population should be screened for polymorphism at the
selected loci. This process is relatively simple because SSCP
profiles of sampled mosquitoes can be quickly compared
against those of the reported alleles. If the seven reported loci
are not sufficiently polymorphic, new marker loci may be
identified by repeating the process we described.

In our study population, only two loci, transferrin precursor
and carboxypeptidase A, conformed to Hardy-Weinberg fre-
cuencies. Vitelline membrane protein 15a, maltase, and
trypsin-Barillas Mury exhibited heterozygote deficiencies,
possibly because of non-amplification of alleles containing
mutations in primer-binding regions. We found no mutations
in the primer-binding sites of the alleles we were able to
identify and sequence. To determine whether such mutations
are present in the population, new primers must be designed
upstream and downstream of the original forward and reverse
primer-binding sites, respectively. Vacuolar ATPase B sub-
unit and salivary vasodilatory protein-sialokinin I were not in
Hardy-Weinberg equilibrium, but no overall excess or defi-
cency of heterozygotes was found. Allele frequencies for
these loci have been examined in only a single population
thus far. Additional populations must be sampled before the
usefulness of these markers for population genetics studies of
*Ae. aegypti* can be assessed.

The markers we characterized will be valuable for finger-
printing *Ae. aegypti* mosquitoes to study the biology of free-
ranging individuals. We plan to use these scnDNA markers
to study mating behavior, particularly to quantify the frequency
of polyandry in natural populations. By dissecting out the
spermatheca from wild-caught females and genotyping the
contents, we can determine the rate at which female *Ae.
aegypti* are inseminated by more than one male. We also plan
to use these markers to examine *Ae. aegypti* oviposition patterns
by tracking the progeny of released females in the field. Other
potential uses for these markers include tracking released
adults to study dispersal and adult survival or tracking re-
leased progeny to determine immature survival and develop-
ment rates in different larval habitats.

In past studies of oviposition behavior, the number and size
of *Ae. aegypti* sibling families in oviposition sites were esti-
mated using RAPDs and RFLPs. These genetic markers
have distinct disadvantages compared with scnDNAs. From a
technical standpoint, RAPDs are difficult to standardize and
reproduce. More importantly, most RAPDs segregate as
dominant alleles. The result is that genotypes for heterozy-
gous and homozygous dominant individuals cannot be dis-
cerned, and strict assumptions regarding identity in state of
null alleles, Mendelian segregation, and Hardy-Weinberg
equilibrium must be made. Although RFLPs segregate as
c-o-dominant alleles and are comparable to scnDNAs in terms
of the number of alleles found per locus, fingerprinting by
RFLPs is limited by the time-consuming steps of identifying
polymorphic loci and the need to extract large amounts
of genomic DNA. scnDNAs are co-dominant, PCR-based
markers that can be amplified from relatively small amounts
of DNA and used to efficiently track individual behavior and
genetic relationships.

Alternative PCR-based genetic markers for *Ae. aegypti*
include microsatellites and single nucleotide polymorphisms
(SNPs). Microsatellites, although considered not abundant in
*Ae. aegypti*, have been successfully applied to study pat-
terns of gene flow among different populations. Some
additional microsatellite loci have recently been discovered
for *Ae. aegypti* and merit exploration for the type of fin-
gerprinting studies we propose. SNPs have recently been used
to study genetic relationships among *Ae. aegypti* popula-
tions, as well as to map regions of the genome associated
with insecticide resistance in this species. We are currently
comparing the utility of microsatellites and SNPs versus
scnDNA markers for genetic profiling of *Ae. aegypti*.

In addition to serving as markers, SSCP analysis of
scnDNA fragments can be used to rapidly identify SNPs in

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* Nucleotide sequences previously published in GenBank.
the *Ae. aegypti* genome. A previous study based on 25 nuclear genes found an average of 12 SNPs per kilobase in *Ae. aegypti*, a frequency similar to that in *Drosophila* and *An. gambiense*. In this study, sequencing of polymorphic alleles showed 111 SNPs in the seven scnDNA markers, with an average frequency of 47 SNPs per kilobase. This elevated SNP frequency is likely a consequence of targeting relatively few small and highly polymorphic gene regions. The distribution of SNPs varies substantially among genes; the highest frequencies are observed in rapidly evolving genes, such as those involved in host–parasite interactions. Quickly-evolving genes could become targets for automated high-throughput SSCP analysis to identify and characterize a large array of SNPs for fine-scale mapping and genotyping studies. The recent publication of the *Ae. aegypti* genome sequence will greatly facilitate the search for additional genetic markers.

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REFERENCES


