ESTIMATES OF GENE FLOW AMONG ANOPHELES MACULATUS POPULATIONS IN THAILAND USING MICROSATELLITE ANALYSIS

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Abstract. We report an analysis of seven microsatellite loci in eight populations of Anopheles maculatus mosquitoes dispersed over a distance of approximately 1,100 km in Thailand. A wide spectrum of genetic variability, with mean heterozygosities ranging from 0.738 to 0.847 were found. Based on microsatellite analysis, geographic populations of An. maculatus can be grouped into two clusters; one includes upper and lower northern populations that extend from approximately 11° to 16° north latitude, and the other (southern populations) extends south from about 7° to 6° north latitude. Wright’s FST and Slatkins’s RST for all seven microsatellite loci indicated low estimates of differentiation among all populations (mean values of FST and RST = 0.0406 and 0.051, respectively, corresponding to the Nm values of 5.91 and 4.65, respectively), and suggested that gene flow occurs among populations. However, there is some restriction of gene flow between the northern and southern populations. Geographic barriers could be limiting factors for greater gene flow between populations.

Anopheles (Cellia) maculatus sensu lato is an important vector of malaria in certain areas of its distribution such as the island of Sumatra, peninsular Malaysia, and southern Thailand. It occurs throughout the Oriental region, ranging from the Indian subcontinent through Southeast Asia and Taiwan. Anopheles maculatus comprises a group of species that are highly variable in both morphology and ability to transmit malaria, and thus it has been recognized as a species complex. These species were identified based on morphologic and cytogentic studies of polytene chromosome rearrangements from ovarian nurse cells of half gravid females. The An. maculatus species complex consists of six closely related species in Thailand and two species in the Philippines.

Among the six species in the Maculatus complex in Thailand, An. maculatus Theobald s.s. has been the most important species because it is the only species that occurs in peninsular Thailand and Malaysia, where it plays a major role in the transmission of human malaria. Two chromosomal forms have been defined and designated B and E. These are found in western and southern peninsular Thailand. Both were recognized from inversion frequencies and geographic distribution. Form B occurs northward of 13° north latitude. Form E occurs south of 12° north latitude to peninsular Malaysia where it has been incriminated as the primary vector of human malaria. Although An. maculatus is of public health importance in peninsular Thailand and Malaysia, there is little information on the population genetics of this mosquito.

Although Anopheles mosquitoes were once thought to have limited gene flow, recent studies have indicated that gene flow in the populations of Aedes spp. and An. gambiae is only weakly restricted. Previous study of An. maculatus in Thailand found no variation in a limited survey of enzyme loci (Green CA, unpublished data). Variation in ribosomal DNA sequence and random amplified polymorphic DNA markers has also been studied and the results have been similar (Rongnoparut P, unpublished data). Limited genetic variation detected using these methods precludes their use in addressing questions of population differentiation. A possible solution to the problem of the low genetic variation detected in An. maculatus is the use of repetitive DNA markers such as microsatellites. Microsatellites are especially useful for studies of population genetics because they exhibit a high degree of polymorphism, abundance throughout the genome, and relative ease of scoring. Microsatellites recently have been developed and used for population genetic studies in a variety of organisms including An. gambiae. We previously developed microsatellite markers for An. maculatus and demonstrated that these markers were polymorphic and therefore suitable for the study of population genetic structure and gene flow among An. maculatus populations in Thailand. In this report we describe the use of seven microsatellite markers to delineate the genetic structure of the An. maculatus populations from eight widely dispersed localities in western and peninsular Thailand. The extent and pattern of population variation in An. maculatus is used to estimate the levels of gene flow between populations.

MATERIALS AND METHODS

Mosquito collection. Female An. maculatus were collected from bovine and human bait in western and peninsular Thailand using methods described by Rattanarthikul and others. Informed consent to participate in the study was obtained from all participants. The study was reviewed and approved by the Ministry of Public Health of Thailand and the Walter Reed Army Institute of Research Human Use Committee. Specimens were collected in Nakorn Sawan, Uthai Thani, Kanchanaburi, Phetchaburi, Prachuap Khiri Khan, Satun, Yala, and Narathiwat Provinces (Figure 1). Mosquitoes were transported alive to the laboratory in Bangkok to be stored frozen at −70°C or to produce F1 progeny as previously described. In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals as promulgated by the Committee on Care and Use of Laboratory Animals.
GENE FLOW IN AN. MACULATUS OF THAILAND

Figure 1. Locations of eight collection sites of *Anopheles maculatus* populations displayed on a National Oceanic and Atmospheric Administration normalized difference vegetation index satellite map of Thailand depicting forest areas as green. Mountain ranges are depicted by 100 meter contour lines shown in red, provincial borders are depicted by black lines, and major drainage systems are shown by blue lines.

of the Institute of Laboratory Animal Resources, National Research Council.

**Extraction of DNA and genotype scoring.** The DNA from individual specimens was extracted using NP40 lysis solution as previously described. Microsatellite assays were performed using polymerase chain reaction (PCR) amplification. A standard PCR was run in a Perkin-Elmer (Norwalk, CT) 9600 thermal cycler. The PCR primers corresponding to unique sequences flanking the repeats were synthesized and purified (Biopolymer Laboratory, University of Maryland, Baltimore, MD). Prior to PCR amplification of specimens, optimal PCR conditions were determined for each pair of primer and actual assays were performed as previously described. After optimized conditions were es-
established, standard PCR amplification was conducted using a primer labeled with γ32P-ATP as described.16 The labeled PCR product was mixed (3:2) with formamide stop solution, denatured at 94°C for 5 min, and subjected to electrophoresis on a 6% polyacrylamide, 7M urea sequencing gel for 1.5–2.5 hr depending on the expected size of the amplified products from different loci. Gels were dried and exposed to x-ray film at −70°C with an intensifying screen for 2 hr. Autoradiographs of gels were visually inspected and allele size was determined.

Data analysis. Microsatellite genotypic frequencies were analyzed with the BIOSYS-1 package of Swofford and Selander.19 Analysis of each population included computation of allele frequencies, heterozygosity per locus, and additional measures of genetic variability. Chi-square values in each of allele frequencies, heterozygosity per locus, and addition-products from different loci. Gels were dried and exposed to x-ray film at −70°C with an intensifying screen for 2 hr. Autoradiographs of gels were visually inspected and allele size was determined.

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**Table 1**

Microsatellite loci in *Anopheles maculatus*; repeat and primer sequences and annealing temperature used in the polymerase chain reactions

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat sequence</th>
<th>Primer sequence (5' → 3')</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT18</td>
<td>(GT)10(T)13</td>
<td>ACGATGATTGCGAGCAACGC</td>
<td>50°C</td>
</tr>
<tr>
<td>GT29</td>
<td>(GA)10</td>
<td>ACTCATCTCATCTAGTAC</td>
<td>50°C</td>
</tr>
<tr>
<td>GT30</td>
<td>(CA)2AG(CA)1</td>
<td>TCTTATTTGACGGCTAGGC</td>
<td>58°C</td>
</tr>
<tr>
<td>GT34</td>
<td>(CA)1</td>
<td>TACGTTTAGAACGGATAAAG</td>
<td>54°C</td>
</tr>
<tr>
<td>GT40</td>
<td>(GT)10AT(GT)10(G)8</td>
<td>GGCTATCTTGGCGCATGC</td>
<td>56°C</td>
</tr>
<tr>
<td>GT67</td>
<td>(GT)5</td>
<td>ATCATGAGAGAGCTACACC</td>
<td>58°C</td>
</tr>
<tr>
<td>GT68</td>
<td>(CA)10GA(CA)5</td>
<td>TGGTTGCTAAACCCTAGGC</td>
<td>54°C</td>
</tr>
</tbody>
</table>

**Table 2**

Estimates of genetic variability at seven microsatellite loci in eight *Anopheles maculatus* populations

<table>
<thead>
<tr>
<th>Population†</th>
<th>Mean sample size per locus</th>
<th>Mean no. of alleles per locus</th>
<th>Mean heterozygosity‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKS</td>
<td>21.6 (0.2)</td>
<td>8.9 (0.5)</td>
<td>0.833 (0.018)</td>
</tr>
<tr>
<td>UTT</td>
<td>21.0 (0.0)</td>
<td>8.4 (0.5)</td>
<td>0.835 (0.013)</td>
</tr>
<tr>
<td>KNB</td>
<td>32.9 (1.2)</td>
<td>10.0 (1.1)</td>
<td>0.842 (0.013)</td>
</tr>
<tr>
<td>PCB</td>
<td>39.6 (0.5)</td>
<td>11.1 (0.9)</td>
<td>0.847 (0.015)</td>
</tr>
<tr>
<td>PKK</td>
<td>25.9 (0.1)</td>
<td>8.7 (0.7)</td>
<td>0.800 (0.027)</td>
</tr>
<tr>
<td>ST</td>
<td>15.7 (0.5)</td>
<td>7.0 (0.9)</td>
<td>0.739 (0.051)</td>
</tr>
<tr>
<td>YL</td>
<td>15.3 (0.8)</td>
<td>6.6 (0.9)</td>
<td>0.748 (0.043)</td>
</tr>
<tr>
<td>NTW</td>
<td>45.3 (0.7)</td>
<td>8.6 (1.1)</td>
<td>0.738 (0.051)</td>
</tr>
</tbody>
</table>

† Values in parentheses are standard errors.
‡ NKS = Nakhon Sawan; UTT = Uthai Thani; KNB = Kanachanaburi; PCB = Petchaburi; PKK = Prachinburi Khiri Khan; ST = Surat; YL = Yala; NTW = Narathiwat.

Analysis of microsatellite data to infer geographic structure differs slightly from that for allozyme loci because, unlike allozymes, microsatellites do not conform to an infinite allele model in which every new mutation creates a novel allele with low mutation rate.10 At microsatellite loci, most mutations involve the gain or loss of only one or two repeat units and thus allow for frequent back mutations, the so-called stepwise mutation model.23 Slatkin recently developed an analog of FST called RST that was designed specifically to take into account the stepwise mutation model.24 The RST values were calculated from the averages of the within-population and total variance components across all seven loci. The 95% confidence intervals were obtained from the standard errors by assuming the t distribution, and these were used to judge whether mean values differed significantly from zero as previously described.25 Gene flow was estimated from the FST values using the relationship Nm = 1/4 (1/FST – 1), in which Nm is the effective size of an isolated, panmictic population (deme), and m is the proportion of the deme that are migrants.26 The Nm values were also derived from RST by substituting RST for FST.

**Map production.** The habitat and site collection map were produced using geographic information software.27,28 The raster base map displayed was produced from digital remote sensing data acquired by the advanced very high resolution radiometer (AVHRR) sensor on polar-orbiting satellites operated by the National Oceanic and Atmospheric Administration (NOAA). The AVHRR records visible, near-infrared, and thermal channels of the electromagnetic spectrum. The characteristics of the NOAA AVHRR have been described previously.29 The normalized difference vegetation index (NDVI) analyzed in this study is a transformation between data from the visible channel and the near-infrared channel. The NDVI data have been shown to be highly correlated with green-leaf biomass and represent the photosynthetic capacity of the area measured. These NDVI data were calculated from NOAA satellite coverages of Thailand for 10 dates between December 1992 and April 1993. Data from

**Figure 2.** Allelic distributions for seven microsatellite loci in populations of *Anopheles maculatus* collected in three geographic regions of Thailand. □ = upper northern populations; □ = lower northern populations; ■ = southern populations. Polymerase chain reaction product lengths of allele classes are indicated as number of basepairs.
GENE FLOW IN *AN. MACULATUS* OF THAILAND

GT18

GT29

GT30

GT34

GT40

GT67

GT68

Allele length (basepairs)
**RESULTS**

**Allele frequencies.** Specimens were collected in three geographic regions (Figure 1): 1) the upper northern provinces (Nakhon Sawan [NKS], Uthai Thani [UTT], and Kanchanaburi [KNB]), 2) the lower northern provinces (Phetchaburi [PCB] and Prachuap Khiri Khan [PKK]), and 3) the southern provinces (Satun [ST], Yala [YL], and Narathiwat [NTW]). The repeat and primer sequences for the seven microsatellite loci used in this study are shown in Table 1. Allele frequencies for seven loci in eight populations were calculated from genotypes of individual mosquitoes (data available from the authors upon request). All seven microsatellite loci examined displayed a high level of polymorphism in all eight populations studied (Table 2), with an average of 8.75 alleles (range = 6.6–11.1) per locus per population. Figure 2 shows the allelic distributions for all loci among populations in three geographic regions. Extensive variability is observed among all populations for loci GT40, GT67, and GT68. The number of alleles detected at some loci varied dramatically among populations (e.g., locus GT68: seven alleles in southern populations and 11 in upper northern populations, Figure 2). The allele compositions of the other four microsatellite loci (GT18, GT29, GT30, and GT34) were more similar among populations. Certain alleles were the same in each population and every allele with a frequency higher than 5% in a given population was also found in all other populations.

The chi-square values for deviations from Hardy–Weinberg equilibrium are shown in Table 3. Comparisons with Hardy–Weinberg equilibrium showed that all populations were in Hardy–Weinberg equilibrium ($P > 0.05$) at all microsatellite loci except PCB, which showed significant deviations.

### Table 3

<table>
<thead>
<tr>
<th>Population</th>
<th>GT18</th>
<th>GT29</th>
<th>GT30</th>
<th>GT34</th>
<th>GT40</th>
<th>GT67</th>
<th>GT68</th>
</tr>
</thead>
<tbody>
<tr>
<td>NKS (n = 22)</td>
<td>36.836 (28)</td>
<td>47.010 (36)</td>
<td>37.625 (45)</td>
<td>12.658 (21)</td>
<td>60.681 (55)</td>
<td>38.330 (28)</td>
<td>25.562 (36)</td>
</tr>
<tr>
<td>UTT (n = 21)</td>
<td>28.891 (21)</td>
<td>20.257 (28)</td>
<td>59.646 (45)</td>
<td>18.743 (21)</td>
<td>44.473 (45)</td>
<td>35.815 (28)</td>
<td>23.349 (36)</td>
</tr>
<tr>
<td>KNB (n = 35)</td>
<td>41.811 (55)</td>
<td>69.669 (78)</td>
<td>22.196 (45)</td>
<td>9.294 (21)</td>
<td>86.943 (91)</td>
<td>23.734 (28)</td>
<td>15.960 (21)</td>
</tr>
<tr>
<td>PCB (n = 40)</td>
<td>40.308 (28)</td>
<td>133.012 (105)</td>
<td>65.559 (66)</td>
<td>41.051 (36)</td>
<td>81.087 (78)</td>
<td>25.927 (45)</td>
<td>50.401 (55)</td>
</tr>
<tr>
<td>PKK (n = 26)</td>
<td>21.238 (28)</td>
<td>61.878 (66)</td>
<td>35.917 (45)</td>
<td>12.166 (21)</td>
<td>25.904 (36)</td>
<td>12.353 (15)</td>
<td>36.036 (36)</td>
</tr>
<tr>
<td>ST (n = 18)</td>
<td>52.615 (45)</td>
<td>47.010 (36)</td>
<td>48.337 (36)</td>
<td>19.746 (21)</td>
<td>6.206 (10)</td>
<td>3.531 (6)</td>
<td>9.679 (10)</td>
</tr>
<tr>
<td>YL (n = 18)</td>
<td>30.420 (36)</td>
<td>46.176 (36)</td>
<td>26.560 (36)</td>
<td>8.222 (6)</td>
<td>4.982 (10)</td>
<td>13.214 (10)</td>
<td>4.754 (10)</td>
</tr>
<tr>
<td>NTW (n = 47)</td>
<td>51.999 (45)</td>
<td>59.760 (91)</td>
<td>26.018 (21)</td>
<td>22.098 (28)</td>
<td>11.359 (36)</td>
<td>7.572 (10)</td>
<td>32.157 (21)</td>
</tr>
</tbody>
</table>

* Values in parentheses are degrees of freedom.
† For definitions of abbreviations, see Table 2. n = number of specimens tested.
‡ $P < 0.05$
parture (0.01 < P < 0.05) from Hardy-Weinberg equilibrium at locus GT29 (Table 3). Since only one of the 56 chi-square values were significant, a value not different from what would be expected by chance alone, it was concluded that observed proportions did not differ from those expected allelic frequencies.

**Genetic structure of subpopulations.** Nei’s unbiased distance matrix was calculated for the eight populations and distance values were clustered by UPGMA to produce a dendrogram shown in Figure 3. The dendrogram showed a correlation between genetic and geographic distances. The eight populations grouped into two clusters: the upper northern (NKS, UTT, and KNB) and lower northern (PCB, PKK) populations grouped together, and the southern (ST, YL, and NTW) populations formed another group.

**Genetic relationships among subpopulations.** Estimates of FST and RST values at the various polymorphic loci in all the geographic populations are compared in Table 4. The F-statistic (FST), a measure of the amount of differentiation among subpopulations, showed a mean value of 0.0406 and a mean index of fixation of individuals relative to the total of subpopulations (FIS) value of 0.0594 when all An. maculatus populations were analyzed (Table 4). The FST values were significantly different from zero for two of seven loci and the RST values were significantly different from zero for one of seven loci (Table 4). The number of migrants calculated from FST and RST values were compared (Table 5) and Nm values were similar. The migrants per generation (Nm) estimated from FST was 5.91 when all populations were considered. This number is greater than that required for fixation of alternative alleles (Nm < 1) and is in agreement with the small differences observed in allele frequencies among all populations.26

Estimates of Nm derived from RST calculations (RST = 0.051) yield a similar value of Nm = 4.65. All Nm values derived from either FST or RST were larger than 4. The RST values of microsatellite loci were higher than their corresponding FST values, as expected for loci with high mutation rates.28

The FST and RST values of populations represented by the upper and lower northern groups indicate minimal differentiation, with the mean values of 0.0115 and 0.019, respectively (corresponding to migration rates of 21.49 and 12.91, respectively, Table 5). High levels of migration suggest that gene flow is only minimally restricted across the upper and lower northern regions with the most distant populations (NKS and PKK) separated by a distance of approximately 550 km. The FST and RST values among subpopulations in the southern group exhibited higher levels of differentiation (with the mean values of 0.0143 and 0.031, respectively, corresponding to migration rates of 17.23 and 7.81, respectively), indicating a lower gene flow rate than that in the upper and lower northern populations. The major contribution to the greater interpopulation diversity in the southern populations was the fewer number of alleles and markedly different allele frequencies for loci GT40, GT67, and GT68 (Figure 2), with FST values of 0.0512, 0.0886, and 0.0896, respectively (Table 4).

The most dramatic differences between population groups was observed at locus GT68; the upper northern populations had several common alleles (142, 144, and 146) at relatively high frequencies, while in the southern populations allele 140 was most common (Figure 2). At locus GT67, allele 106 was most common in the southern populations, and allele 100 was the most common in the upper and lower northern populations (Figure 2). At locus GT40 allele 101, a common allele in all eight populations, was the most common allele in the southern populations (Figure 2). Among all populations, observed RST values indicate high genetic differentiation at the GT68 locus, moderate differentiation at GT18, GT29, and GT67 loci, and lowest differentiation at GT30, GT40, and GT34 loci. Although no fixed differences in microsatellite genotype frequencies at any loci were found for all An. maculatus populations (Figure 2), there was significant differentiation among populations at the locus GT68, as demonstrated by significantly high FST and RST values for this locus. When GT68 was excluded from analysis, FST and RST values decreased to 0.032 and 0.037, respectively, corresponding to Nm values of 7.56 and 6.51, respectively (Table 5).

**DISCUSSION**

Genotypic frequencies in all eight populations were generally in agreement with Hardy-Weinberg equilibrium. The only exception to the Hardy-Weinberg equilibrium was the GT29 locus in the PCB populations. This deviation may have been caused by the occurrence of homozygotes for rare alleles at the GT29 locus.

Microsatellite studies demonstrated an overall similarity in allelic profile of An. maculatus populations separated by approximately 1,100 km, as indicated by low estimates of interpopulation differentiation, and a moderate estimate of average migration rate (Nm > 4). However, some isolation between gene pools of upper-lower northern and southern peninsular populations were observed. Gene flow estimated by Nm was frequent and highest when data from southern
peninsular populations were excluded (Table 5, $N_m (F_{ST}) = 21.49$, $N_e (R_{ST}) = 12.91$). The genetic structure of An. maculatus in southern populations differed from the structure of the northern populations in two respects: 1) there were fewer alleles at certain loci (loci GT40, GT67, and GT68) in southern populations (Figure 2), and 2) southern populations were more genetically divergent from one another than those in upper and lower northern regions, as indicated by their higher $F_{ST}$ and $R_{ST}$ values compared with those of upper and lower northern populations (Table 5).

The NKS, UTT, KNB, PCB, and PKK populations are located along a 550 km section of the eastern slopes of the Tanao Sri mountain range (Figure 1). This mountain range offers a pathway for the dispersal of An. maculatus along interconnecting rivers and streams flowing into the Gulf of Thailand, possibly accounting for extensive gene flow ($N_m (F_{ST}) > 21$ and $N_m (R_{ST}) > 12$) observed among these populations (Figure 1). Genetic distances (Figure 3) suggest that there is some geographic restriction between the upper-lower northern populations and southern populations, and within the southern populations. The geography of the southern peninsular area is complex with mountain ranges interrupting drainage systems and providing somewhat isolated habitats within the area sampled. The Tanao Sri mountain range ends south of PKK at the border of Chumphorn and Ranong Provinces, and two mountain ranges, the Phuket and Nakhon Srithummarat, divide the peninsula between Chumphorn and Satun Provinces (Figure 1). These mountains serve as geographic barriers between the upper-lower northern and southern populations. The discontinuities in habitats might explain the maintenance of two genotypic clusters (the upper-lower northern and the southern populations). Fewer alleles in southern peninsular populations may be indicative of some reproductive isolation as a group. Greater genetic divergence among the southern peninsular populations suggests reproductive isolation between populations. The Sunkarakhiri mountain range separates ST and YL from NTW Provinces (Figure 1). The geographic barrier attributed by the Sunkarakhiri mountain range may be responsible for the moderate genetic heterogeneity and reduced gene flow among southern populations ($N_m (F_{ST}) > 17$, $N_m (R_{ST}) > 7$, Table 5). Furthermore, the ST population is on a drainage system that flows west into the Strait of Malacca, while the YL drainage flows south into Malaysia and the NTW drainage flows into the South China Sea (Figure 1). The fewer alleles found at loci GT40, GT67, and GT68 in southern populations suggest that some alleles were selected for at particular loci in southern populations.

Based on genetic distances and differentiation indices, the three upper northern and two lower northern populations form a discrete group with relatively high gene flow between them. Estimates of interpopulation differentiation among all eight populations were measured by Wright’s $F_{ST}$ and Slatkin’s $R_{ST}$ statistics, and these yielded moderate estimates of the average migration index ($N_m > 4$) across the entire 1,100 km distance. Moderate levels of gene flow observed here may have prevented complete genetic fixation between the upper-lower northern and southern populations. However, levels of gene flow observed in this study may indicate historical rather than ongoing gene flow. Thus, An. maculatus populations of southern regions (ST, YL, and NTW) may eventually form a distinct species through allopatric speciation. At this point there is insufficient information to explain the moderate level of differentiation of An. maculatus populations among southern peninsular populations. Studies of genetic structure of the An. maculatus populations across the southern peninsular area using additional genetic loci and more extensive sampling of field populations could provide more knowledge on the path of gene flow and factors that affect gene flow.

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REFERENCES


