Am. J. Trop. Med. Hyg., 77(2), 2007, pp. 310–315

Analysis of the Population Genetic Structure of the Malaria Vector *Anopheles sinensis* in South Korea Based on Mitochondrial Sequences

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Abstract. The population genetics of *Anopheles sinensis*, a major malaria vector in South Korea, was studied based on the nucleotide sequences of a 238-bp variable region of the mitochondrial control region. Three features of genetic variance were observed. First, the Taebaek and Sobaek mountain ranges may function as genetic barriers between the Northern Group (NG) and the Southern Group (SG). These mountain ranges are associated with the subdivision of the population, and significant and unique population differentiation was observed in the examined area. Second, the genetic cohesiveness observed within each group may have been caused by a recent expansion in the population rather than recurrent gene flow. Third, a marked dissimilarity in the genetic diversity between the two groups may also have resulted from several factors that caused a difference in the effective population sizes.

INTRODUCTION

Various levels of population subdivision of the anopheline mosquito species have been observed from nearly panmictic across a wide geographic range to highly divergent within a short distance. A high rate of recurrent gene flow and/or recency of population expansion leave populations of some anopheline species hardly differentiated.[1–3] However, other species are composed of highly structured populations that are mainly caused by genetic barriers such as mountain chains or arid valleys.[4–6] Because this suggests valuable information about, for instance, the degree of dispersal of insecticide-resistant individuals or genetically modified mosquitoes, population genetic data of vector species could enable us to map out effective strategies for controlling malaria.[7]

Despite its malaria vectorial capacity, *Anopheles sinensis* has not been a focus of concern for population geneticists because this species does not show an active anthrophilic preference.[8] However, population genetic information of this species, at least in South Korea where this species has become a major malaria vector,[9] is needed in controlling malaria for the several hundred cases of this disease occurring every year in this country despite various efforts such as quarantine and treatment with insecticides.[10] Located on a peninsula, South Korean populations of *An. sinensis* may be hardly affected by the gene flow from the continent or nearby islands. Thus, populations in South Korea would be suitable for studying genetic diversity influenced mostly by population substructure within this region with the help of mitochondrial DNA sequences that would be useful to detect subtle genetic divergence because of a quarter of its effective population size compared with that of nuclear DNA. In this study, based on the sequences of the mitochondrial control region, we attempted to elucidate the genetic properties and subdivisions of the South Korean population of *An. sinensis*.

MATERIALS AND METHODS

Sampling and DNA extraction. We collected 290 *An. sinensis* individuals from 19 locations in South Korea (Figure 1; Table 1) by using a CDC Miniature Light Trap (John W. Hock Company, Gainesville, FL). The collected samples were preserved on site using dry ice, and their morphologic characters were studied under a stereomicroscope in the laboratory. DNA was extracted from entire individuals or parts of their bodies by following a standard phenol extraction protocol.[11]

Sequence analysis of the mitochondrial control region. To amplify the 238-bp variable region of the mitochondrial control region, primers (U415, 5′-CCTCCTAATAATTTTC-CCC-3′; L672, 5′-GGGTGATATTAATTATAGACC-3′) were designed based on the complete sequences (GenBank accession numbers, DQ466091–DQ466107) that were amplified and sequenced using the primers DMP5 and DM57.[12] Polymerase chain reaction (PCR) mixtures were made up of 1× *Taq* polymerase buffer, a 1-mmol/L dNTP mix that was AT rich (80% AT content), 2.5 mmol/L $MgCl_2$, 0.5 μmol/L of each primer, 2.5–250 pg total DNA, and 0.04 U/μL of *Taq* polymerase (Promega, Madison, WI). PCR was performed using the GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) under the following cycle conditions: initial denaturation at 94°C for 3 minutes, followed by 30 cycles at 94°C for 30 seconds, 45°C for 50 seconds, and 72°C for 50 seconds, and a final elongation at 72°C for 7 minutes. The amplified products were purified from the PCR mixtures using a QIAquick PCR purification kit (Qiagen, Valencia, CA). Subsequently, the PCR products were sequenced using both the amplifying primers and the Big Dye terminator sequencing kit (Applied Biosystems), according to the manufacturer's instructions. After purification, the reaction products were analyzed on an ABI PRISM Genetic Analyzer 3100 (Applied Biosystems). Bidirectional sequences were aligned and visually examined using Sequence Navigator v1.0.1 (Applied Biosystems). The sequences were aligned with ClustalX[13] and were exported to the NEXUS and PHYLIP formats for data analyses.

Data analyses. DnaSP 4.10[14] was used to calculate the frequency of each haplotype, haplotype diversity,[15] nucleotide diversity,[16] and theta (θ) values based on the number of polymorphic sites (S) and the mean number of pairwise differences (π), respectively. Using TCS 1.18,[17] a 95% set of plausible haplotype networks and the outgroup probability of the haplotypes were computed based on statistical parsimony. To measure the genetic distance between populations, pairwise γ_{ST} and δ_{ST}[18] were calculated using DnaSP 4.10.[14] Subse-

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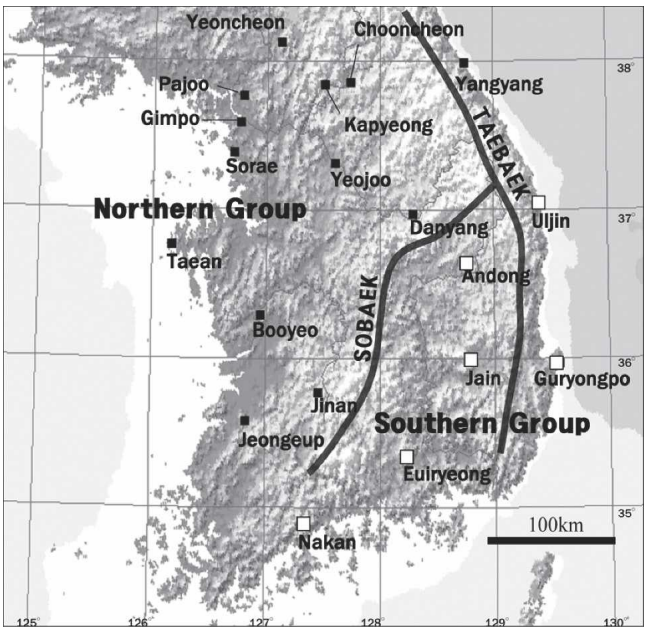

FIGURE 1. Locations where the individuals were collected are identified on a map of Korea. Populations belonging to the NG and SG are represented as ■ and □, respectively. The solid line indicates the Taebaek and Sobaek mountain ranges.

quently, the distance matrices of pairwise γ_{ST} and δ_{ST} were used to determine a relationship among populations by using the neighbor-joining (NJ) method as implemented by the MEGA 3.1[19] software. To evaluate the significance of differentiation between the two populations based on pairwise F_{ST}, the exact test[20] was performed using Arlequin 3.01[21] with a Markov chain length of 10,000 and 1,000 burn-in steps. Using the same software, analysis of molecular variance (AMOVA)[22] was conducted to measure the extent to which genetic variance is assigned to the hierarchical level of population organization. The statistical significance for AMOVA was evaluated using > 10,000 permutations. DnaSP 4.10[14] was used to evaluate the significance of differentiation among the populations within a group, between the groups, or among all the populations using the χ^2 test, and gene flow estimates among the populations were calculated by the method described by Hudson and others.[23] Using Arlequin 3.01,[21] the Mantel test[24] was performed to test any significant relationship between geographical distance and population genetic distance (linearized F_{ST}).[25] Using DnaSP 4.10,[14] population demographic parameters such as tau (τ) and theta initial (θ_0) were estimated assuming theta final (θ_1) as infinite,[26] and the raggedness index[27] was used to show the unimodality of mismatch distribution. The results of the neutrality tests of Tajima[28] and Fu[29] were calculated using Arlequin 3.01.[21] The significance of the D and *F*s values of Tajima and Fu, respectively, was evaluated by comparison with randomly generated values based on the observed θ(S) with 10,000 repeats.

RESULTS

Haplotypes and genetic diversity. From the sequence analysis, 60 haplotypes were identified among the 290 *An. sinensis* individuals of Korea (GenBank accession numbers: DQ445543–DQ445602), and their average guanine and cytosine (GC) content was observed to be 4.45%. The haplotype diversity, i.e., the probability that two randomly selected haplotypes are present in the sample, was 0.8871 ± 0.0122. There were 41 polymorphic sites, and no gaps were present. The nucleotide diversity per site (π) was 0.010317 ± 0.006194 (no gamma correction) based on the mean number of pairwise differences (2.455507 ± 1.332656). The number of individuals containing the seven haplotypes that exhibited the highest frequency was 206, and this accounted for 71% of the total (Figure 2). Although the most frequently occurring haplotype was BY02, CC05 represented the highest outgroup weight probability (0.15). Forty haplotypes (67% of the total number) occurred as private alleles and were present in a single individual. The genetic diversity indices for each population are presented in Table 1. Of the 19 locations, the Nakan and Guryongpo populations displayed the highest gene and nucleotide diversity, respectively. Furthermore, the highest θ(S) and $\theta(\pi)$ values were recorded in the Jain and

TABLE 1
Geographic information, indices of genetic diversity, and theta estimates of each population

Location	Coordinates	No. individuals	No. haplotypes	Gene diversity	Nucleotide diversity	θ(S)	$\theta(\pi)$
Andong (AD)	36°33′32″N 128°46′05″E	11	9	0.964 (±0.051)	0.01217 (±0.00807)	3.76 (±1.80)	3.02 (±1.92)
Booyeo (BY)	36°17′37″N 126°56′57″E	8	4	0.750 (±0.139)	0.00945 (±0.00661)	2.31 (±1.31)	2.25 (±1.57)
Chuncheon (CC)	37°51′06″N 127°43′08″E	14	6	0.681 (±0.132)	0.00905 (±0.00599)	2.52 (±1.24)	2.15 (±1.43)
Danyang (DY)	36°58′00″N 126°16′00″E	17	8	0.846 (±0.062)	0.00865 (±0.00570)	2.66 (±1.25)	2.06 (±1.36)
Euiryeong (ER)	35°20′53″N 128°14′29″E	25	16	0.960 (±0.021)	0.01252 (±0.00754)	3.71 (±1.51)	2.98 (±1.80)
Gimpo (GP)	37°35′41″N 126°45′58″E	12	5	0.727 (±0.113)	0.00980 (±0.00648)	2.65 (±1.33)	2.33 (±1.54)
Guryongpo (GR)	35°58′21″N 129°33′04″E	19	13	0.965 (±0.024)	0.01617 (±0.00950)	4.58 (±1.89)	3.85 (±2.26)
Jinan (JA)	35°46′00″N 127°27′00″E	19	9	0.836 (±0.068)	0.01017 (±0.00645)	2.58 (±1.19)	2.42 (±1.53)
Jain (JI)	35°53′23″N 128°47′04″E	19	13	0.942 (±0.038)	0.01317 (±0.00798)	4.86 (±1.99)	3.13 (±1.90)
Jeongeup (JU)	35°34′00″N 126°49′00″E	9	6	0.833 (±0.127)	0.01354 (±0.00874)	3.68 (±1.86)	3.22 (±2.08)
Kapyong (KP)	37°50′00″N 127°30′00″E	16	8	0.808 (±0.093)	0.01005 (±0.00646)	3.01 (±1.39)	2.39 (±1.54)
Nakan (NA)	34°52′58″N 127°20′52″E	16	14	0.975 (±0.035)	0.01271 (±0.00783)	4.82 (±2.05)	3.03 (±1.86)
Paju (PJ)	37°46′00″N 126°47′00″E	30	14	0.851 (±0.048)	0.01045 (±0.00646)	4.04 (±1.56)	2.49 (±1.54)
Sorae (SR)	37°23′00″N 126°43′00″E	19	7	0.795 (±0.076)	0.01007 (±0.00640)	2.86 (±1.29)	2.40 (±1.52)
Taean (TA)	36°46′30″N 126°09′42″E	13	4	0.603 (±0.131)	0.00453 (±0.00358)	0.97 (±0.63)	1.08 (±0.85)
Uljin (UJ)	37°02′16″N 129°23′23″E	10	7	0.867 (±0.107)	0.00738 (±0.00527)	2.83 (±1.46)	1.76 (±1.26)
Yeoncheon (YC)	38°08′00″N 127°06′00″E	13	8	0.897 (±0.067)	0.01239 (±0.00779)	3.22 (±1.53)	2.95 (±1.85)
Yeojoo (YJ)	37°18′00″N 127°35′00″E	12	6	0.849 (±0.074)	0.00993 (±0.00655)	2.98 (±1.46)	2.36 (±1.56)
Yangyang (YY)	37°58′59″N 128°44′29″E	8	6	0.893 (±0.111)	0.00795 (±0.00576)	1.93 (±1.14)	1.89 (±1.37)

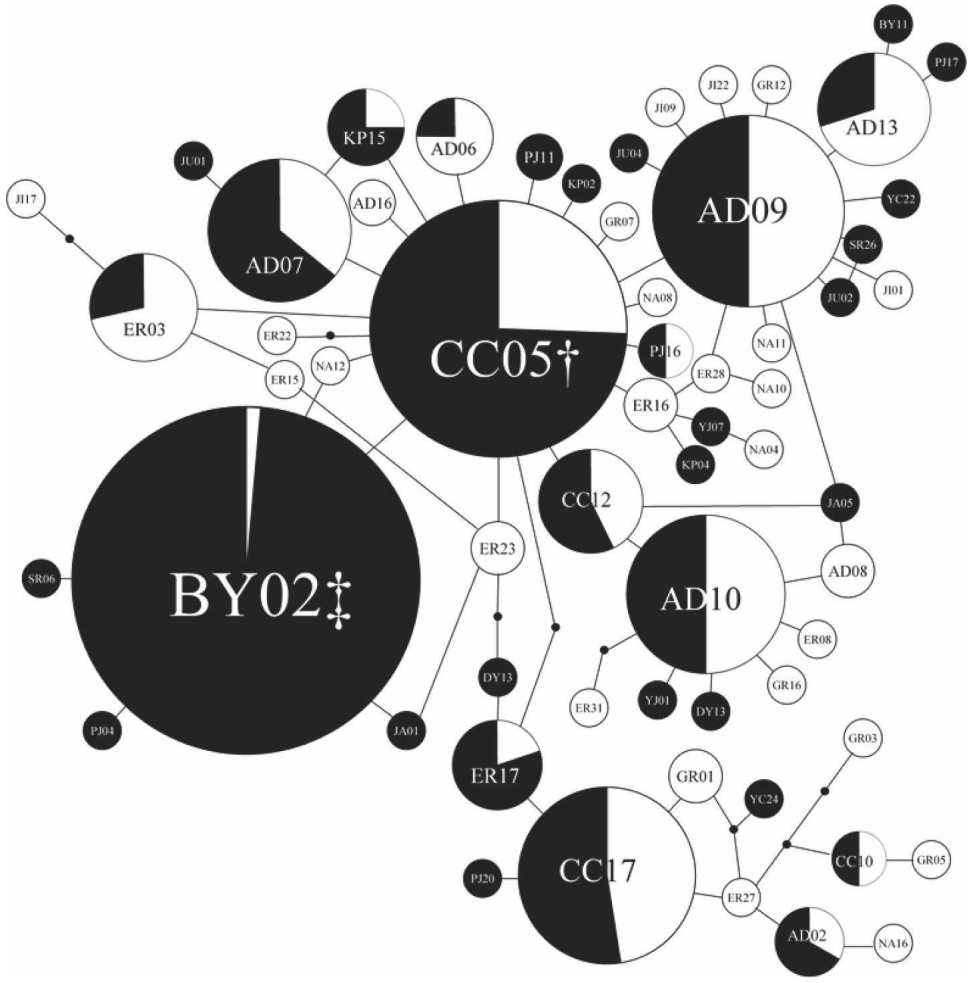

FIGURE 2. The 95% set of plausible haplotype networks as calculated by TCS 1.18.[17] Each circle represents a haplotype, and the numbers indicate haplotype identification. The size of the circles denotes the number of individuals that contain that haplotype, and the shaded portions of the pies indicate the group containing the particular haplotype. Black and white colors of pies represent the NG and SG, respectively. ●, missing haplotypes. †CC05, haplotypes with the highest outgroup probability; ‡BY02, haplotype with the highest frequency in South Korea.

TABLE 2
Pairwise F_{ST} values among populations of *An. sinensis* in South Korea

	AD*	BY	CC	DY	ER*	GP	GR*	JA	JI*	JU	KP	NA*	PJ	SR	TA	UJ*	YC	YJ
BY	0.063																	
CC	**0.1239**	0																
DY	**0.0201**	0.0365	0.0686															
ER*	0	**0.1211**	**0.1498**	0.0138														
GP	0.0308	0	0	0	**0.07**													
GR*	0	0.1169	**0.1252**	**0.0417**	0.0044	0.065												
JA	**0**	0.0244	0.0174	0	**0.0229**	0	**0.0411**											
JI*	0	**0.2129**	**0.2427**	**0.1055**	0.0036	**0.1565**	0	**0.111**										
JU	0	0	0.0126	0.0307	**0.0329**	0	**0.0238**	0	0.0784									
KP	**0.0482**	0	0	0	0.0661	0	**0.0677**	0	**0.1608**	0								
NA*	0	0.1508	**0.1971**	0.0243	0	**0.092**	0.0223	**0.0662**	0.0143	**0.0688**	**0.0937**							
PJ	0.002	0.0214	0.039	0	**0.024**	0	**0.0446**	0	**0.0991**	0	0	**0.0453**						
SR	**0**	0.0026	0.013	0	**0.0263**	0	**0.034**	0	**0.1004**	0	0	**0.0542**	0					
TA	**0.1769**	0	0	0.1159	**0.1953**	0.0175	**0.2019**	0.0678	**0.3125**	0.0583	0.0059	**0.2508**	0.0655	0.0451				
UJ*	0	**0.1611**	**0.1759**	0	0	**0.0677**	0.0114	**0.0214**	0.0379	**0.0669**	**0.045**	0	0	**0.013**	**0.2765**			
YC	0	0.01	0.0296	0	0	0	0	0	0.042	0	0	0.0245	0	0	0.0831	0		
YJ	0	0	0.0072	0	0.0145	0	0.0342	0	**0.1005**	0	0	0.0354	0	0	0.0376	0	0	
YY	0.0137	0	0	0	0.0588	0	0.073	0	**0.1552**	0	0	0.0797	0	0	0	0.0657	0	0

Significant values are in bold. Negative values of F_{ST} are represented as zero.
* Populations belonging to the SG.

Guryongpo populations, respectively. The Taean population displayed the lowest values for all the indices of genetic diversity and the lowest theta estimates (Table 1).

Genetic structure of *An. sinensis* in South Korea. Based on results of the pairwise F_{ST} analysis (Table 2) and NJ analysis of pairwise γ_{ST} and δ_{ST}, the Korean populations of *An. sinensis* were found to be separated into two groups (Figure 3). The Northern Group (NG) was made up of 13 populations that were present in the northern region of South Korea, and the other 6 populations belonged to the Southern Group (SG) (Figure 1). AMOVA revealed that most of the genetic variance lay within the populations, which may have been because of the existence of several private alleles; the 8.78% significant variance observed between the two groups further substantiated the division of the South Korean populations into two groups and also suggested that genetic variance was not structured within the groups (Table 3). The Mantel test supported the AMOVA results and showed that there was a nonrandom association ($P < 0.01$) between the genetic differences (linearized F_{ST}s) and geographic distances among all the 19 populations, but no correlation was observed in each group ($P_{NG} = 0.334$ and $P_{SG} = 0.329$). Significant genetic differentiation among populations was not observed within either group; however, according to the results of the χ^2 test

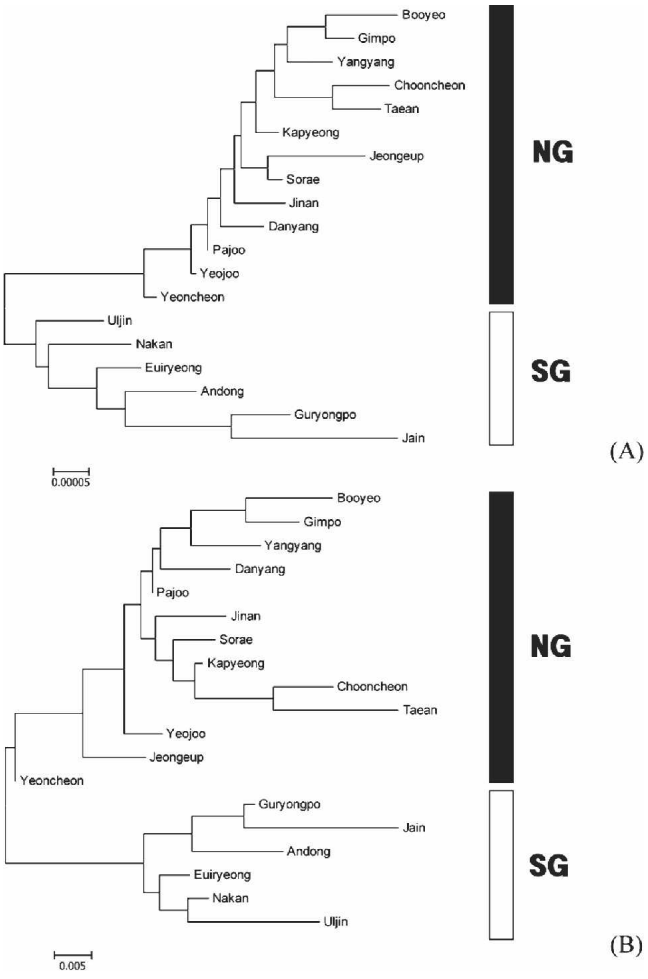

FIGURE 3. Population tree constructed using NJ analysis of pairwise γ_{ST} **A**, and δ_{ST} **B**.

TABLE 3

AMOVA of geographic variation among the *An. sinensis* individuals collected from Korea

Source of variation	df	Sum of squares	Variance components	Percentage of variance (%)	*F*-index
Among groups	1	17.243	0.12317	8.78	0.08783*
Among populations within groups	17	19.244	−0.01051	−0.75	−0.00822†
Within populations	271	349.500	1.28967	91.97	0.08034†
Total	289	385.986	1.40232	100	

* $0.0001 < P < 0.001$; † $0.001 < P < 0.01$.
df, degrees of freedom.

to detect differentiation, it was observed between the two groups (Table 4).

Comparison of the two South Korean groups. All estimates of genetic diversity and theta calculated for the SG were higher than those calculated for the NG; this was observed not only when comparing the populations (Table 1) but also when comparing the average values of the two groups (Table 4). The haplotype composition differed between the two groups. The SG contained a considerable number of private alleles, i.e., 22 of the 40 private alleles found in South Korea, considering that the number of individuals in the SG was nearly one half that in the NG. No remarkable relationship was observed between geographic distribution and haplotype lineage in the haplotype network (Figure 2). Most of the high-frequency haplotypes were observed in both the groups. The NG contained all the individuals carrying BY02, the most frequent haplotype, except one individual belonging to the Andong population present in the SG (Figure 2). Gene flow estimates were high among populations within each group. However, populations belonging to the SG were slightly more differentiated than those belonging to the NG (Table 4). According to the results of the mismatch analysis and neutrality tests, both groups have recently experienced expansion in population size (Figure 4; Table 4). Judging from the smaller θ_0 value and larger τ value of the SG, it may have undergone expansion earlier, with an initial population size that was smaller than that of the NG (Table 4).

DISCUSSION

Anopheline mosquitoes of Africa usually form relatively large geographic demes either because of the extensive gene flow (populations are genetically differentiated in an isolation-by-distance manner) or because of recent range expansion from relatively large, stable populations.[1,2,30] The *An. sinensis* population in South Korea also showed an almost panmictic structure, except for the distinct subdivision into the NG and SG. Geographic features such as mountains and arid valleys have been considered potent genetic barriers for several anopheline species.[4,5] Considering the geographic distribution of the locations of the NG and SG, the Taebaek and Sobaek mountain ranges may play a key role in establishing a genetic barrier that traverses South Korea from the northeast to the southwest (Figure 1). This is supported by the result that all but one individual containing BY02, the most frequent haplotype, were only found in the NG.

Genetic cohesiveness among the populations belonging to each group may be the result of a recent expansion in population size rather than extensive gene flow. According to the

TABLE 4
Comparison of gene flow estimates, genetic diversity, and theta estimates in the SG and NG of the South Korean populations of *An. sinensis*

		Within NG	Within SG	Between NG and SG
P value of genetic differentiation		0.3804	0.5957	0.0000
Hudson and others	F_{ST}	–0.02300	0.00420	0.07859
(1992)	Nm	–22.24	118.57	5.86
Number of individuals		190	100	–
Number of haplotypes		29	40	–
Haplotype diversity		0.798 ± 0.025	0.950 ± 0.009	–
Nucleotide diversity	(π)	0.00830	0.01296	–
Theta	θ (π)	0.00839	0.01319	–
Estimates	θ (S)	0.02241	0.02995	–
Neutrality	Tajima's D	–1.47446*	–1.64116*	
Tests	Fu's *F*s	–24.51570†	–26.42346†	
Mismatch	θ_0	0.727	0.227	
Distribution	τ	1.248	2.858	
	Raggedness	0.0114	0.0234	

* $0.01 < P < 0.05$; † $P < 0.01$.

mark-release-recapture experiment conducted for this species, migration range is not considerably large; 87.6% of the individuals were recaptured within < 6 km from the release point, although some individuals were able to fly ≥ 12 km in one night.[31] These results are in agreement with the fact that almost all malaria cases in South Korea were reported within 10 km of the demilitarized zone (DMZ) during the early days after re-emergence,[32] which was caused by sporozoite-infected mosquitoes that originated from North Korea where malaria has recently been prevalent because of its primitive medical system and long-term poverty.[10] From the mismatch distribution (Figure 4) and neutrality tests (Table 4), it can be inferred that the Korean *An. sinensis* may have expanded. The slight genetic differentiation observed in the SG may be attributed to an earlier expansion in the SG than in the NG, which provided time for the SG to differentiate.

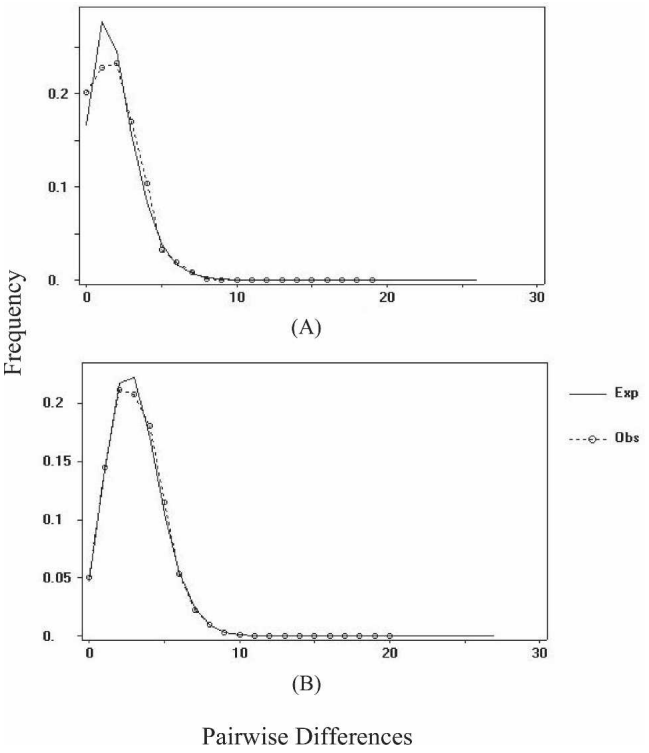

FIGURE 4. Distribution of the pairwise differences (dotted line) in all the individuals of the NG **A**, and SG **B**, in South Korea based on the 238-bp variable sequences in the mitochondrial control region. The expected values (solid line) were estimated according to the population expansion model.

The two groups of the Korean *An. sinensis* population possessed several differences in terms of genetic properties. First, the SG was more genetically diverse than the NG and comparison of the individual values of each population and the average values of each group revealed higher genetic diversity indices for the SG (Tables 1 and 4). Second, the SG contained a larger number of private alleles than the NG (Figure 3). Finally, the theta estimates were higher in the SG than in the NG; assuming an equal mutation rate, the above-mentioned differences indicate that the SG was made up of a relatively large effective population size. This larger effective population size may have resulted from various factors such as less fluctuation in the population size over time and/or less variance in reproductive success, which could be elucidated by further detailed studies.

In summary, the South Korean populations of *An. sinensis* are divided into two groups, i.e., the NG and SG, and the Taebaek and Sobaek mountain ranges may have played a major role in the genetic divergence observed. Compared with the low gene flow rate between the two groups, an extremely high gene flow rate was detected among the populations within each group. This may be attributed to a recent population expansion rather than the recurrence of gene flow. Furthermore, the SG was superior to the NG with regard to genetic diversity and theta estimates, and the various causative factors for this need to be elucidated.

Received April 10, 2006. Accepted for publication April 18, 2007.

Acknowledgments: The authors thank Dr. Yong-Jin Won (Ewha Womans University) and anonymous reviewers for valuable comments on this manuscript.

Financial support: This work was supported by Korea Research Foundation Grant KRF-2002-070-C00080 and the Korea Science and Engineering Foundation (R21-2005-000-10013-0).

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REFERENCES

1. Donnelly MJ, Twonson H, 2000. Evidence for extensive genetic differentiation among populations of the malaria vector *Anopheles arabiensis* eastern Africa. *Insect Mol Biol 9:* 357–367.
2. Lehmann T, Besansky NJ, Hawley WA, Fahey TG, Kamau L, Collins FH, 1997. Microgeographic structure of *Anopheles gambiae* in western Kenya based on mtDNA and microsatellite loci. *Mol Ecol 6:* 243–253.
3. Simard F, Lehmann T, Lemasson JJ, Diatta M, Fontenille D, 2000. Persistence of *Anopheles arabiensis* during the severe dry season conditions in Senegal: an indirect approach using microsatellite loci. *Insect Mol Biol 9:* 467–479.
4. Rongnoparut P, Sirichotpakorn N, Rattanarithiku R, Yaicharoen S, Linthicum KJ, 1999. Estimates of gene flow among *Anopheles maculatus* populations in Thailand using microsatellite analysis. *Am J Trop Med Hyg 60:* 508–515.
5. Lehmann T, Hawley WA, Grebert H, Danga M, Atieli F, Collins FH, 1999. The Rift Valley complex as a barrier to gene flow for *Anopheles gambiae* in Kenya. *J Hered 90:* 613–621.
6. Scarpassa VM, Tadei WP, Suarez MF, 1999. Population structure and genetic divergence in *Anopheles nuneztovari* (Diptera: Culicidae) from Brazil and Colombia. *Am J Trop Med Hyg 60:* 1010–1018.
7. Collins FH, Besansky NJ, 1994. Vector biology and control of malaria in Africa. *Science 264:* 1874–1875.
8. Jung J, Lee E, Kim W, 2006. Isolation and characterization of polymorphic microsatellite markers of *Anopheles sinensis*, a malaria vector mosquito in the East Asia region. *Mol Ecol Notes 6:* 1272–1274.
9. Ree H-I, 2005. Studies on *Anopheles sinensis*, the vector species of vivax malaria in Korea. *Korean J Parasitol 43:* 75–92.
10. Han E-T, Lee D-H, Park K-D, Seok W-S, Kim Y-S, Tsuboi T, Shin E-H, Chai J-Y, 2006. Reemerging vivax malaria: changing patterns of annual incidence and control programs in the republic of Korea. *Korean J Parasitol 44:* 285–294.
11. Sambrook J, Russel DW, 2001. *Molecular Cloning: A Laboratory Manual.* 3rd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
12. Caccone A, Garcia BA, Powelll JR, 1996. Evolution of the mitochondrial DNA control region in the Anopheles gambiae complex. *Insect Mol Biol 5:* 51–59.
13. Thompson JD, Gibson TJ, Plewniak F, Higgins DG, 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res 24:* 4876–4882.
14. Rojas JJ, Sanchez-DelBarrio JC, Messeguer X, Rozas R, 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics 19:* 2496–2497.
15. Nei M, 1987. *Molecular Evolutionary Genetics.* New York: Columbia University Press.
16. Tajima F, 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics 105:* 437–460.
17. Clement M, Posada D, Crandall KA, 2000. TCS: a computer program to estimate gene genealogies. *Mol Ecol 9:* 1657–1659.
18. Nei M, 1982. Evolution of human races at the gene level. Bonne-Tamir B, Cohen T, Goodman RM, eds. *Human Genetics, Part A: The Unfolding Genome.* New York: Alan R. Liss, 167–181.
19. Kumar S, Tamura K, Nei M, 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform 5:* 150–163.
20. Raymond M, Rousset F, 1995. An exact test for population differentiation. *Evolution Int J Org Evolution 49:* 1280–1283.
21. Excoffier L, Laval G, Schneider S, 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol Bioinform Online 1:* 47–50.
22. Weir BS, Cockerham CC, 1984. Estimating F-statistics for the analysis of population structure. *Evolution Int J Org Evolution 38:* 1358–1370.
23. Hudson RR, Slatkin M, Maddison WP, 1992. Estimation of levels of gene flow from DNA sequence data. *Genetics 132:* 583–589.
24. Mantel N, 1967. The detection of disease clustering and a generalized regression approach. *Cancer Res 27:* 209–220.
25. Slatkin M, 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics 139:* 457–462.
26. Rogers AR, 1995. Genetic evidence for Pleistocene population explosion. *Evolution Int J Org Evolution 49:* 608–615.
27. Harpending RC, 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Hum Biol 66:* 591–600.
28. Tajima F, 1989. The effect of change in population size on DNA polymorphism. *Genetics 123:* 597–601.
29. Fu Y-X, 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics 147:* 915–925.
30. Besansky NJ, Lehmann T, Fahey GT, Fontenille D, Braack LEO, Hawley WA, Collins FH, 1997. Patterns of mitochondrial variation within and between African malaria vectors, *Anopheles gambiae* and *An. arabiensis*, suggest extensive gene flow. *Genetics 147:* 1817–1828.
31. Cho S-H, Lee H-W, Shin E-H, Lee H-I, Lee W-G, Kim C-H, Kim J-T, Lee J-S, Lee W-J, Jung G-G, Kim T-S, 2002. A mark-release-recapture experiment with *Anopheles sinensis* in the northern part of Gyeonggi-do, Korea. *Korean J Parasitol 40:* 139–148.
32. Fighner BH, Pak SI, Novakoski WL, Kelsey LL, Strickman D, 1998. Reemergence of Plasmodium vivax malaria in the Republic of Korea. *Emerg Infect Dis 4:* 295–297.