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GENETIC RELATIONSHIPS AMONG POPULATIONS OF *AEDES AEGYPTI* IN TAIWAN BY USING PHENOTYPIC AND RANDOM AMPLIFIED DNA–POLYMERASE CHAIN REACTION MARKERS

YU-CHENG SU,[1] CHI-YI CHANG,[2] ERR-LIEH HSU,[1] CHIH-MING YIN[3] AND CHAU-MEI HO[2,4]

ABSTRACT. An analysis of gene flow was conducted among collections of *Aedes aegypti* from 7 localities along the southwestern and southeastern coasts in Taiwan. Markers include 7 types of scaling patterns and 23 random amplified polymorphic DNA (RAPD) loci amplified by the polymerase chain reaction. Differences in scaling pattern and in the frequencies of RAPD markers were detected among populations and cluster analyses revealed 2 main groups on each side of the Central Mountain Range. Regression analysis of geographic distances and pairwise F_{ST} values estimated from RAPD markers showed that southwestern populations are isolated by distance and that populations within 15 km are panmictic. This is a shorter distance than detected among collections of *Ae. aegypti* in similar published studies from Mexico and Argentina.

KEY WORDS *Aedes aegypti,* scaling pattern, gene flow, F_{ST}, isolation by distance, Taiwan

INTRODUCTION

Taiwan consists of the main island of Taiwan and the Pescadores island group, which contains 64 small islands. The main island is situated between 120° and 122°E. It is 394 km long and 144 km wide at the broadest part, and is cut into almost equal halves, north and south, by the Tropic of Cancer (Fig. 1). Taiwan is bounded on the west by the Taiwan Strait, on the northeast by the East China Sea, on the east by the Pacific Ocean, and on the southwest by the South China Sea. The main Central Mountain Range stretches from the northeastern corner to the southern tip of the island and has many peaks >3,600 m high. Toward the west, the land descends in a succession of terraced fields for tillage. The Taitung Rift Valley, lying on the eastern side of the Central Mountain Range, creates a narrow trough from Hualien to Taitung. Both ranges sharply drop along the coast of the Pacific Ocean, resulting in limited areas of level land in the eastern part of Taiwan. Administratively, the main island includes 2 national cities (Taipei and Kaohsiung), 16 counties, and 331 townships.

The mosquito *Aedes aegypti* L. is a primary vector for dengue fever viruses in Taiwan and other tropical areas. The earliest record of *Ae. aegypti* in Taiwan is in 1901. This species presumably was transported to Taiwan by humans from other Pacific islands (Rosen et al. 1948). Since then, this mosquito has become widely distributed in areas south of 24°50′N, but is more often found in harbors along the coast than in the inland towns and villages of Nantou, Yugen, Guanmau, and Pescadores. In 1954, an antimalaria campaign with island-wide DDT applications was implemented. This campaign also greatly reduced the densities of *Ae. aegypti* and it became limited mostly to the harbors such as Kaohsiung, Tainan, Pingtung, and Pescadores. The use of DDT was banned in 1965 when malaria was eradicated in Taiwan and *Ae. aegypti* gradually reexpanded from harbors to the villages, but remained in the southern areas, between 23°50′N and 22°50′N. The northern and southern boundaries are at Budai in Chiayi County and Linbian in Pingtung County, respectively (Lien 1988, Teng et al. 1996). In the winter of 1987, outbreaks of dengue fever occurred in 3 cities (Tainan, Kaohsiung, and Pingtung) and their neighboring villages. Dengue virus also was isolated from field-collected adults of *Ae. aegypti* (Huang 1991). We found that this mosquito expanded to Taitung, a southeastern harbor, in December 1999.

In southern Taiwan, the density of *Ae. aegypti* in each city fluctuates with local rainfall and dispersal occurs by active flight and passive transportation of eggs, larvae, and adults in various containers along commerce routes (Tabachnick 1991, Reiter et al. 1995). Recent studies have shown that gene flow occurs among populations of *Ae. aegypti* within 90–250 km in northeastern Mexico (Gorrochotegui-Escalante et al. 2000, 2002). Theoretically, the short distances between cities in Taiwan should increase the gene flow between populations, and, thus, the level of genetic variation among cities probably should be much lower in Taiwan. Nevertheless, a high density of residences in the urban areas, intensive control campaign activities that may affect the sizes of the mosquito population, as well as special environmental barriers also may change the level of genetic variation among cities. In addition, this mosquito may occasionally be reintroduced through fishing boats and ships from other countries in Southeast Asia. To better understand the actions of these possibilities, we need to collect certain morphological data with a genetic

[1] Department of Entomology, National Taiwan University, 27 Lane 113, Sec. 4, Roosavelt Road, Taipei, Taiwan, 106.

[2] Department of Parasitology, National Yang-Ming University, 115 Sec. 2, Li-Nung Street, Shih-Pai, Taipei, Taiwan, 112.

[3] Department of Entomology, 102 Fernald Hall, 207 Stockbridge Road, University of Massachusetts, Amhearst, MA 01003.

[4] To whom correspondence should be addressed.

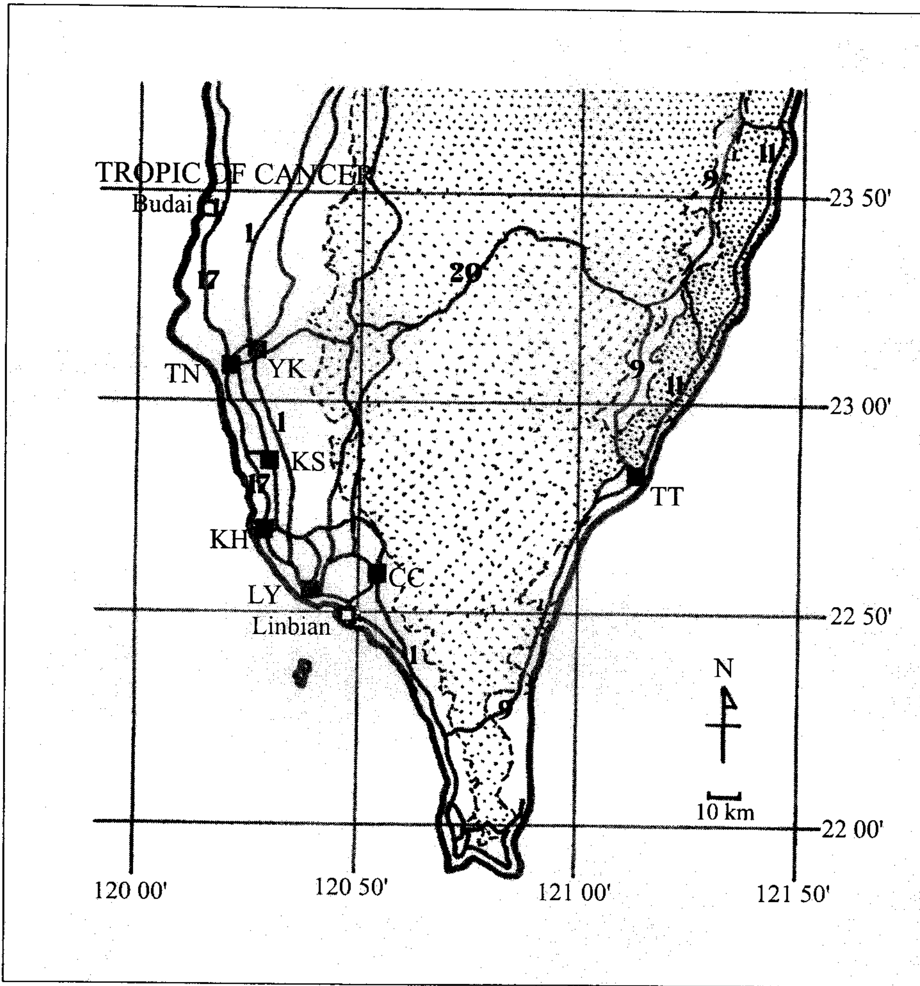

Fig. 1. Map of collection locations of *Aedes aegypti* in Taiwan: CC, Chaochou; KH, Kaohsiung; KS, Kangshan; LY, Liyuan; TN, Tainan; TT, Taitung; YK, Yungkang. The shaded parts in the middle and right side are the Central Mountain Range and East Coast Range, respectively. Taitung Rift Valley lies between these 2 ranges. The main roads between each collection are also indicated. The number beside the main roads indicate the road number.

basis or other genetic data on the natural populations in Taiwan.

Phenotypic characteristics of *Ae. aegypti* initially were used by Mattingly (1957) to differentiate populations. He found that the abdominal scale patterns of adult *Ae. aegypti* are highly variable, and recognized 3 subspecies in *Ae. aegypti. Aedes aegypti aegypti* has pale scales on the 1st tergite, *Ae. aegypti formosus* has markedly black scales on the 1st tergite, and *Ae. aegypti queenslandensis* has increased white scales beyond the 1st abdominal tergite or has a lighter mesonotal color. Mattingly (1957) also stressed that this classification system was not completely constant within distinct populations of *Ae. aegypti.* McClelland (1960) initially studied the genetics of abdominal variation by using selective mating and proposed a multiple factorial mode of inheritance for *Ae. aegypti.* He also described a system for classifying *Ae. aegypti* based on the number of abdominal tergites that have a continuous medial band of white scales from the apical to the distal end. McClelland (1974) developed a pattern value method in which he arbitrarily assigned a letter (F, G, H, J, K, L, M, N, O, P, or Q) to denote 11 color grades, and expanded the system by adding a digit to each letter to indicate the number of tergites with white scales. However, when Hartberg et al. (1986) investigated the genetic basis of scale patterns, they suggested that the number of abdominal tergites with extra white scales (excluding white scales in the basal band and lateral bands) was a better criterion for classification than the grading method of McClelland (1974). Two laboratory strains selected by C. K. Meeks, CARN-LIGHT and CARN-DARK, were used in their study. They formulated the CKM system (given the

Table 1. Collection location of the natural populations of Taiwanese *Aedes aegypti* used in this study for CKM analysis.[1]

City (code)	Date	Latitude	Longitude	No. individuals	
				Male	Female
Tainan	Dec. 3, 1998	22°59′54″N	120°12′07″E	22	18
(TN)	July 14, 1999	22°58′25″N	120°11′02″E	10	15
	Sept. 25, 1999	22°59′20″N	120°11′41″E	14	13
	April 7, 2000	22°58′30″N	120°11′49″E	20	15
Yungkang	July 9, 1998	23°00′20″N	120°13′20″E	14	15
(YK)	April 8, 2000	22°00′05″N	120°15′57″E	15	11
	April 9, 2000	22°00′07″N	120°14′57″E	14	13
Kangshan	July 8, 1998	22°47′47″N	120°17′59″E	5	2
(KS)	July 14, 1998	22°47′47″N	120°17′59″E	3	4
	Sept. 25, 1999	22°47′47″N	120°17′59″E	7	2
	April 4, 2000	22°47′45″N	120°17′04″E	4	2
Kaohsiung	July 8, 1998	22°38′00″N	120°17′56″E	26	12
(KH)	Aug. 25, 1998	22°37′04″N	120°18′19″E	30	15
	July 25, 1999	22°38′00″N	120°17′56″E	23	12
	April 8, 2000	22°38′38″N	120°19′16″E	25	10
Liyuan	July 14, 1998	22°30′09″N	120°22′14″E	35	31
(LY)	Nov. 15, 1998	22°30′09″N	120°22′14″E	29	28
	Sept. 25, 1999	22°30′20″N	120°23′32″E	36	32
	April 7, 2000	22°30′20″N	120°23′32″E	35	27
Chaochou	Nov. 15, 1998	22°30′09″N	120°32′56″E	17	15
(CC)	July 14, 1999	22°29′41″N	120°33′02″E	15	13
	Sept. 25, 1999	22°29′41″N	120°33′02″E	12	13
	April 7, 2000	22°32′50″N	120°32′56″E	16	13
Taitung	May 23, 1999	22°45′15″N	121°09′28″E	30	33
(TT)	July 22, 2000	22°45′15″N	121°09′28″E	49	40
	July 22, 2000	22°45′20″N	121°09′24″E	51	45
	July 22, 2000	22°45′40″N	121°08′34″E	23	27
Total				580	476

[1] A system of classification by and named for C. K. Meeks.

name after C. K. Meeks) for classification. In their CKM system, they considered only the number of abdominal tergites with extra white scales, and disregarded the extent of the white scales on the tergites. In the same study, they also proposed that the abdominal tergal scale pattern is controlled by a polygenic system consisted of 3 loci, tergites white locus (Tw^+/Tw), white scaling locus ($L_1/L_2/l$), and a white intensifier locus (I/i). By applying the CKM system, an adult *Ae. aegypti* can be simply categorized in 1 of the 8 classes based on the number of tergites with extra white scales except in the basal band and lateral spots. Class 0 has no extra white scales in all abdominal tergites. Class 1 adults have extra white scales only on their 1st abdominal tergite. Class 2 adults have extra white scales on the 1st and 2nd tergites. This pattern of classification continues to class 7, in which extra white scales are found on the 1st through the 7th abdominal tergites.

Random amplified polymorphic DNA amplified by polymerase chain reaction (RAPD-PCR) is an efficient technique to generate various genetic markers for the study of population differentiation (Williams et al. 1991). These markers have been used for identifying the origin of infestations and the amount of genetic variation among populations of *Ae. aegypti* from Africa, Australia, and the Americas (Ballinger-Crabtree et al. 1992) as well as natural populations in Mexico and Argentina (Gorrochotegui-Escalante et al. 2000, de Sousa et al. 2001). Apostol et al. (1996) also used RAPD markers to analyze the breeding structure in populations of *Ae. aegypti* from Puerto Rico.

In the present study, we applied 2 kinds of measures, the frequencies of scale pattern and 23 RAPD loci, as genetic markers to estimate the levels of intraspecific polymorphism and genetic relatedness of natural populations of *Ae. aegypti* in Taiwan. The results will aid us in predicting patterns of spread of indigenous populations and in evaluating the effectiveness of control strategies.

MATERIALS AND METHODS

Mosquito collection: Approximately 1,500 eggs of *Ae. aegypti* were collected from 49 ovitraps placed either outside or indoors in 7 areas in southern Taiwan as shown on the map (solid square [■] in Fig. 1). Trapped eggs were taken back to the laboratory, where the eggs hatched after 3–5 days. Larvae were reared in tap water at 27 ± 1°C, and fed a suspension of dried liver and ground rabbit chow. Pupae were collected and allowed to emerge.

Examination of abdominal scale pattern: A total

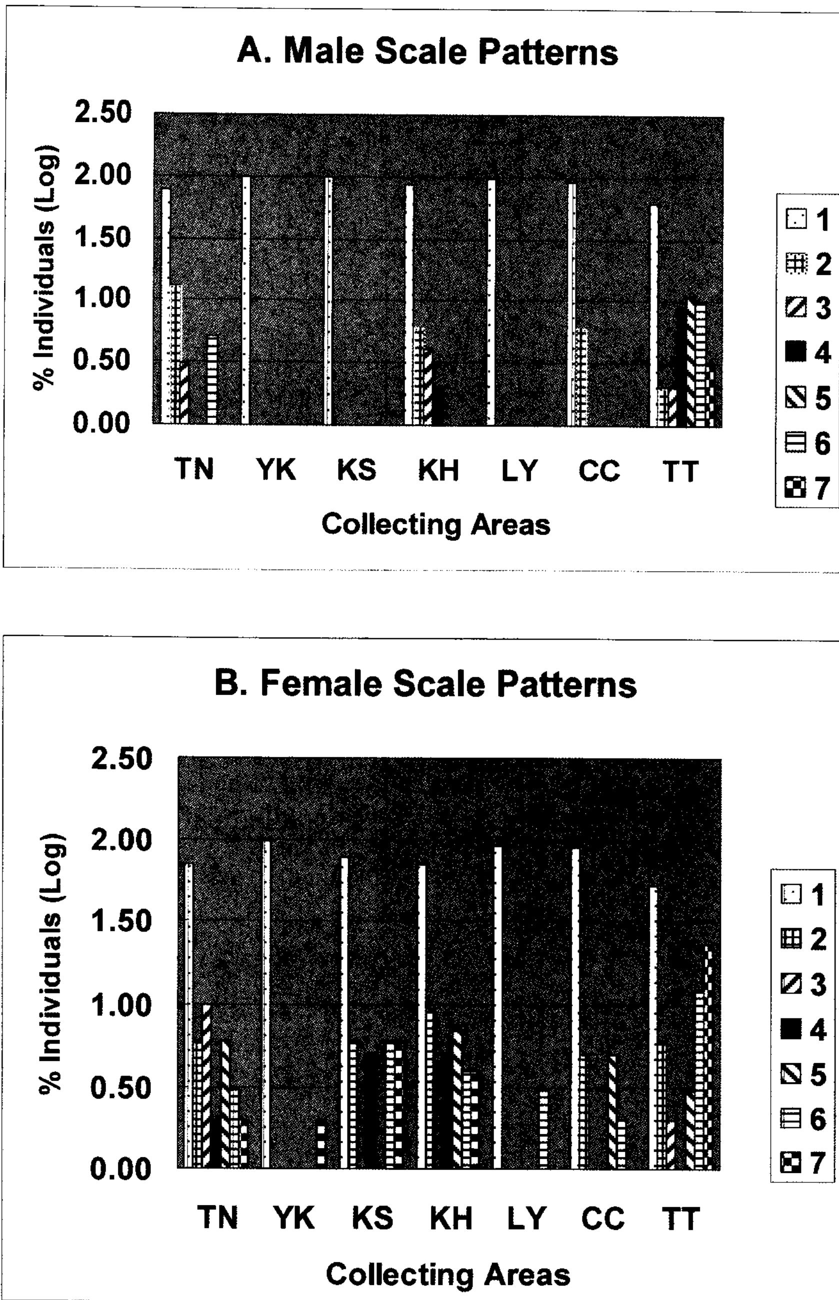

Fig. 2. Scale patterns of *Aedes aegypti* from 7 collection locations in Taiwan in males (A) and females (B). The CKM values, developed by C. K. Meeks, are in Arabic numerals (1–7). TN, Tainan; YK, Yungkang; KS, Kangshan; KH, Kaohsiung; LY, Liyuan; CC, Chaochou; TT, Taitung.

of 1,056 adults were graded for abdominal tergal scale pattern (Table 1). This pattern was graded within 24 h of emergence. Each adult was placed on a glass plate kept at a low temperature by ice. The immobilized mosquito was placed under a stereomicroscope. If the wings blocked the view, a needle was used to move them out of the way and the individuals were classified according to the CKM system described in the Introduction.

DNA preparation: For each location, 20–25 mosquitoes were randomly selected for DNA extraction. The genomic DNA was extracted from single adults (Ballinger-Crabtree et al. 1992). A mosquito was frozen at −70°C and homogenized in

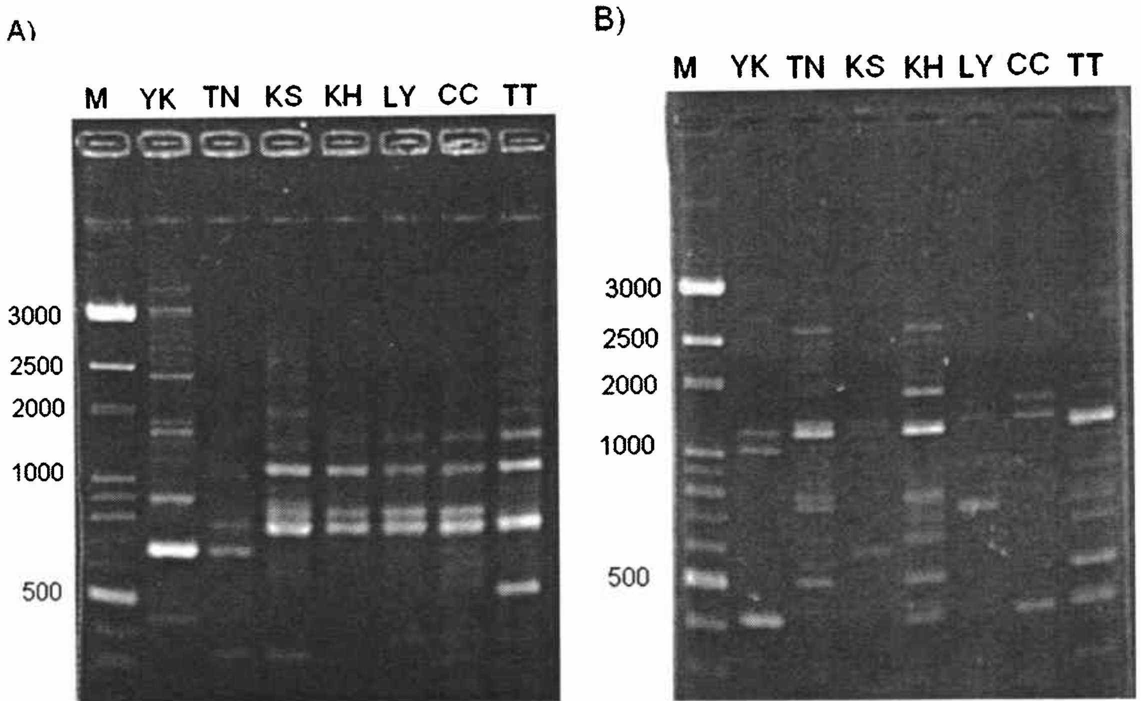

Fig. 3. Examples of random amplified polymorphic DNA markers amplified with primer A2 (A) and primer B3 (B). Lane 1, 100-base pair markers; lanes 2–8, *Aedes aegypti* from different Taiwanese populations. YK, Yungkang; TN, Tainan; KS, Kangshan; KH, Kaohsiung; LY, Liyuan; CC, Chaochou; TT, Taitung.

a microfuge tube containing 300 μl of lysis buffer (100 mM Tris-HCl, pH 8.0, 50 mM NaCl, 50 mM ethylenediaminetetraacetic acid [EDTA], 1% sodium dodecyl sulfate). After adding 2 μl of proteinase K (20 mg/ml) and 1 μl of RNase (10 mg/ml), the suspension was incubated for 2 h at 50°C. Sodium acetate (pH 5.2) was added to the aqueous phase after extraction with Tris-saturated phenol (pH 8.0) and chloroform–iodoacetic acid (IAA) (24:1). The DNA was then precipitated with 95% ethanol (Sambrook et al. 1989). After washing and Speedvac (Savant, Farmingdale, NY) drying, the DNA was resuspended in 20 μl Tris-HCl (10 mM, pH 8.0) and EDTA (1 mM, pH 8.0) and stored at 4°C and DNA concentrations were determined spectrophotometrically.

RAPD-PCR: The working solutions of DNA were prepared at a concentration of 17 ng/μl in sterile water before use for PCR. All primers used were random 10-base oligomers, with guanylic acid and cytidylic acid (GC) content over 70%, synthesized by a local manufacturer (DNAFax, Taipei, Taiwan). Each primer was resuspended at a concentration of 15 ng/μl in sterile water and stored at −20°C. Amplification reactions were performed according to a protocol modified from Williams et al. (1990). Each 25-μl reaction contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.002% gelatin, and 0.1 mM each of deoxynucleoside triphosphates (Promega, Madison, WI), 15 ng of primer, 0.5 units of Taq DNA polymerase (HT Biotechnology LTD, Cambridge, United Kingdom), and 17 ng of mosquito genomic DNA. Each reaction was overlaid with 50 μl of mineral oil and amplified in a temperature cycler (Hybaid, Middlesex, United Kingdom). The program was as follows: 1 cycle at 94°C for 5 min, 45 cycles at 94°C for 30 sec, 36°C for 1 min, 72°C for 2 min, and 1 cycle at 72°C for 5 min.

Electrophoresis: Amplified DNA was analyzed by electrophoresis on 1.5% 7 × 10-cm agarose gels. The 1st lane of each gel contained 100-base pair (bp) DNA ladders (Bayou Biolab, Harahan, LA) as markers in 1 × Tris-borate-EDTA buffer. Electrophoresis was run for 3 h at 50 V. Gels were stained with ethidium bromide and photographed on an ultraviolet transilluminator. Gel photographs were digitized by using the software AABI system (Advanced American Biotech Imaging, Fullerton, CA), which also estimated the sizes for each amplified fragment. Fragments were selected, and size ranges were determined for each selected fragment by comparing calculated sizes for the same band on several different gels. The presence or absence of fragments with a size ranging from 500 to 2,000 bp for each individual was recorded.

Data analysis for RAPD-PCR markers: Band presence and absence were converted to an input file for RAPDPLOT, a Fortran program for analysis of RAPD-PCR data (Black 1997). In this program,

the distances were measured by similarity and percentage of match, and 100 bootstrap replications were performed to obtain distance matrices. These matrices then were analyzed by the programs NEIGHBOR and CONSENSUS in PHYLIP3.5c (Felsenstein 1993) to create a tree with bootstrapping values.

The RAPD-PCR markers were analyzed as genetic markers with the following assumptions: RAPD markers segregate in a Mendelian fashion, genotype frequencies of RAPD loci are in Hardy–Weinberg populations, and recessive band-absent and dominant band-present alleles are identical among and within individuals. Variation in RAPD allele frequencies within and among populations also was examined by using hierarchical analysis in Arlequin ver. 2.000 (Schneider et al. 2000). The programs RAPDDIST and RAPDFST (Black 1997) were used to compute pairwise F_{ST} values, as a standardized measure of variation in allele frequencies among populations. Effective migration rates (*Nm*) also were estimated from F_{ST} ($Nm = (1 - F_{ST})/4F_{ST}$) by assuming an island model of migration among populations (equation 12 in Apostol et al. 1996). Pairwise F_{ST} values were transformed to $F_{ST}/(1 - F_{ST})$ and used to construct a consensus tree among collections with the unweighted pair group method with arithmetic averaging analysis (UPGMA) in PHYLIP 3.5c for testing the consistency of clustering analyses.

Geographic distances were obtained from Easy Road software (1998, ver. 1.52, Systec Company, Taipei, Taiwan). The $F_{ST}/(1 - F_{ST})$ distances were regressed on pairwise geographic distances among populations to test whether distance serves as a barrier to gene flow. This regression was repeated by using a natural logarithm transformation of geographic distance. Transformation, regression analysis, and the Mantel test were performed with MANTEL (Black 1997). The reciprocal of the slope estimated provided an estimate of the average effective population size.

RESULTS

Phenotypic markers

The scaling patterns of field-collected *Ae. aegypti* are shown in Fig. 2. Except for the populations of Liyuan (LY) and Yungkang (YK), the female mosquitoes of other populations exhibited greater variation in scale pattern than males from the same population. This sex-related phenomenon was similar to that reported by others (Mattingly 1957; McClelland 1960; Mogi et al. 1984, 1989; Hartberg et al. 1986; Jupp et al. 1991; Duhrkopf et al. 1993). For each population, the frequency of female CKM values was used in cluster analyses that generated dendrograms (data not shown) depicting the similarity of different populations. The 7 populations assorted into 2 clusters that correspond geographically with southwestern and southeastern areas in Taiwan. The 1st cluster could be classified into 2 groups. Group 1 included YK, Chaochou (CC), and LY, in which more than 90% of females were CKM1 and others ranged from CKM2 to CKM7. Group 2 included 3 populations, Kaohsiung (KH), Kangshan (KS), and Tainan (TN), in which 70–80% of females were CKM1. The 2nd cluster included only the Taitung (TT) population, in which about one half (53%) of the individuals were CKM1, whereas 10% were CKM2 to CKM5 and 37% were CKM6 and CKM7. Because the linear geographic distances from Taitung to other locations is longer than among other cities, these morphological results might indicate that the amount of genetic variation was correlated with geographic distance. We also built dendrograms based on the male scale patterns (data not shown) and these again suggested that the TT population was distantly related to the other 6 populations. Males from these 6 populations formed a single cluster and it appeared that the dendrogram based upon female scale patterns was better resolved.

The correlation of scaling patterns of adults with the egg laying sites also was analyzed. No significant differences were found between mosquitoes from outdoor and indoor sites. However, mosquitoes with CKM4 to CKM7 were collected outdoors more frequently than indoors.

RAPD-PCR markers

Random amplified DNA polymorphisms for 7 populations were studied (Fig. 3). Certain DNA fragments were excluded for clustering analysis based on 2 criteria: fragments occurring at frequencies lower than 60% in 1 or more populations were not used in differentiating the populations and fragments that varied between females and males within a population also were discarded. Clustering analyses were based on the percentage of match for individual phenotype (Fig. 4). Results indicated that individual differences occurred in each location (represented as a lowercase letter after the name of each population, e.g., KHa and KHb in Fig. 4). However, individuals of the TT population were strongly differentiated from all of other populations (bootstrap support = 77%). Collections from TN and YK were closely related (bootstrap support = 71%). Although individuals from KH, LY, and CC showed various phenotypes, they still clustered in a group. The similarity analysis results were consistent with those derived from analysis of percentage match (data not shown).

The RAPD-PCR data were transformed into the measures that included standard indices, molecular diversity, analysis of variance, pairwise genetic distances, and exact test of population differentiation in Arlequin ver. 2000. The frequencies of the dominant RAPD allele at each of 23 loci were subject to a hierarchical analysis to estimate the variance

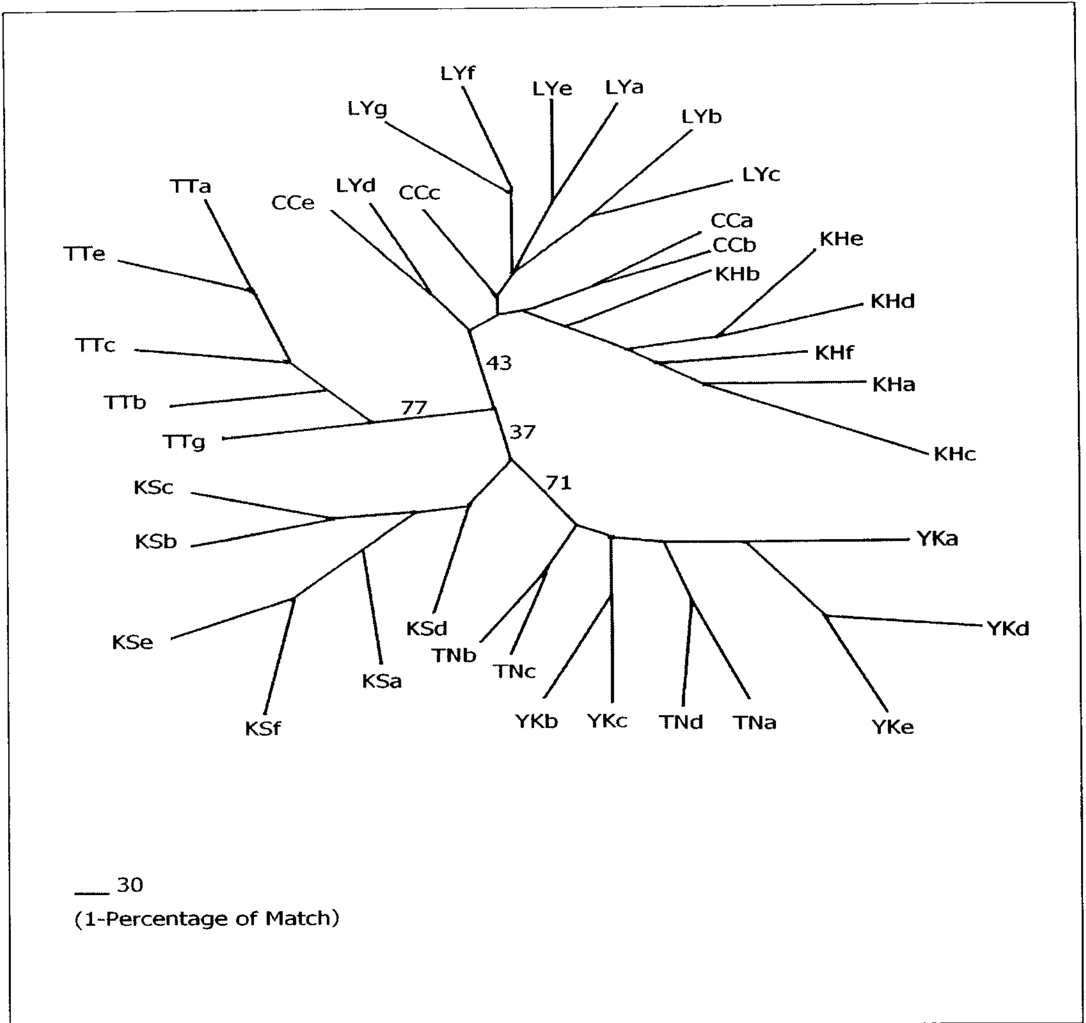

Fig. 4. Cluster analysis of *Aedes aegypti* from 7 locations in Taiwan. Distances were estimated based on the percentages of matched bands with the unweighted pair-group arithmetic average method (UPGMA). Small letters after the name of each location designate different random amplified polymorphic DNA patterns. Numbers above branches indicate the bootstrap support. CC, Chaochou; KH, Kaohsiung; KS, Kangshan; LY, Liyuan; TN, Tainan; TT, Taitung; YK, Yungkang.

among populations (Table 2). Approximately 84% of the total variance was among populations, whereas 16% of the variation arose among collections from different locations. The differentiation test between all pairs of samples indicated that TT was different from other populations at a significance level of 0.05 (data not shown).

Pairwise F_{ST} values were used to examine relationships among our collections. Pairwise $F_{ST}/(1 - F_{ST})$ among collections were regressed against geographic distances (Fig. 5). The $F_{ST}/(1 - F_{ST})$ dendrogram shows that TN, YK, and KS form 1 cluster, whereas KA, LY, and CC form another cluster. However, these collections did not cluster with those from TT, which appeared to be unique.

Pairwise $F_{ST}/(1 - F_{ST})$ among collections were regressed against geographic distance (Fig. 6A) and the natural logarithm of the geographic distances (Fig. 6B) to test whether gene flow among collections is correlated with geographic distances. Results indicated that the level of genetic differences and the geographic distances are positively correlated. The genetic distance became large at a geographic distance greater than 2.7 km (Fig. 6B). This indicated that population become reproductively isolated at distance of ~15 km ($e^{2.7}$). The average effective population size was at ~1 mosquito/km (1 mosquito/0.997 km).

DISCUSSION

In the present study, the frequencies of scaling patterns provided limited information about popu-

Table 2. Partitioning of variation in the frequency of genetic markers among collections of *Aedes aegypti* in southern Taiwan. Hierarchical analysis of random amplified polymorphic DNA allele frequencies among collections and among cities.[1]

Source of variation	df	Sum of squares	Variance components	% of variation
Among cities	6	418.412	3.3405	84.32
Within cities	138	85.740	0.6213	15.68
Total	144	504.152		
Fixation index	F_{ST}: 0.8432	(Nm^2 = 0.0465)		

[1] Significance tests: 1,023 permutations. Variation and F_{ST}: P (random value > observed value) = 0.00000; P (random value = observed value) = 0.00000; P (random value < observed value) = 0.00000 + 0.00000.
[2] *Nm*, effective migration rate.

lation diversity. Even so, our results indicate that *Ae. aegypti* in Taiwan consists of mixed forms of *Ae. aegypti aegypti.* The southeastern population (TT) appears to be distantly related to the 6 southwestern populations. However, the dendrogram was not capable of inferring the relationships between phenotypic frequencies and distances among certain localities, such as the YK, CC, and LY. This method was time consuming and there was a problem with missing scales when specimens were older. Among these adults, only females were useful because their scales are more distinguishable than those of males. These above concerns disallowed many samples from our analyses. Moreover, we found that the scaling pattern is inconstant from generation to generation. When TT mosquitoes were maintained in the laboratory for 3 generations, the percentage of CKM7 in the laboratory colony decreased from 54 to 27%. This observation suggested that in nature the individuals having scales of CKM7 may be adaptive to certain unknown factors. Because the abdominal scale pattern in *Ae. aegypti* appears to be controlled by a polygenic system with modifiers (Hartberg et al. 1986), it is difficult to analyze the breeding structure. On the basis of our analyses and observations, we suggest that the scaling pattern of *Ae. aegypti* may be useful as one way for identifying population diversity but inadequate for population genetics.

In contrast, RAPD markers gave better resolution

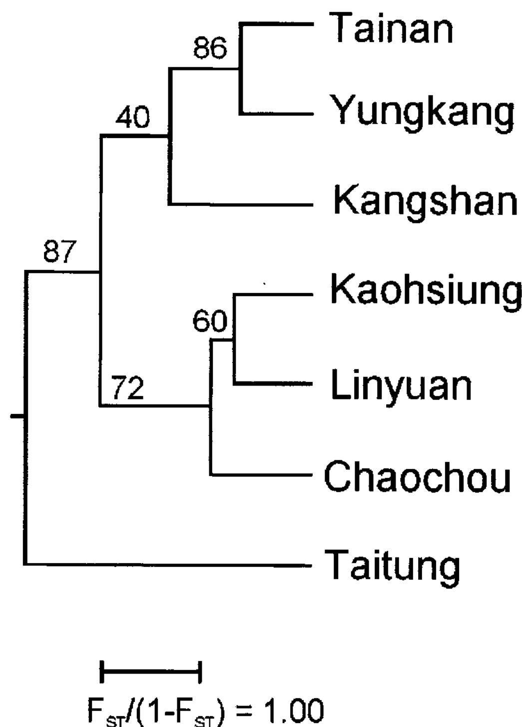

Fig. 5. Relationships among locations by cluster analysis of pairwise $F_{ST}/(1 - F_{ST})$ derived from random amplified polymorphic DNA markers with the unweighted pair-group arithmetic average method (UPGMA). Numbers above branches indicate the bootstrap support.

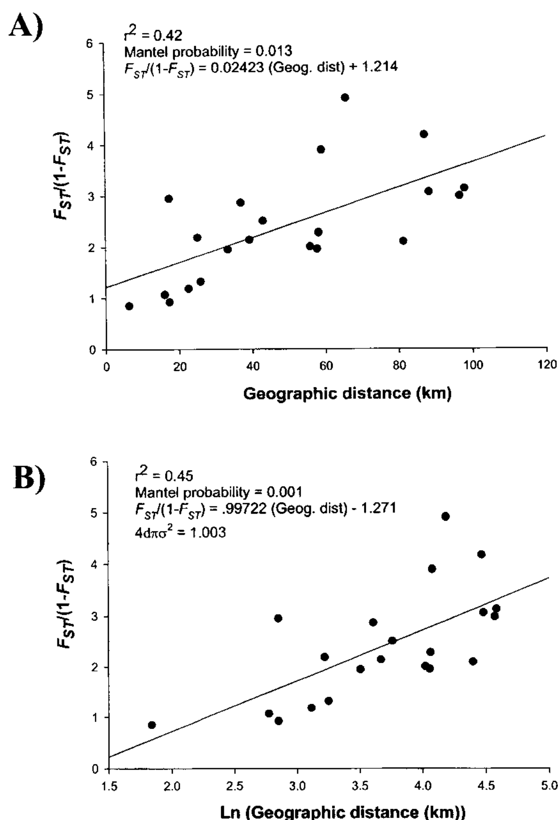

Fig. 6. (A) Regression analysis of pairwise $F_{ST}/(1 - F_{ST})$ regressed on pairwise geographic distances between collections. (B) Regression analysis of pairwise $F_{ST}/(1 - F_{ST})$ regressed on pairwise natural logarithm–transformed geographic distances.

of population. The amount of genetic divergence among populations was positively correlated with geographic distance. Furthermore, limited gene flow was detected among southwestern populations. Teng et al. (1996) reported that larval *Ae. aegypti* grow more frequently in plastic than other types of water containers in Taiwan and that the distribution of this species correlates with distances among houses. Specifically, with a higher density of houses, breeding containers with larval *Ae. aegypti* were more numerous. The dispersal of female *Ae. aegypti* is driven by the location and availability of oviposition sites (Reiter et al. 1995, Apostol et al. 1996). However, the dispersal rates and distance are inversely correlated with the abundance of oviposition sites (Edman et al. 1998).

In this study, the linear geographic distances of our sampling sites ranged from 200 to 150 km. Our results contrast with those of Gorrochotegui-Escalante et al. (2000, 2002) who estimated that for *Ae. aegypti* in Mexico, the genetic isolation distance and average effective population size were 90–250 km and 10–20 mosquitoes/km. The distances over which *Ae. aegypti* was panmictic therefore were much shorter in Taiwan. We believe that the frequency of source reduction may account in large part for the small effective population size, effective migration rate, and geographic range of panmixia. During our study, official survey records indicated that the Breteau indices of *Aedes* ranged from 0 to 2 in most of southern Taiwan, including our collecting sites. The small number of mosquitoes in each city may create high levels of population differentiation because of founder's effects and genetic drift.

Taitung collections had higher homogeneity and were genetically differentiated from other southwestern collections. A similar trend was seen with the Nuevo Laredo collection of *Ae. aegypti* in northeastern Mexico (Gorrochotegui-Escalante et al. 2002). We suggest the following possible explanation for our results. First, the linear geographic distance between TT and other localities is longer than the panmictic distance (15 km; Fig. 1). Second, the Central Mountain Range could serve as an important barrier to the gene flow for *Ae. aegypti.* As indicated in Fig. 1, 2 main highways (9 and 20) connect the southwestern and southeastern cities. Highway 20 is a shortcut route, which features many recreation spots of hot springs and villages of aborigines. Because the Central Mountain Range in southern areas has altitudes of 1,000–3,000 m high, the average winter temperature is much lower than that of areas closer to sea level. Such temperatures are suboptimal for growth and reproduction of *Ae. aegypti.* In contrast, highway 9 is at 300–500 m but has much less traffic than other routes. This lower utilization decreases the transfer opportunities for eggs and larvae of *Ae. aegypti* in artificial containers from west to east. In short, both highways are not optimal for the dispersal of *Ae. aegypti.*

These results have 2 implications for vector control. First, a high rate of gene flow occurs among populations of *Ae. aegypti* in the southwestern regions of Taiwan. This would increase the rate of flow of genes influencing dengue virus susceptibility and insecticide resistance. Second, in southeastern Taiwan, selection appears to be a major driving force to the dispersal of *Ae. aegypti* in this area. This would be interesting to study in more depth.

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