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Genetic Differentiation of the Giant Honey Bee (*Apis dorsata*) in Thailand Analyzed by Mitochondrial Genes and Microsatellites

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Genetic diversity and population differentiation of the giant honey bee (Apis dorsata) *in Thailand were examined. Six PCR-RFLP mitotypes were generated from digestion of the* COI-COII, Cytb-tRNA^{ser}, ATPase6-8, *and* lrRNA *genes with* Dra *I and* Hin *fI. Low genetic diversity (*$h = 0.074$, $\pi = 0.032\%$*) and a lack of genetic population differentiation between A. dorsata originating from geographically different regions were observed from mtDNA polymorphisms (*$P > 0.05$*). In contrast, microsatellite (*A14, A24, *and* A88*) polymorphisms revealed a relatively high level of genetic diversity in* A. dorsata *(*$H_o = 0.68–0.74$*, average number of alleles per locus* $= 6.0–9.0$*). Both* A24 *and* A88 *indicated significant population differentiation between bees from the north-to-central region (north, northeast, and central regions), peninsular Thailand, and Samui Island.*

KEY WORDS: *Apis dorsata*; honey bee; genetic diversity; mtDNA; microsatellites.

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INTRODUCTION

The giant honey bee (*A. dorsata*) is distributed over vast geographic areas in Southeast Asia and is found throughout Thailand. On the basis of morphology, this species can be further classified into three subspecies (*A. dorsata dorsata*, *A. dorsata binghami*, and *A. dorsata breviligula*). In addition, Sakagami *et al.* (1980) recognized the Himalayan giant honey bee as *A. laboriosa*. The giant honey bee plays an important role as the potential pollinator of natural plants (Thapa and Wongsiri, 1997; Wongsiri *et al.*, 2001).

Colony aggregation is a unique characteristic of this taxon. Preferential nesting sites may be shared by more than 150 colonies (Paar *et al.*, 2004). Seasonal migration of 100–200 km is a common behavior of *A. dorsata*, and queens appear to return to their previously occupied sites (Koeniger and Koeniger, 1980; Dyer and Seeley, 1994). Home-site fidelity was recently reported in *A. dorsata* based on microsatellite analysis (*A14*, *A76*, and *A88*; Neumann *et al.*, 2000). Moreover, Paar *et al.* (2000) estimated, based on inferred microsatellite genotypes (*A14*, *A76*, *A88*, and *A107*) of queens heading colonies at two sites in India, that the probability that different queens could have the same genotype by chance alone was approximately 2.24×10^{-5}–6.06×10^{-8}.

In Thailand, colonies of *A. dorsata* reside at the nesting sites between November and April. After April, during the rainy season, *A. dorsata* migrates to forest areas. Newly arrived swarms in the subsequent dry season form colonies, forage, and reproduce until the beginning of the rainy season (May–June) (Wongsiri *et al.*, 1996).

A temporal genetic structure of drone congregation areas (DCA) of *A. dorsata* over an 8-day time window was reported based on microsatellite analysis (*A14*, *A76*, and *A88*). A significant genetic differentiation between 3 sampling days was observed and indicated that the DCA was occupied by at least two subpopulations on all days in varying proportions. The overall effective population size (N_e) was estimated to be as high as 140 (Kraus *et al.*, 2005).

Among honey bee species indigenous to Thailand (*A. cerana*, *A. florea*, *A. andreniformis*, and *A. dorsata*), genetic diversity and population differentiation are well studied only in *A. cerana* using mtDNA (Deowanish *et al.*, 1996; Sihanuntavong *et al.*, 1999; Sittipraneed *et al.*, 2001b) and microsatellite polymorphisms (Sittipraneed *et al.*, 2001a). Knowledge of genetic variation of *A. dorsata* in Thailand is important for the conservation of this species. However, information about intraspecific genetic variability of Thai *A. dorsata* is not available at present.

The local medicinal belief is that *A. dorsata* honey is superior in quality to the honey of other bee species. As a result, a large number of *A. dorsata* colonies are destroyed. In addition, deforestation is a major problem, causing destruction and fragmentation of *A. dorsata* habitat. Appropriate genetic markers can be used to elevate management efficiency of important natural resource species. The

sustainable conservation of these species requires basic knowledge of their genetic population structure and the use of suitable molecular genetic markers to establish appropriate genetic management programs (Avise, 1994).

The objectives of this study were to estimate the level of genetic variability and to identify population differentiation patterns of Thai *A. dorsata* using PCR-RFLP of mtDNA gene segments and microsatellites with large sample sizes. The existence of population subdivisions of Thai *A. dorsata* is reported for the first time. This useful information can be applied for more effective natural resource management and conservation of *A. dorsata* in Thailand.

MATERIALS AND METHODS

Sampling

Adult *A. dorsata* workers were collected from 154 colonies in five geographically different regions covering the species' distribution in Thailand: north ($N = 47$), northeast ($N = 25$), central ($N = 45$), peninsular Thailand ($N = 25$), and Samui Island ($N = 12$) (Fig. 1). Only one bee per colony was subjected to genetic analysis. Specimens were placed in 95% ethanol and kept at 4°C until required.

DNA Extraction

Total DNA was isolated from the thorax of each bee using a phenol-chloroform-proteinase K method (Smith and Hagen, 1997). The concentration of extracted DNA was spectrophotometrically measured and further adjusted using a mini-gel method (Sambrook and Russell, 2001). DNA was kept at 4°C until needed.

PCR-RFLP Analysis

COI-COII, *Cytb-tRNA^{ser}*, *lrRNA*, and *ATPase6–8* gene segments of each *A. dorsata* were amplified in a 25 μL reaction volume containing 20 mM Tris-HCl, pH 8.4, 2.0–5.0 mM $MgCl_2$, 100 μM each dNTP, 0.1 μM each forward and reverse primer, 25 ng genomic DNA, and 0.6 U Amphi*Taq* DNA polymerase (Perkin–Elmer Cetus). PCR was predenatured at 94°C for 1 min followed by 35 cycles consisting of denaturation at 92°C for 1 min, annealing at the optimal temperature (Table I) for 1 min, and extension at 72°C for 2 min. The final extension was performed at the same temperature for 10 min. Approximately 500 ng of each amplification product was separately digested with *Dra* I and *Hin* fI. The digested products were electrophoretically analyzed through 2.0% Metaphor agarose gels prepared in 1 × TBE at 10 V/cm. The bands were visualized under a UV transilluminator after ethidium bromide staining (Sambrook and Russell, 2001).

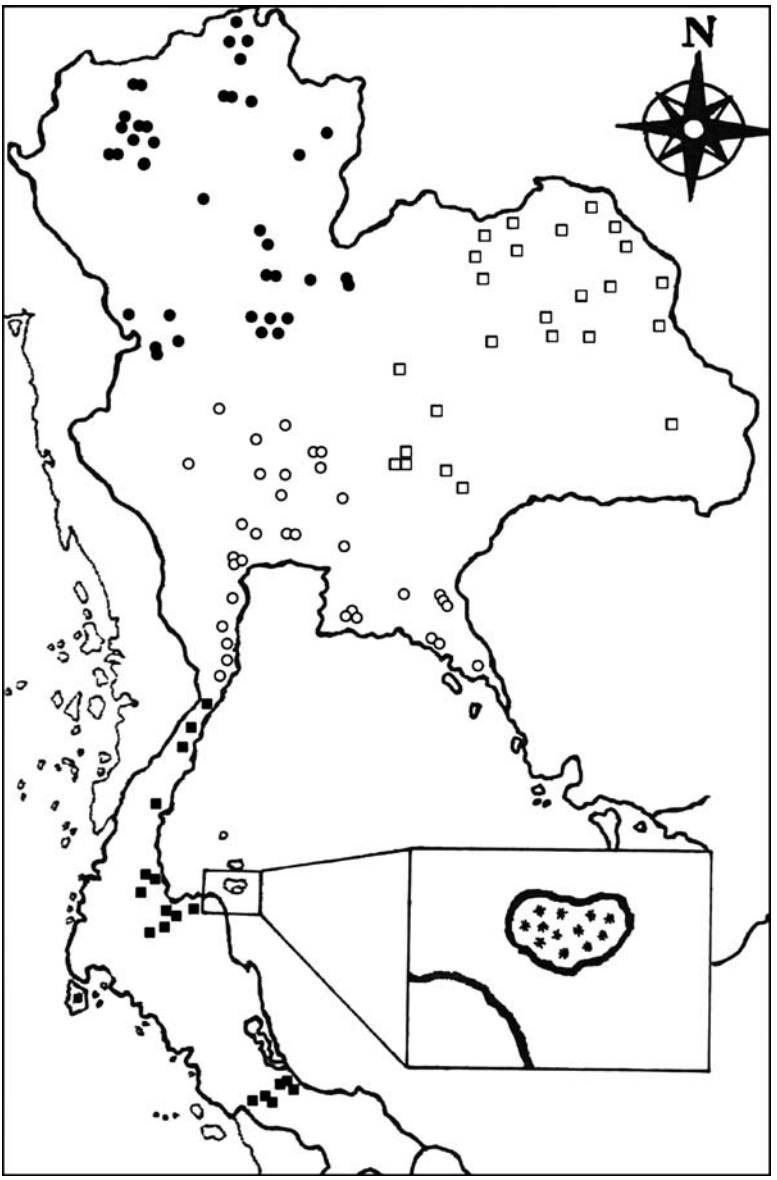

Fig. 1. Collection sites of *A. dorsata* specimens in Thailand. Bees were sampled from the north (*solid circles*; $N = 47$), northeast (*open squares*; $N = 25$), central (*open circles*; $N = 45$), peninsular Thailand (*solid squares*; $N = 25$), and Samui Island (*inset*; $N = 12$).

Table I. Primers Used for Determination of Genetic Diversity of Thai *A. dorsata*

Locus	Primer sequence	$MgCl_2$ (mM)	Annealing temperature	Reference
COI-COII	F: 5′-TCTATACCACGACGTTATTC-3′ R: 5′-CCACAAATTTCTGAACATTGACC-3′	2.0	60	Crozier and Crozier (1993)
Cytb-tRNA^{ser}	F: 5′-TATGTACTACCATGAGGACAAATATC-3′ R: 5′-GAAAATTTTATTTCTATATTATATTTTCA-3′	2.0	48	Delarua *et al.* (1998)
lrRNA	F: 5′-CTATAGGGTCTTATCGTCCC-3′ R: 5′-TTTTGTACCTTTTGTATCAGGGTT-3′	2.5	55	Delarua *et al.* (1998)
ATPase6-8	F: 5′-AAAAATTCCTCAAATAATAC-3′ R: 5′-TTAATTTGATTCAGAGAAAT-3′	5.0	40	Delarua *et al.* (1998)
A14	F: 5′-GTGTCGCAATCGACGTAACC-3′ R: 5′-GTCGATTACCGCTCGTGACG-3′	1.1	50	Estoup *et al.* (1993)
A24	F: 5′-CACAAGTTCCAACAATGC-3′ R: 5′-CACATTGAGGATGAGCG-3′	1.8	58	Estoup *et al.* (1995)
A88	F: 5′-GCGAATTAACCGATTTGTCG-3′ R: 5′-GATCGCAATTATTGAAGGAG-3′	0.6	55	Estoup *et al.* (1995)

Analysis of Microsatellite Polymorphisms

Thirteen heterospecific primer sets of microsatellites (*A7*, *A8*, *A14*, *A24*, *A28*, *A29*, *A35*, *A43*, *A79*, *A81*, *A88*, *A107*, and *A113*; Estoup *et al*., 1993, 1994, 1995; Franck *et al*., 1998) initially developed in *A. mellifera* were screened against representative individuals of *A. dorsata*. Three polymorphic loci (*A14*, *A24,* and *A88*; Table I) were selected for population genetic studies of *A. dorsata* using reaction conditions described in Estoup *et al*. (1995). Microsatellites were size-fractionated in 6% denaturating polyacrylamide gels and visualized by silver staining. Sizes of microsatellites were estimated by comparison to a sequencing ladder of pGEM-3Zf(+).

Data Analysis

For PCR-RFLP analysis, a digestion profile of each bee was coded in order of discovery. Haplotype diversity and nucleotide diversity within each geographic region and nucleotide divergence between pairs of regions were estimated (Nei and Li, 1979; Nei, 1987). Genetic heterogeneity among different geographic regions of *A. dorsata* was analyzed using a Monte Carlo simulation (Roff and Bentzen, 1989) implemented in REAP 4.0 (McElroy *et al*., 1991).

For microsatellite analysis, the number of alleles, allele frequencies, and observed (H_o) and expected (H_e) heterozygosity of each microsatellite locus were estimated for each geographic region (Nei, 1978). The effective number of alleles per locus was calculated (Crow and Kimura, 1965). Exact tests for linkage disequilibrium and Hardy–Weinberg expectations were carried out (Raymond and Rousset, 1995). Genetic heterogeneity in allele distribution frequencies was compared between geographic regions with the Markov chain approach for χ^2 analysis (Guo and Thompson, 1992). The F_{ST} between pairs of regions was tested to determine if F_{ST} was statistically significantly different from zero (Weir and Cockerham, 1984) using GenePop (Raymond and Rousset, 1995). The Cavalli-Sforza and Edwards (1967) chord distance between pairs of regions was calculated because for microsatellites it is among the best genetic distance measures to recover the true tree topology (Takezaki and Nei, 1996). Allele frequencies were bootstrapped 1000 times using SeqBoot. The resulting data were used to calculate the Cavalli–Sforza and Edwards distance using GenDist. A bootstrapped neighbor-joining tree (Saitou and Nei, 1987) was constructed to illustrate the relations among geographic regions using Neighbor and Consense programs. All phylogenetic programs described are routine in Phylip (Felsenstein, 1993).

RESULTS

Restriction analysis of *COI-COII*, *Cytb-tRNA^{ser}*, *lrRNA*, and *ATPase6-8* with *Dra* I and *Hin* fI generated a limited number of restriction patterns (Fig. 2, Table II). Size

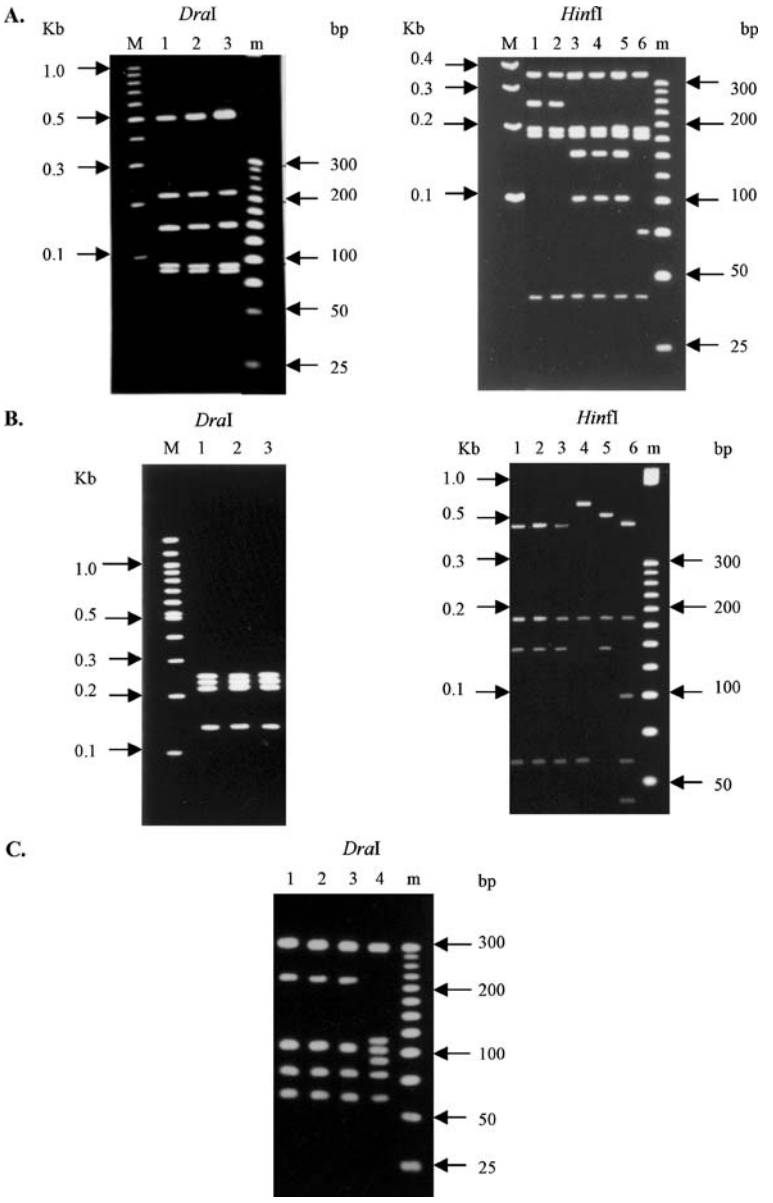

Fig. 2. Examples of restriction patterns of the amplified *COI-COII* (A), *Cytb-tRNA*^{ser} (B), and *lrRNA* gene (C) of *A. dorsata* digested with *Hin* fI and *Dra* I. DNA ladders of 100 (lanes M) and 25 bp (lanes m) were used as the markers.

Table II. Restriction Fragment Patterns Resulting from PCR-RFLP of Four Genes of *A. dorsata* with *Dra* I and *Hin* fI

Gene/Enzyme	Pattern observed (bp)
COI-COII	
Dra I	A: 500, 210, 160, 90, 80
Hin fI	A: 360, 265, 195, 190, 30
	B: 360, 195, 190, 160, 105, 30
	C: 360, 195, 190, 190, 75, 30
Cytb-tRNA^{ser}	
Dra I	A: 250, 235, 220, 140
Hin fI	A: 480, 170, 135, 60
	B: 615, 170, 60
	C: 540, 170, 135
	D: 480, 170, 95, 60, 40
ATPase6-8	
Dra I	A: 520, 300
Hin fI	A: 550, 270
lrRNA	
Dra I	A: 300, 200, 100, 80, 70
	B: 300, 110, 100, 90, 80, 70
Hin fI	Did not cut

heteroplasmy of these gene segments was not observed. A total of 6 composite haplotypes (hereafter called mitotypes) were found across investigated bees. The major mitotype (AAAAAAA) was distributed across geographic regions with approximately identical frequencies (0.920–1.000). The remaining mitotypes were found at relatively low frequencies (0.021–0.080). Low haplotype (0.000–0.153) and nucleotide diversity (0.000–0.059%) within geographic regions was observed in this species (Table III). Distribution frequencies of mitotypes revealed a lack of genetic heterogeneity of *A. dorsata* in Thailand ($P > 0.05$).

Microsatellite analysis (Fig. 3) indicated greater polymorphism than did mitochondrial DNA gene segments (Table IV). Genotypic disequilibrium analysis indicated that genotypes of *A14*, *A24*, and *A88* were associated randomly ($P > 0.05$). The highest polymorphic locus was *A88* ($H_o = 0.781$), followed by *A14* ($H_o = 0.686$) and *A24* ($H_o = 0.671$), with the number of alleles and effective alleles per locus of 20 and 6.66, 8 and 3.95, and 6 and 4.00 respectively. Bees from all investigated regions conformed to Hardy–Weinberg expectations at both *A14* and *A24* ($P > 0.05$). Only the north sample displayed Hardy–Weinberg disequilibrium due to homozygote excess at *A88* ($P < 0.05$).

Low genetic distances were observed in *A. dorsata* from the mainland (0.0192–0.0294), but a greater distance was found between that of the Samui and each of the mainland regions (0.0563–0.0946). Geographic heterogeneity analysis and F_{ST} statistics were generally concordant and indicated significant differentiation across overall samples ($P < 0.05$) at *A24* and *A88* (Table V). Pairwise comparisons indicated significant genetic heterogeneity between bees

Table III. Distribution of Mitotypes Across Geographic Regions of *A. dorsata*

Mitotype[a] and diversity	Frequency					
	North ($N = 47$)	Northeast ($N = 25$)	Central ($N = 45$)	Peninsular Thailand ($N = 25$)	Samui Island ($N = 12$)	Total ($N = 147$)
AAAAAAA	0.957	1.000	0.933	0.920	1.000	0.955
AAACAAA	0.021	–	–	–	–	0.006
ACABAAA	0.021	–	–	–	–	0.066
AAADAAA	–	–	0.022	–	–	0.066
ABAAAAA	–	–	0.022	0.080	–	0.0193
AAAAAAB	–	–	0.022	–	–	0.006
Haplotype diversity ($h \pm$ SE)	0.084 ± 0.055	0.000 ± 0.000	0.130 ± 0.068	0.153 ± 0.092	0.000 ± 0.000	0.074 ± 0.043
Nucleotide diversity (%)	0.053	0.000	0.050	0.059	0.000	0.032

[a]Generated from PCR-RFLP of mitochondrial gene segments with *Dra* I and *Hin* fI.

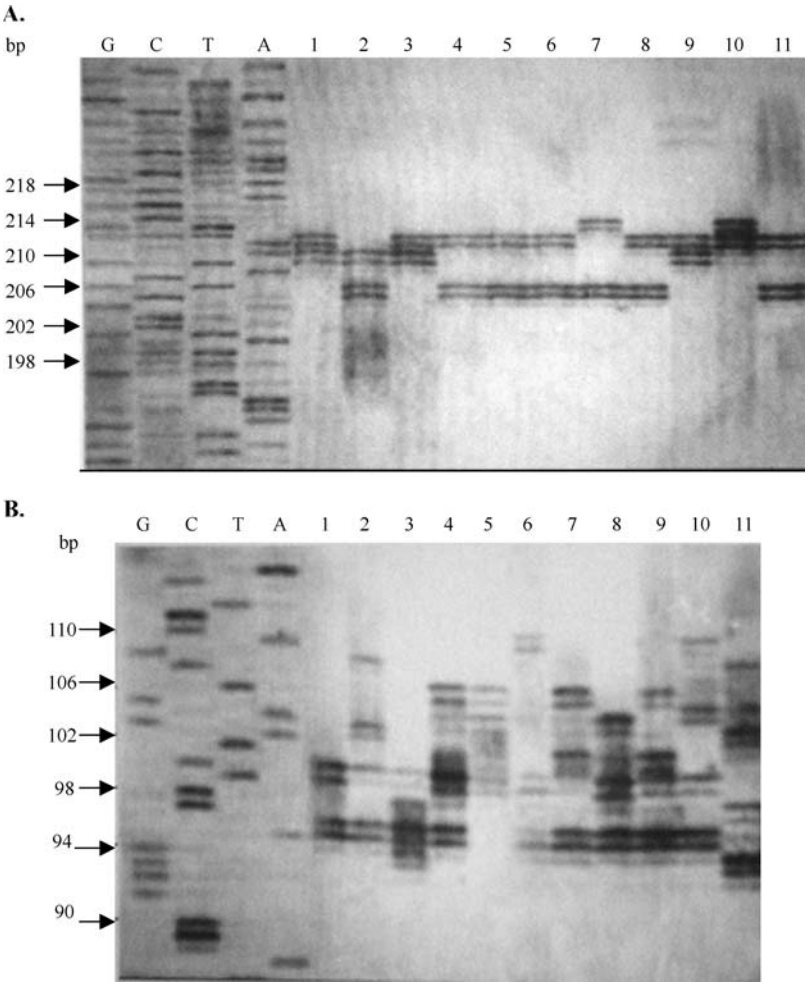

Fig. 3. Examples of microsatellite patterns of nonrelated *A. dorsata* (lanes 1–11) at *A14* (A) and *A24* (B) loci. A sequencing ladder of pGEMZ3f(+) was included as the size standard.

from Samui Island and the mainland (both *A24* and *A88*), the north and the central region (*A24*), and north and peninsular Thailand (*A24* and *A88*). A bootstrapped neighbor-joining tree allocated Thai *A. dorsata* to the north-to-central (north, northeast, and central) region, peninsular Thailand, and Samui populations (Fig. 4).

Table IV. Allele Frequencies at Three Loci in *A. dorsata* Populations of Thailand

	Allele (bp)	North	Northeast	Central	Peninsular Thailand	Samui Island
A14	206	0.244	0.296	0.250	0.260	0.417
	208	0.133	0.111	0.125	0.140	0.042
	210	0.144	0.204	0.205	0.260	0.167
	212	0.400	0.315	0.318	0.280	0.250
	214	0.067	0.074	0.079	0.060	0.083
	216	0.011	–	0.023	–	0.042
Number of alleles		6	5	6	5	6
Number of effective alleles		3.802	4.065	4.386	4.219	3.636
H_o		0.756	0.740	0.659	0.600	0.583
H_e		0.737	0.754	0.772	0.763	0.725
A24	98	0.044	–	0.079	0.020	0.167
	100	0.444	0.423	0.352	0.280	0.042
	102	0.022	0.038	–	0.120	0.167
	104	0.167	0.231	0.341	0.220	0.167
	106	0.278	0.173	0.102	0.160	0.375
	108	0.011	0.096	0.068	0.140	–
	110	0.022	0.038	0.057	0.060	–
	112	0.011	–	–	–	0.083
Number of alleles		8	6	6	7	6
Number of effective alleles		3.279	3.650	3.774	5.263	4.292
H_o		0.711	0.615	0.613	0.680	0.833
H_e		0.695	0.726	0.735	0.810	0.767
A88	132	–	–	–	–	0.042
	133	0.065	0.071	0.125	0.080	–
	134	0.011	0.018	–	–	–
	135	0.054	0.089	0.136	0.040	–
	136	0.043	0.089	0.045	0.010	0.083
	138	0.163	0.143	0.136	0.260	0.333
	139	0.011	–	–	–	–
	140	0.196	0.196	0.091	0.020	–
	142	0.185	0.089	0.170	0.160	0.125
	143	–	–	0.011	–	–
	144	0.141	0.161	0.170	0.280	0.333
	145	0.011	–	0.011	–	–
	146	0.011	0.018	0.023	0.020	–
	148	0.098	–	0.057	0.020	0.083
	149	–	0.018	0.011	–	–
	150	–	0.018	–	–	–
	152	–	–	0.011	0.020	–
	153	–	0.018	–	–	–
	154	0.011	0.054	–	–	–
	157	–	0.018	–	–	–
Number of alleles		13	14	13	10	6
Number of effective alleles		7.246	8.475	8.000	5.525	3.953
H_o		0.696	0.857	0.773	0.880	0.750
H_e		0.862	0.882	0.875	0.819	0.747

Note. H_o, Observed heterozygosity; H_e, Expected heterozygosity.

Table V Genetic Heterogeneity Analysis between Pairs of Geographic Regions of *A. dorsata* Resulting from PCR-RFLP and Microsatellite Analysis

			Microsatellite						
	PCR-RFLP		*A14*		*A24*		*A88*		
Pairwise comparison	Nucleotide divergence	Geographic heterogeneity	Geographic heterogeneity	F_{ST} (*P*-value)	Geographic heterogeneity	F_{ST} (*P*-value)	Geographic heterogeneity	F_{ST} (*P*-value)	Genetic Distance
North–northeast	0.0003	$P = 1.000^{ns}$	$P = 0.748^{ns}$	$P = 0.598^{ns}$	$P = 0.083^{ns}$	$P = 0.232^{ns}$	$P = 0.051^{ns}$	$P = 0.496^{ns}$	0.0254
North–central	0.0005	$P = 0.482^{ns}$	$P = 0.785^{ns}$	$P = 0.631^{ns}$	$P < 0.001^{*}$	$P = 0.005^{*}$	$P = 0.294^{ns}$	$P = 0.033^{*}$	0.0192
North–peninsular Thailand	0.0006	$P = 0.173^{ns}$	$P = 0.524^{ns}$	$P = 0.345^{ns}$	$P = 0.006^{*}$	$P = 0.040^{*}$	$P = 0.007^{*}$	$P = 0.009^{*}$	0.0296
North–Samui	0.0003	$P = 1.000^{ns}$	$P = 0.269^{ns}$	$P = 0.194^{ns}$	$P < 0.001^{*}$	$P = 0.001^{*}$	$P = 0.048^{*}$	$P < 0.001^{*}$	0.0627
Northeast–central	0.0003	$P = 1.000^{ns}$	$P = 0.903^{ns}$	$P = 0.921^{ns}$	$P = 0.128^{ns}$	$P = 0\,405^{ns}$	$P = 0.128^{ns}$	$P = 0.715^{ns}$	0.0291
Northeast–peninsular Thailand	0.0003	$P = 0.492^{ns}$	$P = 0.910^{ns}$	$P = 0.884^{ns}$	$P = 0.709^{ns}$	$P = 0\,744^{ns}$	$P = 0.013^{*}$	$P = 0.146^{ns}$	0.0267
Northeast–Samui	0.0000	$P = 1.000^{ns}$	$P = 0.610^{ns}$	$P = 0.722^{ns}$	$P < 0.001^{*}$	$P = 0\,001^{*}$	$P = 0.041^{*}$	$P < 0.001^{*}$	0.0946
Central–peninsular Thailand	0.0005	$P = 0.813^{ns}$	$P = 0.935^{ns}$	$P = 0.954^{ns}$	$P = 0.024^{*}$	$P = 0.228^{ns}$	$P = 0.099^{ns}$	$P = 0.216^{ns}$	0.0234
Central–Samui	0.0003	$P = 1.000^{ns}$	$P = 0.543^{ns}$	$P = 0.467^{ns}$	$P < 0.001^{*}$	$P = 0.001^{*}$	$P = 0.127^{ns}$	$P = 0.001^{*}$	0.0814
Peninsular Thailand–Samui	0.0003	$P = 0.547^{ns}$	$P = 0.349^{ns}$	$P = 0.511^{ns}$	$P = 0.001^{*}$	$P = 0.001^{*}$	$P = 0.986^{ns}$	$P = 0.081^{ns}$	0.0563

Note. ns, not significant.
$^{*}P < 0.05$.

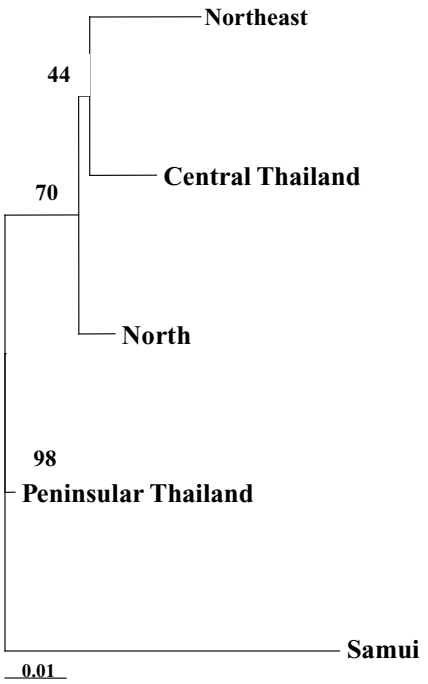

Fig. 4. A bootstrapped neighbor-joining tree summarizing relationships of Thai *A. dorsata* based on microsatellite polymorphism (*A14*, *A24*, and *A88*).

DISCUSSION

As a sampling strategy for population genetic studies of social insects like *A. dorsata*, mtDNA genes have the advantage over nuclear DNA markers because a mitotype of a single specimen represents the matriarchal line of each colony. PCR-RFLP of mitochondrial genes, however, revealed limited genetic diversity and divergence of *A. dorsata* in Thailand. The genetic status of each geographic region is represented by the most common AAAAAAA mitotype distributed across all geographic regions with comparable frequencies. This reflects a lack of genetic heterogeneity based on polymorphisms of mtDNA genes ($P > 0.05$).

In contrast, Sihanuntavong *et al.* (1999) successfully examined genetic diversity and population structure of *A. cerana* in Thailand originating from the north-to-central region, peninsular Thailand, and Phuket and Samui Islands using PCR-RFLP of *srRNA* and *lrRNA* genes and *COI-COII* with *Dra*I. The results were in accord with those from morphometric studies (Sylvester *et al.*, 1998).

Strong population differentiation ($P < 0.0001$) and restricted female gene flow ($F_{ST} = 0.604$, $P < 0.0001$) were observed between different populations of Thai *A. cerana*.

Although the sampling strategy and the laboratory technique for PCR-RFLP are convenient, contribution of the male component cannot be inferred from analysis of mtDNA polymorphism. Microsatellites were then applied for population studies of the same sample set. In contrast to mtDNA polymorphism, relatively high genetic diversity was found in *A. dorsata* (mean alleles per locus = 6.0–9.0, $H_o = 0.68$–0.74). Intraspecific population differentiation of *A. dorsata* was observed at *A24* and *A88*. A limited level of genetic drift may play an important role for genetic differentiation between bees from the mainland and Samui Island ($P = 0.001$). The phylogeography of *A. dorsata* in Thailand was also observed from a bootstrapped neighbor-joining tree constructed from genetic distances between pairs of geographic regions. This further supported the biogeographic differentiation of Thai *A. dorsata* from microsatellite analysis.

Although *A. dorsata* is considered a potentially vagile species, homesite fidelity of colonies to complete their reproductive cycle was reported in this species (Neumann *et al*., 2000; Parr *et al*., 2000). Recently, an examination of genetic differentiation of *A. dorsata* in northern India using eight microsatellite loci indicated that *A. dorsata* aggregations are composed of colonies that share more alleles than expected by chance. The fixation indices revealed significant genetic differentiation among aggregation sites. Nevertheless, the gene flow among aggregations is sufficient to prevent the development of high degrees of relatedness between colonies within aggregations (short-distance migration). The results also indicate significant population differentiation between different geographic regions of northern Indian *A. dorsata*, even though the level of subdivision caused by aggregation exceeded that attributable to geographic origins (Parr *et al*., 2004).

The differing patterns of geographic heterogeneity in *A. dorsata* revealed by different techniques (panmixia from mtDNA polymorphisms but genetic differentiation between geographic regions from microsatellites) may have several possible explanations apart from the migratory behavior of *A. dorsata* alone. Generally, mitochondrial DNA evolves more slowly than microsatellites. Therefore, its polymorphisms may not be variable enough to detect low degrees of genetic differentiation in this species.

Another explanation is that recent bottlenecks may exist and the present Thai *A. dorsata* was recolonized by a small core population. Nevertheless, this is unlikely, because the lack of drastic reduction in the mean number of alleles and heterozygosity of microsatellites in any region examined indicated no recent bottlenecks in Thai *A. dorsata*.

Previously, we examined genetic diversity of *A. cerana* in Thailand using three microsatellite loci (*A28*, *A107*, and *A113*). The results indicated high genetic diversity ($H_o = 0.40$–0.46) in the mainland samples (north, central, northeast,

and peninsular Thailand) but limited diversity ($H_o = 0.18$) in the Samui sample, implying that genetic drift or founder effects may have occurred in *A. cerana* from Samui Island (Sittipraneed *et al*., 2001a).

Under the presumption of selective neutrality of molecular markers, female founder effects rather than gender-biased gene flow may have significantly affected discordant population subdivision patterns when examined by different molecular markers. Since mtDNA is haploid and transmitted maternally, the effective population size estimated from mtDNA is generally smaller than that estimated from nuclear markers (Birky *et al*., 1989). This increases its sensitivity to genetic drift and founder effects compared to nuclear DNA markers (O'Connell, 1998).

Our data do not indicate unambiguously which explanation is correct. Accordingly, results should be further confirmed by a larger number of samples and microsatellite loci. In addition, further DNA sequencing of mitochondrial gene segments will be required to reveal their actual variability in this species.

The ability to identify population differentiation within *A. dorsata* is crucial for the construction of natural resource management and conservation programs for this species. The information suggests that *A. dorsata* from the north-to-central region, peninsular Thailand, and Samui Island should be treated and managed separately. Although the *A24* and *A88* loci showed useful results for levels of genetic variation and differentiation in *A. dorsata,* more loci (e.g., approximately 30 loci recommended by Takezaki and Nei, 1996) are required for understanding genetic relationships of this species accurately.

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