Apidologie 38 (2007) 323–334
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DOI: 10.1051/apido:2007019

Available online at:
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Original article

Matrilineal origins of *Apis mellifera* in Thailand*

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Received 25 September 2006 – Revised 18 January 2007 – Accepted 23 January 2007

Abstract – *Apis mellifera* was imported to Thailand approximately 60 years ago, but the subspecies that contributed to honey bee populations in this country are unknown. We collected 476 colonies from North, Central, Northeast and South Thailand and used PCR-RFLP and direct DNA sequencing to identify mitochondrial lineages and subspecies present. Three common and five rare composite haplotypes were found. Haplotype group ThaiA1 (22% of colonies) and group ThaiA2 (60%) match C or east European lineage *A. m. ligustica* and *A. m. carnica*. Haplotype group ThaiB (18%) belongs to the O or Middle Eastern lineage. Non-coding mitochondrial sequences of ThaiB are similar to those of *A. m. syriaca* and *A. m. lamarckii*, although no published sequence is an exact match. Analysis of Molecular Variation (AMOVA) showed most of the observed genetic variation occurred within individual apiaries, but significant differentiation between North + Central and Northeast + South regions was observed.

***Apis mellifera* / mtDNA variation / RFLPs / DNA sequence / introduced species**

1. INTRODUCTION

Apis mellifera L. was introduced to Thailand in the early 1940s (Wongsiri, 1988) and 1950s (Akratanakul, 2000) for research at Chulalongkorn and Kasetsart Universities in Bangkok, but neither of these early introductions appears to have established lasting populations of *A. mellifera*. In the 1970s large numbers of *A. mellifera* were imported from Taiwan to Lampoon and Chiang Mai in northern Thailand for commercial purposes (Wongsiri et al., 1995). Additional importations may have come from Australia, Europe and Russia (Wongsiri et al., 2000; Kavinseksan et al., 2004). *Apis mellifera* beekeeping spread rapidly in northern Thailand, especially Chiang Mai province, and beekeeping with *A. mellifera* was later extended through the rest of Thailand (Thapa and Wongsiri, 1997). Today there are about 300 000 managed colonies of *A. mellifera* in Thailand, with more than half in the northern provinces (Wongsiri et al., 2000); there is little evidence of a wild or feral population.

Thus, the ancestry of *A. mellifera* currently in Thailand is unknown but could include a mixture of many subspecies. Since subspecies are known to vary in disease resistance, defensive behavior and other economically important traits (Ruttner, 1988), the identity of Thai honey bee populations is potentially of interest for maintenance and improvement of Thai stocks.

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* Manuscript editor: Stefan Fuchs

At least 24 subspecies have been described from *A. mellifera*'s native range in Europe, Africa and the Middle East, based primarily on morphometric variation (Ruttner, 1988; Engel, 1999). Studies of *A. mellifera* mitochondrial DNA have revealed at least 4 major mitochondrial lineages: M or West European, C or Eastern Mediterranean, A or African, (Hall and Muralidharan, 1989; Smith et al., 1989; Cornuet and Garnery, 1991; Arias and Sheppard, 1996) and O or Middle Eastern lineage (Franck et al., 2000; Palmer et al., 2000). A possible fifth lineage, Y, has been described from Ethiopia (Franck et al., 2001).

In recent years, several studies have used microsatellites and mitochondrial DNA restriction site polymorphisms to investigate which subspecies and mitochondrial lineages have contributed to mixed populations, especially Africanized or neotropical African bees in the Americas (e.g., Clarke et al., 2001, 2002; Coulson et al., 2005; Diniz et al., 2003; Pinto et al., 2004, 2005). A combination of mitochondrial restriction site polymorphisms can diagnose major mitochondrial lineages, as shown in Table I (Smith et al., 1989; Hall and Smith, 1991; Palmer et al., 2000). The major *A. mellifera* mitochondrial lineages also exhibit size variation in the mtDNA non-coding region due to the presence of repetitive elements, called "P", "P_0", "P_1", and "Q" that are characteristic of major mitochondrial lineages (Cornuet et al., 1991; Garnery et al., 1992; Palmer et al., 2000).

To clarify the maternal ancestry of *A. mellifera* in Thailand, we used PCR-RFLP analysis of four regions of mtDNA restricted with the 6- base restriction enzymes *Dra* I, *Bgl* II, *Eco*R I, *Hin*c II and a 4-base restriction enzyme, *Hin*f I, as well as DNA sequencing of the non-coding region between $tRNA^{leu}$ and cytochrome *c* oxidase II (COII) genes.

2. MATERIALS AND METHODS

2.1. Collections

Apis mellifera samples were collected between July 2003 and January 2006 from 476 colonies from 4 regions in Thailand (see Fig. 1, Tab. II).

For comparison, 27 samples of honey bees from outside Thailand were also examined: *A. m. ligustica*-derived colonies from Australia (5 colonies), and New Zealand (1); *A. m. carnica* from Gratz (2 colonies), Klagenfurt (1), and Lunz-am-See (3), Austria, from the Institute fur Bienenkunde, Oberursel, Germany (3 colonies) and from Medvode, Slovenia (1); *A. m. mellifera* from Asker, Norway (2 colonies); *A. m. scutellata* from South Africa (2 colonies); and *A. mellifera syriaca* (7 colonies) from Hatay, Turkey. European samples were collected by DRS in 1987 (see Smith, 1991 for details), African by Orley Taylor, Jr. in 1991, and Turkish samples by O. Kaftanoglu in 1997. All samples were kept in 95% ethanol and stored at −4 °C.

2.2. Mitochondrial DNA analysis

Total DNA was extracted from one bee thorax per colony by standard phenol-chloroform extraction methods. Four regions of mtDNA were PCR amplified: $tRNA^{leu}$ to the 5′ end of COII (including the non-coding region between $tRNA^{leu}$ and COII), a portion of cyt-b, a portion of the large ribosomal subunit and the 5′ end of COI. Primers, PCR thermal profiles and restriction enzymes are shown in Table I.

The intergenic region between $tRNA^{leu}$ and COII was sequenced in one example of each major Thai mitotype detected by restriction fragment polymorphisms. PCR product was purified using a gel purification kit (QIAGEN) and sequenced in both directions on an ABI-3100 AVANT sequencer using primers E2 and H2 (Tab. I). The resulting sequences were compared to published sequences and sequence available in Genbank.

2.3. Statistical analysis

Composite haplotypes were generated from the combined restriction patterns of the four mitochondrial regions (Fig. 2, Tab. II). Genetic distances (Nei and Li, 1979) among haplotypes and frequency distribution among populations were calculated using Restriction Enzyme Analysis Package, REAP (McElroy et al., 1992). A dendrogram showing similarity among haplotypes was constructed from the genetic distances using the neighbor-joining method in Mega3 (Kumar et al., 2004). Genetic differentiation among regions and

Table I. Summary of procedures used to identify the mitochondrial DNA of *A. mellifera* in Thailand. M = West European lineage, C = East Mediterranean, A = African, O = Middle Eastern. *Dra*I digestions and size variation of $tRNA^{leu}$-COII are shown in Figure 2.

Regions Amplified	*Primers*	*PCR thermal profile*	*Restriction Enzyme*	*M*	*C*	*A*	*O*
$tRNA^{leu}$-COII	E2: 5′GGCAGAATAAGTGCATTG 3′	94 °C – 60 s, (94 °C – 30 s,	*Hinf* I	$+^{E}$	$+^{C}$, $+^{B}$	$+^{D}$	$+^{A}$
(Garnery et al., 1993)	H2: 5′CAATATCATTGATGACC 3′	55 °C – 30 s, 72 °C – 60 s)[30],					
		72 °C – 10 min					
Cyt b	OLD1: 5′TATGTACTACCATGAGGACAAATATC3′	94 °C – 60 s, (92 °C – 60 s,	*Bgl* II	+	+	–	+
(Crozier et al., 1991)	OLD2: 5′ATTACACCTCCTAATTTATTAGGAAT 3′	50 °C – 60 s, 72 °C – 90 s)[35],	*Dra* I	+	–,+	+	–,+
		72 °C – 5 min	*Hinf* I	$+^{C}$	–	$+^{B}$	$+^{B}$
lsRNA	Ls1: 5′TTTTGTACCTTTTGTATCAGGGTTG 3′	94 °C – 60 s, (94 °C – 30 s,	*EcoR* I	–	+	–	–
(Hall & Smith, 1991)	Ls2: 5′TTTTGTACCTTTTGTATCAGGGTTG 3′	55 °C – 30 s, 72 °C – 60 s)[35],					
		72 °C – 5 min					
COI	COI-1908: 5′ TTAAGATCCCCAGGATCATG 3′	94 °C – 60 s, (94 °C – 60 s,	*Hinc* II	+	–	–	–
(Hall & Smith, 1991)	COI-2932: 5′ TGCAAATACTGCACCTATTG 3′	50 °C – 60 s, 72 °C – 60 s)[35],					
		72 °C – 5 min					

+ indicates restriction site present, – indicates restriction pattern absent, $+^{A}$, $+^{B}$, $+^{C}$, $+^{D}$ and $+^{E}$ indicates different restriction pattern.

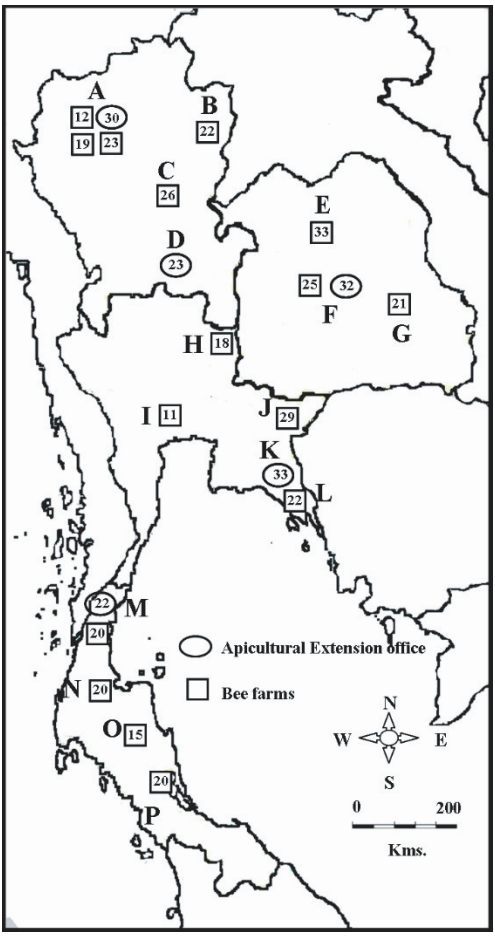

Figure 1. Collection locations for *Apis mellifera* in Thailand. Capital letters indicate collection sites. Circles represent samples collected from Apicultural Extension offices, boxes represent collections from bee farms. Numbers in boxes and circles show number of honeybee colonies sampled at each site.

among apiaries within regions was estimated using AMOVA (Analysis of Molecular Variance; Excoffier et al., 1992) implemented in the program AMOVA1.55 (Excoffier, 1995). Significance testing was carried out by comparison to 1000 random permuations of observed haplotypes into apiaries and regions of the same sizes. Sequence data were aligned by eye and by using Clustal W implemented in Mega 3.1 (Kumar et al., 2004).

3. RESULTS

3.1. RFLP haplotypes

Restriction enzyme digests of PCR products are summarized in Figure 2 and Table II. All Thai samples possess a *Bgl* II restriction site in cytochrome b (pattern "b", Fig. 2B), indicating that none belongs to the African or A mitochondrial lineage. Similarly, all Thai samples lack a *Hin*c II site in the 5′ end of COI (pattern "a", Fig. 2D), indicating that none belongs to the West European or M lineage. The remaining restriction digests indicate the presence of both the Eastern Mediterranean (C) and the Middle East (O) lineages among Thai *A. mellifera*.

Amplification of $tRNA^{LEU}$-COII revealed fragments of two sizes: 573 bp and 837p. The smaller fragment, found in samples designated "ThaiA", is typical of the C lineage. Digestion of this fragment with *Dra* I produced a single pattern identical to that found in C lineage subspecies *A. m. ligustica* and *A. m. carnica* (ThaiA group, pattern "b", Fig. 2A). Digestion of this fragment with *Hin*f I revealed two restriction patterns, "b," found in most of our *A. m. carnica* reference samples, and "c," found in *A. m. ligustica* and a small number of the *A. m. carnica* reference samples. Those with pattern "c" were designated ThaiA1, those with were pattern "b" designated ThaiA2 (Fig. 2A).

Samples with the larger fragment were designated "ThaiB". Digestion of this fragment with *Dra* I produced a single pattern identical to that from Middle Eastern or O lineage (Franke et al., 2000 and our Turkish samples; ThaiB samples, pattern "a"). Digestion of this fragment with *Hin*f I produced only a single pattern ("a"), seen in all non-C lineage reference samples and all samples in the ThaiB group.

The results of the other amplification/digestion combinations (*Dra*I and *Hin*fI digests of cytochrome-b, *Eco*RI digests of the lsRNA region) are summarized in Table II and Figure 2. The patterns revealed by all seven restriction digests were combined into composite haplotypes, e.g., bbbbaba. The composite haplotypes indicate

Table II. Geographic distribution of eight haplotypes from 476 Thai *Apis mellifera* colonies. The seven letters of the composite haplotype indicate restriction fragment patterns for seven gene/enzyme combinations ($tRNA^{leu}$ – COII/*Dra* I, $tRNA^{leu}$ – COII/*Hinf* I, cyt-b/*Bgl* II, cyt-b/*Dra* I, Cytb/*Hinf* I, lsRNA/*EcoR* I and COI/*Hinc* II, respectively). Localities A, B, . . . , P indicate collection sites (apiaries and agricultural offices) as shown in Figure 1.

Regions	Localities	Haplotype								Total
		Eastern Mediterranean lineage					Middle Eastern lineage			
		ThaiA1		ThaiA2			ThaiB			
		ThaiA1.1	ThaiA1.2	ThaiA2.1	ThaiA2.2	ThaiA2.3	ThaiB1.1	ThaiB1.2	ThaiB1.3	
		bcbaaba	bcbbaba	bbbbaba	bbbaaba	bbbbbaa	aabbbaa	aababaa	aabaaba	
North	Chiangmai (A)	7		67		1	8	1		84
(155)	Nan (B)	20	1	1						22
	Utaradit (C)			25			1			26
	Phithsanulok (D)	16		7						23
Northeast	Udornthani (E)	12		16			5			33
(111)	Khon khan (F)	7	6	9			32	1	2	57
	Loi-Ed (G)			15		2	3	1		21
Central	Lopburi (H)			18						18
(113)	Bangkok (I)			11						11
	Srakeaw (J)	10		15	2		2			29
	Chantaburi (K)			33						33
	Trat (L)	20		2						22
South	Chumporn (M)			27		2	13			42
(97)	Surat thani (N)		3	11			6			20
	Nakornsrithammarat (O)		1	10		1	3			15
	Shong-Kla (P)		1	10		1	8			20
	Total	92	12	277	2	7	81	3	2	476

the restriction patterns generated by seven PCR product/restriction enzyme combinations: [$tRNA^{LEU}$–COII/*Dra* I] [$tRNA^{LEU}$–COII/*Hinf* I] [CytB/*Bgl* II] [CytB/*Dra* I] [CytB/*Hinf* I] [lsRNA/*Eco*R I] [COI/*Hinc* II].

Eight composite haplotypes were found among the 476 colonies of Thai *A. mellifera*. The ThaiA1 haplotype group includes haplotypes bcb-aba (dash indicates a or b), the ThaiA2 group includes bbb – – – a, and the ThaiB group includes aab – – – a (Tab. II). Pairwise distances among haplotypes as estimated from restriction site data (Nei and Li, 1979) ranged from 0.006 to 0.06. These distances were used to create a dendrogram by the neighbor-joining method (Fig. 3), which clustered the eight haplotypes into two groups corresponding to the C or Eastern Mediterranean group and the Middle Eastern or O group.

ThaiA2.1 (bbbbaba) was the most common haplotype, found in 58.1% of samples. This was identical to 7 samples of *A. m. carnica* from Austria and Germany. A rarer haplotype, ThaiA1.2 (bcbbaba), matches three samples of *A. m. carnica* from Slovenia and Austria. The second most common haplotype, found in 19.7% of samples, was ThaiA1.1 (bcbaaba). This was identical to putative *A. m. ligustica* from Australia and New Zealand. The patterns of Thai A2.2 (bbbaaba) and ThaiA2.3 (bbbbbaa) were not detected in any of our reference samples, although they clearly are members of the C lineage (Fig. 3) because they possess a *Bgl*II site in Cytb, lack a *Hinc*II site in 5′ portion of COI, the tRNA-COII fragment is small and lacking a P unit, and *Dra*I digests of this fragment produce a pattern seen in *A. m. carnica* and *A. m. ligustica*. Eighteen percent of the samples belong to the ThaiB

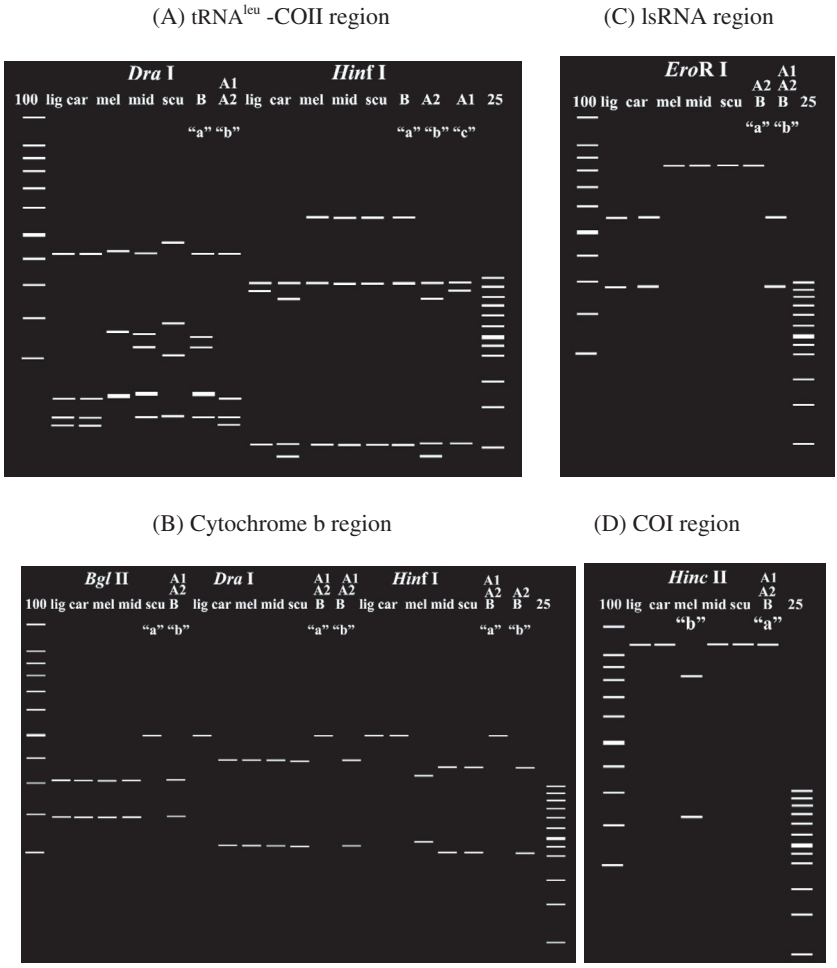

Figure 2. Schematic diagram of restriction patterns found in *A. m. ligustica* (lig), *A. m. carnica* (car), *A. m. mellifera* (mel), Turkish *A. m. syriaca* (mid) and *A. m. scutellata* (scu) reference samples and in Thai honeybees (A1 = ThaiA1 group, A2 = ThaiA2 group and B = ThaiB group). The symbol "a", "b" and "c" represent restriction patterns found in Thai honeybee haplotypes. (A) tRNA^{leu}-COII digested by *Dra* I and *Hinf* I; (B) cytochrome b digested by *Bgl* II, *Dra* I and *Hinf* I; (C) large subunit ribosomal RNA restricted by *EcoR* I; and (D) COI region digested by *Hinc* II. Sizes were determined with 100 bp and 25 bp ladder.

group, which includes haplotypes ThaiB2.1 (aabbbaa, 17.0%), ThaiB2.2 (aababaa) and ThaiB2.3 (aabaaba). The ThaiB2.1 pattern was similar to reference samples of Middle East *A. m. syriaca* from southeastern Turkey.

3.2. Sequence data

We detected no variation among Thai samples in tRNA^{leu} and little in COII (not shown). Sequence of the non-coding intergenic region (Fig. 4) confirms that ThaiA1 and ThaiA2 haplotype groups belong to the C or East Mediterranean lineage as they lack a P sequence, and show high sequence similarity to each other and to the reference sequences of *A. m. carnica* and *A. m. ligustica*. ThaiB haplotypes are excluded from the C lineage because, unlike C lineage, they possess a P sequence, and from the M lineage because their P sequence does not show the 13 bp deletion characteristic of

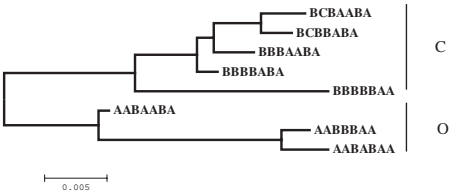

Figure 3. Neighbor-joining dendrogram showing similarity among composite mitochondrial DNA restriction site haplotypes. Haplotypes are as shown in Table II; C and O refer to the East European and Middle Eastern mitochondrial lineages, respectively.

M lineage. Several base substitutions in both the P and Q units also distinguish the ThaiB haplotypes from the A lineage. On the other hand, the ThaiB sequences share features with *A. m. lamarckii* and *A. m. syriaca*, including 3 substitutions in the P element, a 3 bp insertion in Q2, and two substitutions in the Q3 sequence. Our ThaiB sequences show two repeats of the Q element (Q' and Q") while published sequences from *A. m. lamarckii* and *A. m. syriaca* have shown only a single copy of the Q element.

3.3. Distribution and abundance of haplotypes

The three most common haplotypes made up 450 (95%) of the 476 samples. The most common, ThaiA2.1 (bbbbaba), was found at every collection site in all four regions. The second most common haplotype, ThaiA1.1 (bcbaaba), was absent in the South region, but present in the North, Northeast and Central regions. The third most common haplotype, ThaiB1.1 (aabbbaa), was almost absent from the North and Central regions but more common in the South and Northeast; (Fig. 5A). A similar pattern is observed when all eight haplotypes are considered (Fig. 5B).

AMOVA was used to examine differentiation among geographic regions and among apiaries (Tab. III). Two groupings of apiaries into regions were particularly notable. When apiaries were grouped into the North, Central, Northeast and South regions, variance among regions accounted for 19.2% of the total (probability of a more extreme value from random permutations < 0.001). Based on the observed distribution of haplotypes shown in Figure 5, apiaries were regrouped into two larger regions, corresponding to North + Central and Northeast + South; in this configuration variance among regions accounted for 29.9% of the total ($P < 0.001$). In both cases, however, the greatest portion of variance occurred within apiaries (64% and 57%, respectively).

4. DISCUSSION

Thai *A. mellifera* trace their maternal ancestry to the east Mediterranean (C) and Middle Eastern (O) mitochondrial lineages. We infer that the maternal ancestry of Thai *A. mellifera* populations is approximately 60% *A. m. carnica* (ThaiA2.1 haplotype), 22% *A. m. ligustica* (ThaiA1.1) and 18% Middle Eastern or O lineage (the ThaiB haplotype group). We did not find any exact match to our ThaiB sequences, but this may not be surprising since the O or Middle Eastern mitochondrial lineage was identified relatively recently and is still poorly sampled. There was no evidence of either west European (M) or African (A) lineage mtDNAs in Thailand.

Compared to native honey bee species, there is relatively little geographic structure to the distribution of *A. mellifera* matrilines. *Apis cerana* shows clear genetic differentiation among regions of Thailand, particularly between populations north and south of a biogeographic transition in the Isthmus of Kra (e.g., Smith and Hagen, 1996, 1999; Warrit et al., 2006). In contrast, most variation of *A. mellifera* mtDNA haplotypes in Thailand occurs within individual apiaries. This suggests that these introduced bees have been transported widely, and that beekeepers acquire bees of several genetic backgrounds. Despite the variation observed within individual apiaries, a geographic pattern in haplotype distribution does exist in Thailand: the Middle East haplotypes are rare in the North and Northeast, and the *A. m. ligustica*-like haplotypes are rare in the south. These patterns may reflect importation

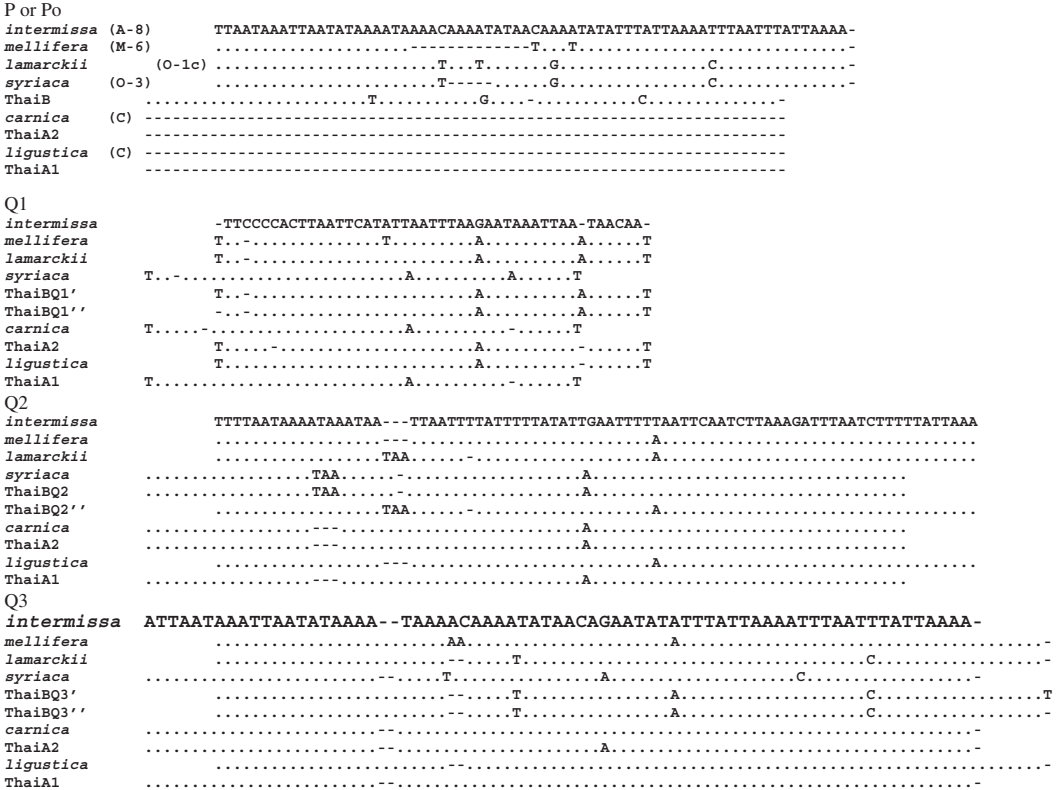

Figure 4. Sequence of the non-coding region between tRNA^{leu} and cytochrome oxidase II in *A. mellifera* mtDNA, including the 3′ end of tRNA^{leu}, P or Po, and Q sequences. Q1, Q2, and Q3 refer to the 5′, middle, and 3′ portions of the Q sequence. The entire Q sequence may be present in 1, 2, 3 or more tandemly repeated copies. Primes (e.g., Q1′, Q1″) are used to indicate the sequence of the first, second, etc. copy of the Q sequence. Thai honeybees (ThaiA1, ThaiA2 and ThaiB) are compared with sequences from other *A. mellifera* subspecies: *A. m. intermissa*, *A. m. mellifera*, *A. m. lamarckii*, *A. m. syriaca* (Franck et al., 2000), *A. m. ligustica* (GenBank accession # L-06178), and *A. m. carnica* (sequence produced in this study). Capital letters in parentheses indicate the mitochondrial lineage of the reference sequences (C, East Mediterranean; M, West European; A, African; O, Middle Eastern). Numbers and small letters indicate which sequence from Franck et al. (2000) is displayed (e.g., O-1c = Middle East sequence 1c). Dots indicate the same base as in the first sequence, dashes indicate a gap in the sequence. East Mediterranean or C lineage haplotypes lack a P unit in the non-coding sequence.

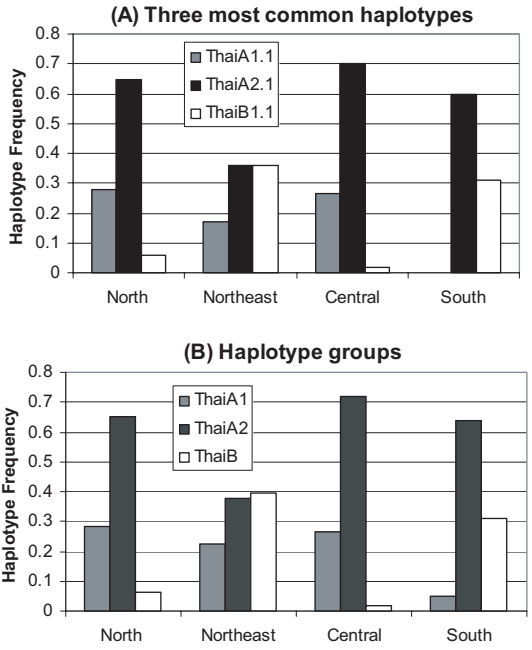

Figure 5. Distribution of *Apis mellifera* mitochondrial haplotypes in 4 areas of Thailand. Bars indicate frequency of colonies of each haplotype. (A) Frequencies of three most common haplotypes. (B) Frequencies of three haplotype groups. ThaiA1 group corresponds to *A. m. ligustica* mtDNA haplotypes, ThaiA2 group corresponds to *A. m. carnica* haplotypes, and ThaiB group corresponds to Middle Eastern mtDNA haplotypes (see text).

Table III. Results of Molecular Analysis of Variance (AMOVA; Excoffier et al., 1992; Excoffier, 1995). (A) Apiaries are grouped into four regions: North, Central, Northeast, and South. (B) Apiaries are grouped into two regions, North + Central, Northeast + South. Significance tested with 1000 random permutations; all F-statistics significant at $P < 0.001$.

		(A)			(B)		
AMOVA analysis	Variance components	Partition of observed variance			Partition of observed variance		
		Variance	%Total	F-statistics	Variance	%Total	F-statistics
Nested analysis	Among regions	0.002	19.20	F ct = 0.192	0.003	29.92	F ct = 0.299
	Among apiaries/within regions	0.001	16.81	F sc = 0.208	0.001	12.93	F sc = 0.184
	Within apiaries	0.006	63.99	F st = 0.360	0.006	57.15	F st = 0.428
Analysis	among apiaries	0.003	33.14		0.003	33.14	
Among apiaries	Within apiaries	0.006	66.86		0.006	66.86	
Analysis	among regions	0.002	22.38		0.003	31.15	
Among regions	Within regions	0.008	77.62		0.007	68.85	

history, patterns of migratory beekeeping, or other factors.

It is rather surprising that up to 20% of the Thai *A. mellifera* population appears to be matrilineal descendants of Middle Eastern honey bees. How and when bees with these mitotypes were introduced remains a mystery. Interviews with Apicultural Extension officers in Chiang Mai, Phisthsanulok, Khonkan, Chantaburi and Chumporn, and with Thai beekeepers indicate researchers and beekeepers in those regions have introduced honeybee queens from the USA, Europe, Australia and Russia, which may have included hybrids with Middle Eastern ancestry. A cooperative project on *A. mellifera* between the Agriculture Department of Thailand and Israel 1990–1995 also may have introduced Middle Eastern queens or colonies from Israel to Thailand (Wongsiri, unpubl. data).

PCR-RFLP polymorphisms in the mitochondrial genome provide a fast method for determining matrilines present in honeybee populations, while sequencing provides finer discrimination of mtDNA genomes characteristic of particular subspecies. In this study we employed both approaches to investigate the maternal ancestry of Thai *A. mellifera*, an exotic species of great economic importance.

ACKNOWLEDGEMENTS

We are grateful for financial support from Naresuan University and the Thailand Research Fund (Royal Golden Jubilee, RGJ: PHD0169/2547 and RTA45008 to S. Wongsiri). We also thank Thai beekeepers, the staff of the Center of Excellence: Bee Biology Biodiversity of Insect and Mites of Chulalongkorn University, the University of Kansas, and Natapot Warrit for their valuable help. Thanks also go to Orley. R. Taylor Jr. (University of Kansas) and Osman Kaftanoglu (Arizona State University) for providing samples of African and Turkish bees.

Origines de l'ascendance maternelle d'*Apis mellifera* en Thaïlande.

***Apis mellifera* / variation ADNmt / séquence ADN / espèce introduite**

Zusammenfassung – Matrilineale Abstammung von *Apis mellifera* in Thailand. *Apis mellifera* wurde vor ungefähr 60 Jahren nach Thailand eingeführt. Heute gibt es mehr als 300 000 Völker in Thailand, allerdings ist ihre Herkunft und genetische Zusammensetzung nicht bekannt. Da die verschiedenen Unterarten von *A. mellifera* sich in ihrer Widerstandsfähigkeit gegen Krankheiten und anderen ökonomisch wichtigen Eigenschaften unterscheiden, ist dies für die Erhaltung und Verbesserung der thailändischen Bienen von Bedeutung. Um die mütterliche Herkunft von *A. mellifera* in Thailand zu bestimmen, benutzten wir PCR-RFLP Analysen und die Sequenzierung des mitochondrialen Genoms. Hierzu wurden Proben von 476 Völkern aus vier thailändischen Regionen genommen: Zentral (113), nördlich (156), südlich (97) und nordöstlich (111). Diese verglichen wir mit 27 für *A. m. carnica*, *A. m. ligustica*, *A. m. mellifera*, *A. m. scutellata* und der türkischen *A. m. syriaca* repräsentativen Referenzproben sowie mit veröffentlichten RFLP Daten. Es wurden vier Regionen der mtDNA mit PCR amplifiziert und mit Restriktionsenzymen verdaut: Ein Fragment der $tRNA^{leu}$ bis zum 5′ Ende von COII mit *Dra* I und *Hinf* I; ein Abschnitt von cyt-b mit *Bgl* II, *Dra* I und *Hinf* I; ein Abschnitt von lsRNA mit *EcoR* I; das 5′ Ende von COI mit *Hinc* II. Wir sequenzierten die nichtkodierende Region zwischen $tRNA^{leu}$ und COII und verglichen sie mit Sequenzen in der Literatur und Genbank.
Wir fanden 3 gewöhnliche und 5 seltene RFLP Haplotypen (Tab. II). Fünf Haplotypen (ThaiA Gruppe) gehörten zu der östlichen mediterranen mitochondrialen Linie (C) und drei (ThaiB Gruppe) zu der Linie (O) des mittleren Ostens. Wie zu vermuten, war die C Linie am häufigsten und wurde in 82 % der Völker gefunden. Es war aber unerwartet, dass 18 % der Völker der mitochondrialen O Linie angehörten. Die drei häufigsten Haplotypen (ThaiA2.1, ThaiA1.1 und ThaiB1.1) umfassten 95 % der Proben. Es wurden aber keine Hinweise auf die Anwesenheit der westeuropäischen (M) oder afrikanischen (A) Linie in der mtDNA in Thailand gefunden. Die Sequenz der nichtkodierenden Region der Thai B Proben ähnelte veröffentlichten Sequenzen von *A. m. syriaca* und *A.m. lamarckii*, entsprach allerdings keiner von beiden ganz genau. Eine Analyse der molekularen Varianz (AMOVA) zeigte signifikante Unterschiede zwischen den geografischen Regionen. Die Haplotypen der O Linie waren im Norden und Nordosten selten, während *A. m. ligustica*-ähnliche Haplotypen im Süden selten waren. Dies könnte die Historie der Einfuhr oder einen Einfluss der Wanderimkerei widerspiegeln. Allerdings wurde der Großteil der Variation innerhalb von Bienenständen gefunden.

***Apis mellifera* / mtDNA Variation / RFLPs / DNA Sequenzen / eingeführte Arten**

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