Population structure of Apis cerana in Thailand reflects biogeography and current gene flow rather than Varroa mite association.

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Abstract:

Concordance between the mitochondrial haplotypes of the Eastern honey bee, Apis cerana, and its ectoparasitic Varroa mites across the Isthmus of Kra in Thailand has suggested that local host–pathogen co-evolution may be responsible for the geographic distribution of particular genotypes. To investigate nuclear microsatellites population structure in A. cerana, single workers of A. cerana colonies from Thailand were genotyped at 18 microsatellite loci. The loci showed intermediate to high levels of heterozygosity and a range of allele numbers. The analyses confirmed a fundamental subdivision of the Thai A. cerana population into the “Asia Mainland” and “Sundaland” regions at the Isthmus of Kra. However, the nuclear microsatellite differentiation was less distinct than mtDNA haplotype differences, suggesting male-biased dispersal and population admixture. Overall, samples showed a weak isolation-by-distance effect. The isolated population on Samui island was most differentiated from the other samples. The results do not support our initial hypothesis of local host–pathogen co-evolution, which predicts a strict correspondence between the nuclear genome and the lineage of parasitic Varroa mite of the A. cerana samples, because the gene flow indicated by our nuclear microsatellite markers should also mix potential Varroa resistance alleles among subpopulations. Instead, our study suggests that the coincidental distribution of Varroa lineages and A. cerana population structure in Thailand are the result of biogeographic history and current migration patterns

Keywords: biogeography | co-evolution | local adaptation | microsatellites | population structure | apis cerana | honey bees | Thailand | Varroa mite association | social insects | biology

Article:

Introduction

Many factors can influence the population genetic structure of a species, including biogeographic history, current population demographics, mating, migration, and local selection patterns, including host–pathogen interactions (Hamilton, 1980; Lively and Dybdahl, 2000). Social insects are subject to a wide range of pathogens and parasites but the population genetic consequences of host–pathogen interactions are poorly understood (Schmid-Hempel, 1998). Social insects are particularly prone to disease because their colonial lifestyle entails close contact among numerous offspring from one or a few reproductive individuals in their colony. Thus, colonies may be genetically homogeneous and represent an additional level of population structure in social insects, particularly noticeable in studies of mitochondrial DNA (mtDNA) variation (Pamilo et al., 1997; Ross et al., 1997).

All honey bee species are characterized by having a single, multiply mated queen per colony that usually returns to her natal nest after mating (Palmer and Oldroyd, 2000). New colonies are initiated by reproductive swarming but it is unclear how far swarms typically disperse. Honey bees are expected to have a pronounced mitochondrial population structure and mtDNA is commonly used to differentiate among subpopulations (Crozier et al., 1991; Ozdil et al., 2009). Population structure in the Western honey bee (Apis mellifera L.) has been largely determined by apicultural practices and human transport (Delaney et al., 2009; Whitfield et al., 2006), except for remaining native honey bee populations in Africa and Europe (El-Niweiri and Moritz, 2010; Ruttner, 1988). In contrast, other Apis species are not cultured and bred by humans to the same extent and are expected to reveal a more natural population structure (Insuan et al., 2007).

Apis cerana is taxonomically close to A. mellifera and the two species share most biological characteristics (Oldroyd and Wongsiri, 2006; Raffiudin and Crozier, 2007). The species has a wide distribution, ranging from Afghanistan to Japan and Indonesia (Hepburn et al., 2001). Across the entire distribution range of A. cerana, considerable variation in mtDNA haplotypes has been described, dividing the species into four principal clusters: the Asian Mainland, yellow or plains bees from India, Sundaland, and the oceanic Philippine islands (Damus and Otis, 1997; de la Rua et al., 2000; Deowanish et al., 1996; Hepburn et al., 2001; Ruttner, 1988; Smith and Hagen, 1996, Smith et al., 2000).

The Asian Mainland and Sundaland populations, as defined by mtDNA haplotypes, are in direct contemporaneous contact at the Isthmus of Kra in Thailand, meeting between 10°34′N and 11°24′N (Deowanish et al., 1996; Sihanuntavong et al., 1999; Sittipraneed et al., 2001b; Smith and Hagen, 1996, 1999; Warrit et al., 2006). An additional variant of the Sundaland mitochondrial haplotype can be found in some bees on the island of Samui (Sittipraneed et al., 2001b; Smith and Hagen, 1996; Warrit et al., 2006). For this reason, as well as its local prevalence and agronomic importance, A. cerana has been studied extensively in Thailand and can serve as a model for the natural population structure of Apis. Morphometric studies of A. cerana in Thailand have reconfirmed the distinction between the Mainland and Sundaland populations, with two additional morphological clusters on the islands of Phuket and Samui (Sylvester et al., 1998). The main distinction between Mainland and Sundaland was also

apparent in an analysis of three microsatellite loci in 257 workers of A. cerana from throughout Thailand (Sittipraneed et al., 2001a). The approach used by Sittipraneed et al. (2001a) differed from other surveys in that they divided the samples into five predetermined geographic sampling regions and compared microsatellite allele frequencies among them. This approach provided evidence for further population subdivisions within the Mainland and Sundaland groups (Sittipraneed et al., 2001a).

In addition to the biogeographic interest, a second important reason for studying A. cerana in Thailand is its long relationship with ectoparasitic Varroa mites, a species complex that infests honey bee colonies and is of considerable apicultural importance (Rosenkranz et al., 2010). A. cerana is presumably the original host of Varroa and thus the source of the global, devastating infestation of A. mellifera (Rosenkranz et al., 2010). In Thailand, mtDNA haplotypes of A. cerana hosts and their Varroa jacobsoni parasites coincide at the Isthmus of Kra (Warrit et al., 2006): The Isthmus of Kra constitutes the boundary between the “NThai” and “Malay” mitochondrial haplotypes of V. jacobsoni and between the corresponding Mainland and Sundaland A. cerana mitochondrial haplotypes. The close correspondence between mite lineages and their host mtDNA haplotypes across the Isthmus of Kra contrasts with the finding that the A. cerana population in northern Thailand hosts both V. jacobsoni and V. destructor (Warrit et al., 2006). In addition, the distinct mtDNA haplotype of V. jacobsoni on the island of Samui is not reflected by the mtDNA haplotypes of their A. cerana hosts (Warrit et al., 2006). These contrasting observations could result from the biogeographic history of the hosts and pathogens, current selection by their mutual co-evolution, or a combination of both. Concordant biogeographic history between Varroa and A. cerana is expected to create a genome-wide, coincidental population structure in mites and bees that weakens in areas of contemporary contact and gene flow. In contrast, local host–pathogen co-evolution may uphold and even reinforce historic population structuring, particularly in genomic regions that mediate host–pathogen interactions.

To distinguish the scenario of concordant biogeography from local co-evolution, we complement a previous study of mtDNA population structure (Warrit et al., 2006) by genotyping a representative subset of their original A. cerana samples at 18 variable microsatellite loci. Due to nearly exclusive selfing in Varroa (Rosenkranz et al., 2010; Sammataro et al., 2000), similar complementary analyses in the corresponding Varroa samples are not expected to give novel insights and are therefore not performed. Specifically, we investigate how the nuclear microsatellite population structure of A. cerana in Thailand corresponds to subdividing this population according to either the Mainland and Sundaland mitochondrial lineages or the type of Varroa hosted by the respective colonies.

Methods and materials

Genotyping

From the original sample set (Warrit et al., 2006), DNA extractions of 44 single workers from 44 independent hives were selected for the analyses (Fig. 1). The concentration of extracted DNA was quantified on a Nanodrop™ spectrophotometer and diluted to 10 ng/µl where possible. The DNA served as template for microsatellite loci amplification using a touchdown PCR protocol in combination with a tailed-primer approach (Meznar et al., 2010; Schug et al., 2004) to label PCR products for size determination on an automated 4300 DNA Analyzer (LI-COR Biosciences, Lincoln, NE, USA).

Fig. 1

Geographic origin of the Apis cerana samples in this study represent a north–south transect through Thailand, crossing the important biogeographic boundary of the Isthmus of Kra

Figure 1 is omitted from this formatted document.

PCRs were set up in a 10 µL reaction volume by combining 10 ng of genomic DNA with a master-mix consisting of dNTPs (final concentration: 200 µM), $MgCl_2$ (2 mM), tailed forward primer (0.25 µM), reverse primer (0.5 µM), IRDye700™ (50 nM), 1× PCR buffer and 0.2 µ Taq Polymerase. The annealing temperature decreased from 68 to 50°C during the initial 10 cycles, followed by an additional 28 cycles with an annealing temperature of 50°C. The products were diluted 1:2–1:10 and mixed with denaturing loading buffer (Blue Stop Solution, LI-COR Biosciences), denatured at 95°C for 3 min and then loaded onto a 12% polyacrylamide gel (length 25 cm, thickness 0.2 mm) and electrophoretic separation was performed for 1–3 h at 1,000 V.

Twelve microsatellite loci published for *A. cerana* (Takahashi et al., *2009*) and 80 existing (Solignac et al.,*2007*) or newly identified loci from the *A. mellifera* genome were screened in a subsample of eight individuals. Based on these screens, 18 primer combinations were selected that reliably amplified one specific, polymorphic microsatellite locus each (Table 1). All 44 individuals were genotyped at these loci, comparing their allelic sizes to a size standard (50–350 bp, LI-COR, NB, USA) and relative to each other.

Table 1

Descriptive statistics of the 18 studied microsatellites and results

Name	Origin	No. of alleles	Size range	H_{obs}/H_{exp}	Deviation from HWE	Fst between the 2 *A. cerana* mt haplotypes	Fst between hosts of the 4*Varroa*lineages
BI125	*A. mellifera*	8	160–174	0.50/0.79	$p <$ **0.001**	Fst = **0.24,**$p <$ **0.001**	Fst = **0.15,**$p <$ **0.001**
K0226	*A. mellifera*	6	210–222	0.63/0.71	$p =$ **0.001**	Fst = 0.08,$p = 0.008$	Fst = **0.09,**$p <$ **0.001**
Ac033	*A. mellifera*	9	180–202	0.76/0.78	$p = 0.264$	Fst = 0.01,$p = 0.160$	Fst = **0.19,**$p <$ **0.001**
At202	*A. mellifera*	8	167–187	0.70/0.78	$p = 0.486$	Fst = 0.09,$p = 0.008$	Fst = 0.08,$p = 0.015$
Ap019	*A. mellifera*	4	153–159	0.85/0.59	$p =$ **0.001**	Fst = 0.08,$p = 0.043$	Fst = 0.06,$p = 0.138$
K0320	*A. mellifera*	4	138–144	0.49/0.56	$p = 0.569$	Fst = 0.00,$p = 0.811$	Fst = 0.01,$p = 0.100$
K1445	*A. mellifera*	15	160–212	0.94/0.93	$p = 0.437$	Fst = 0.01,$p = 0.078$	Fst = 0.02,$p = 0.024$
K0311	*A. mellifera*	10	320–372	0.91/0.90	$p = 0.128$	Insufficient data	Fst = 0.00,$p = 0.169$
UN386	*A. mellifera*	11	248–272	0.91/0.89	$p = 0.298$	Fst = 0.00,$p = 0.727$	Fst = 0.02,$p = 0.110$
SV168	*A. mellifera*	11	216–238	1.00/0.91	$p = 0.658$	Fst = **0.04,**$p <$ **0.001**	Fst = 0.01,$p < 0.107$
OR8_29a	*A. mellifera*	16	240–274	0.77/0.90	$p <$ **0.001**	Fst = **0.08,**$p <$ **0.001**	Fst = **0.09,**$p <$ **0.001**
AC1	*A. cerana*	7	207–221	0.61/0.54	$p = 0.529$	Fst = 0.03,$p = 0.062$	Fst = 0.00,$p = 0.681$
AC3	*A. cerana*	10	332–350	0.92/0.88	$p = 0.019$	Fst = 0.00,$p = 0.754$	Fst = 0.00,$p = 0.608$
AC26	*A.*	7	148–	0.78/0.80	$p = 0.178$	Fst = 0.08,$p = 0.009$	Fst = 0.11,$p = 0.004$

Name	Origin	No. of alleles	Size range	H_{obs}/H_{exp}	Deviation from HWE	Fst between the 2 *A. cerana* mt haplotypes	Fst between hosts of the 4*Varroa*lineages
	cerana		180				
AC27	*A. cerana*	14	114–146	0.80/0.90	$p = 0.066$	Fst = 0.01,$p = 0.085$	Fst = 0.00,$p = 0.067$
AC30	*A. cerana*	12	224–256	0.67/0.79	$p = 0.007$	Fst = 0.02,$p = 0.190$	Fst = 0.16,$p = 0.005$
AC34	*A. cerana*	8	149–165	0.79/0.81	$p = 0.241$	Fst = 0.05,$p = 0.183$	Fst = **0.12,**p = **0.001**
AC35	*A. cerana*	9	141–157	0.79/0.78	$p = 0.470$	Fst = 0.04,$p = 0.005$	Fst = **0.11,**$p <$ **0.001**

Original probabilities are given but cells are marked bold only when the reported effect is significant after Bonferroni correction

Analyses

The data were evaluated with the software packages GENEPOP 4.0 (Rousset, 2008) and STRUCTURE 2.3.3 (Hubisz et al., 2009; Pritchard et al., 2000). GENEPOP was used for global and locus-specific tests of Hardy–Weinberg Equilibrium (HWE), studying pair-wise linkage disequilibrium between loci, and calculating expected and observed heterozygosity for each locus across the entire data set. Furthermore, GENEPOP with program defaults was used to calculate isolation-by-distance between individual samples, as well as Fst values and genic differentiation (by G-test) between the Sundaland and Mainland mitochondrial haplotype groups and between groupings of bees based on the lineage of their parasitic mites (V. destructor, V. jacobsoni NThai, V. jacobsoni Malay, V. jacobsoni Samui).

The program STRUCTURE was used to detect discontinuities in the genetic data and estimate the most likely number of populations (evaluating K = 1–6 populations) using no a priori information about the samples. STRUCTURE uses a Bayesian model approach to estimate simultaneously the most likely number of populations in HWE and population origin of individual samples (Pritchard et al., 2000). Each K was evaluated 10 times with and without assuming population admixture. We used a burn-in period and iteration number of 10,000 each and assumed correlated allele frequencies, with program defaults for all other parameters and options (Pritchard et al., 2000). The natural logarithm of the probability of the Bayesian model

(Ln P(X|K), abbreviated as ln(P) in the following) was averaged over the 10 replicates of each scenario and the ΔK method (Evanno et al., 2005) used for determining the most likely number of populations. The ΔK approach relies on the rate of change in model probability with increasing K and detects population structure more accurately than a simple comparison of the model likelihood ln(P) (Evanno et al., 2005). For the most likely model, population membership coefficients (Q) were estimated for each individual.

The calculations of ln(P) and (Q) were repeated for a priori divisions of the data set based on mtDNA haplotype and Varroa mite infestation (Hubisz et al., 2009). The results from the a priori "mtDNA" and "Varroa" models were then compared to the non-parameterized scenarios with two and four populations, respectively. These analyses were performed as described above assuming admixture but six individuals were removed from the Varroa analysis because their colonies did not contain any Varroa.

Additionally, all analyses were repeated with 14 loci, excluding four loci that showed significant deviations from HWE. Furthermore, the STRUCTURE analysis was repeated with a subset of the nine least polymorphic loci but results did not significantly differ from those obtained with the full data set and are therefore not reported. Unless indicated otherwise, mean value ± standard deviation and original probabilities are reported.

Results

The 18 selected microsatellite loci displayed between 4 and 16 alleles and accordingly, high expected heterozygosity values (Table 1). Overall, the multi-locus genotypes did not deviate significantly from HWE ($p = 0.068$), but four individual loci showed significant deviations after Bonferroni correction (Table 1). After adjusting significance values for multiple comparisons (using Bonferroni correction), three of the 144 tested pairings of loci tested showed significant linkage disequilibrium: AT202–K1445 ($p < 0.001$), AC27–OR8_29a ($p < 0.001$), and UN386–Ap019 ($p = 0.006$). The linkage disequilibrium was not caused by physical proximity because none of these pairs consisted of two loci on the same chromosome.

The microsatellite differentiation between bees with different mtDNA haplotypes was modest (overall $Fst = 0.05$, $p < 0.001$). Three loci showed a significant Fst after Bonferroni correction and several more were close to statistical significance (Table 1). Microsatellite differentiation between hosts of different Varroa was significant (overall $Fst = 0.08$, $p < 0.001$) but was also variable among loci (Table 1) and population pairs (Table 2). Little difference existed between bees in northern Thailand that hosted V. destructor and V. jacobsoni NThai, and the strongest differences were found between bees that hosted V. jacobsoni Samui and all other groups (Table 2). Across all loci, only 27% of all alleles were found in the Samui population. Overall, there was a significant isolation-by-distance effect ($p = 0.002$; genetic distance = $0.026 \times \ln$ (geographic distance) + 0.029).

Table 2
Pairwise genetic differentiation between groups based on mite parasite lineage

	V. jacobsoni **NThai hosts**	***V. jacobsoni*** **Malay hosts**	***V. jacobsoni*** **Samui hosts**
V. destructor hosts	Fst = 0.02, $p = 0.006$	Fst = 0.07, $p < 0.001$	Fst = 0.17, $p < 0.001$
V. jacobsoni NThai hosts	–	Fst = 0.06, $p < 0.001$	Fst = 0.16, $p < 0.001$
V. jacobsoni Malay hosts	–	–	Fst = 0.14, $p < 0.001$

Excluding the four loci that deviated significantly from HWE reduced the differentiation between mtDNA haplotypes (Fst = 0.02, p < 0.001) and among groupings of A. cerana based on their Varroa parasites (Fst = 0.07, p < 0.001). The Samui island population was still most differentiated from all other samples. The isolation-by-distance effect was also slightly weaker (p = 0.002; genetic distance = 0.021 × ln(geographic distance) + 0.020).

ΔK analyses suggested that a division of the data set into two populations was most likely among the considered scenarios (1–6 populations) when assuming admixture (Table 3). In this case the maxima for ΔK and ln(P) coincided (Evanno et al., 2005). Without admixture, no particular number of populations was favored over the alternatives. The two suggested populations were significantly associated with mtDNA haplotype (Fisher's exact p < 0.001) but not perfectly (Fig. 2a). The average assignment probability (Q) for these two populations was 0.86 (±0.12) assuming admixture and 0.92 (±0.11) assuming no admixture. Using the two alternative mtDNA haplotypes as an informative prior, population separation was almost complete (Fig. 2b), and Q increased to 0.96 (±0.06).

Table 3

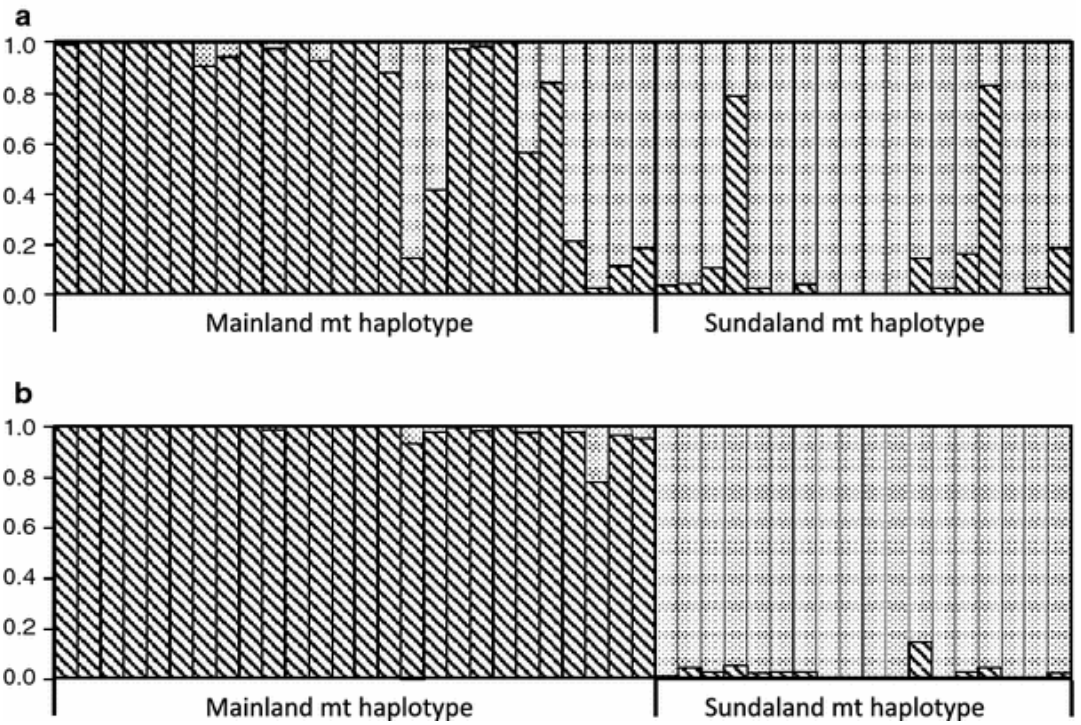
STRUCTURE estimations of the probability of different numbers of population subdivisions (K) in the dataset, with and without assuming population admixture

Assumed number of populations (*K*)	**Posterior probability with admixture (average ln(*P*) ± SD)**	**2nd order rate of change in ln(*P*) (Δ*K*)**	**Posterior probability without admixture (average ln(*P*) ± SD)**	**2nd order rate of change in ln(*P*) (Δ*K*)**
1	−2,176 ± 2	N/A	−2,174 ± 1	N/A

Assumed number of populations (*K*)	**Posterior probability with admixture (average ln(*P*) ± SD)**	**2nd order rate of change in ln(*P*) (Δ*K*)**	**Posterior probability without admixture (average ln(*P*) ± SD)**	**2nd order rate of change in ln(*P*) (Δ*K*)**
2	−2,143 ± 12	14.0	−2,173 ± 91	1.4
3	−2,284 ± 76	2.5	−2,223 ± 57	3.5
4	−2,238 ± 79	2.5	−2,354 ± 223	1.4
5	−2,394 ± 217	0.6	−2,301 ± 83	1.7
6	−2,412 ± 317	–	−2,259 ± 89	–

Fig. 2

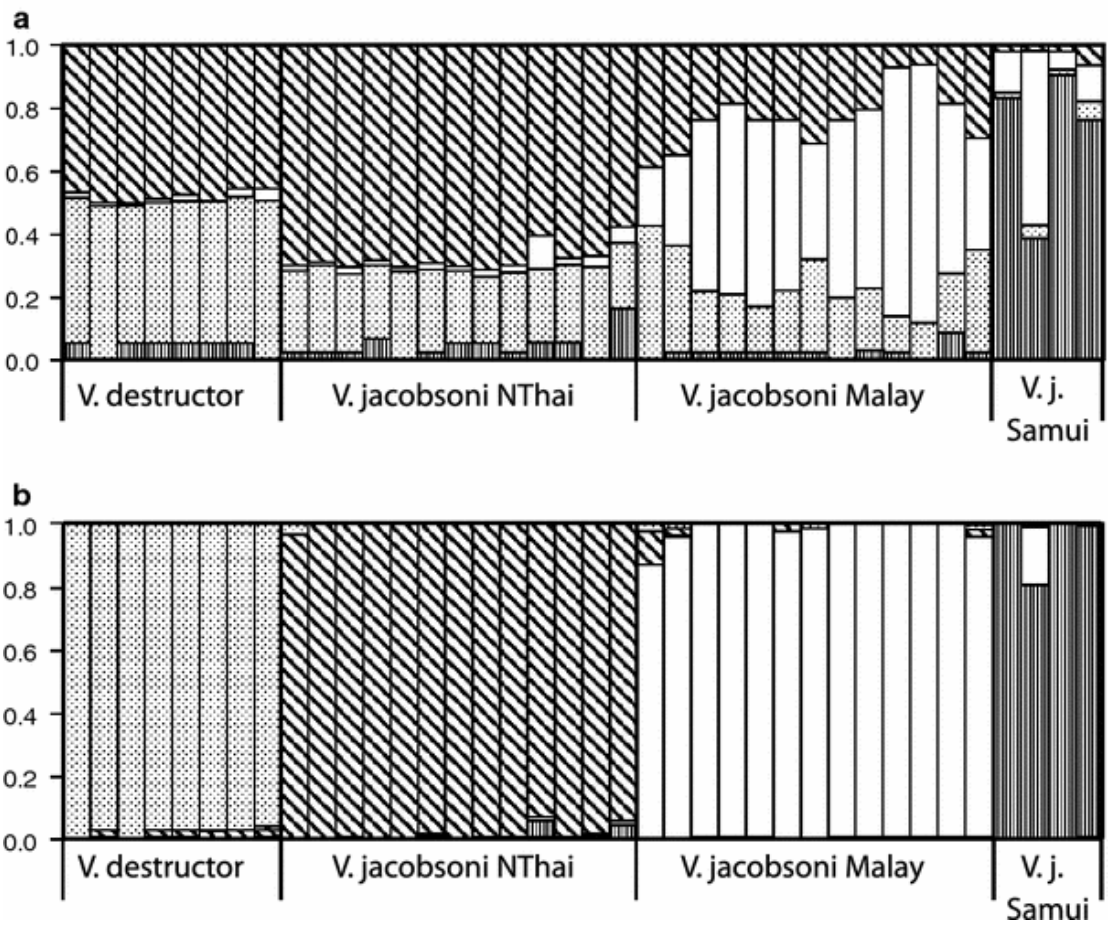
The most probable microsatellite population structure of A. cerana in Thailand is a subdivision into two subpopulations that correspond approximately to the Mainland and Sundaland mtDNA lineages (a). When the mtDNA haplotype distinction is used as a prior, the computer program STRUCTURE assigned the individuals correctly to haplotype groups (b)



A subdivision of the data set into four populations without prior information did not result in four discrete subpopulations (Fig. 3a) and Q was on average 0.67 (±0.15). In particular, bees from northern Thailand were not clearly distinguishable between hives that were infected with V. destructor and with V. jacobsoni (NThai). The bees that were infected with V. jacobsoni (Malay) showed different assignment probabilities from the northern bees and the individuals from colonies that were infected with V. jacobsoni (Samui) formed the most distinct cluster (Fig. 3a). When the four Varroa mite types present in the colonies were used as prior, four distinct subgroups were delineated (Fig. 3b) with an average Q of 0.95 (±0.06).

Fig. 3

With four populations assumed, sampled bees are mostly assigned to populations according to the mite lineage found in their hive without a priori information (a). Using the mite lineage as a priori information, STRUCTURE can assign individuals into four populations that correspond almost perfectly to the mite lineages (b)



When the four loci that deviated significantly from HWE were excluded, ΔK showed a maximum for four populations, assuming admixture. However, these four populations did not

correspond to geographic location or the pattern of Varroa parasites, and Q was only 0.37 (±0.14). No clear maximum for ΔK emerged without assuming admixture.

Discussion

Thailand is a biogeographic region of significant importance because it is the principal contact zone between Sundaland and the Asian Mainland (Smith et al., 2000). Potential local co-evolution of A. cerana with its Varroa mite parasites (Warrit et al., 2006) also makes the genetic differences between Thai A. cerana populations apiculturally relevant. This study complements an earlier investigation of the mitochondrial population structure of A. cerana in Thailand that suggested local co-evolution between this honey bee and its parasitic Varroa mites (Warrit et al., 2006). In contrast to the previous data, our results provide evidence for significant nuclear gene flow in A. cerana across the major ecotone that separates Mainland from Sundaland mitotypes of A. cerana and V. jacobsoni, weakening the argument for their local co-evolution. However, our results agree with Warrit et al. (2006) in two other regards. First, no genetic differentiation is apparent between the A. cerana colonies hosting Varroa destructor and those that host V. jacobsoni in northern Thailand. Second, the population on the island of Samui is genetically distinct. Together, the results suggest that current Thai A. cerana population structure and Varroa distribution patterns may be shaped by biogeographic history and migration.

Although we found evidence for gene flow, our results also confirm previous reports of a fundamental biogeographic demarcation between A. cerana in northern Thailand and its conspecifics south of the Isthmus of Kra on the Malay Peninsula. This distinction was first noted based on mtDNA haplotype analysis (Smith and Hagen, 1996) and subsequently confirmed by morphometric methods (Sylvester et al., 1998) and further mtDNA haplotype data (Sihanuntavong et al., 1999; Sittipraneed et al., 2001b; Songram et al., 2006). The only other study of microsatellite differentiation also found a clear separation along the Isthmus of Kra in two of three loci (Sittipraneed et al., 2001a). Compared to Sittipraneed et al. (2001a), we investigated a smaller number of individual samples with more markers and used different analytical methods. The larger number of markers allowed us to repeat our analyses with different subsets of markers, confirming that our main conclusions were not influenced by particular loci. Excluding four loci that deviated from HWE, possibly due to selection, changed the results slightly but did not offer a meaningful alternative interpretation of the data. In sum, our results are robust and our main conclusions of a population division across the Isthmus of Kra and the strong genetic differentiation of the island population on Samui are concordant with the study of Sittipraneed et al. (2001a).

The genetic isolation between the general northern and southern Thai A. cerana in our data was relatively small, suggesting only a weak, partial genetic isolation. The pattern might also reflect historic differences, which are currently eroding by natural or human-assisted dispersal. In

contrast, the mtDNA of the samples showed a distinct difference between bees that were sampled north and south of the Isthmus of Kra (Warrit et al., 2006). The contrast between the maternally inherited mtDNA and the biparentally inherited microsatellites indicates male-biased population admixture. This bias suggests that translocated drones mate successfully but queens and colonies do not establish themselves. Human-mediated gene flow is unlikely to result in such a pattern. Instead, the pattern may result from ecological factors: the northern end of the Isthmus of Kra (11–13°N latitude) corresponds to the transition from wet, seasonal, evergreen, dipterocarp rain forest in the south to moist, mixed deciduous forest in the north (Woodruff, 2003).

The strongest genetic isolation was found between the few samples from the island of Samui and all remaining samples. The distinction of Samui island bees confirms earlier studies based on morphology (Nakamura et al., 1991; Sylvester et al., 1998), mtDNA (Sihanuntavong et al., 1999; Sittipraneed et al., 2001b; Songram et al., 2006), and microsatellites (Sittipraneed et al., 2001a). The morphological distinction of the Samui population has been explained by a founder effect (Hepburn et al., 2001; Sylvester et al., 1998). Although this explanation is not corroborated by the existence of two distinct mtDNA haplotypes on Samui (Songram et al., 2006; Warrit et al., 2006), a low allelic diversity on Samui island at our microsatellite loci supports the suggested founder effect. In contrast, the overall gene pool of Thai A. cerana seems diverse and suffers little from the assumed small effective population size of social insects (Pamilo et al., 1997).

Although STRUCTURE was able to sort our bee samples into four populations corresponding to the mtDNA genotypes of their Varroa mites with a priori information, it did not do so without a priori information. In particular, the bees that were infected with V. destructor and V. jacobsoni in Northern Thailand were not distinguished, and the Fst value between the bees that hosted “NThai” and “Malay” Varroa strains was very small. Thus, our overall measure of nuclear microsatellite differentiation did not correspond to differential mite presence in the absence of common biogeographic barriers. This finding does not support the hypothesis of local host–pathogen co-evolution. Other common factors, such as climatic selection or biogeographic history, may better explain the coincidence between Varroa and A. cerana mtDNA lineages in Thailand (Warrit et al., 2006). However, field data are still insufficient to determine whether genetic variation in susceptibility to V. destructor and V. jacobsoni exists in Thai A. cerana. Based on our results, it cannot be excluded that specific mite resistance genes differ systematically between Thai A. cerana populations. However, this is unlikely because it would be expected that these specific genes mix with the gene flow indicated by our microsatellite markers. Our results indicate that the island population of A. cerana on Samui is most likely to harbor some unique genetic material.

Loci were genotyped with primer pairs that were designed for A. mellifera (Solignac et al., 2007) and with some that were specifically designed for A. cerana (Takahashi et al., 2009). While the success rate of amplification was higher in the specific A. cerana primers, no significant differences existed between these two groups with regard to allele number, observed and

expected heterozygosity, or the ratio of observed to expected heterozygosity. Most loci showed a heterozygote deficiency, which could be explained by the substructure of the Thai A. cerana population. However, at least one locus (Ap019, located on chromosome two, group 2.35, in Amel4.0) showed a strong heterozygote excess, which may indicate selection in this genome region. The locus BI125 was also noticeable because it showed an exceptionally strong differentiation between sample groups, which could also indicate selection. In A. mellifera, this microsatellite is located on chromosome 12 (group 12.26).

In conclusion, this study suggests a fundamental, but partial differentiation between Asia Mainland and Sundaland A. cerana populations in Thailand based on data from 18 nuclear microsatellites. No strong evidence was found for a correlation between microsatellite genetic differentiation in A. cerana and their parasitic Varroa mite lineages that would have suggested resistance mechanisms based on local host–pathogen co-evolution. However, the possibility that a few specific genes mediating mite resistance differ systematically between Thai A. cerana populations cannot be excluded.

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