

Morphometric studies of *Apis cerana* in Thailand and the Malaysian peninsula

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SUMMARY

Samples of *Apis cerana* were collected from 44 locations in 12 regions of Thailand and peninsular Malaysia. Morphometric measurements were made on 58 characters. Statistical analysis showed that these samples could be separated into four groups: northern to central Thailand, southern Thailand to the end of the Malaysian peninsula, Samui Island and, less distinctly, Phuket Island. These differences support the interpretation that *A. cerana* has spread its range into south-east Asia in recent geological times.

Keywords: *Apis cerana*, eastern honey bee, morphometrics, Thailand, Malaysia

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INTRODUCTION

Modern beekeeping techniques together with the knowledge obtained from several years studying *Apis cerana* have enhanced the success of *A. cerana* beekeeping in the People's Republic of China (Wongsiri *et al.*, 1986). Selection and breeding have been done in Thailand in order to obtain improved strains of *A. cerana* for commercial beekeeping (Wongsiri, 1995; Pothichot, 1989; Wongsiri *et al.*, 1989).

Morphometric studies of *A. cerana* have been reported by a few authors (Kshirsagar, 1981; Mattu & Verma, 1983, 1984a, 1984b; Ruttner, 1985, 1988). Diniz-Filho *et al.* (1993) reanalysed Kshirsagar's (1981) data using spatial autocorrelation analysis. This first morphometric study of *A. cerana* samples from Thailand and the Malaysian peninsula was conducted to obtain quantitative information on *A. cerana* morphology. This information will help to delimit *A. cerana* ecotypes that may be useful in breeding applications. Further details of this study are provided in Limbipichai (1990).

MATERIALS AND METHODS

Samples from 128 colonies of *A. cerana* were collected from 44 locations in 12 regions from northern Thailand to southern Malaysia (table 1, fig. 1). The regions in Thailand were chosen on the basis that they are reasonably contiguous geographical areas which may have some relationship to ecological variation. Region 4 consisted of only one sample and was therefore excluded from the analyses. Ten bees from the inner cover or a comb of each colony were randomly collected as a

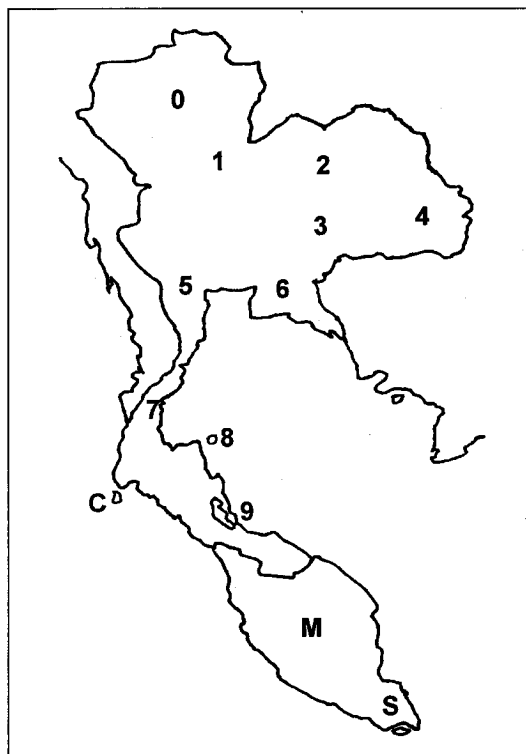


FIG. 1. Map of Thailand and the Malaysian peninsula showing the regions from which samples were collected. (0–6 = northern group (N); 7, 9, M, S = southern group (S); C = Phuket Island; and 8 = Samui Island).

TABLE 1. Sampling sites in Thailand and the Malaysian peninsula.

Region	No. of colonies	No. of locations	Province	Country
0	19 (17) ¹	6	Chiang Rai, Chiang Mai, Lamphun, Lampang, Phrae	Thailand
1	10	4	Uttaradit, Phitsanulok	Thailand
2	18 (14) ¹	7	Udonthani, Nongkhai, Khonkaen	Thailand
3	5	2	Nakhon Ratchasima	Thailand
5	26	7	Samutsongkhram, Ratburi, Phetchaburi	Thailand
6	20	7	Chonburi, Rayong, Chanthaburi	Thailand
7	5	2	Chumporn	Thailand
8	5	1	Suratthani (Samui Island)	Thailand
9	5	1	Songkhla	Thailand
C	5	1	Phuket	Thailand
M	5	2	Selangor	Malaysia
S	5	4	Johor	Malaysia

¹The numbers in brackets are the numbers of colonies analysed by canonical analysis, where this differs due to lost data

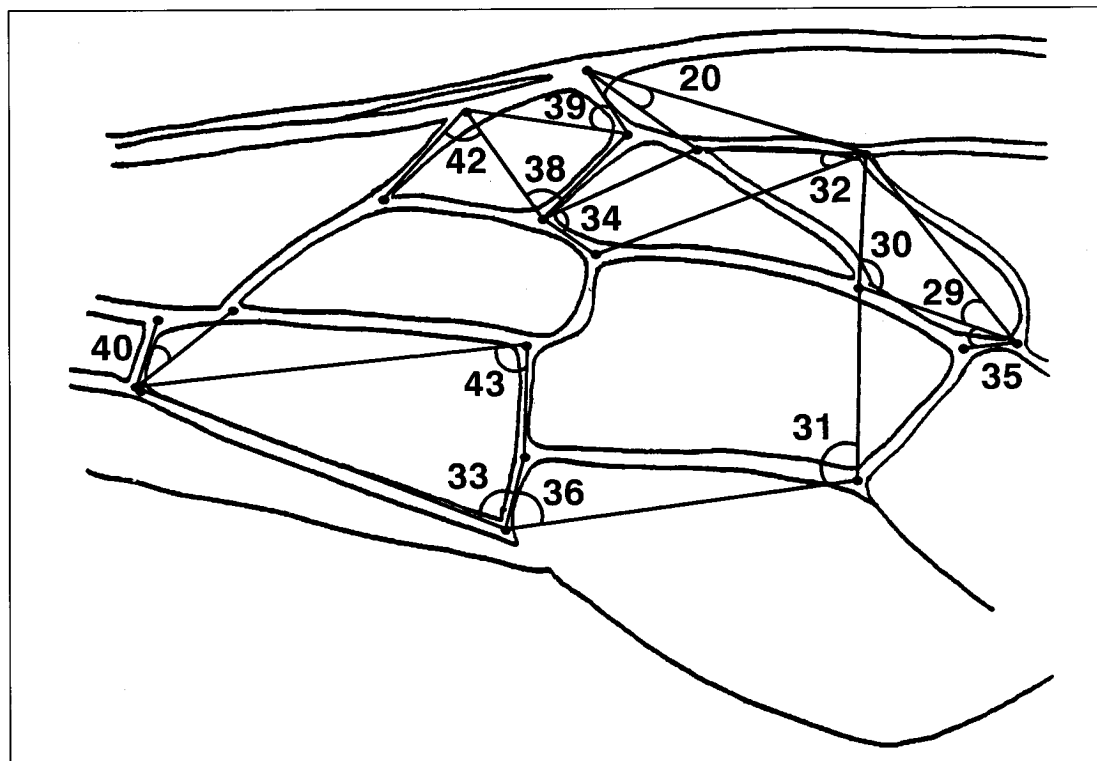


FIG. 2. Wing venation angles.

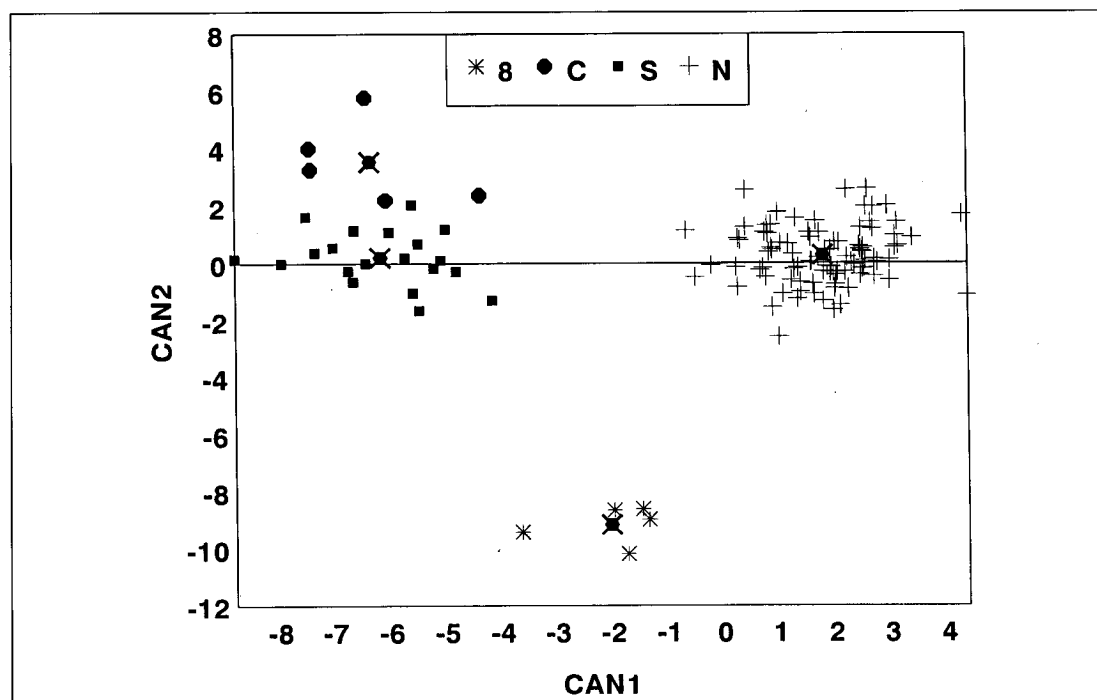


FIG. 3. Factors CAN1 \times CAN2 from a canonical discriminant analysis of 35 structural characters, excluding tongue characters, on 122 colonies of *Apis cerana* (N = northern, S = southern, C = Phuket Island and 8 = Samui Island).

TABLE 2. Characters of *Apis cerana* measured in Thailand and the Malaysian peninsula.

Variable	Character	Variable	Character
FWLN	fore wing length	ST6L	sternite 6 longitudinal
FWWD	fore wing width	ST6T	sternite 6 transversal
HWLN	hind wing length	PIG2	pigmentation of tergite 2
HWWD	hind wing width	PIG3	pigmentation of tergite 3
HAMU	hamuli	PIG4	pigmentation of tergite 4
AN20-AN43	wing angles 20–43 (fig. 2)	PIGS	pigmentation of sclerite S
TBLN	tibia length	PIGK	pigmentation of sclerite K
FELN	femur length	PIGB	pigmentation of sclerite B
TRLN	basitarsus length	CUBINDEX	cubital index (a/b)
TRWD	basitarsus width	LPALP	left labial palpus
STLN	sternite 3 length	RPALP	right labial palpus
WXLN	wax mirror length	TONGUE	tongue length
WXWDA	wax mirror width	FWINDEX	fore wing index = FWLN/FWWD
WXWDB	distance between wax mirrors	HWINDEX	hind wing index = HWLN/HWWD
POST	postmentum	TRINDEX	basitarsus index = TRLN/TRWD
GLOS	glossa + mentum	WXINDEX	wax mirror index = WXLN/WXWDA
LPSEG	labial palpi left proximal segment	LPINDEX	left labial palpus index = LPSEG/LDSEG
LDSEG	labial palpi left distal segment	RPINDEX	right labial palpus index = RPSEG/RDSEG
RPSEG	labial palpi right proximal segment	PIINDEX	labial palpi index
RDSEG	labial palpi right distal segment	TER3+4	TER3 + TER4
TER3	tergite 3 width	LEG	hind leg length = TBLN+FELN+TRLN
TER4	tergite 4 width		
TOMA	tomentum A		
TOMB	tomentum B		

sample and preserved in 70% ethanol. All samples were measured according to the computer-assisted procedures outlined by Daly *et al.* (1982). The 58 morphological characteristics considered, as listed in table 2, were measured and calculated for each bee. The characters examined were described by Daly and Balling (1978) and Ruttner (1988), except for the labial palpi that follow the description of Morimoto (1968).

The statistical procedures were done in two phases, morphometric character analysis and then grouping analysis by discriminant analysis using canonical variates (SAS, 1987). The character analyses were done on 128 colonies by two different statistical procedures: *F* test and Student-Newman-Keuls (SNK) multiple range test, to compare each character to find the differences among the 12 regions. Although the SNK test gives some protection against high type 1 errors on a per variable basis, applying this test to 58 variables may result in an inflated type 1 error rate. As a result, the results of the SNK should be viewed as exploratory in nature. Grouping analyses were conducted on 122 colonies by canonical discriminant analysis (SAS, 1987). The grouping analyses were done on only 35 structural

characters, excluding pigmentation, tongue characters and indices. The 35 characters used were chosen based on the results of the stepwise discriminant analyses. Although individual characters may not be distributed normally, the use of these multivariate techniques has been successfully applied to like characters in previous research on *Apis mellifera* using similar sample sizes (Rinderer *et al.*, 1990).

RESULTS

The SNK multiple range tests on the means of 58 characters among the 12 regions are given in tables 3, 4 and 5. The bees can be divided, by using a combination of body size and wing venation angles, into three groups, with a gradual gradient of each character from north to south. The bees from northern to central Thailand (table 1 and fig. 1: regions 0, 1, 2, 3, 5 and 6) constitute the 'northern bees', which are larger in size. The bees from southern Thailand to the end of the Malaysian peninsula (7, 9, M and S), including Phuket Island (C) constitute the 'southern bees'. The last group is the

TABLE 3. Results of the analysis of wing characters of *Apis cerana* in 12 regions of Thailand and the Malaysian peninsula.

Variable	F value	Highest mean (\pm s.d.)	Lowest mean (\pm s.d.)	SNK test among 12 regions ¹
FWLN	10.74**	7.07 \pm 0.09	7.50 \pm 0.09	<u>0 2 5 1 3 6 C 8 9 S M 7</u>
FWWD	8.52**	2.74 \pm 0.05	2.61 \pm 0.03	<u>0 5 1 2 3 6 8 C 9 S M 7</u>
HWLN	9.13**	3.74 \pm 0.06	3.55 \pm 0.06	<u>0 2 5 1 3 6 8 C 9 S M 7</u>
HWWD	8.29**	1.54 \pm 0.04	1.44 \pm 0.04	<u>5 1 0 2 3 6 8 C S 9 7 M</u>
CUBINDEX	1.92*	4.22 \pm 0.32	3.53 \pm 0.49	<u>8 9 0 1 2 S M 5 7 6 C 3</u>
FWINDEX	1.64ns	2.87 \pm 0.02	2.86 \pm 0.05	<u>9 C M S 6 7 0 2 8 5 3 1</u>
HWINDEX	4.74**	2.47 \pm 0.02	2.39 \pm 0.05	<u>M C 9 8 S 0 7 3 2 6 1 5</u>
HAMU	6.27**	17.81 \pm 0.75	16.10 \pm 0.61	<u>2 3 0 6 8 5 1 9 7 C S M</u>
AN20	1.44ns	14.67 \pm 0.72	13.82 \pm 0.53	<u>9 C 7 5 5 A M 3 1 6 2 0</u>
AN29	11.04**	33.88 \pm 0.62	30.75 \pm 1.13	<u>7 9 M C S 3 8 1 6 2 5 0</u>
AN30	4.29**	110.59 \pm 2.13	105.97 \pm 1.41	<u>5 0 2 6 1 3 C 8 S 7 9 M</u>
AN31	3.56**	98.67 \pm 1.59	94.48 \pm 1.51	<u>C M 7 9 3 8 S 0 5 1 2 6</u>
AN32	4.15**	21.49 \pm 0.66	19.87 \pm 0.57	<u>5 6 C 0 1 3 S 2 8 7 9 M</u>
AN33	2.10*	90.96 \pm 2.06	87.77 \pm 2.26	<u>M S 7 C 2 6 8 5 1 9 0 3</u>
AN34	3.69**	51.80 \pm 1.62	47.43 \pm 1.59	<u>M 7 1 9 8 6 C S 2 5 3 0</u>
AN35	2.46**	32.73 \pm 3.42	27.49 \pm 1.85	<u>S M 7 1 C 5 2 9 0 6 8 3</u>
AN36	1.16**	68.08 \pm 1.35	65.09 \pm 1.79	<u>9 0 3 1 6 5 C 8 7 2 S M</u>
AN38	1.57ns	99.81 \pm 1.07	97.36 \pm 1.49	<u>M 0 9 8 C 1 3 2 5 7 6 S</u>
AN39	3.26**	38.77 \pm 1.24	36.23 \pm 0.60	<u>7 9 S C M 5 8 1 6 2 0 3</u>
AN40	3.10**	35.16 \pm 1.04	30.28 \pm 1.44	<u>8 9 0 6 2 S C 1 5 M 7 3</u>
AN42	12.11**	112.56 \pm 0.63	107.19 \pm 1.20	<u>8 9 S 0 M 3 7 6 1 C 2 5</u>
AN43	4.73**	76.68 \pm 1.23	73.19 \pm 0.87	<u>2 M 6 S 0 1 3 5 9 7 C 8</u>

¹Regions in the same range are not significantly different ($\alpha = 0.05$)
**P < 0.01
*P < 0.05
ns = not significant at the 5% level

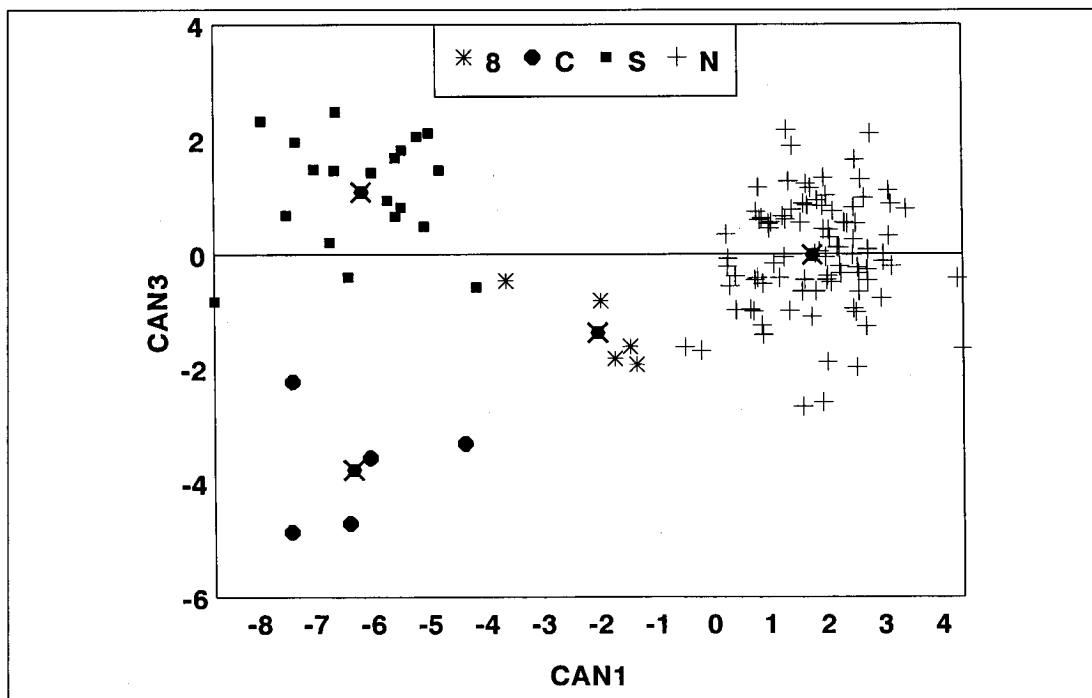


FIG. 4. Factors CAN1 \times CAN3 from a canonical discriminant analysis of 35 structural characters, excluding tongue characters, on 122 colonies of *Apis cerana* (N = northern, S = southern, C = Phuket Island and 8 = Samui Island).

bees from Samui Island (8), which are intermediate, but are more similar to the southern bees.

The canonical discriminant analysis showed that the bees from these 122 colonies can be discriminated into the same three groups given above, but that the bees from Phuket Island can also be separated into a fourth group. The scattergrams from the canonical discriminant analysis were plotted between canonical discriminant factors CAN1, CAN2 and CAN3. The scattergram of CAN1 \times CAN2 (fig. 3) shows three groups. Although the colonies from Phuket Island are grouped, they appear to be part of the southern group. However, the scattergram of CAN1 \times CAN3 (fig. 4) shows *A. cerana* from Phuket Island as a distinct group, with the bees of Samui Island forming a clear group intermediate in morphology between the southern and northern groups. An analysis of the Mahalanobis distances between the centroids of these four groups for all 35 variables revealed that each group was significantly different ($P < 0.0001$) from the other three groups. The Mahalanobis distances are: Samui Island to Phuket 185.55, to southern 110.57, to northern 105.19; Phuket Island to southern 34.74, to northern 90.59; and southern to northern 63.97.

Most of the variation in the 35 variables is correlated with CAN1. However, wing angles 40 and 42, and basitarsus length are primarily correlated with CAN2 and wing angle 38 and, to some extent, angle 43 are correlated with CAN3.

The northern bees have larger bodies, larger wings and longer legs than the bees from the other two groups (tables 3, 4 and 5). The hamuli of the hind wing were significantly fewer in the southern bees than in the northern bees (table 3). The Samui Island bees had a large number of hamuli like the northern bees, but a shorter hind wing like the southern bees.

The pattern of wing venation can be used to discriminate the bees. The northern bees were characterized by larger means of angles 29, 30 and 32 and smaller means of angles 31, 33 and 39 than the southern bees (table 3). These six angles were intermediate in the Samui Island bees. The Samui Island bees showed greater means in angles 40 and 42 and smaller means in angle 43. The Samui Island bees showed different hind leg proportions from the northern and southern bees as they have a relatively small tibia and femur, like the southern bees, but a relatively large basitarsus like the northern bees.

The labial palpi were longest in the bees from Johor (region S) while the labial palpi of the other southern bees are relatively shorter than those of the northern bees and the Samui Island bees (table 4). The labial palpi of the Samui Island bees are longer than those of the northern and southern bees (except the bees from Johor), which made the Samui Island bees different from the bees from the other groups. Although the Phuket Island bees are statistically distinguishable using

TABLE 4. Results of the analysis of the mouthpart and hind leg characters of *Apis cerana* in 12 regions of Thailand and the Malaysian peninsula.

Variable	F value	Highest mean (\pm s.d.)	Lowest mean (\pm s.d.)	SNK test among 12 regions ¹
TBLN	6.55**	2.83 \pm 0.04	2.72 \pm 0.04	<u>2 0 5 6 3 1 C S 9 M 8 7</u>
FELN	8.32**	2.24 \pm 0.03	2.15 \pm 0.03	<u>2 0 5 3 6 1 C S 9 M 7 8</u>
TRLN	1.76ns	1.72 \pm 0.01	1.70 \pm 0.02	<u>8 2 3 0 5 1 6 M S 9 C 7</u>
TRWD	7.36**	0.97 \pm 0.02	0.91 \pm 0.01	<u>2 8 3 5 6 0 1 M 9 S 7 C</u>
LEG	4.92**	6.77 \pm 0.09	6.54 \pm 0.09	<u>2 0 5 3 6 1 S C 8 9 M 7</u>
TRINDEX	6.35**	1.83 \pm 0.01	1.75 \pm 0.02	<u>C S 7 9 M 8 1 0 2 3 5 6</u>
POSI	16.50**	0.27 \pm 0.003	0.16 \pm 0.01	<u>S M 5 7 9 C 6 2 1 0 3 8</u>
GLOS	12.01**	4.26 \pm 0.19	3.17 \pm 0.10	<u>S 5 6 M C 2 1 0 3 8 7 9</u>
TONGUE	12.00**	4.52 \pm 0.19	3.41 \pm 0.10	<u>S 5 6 M C 2 1 0 3 8 7 9</u>
LPSEG	6.77**	1.14 \pm 0.05	1.04 \pm 0.02	<u>S 8 0 5 1 6 M 2 3 C 9 7</u>
LDSEG	7.94**	0.44 \pm 0.02	0.40 \pm 0.01	<u>S 6 5 0 1 8 2 3 7 M 9 C</u>
RPSEG	7.63**	1.16 \pm 0.05	1.04 \pm 0.02	<u>S 8 0 5 M 1 6 2 3 C 9 7</u>
RDSEG	9.07**	0.44 \pm 0.02	0.40 \pm 0.01	<u>S 5 6 2 0 1 3 8 7 M 9 C</u>
LPALP	8.74**	1.58 \pm 0.07	1.45 \pm 0.03	<u>S 8 0 5 6 1 2 M 3 C 9 7</u>
RPALP	8.78**	1.58 \pm 0.07	1.45 \pm 0.03	<u>S 8 0 5 6 1 2 3 M C 9 7</u>
LPINDEX	3.76**	2.68 \pm 0.04	2.52 \pm 0.08	<u>C M 8 S 9 0 1 2 5 3 6 7</u>
RPINDEX	6.71**	2.71 \pm 0.06	2.52 \pm 0.08	<u>8 C M S 9 0 1 6 2 5 3 7</u>
PIINDEX	5.74**	2.69 \pm 0.05	2.52 \pm 0.08	<u>C 8 M S 9 0 1 2 5 3 6 7</u>

¹Regions in the same range are not significantly different ($\alpha = 0.05$)

** $P < 0.01$

* $P < 0.05$

ns = not significant at the 5% level

multivariate techniques, no single character is distinctive for this island population (tables 3, 4 and 5).

DISCUSSION

The distinctness of the two island populations may be due to their long geographical isolation after sea levels rose and ended their connection to the mainland. This may have been since the end of the Pleistocene, 8000–10 000 years ago (Ruttner, 1988). Samui Island is much further from mainland Thailand (30 km) than is Phuket Island (2 km). The short distance separating Phuket probably allows swarms from the mainland to reach the island, but not with such frequency as would occur if it were a contiguous land area. It can be expected that there would be more gene flow from the mainland population to Phuket Island than to Samui Island resulting in a less distinct population.

The Samui Island bees show a quite distinct array of characteristics, which is not just a subset of the

southern range of characteristics. This indicates that they have evolved separately for some time, rather than simply being a recent introduction from elsewhere. Even though only five samples from Samui Island were examined, it is likely that their distinctness is representative of the population. This distinctness of the *A. cerana* population of Samui Island is supported by an analysis of DNA characteristics of Thai *A. cerana*, where the Samui Island RFLP pattern was found to be completely different from the other parts of Thailand (Pratum, 1994).

The distinctness of these island populations may be due to a founder effect from there having been only a few colonies which arrived on these islands through the time of their occupation by *A. cerana*. If *A. cerana* arrived in southern Thailand and Malaysia after these islands had separated from the mainland, natural or human-assisted colonization would likely have been by only a few colonies. If *A. cerana* arrived after Samui Island had been separated, but before Phuket Island was separat-

TABLE 5. Results of the analysis of the sternite and tergite characters of *Apis cerana* in 12 regions of Thailand and the Malaysian peninsula.

Variable	F value	Highest mean (\pm s.d.)	Lowest mean (\pm s.d.)	SNK test among 12 regions ¹
STLN	10.19**	2.26 \pm 0.05	2.13 \pm 0.05	<u>5 2 0 3 1 6 S C 8 M 9 7</u>
WXLN	5.93**	0.90 \pm 0.01	0.82 \pm 0.01	<u>3 2 0 1 C 5 6 M 8 5 9 7</u>
WXWDA	11.48**	1.87 \pm 0.03	1.74 \pm 0.02	<u>0 5 2 1 6 3 M C 8 9 5 7</u>
WXWDB	2.98**	0.32 \pm 0.03	0.26 \pm 0.01	<u>S 9 C 7 M 5 2 6 1 3 0 8</u>
WXINDEX	2.14*	0.49 \pm 0.004	0.47 \pm 0.01	<u>3 C 2 1 S 8 0 9 M 6 7 5</u>
TER3	2.72**	1.72 \pm 0.04	1.66 \pm 0.02	<u>5 S 2 0 1 6 3 M 9 8 C 7</u>
TER4	5.72**	1.71 \pm 0.04	1.62 \pm 0.02	<u>5 2 1 0 S 6 3 M 8 9 C 7</u>
TER3+4	4.17**	3.43 \pm 0.08	3.28 \pm 0.04	<u>5 2 S 0 1 6 3 M 8 9 C 7</u>
TOMA	9.08**	0.22 \pm 0.04	0.11 \pm 0.02	<u>5 3 0 2 1 8 C 6 S M 7 9</u>
TOMB	5.92**	0.92 \pm 0.03	0.80 \pm 0.05	<u>9 M 7 S 8 6 2 0 C 1 3 5</u>
ST6L	8.05**	2.08 \pm 0.05	1.96 \pm 0.04	<u>5 2 0 1 3 6 S C M 8 9 7</u>
ST6T	4.39**	2.47 \pm 0.05	2.34 \pm 0.04	<u>2 0 5 6 C 1 M 3 S 9 8 7</u>
PIG2	2.80**	7.39 \pm 0.50	6.96 \pm 0.13	<u>2 0 5 C 3 6 7 8 9 S M 1</u>
PIG3	2.09*	7.28 \pm 0.46	7.00 \pm 0	<u>2 0 5 1 3 6 7 8 9 C M S</u>
PIG4	1.94*	7.22 \pm 0.42	7.00 \pm 0	<u>0 5 1 3 2 6 7 8 9 C M S</u>
PIGS	2.78**	7.02 \pm 0.08	5.46 \pm 1.40	<u>3 8 7 5 0 2 C 6 9 M 1 S</u>
PIGK	3.11**	3.66 \pm 0.26	1.48 \pm 1.11	<u>8 3 7 6 5 0 2 C 9 1 M S</u>
PIGB	2.21*	2.32 \pm 0.46	0.78 \pm 0.38	<u>8 3 7 6 5 9 2 0 1 C S M</u>

¹Regions in the same range are not significantly different ($\alpha = 0.05$)
 ** $P < 0.01$
 * $P < 0.05$

ed, more founder colonies would have arrived on Phuket Island than on Samui Island. Thus, the founder effect would be more pronounced for Samui Island.

Rinderer *et al.* (1993) suggest that the overlap of drone flight times of *A. cerana* and *A. florea* in SE Thailand indicates that *A. cerana* may be a more recent arrival in SE Thailand.

An analysis combining results across the range of *A. cerana* may resolve the competing hypotheses regarding the spread of *A. cerana*. This will contribute to solving the conundrum of the sympatric occurrence of several generalist pollinators of the genus *Apis* in Asia.

Size differences between northern and southern bees may be explained by animal thermoregulation, where the surface area to volume ratio is reduced in cooler areas, and also by hydroregulation, where the surface area to volume ratio is reduced to preserve water content in dry climatic zones (Daly, 1985). The larger body size of the northern bees agrees with Bergmann's rule that, all else being equal, tropical animals should be

smaller than those living in the Arctic. But their larger wings and longer legs do not seem to agree with Allen's rule that extremities in Arctic species should be shorter than those in individuals inhabiting warmer areas (cited in Emlen, 1973). However, the northern area is both cooler and drier than the constantly moist, tropical rainforest of the southern area. Thus, the climatic gradient from northern Thailand to southern peninsular Malaysia is complex, rather than a simple change in temperature or rainfall. To the extent that these rules apply in such complex gradients, these climatic differences may account for the larger and longer extremities of the northern bees.

Diniz-Filho *et al.* (1993) were able to reanalyse the data of Kshirsagar (1981) using spatial autocorrelation analysis. Unfortunately, for this data set the choice of sampling sites was not made with sophisticated statistical analyses in mind, and insufficient collection data were gathered to allow analyses such as a canonical trend surface analysis (Diniz-Filho, 1995). In particular, data are not available on the elevation and exact latitude and

longitude in order to properly specify the microclimate at the collection sites. There is a significant amount of local climatic variation in the areas where the samples were collected, particularly due to elevation changes between valleys and mountains. Thus, there are probably at least some cases where colonies which are nearby geographically, but at significantly different elevations, are ecologically distinct. Unfortunately, this cannot be detected in this data set. When the 44 locations were individually taken as the groups and a discriminant analysis was performed on the pairwise squared distances between groups (not reported), locations often did not appear to be too similar to nearby locations. This indicates that the broad geographic trends in morphometric variation are complicated by local variation. The 12 sample groups based on contiguous geographic areas were therefore retained for these analyses.

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