

Distinctive hydrocarbons among giant honey bees, the *Apis dorsata* group (Hymenoptera: Apidae)

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Summary — Cuticular hydrocarbon pattern (CHP) analysis was performed on giant honey bees (the *Apis dorsata* group) including: 1), those occasionally given species status—Himalayan honey bees, Philippine honey bees, Sulawesi honey bees; 2), those separated since the Pleistocene—common *A dorsata* of the Indian and Asian lowlands and islands on the continental shelf (India and Sri Lanka, Thailand and Sumatra); and 3), giant honey bees of Borneo and Palawan, potential stepping-stones to the Philippines and Sulawesi. Four groups were found among giant honey bees by this CHP analysis. Most distinctive were those of Palawan and Nepal. The widespread lowland *Apis dorsata* differed very little among mainland and island populations, whereas those of Borneo, Sulawesi, and Philippines proper formed a single group. Those of the Himalayas appear to have diverged from *A dorsata*.

Apis dorsata / *Apis laboriosa* / systematics / hydrocarbon / gas chromatography

INTRODUCTION

Taxonomic problems having evolutionary implications and applied importance abound in honey bees (Ruttner, 1988; Roubik, 1989). Geographic races or subspecies, although often purely artificial in taxonomic work, have special value in studies of organisms such as honey bees because they may lead to recognition of bionomic characteristics that differ among populations (Mayr, 1970; Ruttner, 1988).

The giant honey bees, formerly subgenus *Megapis* (Ruttner, 1988) apparently contain cryptic species, notably *Apis laboriosa*, the Himalayan honey bee (Maa, 1953; Sakagami *et al*, 1980; Roubik *et al*, 1985; McEvoy and Underwood, 1988). New species have also been confirmed for other Asian honey bees. *Apis andreniformis* (F Smith, 1858), proved to be distinct from the widespread "little honey bee" *A florea* (Wu and Kuang, 1986; Roubik, 1989). Also, a distinctive Saban honey bee, *Apis*

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koschevnikovi (Buttel-Reepen, 1906), is found in northeastern Borneo and shares its habitat with the closely similar *Apis cerana* (Koeniger *et al*, 1988; Tingek *et al*, 1988). These last 2 species (*A koschevnikovi* was called *Apis vechti*) not only have distinctive male genitalic structure but also different times of mating flight activity—both features that would prevent interspecific mating. Recently, McEvoy and Underwood (1988) reported no differences in the male endophallus of Himalayan honey bees and the common giant honey bee *Apis dorsata*, but believed they could be distinct species. The Himalayan honey bee and other populations of giant honey bees are still under study because we do not know which represent races and which are species. As a step toward definitive analysis, we studied the giant honey bees using a chemoanalytical technique that can be applied to dead pinned museum specimens without damaging them—cuticular hydrocarbon pattern analysis (Carlson and Bolten, 1984; Francis *et al*, 1985; Lockey, 1988; Smith, 1988). Based on the analysis presented here, we conclude that 4 natural groups and at least 2 species may exist. These hypotheses are corroborated by a biogeographic analysis.

MATERIALS AND METHODS

The names of candidate giant honey bee species first suggested by Maa (1953) will appear within quotation marks throughout the present text. Pinned specimens of worker bees are now in the collection of DWR or SF Sakagami, and were supplied from the collections of CK Starr (Philippine bees), B Sutton (Borneo bees), SF Sakagami (Palawan, Nepal, Sulawesi bees), or were collected by DWR. These specimens included workers collected up to 18 yr before our analysis. The bees, identified by SF Sakagami, were from: central Nepal (*A 'laboriosa'* from Kalli La, Drandikhola, Trubkin Kharka, Tukucha Palpa and Tharepati-Melemchi), Sulawesi (for-

merly Celebes; *A 'binghami'* from Minahassa; Dumoga), the Philippine Islands (*A 'breviligula'* from Laguna: Los Banos, and Leyte: Visca). Samples of *Apis dorsata* were collected from much of its geographic range and several islands and included: 1), Palawan Islands (collected by SF Sakagami and T Inoue); 2), Sabah, Borneo (collected by B Sutton); 3), New Delhi, India (DWR); Chaing Rai, northern Thailand (DWR); Sri Lanka; Monaragala (DWR), and Sumatra; Padang, western Indonesia (DWR). From 4 to 13 bees were assayed from each giant honey bee group (table I), ie a total of 41 bees. All were collected at flowers or had flown to lights at night, making it likely that they were older bees. This is important for cuticular hydrocarbon study, since younger bees, those that do not leave the nest or forage, have different chemical traits than older worker bees (D Carlson, unpublished observations).

The identification of 3 classes of cuticular hydrocarbons—alkanes, alkenes, and methyl-branched alkanes—was made solely on the basis of gas chromatograph retention times. Following the methods of Carlson and Service (1980) lipids were extracted from individual bees during overnight immersion in hexane, and then fractionated by liquid chromatography using small columns made from disposable 0.4 x 5.0 cm pipettes packed with silica gel. The hydrocarbon fraction of each extract was eluted with hexane, then subjected to gas chromatography. A varian Model 3700 flame ionization instrument was used, fitted with a DB-1 fused silica capillary column (15 m length x 0.32 mm internal diameter; J & W Scientific, Folsom, CA). An OCI-3 glass on-column injector (Scientific Glass Engineering Inc, Austin, TX) was used in the splitless mode with the column oven at 40 °C. It was then temperature-programmed from 40 °C to 320 °C, at a 20 °C increase per min. The samples were evaporated to dryness and taken up in 50 µl of hexane solvent just before the injection of one µl. The gas chromatograph was coupled through a 760-series interface and a Nelson Analytical system to an IBM PC/XT computer, an Epson FX80+ printer, and a Hewlett-Packard 7470A plotter for data output. The column had ≈ 60 000 theoretical plates with a C13 *n*-alkane standard. The samples were the equivalent of 2% of the amount obtained from one bee. Each was injected together with alkane standards for determination of Kovats retention indices (KI) (Kovats, 1966). KI values were con-

Table I. Composition of hydrocarbons of giant honeybee workers: mean percentage composition (\pm SD).

KI	Compound class ^a	A dorsata (P) ^b n = 5 ^c	A dorsata (B) ^c n = 6	A dorsata (I) ^d n = 5	A 'labriosa' n = 13	A 'binghami' n = 4	A 'breviligula' n = 8
2275	23:1	0.3 (\pm 0.1)	4.0 (\pm 2.8)	0.4 (\pm 0.5)	0.4 (\pm 0.3)	0.8 (\pm 0.4)	0.4 (\pm 0.2)
2300		1.7 (\pm 0.0)	7.9 (\pm 3.2)	1.7 (\pm 1.6)	5.2 (\pm 1.7)	9.0 (\pm 3.9)	2.8 (\pm 1.8)
2475	25:1	2.1 (\pm 0.1)	8.6 (\pm 2.8)	0.8 (\pm 0.7)	13.5 (\pm 6.0)	7.1 (\pm 3.0)	6.2 (\pm 4.2)
2500		11.0 (\pm 1.2)	18.2 (\pm 3.9)	15.7 (\pm 5.3)	40.6 (\pm 15.9)	17.8 (\pm 6.5)	20.1 (\pm 6.3)
2535	25A	2.1 (\pm 0.1)	0.1 (\pm 0.04)	1.4 (\pm 0.8)	1.5 (\pm 0.8)	0.9 (\pm 0.2)	0.9 (\pm 0.5)
2675	27:1	3.9 (\pm 0.3)	7.0 (\pm 1.1)	1.2 (\pm 0.6)	6.5 (\pm 3.1)	6.0 (\pm 0.8)	6.4 (\pm 3.2)
2700		11.2 (\pm 2.2)	15.3 (\pm 1.9)	18.4 (\pm 7.9)	14.0 (\pm 5.4)	8.1 (\pm 1.7)	18.5 (\pm 8.4)
2735	27A	7.8 (\pm 0.6)	2.1 (\pm 0.9)	4.3 (\pm 1.8)	0.7 (\pm 0.5)	3.3 (\pm 1.8)	3.2 (\pm 1.3)
2752	27B	1.0 (\pm 0.3) ^c		0.9	0.1	0.3	0.2
2875	29:1	6.3 (\pm 0.3)	2.9 (\pm 0.3)	0.9 (\pm 0.5)	1.9 (\pm 1.8)	8.2 (\pm 3.1)	2.7 (\pm 2.1)
2900		4.1 (\pm 0.8)	5.8 (\pm 1.7)	7.4 (\pm 2.9)	2.9 (\pm 1.5)	9.5 (\pm 1.7)	8.2 (\pm 3.7)
2935	29A	9.6 (\pm 0.6)	3.1 (\pm 1.6)	7.3 (\pm 1.4)	0.2 (\pm 0.1)	7.4 (\pm 3.4)	4.5 (\pm 1.8)
2952	29B			1.5	0.1	0.3	0.2
3075	31:1	6.6 (\pm 0.3)	1.9 (\pm 0.6)	0.8 (\pm 1.2)	0.8 (\pm 1.2)	2.6 (\pm 1.1)	2.1 (\pm 2.3)
3100		2.5 (\pm 0.4)	2.9 (\pm 1.0)	6.3 (\pm 1.0)	0.9 (\pm 0.9)	2.5 (\pm 1.0)	2.0 (\pm 1.2)
3135	31A	8.0 (\pm 0.6)	3.8 (\pm 1.6)	10.1 (\pm 3.7)	0.5 (\pm 0.4)	4.0 (\pm 2.6)	3.8 (\pm 1.5)
3152	31B	2.0		1.5	0.1	0.3	0.2
3243	33:2	0.7 (\pm 0.1)	0.3 (\pm 0.1)	0.8 (\pm 0.3)	0.3 (\pm 0.3)	0.3 (\pm 0.2)	0.5 (\pm 0.4)
3265	33:1	2.9 (\pm 0.5)	1.0 (\pm 0.3)	2.6 (\pm 1.0)	1.4 (\pm 1.6)	0.7 (\pm 0.6)	1.5 (\pm 0.9)
3300		0.6 (\pm 0.2)	0.9 (\pm 0.3)	0.5 (\pm 0.2)	0.4 (\pm 0.3)	0.2 (\pm 0.2)	0.7 (\pm 0.4)
3443	35:2	0.2 (\pm 0.1)	0.1 (\pm 0.02)	0.2 (\pm 0.1)	0.3 (\pm 0.5)	0.1 (\pm 0.0)	0.2 (\pm 0.2)
3465	35:1	0.4 (\pm 0.2)	0.3 (\pm 0.1)	0.7 (\pm 0.3)	1.0 (\pm 1.4)	0.1 (\pm 0.1)	1.4 (\pm 0.7)
3643	37:2	**	0.2 (\pm 0.06)	**	0.2 (\pm 0.4)	**	0.2 (\pm 0.2)
3665	37:1	**†	0.2 (\pm 0.07)	**	0.4 (\pm 0.5)	**	0.8 (\pm 0.4)

^a 33:1 = alkene; 33:2 = alkiadiene; 33A = internal monomethylalkane; 33B = internal dimethylalkane; ^b Palawan; ^c Borneo; ^d DWR collections from India, Sri Lanka, Thailand, Sumatra. ^e Number of samples; [†] none detected.

verted to equivalent chain lengths (ECL) by dividing by 100. The bee specimens used here were undamaged by hydrocarbon extraction. Five ratios were derived for each specimen by dividing the recorded integrated areas of 2 selected alkenes (2 475 and 2 675 retention times), and 3 methyl alkanes (2 735, 2 935, 3 135) by the area of the 2 700 alkane peak. The areas were calculated for the peaks printed out by the plotter, as shown in the following results.

Precautions were taken to screen for the extraction technique and possible effects of other hydrocarbons found on the bodies of worker bees. Pollens on the bodies of *Apis mellifera* (outside of the load carried in the corbicula) were found to contribute insignificant amounts of hydrocarbon compared to that extracted from a bee specimen. The quality of hydrocarbons as shown by GC patterns did not appear to differ appreciably between honey bees extracted overnight in hexane, compared to nest mates that were placed in hexane for 10 min.

RESULTS

Visual comparison

Alkanes, alkenes and internally methyl-branched alkanes in each sample contained 23 to 36 carbons (fig 1a-f). In general, the bees exhibited variation in 4 sets of triple peaks, with the center peak always an *n*-alkane of 25, 27, 29 or 31 carbons. In addition, a series of larger alkenes was found, having 34- to 37-carbon "backbones" (KI 3 365 to 3 665). A series of alkadienes with the same 'backbone' (KI 3 343 to 3 643) was also found in the descendants of African honey bees in Venezuela (Carlson and Bolten, 1984). Each of the bee groups we examined was evaluated as follows:

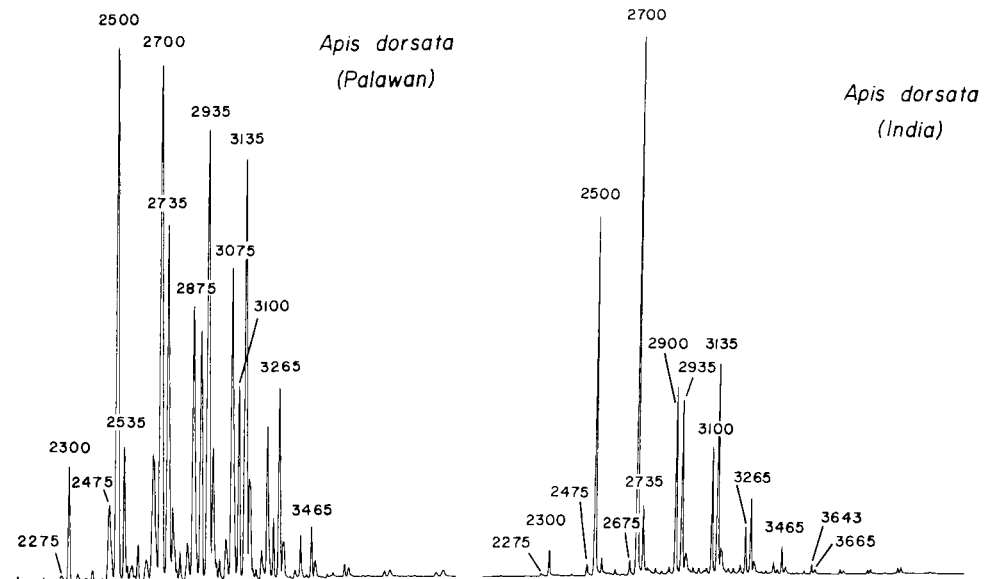
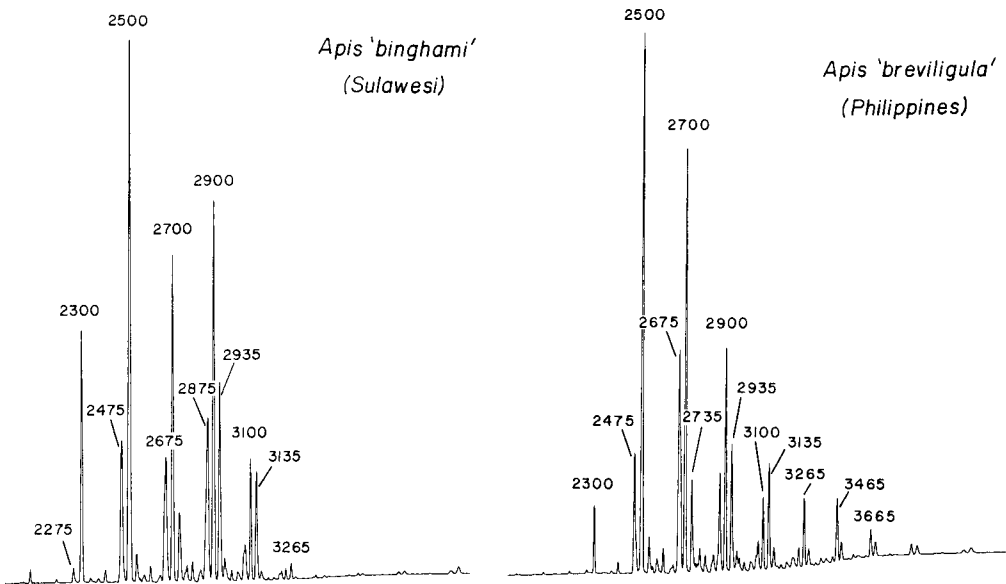
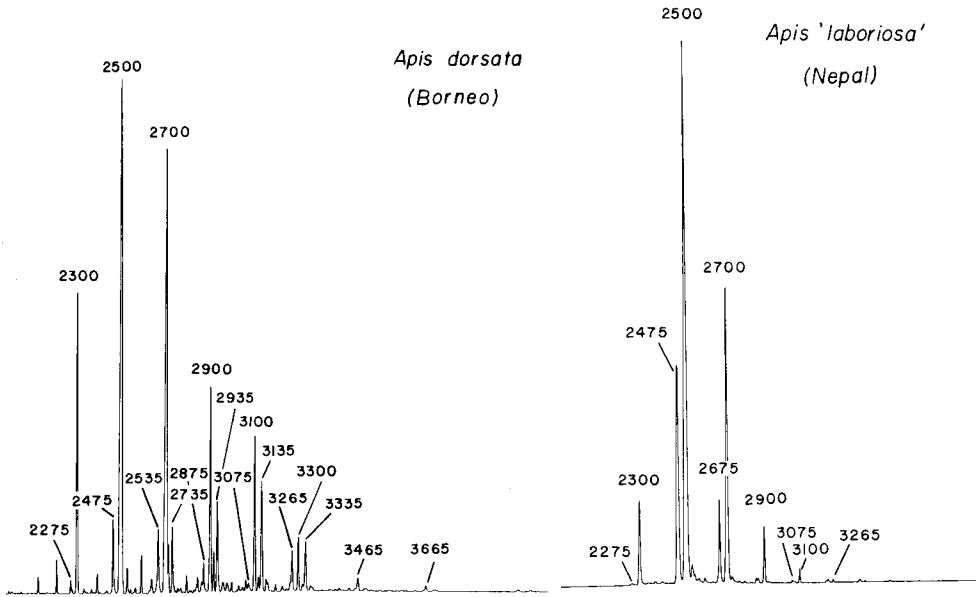


Fig 1. Gas chromatograms of extracted hydrocarbons from individual workers of giant honey bees from : (a) Palawan; (b) northern India; (c) Borneo; (d) Nepal; (e) Sulawesi; (f) the Philippines proper.



Apis dorsata

The 5 worker bees from Palawan exhibited consistent CHPs, comprised primarily of 4 sets of triple peaks with a smaller fourth peak (fig 1a). The Kovats retention indices of 2 675, 2 700 and 2 735 here correspond to respective alkene, *n*-alkane and internally methyl-branched alkane peaks in all the triplets. The third peak of such triplets was nearly as large or larger than the center peak at KI 2 735, 2 935 and 3 135. The alkene peak was the smallest in triplets of carbon numbers up to KI 2 675 but was the second largest peak in the triplet at KI 3 075. Two alkenes (KI 3 075 and 3 265) were double in composition in Palawan bees; compared to those found in the other *A dorsata* (9.5% compared to 1.5 to 5%, respectively). The patterns from the Bor-

neo bees (fig 1c and fig 2) were substantially similar to those of the Philippine bees and Sulawesi, but unlike those of the Asian mainland or Palawan. The CHPs of bees from Thailand, Sri Lanka, and India were very similar to each other (see Indian bee, fig 1b), and to an *A dorsata* worker from Pakistan (Francis *et al*, 1985).

Apis 'laboriosa'

Thirteen bees exhibited doublets of alkanes and alkenes at KI 2 500 to 2 900, as the methyl-branched alkanes were consistently minor peaks. More complex patterns were seen at KI 3 100 to 3 200 (fig 1d). The quantities of alkenes in the peaks at KI 2 475 and 2 675 were consistently the largest in this bee.

Composition of Hydrocarbons

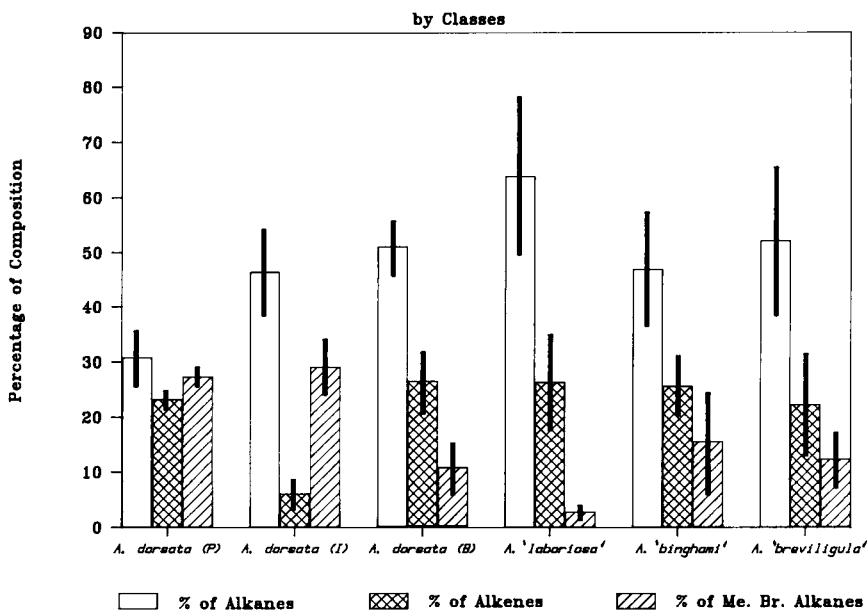


Fig 2. Composition of hydrocarbon classes from giant honey bees. Bars show standard deviations from samples of 4–13 bees of each kind (see table I). *Apis dorsata* from 3 localities are shown: (P) = Palawan; (I) = northern India; (B) = Borneo.

Apis 'binghami'

Four sets of triple peaks appeared in the CHP of the 4 bees that were analyzed (fig 1e). The triplets contained nearly constant and then gradually decreasing proportions of alkenes from KI 2 475 to 3 075 and showed a dominating alkane. Increasing proportions of methyl alkanes occurred at KI 2 535, 2 735, and 2 935, with only the KI 3 135 peak averaging larger than the alkane.

Apis 'breviligula'

The 8 bees had CHPs most similar to those of *A. binghami*, showing 4 repeated sets of triple peaks, probably containing similar compounds (fig 1f). Neither the alkenes nor the methyl-alkane peaks of each triplet were larger than the alkane, except for the KI 3 135 peak.

HYDROCARBON COMPOSITION BY CLASSES

Comparisons of all 6 groups (fig 2) show that each of the 3 major classes of hydrocarbon components can be combined to yield a CHP profile and standard error. They give the summed proportion of *n*-alkanes, alkenes and methyl-branched alkanes presented in table I, which considers the average composition of 24 distinct hydrocarbons for each bee group. Another representation of the data is given in figure 3, emphasizing the individual compounds of the 3 hydrocarbon classes. This graph in particular shows that the bees of Palawan are distinct from those of mainland Asia, while the bees of Borneo are close to *Apis* 'breviligula', *A. binghami* and also *A. laboriosa*. The relatively large distance between *A. laboriosa* and the *A. dorsata* from India is particularly clear in figure 3. The Himalayan honey bee CHP patterns show an excess of alkenes and a general

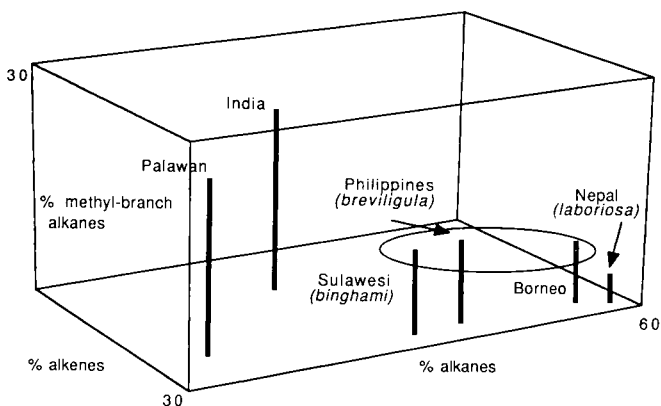


Fig 3. Clustering of giant honey bees by cuticular hydrocarbon traits (see *Results*). The circled groups include bees from Sulawesi, Philippines proper and Borneo, which were found by statistical analysis to share traits more closely than indicated by the percentages of 3 classes of compounds in figure 2.

Table II. Peak ratios from hydrocarbon composition in the cuticle of giant honeybee workers, (\pm SD).

Peak ratios	A dorsata Palawan	A dorsata Borneo	A d dorsata	A 'laboriosa'	A 'inghami'	A 'breviligula'
R1*	0.2 (\pm 0.1) a	0.6 (\pm 0.3) ab	0.1 (\pm 0.1) a	1.2 (\pm 0.9) b	1.0 (\pm 0.6) ab	0.5 (\pm 0.5) ab
R2	0.4 (\pm 0.1) ab	0.5 (\pm 0.1) ab	0.1 (\pm 0.1) a	0.5 (\pm 0.3) b	0.8 (\pm 9.3) b	0.5 (\pm 0.4) ab
R3	0.7 (\pm 0.1) a	0.13 (\pm 0.04) bc	0.4 (\pm 0.1) b	0.1 (\pm 0.0) c	0.4 (\pm 0.2) b	0.2 (\pm 0.1) bc
R4	0.9 (\pm 0.2) a	0.2 (\pm 0.08) b	0.6 (\pm 0.2) ab	0.01 (\pm 0.0) c	0.9 (\pm 0.4) a	0.3 (\pm 0.2) b
R5	0.7 (\pm 0.2) a	0.2 (\pm 0.08) c	0.7 (\pm 0.3) a	0.04 (\pm 0.0) b	0.5 (\pm 0.3) ac	0.3 (\pm 0.2) c

* R1 = 2475/2700; R2 = 2675/2700; R3 = 2735/2700; R4 = 2935/2700; R5 = 3135/2700. Means not followed by the same letter are significantly different from each other at the 1% level of significance by the one-way ANOVA F-test.

lack of branched alkanes. The individual bees, taken from a variety of localities, were quite uniform in these traits. The *n*-alkanes appear to have some chemotaxonomic value, despite their variability due to diet (Smith, 1988). In addition, the mainland and island localities of the typical lowland, continental *A dorsata* (Sumatra, Sri Lanka, northern India, northern Thailand) all showed highly consistent CHP traits, with little more variance than bees all from a single locality (eg *A dorsata* from Palawan or *A 'binghami'*; see table I).

Peak ratios

The second analytical technique applied to the CHPs was that comparing areas under some of the peaks shown in figure 1. Numerical and statistical methods could thus be applied (table II). The 5 peaks selected for the analysis (see *Methods*) allowed 5 comparisons of peak ratios or 'R values'. This analysis produced a picture of group similarities and differences very similar to that given in figure 3 with some modifications. First, the bees of Borneo and those of the Philippines had exactly the same peak ratios, and those of the Borneo and the Sulawesi bees were also quite similar. Second, the Himalayan honey bees shared none of the 5 traits with the typical lowland *A dorsata*, and relatively few either with Sulawesi or with Palawan bees. In addition, most paired comparisons from table II show that about half of the traits were shared between any 2 bee groups (two-thirds of all comparisons), while the remainder had either very high or very low similarity. These statistical results are added to figure 3 by grouping '*binghami*', '*breviligula*' and *A dorsata* from Borneo in a single cluster.

DISCUSSION

The chemical classes described here were determined from retention times because of the similarity of these components to those present in *Apis mellifera*. Honeybee hydrocarbons have been extensively studied by mass spectrometry, and include internally branched monomethyl alkanes and internally dimethyl branched alkanes (McDaniel *et al*, 1984), and a similar series of alkenes and alkadienes (Carlson *et al*, 1989). In the latter work, the positions of unsaturation were variable in alkene mixtures found in each peak. In compounds with the same backbone, double bonds were located mostly at 7- and 9- below C29, and 8-, 10- and 12- in longer alkenes. These compounds have slightly different retention times (Smith, 1988) and are separated from each other on a high resolution column, which may split each alkene peak. Here, using a short column of less resolving power, only the center of each peak is measured and reported.

The study reveals 4 groups among the giant honey bees (fig 3). These are: 1), the widespread lowland *Apis dorsata*, which our data suggest consists of bees on the continent and on islands connected to the mainland during glaciations and lowering of the sea level (Audley-Charles, 1981); 2), the giant honey bees of the Philippines proper, Sulawesi and Borneo, between which many animals and plants have migrated (Heaney, 1986; Whitmore, 1987); 3), the giant honey bees of the Himalayas; and 4), giant honey bees on the Philippine island of Palawan, which lies between northeastern Borneo and the Philippines proper. Island-hopping and migration during the Pliocene and Pleistocene glaciations probably permitted gene exchange, resulting in (1) and (2).

Conservatively, bees of group (2) might be considered a single geographic race (*A dorsata binghami* by the rule of taxonomic priority) reflecting their past distribution rather than current isolation. However, at present it appears that any of the giant honey bees east of Wallace's line might be specifically distinct from *Apis dorsata*.

Biochemical variation among the highly distinctive bee species, *A cerana*, *A dorsata* and *A florea* (Francis *et al*, 1985; Sheppard and Berlocher, 1989) is apparently greater than that detected here among the *A dorsata* group. The biochemical variation detected within our *A dorsata* collection is similar to that seen among subspecies of *A mellifera* (Sylvester, 1986; Sheppard and Huettel, 1988). These studies do not, however, establish a general criterion for differentiating honey bee species from races, and no biochemical comparisons have been published for the species pairs formerly regarded as cryptic: *A florea* and *A andreniformis*, and *A cerana* and *A koschevnikovi*. Until such comparisons are made, biogeographic analysis can guide additional interpretation of the biochemical variation and evolution of giant honey bees.

Historical biogeography suggests that inter-island dispersal by giant honey bees beyond continental southeast Asia has occurred rarely since the upper Pliocene (3 million yr before the present), and genetic exchanges between Himalayan populations and those of adjacent lowlands were likely even less frequent. Glacial periods have constituted $\approx 90\%$ of the past 3 million yr (Graham, 1986). The global drops in temperature during glaciations likely produced a greater separation between Himalayan honey bees and lowland *A dorsata* than that seen today. Cooler temperatures in the Nepalese foothills and adjacent areas likely pushed tropical *A dorsata*

farther south. Evolution in allopatry and strong natural selection for traits allowing survival in a cold or temperate climate may have led to species formation for *A 'laboriosa'*. Similar divergence of a highland species from a widespread lowland species has occurred in another eusocial bee, a *Melipona* of the Peruvian Andes, although male genitalic differences have also evolved there (Nates and Roubik, 1990). Weak sympatry between *laboriosa* and *dorsata* has been reported from Assam (Sakagami *et al*, 1980), but in Nepal and northern India the 2 giant honey bee varieties appear to be strictly allopatric (Ruttner, 1988). There are still no known hybrids and we doubt that fertile and viable hybrids exist in nature.

ACKNOWLEDGMENTS

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Résumé — Différences dans la composition en hydrocarbures au sein des abeilles géantes (groupe *Apis dorsata*, Hymenoptera, Apidae). Le spectre des hydrocarbures cuticulaires (CHP) a été analysé chez les abeilles géantes (groupe *Apis dorsata*) afin d'obtenir des informations sur leur taxonomie et leur évolution. Quatre à 13 abeilles ont été analysées par groupe d'abeilles géantes. Celles-ci comprenaient :

– des abeilles ayant parfois reçu le statut d'espèce—abeilles de l'Himalaya, des Philippines et de Sulawesi (Célèbes);

– l'abeille géante «commune», répandue en Inde, dans les régions basses d'Asie continentale et dans les îles du plateau continental (Inde et Sri Lanka, Thaïlande et Sumatra);

– des abeilles géantes de Bornéo et de Palawan, intermédiaires possibles avec celles des Philippines et de Sulawesi (tableau I).

Trois techniques analytiques ont été employées en fonction des temps de rétention de la chromatographie en phase gazeuse : analyse visuelle des chromatogrammes (fig 1a–f), analyse d'après le pourcentage de 3 classes d'hydrocarbures (alkanes, alkènes et méthyl-alkanes, fig 2) et «rapport des pics», où les surfaces situées sous 5 pics du chromatogramme sont comparées à un pic standard (tableau II). Cette nouvelle méthode analytique, qui permet d'utiliser des spécimens de musée tout en les conservant intacts, a montré que :

– il existe 4 groupes parmi les abeilles géantes (fig 3);

– les groupes les plus distincts sont ceux de Palawan et de l'Himalaya;

– les *Apis dorsata* du continent et des îles proches sont très peu différentes; et

– celles de Bornéo, du Sulawesi et des Philippines forment un seul et même groupe (figs 2 et 3).

Des réflexions d'ordre biogéographique et les spectres CHP laissent penser qu'*Apis laboriosa* a fortement divergé des abeilles géantes des régions basses et existe maintenant en tant qu'espèce biologique distincte. Le statut d'espèce est également possible pour les abeilles géantes de Palawan et de Bornéo–Sulawesi–Philippines.

***Apis dorsata* / *Apis laboriosa* / systématique / hydrocarbure / chromatographie en phase gazeuse**

Zusammenfassung — Unterschiede in den Hydrocarbonen innerhalb von Riesenhonigbienen, der *Apis dorsata*-Gruppe (Hymenopteren-Apiden). Es wurden Analysen des Hydrocarbon-Musters (CHP) der Cuticula (Körperdecke) von Riesenhonigbienen (der *Apis dorsata*-Gruppe) durchgeführt, um Einblick in ihre Taxonomie und Evolution zu erhalten. Vier bis 13 Arbeiterinnen pro Gruppe umfaßten folgende Herkünfte: 1.- Bienen, denen manchmal der Status einer eigenen Art gegeben wurde - Honigbienen vom Himalaya, Honigbienen von den Philippinen und von Sulawesi (Celebes); 2.- Die "gewöhnliche" Riesenhonigbiene, allgemein verbreitet im Tiefland von Indien und dem asiatischen Kontinent, sowie auf den Inseln des Kontinentalschelfs (Indien und Sri Lanka, Thailand und Sumatra); 3.- Riesenhonigbienen von Borneo und Palawan, mögliche Zwischenglieder nach den Philippinen und Sulawesi (tab I).

Es wurden drei analytische Techniken zur Beurteilung der Resultate der Retentionszeiten am Gaschromatographen angewandt: Visuelle Inspektion der Chromatogramme (fig 1a-f), Analyse nach dem Prozentsatz von drei Hydrocarbon-Klassen (Alkane, Alkene, methylverzweigte Alkane; fig 2) und "Peak-Verhältniswerte", bei welchem Verfahren die Flächen unter fünf Chromatogramm-Peaks mit einem Standard-Peak verglichen werden (tab 2).

Mit dieser neuen Technik, die auch an Museumsexemplaren angewandt werden kann, weil sie diese intakt läßt, wurde folgendes festgestellt: 1.- Es existieren innerhalb der Riesenhonigbienen vier Gruppen (fig 3); 2.- am stärksten verschieden sind die Riesenhonigbienen von Palawan und

vom Himalaya; 3.- *Apis dorsata* vom Festland und von den nahegelegenen Inseln sind nur sehr wenig voneinander verschieden; 4.- die Bienen von Borneo, Sulawesi und von den Philippinen selbst bilden eine einzige Gruppe (fig 2, 3). Biogeographische Überlegungen und die Daten vom CHP-Muster lassen vermuten, daß *Apis laboriosa* beträchtlich von der Riesenhonigbiene des Tieflands abweicht und jetzt als eigene biologische Art existiert. Den Rang einer Art besitzen möglicherweise auch die Riesenhonigbienen von Palawan und von Borneo - Sulawesi und den eigentlichen Philippinen.

***Apis dorsata* / *Apis laboriosa* / Taxonomie / Hydrocarbone / Gaschromatographie**

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