The wing coupling apparatus and the morphometric analysis of honeybee populations

H.R. Hepburn[a*] and S.E. Radloff[b]

Significant differences between countries were found in the distribution of the number of hamuli within *Apis andreniformis, A. florea, A. cerana* and *A. koschevnikovi.* The mean hamuli numbers for *Apis mellifera intermissa* differed significantly among localities in Algeria. Significant differences in intercolonial variability between countries were found within *A. cerana.* There was no significant infraspecific variability within *A. andreniformis*, *A. florea*, *A. koschevnikovi* and *A. m. intermissa.* Significant differences in the mean number of hamuli occur between *A. m. intermissa* and *A. andreniformis, A. florea* and *A. cerana*; also between *A. cerana*/*A. koschevnikovi* and *A. andreniformis* and *A. florea.* Significant differences were found in the distribution and variability of the number of hamuli between species (populations). The mean numbers of hamuli for *A. andreniformis* differed from those of *A. florea.* Both these population means differed from those of *A. cerana, A. koschevnikovi* and *A. m. intermissa.* No significant differences were found between *A. cerana* and *A. koschevnikovi.* When the analysis included data for *A. dorsata, A. nigrocincta, A. m. carnica, A. m. caucasica* and *A. m. ligustica*, the results showed significant differences in hamuli numbers between *A. andreniformis*/*A. florea* and *A. cerana*/*A. koschevnikovi*/*A. nigrocincta* and *A. m. intermissa*/*A. m. carnica*/*A. m. caucasica*/*A. m. ligustica.* Hamuli numbers in *A. dorsata* significantly differed from those of other populations except *A. m. intermissa.* These results show that hamuli numbers are useful in the classification of honeybee populations. Whether hamuli would be useful in multivariate analysis depends on the correlation between the number of hamuli and the other characters used.

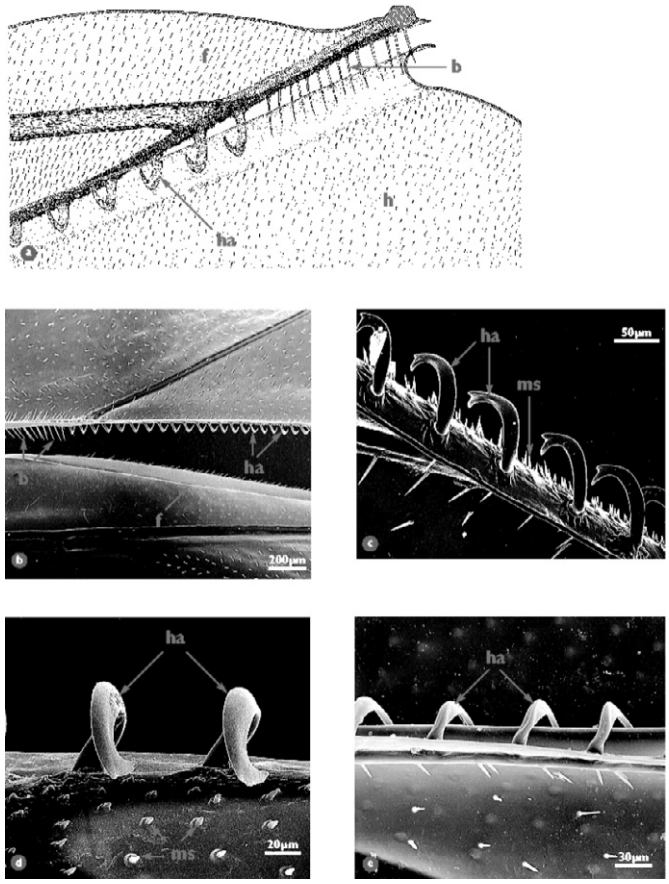

Fig. 1. Honeybee wing coupling mechanism: **a**, diagrammatic view from below forewing, hindwing, hamulus, and wing bristles; **b**, SEM micrographs of wings separated, hindwing at top, hamuli and bristles on leading edge of hindwing with fold in trailing edge of forewing; **c**, hamuli and small mechanoreceptor setae; **d**, hamuli and mechanoreceptor setae viewed from above; e) hamuli coupled with fold in forewing (symbols: f = forewing, h = hindwing, ha = hamulus, ms = mechanoreceptor setae). From Goodman[13] with permission.

Introduction

The history of the classification of honeybees reflects several important changes in the definitions of categories, forms of measurement, and methods of analysis. A major advance was the formulation of a metrical set of morphological characters and the application of univariate statistical methods of analysis that emerged from the Russian school early in the last century.[1–3] Subsequently, some 60-odd morphological characters were gradually introduced into the literature on honeybee morphometrics, but the relative value or weight of characters had necessarily remained blurred until the polyandrous mating of honeybee queens was demonstrated,[4] the genetic theory of natural selection developed,[5] and the general application of multivariate methods of analysis[6] had become commonplace.[7,8]

One particular character, the hamuli, or wing-hooks, has become something of an enigma in honeybee taxonomy.[9] These structures are located near the proximal end of the anterior margin of each hindwing and which couple it to a fold in the forewing to form a single, functional aerofoil during flight[10–13] (Fig. 1). Long used in classification studies of honeybees, they were judged to be of no use for taxonomic discrimination because within-bee variation was significantly greater than between-bees variation,[9] despite having a high genetic heritability.[14,15] Here we reassess and re-evaluate all of the relevant data (some previously published, other new) on the hamuli of the honeybee wing in terms of their suitability as probes in honeybee population structure as well as classification. The cardinal points of interest in these structures include developmental homeostasis, environmental effects, genetics, sexual and caste dimorphism, classification and population biology.

Materials and methods

Honeybees

The numbers of hamuli of worker honeybees used for the analyses in this study derive from: (1) raw data from the Institut für Bienenkunde at Oberursel; (2) new material collected for *A. andreniformis, A. florea, A. cerana* and *A. koschevnikovi* from

[a]Department of Zoology and Entomology, and [b]Department of Statistics, Rhodes University, Grahamstown 6140, South Africa.
*Author for correspondence. E-mail: r.hepburn@ru.ac.za

Table 1. Mean number of hamuli and standard deviations (s.d.), coefficients of variation (CV), ranges and extent of hamuli from previously published papers.

Species	Form[#]	No. of bees	Mean ± s.d.	CV (%)	Range	Extent (mm)	Extent CV (%)	Region	References
A. cerana	W	40	18.50 ± 1.38	7.45	15–21			Japan	18
	W	1075	18.23 ± 1.87	10.27	13–24			E. China	18
	W	>25	18.09 ± 0.90	4.97				India	39
	W	?	18.04 ± 0.65	3.60				India	40
	W	420	18.05 ± 1.55	8.59		1.15 ± 0.05	4.28	Nagaland, NE India	61
	W	420	18.52 ± 2.13	11.50		1.14	5.36	Brahmaputra Valley, NE India	61
	W	300	18.29 ± 1.48	8.09		1.16	4.20	Himalayas, NE India	61
	W	40	17.20 ± 1.04	6.05	16–19			N. Borneo	19
	W	?	19.35 ± 0.83	4.27		1.25 ± 0.02	1.63	N. India	59
	W	3	18.16 ± 0.39	2.15				Sulawesi, Indonesia	21
	W	200	17.90 ± 1.47	8.24	14–21			N. India	10
*A. dorsata**	W	410	25.31 ± 0.58	2.30	20–30	1.65 ± 0.03	1.82	India	58
	W	?	24.43		18–29			Central Myanmar	64
	W	200	24.62 ± 1.73	7.00	21–29			N. India	10
A. andreniformis	W	180	10.38 ± 0.92	8.86				Thailand	20
	W	40	10.88 ± 1.07	8.38				Philippines	20
A. florea	W	200	11.81 ± 1.14	9.63	9–15			N. India	10
A. koschevnikovi	W	90	17.70 ± 0.60	3.39				N. Borneo	19
*A. nigrocincta**	W	10	17.86 ± 0.68	3.80				Sulawesi, Indonesia	21
*A. m. carnica**	W	148	19.53 ± 1.62	8.29	16–24			E. USA	31
	W	154	19.60 ± 1.60	8.15	16–24			Europe	18
*A. m. caucasica**	W	106	20.69 ± 0.42	2.01	16–29			Central Russia	31
	W	474	21.08 ± 0.31	1.46	17–26			S. Russia	31
	W	201	20.38 ± 1.84	9.02	16–26			Russia	36
	W	256	20.61 ± 1.49	7.24	16–25			N. Caucasus, Russia	31
	W	142	21.37 ± 1.61	7.54	18–26			Caucasus Mtns, Russia	31
	W	212	20.91 ± 1.68	8.05	14–25			Abchasia, Russia	31
	W	69	21.71 ± 1.56	7.19	18–27			Transcaucasia, Russia	31
	W	506	21.49 ± 0.36	1.65				Bulgaria	31
*A. m. ligustica**	W	150	21.51 ± 1.54	7.18	17–25			China ex Italy	18
	W	402	21.01 ± 1.64	7.83	17–25			E. USA ex Italy	31
	W	?	22.64 ± 1.74	7.69		1.42 ± 0.07	4.72	India	59
	W	699	20.56	8.10	16–26			Australia ex Italy	36
	W	374	21.45 ± 1.60	7.47	17–26			Manchuria ex Italy	18
	W	200	20.09 ± 1.72	8.56	15–25			India ex Italy	10
A. cerana	Q	100	16.10		15–17			India	39
A. dorsata	Q	3	23.00		22–27			India	38
A.m.caucasica ?	Q	139	18.46 ± 1.89	10.25				Bulgaria	31
	Q	27	18.67					Russia	31
A. m. carnica	Q	8	16.25					Germany	31
A. m. ligustica	Q	10	18.00					Canada	31
A.m.caucasica	D	118	20.57 ± 0.28	1.37				Russia	31
	D	997	21.39 ± 1.20	1.65				Bulgaria	31
	D	200	20.91 ± 2.13	10.17				Australia ex Russia	36

*Data used in the analysis. [#]W, workers; Q, queens; D, drones.

Borneo, Cambodia, Malaysia, Myanmar, Nepal, the Philippines, Thailand and Vietnam – referred to as the Grahamstown database; (3) raw data from a previous study of *Apis mellifera intermissa* from Algeria were obtained from Barour *et al.* (unpubl.); (4) data from previously published papers (Table 1). Morphometric analyses were performed on 11 065 individual worker bees from 639 colonies across the distributional areas of *A. andreniformis, A. florea, A. cerana, A. koschevnikovi* and *A. m. intermissa*.

Data analysis

Colony sample means and inter- and intracolonial coefficients of variation were computed for hamuli numbers from 15–20 bees per colony. Univariate statistical analysis of the data included ANOVA and Tukey multiple comparison procedures for means and Levene's test procedure for variances.[16]

Results and discussion

Classification

The number of hamuli, their frequency distribution and linear extent on the edge of honeybee wings have generally been an adjunct to classification, since the work of Koschevnikov.[1] Hamuli numbers were also shown to be species-specific and the frequency distributions bimodal in *A. cerana* and *A. mellifera* workers.[17,18] This point was also established for *A. florea* and

Table 2. Mean and range of hamuli numbers, inter- and intra-colonial variations by species.

Species	Mean*	*n*	Intercolonial CV (%)	No. of bees	Intracolonial CV (%)	Range
A. andreniformis	10.29[a]	16	4.19[a]	310	9.04[a]	7–14
A. florea	11.18[b]	104	5.51[b]	1 872	8.87[ab]	8–16
A. cerana	17.56[c]	475	4.96	8 027	7.85[ac]	10–24
A. koschevnikovi	18.40[c]	5	5.40	100	8.36[a]	14–22
A. mellifera intermissa	22.56[d]	39	5.11	756	7.84[a]	16–29
	$F_{4,634}$ = 1910.6	639	$F_{4,634}$ = 9.8		$F_{4,634}$ = 7.3	
	P < 0.0001		P < 0.0001	11 065	P < 0.0001	

*Means with the same superscript letter do not differ significantly (P > 0.05).

A. dorsata.[10] Similarly, Rinderer *et al.* showed that the hamuli means and coefficients of variation differ significantly between *A. florea* and *A. andreniformis,* but did not differ significantly between *A. koschevnikovi* and *A. cerana* (Table 1).[19,20] The lack of significant differences in hamuli numbers between *A. cerana* and *A. nigrocincta* has also been reported (Table 1).[21] Somewhat surprisingly, Gromisz[22] and Ruttner[9] excluded hamuli from their morphometric character suites because they did not obtain clear-cut geographical variation in very large samples of *A. mellifera* and A. *cerana* and concluded that they have little value in subspecific classification ('subspecies' in honeybees is a highly suspect concept anyway).[23,24] Perhaps so, but in studies of honeybee population structure they are a rich source of information, particularly if one considers their genetic properties (see below).

Between-species analysis

In this study, we confirm these results for the mean number of hamuli (Table 2). Significant differences between the mean number of hamuli were also found between *A. m. intermissa* and *A. andreniformis, A. florea* and *A. cerana*; also between *A. cerana/A. koschevnikovi* and *A. andreniformis and A. florea* (Table 2). Significant differences were found in the distributions in the number of hamuli and variability between species (populations) (ANOVA: means: $F = 1910.6$, (4, 634) d.f., $P < 0.0001$; Levene: variability: $F = 9.9$, (4, 634) d.f., $P < 0.0001$). The mean number of hamuli of *A. andreniformis* hamuli mean numbers differed from those of *A. florea.* Both these population means differed from those of *A. cerana, A. koschevnikovi* and *A. m. intermissa* (Table 2). No significant differences were found between *A. cerana* and *A. koschevnikovi.*

When the analysis included data for *A. dorsata, A. nigrocincta, A. m. carnica, A. m. caucasica* and *A. m. ligustica* (Table 1), the results showed significant differences in hamuli numbers between *A. andreniformis/A. florea* and *A. cerana/A. koschevnikovi/ A. nigrocincta* and *A. m. intermissa/A. m. carnica/A. m. caucasica/A. m. ligustica* (ANOVA: $F = 150.3$, (9, 56) d.f., $P < 0.0001$). Hamuli numbers of *A. dorsata* significantly differed from those of other populations except *A. m. intermissa.*

Within-species analysis

Significant differences were found in the distribution in the number of hamuli between countries within *A. andreniformis, A. florea, A. cerana* and *A. koschevnikovi* (ANOVA: *andreniformis*: $F = 3.7$, (4, 11) d.f., $P < 0.0373$; *florea*: $F = 14.9$, (11, 92) d.f., $P < 0.0001$; *cerana*: $F = 11.0$, (23, 451) d.f., $P < 0.001$; *koschevnikovi*: $F = 124.4$, (1, 3) d.f., $P = 0.0015$; Table 3(a)–(d)). The mean numbers of hamuli for *A. m. intermissa* differed significantly among locali-

Table 3. Mean numbers of hamuli, inter- and intra-colonial variations for within-species analysis.

Country	Mean*	No. of bees	Intracolonial CV (%)	*n*	Intercolonial CV (%)
(a) *A. andreniformis*					
Malaysia	9.83[a]	60	9.58	3	1.78
Myanmar	10.03[a]	60	9.17	3	2.92
Borneo	10.23[a,b]	60	9.06	3	1.57
Vietnam	10.62[b]	110	9.46	6	4.01
Philippines	10. 85[b]	20	8.60	1	–
	$F_{4,305} = 9.5$	310	$F_{4,305} = 1.3$	16	$F_{3,11} = 1.76$
	$P < 0.0001$				$P = 0.2569$
	$P = 0.2129$				
(b) *A. florea*)					
Saudi Arabia	12.93	15	6.18	1	–
Oman	13.15	60	8.26	3	3.63
Iran	12.20	49	11.59	3	7.46
Pakistan	11.67	6	7.00	1	–
Nepal	11.40	20	6.61	1	–
India	10.50	18	7.48	1	–
Sri Lanka	11.43	40	8.38	2	2.17
Myanmar	10.62	220	10.86	11	4.50
Thailand	11.19	895	9.13	52	2.94
Cambodia	11.38	120	8.69	6	2.66
Vietnam	10.98	409	9.46	22	3.77
Malaysia	10.10	20	9.58	1	–
	$F_{11,1860} = 38.0$	1872	$F_{11,1860} = 2.1$	104	$F_{6,92} = 1.5$
	$P < 0.0001$		$P = 0.0166$		$P = 0.1937$
(c) *A. cerana*					
Afghanistan	18.20	137	8.08	8	3.82
Japan	18.50	160	8.42	8	3.92
Pakistan	19.00	120	9.58	6	2.66
Korea	19.45	20	6.35	1	–
Hong Kong	18.59	240	8.59	12	3.80
China	18.06	471	8.86	31	4.21
Nepal	17.86	91	10.14	6	5.78
N. India	18.09	296	8.34	17	3.19
Myanmar	17.48	1201	10.95	63	5.25
Vietnam	17.64	1270	8.64	57	3.59
Java	18.21	365	7.69	9	2.97
Bali	18.37	97	6.95	5	2.04
Cambodia	17.46	325	8.88	17	4.34
Papua New Guinea	17.37	270	7.22	11	2.52
Thailand	17.96	97	7.71	6	2.59
Sri Lanka	17.87	159	7.94	19	2.92
Sumatra	17.83	120	6.93	6	1.53
Sulawesi	17.74	90	8.34	6	3.83
Indonesia	17.20	119	7.00	21	4.16
Philippines	17.03	1788	8.68	126	4.39
Malaysia	16.75	128	8.54	6	3.23
Borneo	16.70	463	8.02	34	3.29
	$F_{21,8005} = 43.8$	8027	$F_{21,8005} = 9.2$	475	$F_{20,453} = 1.7$
	$P < 0.0001$				$P = 0.2569$
	$P = 0.0310$				
(d) *A. koschevnikovi*					
Malaysia	19.48[a]	40	7.80	2	0.91
Borneo	17.68[b]	60	8.62	3	0.99
	$F_{1,98} = 33.3$	100	$F_{1,98} = 0.003$	5	$F_{1,3} = 0.002$
	$P < 0.0001$		$P = 0.9543$		$P = 0.9702$
(e) *A. m. intermissa*					
Berrahel	24.15	100	8.25	5	3.26
Selawa	22.72	36	7.77	3	5.41
Ain Essel	21.97	60	7.16	3	2.81
Drean	23.17	60	7.79	3	5.11
Tawra	23.00	60	10.00	3	1.88
Souk Ahras	22.52	60	8.40	3	3.34
Mewna	22.45	60	10.19	3	3.67
Bri Metrane	22.55	60	7.98	3	1.77
Serraidi	22.60	80	8.01	4	1.41
El Hadeykes	23.03	40	5.52	2	2.30
Bni Isguen	21.50	100	9.08	5	4.27
Bni Isguen	20.10	40	8.72	2	3.87
	$F_{11,744} = 16.9$	756	$F_{11,744} = 2.4$	39	$F_{11,27} = 1.4$
	$P < 0.0001$				$P = 0.0058$
	$P = 0.2180$				

*Means with the same superscript letter do not differ significantly ($P > 0.05$).

Table 4. Correlation coefficients (*r*) between hamuli numbers and altitude and forewing length.

Species	Altitude		Forewing	
	r	No. of colonies	*r*	No. of bees
A. andreniformis	–0.460	6	0.085	310
A. florea	–0.135	95	0.149	1 863
A. cerana	0.045	414	0.250	8 027
A. koschevnikovi	–	–	0.168	100
A. m. intermissa	–0.308	27	0.176	756
All groups	0.089	544	0.898**	11 056

**$P < 0.01$.

ties within Algeria (ANOVA: $F = 4.9$, (11, 27) d.f., $P = 0.0004$; Table 3(e)). Significant differences in intercolonial variability between countries were found within *A. cerana* (Levene: $F = 1.6$, (23, 451) d.f., $P = 0.0337$), whereas no significant differences in variability were found within *A. andreniformis, A. florea, A. koschevnikovi* and *A. m. intermissa.*

Sexual and caste dimorphism

Casteel and Phillips reported that variability in the number of hamuli was greater in drones than in workers of *A. mellifera,* which they prematurely ascribed to environmental effects.[25] While others established the breadth of individual variation,[18,26–30] substantial progress was made by Alpatov[2,31] and Narayanan,[32–34] who analysed large samples of *A. mellifera* and showed that the intercolonial means (all normally distributed) and coefficients of variation in hamuli number for drones, workers and queens were statistically significantly different and that greatest variability occurred in drones (Table 1). But, while queens had significantly fewer hamuli than drones or workers, their coefficients of variation were very similar to drones'. Despite their greater size, the numbers of hamuli in drones and workers are much the same.[2] Woyke[35] demonstrated that while most structures were larger in diploid drones than haploid ones, the number of hamuli in the former was significantly lower than the latter and workers' were significantly lower than queens'.[35]

Experiments by Lee[36] confirmed that variation in the number of hamuli is greater in drones than in workers[2,25] and also that drones raised in worker cells did not significantly differ from those of drone cells, a result pointing to a lack of environmental influence over the phenotype and a lack of dominance homeostasis in haploids. Contradictory results were reported for a correlation between hamuli and the cubital index,[10,30] but it was established that hamuli number is proportional to hindwing size.[30] That queens and workers showed caste dimorphism for both means and variances of a quantitative character like hamuli suggests developmental differences (see below). The numbers, frequency distributions, linear extent of hamuli distribution along the edge of the hindwing and coefficients of variation for hamuli in *A. cerana* differ significantly for queens and workers. Likewise, queens of *A. dorsata, A. mellifera* and *A. cerana* differ significantly in these respects (cf. Table 1).[31,37–41]

Environmental effects

Kellogg[26] reported that variation in several features of the wings of drones, reared in large drone or small worker cells, did not differ significantly; in more comprehensive experiments, however, Grout[42] noted significant changes in the wing length and width in workers bees reared under similar conditions. But, there remained questions about wing symmetry and the possibility of environmental and/or developmental effects in the final expression of the phenotype. Petrov and Ankinovich calculated

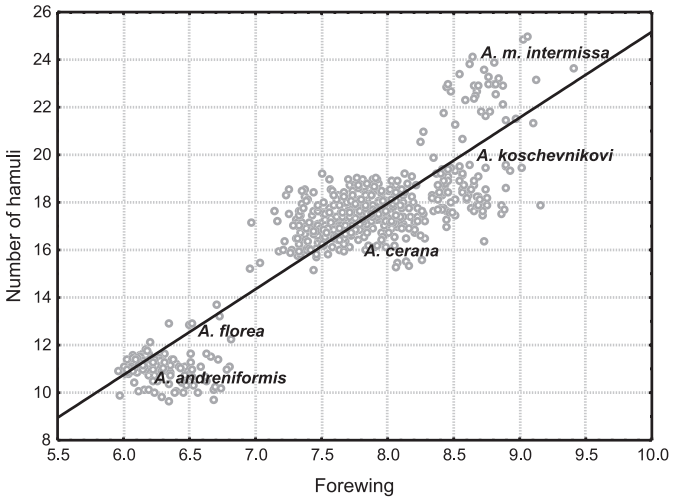

Fig. 2. Number of hamuli versus forewing length in five *Apis* species.

that the symmetry of the hamuli between left and right wings had a correlation close to unity (0.996).[43]

Petrov subsequently reported that the numbers of hamuli in both drones and workers of *A. mellifera* were not affected by queen age, or the age of nurse bees[44] (but nutrition significantly affects hamuli number),[31] nor did they vary seasonally as do other traits in honeybees such as pigmentation.[45]

The susceptibility of wing size in *A. mellifera* to environmental effects was analysed by Alpatov for bees that were normally fed and under-fed; the coefficients of variation did not differ significantly but underfed bees had significantly fewer hamuli than the others.[31] Further analyses of wing size and number of hamuli in different species of *Apis* have been developed. Saini *et al.* reported that the mean numbers of hamuli in workers of a given species are simply proportional to the size of the hindwing.[10] Lee had earlier calculated the correlation between the number of hamuli and the size of the forewing in workers of *A. mellifera* and found it to be remarkably low (0.103),[36] although the correlation for left–right wing symmetry approached unity.[43] Our findings confirmed low correlations between forewing length and hamuli numbers within species ($0.09 < r < 0.25$), whilst a significantly high correlation was found across species (Table 4, Fig. 2). Finally, Diniz-Filho *et al.* investigated genetic and within-colony environmental components in the number of hamuli in *A. m. scutellata* modified to control for environmental variation.[15] He estimated the heritability for the number of hamuli in the worker progeny of a queen inseminated by 17 drones and calculated heritability to be 0.6768 ± 0.2991. The within-colony environmental variance component was not significant, hence the heritability values are dependably accurate and identical to those of Oldroyd and Moran.[14]

Genetics

Using inbred lines and hybrids of *A. mellifera* that were instrumentally inseminated, Roberts found that heritability for hamuli is high and that there were significant differences among the inbred lines and the hybrids;[46] results confirmed by others.[14,15,36,47–49] Subsequently, Lee established that hamuli number was normally distributed, but independent in drones and workers, and that queens had fewer hamuli than their sister workers.[36] There was also high variability between different lines in Lee's experiments,[36] which indicated a high response to selection. This point was pursued by Goncalves,[50–52] Paulino,[53] and Petrov,[44] who developed a directional selection programme for high and low hamuli numbers in two inbred lines of *A. mellifera.* The

inbreeding coefficients of workers and queens, the variance components, coefficient of variance among workers, correlation analysis, and regression of offspring on mid-parents allowed an estimation of heritability. There was a high correlation between the means of mid-parents and offspring of both lines. The symmetry of the parents had no effect on offspring variability in workers, drones or queens. Interestingly, there was also a reduction in the genotypic variance of drones and workers.[49]

While the above results are very telling, two points of interest cannot be determined from them: (1) the natural frequency distribution for hamuli number in a natural population and (2) highly accurate values for heritability (this is because single drone inseminations suffer bias of the effect of dominance variance to covariance in sib worker bees). Oldroyd and Moran developed a method to determine heritability of hamuli numbers in a natural population of *A. mellifera*.[14] Relatedness among workers was estimated as a function of the number of drone matings by the queen, and the relatedness among these drones. Heritability values were obtained by dividing the interclass correlation for hamuli number by the relatedness among workers within colonies. They estimated the heritability for hamuli number in a natural population of honeybees to be 0.68 ± 0.18; using different experimental methods, Diniz-Filho *et al.* calculated heritability for hamuli number to be 0.677 ± 0.29.[15]

Developmental homeostasis

The finding that the correlation for left–right wing symmetry of hamuli was more variable in queens than worker honeybees[43] invited the possibility of important developmental effects. Because constancy of a character is a measure of homeostasis, it is necessary to ask whether developmental homeostasis is linked with heterozygosity in haplo-diploid insects? One study on the effects of gene dosage on variability in hamuli number concluded that a higher variance occurs in haploid drones;[36] homeostasis of wing symmetry is generally enhanced in haplo-diploid systems such as honeybees.[54] Recent studies on the genetic basis of wing symmetry in relation to developmental stability showed that the symmetry of distribution of hamuli on the wings of workers of *A. mellifera* did not exhibit any statistical deviation from normality.[55] Statistically, as the number of hamuli approaches the mean, the greater the probability of symmetry in hamuli numbers in both pairs of wings.[12] However, the genetic component of this system and possible environmental effects require more detailed analysis.

Population studies and clines

Clinal variation in honeybees was first demonstrated by Alpatov, who measured hamuli frequencies for *A. mellifera* between 50°N and 55°N latitude in central Russia and recorded an increase in the average number of hamuli for worker bees from south to north.[56] He also detected what is now termed an altitudinal cline in the Caucasus Mountains (Table 1).[31] It is of historical interest to note that the term 'cline' was first proposed by Huxley to describe geographical variation[57] on the basis of Alpatov's data. Similar clines have been documented for *A. dorsata*[58] and *A. cerana* in the sub-Himalayan region[32–34,59–61] and in central Europe[62] (cf. Table 1). In this study, no significant correlations were found between altitude and hamuli numbers within species or between species (Table 4). No altitudinal clines were found.

Of equal importance from a population perspective, Kellogg showed that the average coefficient of variability for *A. cerana* differed significantly from *A. mellifera*.[18] This suggests a greater heterogeneity in the natural populations of the native *A. cerana*

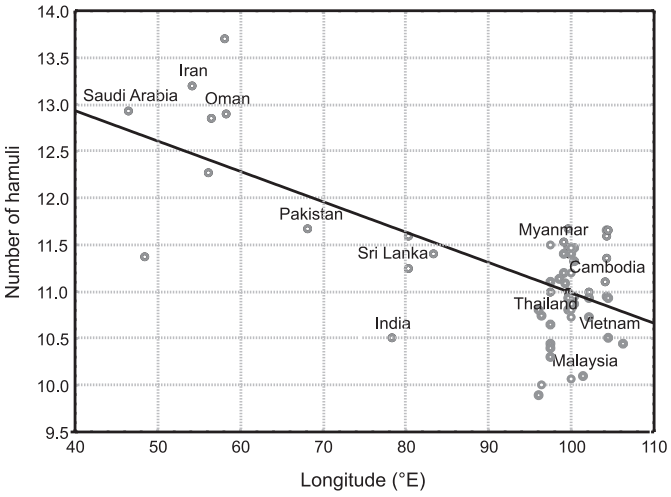

Fig. 3. Longitudinal cline in the number of hamuli in *Apis florea*.

of China against those imported from a smaller European gene pool of A. mellifera. Finally, Norkina and Korolev calculated the incidence of discrete phenotypic traits (phenes) that could define two populations within Grey and central Russian bees (both *A. m. caucasica*) because the drones of two groups differed significantly for a spectrum of phenes including hamuli.[63] Our analysis of the most complete data sets shows that hamuli numbers exhibit significant longitudinal and latitudinal clines over large distances in *A. florea* and *A. cerana* worker honeybees (*A. florea*: longitude: $r = -0.68$, $P < 0.0001$; latitude: $r = 0.51$, $P < 0.0001$; *A. cerana*: longitude: $r = -0.34$, $P < 0.0001$; latitude: $r = 0.35$, $P < 0.0001$, Fig. 3). The number of hamuli decreases along a sloping cline from 30°34′N in northwestern Iran to 9°20′N in southeastern Vietnam.

Conclusions

The number of hamuli and their linear extent on the edge of the hindwings of honeybees have high heritability values and are readily modified by genetic selection. They differ significantly between the sexes and female castes. They vary among most species of *Apis* as well as in infraspecific categories (workers and queens of *A. m. caucasica* and *A. m. carnica* versus *A. m. ligustica*). The frequency distributions of hamuli and their extent on hindwings significantly vary at the population level and exhibit latitudinal and longitudinal clines over large distances. Likewise, there are geographical oscillations in the occurrence of high and low values for the coefficient of variation that indicate the relative homogeneity or heterogeneity of local populations. These results show that hamuli numbers are useful in the classification of honeybee populations. Whether hamuli would be useful in multivariate analysis depends on the correlation between hamuli numbers and the other characters used. If hamuli numbers are highly correlated with a character that has greater discriminatory power of populations, then hamuli numbers may not be selected in the character suite.

We thank S. Fuchs, Institut für Bienenkunde at Oberursel, Germany, for the Oberursel database and C. Barour, Université Badji Mokhtar, Annaba, Algeria, for the Algerian data. We are grateful to Colleen Hepburn for the measurements that form the Grahamstown database. We are particularly grateful to the International Bee Research Association and the L.J. Goodman Insect Physiology Research Trust, copyright-holders, for permission to reproduce Fig. 1 from *Form and Function in the Honeybee* by L. Goodman.

1. Koschevnikov G.A. (1900). *Materials on the natural history of the honeybee.* Société des Amateurs des Sciences Naturelles, Moscow. (In Russian).
2. Alpatov V.V. (1929). Biometrical studies on variation and races of the honey bee (*Apis mellifera* L.). *Quart. Rev. Biol.* **4**, 1–58.

3. Alpatov V.V. (1948). *The Races of Honeybees and their Use in Agriculture.* Moscow Naturalist Society, Moscow. (In Russian).
4. Roberts W.C. (1944). Multiple mating of queen bees proved by progeny and flight tests. *Glean. Bee Cult.* **72**, 281–283.
5. Fisher R.A. (1930). *The Genetic Theory of Natural Selection.* Clarendon Press, Oxford.
6. DuPraw E.J. (1964). Non-Linneaen taxonomy. *Nature* **202**, 849–852.
7. Daly H.V. and Balling S.S. (1978). Identification of Africanized honeybees in the western hemisphere by discriminant analysis. *J. Kans. Ent. Soc.* **51**(4), 857–869.
8. Ruttner F., Tassencourt L. and Louveaux J. (1978). Biometrical-statistical analysis of the geographic variability of *Apis mellifera* L. 1. Material and methods. *Apidologie* **9**, 363–381.
9. Ruttner F. (1987). *Biogeography and Taxonomy of Honeybees.* Springer, Berlin.
10. Saini M.S., Aggarwal R. and Dhillon S.S. (1983). Functional morphology of the wing coupling apparatus and its bearing on the taxonomy of genus *Apis* Linnaeus. *Uttar Pradesh J. Zool.* **3**(1), 10–14.
11. Petrov P. (1994). Symmetry of the wing-coupling apparatus of the honeybee *Apis mellifera* (Hymenoptera: Apidae). *Nauchni Tr. Vissh. Selskostop. Inst. (Plovdiv)* **39**(81), 85 (In Bulgarian).
12. Es'kov E.K. (1997). The dependence of the asymmetry of wing coupling apparatus on the variability of the number of frenula in honey bees. *DKBSA* **352**(1/6), 71–72.
13. Goodman L. (2003). *Form and Function in the Honey Bee.* International Bee Research Association, Cardiff.
14. Oldroyd B.P. and Moran C. (1983). Heritability of worker characters in the honeybee (*Apis mellifera*). *Aust. J. Biol. Sci.* **36**, 323–332.
15. Diniz-Filho J.A.F., Malaspina O. and Cavalheri D.H. (1994). Genetic and within colony environmental components of variation in the hamuli number of Africanized honey bees (*Apis mellifera* L.). *Rev. Brasil. Genét.* **17**, 255–258.
16. Rao P.V. (1998). *Statistical Research Methods in Life Sciences.* Brooks/Cole Publishing Company, Pacific Grove, CA.
17. Kellogg C.R. and Yung L.L. (1929). Studies in the variation in number of hooks on the hind wings of the Chinese honey bee (*Apis indica*). *Proc. Natl Hist. Soc. Fukien Christ. Univ.* **2**, 57–59.
18. Kellogg C.R. (1930). The use of biometry to distinguish between *Apis indica* and *Apis mellifica. Bee Wld* **11**, 127–130.
19. Rinderer T.E., Koeniger G., Tingek S. and Mardan M.B. (1989). A morphological comparison of the cavity dwelling honeybees of Borneo *Apis koschevnikovi* (Buttel Reepen, 1906) and *Apis cerana* (Fabricius, 1793). *Apidologie* **20**, 405–411.
20. Rinderer T.E., Oldroyd B.P., Wongsiri S., Sylvester H.A., De Guzman L.I., Stelzer J.A. and Riggio R.M. (1995). A morphological comparison of the dwarf honey bees of southeastern Thailand and Palawan, Philippines. *Apidologie* **26**, 387–394.
21. Hadisoesilo S., Otis G.W. and Meixner M. (1995). Two distinct populations of cavity-nesting honey bees (Hymenoptera: Apidae) in South Sulawesi, Indonesia. *J. Kans. Ent. Soc.* **68**, 399–407.
22. Gromisz M. (1967). Comparison of bees from the Carpathian mountains district with bees from northern Poland (*A. m. mellifera*) and the Danube basin (*A. m. carnica). Pszczeln. Zesz. Nauk.* **11**, 1–35 (In Polish).
23. Wilson E.O. and Brown W.L. (1953). The subspecies concept and its taxonomic application. *Sys. Zool.* **2**, 97–111.
24. Engel M.S. (1999). The taxonomy of recent and fossil honey bees (Hymenoptera: Apidae; *Apis*). *J. Hymenop. Res.* **8**, 165–196.
25. Casteel D.B. and Phillips F.B. (1903). Comparative variability of drones and workers of the honey bee. *Biol. Bull.* **6**, 18–37.
26. Kellogg V.L. (1906). Variation in parthenogenic insects. *Science* **24**, 695–699.
27. Wright A., Lee A. and Pearson R. (1907). A cooperative study on queens, drones and workers in *Vespa vulgaris. Biometry* **5**, 407–422.
28. Bachmetjew P. (1910). Statistical analysis of the number of hamuli in honeybees and the consequence thereof. *Z. Wiss. Zool.* **94**. (In German, cited from Alpatov, 1929).
29. Pearl R. (1910). Recent quantitative studies in variation in social insects. *Am. Nat.* **44**, 521.
30. Phillips E.F. (1929). Variation and correlation in the appendages of the honey bee. *Mem. Cornell Univ. Agric. Exp. Sta.* **21**.
31. Alpatov V.V. (1928). Variation in hooks on the hind wing of the honey bee (*Apis mellifera* L.). *Biol. Bull.* **60**, 209–234.
32. Narayanan E.S., Sharma P.L. and Phadke K.G. (1960). Studies on biometry of the Indian bees I. Tongue length and number of hooks on the hind wings of *Apis indica* F. *Indian Bee J.* **22**(7/9), 58–63.
33. Narayanan E.S., Sharma O.P. and Phadke K.G. (1961). Studies on biometry of the Indian bees IV. Tongue length and number of hooks on the hind wings of *Apis indica* F. collected from Uttar Pradesh. *Indian Bee J.* **23**(10/12), 69–74.
34. Narayanan E.S., Sharma P.L. and Phadke K.G. (1961). Studies on biometry of the Indian bees. III. Tongue length and number of hooks on the hind wings of *Apis indica* F collected from Madras State. *Indian Bee J.* **23**(1/3), 3–9.
35. Woyke J. (1978). Comparative biometrical investigation on diploid drones of the honeybee. II. The thorax. *J. Apic. Res.* **17**,195–205.
36. Lee G.L. (1974). The effect of gene dosage on variability in the honeybee. 2. Wing hook number. *J. Apic. Res.* **13**, 257–263.
37. Okada I., Sakai T. and Hasegawa M. (1956). Notes on some morphological characters of the Japanese honeybee (*Apis indica japonica* Rad.). Proc. XVI Int. Beekeep. Congr. Prelim. Sci. Meeting.
38. Kshirsagar K.K. (1969). Preliminary observations on the biometry of *Apis dorsata* Fab. queen. *Indian Bee J.* **31**(1), 12–16.
39. Kshirsagar K.K. (1973). Comparative biometric studies on Indian honeybees. III. Preliminary observations on biometry of *Apis cerana* F. queen. *Indian Bee J.* **35**(1/4), 21–26.
40. Kshirsagar K.K. and Ranade D.R. (1981). Morphometric characterization of Indian hive bee *Apis cerana indica* F. (Apidae, Hymenoptera), worker. *J. Univ. Poona, Sci. Tech.* Section **54**, 101–120.
41. Gromisz M. (1969). Experimental morphometric definition of queen honeybees. *Pszczeln. Zesz. Nauk.* 13(1/3), 52–72. (In Polish).
42. Grout R.A. (1937). The influence of size of brood cell upon the size and variability of the honeybee (*Apis mellifera* L.). *Res. Bull. Iowa Agric. Exp. Sta.* **218**, 261–280.
43. Petrov S.G. and Ankinovich G.B. (1968). The hamuli on the wings of honeybees. *IzvestiyaTimiryazevsk. Sel'skok. Akad.* **3**, 226–232. (In Russian).
44. Petrov S.G. (1975). Genetics of the wing-hook apparatus of honeybees. *Dokl. Timiriazevsk. S-Kh. Akad.* **211**, 95–202. (In Russian).
45. TsurutaT., Matsuka M. and Sasaki M. (1989). Temperature as a causative factor in the seasonal colour dimorphism of *Apis cerana japonica* workers. *Apidologie* **20**, 149–155.
46. Roberts W.C. (1961). Heterosis in the honey bee as shown by morphological characters in inbred and hybrid bees. *Ann. Ent. Soc. Am.* **54**, 878–882.
47. Goetze G.K.L. (1964). *Honeybees under Natural and Artificial Selective Breeding.* Paul Parey, Hamburg. (In German).
48. El-Banby M.A. (1968). Inheritance of some quantitative characters in the Carnio-Egyptian honey-bee hybrid. *Bull. Soc. Ent. Egypte* **52**, 527–534.
49. Goncalves L.S. (1978). Honey bee improvement through behavioral genetics. *Ann. Rev. Ent.* **31**,197–213.
50. Goncalves L.S. (1972). Investigations on the morphological character number of hamuli in *Apis mellifera.* In *Int. Bienenzüchter Kongr.* Apimondia, Lunz am See. (In German).
51. Goncalves L.S. (1976). *Directional selection for two inbred lines of* Apis mellifera *L.* Ph.D. thesis, Ribeirao Preto University, Ribeirao Preto. (In Portuguese).
52. Goncalves L.S. (1977). Directional selection in two inbred lines of *Apis mellifera* L. In *III Congr. Lat. Am. Genet.,* Montevideo. (In Portuguese).
53. Paulino Z.L. (1975). Study of the variability of *Apis mellifera mellifera* colonies. In Anais 3 Congr. Brasil. Apic., pp. 137–144. Piracicaba, Brazil. (In Portuguese).
54. Brückner D. (1976). The influence of genetic variability on wing symmetry in honeybees (*Apis mellifera*). *Evolution* **30**, 100–108.
55. Clarke G.M. and Oldroyd B.P. (1996). The genetic balance of developmental stability in *Apis mellifera.* II. Relationships between character size, asymmetry and single-locus heterozygosity. *Genetica* **97**(2), 211–224.
56. Alpatov V.V. (1927). On the variability of the hamuli on the hind wings of the honeybee. *Ptschelowodnoje Djelo.* 1–7. (In Russian).
57. Huxley J. (1938). Clines: an auxillary taxonomic principle. *Nature* **142**, 219–220.
58. Mujumdar S. and Kshirsagar K.K. (1986). Morphometric characterization of *Apis dorsata* Fabr. workers. *Indian Bee J.* **48**, (1/4) 25–29.
59. Mattu V.K. and Verma L.R. (1983). Comparative morphometric studies on introduced European bee *Apis mellifera* L. and Indian honeybee *Apis cerana indica* F. in Himachal Pradesh. In *Proc. 2nd Int. Conf. Apic. Trop. Clim.,* pp. 262–277. IBRA, New Delhi.
60. Mattu V.K. and Verma L.R. (1984). Comparative morphometric studies on the Indian honeybee of the northwest Himalayas 2. Wings. *J. Apic. Res.* **23**, 3–10.
61. Singh M.P., Verma L.R. and Daly H.V. (1990). Morphometric analysis of the Indian honeybee in the northeast Himalayan region. *J. Apic. Res.* **29**, 3–14.
62. Gromisz M. and Bornus L. (1971). A morphological-mathematical model of the local honeybee population. *Pszczeln. Zesz. Nauk* **15**(1/2), 1–14. (In Polish).
63. Norkina N.A. and Korolev V.G. (1982). Particular phenetics of *Apis mellifera* L. I. Colonial and population variability of the 3rd cubital cell venation phenes and of phenes in structure and number of hooks. *SOGEB* **18**, 556–560.
64. Mar T.T. (1996). *Morphological study of* Apis dorsata *and the analysis of its honey quality.* Thesis, University of Yangon, Yangon, Myanmar.