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Population structure and the interface between *Apis mellifera capensis* and *Apis mellifera scutellata*

H.R. Hepburn[a]*, S.E. Radloff[b], S. Fuchs[c]

[a] Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 South Africa
[b] Department of Statistics, Rhodes University, Grahamstown, 6140 South Africa
[c] Institut für Bienenkunde, Polytechnische Gesellschalt, JW Goethe Universität, Frankfurt am Main, Karl-von-Frisch-Weg 2, D-61440 Oberursel, Germany

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Abstract – Honeybees of southern Africa below 28° latitude south were analysed morphometrically. Based on a combined data set from the morphometric data bank in Oberursel and that of the Apicultural Group of Rhodes University, the distribution of the morphoclusters of *Apis mellifera capensis* and *A. m. scutellata*, as well as the extent of the hybrid zone were re-established for 8 743 worker bees from 442 colonies covering 104 localities. This distribution was matched against particular traits such as thelytokous parthenogenesis, mitochondrial and nuclear DNA profiles, and sting alarm pheromone variability known from previous investigations. The striking incongruences in the geographical distribution of these traits demonstrate a dynamic and independent pattern of gene flow. They also create considerable disagreement between morphometric group definitions and those derived from other biological characteristics. © Inra/DIB/AGIB/Elsevier, Paris

***A. m. capensis* / *A. m. scutellata* / hybridization / morphometrics / pheromones / mtDNA / nuclear DNA**

1. INTRODUCTION

Two discrete morphoclusters of honeybees, based on multivariate analysis, have been described from southern Africa [4]. Following Ruttner [22], they were designated as *Apis mellifera capensis* and *A. m. scutellata* and their distributions and an area of hybridization were defined [4]. However, parallel studies of the same populations revealed extensive variations in their alarm pheromones [13] and the frequency distributions of their mitochondrial and nuclear DNA profiles [17, 18]. These and other biological traits, such as ovariole number and thelytokous parthenogenesis in worker bees [9], also have geographic distributions which are coincident but not concordant with those

* Correspondence and reprints
E-mail: zohh@warthog.ru.ac.za

of the relevant morphoclusters. We have now further explored the relationships between the phenotypic expression of both morphometric and non-morphometric traits and the implications for gene flow in natural populations of these honeybees. We then comment on the usefulness of morphometric categories traditionally used for definitions of subspecies.

2. MATERIALS AND METHODS

Recently (1997) the morphometric databases on honeybees of the Institut für Bienenkunde (Ruttner collection, Oberursel, Germany) and of the Apiculture Group at Rhodes University (Hepburn and Radloff collection, Grahamstown, South Africa) were amalgamated to form a single database for Africa. The merger now allows a sampling distance resolution of about 50 km for bees of the southern Cape and a three-fold increase in the numbers of honeybees, colonies and localities that have been sampled since the study of Crewe et al. [4].

Worker honeybees were sampled from the colonies of small-scale, fixed-site beekeepers at 104 localities throughout southern Africa almost all of which are below 24° latitude and including Namibia and South Africa (*table I*). While 'captive colonies' were sampled, it must be understood that the bees are simply attracted to empty hives from the wild population. They are not transported nor is bee breeding practised in southern Africa; thus the samples used for analysis constitute authentic subsamples of the wild population. Morphometric measurements were usually taken on 20 worker bees per colony from a variable number of colonies per locality (*table I*). A total of 8 743 bees was used in the morphometric analysis.

The same nine characters used in previous studies of honeybees in Africa were measured [4, 10, 19]. Their Ruttner [22] numbers are given in brackets as follows: length of cover hair on tergite 5 (1), width of wax plate on sternite 3 (11), transverse length of wax plate on sternite 3 (13), pigmentation of scutellum (35), pigmentation of scutellar plate (36), pigmentation of tergite 2 (32), wing angle B4 (22), wing angle N23 (30) and wing angle O26 (31).

Multivariate statistical analysis of the colony mean data included factor analysis and linear discriminant analysis. The last procedure may provide an overly optimistic estimate of the probability of correct classification. A jackknife procedure was therefore carried out that classified each colony into a group with the highest a posteriori probability according to the discrimination functions computed from all the data except the colony being classified [14]. Wilk's lambda test was used to compare multivariate population means between groups. The distribution of the statistic was approximated by the F distribution [15]. 100(1–a) % confidence ellipses were constructed for each group for various values of α [2]. Levene's F statistic for testing the equality of the variances between groups was also used in the analysis. For the morphometric analyses, colony means, standard deviations and covariances of the morphometric characters were analysed.

3. RESULTS

In a factor analysis of the morphometric characters of 8 743 worker honeybees from 442 colonies from 104 localities, three factors with eigenvalues greater than one were isolated: factor 1, pigmentation of scutellum (35) and abdominal tergite 2 (32), length of hair on tergite 5 (1); factor 2, width and length of wax plate on sternite 3 (11) and (13); factor 3, angles of wing venation O26 (31) and N23 (30), pigmentation of scutellar plate (36). These factors accounted for 63.9 % of the variance in the data of which 27.5 % is attributable to factor 1. The factor loadings for each character had absolute values greater than 0.75. The graph of the factor scores from factors 1 and 2 showed two morphoclusters: colonies from the south western Cape to Port Elizabeth forming a cluster (group 1) in the left-hand half of the plot and colonies from the rest of southern Africa forming a cluster (group 2) in the right-hand half (*figure 1*).

A stepwise discriminant analysis using the colony means of the morphometric characters confirmed the separation of the two clusters. The linear discriminant functions obtained using the most discriminatory characters classified 91.1 % of the colonies from the western Cape into group 1 (*capen-*

Table I. Distribution of the morphoclusters, morphometric and pheromonal variance, ovariole number, thelytokous parthenogenesis and mtDNA haplotype frequencies of worker honeybees and nuclear DNA allele polymorphism in queens and drones in southern Africa.

Locality	Coordinates	Sample sizes		Morpho-cluster[1]	Morphometric variance	Pheromonal variance[2]	Ovariole number[3]	DELW[4]	Nuclear DNA[5]	mtDNA[6]	
		colonies	bees						queen drone	P_0Qa	P_0QQa
1 Alexander Bay	28.40S,16.30E	6	120	S	5.029		2.6 ± 1.7	–		0.25	0.5
2 Karasburg	28.00S,18.43E	5	89	S	2.347			–?			
3 Nababeep	29.36S,17.46E	4	80	S	4.182			–			
4 Springbok	29.43S,17.55E	1	10	S	1.840			–			
5 Mesklip	29.52S,17.53E	1	10	S	1.471			–			
6 Garies	30.30S,18.00E	4	79	S	3.650		2.6 ± 2.7	–?		0.0	1.0
7 Bitterfontein	31.03S,18.16E	3	60	S	4.807	190		–?			
8 Lutzville	31.46S,18.21E	5	100	S	4.208	136		+?			
9 Elandsbaai	32.17S,18.25E	5	100	H	6.224*	247		+			
10 Velddrif	32.47S,18.10E	2	40	H	3.871			+			
11 Laaiplek	32.47S,18.09E	3	60	H	4.249	363		+			
12 Langebaan	33.06S,18.03E	3	60	C	4.817	382		+			
13 Darling	33.23S,18.23E	5	100	C	5.401	100		+			
14 Cape Town	33.56S,18.28E	1	20	C	4.081		17.4 ± 4.9	+		0.0	1.0
15 Ariamsvlei	28.08S,19.05E	4	80	S			3.0 ± 1.1	–		0.5	0.5
16 Nieuwoudtville	31.24S,19.06E	5	100	S	6.926*	376		–			
17 Calvinia	31.25S,19.45E	5	100	S	4.346	51	4.3 ± 2.3	+		0.0	1.0
18 Botterkloof	31.49S,19.17E	3	60	S	7.411*	199		+?			
19 Sonop	31.57S,19.44E	3	60	S	5.190	88		–?			
20 Clanwilliam	32.11S,18.54E	6	120	S	4.674	273	11.5 ± 5.1	+			
21 Elandsvlei	32.20S,19.33E	4	80	H	6.209*	47		+			
22 Citrusdal	32.36S,19.00E	5	100	C	5.023	200		+		0.0	1.0
23 Piketberg	32.54S,18.46E	5	100	C	4.987	140		+			
24 Tweeriviere	33.10S,19.48E	4	80	C	4.968	9		+			
25 Ceres	33.21S,19.18E	3	60	C	5.441	2		+			
26 Sandvlei	33.36S,19.52E	5	100	C	4.643	22		+			

Locality	Coordinates	Sample sizes		Morpho-cluster[1]	Morphometric variance	Pheromonal variance[2]	Ovariole number[3]	DELW[4]	Nuclear DNA[5]		mtDNA[6]	
		colonies	bees						queen	drone	P_0Qa	P_0QQa
27 Malmesbury	33.28S,18.44E	5	100	C	5.683*	70		+				
28 Worcester	33.39S,19.27E	5	100	C	4.434	26		+				
29 Paarl	33.45S,18.56E	5	100	C	4.781	41		+				
30 Kraaifontein	33.50S,18.43E	6	120	C	4.499			+				
31 Stellenbosch	33.56S,18.51E	7	135	C	3.929		18.2	+				
32 Villiersdorp	33.59S,19.17E	5	100	C	5.801*	156		+?				
33 Somerset West	34.08S,18.50E	5	100	C	4.734	60	12.7	+				
34 Riviersonderend	34.10S,19.55E	3	60	C	4.614	86		+				
35 Hermanus	34.25S,19.16E	4	80	C	4.745	57		+				
36 Napier	34.28S,19.54E	5	100	C	3.682	80		+				
37 Gansbaai	34.35S,19.20E	1	20	C	3.839			+				
38 Upington	28.25S,21.15E	5	97	S	3.415		2.8 ± 2.7	–			0.0	1.0
39 Tontelbos	30.56S,20.23E	3	60	S	7.434*			–?			0.0	1.0
40 Sutherland	32.24S,20.40E	6	120	S	4.102		6.4 ± 3.0	+			0.0	1.0
41 Touwsrivier	33.20S,20.00E	5	100	H	4.679	14		+				
42 Bonnievale	33.55S,20.05E	5	100	C	5.122	37		+?				
43 Swellendam	34.02S,20.26E	5	100	C	5.512*	53	10.7	+				
44 Heidelberg	34.06S,20.59E	6	120	C	4.137		12.6 ± 4.1	+			0.0	1.0
45 Bredasdorp	34.32S,20.02E	1	20	C	3.969			+				
46 Skipskop	34.33S,20.24E	5	100	C	4.503	69		+				
47 Booiskraal	31.50S,22.36E	2	40	S	4.653	30		+?				
48 Vonkfontein	31.56S,21.50E	2	40	S	2.320			+?				
49 Beaufort West	32.18S,22.36E	10	199	H	4.618	15	7.9 ± 3.0	+			0.0	1.0
50 Middelwater	32.25S,22.04E	2	40	S	3.254	32		+?				
51 Mosselbaai	34.12S,22.08E	2	37	C	2.423		6.8	+				
52 Postmasburg	28.18S,23.05E	4	80	S	4.760		3.9 ± 2.9	–			0.0	0.5
53 Britstown	30.37S,23.30E	4	80	S	5.030		2.7 ± 1.9	–	2	6	0.0	1.0
54 Victoria West	31.25S,23.04E	3	60	S	4.225	36	4.2	+?	3	7	0.0	1.0
55 Murraysburg	31.58S,23.47E	1	20	S	3.725	174		+?				

Locality	Coordinates	Sample sizes		Morpho-cluster[1]	Morphometric variance	Pheromonal variance[2]	Ovariole number[3]	DELW[4]	Nuclear DNA[5]		mtDNA[6]	
		colonies	bees						queen	drone	P_0Qa	P_0QQa
56 Nelspoort	32.07S,23.01E	2	40	S	4.152	15	8.9	–?				
57 Boesmanskop	32.02S,24.19E	3	60	S	3.085	36		–?				
58 Aberdeen	32.29S,24.03E	3	44	S	0.663	15		+				
59 Wiegenaarspoort	32.38S,23.12E	2	40	S	4.444	17		–?				
60 Knysna	34.03S,23.03E	1	20	C	3.463		11.8 ± 2.9	+	3	7	0.14	0.86
61 Wittedrif	34.01S,23.22E	6	120	C	4.221			+				
62 Warrenton	28.09S,24.47E	6	120	S	4.990		3.3 ± 1.9	–			0.5	0.5
63 Cradock	32.08S,25.36E	6	120	H	6.088*	63		+	3	6		
64 Kendrew	32.31S,24.30E	6	120	H	4.333		10.2 ± 3.1	+				
65 Addo	33.29S,25.46E	5	100	H	5.843*	132		+	4	6		
66 Port Elizabeth	33.58S,25.36E	7	140	C	3.448		12.5 ± 2.8	+				
67 Springfontein	30.19S,25.36E	6	120	S	3.458		4.4 ± 1.2	–				
68 Smithfield	30.09S,26.30E	4	80	S	5.251	63		–	3	5		
69 Venterstad	30.47S,25.48E	2	40	S	3.935	36		–?				
70 Aliwal North	30.45S,26.45E	6	120	S		96		–?	5	8		
71 Burgersdorp	30.59S,26.20E	4	80	S	4.972	50		+?	3	4		
72 Jamestown	31.07S,26.48E	3	60	S	4.368	69		+?	5	7		
73 Steynsburg	31.20S,25.50E	3	60	S	5.299	35		+?	3	7		
74 Molteno	31.22S,26.22E	4	79	S	5.305	393		+	4	4		
75 Dordrecht	31.20S,27.03E	6	120	S	5.536*	122		+	6	10		
76 Hofmeyr	31.39S,25.50E	3	60	S	5.015	68		+?	4	7		
77 Sterkstroom	31.34S,26.33E	6	120	S	7.767*	71		+?	9	12		
78 Queenstown	31.52S,27.00E	11	219	S	4.759	710	6.0 ± 3.2	+	4	9	0.0	1.0
79 Tarkastad	32.01S,26.16E	6	120	S	6.649*	60		+?	6	10		
80 Fort Beaufort	32.48S,26.38E	4	80	S	6.786*	160		+	4	6		
81 Winburg	28.37S,27.00E	6	120	S	3.367		2.3 ± 2.1	–				
82 Zastron	30.18S,27.07E	6	120	S	3.468		3.8 ± 2.7	–	4	6	0.0	0.5
83 Stutterheim	32.33S,27.28E	6	120	H	4.971	779		+	6	9		
84 East London	32.58S,27.55E	6	120	S	3.931		7.1 ± 2.0	+				

Locality	Coordinates	Sample sizes		Morpho-cluster[1]	Morphometric variance	Pheromonal variance[2]	Ovariole number[3]	DELW[4]	Nuclear DNA[5] queen drone	mtDNA[6]	
		colonies	bees							P_0Qa	P_0QQa
85 Harrismith	28.18S,29.03E	6	120	S	3.490		2.6 ± 1.1	–			
86 Underberg	29.50S,29.22E	1	20	S	3.369		1.6 ± 1.2	–		0.0	1.0
87 Richmond	29.45S,30.56E	2	40	S	3.593	21		–?			
88 Durban	29.55S,31.00E	5	100	S	4.120		3.2 ± 1.7	–		0.33	0.0
89 Ixopo	30.08S,30.00E	5	100	S	3.214		2.9 ± 1.1	–		0.0	0.75
90 Badplaas	25.58S,30.34E	6	120	S	3.277		3.5 ± 1.8	–			
91 Hoedspruit	24.21S,30.57E	6	120	S	2.766		2.8 ± 1.2	–		0.75	0.25
92 Johannesburg	26.10S,28.02E	1	20	S	3.630			–			
93 Keetmanshoop	26.36S,18.08E	4	80	S	3.785			–?			
94 Klerksdorp	26.58S,26.39E	6	120	S	3.777		4.6 ± 2.3	–		0.0	0.67
95 Magaliesberg	26.00S,27.33E	5	100	S	3.794			–			
96 Maltahohe	24.50S,17.00E	1	18	S	2.622			–?			
97 Mariental	24.36S,17.59E	4	80	S	3.943		3.3 ± 1.4	–?			
98 Nigel	26.30S,28.28E	6	120	S	4.256			–		0.67	0.33
99 Pretoria	25.45S,28.12E	4	60	S	2.856			–			
100 Sodwana Bay	27.20S,32.45E	1	7	S	1.759		2.9 ± 1.6	–			
101 Thabazimbi	24.41S,27.21E	5	100	S	3.941			–		0.25	0.75
102 Tsabong	26.28S,21.35E	1	20	S	2.359		3.1 ± 1.5	–?			
103 Vryheid	27.52S,30.38E	6	120	S	3.655		3.1 ± 1.5	–		0.25	0.75
104 Warmbaths	24.53S,28.17E	6	120	S	3.581			–		0.25	0.5

[1] Morphocluster analyses include previously published data [4, 22]; C = *capensis*, H = hybrid, S = *scutellata*.
[2] From Hepburn et al. [7].
[3] From Hepburn and Crewe [9].
[4] DELW = diploid eggs from laying workers [7].
[5] Nuclear DNA alleles at the Z-locus [18].
[6] Frequency distribution of two mitochondrial haplotypes [17].
* Significant ($P < 0.05$).

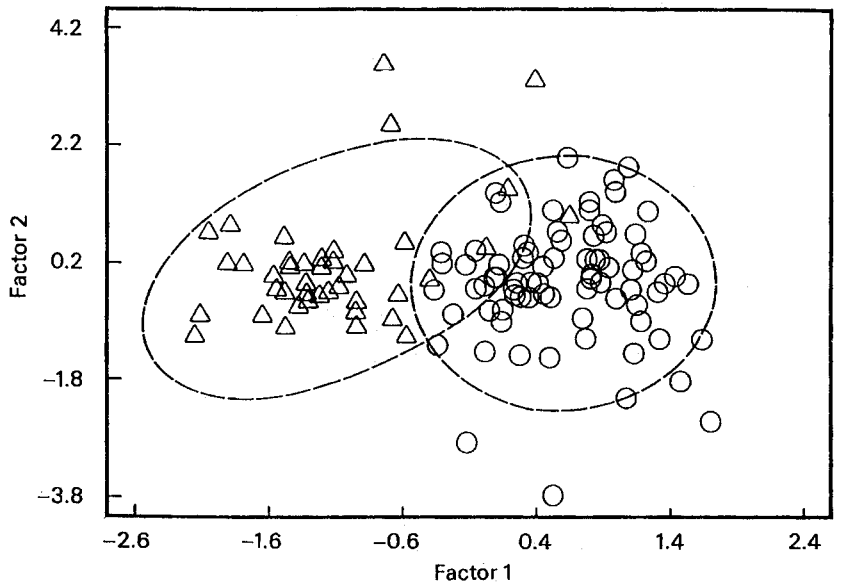

Figure 1. Factor analysis plot using the colony means of the morphometric data: cluster 1 = *capensis* morphocluster; cluster 2 = *scutellata* morphocluster. Confidence ellipses are at the 90 % level.

sis) and 97.7 % of the colonies from the rest of southern Africa into group 2 (*scutellata*).

Colonies from Beaufort West, Cradock, Fort Beaufort, Elandsbaai, Elandsvlei, Clanwilliam and Skipskop exhibited intermediate discriminant scores indicating hybridization in these regions (*table I*). A jackknife procedure gave the same classification results except that one colony from Clanwilliam was misclassified into group 2. A significant difference between the group means was found ($\lambda = 0.21$ with 4,1,122 df, $F = 109.66$ with 4,119 df, $P < 0.0001$).

The variances of the factor scores were used to test for homogeneity of the variances at each locality. A significant difference was found between the variances over all the localities (*table I*, Levene's test $F = 6.16$ with 123,8365 df, $P < 0.0001$). Significantly larger variances were obtained in the morphometrically defined hybrid zone (*tables I* and *II*, *figure 2*). Also a larger percentage of significantly high morphometric variances was found in the hybrid zone than in the two morphocluster areas (*table II*).

4. DISCUSSION

The results of the discriminant function analysis clearly demonstrate that the honeybees of southern Africa below 28° of latitude south resolve into two discrete clusters based on their morphometric characteristics (*figure 1*). Traditionally, these morphoclusters would be designated as the subspecies *A. m. capensis* and *A. m. scutellata* while those honeybees which fall outside of the confidence ellipses would be considered hybrids and their native localities a hybrid zone [22]. Although multivariate techniques provide probability contours of high predictive value in morphometric analyses of populations, they may also mask important indices of genetic flux.

As examples, in the area of the *capensis* morphocluster, the bees of Malmesbury

Table II. Summary statistics of the morphometric and non-morphometric traits when analysed in terms of the morphocluster distributions.

	capensis	Hybrids	*scutellata*	Tests
Sample size *n*	28	10	66	
Mean morphometric variance	4.53	5.11	4.13	$F = 3.36$ (2,99df) $P = 0.039$*
No. of high morphometric variances	3 ($n = 28$)	4 ($n = 10$)	7 ($n = 64$)	$\chi^2 = 6.46$ (2df) $P = 0.039$*
No. of high pheromonal variances	2 ($n = 18$)	3 ($n = 8$)	4 ($n = 29$)	$\chi^2 = 3.11$ (2df) $P = 0.211$
Ovariole number	8/0 high/low	2/0 high/low	5/23 high/low	$\chi^2 = 20.81$ (2df) $P < 0.001$**
DELW[1] frequencies	26/0 yes/no	10/0 yes/no	9/29 yes/no	$\chi^2 = 45.18$ (2df) $P < 0.001$**
Nuclear DNA allele mean frequencies	3.0/7.0 ($n = 1$) queen/drone	4.3/7.0 ($n = 3$) queen/drone	4.3/7.2 ($n = 15$) queen/drone	$F_{queen} = 0.29$ (2,16df) $P = 0.750$ $F_{drone} = 0.01$ (2,16df) $P = 0.987$
mtDNA haplotype frequencies	3/1/0 QQ/mixed/Q	1/0/0 QQ/mixed/Q	13/8/1 QQ/mixed/Q	$\chi^2 = 1.07$ (4df) $P = 0.899$

[1] DELW = diploid eggs from laying workers.
Significant at 0.05*, 0.01**.

(*figure 2*, site 27; *table I*) are morphometrically significantly more variable than those of Stellenbosch (*figure 2*, site 31; *table I*) only 50 km away; in the area of the *scutellata* morphocluster, the same applies to the bees of Hofmeyr (*figure 2*, site 76; *table I*) and Sterkstroom (*figure 2*, site 77; *table I*). Similarly, morphometric variance at Swellendam (*figure 2*, site 43) in the *capensis* morphocluster area is significantly greater than that at Touwsrivier (*figure 2*, site 41) in the morpho-hybrid zone (*figure 2*, *table I*). It is equally evident that an area which is defined by a specific morphocluster may not coincide with the area of distribution of some particular character (for example thelytokous parthenogenesis, *figure 2*, sites 9, 17, 40, 58, 63, etc.) which is generally regarded as a unique property of honeybees in the morphocluster 'mother area' (*figure 2*).

The distributions of mitochondrial and nuclear DNA profiles further illustrate this last point. Moritz et al. [17, 18] reported the spectrum of haplotypes from the COI-COII region of mtDNA from worker bees along a transect from the Cape Peninsula to the northeastern Transvaal. Of these, the haplotype P_0QQa occurs with a frequency of 100 % (with the anomalous exception of site 60) in the area of the *capensis* morphocluster and only begins to decrease well within the area of the *scutellata* morphocluster (*figure 2*, sites 1, 15, 88, 89 and other localities north of 28°; *table I*). Conversely, the haplotype P_0Qa, a northern trait, only begins to disappear some 300 km short of the southern limit of the *scutellata* morphocluster area (*figure 2*, sites 15, 38, 62). (Similar incongruences have been reported elsewhere [5, 24].) No significant differences were found in the frequency distributions of the spectrum of

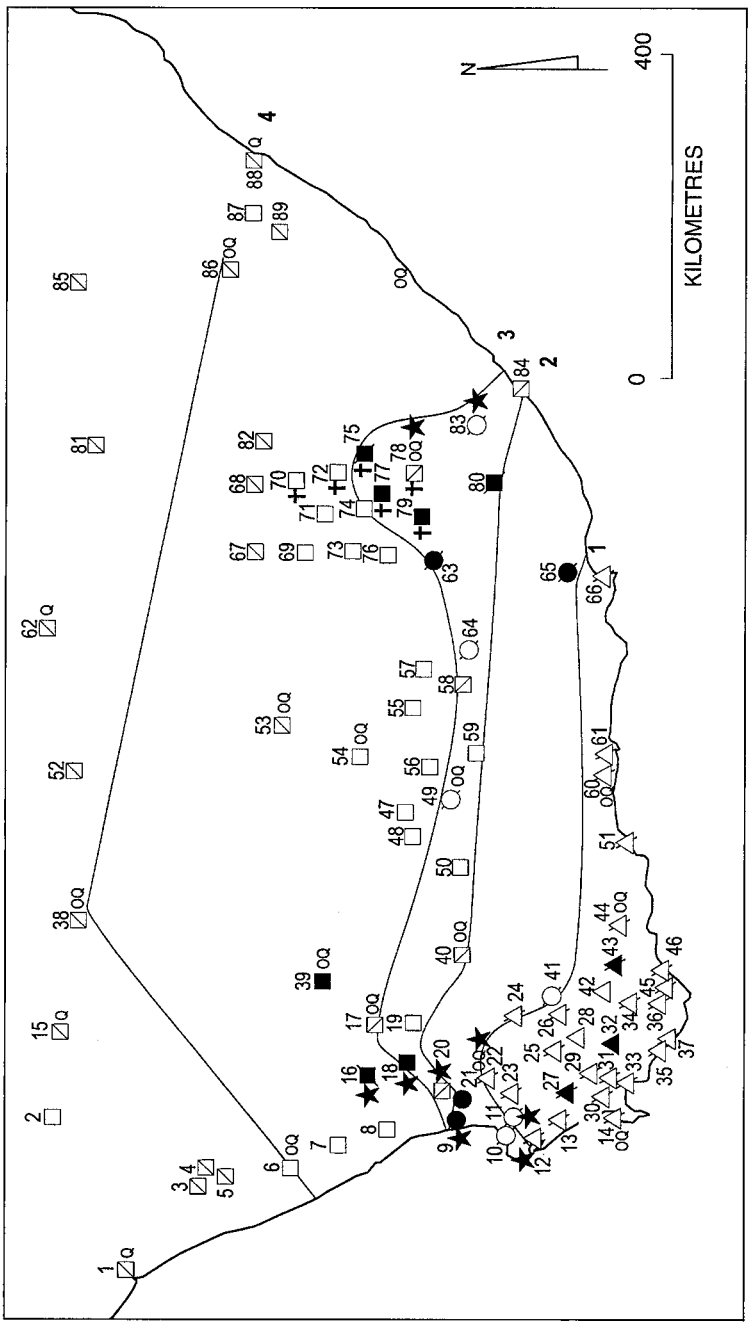

Figure 2. Map of southern Africa illustrating distributions of morphometric and non-morphometric features of *capensis, scutellata* and zone of introgression. Line 1 = northern limit of *capensis* morphocluster; line 2 = southern limit of *scutellata* morphocluster; line 3 = northern limit for thelytokous parthenogenesis; line 4 = northern limit for 100 % frequency of the *capensis* haplotype P_0QQa. Open triangles = *capensis* morphocluster, closed triangles = high morphometric variance; open squares = *scutellata* morphoclusters, closed squares = high morphometric variance; open circles = morphometric hybrids, closed circles = high morphometric variance in hybrids; stars = high sting pheromone variance; Q = *scutellata* P_0Qa haplotype; QQ = *capensis* P_0QQa haplotype; crosses = area of high mitochondrial and nuclear DNA variance. Oblique line \ = thelytokous parthenogenesis present, / = absent. Details by locality are given in *table 1*.

mtDNA haplotypes when compared between the morphocluster groups (*table II*). Moreover, maximal heterogeneity in allele frequencies for the Z-locus [1] in the nuclear DNA of queens and drones [18] occurs within the domain of the *scutellata* morphocluster (*figure 2*, sites 75, 77, 78, 79) and not within the morphocluster hybrid zone (with the exception of site 83, *figure 2*; *table I*). When the mean numbers of allele frequencies of queens and drones were compared between the morphocluster distributions no significant differences were found (*table II*). It must be acknowledged that a possible bias could derive from the fact that only about 20 % of the localities sampled were analysed in Z-locus terms.

The reproductive features of Cape worker bees have brought them considerable attention. Yet, Cape bees are further unusual in taxonomic terms because they constitute an exceptional case among honeybee subspecies precisely because non-morphometric features have been incorporated in the definition of *A. m. capensis* [4, 9, 20, 22]. In this context there are two genetic traits of interest: worker ovariole number and thelytokous parthenogenesis. Mean ovariole number in the *capensis* morphocluster area and hybrid zone is significantly greater than that of the *scutellata* morphocluster area (*table II*; [8]). Similarly, thelytokous parthenogenesis occurs with the highest female/male progeny ratios in the *capensis* morphocluster area but nonetheless is also expressed in the morpho-hybrid zone [9]. Thus the mean ovariole number typical of the *capensis* morphocluster area and the trait 'diploid eggs from laying workers' are both phenotypically expressed in the morphocluster hybrid zone well beyond the northern boundary of the *capensis* morphocluster area (*figure 2*, *table I*, also Hepburn and Crewe [8, 9]). However, significant differences in the ovariole number and the frequency of thelytokous parthenogenesis were also established between the *capensis* and hybrid morphocluster zones and the *scutellata* morphocluster zone (*table II*).

Compositional variability in another trait, sting alarm pheromones, is similarly problematic. Domains of significantly high pheromonal variance occur both within the morphometrically defined hybrid zone as well as the *capensis* morphocluster area in the west (*figure 2*, sites 9, 11, 12). However, to the east, high pheromone variance domains (sites 74, 78, 83) are closely associated with similar domains of DNA and occur within the area of the *scutellata* morphocluster as well as the hybrid zone (*figure 2*). No significant differences in the number of high pheromonal variances were found between the morphocluster zones (*table II*). Other population probes, such as allozymes of malate dehydrogenase are fixed throughout southern Africa [6, 23] and so do not contribute to the discussion in the same way as they would do for the three morphoclusters of the *iberica* region [10, 25]. Having outlined the distribution areas of the morphoclusters and a morphometrically defined hybrid zone, as well as the distributions of various non-morphometric traits, it is apparent that there is little geographical congruence between them (*figure 2*).

From a biological as opposed to strictly morphometric point of view, the honeybees below 28° latitude can be resolved into about 17 distinct groups based on the presence or absence of different traits in their various permutations. More groups would probably form as further traits are analysed and would result in an unworkable nomenclatural system which, in any event, would obscure the continuous flow of character traits in the population. Alternatively, two major groups and four areas could be delineated: *capensis* morphocluster, *scutellata* morphocluster, one hybrid zone where thelytokous parthenogenesis is expressed and another where it is not expressed. Finally, if areas of greatest overall variability were designated as hybrid zones this would result in a western hybrid zone in keeping with morphometric results, and an eastern hybrid zone within the morphometrically defined *scutellata* area (*figure 2*), a contradiction in terms.

It must also be remembered that, in addition to thelytokous parthenogenesis, Cape worker bees also have the ability to reversibly alter the pheromones of the mandibular gland from 'worker-like' to 'queen-like' [3, 7]. To designate honeybees as *A. m. capensis* solely on the grounds of their morphocluster membership (established through multivariate analysis) would effectively restrict the fully 'biological *capensis*' to one-fourth of the area in which it actually occurs. The empirical data demonstrate the dynamic nature of gene flow in a continuous population of honeybees: different traits have moved different distances (*table I*, *figure 2*). The result is a significant lack of concordance between a large suite of biological features and morphocluster (or subspecies) membership.

Those domains of greatest overall variability occur at the interface of different ecological–climatological zones. In the west this is between the winter and summer rainfall regions, the former coinciding with the mediterranean eco-climatic zone to the south and sahelian (Karoo of southern Africa) to the north; in the east, the transition is between dry tropical and sahelian (Karoo of southern Africa) regions (*figure 2*). Thus, the *capensis/scutellata* interface further documents the differential nature of gene flow in continuous populations of honeybees in Africa. Morphometric clusters may well be obtained from multivariate analyses, but there are always transitional forms between them. The apparent absence of efficient barriers to gene flow results in the introgression of traits from one population to another. Instead of the evolution of homotypically distinct types, the observable patterns of character distribution are probably the result of permanent hybridization [11, 12, 21].

The non-concordant distributions of the morphoclusters and other biological traits are ascribed to patterns of gene flow among different genes coding for the various characters. This does not imply that the characters discussed are considered as selectively neutral. With the possible exceptions of the Z-locus and the COI-COII mtDNA regions, all other character traits are potentially subject to selection pressure. Other possible explanations for the observed patterns of character distribution include gametic and zygotic gene flow. In the former case drones transmit only nuclear genes, but in the latter swarms transmit both nuclear and mitochondrial genes [16]. It is not yet possible to accurately discriminate between these mechanisms.

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Résumé – Structure de la population et interface entre *Apis mellifera capensis* et *Apis mellifera scutellata*. Les abeilles africaines au sud du 28^{e} parallèle ont été étudiées du point de vue morphométrique à partir d'un ensemble de données regroupant la base de données de l'Institut für Bienenkunde à Oberursel et celle du Groupe d'Apiculture de l'Université de Rhodes. Cet ensemble de données portait sur un total de 8 743 ouvrières issues de 442 colonies, qui représentaient 104 sites de prélèvement. Toutes les analyses ont porté sur neuf caractères morphométriques (n° 1, 11, 13, 35, 36, 32, 22, 30 et 31, d'après Ruttner, 1988). Une analyse discriminante pas à pas [14] a classé 91,1 % des échantillons provenant de la province occidentale du Cap comme *A. m. capensis* et 97,7 % des échantillons du reste de l'Afrique du Sud comme *A. m. scutellata*. Huit des échantillons n'ont pas été classés de façon claire et ont été considérés comme des hybrides. Les variances des caractères ont présenté des différences significatives (*tableau I*, $p < 0{,}0001$) et sont plus élevées dans la zone définie morpho-

métriquement comme zone d'hybridation (*tableau II*). La répartition géographique des groupes définis par la morphométrie a été comparée à celle des mêmes échantillons classés en fonction de leurs caractéristiques biologiques et génétiques, qui ont été publiées antérieurement. Il en ressort que l'aire de répartition de la parthénogenèse thélytoque, en particulier dans la région occidentale, pénètre dans l'aire d'*A. m. scutellata* au-delà de la zone d'hybridation morphométrique (*figure 2* ; *tableau II*). L'haplotype génétique P_0QQa dépasse largement la zone d'hybridation et s'étend jusque dans l'aire d'*A. m. scutellata* sans relation avec la limite entre les deux morphogroupes. De même les zones de forte variance présentent une image variée. La variance élevée de l'ADN se trouve dans la région orientale, presqu'exclusivement dans l'aire du morphogroupe *scutellata*. Dans la région occidentale, la variance élevée de la composition de la phéromone d'alarme coïncide en partie avec la variance élevée de l'ADN et s'étend dans l'aire d' *A. m. scutellata* ainsi que dans la zone d'hybridation; en revanche, dans la région occidentale, on la trouve dans les deux morphogroupes ainsi que chez les hybrides et sans être associée à une variance élevée de l'ADN, mais là elle suit la variance morphométrique élevée. Ces résultats montrent que les caractéristiques génétiques ou biologiques spécifiques sont largement indépendantes de la division en groupes obtenue par la morphométrie. Cela peut conduire à une forte contradiction entre la définition biologique de groupes, comme celui basé sur la parthénogenèse thélytoque, et la définition morphométrique de sous-espèces. L'association d'une variabilité élevée avec des zones de transition climato-écologique (à l'ouest des zones de pluviométrie hivernale ou estivale, à l'est des zones sahéliennes à climat tropical sec) montre que dans ces aires les caractéristiques génétiques individuelles peuvent pénétrer d'une population à l'autre de façon indépendante et différente.

***A. m. capensis* / *A. m. scutellata* / hybridation / morphométrie / phéromone / mtDNA / nDNA**

Zusammenfassung – Populationsstruktur der Schnittstelle zwischen *Apis mellifera capensis* und *Apis mellifera scutellata*. Die afrikanischen Honigbienen südlich 28° südlichen Breite wurden auf Grundlage eines zusammengefassten Datensatzes des Instituts für Bienenkunde in Oberursel und der Gruppe für Bienenzucht der Rhodes Universität morphometrisch untersucht. Dieser Datensatz umfasste insgesamt 8 743 Arbeiterinnen aus 442 Völkern, die 104 verschiedene Sammelorte repräsentierten. Alle Analysen wurden auf Grundlage von 9 morphometrischen Merkmalen (Nummern nach Ruttner 1988: 1,11,13,35,36,32,22,30,31) durchgeführt. Eine Diskriminanzanalyse mit schrittweiser Zuordnung der Proben [14] klassifizierte 91.1 % der Proben aus der westlichen Kapprovinz als *A. m. capensis* und 97.7 % der Proben des übrigen Gebietes als *A. m. scutellata*. 8 der Proben wurden nicht eindeutig zugeordnet und als Hybriden klassifiziert. Die Varianzen der Merkmale zeigten deutlich Unterschiede (*Tabelle I*, $P < 0.0001$) und waren in der morphometrisch definierten Hybridisierungszone am höchsten (*Tabelle II*). Die geographische Verbreitung der morphometrisch begründeten Gruppen wurde mit aus früheren Publikationen bekannten biologischen und molekulargenetischen Eigenschaften der gleichen Proben in Beziehung verglichen. Es zeigte sich, daß die Verbreitung der thelytoken Parthenogenese insbesondere im westlichen Bereich deutlich über die morphometrische Hybridisierungszone hinweg in den Bereich von *A. m. scutellata* hineinreicht (*Abb. 2*, *Tabelle II*). Der genetische Haplotyp P_0QQa reicht ohne eine Beziehung zur Grenze zwischen den Morphoclustern weit über die Hybridisierungszone in den Bereich von *A. m. scutellata* vor. Die Zonen hoher Varianz zeigen ebenfalls ein uneinheitliches Bild. Hohe Varianz der

DNA findet sich im östlichen Gebiet fast ausschließlich im Bereich des *Scutellata*-Morphoclusters. Während im östlichen Gebiet hohe Varianz in der Zusammensetzung der Alarmpheromone mit hoher Varianz der DNA teilweise zusammenfällt und sich auf den Bereich von *A. m. scutellata* sowie der Hybridisierungszone erstreckt, findet sich solche im westlichen Gebiet in beiden Morphoclustern sowie bei den Hybriden ohne eine Assoziation zu hoher Varianz der DNA, geht hier aber weitgehend mit hoher morphometrischer Varianz parallel. Diese Ergebnisse zeigen einen hohen Grad von Unabhängigkeit spezifischer genetischer oder biologischer Eigenschaften von der morphometrisch gewonnenen Gruppeneinteilung. Diese kann zu deutlichen Widersprüchen zwischen biologischen Gruppendefinitionen, wie etwa auf Grund der thelytoke Parthenogenese, und der morphometrischen Definition von Unterarten führen. Die Assoziation von hoher Variabilität mit ökologisch-klimatischen Übergangszonen (im Westen von Zonen winterlichen oder sommerlichen Regenfalls, im Osten von trocken-tropischer zu Sahelzonenbereichen) weist darauf hin, daß in diesen Bereichen einzelne genetische Eigenschaften unabhängig und unterschiedlich weit von einer Population in die andere vordringen können.

***A. m. capensis* / *A. m. scutellata* / Hybridisierung / Morphometrie / Pheromone / mitochondriale Dns / nucleare Dns**

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