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Original article

Morphometric analysis of 2 southern African races of honeybee

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Summary — A suite of 10 morphological characters was identified that discriminated between the 2 southern African honeybee races, *Apis mellifera capensis* and *A m scutellata*. Collections of samples from 32 localities which spanned the sub-continent from the west coast to the east coast and ranged from Cape Town in the south to north of Johannesburg were used to define the current distribution of the 2 races and the hybrid zone between them. The data obtained from the morphometric analysis are in good agreement with data on laying worker reproduction.

***Apis mellifera capensis* / *Apis mellifera scutellata* / morphometry / race / South Africa**

INTRODUCTION

The extensive area over which the species *Apis mellifera* is distributed has resulted in the production of substantial geographic variability, and the identification of numerous races within the species (Ruttner, 1988). The races are now generally defined on the basis of quantitative measures of morphological characters. Ruttner (1988) has reviewed the history and development of these sophisticated techniques. Quantitative analysis of morphological characters was used to define races of African honeybees by Smith (1961) when he studied honeybees in Tanzania.

Particular interest in the southern African races of honeybees has arisen because the Cape honeybee, *Apis mellifera capensis*, has workers that exhibit thelytokous parthenogenesis (Onions, 1912; Hepburn and Crewe, 1991) and queens of the other race, *A m scutellata*, from South Africa and Tanzania were sent to Brazil and formed the basis of the Africanized bee population. *A m capensis* was named by Echscholtz in the last century and its existence as a distinct race confirmed by Alpatov (1933) and Ruttner (1977a,b). The other southern African race had for many years been classified as *Apis mellifera adansonii*, until Ruttner (1988) conducted a thorough morphometric analysis of Afri-

can races of honeybees and indicated that it was *Apis mellifera scutellata* and quite distinct from the west African *adansonii*.

Resolution of the nomenclatural status of the 2 southern African bee races did not address the question of their current distribution or the way in which they interacted. Work by Ruttner (1977b) and Moritz and Kauhausen (1984) suggested that the Cape honeybee was restricted to a small region of the western Cape in the vicinity of Cape Town. However, the discovery that honeybees in the eastern Cape had *capensis*-like qualities (Hepburn *et al*, 1988; Hepburn, 1989) led to our conducting an extensive sampling of the southern African populations of honeybees (Hepburn and Crewe, 1990) in an attempt to define the limits of distribution of the 2 races and their hybrids. Using a combination of the number of ovarioles per ovary and the sex ratio of offspring of laying workers, it was possible to define the geographic location of the 2 races and the broad hybrid zone between them (Hepburn and Crewe, 1990, 1991). However, the use of a small number of characters made it difficult to describe the variation within the hybrid zone. Use of multivariate methods based on many characters was considered to be the best approach to resolving difficulties of interpretation.

To determine whether the analysis of additional morphological characters would support the distributions defined by ovary structure and sex of laying worker offspring, we carried out a thorough morphometric analysis of the bees collected from the colonies that were sampled for that study.

Table I. List of the localities at which bees were sampled for morphometric analysis, the number of hives sampled and their classification into race (c = *capensis*, h = hybrid, s = *scutellata*).

Locality	*No of hives sampled*	*Race*
Cape Town	6	c
Knysna	6	c
Heidelberg	6	c
Port Elizabeth	6	c
Clanwilliam	6	h
Garies	4	h
Beaufort West	6	h
East London	6	h
Graff Reinet	6	h
Sutherland	6	h
Calvinia	3	h
Ariamsvlei	4	s
Alexander Bay	6	s
Badplaas	6	s
Britstown	4	s
Durban	5	s
Harrismith	6	s
Hoedspruit	6	s
Ixopo	5	s
Underberg	1	s
Klerksdorp	6	s
Nigel	6	s
Posmasberg	4	s
Queenstown	6	s
Sprinfontein	6	s
Thabazimbi	5	s
Upington	5	s
Vryheid	6	s
Warmbaths	6	s
Warrenton	6	s
Winburg	6	s
Zastron	6	s
Total number of colonies investigated	172	

MATERIALS AND METHODS

The bees were sampled from 32 different localities which form a subset of those listed by Hepburn and Crewe (1991). The localities are listed in table I. They ranged from the west coast to the east coast, and from Cape Town in the south to north of Johannesburg.

At each locality, 20 bees were collected from each colony sampled. At most localities, 6 colonies were sampled. The selection of the colonies for sampling was done following the advice given by Ruttner (1988) that he encapsulated in the following way "the more primitive the bee-

keeper, the purer the race". What is meant by primitive in this case is that the colonies were established by trapping feral swarms, and had not been moved from the localities where they were collected. For each of the bees, 36 characters were measured. The characters used are listed in table VI.1 of Ruttner (1988). Complete sets of voucher material have been deposited at the Institut für Bienenkunde in Oberursel, Albany Museum in Grahamstown, and the National Collection of Insects in Pretoria.

To undertake the statistical analysis of the samples, the means and standard deviations of the characters measured from the 20 bees were used to give colony characters. These colony characters were used in the subsequent analysis. Multivariate statistical analysis of this data was undertaken using principal components analysis, stepwise discriminant function analysis and discriminant analysis. The statistical package SAS–PC Version 6.04 (SAS Institute, 1988) was used for the data analysis.

RESULTS

Principal components analysis

Preliminary exploration of the data set was undertaken using a principal components analysis to determine whether the colonies that had been identified as *capensis, scutellata* or hybrids formed distinct clusters. In this procedure all 36 characters that had been measured from each bee were used. The first 2 principal components that were extracted by this procedure accounted for 31.75% of the variance in the data set. Figure 1 shows the clusters that are formed when these 2 factors are plotted against each other. The *capensis* colonies (■) form a cluster in the upper-left-hand quadrant of the plot, while *scutellata* colonies (◆) cluster in the other 3 quadrants, with the exception of a single colony from Durban which had anomalously dark pigmentation. The hybrid colonies show a great deal of variability and overlap both of the clusters of their parental races, with a group of them falling into the diagonal space between the 2 main racial clusters. Hybrid colonies were defined using the criteria given in Hepburn and Crewe (1991).

The characters that load most heavily on factor 1 (eigenvectors > 0.23) are length of femur, length of tibia, metatarsus length, metatarsus width, length of sternite 3, transverse width of wax plate on sternite 3, width of sternite 6, forewing length and forewing width. These characters give some measure of the relative size of the bees and very probably relate to pollen loading ability. The characters that loaded least heavily on factor 2 (eigenvectors > –0.23) were the length of the hair on tergite 5, proboscis length, pigmentation of tergites 2, 3 and 4, and pigmentation of the *scutellum*. This pigmentation factor appears to be related to the colour of the bees. In figure 1, moving from left to right on the *x*-axis, results in moving from relatively smaller individuals to larger ones, while moving from the bottom to the top of the *y*-axis, results in a progression from lighter to darker individuals.

Discriminant analysis

Having established *via* the principal components analysis that the southern African races of honeybees formed clusters, we proceed to conduct a discriminant analysis. The first step in this process was to conduct a stepwise discriminant function analysis (significance level to enter and to stay = 0.15 see table II) to determine which of the 36 characters that had been measured best discriminated between the races. This procedure indicated that 12 of the 36 characters could be used to distinguish between the 2 races (table II). These characters were compared with those that Ruttner (1988) used to distinguish between all

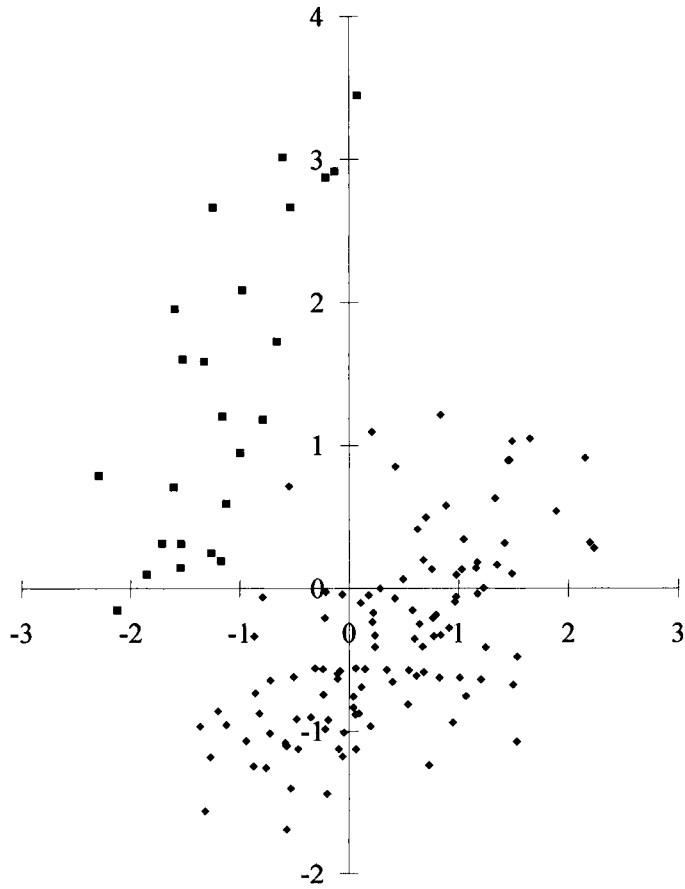

Fig 1. Scatterplot of the first (*x*-axis) and second (*y*-axis) principal components obtained from the colony means for the 36 characters measured on each bee. The *capensis* colonies are indicated by the symbol (■) and *scutellata* colonies by the symbol (◆). The hybrid colonies (not presented on the graph) do not cluster, some fall between the 2 main racial clusters and others overlap them.

of the tropical African honeybee races. Although both studies used a suite of 12 characters to discriminate between the races being examined, only 4 of these characters are common to both investigations. The descriptive statistics for the 12 characters that were chosen by the stepwise discriminant function analysis are presented in table III. Two of these characters, pigmentation of the *scutellum* (Sc) and the width of stripe posterior of tomentum, were not used in the generation of the

Table II. Results of the stepwise discriminant function analysis of the data set, which indicated that 12 of the 38 variables measured could be used to discriminate between *A m capensis* and *A m scutellata*. *

Step	*Variable*	*Partial R2*	F *statistic*	*Prob* > F
1	Pigmentation of tergite 2	0.784	482.65	0.0001
2	Length of cover hair tergite 5	0.119	17.80	0.0001
3	Wing angle J16	0.075	10.68	0.0014
4	Wax plate sternite 3, transverse	0.067	9.27	0.0028
5	Wing angle N23	0.046	6.20	0.0140
6	Pigmentation of scutellum (Sc)	0.062	8.43	0.0044
7	Pigmentation of scutellum (B,K)	0.039	5.14	0.0250
8	Proboscis	0.028	3.61	0.0597
9	Sternite 3, longit	0.030	3.85	0.0520
10	Fore wing, transverse	0.039	5.07	0.0260
11	Wing angle B4	0.022	2.80	0.0971
12	Pigmentation of scutellum (B,K)	0.016	2.00	0.1601 removed
13	Width of stripe posterior tomentum	0.018	2.30	0.1320
14	Wing angle O26	0.021	2.63	0.1077

* The variable entered at step 7 is removed at step 12.

Table III. Descriptive statistics of the variables used in the discriminant function analysis to classify colonies into the 2 southern African honeybee races. *

Character	Capensis *(N = 24)*			Scutellata *(N = 111)*		
	Mean	*SD*	*CV*	*Mean*	*SD*	*CV*
Pigmentation of tergite 2	4.89	1.24	25.30	8.32	0.51	6.14
Length of cover hair tergite 5	1.42	0.15	10.69	1.77	0.16	8.88
Wing angle J16	22.34	0.91	4.07	21.42	1.10	5.12
Wax plate sternite 3, transverse	2.12	0.04	1.70	2.17	0.05	2.18
Wing angle N23	76.30	2.15	2.82	74.55	2.06	2.77
Pigmentation of scutellum (Sc)	2.57	1.13	44.11	3.66	1.60	43.72
Proboscis	5.47	0.23	4.14	5.73	0.24	4.21
Sternite 3, longit	2.57	0.04	1.72	2.60	0.07	2.60
Fore wing, transverse	2.88	0.06	1.93	2.96	0.06	2.03
Wing angle B4	99.69	3.12	3.13	101.36	1.18	1.86
Width of stripe posterior tomentum	0.54	0.51	94.48	0.30	0.18	61.61
Wing angle O26	37.50	1.82	4.85	37.65	1.77	4.71

* *N* is the number of colonies from which 20 bees were sampled for analysis.

linear discriminant function used to classify the colonies into the 2 races since measurement of these characters was equivocal.

The linear discriminant function that was developed using the 10 characters listed in table IV, classified all of the *scutellata* colonies (table I, 111 colonies) into *scutellata* with a probability > 0.99, while all but 3 of the *capensis* colonies (24 colonies) were classified into *capensis* with a probability of 1.0. Of the remaining colonies, 2 were classified as *capensis* with $p > 0.99$ and 1 with $p > 0.78$.

The hybrid colonies (37 colonies) from 7 localities were analysed using the discriminant function developed from the data collected from racially homogeneous colonies and classified as indicated in table V. All of these hybrid colonies, with the exception of those from East London and Graff Reinet, are found between factor score contours –0.75 and –0.25 as indicated in figure 2.

To obtain a better understanding of the actual geographic distribution of the subspecies and their hybrid zone, we plotted the scores of factor 1 (fig 2) and the intercolonial variance (fig 3) at each sample location on the map of southern Africa. In this analysis, additional samples from Port St Johns (3 colonies), Molteno (5 colonies), Stutterheim (7 colonies) and Fort Beaufort (5 colonies) were included. The area with particularly low factor scores ranges from Cape Town in the west to Port Elizabeth in the east. The samples collected at Graaf Reinet were also within the range of low factor scores which results in a bulge-shaped distribution of *capensis*-like honeybees. Colonies sampled in coastal regions had a general trend for low factor scores and bees of smaller size.

A particularly steep cline is found along the west coast indicating a narrow hybrid zone between 2 racial types. The map in figure 3 corresponds well with the data presented in figure 2. Areas with steep clines are also areas with a high intercolonial variance. We calculated an estimate for the slope of the cline from the average factor score difference/geographic distance between sample location and all adjacent sample locations. The correlation between estimated cline and intercolony variance is highly significant ($r = 0.56$; $p < 0.001$; df = 35).

Table IV. The discriminant coefficients for each of the 10 characters used to generate the linear discriminant function used to classify of the colonies sampled.

Character	Capensis	Scutellata
Constant	–3872	–4026
Length of hair cover on tergite 5	22.42798	35.68944
Proboscis	63.04463	67.74426
Sternite 3, longit	–291.90016	–349.43507
Wax plate of sternite 3, transverse	467.41266	530.27797
Fore wing, transverse	898.52625	937.54516
Wing angle B4	23.06224	23.36596
Pigmentation of tergite 2	7.67941	15.86348
Wing angle J16	8.38750	6.41414
Wing angle N23	18.79714	18.02424
Wing angle O26	15.31983	15.94741

Table V. Classification of the hybrid colonies into races using the discriminant function developed from the data from racially homogeneous colonies. *

Locality	*Race*		
	Scut	*Int*	Cap
Beaufort West	3	0	3
Clanwilliam	5	1	0
East London	5	1	0
Garies	4	0	0
Graaf Reinet	2	0	4
Calvinia	3	0	0
Sutherland	6	0	0

* Classification into a particular race occurs when the posterior probability of membership is > 0.75. If $p < 0.75$ then the colony is classified as intermediate (int).

DISCUSSION

The sample sizes used in this investigation of the southern African races of honeybees are large, and the localities from which the material was collected extend over the whole subcontinent. As a consequence, we have been able to confirm by means of morphometric analysis the division of honeybee populations in southern Africa into 2 races with a hybrid zone between them. The morphometric data are in agreement with reproductive and other biological data (Hepburn and Crewe, 1990, 1991).

The principal components analysis provides a clear indication that the 2 races form distinct clusters that are dependent on a complex interaction between size and colour, with *capensis* being generally smaller and darker then *scutellata*. This result is in accord with previous less exten-

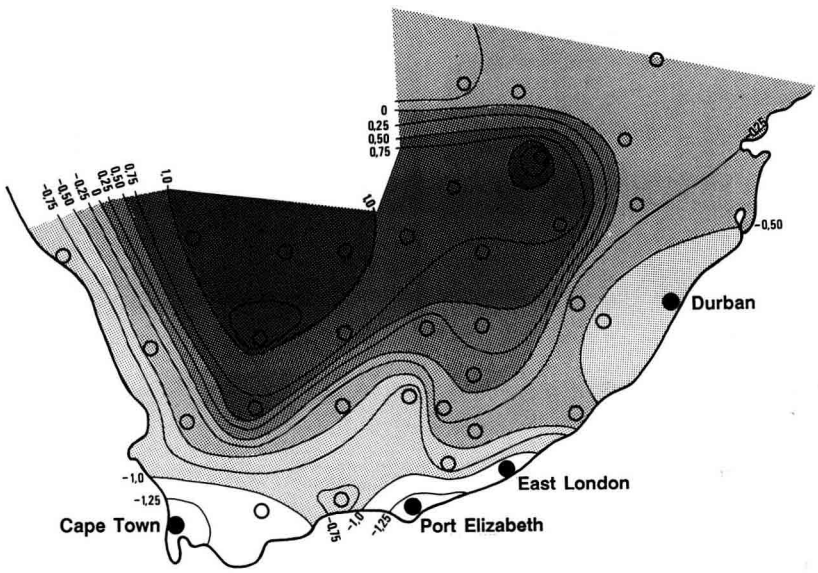

Fig 2. Graphic representation of the scores of factor 1 on a map of South Africa. The factor scores of colony means are low for sample locations along the southern coast line. The shading indicates the height of the score value in steps of 0.5.

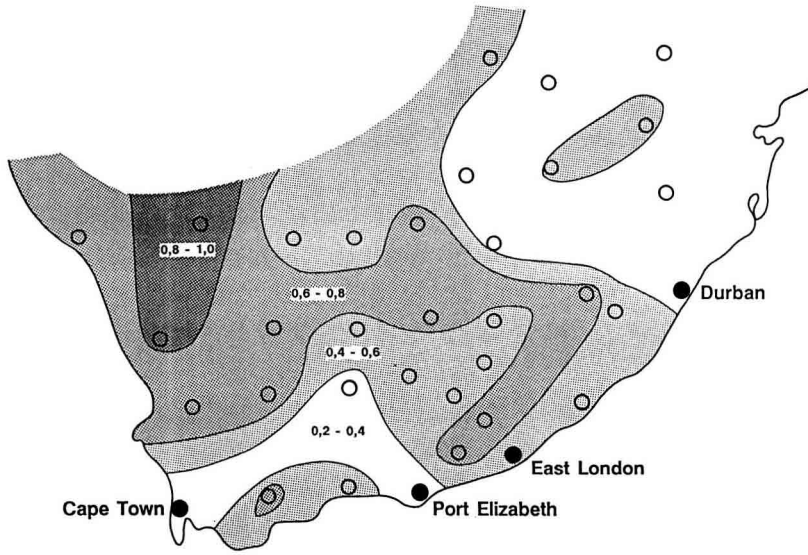

Fig 3. Intercolonial variance at each sample location for factor 1. The darker shades indicate a higher variance.

sive investigations of the relationship between the races (Ruttner, 1988). The hybrid colonies display a much greater variation in characteristics (fig 1) than the 2 parental races, as would be expected from their hybrid origin (Moritz and Kauhausen, 1984).

The linear discriminant function that was developed using our data set appears to be able to discriminate effectively between colonies of the 2 races. The fact that we used characters that were different from those used by Ruttner (1988) is probably a consequence of having to discriminate between only 2 populations of bees with a much more restricted geographic distribution.

The distribution of *capensis* in the eastern Cape province is more extensive than was previously thought to be the case (Ruttner, 1988). It extends along the southern coast, eastwards to beyond Port Elizabeth and inland as far as Graaf Reinet. The hybrid zone, as revealed by an increasing population variability, covers a vast geographic area. This is surprising in the light of the constraints under which stable equilibria between both reproductive strategies can be obtained in population genetic models (Moritz 1986, 1989). The relatively wide zone with increased intercolonial variance may be due, in part, to the extensive migratory bee keeping that has taken place over the last 20 yr (Allsopp, 1992). Nevertheless, racially pure areas with reduced intercolonial variance can be identified clearly and it remains fascinating to speculate on the reasons for the existence of this hybrid zone, particularly since *capensis* workers have the potential to act as social parasites in *scutellata* colonies and usurp the reproductive function of the *scutellata* queen.

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Résumé — Analyse morphométrique de 2 races d'abeilles sud-africaines. Les populations d'abeilles (*Apis mellifera* L) d'Afrique du Sud ont été échantillonnées dans 32 localités différentes (liste dans le tableau I). Vingt abeilles ont été prélevées par colonie à raison de 6 colonies par localité. On a mesuré 36 caractères sur chaque abeille (tableaux II, III). Les caractères retenus sont ceux décrits par Ruttner (1988). On a ensuite soumis à l'analyse factorielle et à l'analyse discriminante les moyennes de chaque colonie. L'ensemble des données obtenues à partir des mesures a été soumis à une analyse en composantes principales. Celle-ci a montré que les localités qui avaient des colonies identifiées comme étant de la race *capensis* étaient regroupées en un amas, que celles qui avaient des colonies identifiées comme *scutellata* étaient regroupées en un autre amas et que les colonies hybrides étaient variables (tableau V) et formaient un amas qui chevauchait ceux de leurs 2 parents (fig 1). Une analyse discriminante pas à pas indique qu'il est possible d'utiliser 12 caractères pour séparer les 2 races (tableau II). La fonction discriminante linéaire sur la base de 10 caractères (tableau III) a discriminé toutes les colonies de *scutellata*, avec une probabilité comprise entre 0,99 et 1, tandis que les colonies *capensis* ont été discriminées avec une probabilité comprise entre 0,78 et 1. Les données morphométriques présentées ici confirment la division des populations d'abeilles d'Afrique du Sud en 2 races, avec présence d'une zone hybride entre elles. Par ailleurs, la répartition de *capensis* dans la partie est de la province du Cap est plus étendue qu'on ne le pensait auparavant.

***Apis mellifera capensis* / *Apis mellifera scutellata* / morphométrie / Afrique du Sud / race**

Zusammenfassung — Morphometrische Analyse von zwei südafrikanische Rassen der Honigbiene. Tabelle I zeigt 32 verschiedene Sammelorte, an denen Stichproben in Honigbienenpopulationen genommen wurden. Je Ort wurden 20 Arbeiterinnen von jeweils sechs Völkern gesammelt. Insgesamt wurden 36 morphologische Merkmale je Biene gemessen wie bei Ruttner (1988) beschrieben. Die Mittelwerte jeder Kolonie wurden mit Hilfe der Faktorenanalysen und der Diskriminanzanalyse ausgewertet.

Eine Principal Component Analyse zeigte, daß die Proben, die als zur Rasse *Apis mellifera capensis* und *Apis mellifera scutellata* zugehörig klassifiziert waren, klare Cluster bilden, während die Bienen der Hybridzone eine große Variabilität in ihren morphologischen Merkmalen aufwiesen (Tabelle V). Sie bildeten einen Cluster, der mit den beiden reinen Rassen überlappte (Abb 1). Eine schrittweise Diskriminanzanalyse zeigte, daß 12 Merkmale zur Unterscheidung zwischen den beiden reinen Rassen herangezogen werden konnten (Tabelle II). Bereits die Diskriminanzfunktion auf einer Basis von nur 10 Merkmalen (Tabelle III) trennte die *scutellata* Kolonien von den restlichen Proben mit einer Wahrscheinlichkeit zwischen 0,99 und 1, während diese Wahrscheinlichkeit bei den *capensis* Stichproben zwischen 0,78 und 1 lag.

Die morphologische Analyse bestätigt die Teilung der Honigbienen Südafrikas in zwei Rassen mit einer klar definierten Hy-

bridzone. Die Ausbreitung der Kaphonigbiene in der Kapprovinz ist wesentlich weiter als in früheren Arbeiten angenommen.

***Apis mellifera capensis* / *Apis mellifera scutellata* / Morphometrie / Rasse / Südafrika**

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