Discriminant analysis of the honeybee populations of southwestern Africa

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The morphometric characters and sting pheromones of worker honeybees, *Apis mellifera* Linnaeus, were analysed by multivariate methods to characterize their populations in southwestern Africa. There is a discrete homogeneous population in northern South Africa and southern Namibia recognized as the subspecies *A. m. scutellata*, a discrete population in northern Namibia recognized as *A. m. adansonii* and a hybrid population between them.

Key words: *Apis mellifera*, southwestern Africa, morphometrics, sting pheromones.

INTRODUCTION

The subspecies of *Apis mellifera* Linnaeus of Africa have been characterized morphometrically, but very few honeybees from Namibia, and none from Angola, were included in the analyses (Ruttner 1988, 1992). These two countries are geographically poised between the ranges *A. m. scutellata* Lepeletier to the southeast (Hepburn & Crewe 1991; Crewe *et al.* 1994) and *A. m. adansonii* Latreille to the northwest (Ruttner 1988, 1992). The colour patterns of drones from northern Namibia (Johannsmeier 1973) and worker bees from Angola (Portugal Araujo 1956; Rosario Nunes & Tordo 1960) suggest that these bees could be *A. m. adansonii* or their hybrids with *A. m. scutellata*. Conversely, sparse morphological data on bees from southern Namibia (Moritz & Kauhausen 1984; Ruttner 1992) suggest affinities with *A. m. scutellata* of Botswana and the northern Cape Province of South Africa. Honeybees of southwestern Africa were studied morphometrically and pheromonally to characterize the populations and establish their subspecific characteristics.

MATERIAL AND METHODS

Worker honeybees were collected during January 1994 at seven localities extending from Nababeep (29.36S 17.46E) in northwestern South Africa through Karasburg (28.00S 1843E), Keetmanshoop (26.36S 18.08E), Mariental (24.36S 17.59E), Windhoek (22.43S 17 06E), Okahandja (21.59S 16.58E) to Otjiwarongo (20.29S 16.36) in Namibia (Fig. 1).

Morphometric measurements were made on 20 alcohol-preserved workers from each colony and four colonies were sampled at each locality. The same morphometric characters used in a previous study that successfully classified colonies into two southern African races of honeybees were selected for this present analysis (Crewe *et al.* 1994). Their Ruttner (1988) numbers are given in brackets except the wing angle MJI (see Ruttner 1988, Fig. 6.9): length of cover hair on tergite 5 (1), pigmentation of scutellum (35), pigmentation of scutellar plate (36), wing angle B4 (22), wing angle MJI, wing angle N23 (30), wing angle O26 (31), length of proboscis (4), width of wax plate on sternite 3 (11), transverse length of wax plate on sternite 3 (13), pigmentation of tergite 2 (32).

For the pheromone analyses, guard bees were sampled from five to six colonies per locality. Four of these colonies at each locality were the same as those used for the morphometric analysis. The stings of live bees were removed and individually placed in vials containing dichloromethane. Sting samples were concentrated with nitrogen for gas chromatographic analysis. Five sting samples per colony were analysed using a Hewlett Packard 5890 Series II gas chromatograph fitted with a bonded methyl silicone fused silica column, 0.3 mm × 25 m. Signals from the flame ionization detector were quantified with a Hewlett Packard 3396 Series II integrator and calibrated against authentic standards. The following pheromone compounds were identified: isopentyl alcohol (1), n-butyl acetate (2), isopentyl acetate (3), benzyl alcohol (4), n-octyl acetate (5), 2-nonanol (6), benzyl acetate (7), n-decyl acetate (8).

Multivariate statistical methods, including principal component analysis, factor analysis and linear discriminant analysis were used to analyse

the data. In the morphometric analyses, the means, standard deviations and covariances of the 11 characters from the 20-bee samples were used. For the pheromones, the means, standard deviations and covariances of the percentages of the eight compounds were used for analysis.

RESULTS

Morphometric analysis

In a principal component analysis of the morphometric measurements, three factors were isolated: Factor 1, pigmentations of scutellum (35) and scutellar plate (36), angles of wing venation MJI and B4 (22); Factor 2, width/length of wax plate on sternite 3 (11) and (13), angle of wing venation O26 (31); Factor 3, length of proboscis (4), length of hair on tergite 5 (1), angle of wing venation N23 (30). These factors accounted for 60.5 % of the variance in the data. The loadings for each character had absolute values greater than 0.60. The graph of factors 1 and 2 scores showed colonies from Otjiwarongo forming a cluster in the right-hand-half of the plot, while the colonies from Nababeep, Karasburg and Keetmanshoop clustered in the left-hand-half. Colonies from Mariental, Windhoek and Okahandja were evenly scattered.

A stepwise discriminant analysis was used to verify the separation of the two clusters obtained from the factor analysis (Fig. 2). The Mahalanobis distance between the two groups was 13.48. Table 1 gives the characters entered into the linear discriminant functions ranked according to their discriminatory power. Each case was assumed to have equal prior probability of being in any group.

The discriminant analysis revealed that pigmentation of the scutellum best discriminated between the two groups. The second character to enter the discriminant function was angle MJI of the wing venation. Both these

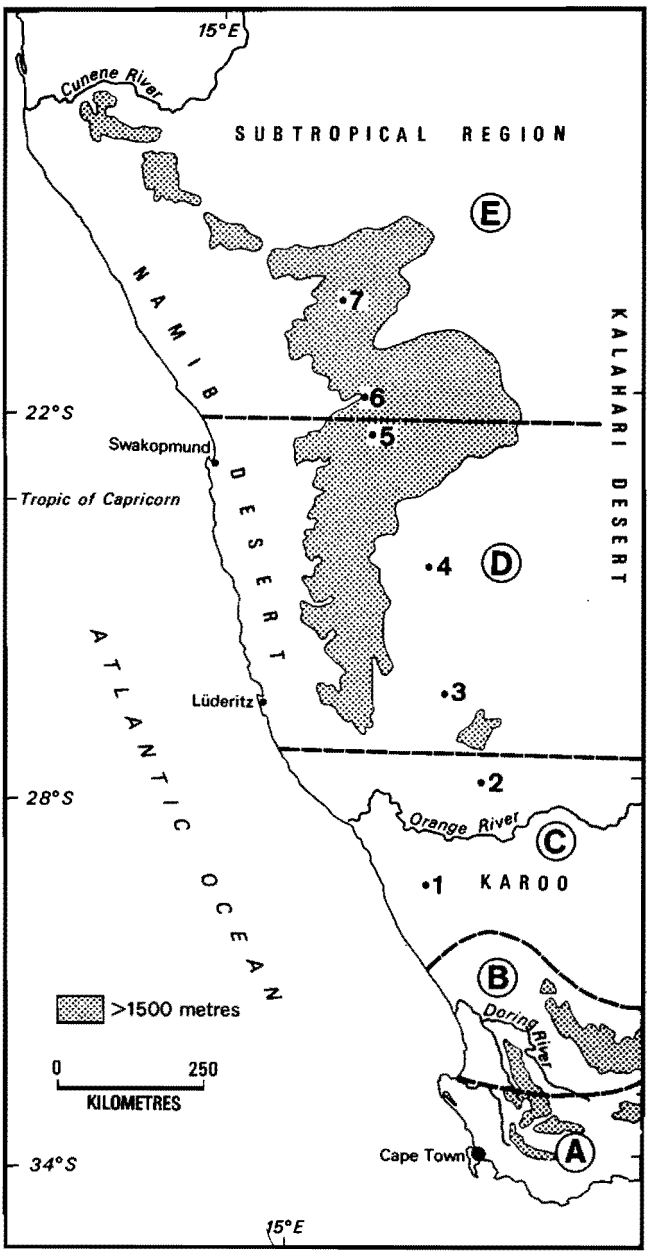

Fig. 1. Honeybee populations of southwestern Africa. Numbers 1–7 are the localities sampled from Nababeep to Otjiwarongo. Indentifiable populations based on morphometric and pheromonal variance indicated are: *Apis mellifera capensis* (**A**), *A. m. capensis* × *A. m. scutellata* hybrid zone (**B**) (Hepburn *et al.* 1994), *A. m. scutellata* (**C**), *A. m. scutellata* × *A. m. adansonii* hybrid zone (**D**) and *A. m. adansonii* (**E**).

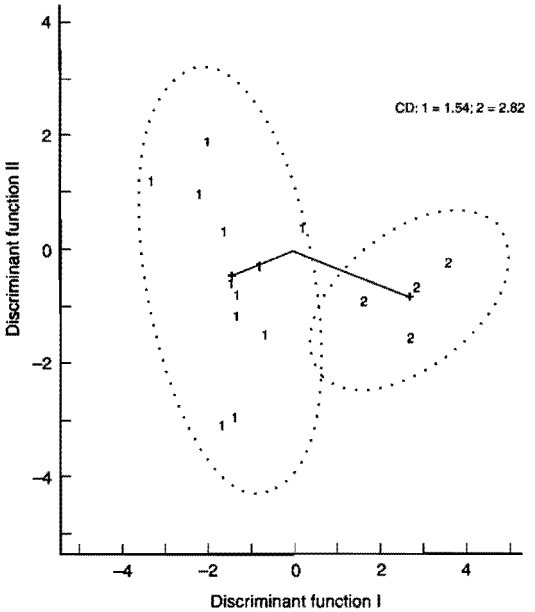

Fig. 2. Discriminant analysis plot using the colony means of the morphometric data. Cluster 1 comprises colonies from Nababeep, Karasburg, Keetmanshoop and cluster 2 reflects colonies from Otjiwarongo. Confidence ellipses at the 90 % level. CD = canonical distances from common centroid of clusters.

characters had high factor loadings in factor 1 of the factor analysis. The next three characters to enter the discriminant function were wing angle O26 (31), width/length of wax plate on sternite 3 (13) and (11) were heavily loaded in factor 2.

The linear discriminant functions obtained using the five characters in Table 1 correctly classified all the colonies from Nababeep, Karasburg and Keetmanshoop into group 1 with *a posteriori* probability $P = 1.0$ for 11 cases and $P = 0.97$ in the remaining case. All the colonies from Otjiwarongo were correctly classified into group 2 with *a posteriori* probability $P = 1.0$. Six colonies from Mariental, Windhoek and Okahandja were classified into group 1 and six into group 2. This classification procedure may provide an optimistic estimate of the probability of correct classification. A jackknife procedure was therefore carried out which classifies each colony into a group with the highest *a posteriori* probability according to the discriminant functions computed from all the data except the colony being classified. This is repeated for each colony omitted in turn (Lachenbruch & Mickey 1968). The same classification results were

Table 1. Morphometric characters entered into the discriminant function ranked according to their discriminatory power.

Character entered	*F*-value	*d.f.*	*P*-value
Pigmentation (35)	20.904	1,14	0.0004
Wing angle MJI	3.103	1,13	0.1016
Wing angle (31)	4.451	1,12	0.0565
Length of wax plate (13)	2.872	1,11	0.1182
Width of wax plate (11)	5.769	1,10	0.0372

obtained except for specimens from one colony from Keetmanshoop that were misclassified into group 2.

Another method was also used to check the estimate of probability of correct classification of the discriminant function. The data were divided at random into two samples. The first half of the data was used to fit the discrimination function while the remainder was used to test the classification performance of the method. Linear discriminant analysis using the five characters specified, did not misclassify any cases in the training data set or any in the test data set.

To test for the equality of the group means for the characters used in the discriminant function, Wilks' lambda approximated by the *F*-statistic was determined. Means of the two groups differed significantly ($\Lambda = 0.1188$, *d.f.* 5,1,14; $F = 14.838$, *d.f.* 5,10; $P = 0.0002$). The honeybees of southwestern Africa thus resolve morphometrically into two distinct and homogeneous populations, one in the north and one in the south with a heterogeneous population between them. The means and standard deviations of the 11 morphometric characters are shown in Table 2 for the two groups and the transition group using all 560 bees from seven localities.

A stepwise regression analysis was used to assess the relationship between the morphometric characters (independent variables) and geographical latitude of the seven localities (dependent variable). The procedure entered four of the five characters obtained in the discriminant analysis together with the length of cover hair on tergite 5 (1) into the model (Table 3). Four of the characters were significantly correlated with latitude and 68.2 % of the variation in latitude was accounted for by these five characters. Finally, a correlation analysis of pigmentation and latitude yielded a significant correlation coefficient ($r = -0.6116$, $P = 0.0005$) indicating that pigmentation is strongly graded

Table 2. Means and standard deviations of morphometric characters (measurements in mm, angles in degrees).

Character	Group 1 $n = 240$	Transition group $n = 240$	Group 2 $n = 80$
Hair length (1)	0.23 (0.03)	0.22 (0.03)	0.22 (0.03)
Proboscis length (4)	5.69 (0.37)	5.71 (0.31)	5.87 (0.16)
Width of wax plate (11)	2.62 (0.08)	2.57 (0.05)	2.60 (0.10)
Length of wax plate (13)	2.16 (0.08)	2.15 (0.06)	2.14 (0.07)
Pigmentation (35)	4.92 (1.81)	6.02 (1.52)	7.36 (1.63)
Pigmentation (36)	1.58 (2.11)	3.05 (2.50)	4.20 (1.69)
Pigmentation (32)	7.95 (0.48)	8.24 (0.53)	8.19 (0.79)
Wing angle B4 (22)	104.98 (6.08)	103.44 (6.20)	103.33 (6.02)
Wing angle MJI	20.47 (2.57)	21.01 (2.17)	23.25 (2.61)
Wing angle N23 (30)	85.45 (3.55)	85.14 (3.57)	84.77 (3.72)
Wing angle O26 (31)	37.35 (3.92)	36.81 (4.06)	37.30 (3.38)

Table 3. Morphometric characters entered into the regression model.

Independent variable	Coefficient	*t*-value	*P*-value
Constant	27.16	1.04	0.3096
Pigmentation (35)	–1.70	–4.19	0.0004
Length of wax plate (11)	31.85	3.25	0.0038
Width of wax plate (13)	–44.48	–3.62	0.0016
Hair length (1)	69.97	2.50	0.0210
Wing angle (31)	0.43	2.04	0.0539

Table 4. Sting pheromone compounds entered into the discriminant function ranked according to their discriminatory power.

Pheromone entered	*F*-value	*d.f.*	*P*-value
benzyl acetate	16.286	6,33	<0.0001
benzyl alcohol	5.970	6,32	0.0003
2-nonanol	5.743	6,31	0.0004
isopentyl alcohol	4.316	6,30	0.0030
isopentyl acetate	2.414	6,29	0.0514

from black in the south with a transition to yellow in the north.

Pheromone analysis

In a principal components analysis of the eight compounds of the sting pheromone complex, three factors were isolated: factor 1, 2-nonanol (6), n-octyl acetate (5), n-butyl acetate (2); factor 2, isopentyl acetate (3), isopentyl alcohol (1), benzyl acetate (7); factor 3, n-decyl acetate (8), benzyl alcohol (4). These factors accounted for 66.39 % of the sample variance. The scattergram of the factors 1 and 2 scores showed colonies from Karasburg forming a cluster in the upper half of the plot. To a lesser extent the colonies from Mariental formed a cluster in the right-hand-half of the plot.

A stepwise discriminant analysis of the sting pheromone compounds was performed and the compounds ranked according to their discriminatory power (Table 4).

The linear discriminant functions obtained using the five pheromones (Table 4) correctly classified 83.3 % of the colonies from Nababeep, 100 % of the colonies from Karasburg and Keetmanshoop, 83.33 % from Mariental, 66.67 % from Windhoek and 100 % from Okahandja and Otjiwarongo.

DISCUSSION

The principal component analysis of the morphological characters of the honeybees of southwestern Africa established two distinct groups. The southern group includes bees from Nababeep, Karasburg and Keetmanshoop while the northern group occurs at Otjiwarongo. In central Namibia the bees from Mariental, Windhoek and Okahandja are heterogeneous. These population differences are also indicated in the discriminant analysis. Similarly, discriminant analysis of the percentage occurrence of sting pheromone compounds produced distinct northern and southern groups with highly heterogeneous intermediate populations. Combining the morphometric and pheromonal data, northern and southern groups emerge which are similar but not identical with respect to locality.

Table 5. The mean coefficient of variation of the 11 morphometric characters at each locality.

Locality	C.V.
Nababeep	0.0355 low
Karasburg	0.0393
Keetmanshoop	0.0686
Mariental	0.0445 high
Windhoek	0.0467
Okahandja	0.0409
Otjiwarongo	0.0358 low

Table 6. The mean intracolonial variance and mean interlocality variance of the eight pheromone compounds at each locality.

Locality	Intracolonial	Interlocality
Nababeep	72.59	102.93
Karasburg	62.99	87.89
Keetmanshoop	122.55	130.18
Mariental	129.59	131.62
Windhoek	96.38	115.34
Okahandja	83.64	114.33
Otjiwarongo	72.53	83.10

There is no geographical barrier to gene flow between northern South Africa and southern Angola and gene flow should be facilitated by the complete cross-fertility of the races of *A. mellifera* (Moritz & Southwick 1992; Ruttner 1992). Variance patterns should consequently reflect continuous changes in certain characters (clines) along a transect between significantly differing terminal populations to delineate a zone of hybridization. This should result in clines with especially large variance values in the putative hybrid area (Falconer 1989; Moritz & Kauhausen 1984).

The genetic requirements are met in the case of the morphometric features by considering the mean coefficients of variation for the relevant localities (Table 5). The northern and southern areas have lower coefficients of variation than the high variance group: Keetmanshoop, Mariental, Windhoek. Similarly, intracolonial variance for the sting pheromones is higher in the middle group of localities than for the end-points (Table 6). The total variance values obtained from the analyses point to two distinct populations of honeybees in the region with a broad zone of hybridization between them (Fig. 1).

The morphometric data of the present study, together with similar data for South Africa (Crewe *et al.* 1994; Moritz & Kauhausen 1984), indicate that the bees of the southern group are *A. m. scutellata.* The northern group is more problematic because the honeybees of Angola have not been classified. Ruttner (1992) delineated northern Namibia as probably being *A. m. adansonii* but gave no morphometric data. However, morphometric data for three features of *A. m. adansonii* honeybees from Zambia, Zaire and the Central African Republic (Ruttner, pers. comm.) and shared in common with the present data set correspond well with results obtained from northern Namibia. This group is consequently classified as *A. m. adansonii, sensu* Ruttner (1988) *A. m. scutellata* ecotype *adansonii* according to Kerr (1992).

Studies of natural introgression in native populations of honeybees are relatively few but they show that races or subspecies of honeybees investigated to date can be resolved into distinct populations with significant hybrid zones between them (Cornuet & Fresnaye 1989; Moritz *et al.* 1994; Smith *et al.* 1991). The same is true of *A. m. capensis* and *A. m. scutellata* in South Africa (Hepburn *et al.* 1994). Considering the latter with the present data one obtains a transect (Fig. 1) extending from Cape Algulhas (34.50S 20.01E) into northern Namibia along which a well-defined homogeneous population of *A. m. capensis* (A) gives way to a heterogeneous hybrid zone (B) before the next homogeneous population (C) of *A. m. scutellata.* Further northward, this *A. m. scutellata* population gives way to another hybrid zone (D) in central Namibia, finally reaching another relatively homogeneous population (E) which we have determined as *A. m. adansonii.*

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