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Population structure and morphometric variance of the *Apis mellifera scutellata* group of honeybees in Africa

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Abstract

The honeybee populations of Africa classified as *Apis mellifera scutellata* Lepeletier were analysed morphometrically using multivariate statistical techniques. The collection consisted of nearly 15,000 worker honeybees from 825 individual colonies at 193 localities in east Africa, extending from South Africa to Ethiopia. Factor analysis established one primary cluster, designated as *A. m. scutellata*. Morphocluster formation and inclusivity (correct classification) are highly sensitive to sampling distance intervals. Within the *A. m. scutellata* region are larger bees associated with high altitudes of mountain systems which are traditionally classified as *A. m. monticola* Smith, but it is evident that these bees do not form a uniform group. Variance characteristics of the morphometric measurements show domains of significantly different local populations. These high variance populations mostly occur at transitional edges of major climatic and vegetational zones, and sometimes with more localised discontinuities in temperature. It is also now evident that those *A. m. scutellata* introduced nearly fifty years ago into the Neotropics were a particularly homogenous sample, which exhibited all the traits expected in a founder effect or bottleneck population.

INTRODUCTION

Modern studies of the classification of the honeybees of Africa stem from the work of Kerr and Portugal-Araújo (1958). They recognised five subspecies, the most widely spread of which was *A. m. scutellata* (then called *A. m. adansonii*) and its various ecotypes (Kerr, 1992). Details of the nomenclatural history are reviewed by Ruttner (1988) and Daly (1991). Further analyses of the bees falling under the *A. m. scutellata* umbrella of distribution were provided by Smith (1961) and in a series of studies summarised by Ruttner (1988). Recently the large morphometric databases on the honeybees of Africa that had been separately assembled in Germany and South Africa were amalgamated into a single unit of considerable depth and breadth and the apifauna of the whole continent morphometrically re-analysed (Hepburn and Radloff, 1998).

In general terms the results of Hepburn and Radloff (1998) supported many of the interpretations of Ruttner (1988), but some not unexpected refinements were made. One was the fact that all of the bees classified as *A. m. scutellata* on the basis of multivariate morphometric analyses by Ruttner (1988) resolved into two distinct morphoclusters in the analysis of Hepburn and Radloff (1998). The honeybees of southern Ethiopia and northern Kenya formed one *A. m. scutellata*-like morphocluster that differed from the morphocluster to the south, in Zimbabwe and South Africa (whence the Neotropical introductions originated). These morphoclusters were simply designated as "*scutellata* 1" and "*scutellata* 2" pending the acquisition of additional data.

This *A. m. scutellata* problem is interesting for several different reasons. Firstly, the geographic distribution of the Ruttner *A. m. scutellata* was more or less rectangular, extending the length of the eastern highlands of Africa from South Africa to Ethiopia (hence the name "highland bee"). In the Hepburn-Radloff analysis this rectangle was somewhat crimped or narrow-waisted where *A. m. adansonii* pushed eastwards from Zambia and *A. m. litorea* westwards from Mozambique. Secondly, in both the Ruttner (1988) and Hepburn and Radloff (1998) studies there was an extreme paucity of material then available from northwestern Mozambique, western Zimbabwe, eastern Botswana and southern Kenya. Tanzania, Malawi and Lesotho were simply dataless gaps. Thirdly, until now there was not sufficient information on the original mother populations of the bees subsequently introduced into the Neotropics, from which reasonable inference might have been made.

It has recently been established for the African apifauna that morphocluster formation resulting from multivariate analyses can be very sensitive to sample distance intervals as well as to levels of statistical confidence employed to make interpretations (Radloff and Hepburn, 1998). Very recently a considerable amount of new material has been obtained from Botswana, Zimbabwe, Malawi, Lesotho and Tanzania. Here we report the results of fresh analyses of a considerably (30%) enhanced database to re-examine the honeybee populations of the *A. m. scutellata* group, their various characteristics, biogeographical relationships and areas of hybridisation with neighbouring populations of other sub-

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species. The geographical area investigated was the whole of Ruttner's original *A. m. scutellata* rectangle, extending from South Africa to Ethiopia (Figure 1). Also, to the extent possible, Palaeotropical *A. m. scutellata* were compared with recent Neotropical (but non-morphometric) data to estimate the extent of bottlenecking (expression of a founder effect of the original introductions) in the latter region.

MATERIAL 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. A new multivariate morphometric analysis of

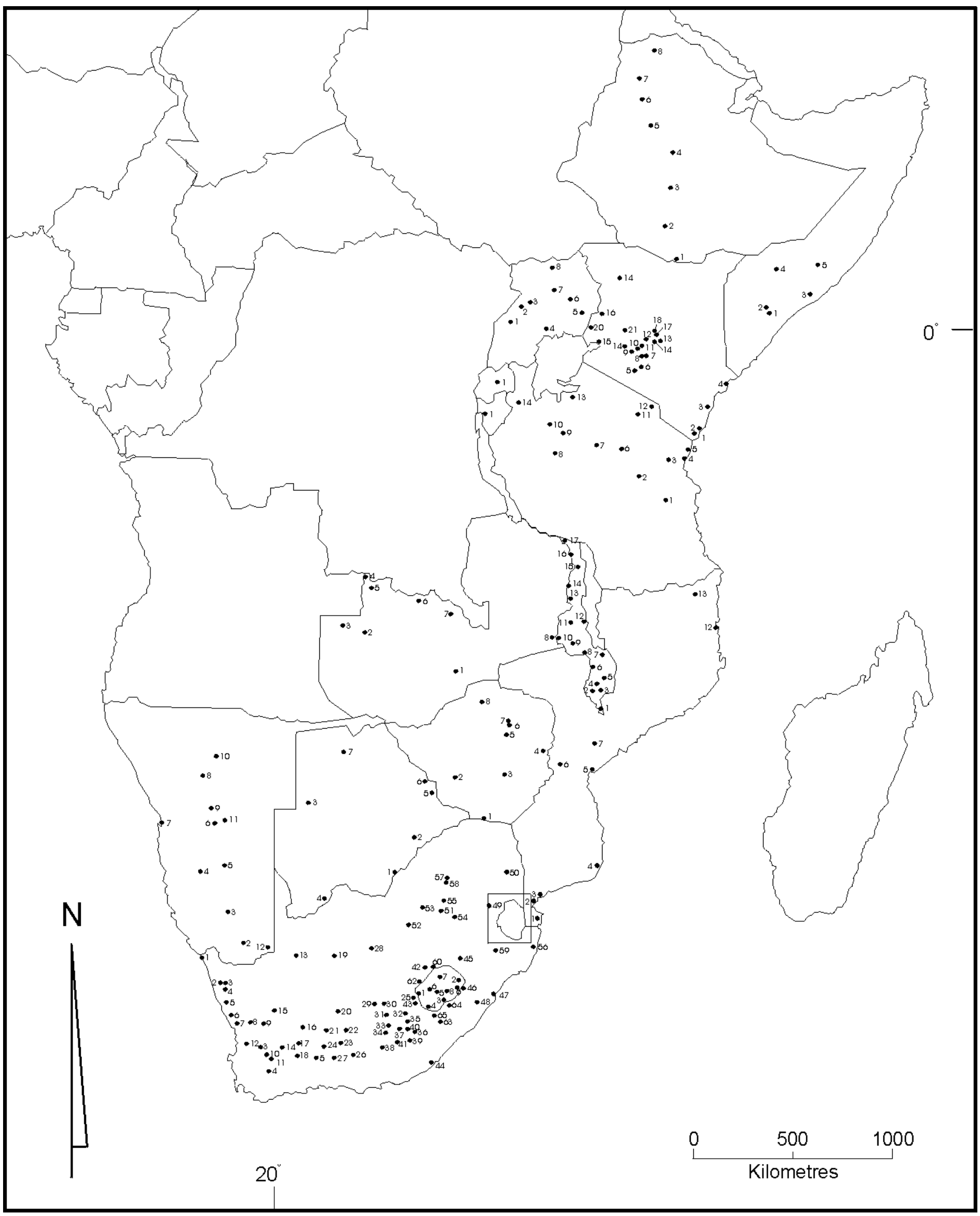

Figure 1 - Map of eastern Africa indicating the localities at which worker honeybees were collected for analysis in our study. Note that for each country the numbering system for localities always begins with "1" to avoid overlapping. Map numbers for each country correspond to the geographical and biological information in Table I.

this material was recently published (Hepburn and Radloff, 1998). In the interim, important new collections of honeybee workers were obtained from Botswana, Lesotho, Malawi, Tanzania and Zimbabwe, that increased the original database by 30%. This material has been measured, inserted into the database and a fresh multivariate morphometric analysis of the honeybees of the eastern side of Africa made.

All of the worker bees used in the study were sampled from the colonies of small-scale, fixed-site beekeepers at 193 localities, extending the length of eastern Africa from South Africa to Ethiopia (Table I). While "captive" colonies were often sampled it must be understood that the bees are simply attracted to empty hives from the wild population. Bees in Africa are very rarely transported and bee breeding is virtually non-existent. Thus, the samples used in the analysis constitute authentic subsamples of the wild population whence they came. Morphometric measurements were usually taken on 20 worker bees per colony from a variable number of colonies per locality, total colonies sampled being 825 individual colonies. A total of 14,973 individual worker honeybees was measured morphometrically (Table I).

Table I - Distribution of the localities, co-ordinates, altitudes, sample sizes and intercolonial variances of worker honeybees analysed morphometrically.

Countries and localities	Co-ordinates		Altitude (m)	Sample sizes		Variance	Map Ref.
				Colonies	Bees		
Botswana							
Francistown	21.11S	27.32E	900	4	80	13.7	5
Gaborone	24.45S	25.55E	1000	5	100	6.2	1
Ghanzi	21.39S	21.39E	1137	1	20	-	3
Irdbridge	19.15S	23.30E	945	1	8	-	7
Mahalapye	23.05S	26.51E	1000	5	100	16.5	2
Tsabong	26.28S	21.35E	1034	1	20	-	4
Tutume	20.26S	27.02E	1100	4	80	23.2	6
Burundi							
Bujumbura	03.22S	29.19E	800	6	120	74.3*	1
Ethiopia							
Addis Ababa	09.03N	38.42E	2842	9	180	22.6	4
Adi Arkay	13.35N	37.57E	950	5	100	36.8	8
Agere Maryam	05.13N	38.20E	2000	5	100	18.5	2
Bahir Dar	11.33N	37.25E	2400	5	100	12.7	6
Debre Markos	10.19N	37.41E	2000	5	99	13.3	5
Gonder	12.39N	37.29E	2121	6	120	22.1	7
Mega	04.02N	31.19E	2100	5	98	18.9	1
Shashemene	07.13N	38.33E	1800	6	119	18.1	3
Kenya							
Aberdare	00.35S	36.38E	2666	1	20	-	9
Chepkitale	00.58N	34.33E	2986	1	10	-	16
Chiokariga	00.17S	37.55E	762	3	45	41.4*	8
Chuka	00.20S	37.38E	1401	1	15	-	7
Gatimbi	00.01N	37.39E	1584	2	30	37.0	17
Kaaga	00.04N	37.39E	1600	3	45	3.2	18
Kerio Valley	02.24N	36.21E	450	1	20	-	19
Kimbo/Meru	00.06N	37.29E	2437	12	177	28.9	10
Kimititi	00.34N	34.34E	1534	6	90	28.0	20
Kiria	00.12S	37.39E	1371	1	15	-	13
Lake Baringo	00.38N	36.03E	980	1	12	-	21
Lamu	02.15S	40.50E	0	1	20	-	4
Malindi	03.14S	40.05E	0	1	20	-	3
Meru (township)	00.04N	37.39E	1554	4	65	42.2*	11
Mombasa	04.04S	39.40E	0	1	20	-	2
Mt. Elgon	01.07N	34.31E	4320	8	125	28.2	15
Mt. Kenya	00.32S	37.28E	1320	5	77	38.6*	14
Nairobi	01.17S	36.50E	1576	3	56	27.9	6
Nakuru	00.16S	36.04E	1860	1	20	-	14
Nanyuki	00.05S	37.10E	2220	2	40	44.0*	12
Ngong Hills	01.24S	36.38E	2460	13	195	20.4	5
Shimba Hill	04.12S	39.28E	448	1	13	-	1
Soudu Kisumu	00.08S	34.47E	1151	1	20	-	15
Tunyai	00.10S	37.50E	1029	1	15	-	12

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Table I - Continued

Countries and localities	Co-ordinates		Altitude (m)	Sample sizes Colonies	Bees	Variance	Map Ref.
Lesotho							
Ha Lejone	29.10S	28.27E	1479	2	40	24.4	7
Mafeteng	29.48S	27.13E	1616	7	140	24.5	1
Marakabei	29.32S	28.08E	1977	7	140	35.9	6
Mokhotlong	29.35S	29.17E	2133	6	120	30.5	2
Quachasnek	30.06S	20.40E	1878	7	140	14.6	3
Quiteng	30.25S	27.42E	1578	7	140	12.1	4
Sani Top	29.33S	29.13E	2496	1	20	-	9
Semonkong	29.50S	28.05E	2200	7	140	33.0	8
Thaba-Tseka	29.31S	28.35E	2286	3	60	23.4	5
Malawi							
Blantyre	15.46S	35.00E	1000	1	15	-	4
Chikwawa	16.02S	34.54E	100	6	120	25.4	2
Chilinda	10.36S	33.48E	2600	26	420	45.6*	16
Chitipa	09.41S	33.19E	1300	6	120	31.2	17
Dedza	14.20S	34.24E	1600	6	120	17.3	8
Kasungu	13.04S	33.29E	1070	7	135	16.4	11
Lilongwe	13.58S	33.49E	1067	1	15	-	9
Mangochi	14.30S	35.15E	450	6	120	7.0	7
Mbalachanda	11.21S	33.22E	1336	6	120	21.3	14
Mchinji	13.48S	32.53E	1200	6	120	17.2	10
Mzimba	11.55S	33.39E	1330	6	120	10.1	13
Nkhotakota	12.55S	34.19E	500	6	120	39.2*	12
Nsanje	16.47S	35.15E	70	6	120	20.2	1
Ntcheu	14.49S	34.38E	1100	6	120	25.6	6
Rumphi	10.59S	33.50E	1050	6	120	27.3	15
Thyolo	16.04S	35.09E	900	6	120	28.3	3
Zomba	15.22S	35.22E	950	6	120	18.7	5
Mozambique							
Beira	19.49S	34.52E	0	3	60	6.2	5
Inhaminga	18.24S	35.00E	327	1	11	-	7
Manhica	25.23S	32.49E	61	1	13	-	3
Marrocuene	26.15S	32.40E	10	1	12	-	2
Maxixe	23.51S	35.21E	76	1	18	-	4
Mueda	11.40S	39.31E	439	1	20	-	9
Pemba	13.00S	40.29E	0	1	16	-	8
Salamanga	26.29S	32.40E	45	1	12	-	1
Sussendenga	20.35S	33.09E	585	1	8	-	6
Namibia							
Ariamsvlei	28.08S	19.05E	774	4	80	31.8	12
Karasburg	28.00S	18.43E	1013	5	89	23.6	2
Katima Molilo	17.27S	24.10E	946	1	15	-	1
Keetmanshoop	26.36S	18.08E	1773	4	80	14.0	3
Maltahöhe	24.50S	17.00E	1340	1	18	-	4
Mariental	24.36S	17.59E	1180	4	80	23.8	5
Okahandja	21.59S	16.58E	1439	4	80	17.6	9
Otavi	19.39S	17.20E	1414	2	30	6.9	10
Otjiwarongo	20.29S	16.36E	1565	6	110	46.6*	8
Seeis	22.29S	17.39E	1610	1	3	-	11
Swakopmund	22.40S	14.34E	0	4	80	25.9	7
Windhoek	22.43S	17.06E	1779	5	100	17.3	6
Rwanda							
Kigali	01.56S	30.04E	1400	4	65	16.3	1
Somalia							
Afgoi	02.07N	45.02E	86	1	20	-	3
Baidoa	03.04N	43.48E	485	3	60	15.4	4
Buale	01.14N	42.36E	63	4	80	51.3*	1
Bulo Burti	03.50N	45.33E	158	2	40	23.9	5
Dugiuma	01.20N	42.34E	63	2	40	9.8	2
South Africa							
Aberdeen	32.29S	24.03E	732	3	44	9.9	26
Alexander Bay	28.40S	16.30E	0	6	120	29.9	1
Aliwal North	30.45S	26.45E	1317	6	120	40.6*	31

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Table I - Continued

Countries and localities	Co-ordinates		Altitude (m)	Sample sizes Colonies	Bees	Variance	Map Ref.
South Africa (cont.)							
Badplaas	25.58S	30.34E	1067	6	120	9.9	49
Bitterfontein	31.03S	18.16E	354	3	60	4.8	6
Boesmanskop	32.02S	24.19E	1677	3	60	26.7	25
Booiskraal	31.50S	22.36E	1360	2	40	45.4*	16
Botterkloof	31.49S	19.17E	720	3	60	35.8	10
Britstown	30.37S	23.30E	1122	4	80	28.2	20
Burgersdorp	30.59S	26.20E	1481	4	80	15.9	33
Calvinia	31.25S	19.45E	980	5	100	29.1	9
Clanwilliam	32.11S	18.54E	75	6	120	12.7	12
Dordrecht	31.20S	27.03E	1637	6	120	13.2	36
Durban	29.55S	31.00E	0	5	100	18.2	47
East London	32.58S	27.55E	0	6	120	12.7	44
Ficksburg	28.51S	27.43E	1575	7	139	17.9	60
Fort Beaufort	32.48S	26.38E	456	4	80	43.8*	61
Garies	30.30S	18.00E	227	4	79	3.7	5
Harrismith	28.18S	29.03E	1642	6	120	8.8	45
Hoedspruit	24.21S	30.57E	603	6	120	9.8	50
Hofmeyr	31.39S	25.50E	1251	3	60	10.0	38
Ixopo	30.08S	30.00E	992	5	100	20.1	48
Jamestown	31.07S	26.48E	1617	3	60	2.5	35
Johannesburg	26.10S	28.02E	1753	1	20	-	51
Klerksdorp	26.58S	26.39E	1301	6	120	20.4	52
Ladybrand	29.12S	27.27E	1569	7	140	28.5	62
Lutzville	31.46S	18.21E	150	5	100	33.2	7
Maclear	31.04S	28.29E	1359	7	140	18.4	63
Magaliesberg	26.00S	27.33E	1432	5	100	10.6	53
Matatiele	30.21S	28.51E	1466	7	140	14.6	64
Mesklip	29.52S	17.53E	759	1	10	-	4
Middelwater	32.25S	22.04E	720	2	40	14.6	18
Molteno	31.22S	26.22E	1580	4	79	52.6*	37
Murraysburg	31.58S	23.47E	1158	1	20	-	23
Nababeep	29.36S	17.46E	915	4	80	16.5	2
Nelspoort	32.07S	23.01E	1015	2	40	6.9	24
Nieuwoudtville	31.24S	19.06E	719	5	100	47.2*	8
Nigel	26.30S	28.28E	1606	6	120	33.1	54
Postmasburg	28.18S	23.05E	1311	4	80	28.4	19
Pretoria	25.45S	28.12E	1400	4	60	3.3	55
Queenstown	31.52S	27.00E	1077	11	219	21.2	39
Rhodes	30.47S	27.57E	1700	5	100	18.1	65
Richmond	31.23S	23.56E	856	2	40	4.1	22
Smithfield	30.09S	26.30E	1400	4	80	21.3	30
Sodwana Bay	27.20S	32.45E	0	1	7	-	56
Sonop	31.57S	19.44E	450	3	60	27.2	11
Springbok	29.43S	17.55E	1400	1	10	-	3
Springfontein	30.19S	25.36E	1519	6	120	26.6	29
Sterkstroom	31.34S	26.33E	1343	6	120	75.5*	40
Steynsburg	31.20S	25.50E	1448	3	60	60.7*	34
Sutherland	32.24S	20.40E	1459	6	120	25.7	14
Tarkastad	32.01S	26.16E	1290	6	120	13.8	41
Thabazimbi	24.41S	27.21E	1026	5	100	18.0	57
Tontelbos	30.56S	20.23E	1122	3	60	49.7*	15
Underberg	29.50S	29.22E	1550	1	20	-	46
Upington	28.25S	21.15E	836	5	97	13.6	13
Venterstad	30.47S	25.48E	1340	2	40	26.3	32
Victoria West	31.25S	23.04E	1269	3	60	0.7	21
Vonkfontein	31.56S	21.50E	1369	2	40	7.6	17
Vryheid	27.52S	30.38E	1189	6	120	19.1	59
Warmbaths	24.53S	28.17E	1116	6	120	5.8	58
Warrenton	28.09S	24.47E	1198	6	120	18.1	28
Wiegenaarspoort	32.38S	23.12E	853	2	40	8.5	27
Winburg	28.37S	27.00E	1433	6	120	22.3	42
Zastron	30.18S	27.07E	1661	6	120	26.6	43

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Table I - Continued

Countries and localities	Co-ordinates		Altitude (m)	Sample sizes Colonies	Bees	Variance	Map Ref.
Tanzania							
Handeni	05.25S	38.04E	1040	5	25	31.1	3
Kahama	03.48S	32.36E	1380	5	25	19.2	10
Kiteto	05.50S	36.50E	1750	5	25	22.2	2
Kwamtoro-Kondoa	04.14S	35.23E	1200	5	25	27.3	6
Lusahanga	02.55S	31.12E	1500	5	25	27.5	14
Mt. Kilimanjaro	03.10S	37.30E	2500	8	140	60.6*	12
Magu	02.31S	33.28E	1138	5	75	12.1	13
Mlali	06.19S	36.48E	1300	1	15	-	1
Njiro/Arusha/Tengeru	03.23S	36.40E	1390	21	328	6.9	11
Nzega	04.13S	33.11E	1200	5	25	18.9	9
Pangani	05.27S	39.00E	0	5	25	24.3	4
Singida	04.50S	34.45E	1524	5	25	16.7	7
Tabora	05.02S	32.50E	1188	1	15	-	8
Tanga	05.07S	39.05E	0	3	60	28.2	5
Uganda							
Arapai	01.45N	33.38E	1143	1	15	-	6
Bugoye	00.17N	30.06E	1400	1	15	-	1
Hoima	01.25N	31.22E	1300	1	15	-	2
Kampala	00.19N	32.35E	1198	1	20	-	4
Kitgum	03.17N	32.54E	1000	1	15	-	8
Lira	02.15N	32.55E	1101	1	15	-	7
Masindi	01.41N	31.45E	1147	2	27	9.9	3
Mbale	01.04N	34.12E	1300	1	15	-	5
Zambia							
Chipata	13.40S	32.42E	1104	2	30	43.1*	8
Ikelenge	11.30S	24.05E	1372	5	100	5.7	4
Kabompo	15.25S	24.13E	1100	2	30	8.6	2
Kitwe	12.48S	28.14E	1288	7	135	7.7	7
Lusaka	15.26S	28.20E	1296	3	60	18.1	1
Mwinilunga	11.44S	24.24E	1250	3	45	7.1	5
Nguru/Balovale	13.30S	23.06E	1078	1	15	-	3
Solwezi	11.28S	26.23E	1299	5	90	31.5	6
Zimbabwe							
Beit Bridge	15.00S	30.15E	440	5	100	18.9	1
Bulawayo	20.10S	28.43E	1390	5	100	35.7	2
Glendale	17.22S	31.05E	1150	5	100	20.6	7
Harare	17.43S	24.13E	1478	14	280	15.7	6
Karoi	16.46S	29.45E	1251	6	120	24.0	8
Marondera	18.11S	31.33E	1688	4	80	10.1	5
Masvingo	20.05S	30.50E	1099	5	100	10.8	3
Mutare	19.00S	32.40E	338	6	120	17.0	4

The same nine characters used in previous studies of honeybees in Africa were measured (Crewe *et al.*, 1994; Radloff, 1996; Hepburn and Radloff, 1998). Their Ruttner (1988) numbers are given in parentheses 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 latter 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 (Lachenbruch and Mickey, 1968). Wilk's lambda test was used to compare multivariate population means between groups. The distribution of the statistic was approximated by the F distribution (Mardia *et al.*, 1979). 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.

RESULTS

Mesolevel analysis

In a factor analysis of the morphometric characters of worker honeybees from 825 colonies with a mean sampling distance resolution of 210 km, three factors with eigenvalues greater than one were isolated: factor 1, pigmentation of the scutellum (35) and abdominal tergite 2 (32); factor 2, width and length of wax plate on sternite 3 (11) and (13), factor 3, angles of wing venation N23 (30) and O26 (31). These factors accounted for 58.8% of the variance in the data. The factor loadings for each character had absolute values greater than 0.65. The graph of the factor scores from factors 1 and 2 showed one main morphocluster with colonies from Ethiopia scattered to the left of the main cluster (Figure 2). This result confirms that the honeybees of Ethiopia have darker pigmentation than those from the *A. m. scutellata* subspecies (Radloff and Hepburn, 1997).

A stepwise discriminant analysis using the colony means of the morphometric characters confirmed the separation of the colonies from Ethiopia from the main cluster. The linear discriminant functions obtained using the most discriminatory characters classified 90.2% (four misclassified) of the colonies from Ethiopia correctly with *a posteriori* probabilities $0.69 \leq P \leq 0.98$ for six colonies and P = 1.0 for the remaining colonies and 96.2% (27 misclassified) of the colonies correctly from the rest of the data set. A jackknife procedure gave the same classification results except that one more colony from the main group was misclassified into the group from Ethiopia. A significant difference was found between the means of the two groups ($\Lambda = 0.52$ with 7,1,752 d.f., F = 93.97 with 7,746 d.f., P < 0.0001). The variances of the factor 1 scores and factor 2 scores were used to test for the homogeneity of the colony variance at each locality. A significant difference was found between the intercolonial variances over all the localities (Levene's test, F = 2.54 with 184,551 d.f., P < 0.0001). Those localities with significantly higher variances are indicated in Table I.

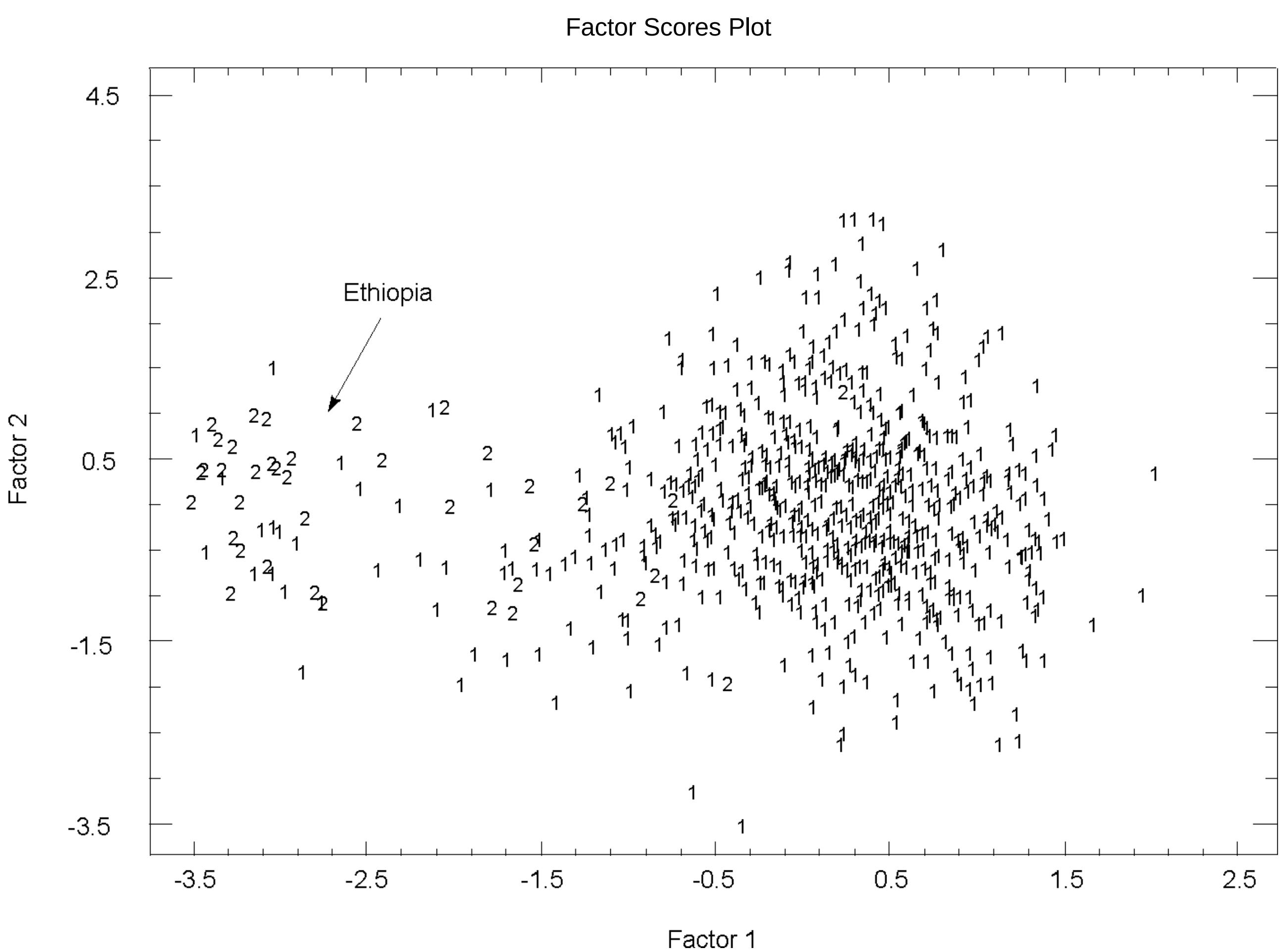

Figure 2 - The graph of the factor scores from factors 1 and 2 shows a large morphocluster to the right of the figure that represents *Apis mellifera scutellata* bees, and the small cluster to the left represents the honeybees of Ethiopia.

Distance effects

The length of the transect may obscure small biometric groups if the between-group variation is considerably larger than the within-group variation (Table II). When the colonies from Ethiopia alone were analysed, three morphoclusters were isolated, namely *A. m. jemenitica*, *A. m. bandasii* and *A. m. scutellata* (Radloff and Hepburn, 1997). Also when the colonies from Kenya, Uganda, Tanzania, Rwanda, Burundi, Malawi, Zambia and northern Mozambique were analysed, three morphoclusters were delineated, namely *A. m. scutellata*, *A. m. monticola* (black) from the high altitudes of Kenya and Tanzania and *A. m. monticola* (yellow) from the high altitudes of the Nyika Plateau, Malawi (Hepburn and Radloff, 1998).

Because the honeybees at higher altitudes in other African mountains (e.g. Mt. Kilimanjaro, Mt. Kenya) differ from lower surrounding populations in both size and pigmentation (Ruttner, 1988), these traits were specifically examined for the honeybees of the Nyika Plateau in Malawi. In this case, there was a significant correlation between size (1) and altitude, that is, bees become increasingly larger with increasing altitude ($r = 0.59$, $P < 0.0001$). There was also a significant correlation between pigmentation (35) and altitude; with increasing altitude the bees became lighter in colour ($r = 0.44$, $P < 0.0001$).

Finally, statistical comparisons of high-altitude bees from the Nyika Plateau with others from Mt. Kilimanjaro (Tanzania), Mt. Kenya (Kenya) and the Drakensberg mountains revealed all of these bees to be larger in size than their lower-altitude counterparts ($F = 10.75$ with 4,297 d.f., $P < 0.0001$); however, the honeybees of the Drakensberg and Nyika Plateau are significantly lighter in colour than the more northerly mountain bees ($F = 109.00$ with 3,82 d.f., $P < 0.0001$).

Variance characteristics

The intercolonial variance values of the populations sampled are listed in Table I. Only 19 of the 193 localities (about 10%) exhibited statistically significant elevated values of variance. It is of interest to consider the sources of the high variance domains. The intercolonial variance is derived from mean values of whole colonies (between colonies) for each locality (Table I) but it is not immediately obvious what different components give rise to the variance. A plot of those high variance colonies on an intracolonial (within colonies) basis shows that high variance values arise in two different ways. Of 19 high intercolonially variant colonies, 10/19 exhibited a range of variances that statistically yield high variance, but there was no single colony in the locality set which exhibited significantly high within-colony variance (Table III). Thus, for these colonies the localised population was highly heterogeneous. The remaining 9/19 colonies yielded high intercolonial variances because particular individual colonies were themselves highly variant. In fact all nine of this second group of localities were correlated with hybrid zones based on morphocluster analysis and were indicative of hybridisation. Thus there are two separate origins for high

Table II - The effects of sampling distance intervals on morphocluster inclusiveness.

1.	Distance approximately	4450 km	Countries: South Africa and Ethiopia
	Eigenvalue	$\lambda = 2.2722$	
	Classification:		
	Ethiopia	87.8% correctly classified (5 misclassified)	
	South Africa	99.6% correctly classified (1 misclassified)	
2.	Distance approximately	2105 km	Countries: South Africa, Malawi and Ethiopia
	Eigenvalue	$\lambda = 2.2216$	
	Classification:		
	Ethiopia	87.8% correctly classified (5 misclassified)	
	Malawi	74.8% correctly classified (27 misclassified)	
	South Africa	78.6% correctly classified (59 misclassified)	
3.	Distance approximately	1050 km	Countries: South Africa, Zimbabwe, Malawi, Tanzania and Ethiopia
	Eigenvalue	$\lambda = 2.0360$	
	Classification:		
	Ethiopia	82.9% correctly classified (7 misclassified)	
	Tanzania	64.7% correctly classified (18 misclassified)	
	Malawi	71.6% correctly classified (27 misclassified)	
	Zimbabwe	60.5% correctly classified (17 misclassified)	
	South Africa	58.3% correctly classified (115 misclassified)	
4.	Distance approximately	210 km	Countries: All 15
	Eigenvalue	$\lambda = 1.90$	
		$r = 0.91$, $r^2 = 82.51\%$, $P < 0.0001$	

Table III - Intracolonial variances of worker honeybees at localities with high intercolonial variances.

Countries and localities	Individual colony variances	Combined colony variance
Burundi		
Bujumbura	38.72 / 29.98 / 31.98 / 41.76 / 56.74* / 54.67*	72.3
Kenya		
Chiokariga	32.16 / 26.89 / 31.13	46.1
Meru	33.90 / 27.43 / 49.52* / 32.24	42.2
Mt. Kenya	27.02 / 34.97 / 26.05 / 25.13 / 22.83	42.4
Nanyuki	20.14 / 19.19	37.1
Malawi		
Chilinda	34.70 / 27.29 / 19.49 / 25.67 / 33.25 / 33.58 / 29.83 43.52 / 37.77 / 39.83 / 27.47 / 36.78 / 31.64 / 19.97 23.19 / 35.47 / 39.39 / 30.05 / 23.53 / 34.11 / 29.62 31.22 / 34.08 / 18.12 / 41.97 / 26.48	41.9
Nkhotakota	31.43 / 35.47 / 38.87 / 27.38 / 33.40 / 23.16	44.9
Namibia		
Otjiwarongo	31.34 / 28.78 / 25.47 / 29.77 / 43.61 / 37.47	53.0
Somalia		
Buale	23.09 / 35.29 / 29.69 / 27.47	53.1
South Africa		
Aliwal North	39.32 / 25.65 / 60.98* / 62.19* / 67.55* / 37.49	62.7
Booiskraal	33.34 / 67.00*	61.5
Fort Beaufort	75.86* / 51.88* / 43.62 / 63.58*	69.9
Molteno	48.46 / 73.34* / 40.37 / 59.91*	72.4
Nieuwoudtville	27.40 / 53.25* / 31.92 / 33.55 / 28.74	55.4
Sterkstroom	56.69* / 35.37 / 41.99 / 42.56 / 49.44* / 341.57*	158.4
Steynsburg	38.84 / 40.24 / 44.57	64.4
Tontelbos	34.40 / 65.94* / 89.88*	76.7
Tanzania		
Mt. Kilimanjaro	26.54 / 35.68 / 37.06 / 24.17 / 48.48 / 37.55 / 30.59 / 47.82	60.4
Zambia		
Chipata	32.45 / 34.92	45.4

* Significantly higher variance ($P < 0.05$).

intercolonial variance: those colonies in which the bees are themselves heterogeneous (intracolonial variance) and other colonies where the within-colony variance is not significantly high but collectively the colonies of bees of the locality are significantly more variant than others from neighbouring localities. Thus, of the 193 colonies of honeybees analysed in this way nearly 90% of them are fairly homogeneous as morphocluster entities.

The geographical distributions of these high intercolonial variances demonstrate distinct patterns. For example, all of the localities marked with an asterisk in Table I in Burundi, Kenya, Tanzania and Malawi are associated with mountain systems for which the "*A. m. monticola*-like" bees occur in an archipelago surrounded by the morphometrically more uniform and distinct *A. m. scutellata*. In the case of South Africa, similarly marked variances (except two unexplained cases) all came from the natural hybrid zone between *A. m. capensis* and *A. m. scutellata* in mountainous countryside. Those colonies of Zambia and Namibia with high variances are in hybrid zones between *A. m. scutellata* and *A. m. adansonii*. Too little information is available on the bees of Somalia (Buale) to even venture comment at this stage.

Those colonies exhibiting statistically high levels of intercolonial variance between localities could also be related to geophysical parameters. Figure 3 depicts regions of high variance against altitudinal relief in the *A. m. scutellata* area. It is evident that the majority of high variance localities are associated with areas of greatest rate of altitudinal change throughout eastern Africa. Altitude of course reflects modification of climatic systems and consequential to this are changes in vegetation structure of differing biomes and to the bees themselves (Hepburn *et al*., 1998).

Figure 4 depicts the high variance localities of *A. m. scutellata* in eastern Africa on a map of the major climatic zones of the continent. Here it becomes strikingly evident that high variance is typically associated with regions of climatic transition in most instances. Those high variance localities not in such transitional zones are nonetheless associated with more localised discontinuities in climate for which there are significant differences in heat and/or cold intensity regimes. Figure 5 depicts the high variance localities against the major vegetation zones of eastern Africa and again it can be seen that the local populations of these bees are associated with edge effects, which are transitional regions between the major biomes.

In a final biogeographical composite the regions of

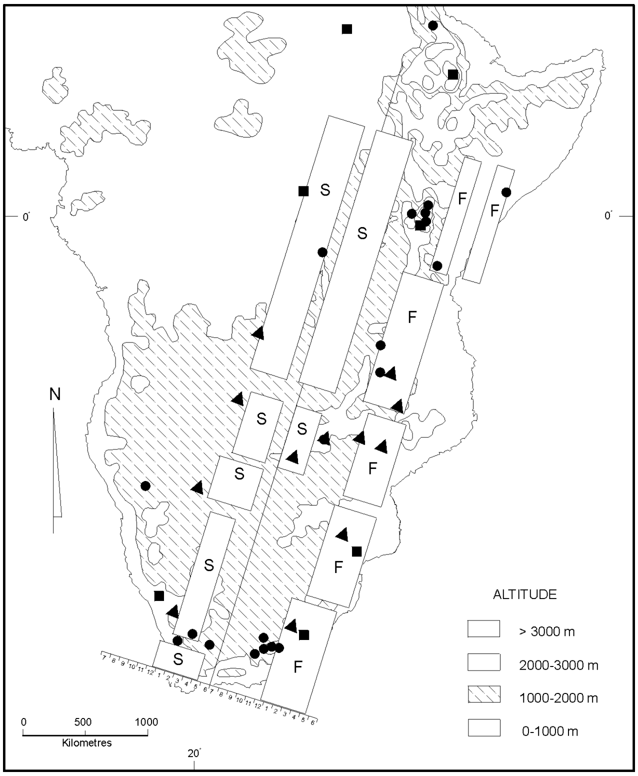

Figure 3 - Relief map of eastern Africa indicating areas (circles) of significantly high values of morphometric variance within the *Apis mellifera scutellata* populations. (Map modified from van Chi-Bonnardel, 1973). Triangles denote localised regions of high temperature changes, squares denote low temperature discontinuities. Principal reproductive swarming (S) and major flowering (F) periods of the relevant bee flora are indicated to the left and right of the vertical line, respectively. Horizontal scale units are months of the year beginning on both sides of the vertical line with July (month 7) and running through June (month 6) because July is mid-winter and the end of the annual colony cycles. The map is diagrammatic for swarming and flowering as they change with latitude.

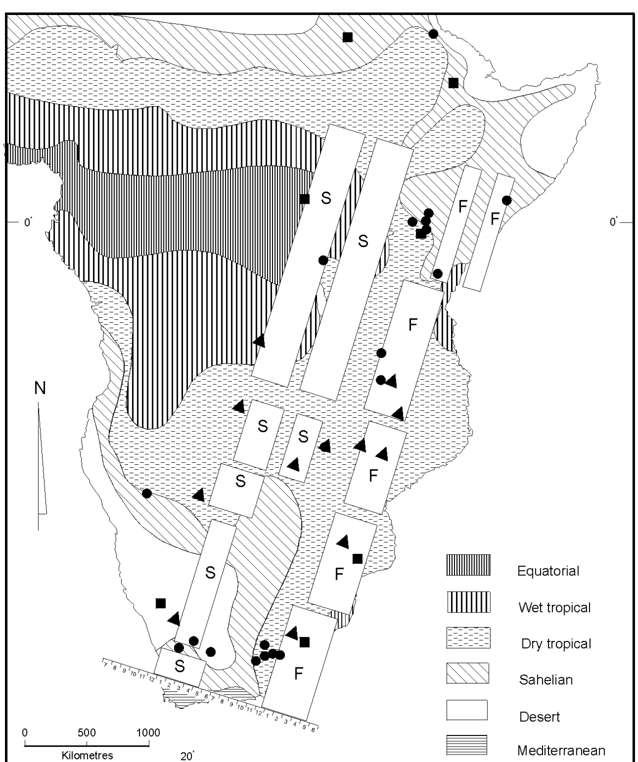

Figure 4 - Map of the regions of high morphometric variance in relation to the major climatic zones of east Africa. Symbols as in Figure 3. (Map modified from van Chi-Bonnardel, 1973).

high variance for the *A. m. scutellata* populations are illustrated in terms of localised discontinuities of climate (rapid changes in hot or cold in a small region) and also in relation to the principal swarming seasons and the principal flowering periods for the relevant major honeybee flora of eastern Africa. The scale at the bottom of the map represents months of the year for swarming (left side of vertical line) and for flowering (right side of vertical line) and both are expressed as months of the year beginning with July and ending with June (months 7 to 6) because July is the winter end of the annual cycle. The drawing is diagrammatic for an imaginary vertical line running north-south through the region so that swarming and flowering are averaged for the eastern part of the continent. Each must be read as running horizontally across the vertical line for any particular latitude in eastern Africa. Seen in this way the honeybee populations of southern Africa (8 high variance regions) enjoy more or less equality of flowering periods but a narrower window of reproductive swarming. However, note that swarming and seasonal flowering are geographically related to localised climatic discontinuities which result in ecological instability at the edges. This should in turn lead to selective pressure for a high turnover in gene flow and partially explain the high levels of variance associated with such regions.

DISCUSSION

Two principal conclusions about the morphocluster analysis emerge in this study. Firstly the graph of the fac-

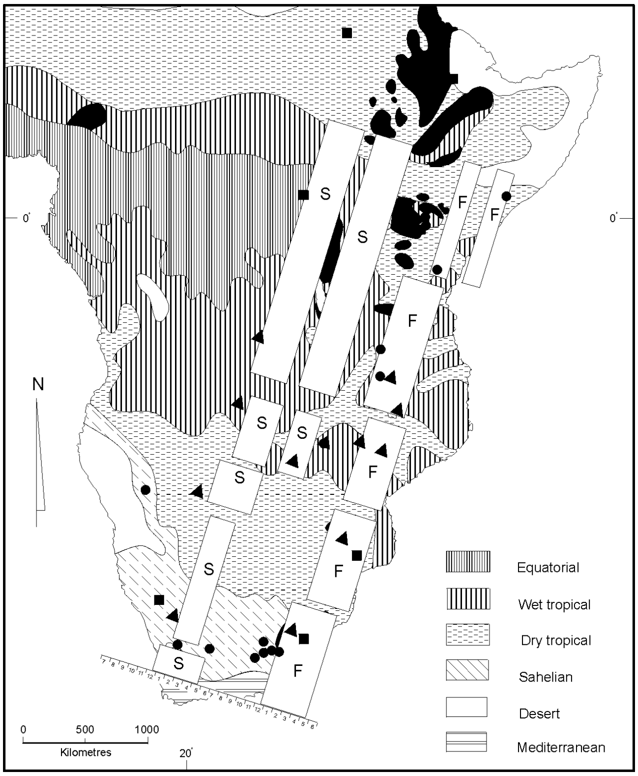

Figure 5 - Map of regions of high morphometric variance in relation to the major zones of vegetation (biomes) in eastern Africa. High variance domains are primarily associated with transitional areas between biomes. Symbols as in Figure 3. (Map modified from van Chi-Bonnardel, 1973).

tor scores (Figure 2) confirm that the honey bees of Ethiopia are morphometrically distinguishable from all of the other more southerly bees, all of which have been previously defined as *A. m. scutellata*. Secondly, detailed and localised analyses of the mountain populations of the honeybees of eastern Africa show that they can indeed be differentiated, to a greater or lesser extent, from the *A. m. scutellata* that surround the mountain archipelago bees. However, it is now evident that the mountain populations themselves can in fact be further differentiated into different groups. Because of the lack of precision in the traditional usage of terms such as "subspecies" or "ecotype" we simply note what morphoclusters can be formed and do not assign names to them.

The results of Table II show that the greater the distance between countries, the greater the extent of variation in morphometric characters. As a corollary, the greater the distance between countries, the higher the probability of "correctly" assigning colonies to specific morphoclusters. This conforms exactly with conclusions reached in studies on the effects of sampling distance and variable confidence limits (Radloff and Hepburn, 1998): the greater the distance between samples, the more distinct the morphoclusters.

The significance of the sampling distance interval is clearly demonstrated in analyses of localised regions. For example, the discriminant analysis of the morphometric characters of the honeybees of eastern Africa unequivocally established the occurrence of two distinct morphoclusters. One morphocluster comprises honeybees living at high altitudes (± 2500 m) on the Nyika Plateau (Malawi) while a second morphocluster comprised all of the bees at altitudes below ± 1600 m throughout Malawi from the borders of Tanzania to Mozambique. Following the system of classification of Ruttner (1988) the lower altitude bees are *A. m. scutellata* Lepeletier and the high ones *A. m. monticola* Smith.

The mountain bees of Nyika bear close morphological similarities to those of other mountains such as Mt. Kenya, Mt. Meru and Mt. Elgon to the north and to others of the more southerly Drakensberg (Hepburn and Radloff, 1998; Radloff and Hepburn, 1998). The Nyika bees are significantly larger in size than those of lower altitude. However, pigmentation presents some interesting problems. With increasing altitude the southern mountain bees (Nyika and Drakensberg) become more yellow in overall colouration while in the northern mountain bees the trend is to darker colouration with increasing altitude. It is possible that the high mountain bees do in fact constitute a unique subspecies *A. m. monticola* distinct from *A. m. scutellata* at lower altitudes as proposed by Ruttner (1988) and Meixner *et al*. (1989). However, final resolution of this problem will require a critical DNA analysis.

Morphometric variance among colonies of a natural honeybee population can be attributed to two proximate causes. Because queen honeybees are polyandrous (Adams *et al*., 1977; Neumann *et al*., 1999, 2000), honeybee colonies may consist of several to many patrilines. The effect is that regions of high variance of either or both high intracolonial variance among workers as well as intercolonial variance may occur. However, variance must be seen in the broader context of frequency distributions of character states.

The genetics of metric character states such as can be derived from morphometrics of honeybee centres around the analysis of the frequency distribution patterns of variation for it is in terms of variation that primary population genetic questions can be formulated (Wright, 1969, 1978; Falconer and Mackay, 1997). The basic premise underlying the analysis of variation is that it can be partitioned into components of differing probable cause. The relative magnitude of these components determine the genetic properties or structure of populations and the extent of this variation is expressed in terms of variance.

A final comment on the *A. m. scutellata* that were introduced into the Neotropics can now be made. Kerr (1992) clearly stated where all of this original honeybee livestock originated, principally the Transvaal region of South Africa. It can be noted in the present set of results that the *A. m. scutellata* bees of that area are extremely homogeneous and display low variance values, and are uniformly aggressive and virulently invasive. Thus, it can be concluded from the analysis of the mother African material alone that those *A. m. scutellata* that spread through the Neotropics did so on the basis of a founder effect. This conclusion is absolutely compatible with the identical conclusion reached on mitochondrial studies of *A. m. scutellata* in the Neotropics (Smith *et al*., 1999; Del Lama, 1999).

ACKNOWLEDGMENTS

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RESUMO

Populações africanas de abelhas comuns classificadas como *Apis mellifera scutellata* Lepeletier foram analisadas morfometricamente usando-se técnicas estatísticas multivariadas. A população consistia de aproximadamente 15.000 abelhas operárias provenientes de 825 colônias individuais de 193 localidades do leste da África, estendendo-se da África do Sul até a Etiópia. A análise de fatores estabeleceu um agrupamento primário designado *A. m. scutellata*. A formação de agrupamento morfológico e a inclusividade (classificação correta) são altamente sensíveis aos intervalos de distância da amostragem. Dentro da região de *A. m. scutellata* há abelhas maiores associadas às altas altitudes montanhosas, que são tradicionalmente classificadas como *A. m. monticola* Smith, mas é evidente que estas abelhas não formam um grupo uniforme. As características de variação das medidas morfométricas mostram domínios de populações locais significantemente diferentes. Estas populações altamente variáveis ocorrem em sua maioria em margens de transição de zonas climáticas e de vegetação, e algumas vezes com alterações mais localizadas de temperatura. Agora também é evidente que as *A. m. scutellata* introduzidas há aproximadamente 50 anos na região neotropical constituíam uma amostra particularmente homogênea que exibia todos os caracteres esperados em uma população com efeito fundador ou “gargalo”.

REFERENCES

Adams, J., Rothmann, E.D., Kerr, W.E. and **Paulino, Z.L.** (1977). Estimation of the number of sex alleles and queen matings from diploid male frequencies in a population of *Apis mellifera*. *Genetics 86*: 583-596.

Crewe, R.M., Hepburn, H.R. and **Moritz, R.F.A.** (1994). Morphometric analysis of 2 southern African races of honeybees. *Apidologie 25*: 61-70.

Daly, H.V. (1991). Systematics and identification of Africanized honey bees. In: *The “African” Honey Bee* (Spivak, M., Fletcher, D.J.C. and Breed, M.D., eds.). Westview Press, Boulder, pp. 13-44.

Del Lama, M.A. (1999). Population genetics of the Africanized honeybees from South America. In: *Proceedings of the XIII International Congress of IUSSI*, Adelaide, p. 133.

Falconer, D.S. and **Mackay, T.F.C.** (1997). *Introduction to Quantitative Genetics*. Longman, Essex.

Hepburn, H.R. and **Radloff, S.E.** (1998). *Honeybees of Africa*. Spinger Verlag, Berlin.

Hepburn, H.R., Radloff, S.E., Brown, R.E., Youthed, C. and **Illgner, P.** (1998). Production of aerodynamic power in mountain honeybees (*Apis mellifera*). *Naturwissenschaften 85*: 389-390.

Kerr, W.E. (1992). Abejas Africanas su introduccion y expansion en el continente Americano. Subespecies y ecotipos Africanos. *Ind. Apic. 13*: 12-21.

Kerr, W.E. and **Portugal-Araújo, V. De** (1958). Raças de abelhas da Africa. *Garcia de Orta 6*: 53-59.

Lachenbruch, P. and **Mickey, R.M.** (1968). Estimation of error rates in discriminant analysis. *Technometrics 10*: 1-11.

Mardia, K.V., Kent, J.T. and **Bibby, J.M.** (1979). *Multivariate Analysis*. Academic Press, London.

Meixner, M., Ruttner, F., Koeniger, N. and **Koeniger, G.** (1989). The mountain bees of the Kilimanjaro region and their relation to neighbouring bee populations. *Apidologie 20*: 165-174.

Neumann, P., Moritz, R.F.A. and **Van Praagh, J.** (1999). Queen mating-frequency in different types of honeybee mating apiaries. *J. Apic. Res. 38*: 11-18.

Neumann, P., Moritz, R.F.A., Radloff, S.E. and **Kryger, P.** (2000). Heritability for polyandry in the honeybee (*Apis mellifera* L.). *Heredity* (in press).

Radloff, S.E. (1996). Multivariate analysis of selected honeybee populations in Africa. PhD thesis, Rhodes University, Grahamstown.

Radloff, S.E. and **Hepburn, H.R.** (1997). Multivariate analysis of honeybees, *Apis mellifera* Linnaeus (Hymenoptera: Apidae) of the Horn of Africa. *Afr. Entomol. 5*: 57-64.

Radloff, S.E. and **Hepburn, H.R.** (1998). The matter of sampling distance and confidence levels in the subspecific classification of honeybees, *Apis mellifera* L. *Apidologie 29*: 491-501.

Ruttner, F. (1988). *Biogeography and Taxonomy of Honeybees*. Springer Verlag, Berlin.

Smith, F.G. (1961). The races of honeybees in Africa. *Bee World 42*: 255-260.

Smith, D.R., Palmer, M.S., Orive, M.E. and **Taylor, O.R.** (1999). Modeling founder effects in Neotropical African honey bees. In: *Proceedings of the XIII International Congress of IUSSI*, Adelaide, p. 449.

van Chi-Bonnardel, R. (1973). *The Atlas of Africa*. Jeune Afrique, Paris.

Wright, S. (1969). *Evolution and Genetics of Populations. Vol. 2. The Theory of Gene Frequencies*. University of Chicago Press, Illinois.

Wright, S. (1978). *Evolution and Genetics of Populations. Vol. 4. Variability Within and Among Natural Populations.* University of Chicago Press, Illinois.

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