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 bottlenecks (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

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**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” colo-

### 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) | Colonies | Bees | Variance | Map Ref. |
|--------------------------|--------------|--------------|----------|------|----------|----------|
| 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        |
| Irirbridge               | 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        |
| Chepkikale               | 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/Mera               | 00.06N 37.29E | 2437         | 12       | 177  | 28.9     | 10       |
| Kimutti                  | 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  | 204      | 5        |
| Shimba Hill              | 04.12S 39.28E | 448          | 1        | 13   | -        | 1        |
| Soudi Kismu              | 00.08S 34.47E | 1151         | 1        | 20   | -        | 15       |
| Tunayi                   | 00.10S 37.50E | 1029         | 1        | 15   | -        | 12       |

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| Countries and localities | Co-ordinates | Altitude (m) | 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 33.15E| 480          | 6        | 120  | 7.0      | 7        |
| Mbhalachanda             | 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        |
| Ruphiphi                 | 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   | 62.4     | 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        |
| Pembra                   | 13.00S 40.29E| 0            | 1        | 16   | -        | 8        |
| Salamanga                | 26.29S 32.40E| 45           | 1        | 12   | -        | 1        |
| Sussendenga              | 20.55S 33.09E| 585          | 1        | 8    | -        | 6        |
| **Namibia**              |              |              |          |      |          |          |
| Ariasvlei                | 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        |
| Okahandjia               | 21.59S 16.38E| 1439         | 4        | 80   | 17.6     | 9        |
| Otavi                    | 19.39S 17.20E| 1414         | 2        | 30   | 6.9      | 10       |
| Otijwarongo              | 20.20S 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 42.56E| 63           | 4        | 80   | 51.3*    | 1        |
| Bulo Buri                | 03.50N 45.33E| 158          | 2        | 40   | 23.9     | 5        |
| Dugiysma                 | 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) | Colonies | Bees | Variance | Map Ref. | Sample sizes |
|--------------------------|--------------|--------------|----------|------|----------|----------|--------------|
| South Africa (cont.)     |              |              |          |      |          |          |              |

|             | 25.58S 30.34E | 1067 | 6 | 120 | 9.9 | 49 |
| Badplaas    | 31.03S 18.16E | 354  | 3 | 60  | 4.8 | 6  |
| Bitterfontein| 32.02S 24.19E | 1677 | 3 | 60  | 26.7| 25 |
| Boesmanskop | 31.50S 22.36E | 1360 | 2 | 40  | 45.4*| 16 |
| Booskrant   | 31.49S 19.17E | 720  | 3 | 60  | 35.8| 10 |
| Botterkloof | 30.37S 23.30E | 1122 | 4 | 80  | 28.2| 20 |
| Britstown   | 30.59S 26.20E | 1481 | 4 | 80  | 15.9| 33 |
| Burgersdorp | 31.25S 19.45E | 980  | 5 | 100 | 29.1| 9  |
| Calvina     | 32.11S 18.54E | 75   | 6 | 120 | 12.7| 12 |
| Clanwilliam | 31.20S 27.03E | 1637 | 6 | 120 | 13.2| 36 |
| Dordrecht   | 29.55S 31.00E | 0    | 5 | 100 | 18.2| 47 |
| Durban      | 32.58S 27.55E | 0    | 6 | 120 | 12.7| 44 |
| East London | 28.51S 27.43E | 1575 | 7 | 139 | 17.9| 60 |
| Ficksburg   | 32.48S 26.38E | 456  | 4 | 80  | 43.8*| 61 |
| Fort Beaufort| 30.30S 18.00E | 227  | 4 | 79  | 3.7 | 5  |
| Garies      | 28.18S 29.03E | 1642 | 6 | 120 | 8.8 | 45 |
| Harrismith  | 24.21S 30.57E | 603  | 6 | 120 | 9.8 | 50 |
| Hofmeyr     | 31.39S 25.50E | 1251 | 3 | 60  | 10.0| 38 |
| Iscopo      | 30.08S 30.00E | 992  | 5 | 100 | 26.1| 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| 26.09S 27.33E | 1432 | 5 | 100 | 10.6| 53 |
| Matatiele   | 30.21S 28.51E | 1466 | 7 | 140 | 14.6| 64 |
| Moskop      | 29.52S 17.53E | 759  | 1 | 10  | -   | 4  |
| Middelwater | 32.55S 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 |
| Nieuwoudville| 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 |
| Sterksboom  | 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 |
| Vonkerfontein| 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 |
| Wiegenaarsoort | 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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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.

### Table I - Continued

| Countries and localities | Co-ordinates | Altitude (m) | 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   |      |
| Kwanamo-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  |      |
| Milali                   | 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 32.59E| 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   |      |
| Ikeleenge                | 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 23.00E| 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   |      |
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.

| Distance          | Countries                          | Eigenvalue | Classification |
|-------------------|------------------------------------|------------|----------------|
| 1. Distance       | South Africa and Ethiopia          | λ = 2.2722 | **Ethiopia** 87.8% correctly classified (5 misclassified)  |
| 2. Distance       | South Africa, Malawi and Ethiopia   | λ = 2.2216 | **Ethiopia** 87.8% correctly classified (5 misclassified)  |
| 3. Distance       | South Africa, Zimbabwe, Malawi,    | λ = 2.0360 | **Ethiopia** 82.9% correctly classified (7 misclassified)  |
| 4. Distance       | All 15                             | λ = 1.90   | **Zimbabwe** 60.5% correctly classified (17 misclassified) |
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 \textit{A. m. monticola}-like bees occur in an archipelago surrounded by the morphometrically more uniform and distinct \textit{A. m. scutellata}. In the case of South Africa, similarly marked variances (except two unexplained cases) all came from the natural hybrid zone between \textit{A. m. capensis} and \textit{A. m. scutellata} in mountainous countryside. Those colonies of Zambia and Namibia with high variances are in hybrid zones between \textit{A. m. scutellata} and \textit{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 \textit{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 \textit{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

| 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 | 41.9                     |
| Nkhotakota              | 31.43 / 35.47 / 38.87 / 27.38 / 33.40 / 23.16 | 44.9                     |
| Namibia                 |                             |                          |
| Otijwarongo             | 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                     |
| Booskrail               | 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                     |
| Nieuwoudville           | 27.50 / 13.25* / 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                     |
| Tontelbros              | 34.40 / 65.94* / 89.88*     | 76.7                     |
| Tanzania                |                             |                          |
| Mt. Kilimanjaro         | 26.54 / 35.06 / 37.06 / 24.17 / 48.48 / 37.53 / 30.59 / 47.82 | 60.4                     |
| Zambia                  |                             |                          |
| Chipata                 | 32.45 / 34.92               | 45.4                     |

* Significantly higher variance (P < 0.05).
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-
Population structure and morphometric variance

315 Population structure and morphometric variance (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* Lepetier 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).

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