

# Parasitic Cape bees in the northern regions of South Africa: source of the founder population

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**Multivariate discriminant analyses of nine standard morphometric characters of honeybee workers were used to track the origin of a social parasitic pseudo-clone of thelytokous laying workers that have invaded colonies of *Apis mellifera scutellata* in South Africa. Twenty social parasitic workers were sampled from both of two infested *A. m. scutellata* colonies at two distant apiaries (Graskop and Heilbronn, about 390 km apart) and compared with data obtained from 80 colonies in four different geographical zones (zone I: thelytokous *A. m. capensis* morphocluster; zone II: natural thelytokous hybrids between *A. m. capensis* and *A. m. scutellata*; zone III: thelytokous *A. m. scutellata* morphocluster; zone IV: an arrhenotokous *A. m. scutellata* morphocluster). Thelytokous laying workers occur naturally in zones I–III. Highly significant morphometric differences were found among the bees in the four zones. The data support the conclusion that the social parasitic workers belong to the thelytokous *A. m. capensis* morphocluster. It is most likely that the social parasitic workers originated from the heart of the Cape bee's distribution range in the Western Cape region in zone I. Morphometric analysis makes it feasible to restrict the possible origin of the social parasitic workers from the natural distribution range of thelytoky (approximately 240 000 km<sup>2</sup>) down to about 12 000 km<sup>2</sup>, which represents a resolution capacity of about 95%.**

## Introduction

The recent introduction by humans of honeybee colonies with thelytokous (female producing) laying workers, thought to be *Apis mellifera capensis*, into the region of the neighbouring arrhenotokous (male producing) subspecies *A. m. scutellata* in northeastern South Africa, resulted in the usurpation and subsequent loss of many thousands of colonies of the latter.<sup>1–3</sup> This followed from intraspecific social parasitism of the thelytokous laying workers.<sup>4,5</sup> Recent genetic analyses<sup>6–10</sup> confirmed that a single matriline of thelytokous laying workers, constituting an almost genotypically identical so-called pseudo-clone,<sup>6,7</sup> is invading large areas of the range of *A. m. scutellata*.

Because the social parasitic pseudo-clone workers are thelytokous and black, it was assumed that they were Cape bees.<sup>1,11</sup> This view is problematic because thelytoky occurs naturally in bees that are morphometrically defined as *A. m. capensis*, *A. m. scutellata* and their natural hybrids.<sup>12</sup> Moreover, colour is a subjective and unreliable indicator of intra-subspecific categories.<sup>10</sup>

We now report on the effectiveness of morphometric analysis as a forensic probe to track the founder population of the

pseudo-clone of social parasitic thelytokous workers invading the neighbouring subspecies, *A. m. scutellata*.

## Materials and methods

Sealed brood frames containing social parasitic worker offspring were obtained from four *A. m. scutellata* colonies heavily infested with thelytokous laying workers, at two distant localities (Graskop and Heilbronn, about 390 km apart) in the natural distribution area of *A. m. scutellata* in South Africa. In a previous study,<sup>10</sup> we showed that the discriminant analysis produced only one cluster for the parasitic clone bees. That means that these bees are morphometrically inseparable despite very varied environmental conditions at the two sampling localities.

The samples were taken at late stages of infestation,<sup>5</sup> when the host queen was already absent for more than two weeks. Thus, these frames definitely contained parasitic worker offspring because laying workers of *A. m. scutellata* produce drone offspring.<sup>13</sup> These brood frames were individually confined in gauze-covered cages and placed in an incubator until emergence of adults. Twenty social parasitic workers from each of the four colonies were morphometrically analysed using nine standard characters in honeybee morphometrics.<sup>14,15</sup> Their Ruttner<sup>14</sup> numbers are given in brackets as follows: length of cover hair on tergite 5 (1); sternite 3, longitudinal (11); wax plate of sternite 3, transversal (13); wing angle 34 (22); wing angle N23 (30); wing angle O26 (31); pigmentation of tergite 2 (32); pigmentation of scutellum (35), and pigmentation of scutellar plate (36). The genotypic composition of the workers was verified by DNA microsatellite analysis.<sup>8</sup>

These morphometric data were combined with previous information from 80 localities in southern Africa south of 28°S<sup>16</sup> and analysed. This region consists of three zones with morphometrically distinct groups of honeybees where reproduction of thelytokous workers naturally occurs (zones I–III; with a total area of approximately 240 000 km<sup>2</sup>) and a fourth zone with arrhenotokous worker reproduction (Fig. 1). Thelytokous workers were collected from 24 localities in zone I (~70 000 km<sup>2</sup>), for which the bees are morphometrically defined as *A. m. capensis*. Thelytokous bees were also collected from five localities in zone II (~80 000 km<sup>2</sup>), an area of morphometrically defined natural hybrids between *A. m. capensis* and *A. m. scutellata*, and from 19 localities in zone III (~80 000 km<sup>2</sup>), where bees are morphometrically defined as *A. m. scutellata*. Finally, samples were taken from 32 localities in zone IV, which consists of arrhenotokous bees morphometrically defined as *A. m. scutellata* and which extends several million km<sup>2</sup> northwards into eastern Africa.<sup>15,16</sup>

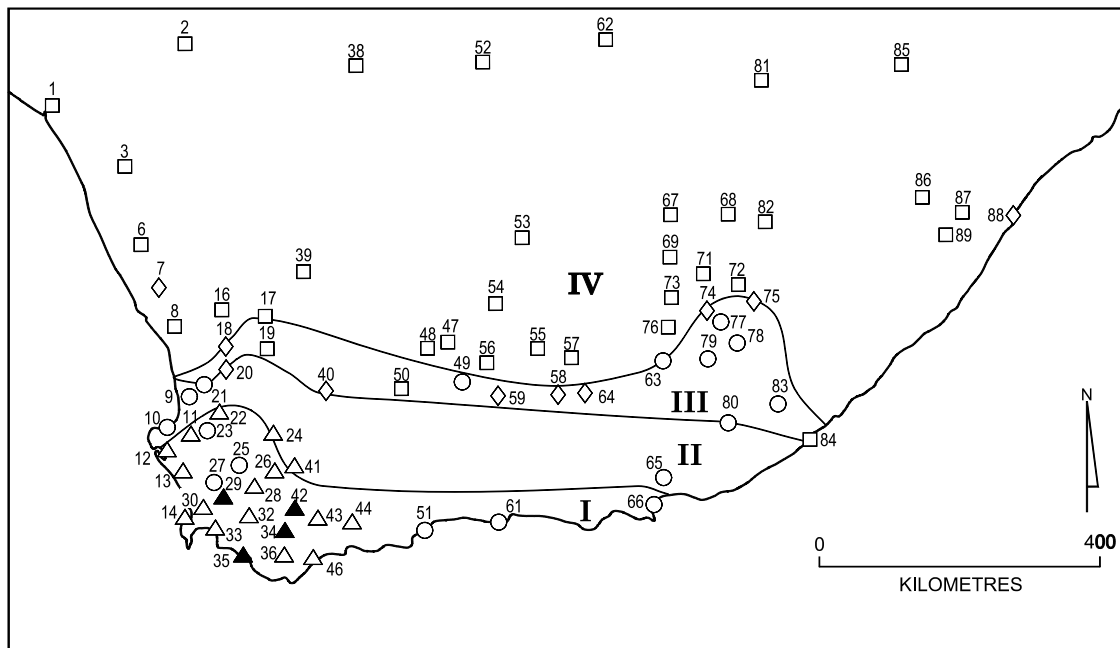
Multivariate discriminant analyses using nine morphometric characters were carried out to determine cluster formations of the colonies at each locality and of the parasitic clone workers. The Mahalanobis squared distances between the clusters of each locality and between the parasitic clone cluster were calculated separately. The Mahalanobis distance ( $D$ ) is that between the centroids of the clusters in a multidimensional space.<sup>17</sup> The Mahalanobis squared distances failed tests of normality (Kolmogorov-Smirnoff  $d = 0.174$ ,  $P < 0.05$ ) and hence the non-parametric Kruskal-Wallis test was used to test for significant differences in the distances between the four zones. Mann-Whitney  $U$  tests with Bonferroni adjustments to the levels of significance ( $\alpha^* = 0.05/6 = 0.0083$ ) were used for the pair-wise comparison of the distances between the zones. Box-and-whisker plots were used to check for any extreme values of the Mahalanobis squared distances within the *A. m. capensis* morphocluster.

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**Fig. 1.** Distribution of the Mahalanobis squared distances among the four zones investigated. **Zone I:** thelytokous *A. m. capensis* morphocluster; **zone II:** natural thelytokous hybrids between *A. m. capensis* and *A. m. scutellata*; **zone III:** thelytokous *A. m. scutellata* morphocluster; **zone IV:** arrhenotokous *A. m. scutellata* morphocluster; ref. 16; open squares = 30+, open diamonds = 20–30, open circles = 10.5–20, open triangles = 6–10.5, filled triangles = <6).

**Results**

The values of  $D^2$  from the centroid of the parasitic clone cluster to each of the locality clusters are given in Table 1; their distribution among the four zones is shown in Fig. 1. Highly significant differences were found between the four zones ( $H = 62.60, 3 \text{ d.f.}, P < 0.0001$ ). Multiple pair-wise comparisons revealed significant differences between zone IV and zones I, II and III (zone I:  $U = 0.0, P < 0.0001$ ; zone II:  $U = 0.0, P = 0.0004$ ; zone III:  $U = 42.0, P < 0.0001$ ). No significant difference (using Bonferroni adjustments) was found between zones II and III ( $U = 17.5, P = 0.0329$ ) and between I and II ( $U = 21.0, P = 0.0244$ ). The Mahalanobis squared distances between zones I and III were significantly different ( $U = 26.0, P < 0.0001$ ). Three extreme outliers in the Mahalanobis squared distances were found within zone I at Piketberg (23), Mosselbaai (51) and Port Elizabeth (66, cf. Fig. 1). When these extreme values were removed from the analysis, a significant difference in  $D^2$  was found between zones I and II ( $U = 6.00, P = 0.0025$ ). Four localities in the Western Cape (29, 34, 35 and 42) had  $D^2$  values below 6.

**Discussion**

The data clearly show that the pseudo-clone of social parasitic thelytokous workers invading *A. m. scutellata* originates from the *A. m. capensis* morphocluster and further indicates that the pseudo-clone probably originated from the Western Cape, the heart of the range of the Cape bee. Thus, we were able to confirm several earlier statements<sup>1,3,11</sup> that the social parasitic workers invading *A. m. scutellata* in its native range are indeed Cape bees and not thelytokous hybrids nor *A. m. scutellata*. Our data also confirm that morphometric analysis can be used as an inexpensive forensic

**Table 1.** Mahalanobis squared distances ( $D^2$ ) between the centroids of the parasitic clone cluster and the morphoclusters at each locality (locality numbers as in ref. 16; zone I: thelytokous *A. m. capensis* morphocluster; zone II: natural thelytokous hybrids between *A. m. capensis* and *A. m. scutellata*; zone III: thelytokous *A. m. scutellata* morphocluster; zone IV: arrhenotokous *A. m. scutellata* morphocluster; ref. 16; asterisks indicate extreme outliers found within zone I).

Locality	$D^2$	Locality	$D^2$
<b>Zone I</b>			
12. Langebaan	9.41	58. Aberdeen	29.34
13. Darling	8.89	59. Wiegenaarspoort	28.49
22. Citrusdal	6.91	63. Cradock	11.32
23. Piketberg*	16.47	78. Queenstown	14.21
24. Tweeriviere	7.43	79. Tarkastad	12.58
25. Ceres	11.31	80. Fort Beaufort	11.55
26. Sandvlei	6.38	83. Stutterheim	13.10
27. Malmesbury	10.52	84. East London	36.06
28. Worcester	7.15	Mean ± s.d.	23.70 ± 12.41
29. Paarl	5.64	<b>Zone IV</b>	
30. Kraaifontein	8.64	1. Alexander Bay	71.08
32. Villiersdorp	9.24	2. Karasburg	69.94
33. Somerset West	7.85	3. Nababeep	50.74
34. Riviersonderend	5.57	6. Garies	112.40
35. Hermanus	5.36	7. Bitterfontein	23.01
36. Napier	7.42	8. Lutzville	43.25
41. Touwsrivier	8.85	16. Nieuwoudtville	39.28
42. Bonnievale	5.34	38. Upington	134.28
43. Swellendam	7.61	39. Tontelbos	97.58
44. Heidelberg	8.25	47. Booiskraal	62.06
46. Skipskop	10.26	48. Vonkfontein	88.63
51. Mosselbaai*	18.84	52. Postmasburg	132.23
61. Witte drif	11.00	53. Britstown	66.41
66. Port Elizabeth*	16.58	54. Victoria West	66.44
Mean ± s.d.	9.21 ± 3.59	55. Murraysburg	36.45
Mean ± s.d. without*	8.05 ± 1.84	56. Nelspoort	33.79
<b>Zone II</b>			
9. Elandsbaai	10.38	57. Boesmanskop	63.14
10. Velddrif	14.68	62. Warrenton	52.47
11. Laaiplek	10.30	67. Springfontein	51.74
21. Elandsvlei	14.36	68. Smithfield	50.33
65. Addo	11.55	69. Venterstad	55.51
Mean ± s.d.	12.25 ± 2.13	71. Burgersdop	37.55
<b>Zone III</b>			
17. Calvinia	48.25	72. Jamestown	40.60
18. Botterkloof	23.66	73. Steynsburg	44.29
19. Sonop	36.26	76. Hofmeyr	33.68
20. Clanwilliam	10.85	81. Winburg	79.90
40. Sutherland	20.03	82. Zastron	110.89
49. Beaufort West	10.81	85. Harrismith	36.87
50. Middelwater	51.25	86. Underberg	68.45
		87. Richmond	62.13
		88. Durban	27.50
		89. Ixopo	46.42
		Mean ± s.d.	62.16 ± 29.06

probe to track the origin of introduced honeybees.<sup>18</sup> In the particular case of South African bee populations, nuclear DNA markers are too variable to resolve the origin of introduced bees. For example, intrapopulation variation among several DNA microsatellite loci was shown to be as high as interpopulation variation between *A. m. capensis* and *A. m. scutellata*.<sup>19</sup> Unlike the thelytokous parasitic pseudo-clones, however, other introduced bees will reproduce with the local population and morphometric characters will become difficult to distinguish within a short time. So, morphometric analysis can be used for the originally introduced bees, but for later generations its use may be difficult.

Obviously, the social parasitic honeybee workers could have originated from only the natural distribution area of thelytoky. Indeed, the data show that the parasites certainly do not originate from the region of arrhenotokous *A. m. scutellata* (Fig. 1). Moreover, smaller Mahalanobis squared distances were obtained for the pure *A. m. capensis* morphocluster as opposed to the natural hybrid zone and the more southerly *A. m. scutellata* localities, where thelytoky also occurs naturally (Fig. 1). It is therefore evident that the parasitic workers originated from the *A. m. capensis* morphocluster and not from other areas, where thelytoky naturally occurs.

Within the *A. m. capensis* morphocluster, the results indicate that it is unlikely that the parasitic worker clone originated from east of Heidelberg or north of Ceres/Malmesbury. Moreover, there is a region of four localities with  $D^2$  less than 6 (Paarl, Riviersterend, Hermanus and Bonnievale), which indicates that the social parasitic clone originates from the heart of the Cape bee distribution range in the Western Cape. Thus, morphometric analysis restricts the potential area of origin from the natural distribution range of thelytoky (~240 000 km<sup>2</sup>) down to about 12 000 km<sup>2</sup>, which corresponds to a resolution of 95%.

Considering the clinal structure of the distribution of the naturally occurring hybrid between *A. m. capensis* and *A. m. scutellata*,<sup>16</sup> it seems most likely that the social parasites with the highest within-host virulence<sup>4,5</sup> originated from the heart of the Cape bee's distribution range. In this region, almost all laying workers reproduce thelytokously and have high reproductive potential. Indeed, what is known of beekeeping activity seems to support our findings.<sup>3</sup> Incidentally, *A. m. capensis* colonies were brought from the western Cape to the then Transvaal in about 1991, just before the usurpation of *A. m. scutellata* colonies by the social parasitic *A. m. capensis* reached epidemic levels.<sup>1,11</sup>

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### New reference work on science indicators

Reliable facts and figures are indispensable for monitoring the consequences of policy in all walks of life, especially where change is happening and wanted. The last substantial suite of statistics to be published on the S&T system in South Africa was the FRD's *South African Science and Technology Indicators 1996*. An abbreviated version of these measures, citing the most recent information available, called *South African Science and Technology: Key Facts and Figures 2002*, has now been produced by the National Advisory Council on Innovation (NACI) in conjunction with the Department of Science and Technology. It summarizes policy and strategic issues, and offers data on selected S&T criteria.

The compilers chose the material that they believed is most often used and details of which have not been readily available. Here you will find information on the structure and size of the S&T system, with particular emphasis on the higher education sector; R&D expenditure and income; outputs (such as patents and research publications) compared with the performance of other countries; and how South Africa rates with Australia, Malaysia and South Korea in terms of the Technology Achievement Index, a measure devised by the United Nations Development Programme.

For a developing country, South Africa has a well-established S&T infrastructure. On the other hand, *Key Facts and Figures* highlights reasons for concern about the demographics of our student body and workforce in S&T. The population that produces research results is ageing (in a large database, the proportion of publications by authors over the age of 50 grew from 18% in 1990 to 45% in 1998; in the 1990s, 94% of scientific publications in the same database was authored by white academics). On the other hand, the number of enrolments for mathematics and science at school and at tertiary education institutions remains low. Matriculation pass rates with university exemption are inadequate for the country's future needs, and the proportion of science, engineering and technology students in the higher education sector has remained essentially constant at 27% over the past five years.

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