The value of hindleg banding patterns in the identification of species of the *Anopheles gambiae* Giles complex (Diptera: Culicidae) in Natal, South Africa

by

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ABSTRACT. The validity of using hindleg banding patterns for the identification of *Anopheles merus*, *An. quadriannulatus* and *An. arabiensis* was investigated in Natal. A high percentage of *An. merus* and *An. quadriannulatus* were correctly identified. Of two geographically separated *An. arabiensis* populations only one closely conformed to the identification criteria.

INTRODUCTION

Species identification of members of the *Anopheles gambiae* complex is vital to the efficient management of malaria vector control programs in Africa. Due to lack of suitable morphological characters, species identification within this complex of mosquitoes has mainly depended on cytological (Coluzzi 1968) and biochemical (Miles 1978, 1979) techniques. Coetzee et al. (1982) and Coetzee (1986) demonstrated that *An. gambiae/An. arabiensis* could be distinguished from *An. merus/An. quadriannulatus* by the width of the pale band at the apex of hind tarsomeres 3 and 4. Coetzee (1986) cautioned that the reported measurements might only apply to the localities sampled, and not to other areas in Africa. The reasoning behind this statement was not outlined by the author. Due to the practical implications of this technique for the field entomologist, an investigation was launched to evaluate the effectiveness of this method of identification of *An. gambiae* sensu. lato. species in Natal.

MATERIALS AND METHODS

The legs of wild caught females, their adult female progeny and adult females raised from wild caught larvae were mounted according to the technique of Hunt and Coetzee (1986). No more than 5 F1 females per family or 5 females from larvae collected in any one pool were used in scoring leg banding.

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Measurements were taken of the pale band at the junction of hindtarsomeres 3 and 4 using a compound microscope (magnification x100) fitted with an eyepiece micrometer. The maximum and minimum length of pale scaling were measured and the mean calculated. For each species, specimens were treated as two groups; (a) wild caught adults (WCA) and, (b) rearings from wild caught larvae or adult progeny of wild caught females (LE).

All material was collected in the Natal province of the Republic of South Africa at the following grid references: *An. arabiensis* Dondota (31°58'E, 28°34'S), Mamfene (32°15'E, 27°22'S); *An. merus*, Ophansi (32°16'E, 27°32'S), Chubu (32°51'E, 28°2'S), *An. quadriannulatus*, Numaneni (32°16'E, 26°58'S), Sandspit road (30°57'E, 30°48'S), Ophansi (32°16'E, 27°32'S), Mamfene (32°15'E, 27°22'S), Lumbongwenya stream (32°12'E, 27°51'S), Numaneni (32°15'E, 26°58'S), Buwensi (32°11'E, 27°12'S), Makhanis Drift (32°17'E, 27°1'S), Sihangwane (32°33'E, 27°5'S).

Species were identified by isoenzyme electrophoresis (Miles 1978, 1979), polytene chromosome analysis (Coluzzi 1968; Hunt 1973) and the physiological salt tolerance method (Muirhead-Thomson 1951).

RESULTS

A total of 440 females viz. 206 *An. arabiensis* 118 *An. merus* and 116 *An. quadriannulatus* were examined. Measurements were subjected to the Coetzee (1986) method in order to determine percentage correct identification. Where the pale band at the junction of hind tarsomeres 3 and 4 measures less than 0,099 mm in *An. gambiae/An. arabiensis*, and more than 0,1 mm in *An. merus/An. quadriannulatus*.

The percentage of *An. merus* correctly identified was >92% for both reared and wild caught females and >79% for *An. quadriannulatus* (Table 1). The Dondota *An. arabiensis*, population (A), revealed correct identification in excess of 86%, whereas only 19.6% were correctly identified in the case of the Mamfene *An. arabiensis*, population (B).

The distribution of leg banding measurements for *An. arabiensis* populations (A) and (B) are presented in Figure 1. In population (A) peak frequency was at 0,07 mm and 0,1 mm in population (B). 68% of the leg-band measurements in population (B) were wrongly identified using the Coetzee (1986) method in contrast with the 7.6% in population (A). This difference was statistically significant (Chi-square = 136.842, $P < 0.001$).

The percentage WCA and LE legs incorrectly identified according to the Coetzee (1986) method is shown in Table 2. Within species, the number of specimens in the WCA and LE groups wrongly identified by the method were not significantly different (Table 2).

DISCUSSION

A high percentage of measurements conformed to the Coetzee (1986) criteria in *An. merus*, *An. quadriannulatus* and *An. arabiensis* population (A). In all cases this was lower than the 94,0% correct grouping found by Coetzee (1986). This may be due to the use of greater magnification, the method of measurement or the use of a calculated mean measure in this study. Since the scaling of the leg-band may become rubbed with age there is the possibility that this might affect identification. To investigate this possibility all samples within species were separately treated as either freshly emerged or wild caught adults prior to pooling the data. None of the three species showed statistical differences between these groups with respect to specimens that were wrongly identified by the Coetzee (1986) method.

Two populations of *An. arabiensis*, 120 km apart were investigated during this study: population (A) from an area that had never been subjected to the intra-domicillary application of D.D.T. and population (B), from an area which is sprayed annually. A much higher percentage of measurements from population (A) fitted the Coetzee criteria than from population (B). The peak in distribution of the leg-banding measurements in population (B) was 0,1 mm, coincident with the measurement used by Coetzee (1986) for *An. merus/An. quadriannulatus*.

Coetzee (1986) states that of the four species examined, *An. arabiensis* showed the greatest variability. This finding is highlighted by the results of the present study. We speculate that the major difference between these two collection areas is the application of D.D.T.. The majority of the areas from which *An. arabiensis* were collected by Coetzee (1986) were not subject to a malaria vector control program. These data compare well with those collected in the present study from the unsprayed area.

The pooling of the *An. arabiensis* data from this study resulted in only 56% correct identification. This result seriously detracts from the use of leg-banding for the separation of the *An. gambiae* complex member species in the Natal region and highlights the necessity of confirming morphological identifications by biochemical and/or cytological means.

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Table 1. Percentage correct identification of *An. gambiae* complex species using the Coetzee (1986) method.

Species	Status of Adult	Number of Adults	% Correct Identification
An. merus	WCA	52	92,3
	LE	66	93,9
	Total	118	93,2
An. quadriannulatus	WCA	48	81,3
	LE	68	79,4
	Total	116	80,2
An. arabiensis (A)	WCA	33	93,9
	LE	76	86,8
	Total	109	89,0
An. arabiensis (B)	WCA	97	19,6

Table 2. Comparisons of WCA and LE leg measurements within species wrongly identified by the Coetzee (1986) method.

	Percentage of leg measurements wrongly identified.			
Species	WCA	LE	Chi sq.	P=
An. merus	4.2	3.2	0.009	N.S.
An. quadriannulatus	6.1	13.6	2.599	N.S.
An. arabiensis (A)	4.6	7.8	0.142	N.S.

Figure 1. Distribution of the leg-banding measurements of *Anopheles arabiensis* populations (A) and (B).