Use of the Palpal Ratio and the Number of Pale Bands
on the Palps in Separating *Anopheles gambiae* Giles s.s.
and *Anopheles melas* Theobald (Diptera: Culicidae)

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ABSTRACT. In a study in The Gambia, West Africa, it was found that 96.2% of *Anopheles melas* Theobald and 91.59% of *An. gambiae* s.s. occurring sympatrically and in the absence of other sibling species of the *An. gambiae* complex could be correctly identified using the palpal ratios. All ratios of 0.81 and above were ascribed to *An. melas* and all below to *An. gambiae* s.s. Up to 42% of *An. melas* had 4 pale palpal bands whereas all *An. gambiae* scored were 3-banded.

INTRODUCTION

The *Anopheles gambiae* Giles complex is presently known to consist of 6 sibling species (Davidson et al., 1967; Davidson & Hunt, 1973). The larvae of two of these, *An. merus* Dönitz in East Africa, and *An. melas* Theobald in West Africa inhabit saltwater, while the larvae of *An. gambiae* s.s., *An. arabiensis* and *An. quadriannulatus* (Theobald) inhabit fresh ground pools. The sixth species has as yet no valid formal name and is referred to as species D of the *An. gambiae* complex. Its larvae have so far only been found in hot mineral spring water in Uganda (Davidson & Hunt, 1973; White, 1973). In many areas of Africa, 2 or more of the species are sympatric. As marked behavioral differences exist between the species which influence their importance in disease transmission, identification of individual mosquitoes is crucial to an understanding of the epidemiology of the diseases for which they are vectors, e.g., malaria, bancroftian filariasis and some arboviruses (White, 1974).

At present, reliable identification is possible cytogenetically, using the morphology of the polytene chromosomes of fourth instar salivary gland cells or the ovarian nurse cells of female adults (Coluzzi, 1968; Coluzzi & Sabatini, 1967, 1968, 1969; Green, 1972; Davidson & Hunt, 1973). Electrophoretic studies on iso-enzymes have revealed differences between the species which are of taxonomic value (Bullini & Coluzzi, 1973; Mahon et al., 1976; Miles, 1978). In the past, extensive use has been made of crossing studies (Davidson et al., 1967; Coz, 1973). Unknown specimens are crossed to reference strains; conspecific crosses produce fertile hybrids while the F_1 males from interspecific crosses are sterile (Burgess, 1962; Davidson et al., 1967; Davidson & Hunt, 1973). Physiological differences between the species have also been exploited taxonomically, e.g., tolerance to saline conditions (Ribbands, 1944; Muirhead-Thomson, 1950).

All of these methods are time consuming and require, particularly in the case of the cytogenetic technique, considerable expertise. Therefore, many studies have been carried out to find reliable morphological differences between the species (Coluzzi, 1964; Chauvet & Déjardin, 1968; Zahar et al., 1970; Clarke, 1971; Green, 1971; White & Muniss, 1972; Reid, 1973, 1975a, 1975b). Although, except in very restricted areas (Green, 1971) it is not possible to distinguish *An. gambiae*, *An. arabiensis* and *An. quadriannulatus* morphologically, these 3 species can often be separated from *An. melas* and *An. merus*. The eggs of the latter two species have wide decks while the freshwater species have eggs with narrow decks (Coluzzi, 1964). In adults, the palpal ratio (the length of the fourth and fifth segments divided by the length of the third) can be used to separate the two groups. If the ratio is 0.75 or less, the mosquito belongs to one of the freshwater species; a ratio of 0.85 or more is diagnostic for *An. merus* in East Africa and *An. melas* in West Africa (Coluzzi, 1964). In many specimens the ratio lies between 0.75 and 0.85 and thus they cannot be identified. The number of pale bands on the palps of *An. gambiae sensu lato* is usually three (Gillies & De Meillon, 1968) but 4 bands also occur and are often more frequent in *An. melas* and *An. merus* than in the other species (Holstein, 1952; Ribbands, 1944; Gelfand, 1955).

Recently, a study has been undertaken in The Gambia, West Africa, to see if the palpal ratio and the number of pale bands on the palps could be used to distinguish between the local *An. gambiae* and *An. melas* with greater accuracy than the criteria of Colluzzi (1964) allow.

Mosquitoes for this study were obtained from a village where the *An. gambiae* complex has been surveyed for over two years (Bryan, 1979). Both *An. melas* and freshwater *An. gambiae* are present. Of 884 freshwater specimens identified cytogenetically 882 (99.8%) were *An. gambiae* s.s. and only 2 (0.2%) were *An. arabiensis*. In view of the small numbers of *An. arabiensis* it was not considered necessary to identify all the freshwater specimens used in this study.

MATERIALS AND METHODS

Mosquitoes were obtained from the village of Brefet (about 13.15N 16.20W) in the Western District of The Gambia. This village is situated within 2 kilometers of The Gambia River, which is saline in this area and bordered by mangroves and tidal salt flats, the larval habitat of *An. melas*. During the rainy season, many ground pools form in and around the village, providing freshwater breeding sites for *An. gambiae*.

Freshly fed and half gravid females of *An. gambiae* s.l. were collected during the day inside houses. They were taken to an insectary, tubed individually and allowed to oviposit. After oviposition the palps of the females were removed and the last three segments measured using a micrometer eyepiece at a magnification of X 500 and the palpal ratio determined. Many of the palps were also scored for the number of pale bands.

Initially the mosquitoes were identified by the morphology of their eggs; those laying eggs with wide decks were regarded as *An. melas* and those whose eggs had narrow decks as *An. gambiae*. To confirm the validity of these identifications, first instar larvae from 747 batches of *An. melas* and 20 from *An. gambiae* were exposed to saline (23.5g NaCl/1) for two hours. These conditions are fatal to the freshwater larvae but not to the saltwater larvae (Muirhead - Thomson, 1950). Of the females laying narrow-decked eggs, 367 were refed and 20-24 hours later their ovarian chromosomes were scored following the method of Coluzzi (1968).

RESULTS

Palpal Ratios. The results of the palpal measurements are given in Table 1. The results using different discriminating points of the palpal ratios for the separation of *An. melas* and *An. gambiae* are shown in Table 2.

Palpal Banding. All 684 *An. gambiae* examined for the number of pale bands on the palps were 3-banded. In contrast 109 (14.3%) of 763 *An. melas* were 4-banded; the rest were 3-banded. A subsequent study of a further 81 *An. melas* showed a much higher percentage of 4-banded palps, 42%.

Salinity Testing. Larvae from all 747 batches of *An. melas* survived the salinity test, whereas this test killed all larvae from 20 batches of *An. gambiae* eggs.

DISCUSSION

That egg morphology can be used reliably to separate *An. melas* and *An. gambiae* in the study area was confirmed by salinity tests and cytogenetically. However, this may not be true for all populations of these species as *melas*-type eggs have been recorded from areas in which this species does not occur (Bruce-Chwatt & Service, 1957; Ramsdale & Le Port, 1967).

Using the palpal ratio as described by Coluzzi (1964) with a ratio of 0.85 or higher being indicative of the salt-water species and 0.75 and less being diagnostic for the fresh-water species, 72.6% of *An. melas* and 60.5% of *An. gambiae* would have been correctly identified. However, 27.4% of *An. melas* and 39.6% of *An. gambiae* would not have been identified as their palpal ratios were in the overlap range (0.76 to 0.84).

More satisfactory separations can be achieved by altering the discriminating points. The highest percentages of correct identifications occur if all ratios of 0.81 and above are ascribed to *An. melas* and all below to *An. gambiae* (Table 2), with 96.2% of *An. melas* and 94.25% of *An. gambiae* being correctly identified. However, the number of misidentified specimens is increased to 3.8% for *An. melas* and 5.75% for *An. gambiae*. The results obtained if 0.80 alone or 0.80 to 0.81 are regarded as being in the overlap range are shown in Table 2.

The choice of discriminating point will obviously depend on the degree of accuracy required. Certainly, the large number of unidentified specimens in the study area using Coluzzi's (1964) criteria would be unacceptable (Table 2, column 1). For many behavioral and epidemiological studies the use of 0.81 as the only discriminating point would provide sufficiently accurate data (Table 2, column 2). Greater accuracy would be required in genetic studies.

Further help in the identification of *An. melas* can be obtained from the number of pale palpal bands. At Brefet, at the time of the study, any *An. gambiae* s.l. with 4-banded palps could be ascribed to *An. melas*. Of the 73 *An. melas* with a palpal ratio of 0.81 or less, 10 (13.7%) had 4-banded palps. At times, the percentage of 4-banded *An. melas* seems to increase, which should allow even more *An. melas* to be identified. This characteristic may not be so useful in other areas; although the percentage of 4-banded palps is not strictly clinal (Gillies & De Meillon, 1968), the percentage is higher in the western part of the range of *An. melas*. Holstein (1952) found that in Senegal up to 90% of *An. melas* were 4-banded, but the percentage falls to 5% in Nigeria (Chwatt, 1949). There are also variations in the number of bands in the other species of the complex. White et al. (1972), found that at Segera, Tanzania, 28% of *An. gambiae* and 4% of *An. arabiensis* were 4-banded.

The palpal characters could be used in conjunction with cytogenetic identification or iso-enzyme studies, with the latter two techniques being used in cases of doubt. The advantages of using the palpal characters are that they are relatively quick to score compared with the other techniques and they require little expertise or expensive equipment and females in all stages of ovarian development can be scored; the cytogenetic technique is only suitable when the ovaries have reached Christopher's Stage III.

The palpal characters may vary from population to population as already shown above. However, Reid (1973; 1975a; 1975b) and Green (1971) have already shown that detailed morphological studies of sympatric populations of *An. gambiae* and *An. arabiensis* may reveal diagnostic features, and the present study indicates that for sympatric populations of *An. melas* and *An. gambiae* palpal characters could be used to advantage. The results presented here should not be regarded as applicable to other areas until further local studies have been undertaken.

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T A B L E   1

Palpal ratios of *An. melas* and *An. gambiae* from The Gambia

Palpal ratio	An. melas	An. gambiae	Palpal ratio	An. melas	An. gambiae
0.66		3	0.84	74	3
0.67		4	0.85	106	2
0.68		13	0.86	85	
0.69		16	0.87	96	
0.70		31	0.88	70	
0.71		48	0.89	73	
0.72		58	0.90	50	
0.73	1	79	0.91	41	
0.74		75	0.92	40	
0.75	1	104	0.93	30	
0.76	1	79	0.94	17	
0.77	1	57	0.95	9	
0.78	6	53	0.96	9	
0.79	7	26	0.97	2	
0.80	16	26	0.98	0	
0.81	40	20	0.99	1	
0.82	37	7	1.00	1	
0.83	54	9			

T A B L E   2

The results obtained when using different values of the palpal ratio to distinguish between *An. melas* and *An. gambiae*

Column	1*	2	3	4
Ratios for *An. melas*	>0.85*	>0.81	>0.81	>0.81
Ratios for *An. gambiae*	<0.75	<0.81	<0.80	<0.79
Overlap range	0.76 to 0.84	None	0.80	0.80 to 0.81
Results obtained				
Correctly identified *An. melas*	72.58%	96.2%	91.59%	91.59%
Correctly identified *An. gambiae*	60.49%	94.25%	94.25%	90.60%
Unidentified *An. melas*	27.19%	0	4.61%	6.45%
Unidentified *An. gambiae*	39.27%	0	2.81%	6.45%
Mis-identified *An. melas*	0.23%	3.8%	3.8%	1.96%
Mis-identified *An. gambiae*	0.28%	5.75%	2.95%	2.95%

*Criteria used by Coluzzi (1964).