

Pupal Differences between Species A and B of
the *Anopheles gambiae* group from Kisumu,
East Africa

J. A. Reid

4 Glenwood, Dorking, Surrey, England

ABSTRACT. A combination of setal characters identifies 96 per cent, of a sample of pupae of species A and B of the *Anopheles gambiae* group from Kisumu, Kenya.

Introduction

This is the second paper recording the results of an attempt to find external morphological differences between species A and B of the *Anopheles gambiae* species group. The background and methods were described in the first paper (Reid, 1973) and for the most part need not be repeated here. That paper was on sympatric larvae of the two species, originating from Kaduna, Nigeria, West Africa, and showed that a combination of characters identified 93 per cent of the specimens.

Methods

In one respect the methods differed from those used on the Kaduna larvae. The first examination of all the numbered setae did not show any promising differences other than in seta 9, VIII. However, by a fortunate chance, a question from Dr. G. B. White necessitated comparing the sum of the branches on seta 2 on abdominal segments IV-VII (Coluzzi, 1964, p. 213) in species A and B, using either one seta per segment or the pair of setae. This showed that particularly when the number of branches on the segmental pairs, rather than on single setae, were summed, there was no overlap in the small samples used. Accordingly work was recommenced, using an initial sample of five pairs of 22 different setae. When completed this gave a number of pairs of setae in which the sum of the branches showed promising differences between A and B. Larger samples of these pairs, or combinations of them, were then examined.

The setal notation system of Belkin (1962) has been used, but with one small variation. Instead of separating the setal number from the Roman numeral by a hyphen, a comma has been used. This is chiefly to avoid confusion between hyphens and the minus signs necessary in this work (Table I, combinations 7 & 8).

Materials

Eggs of several females were received by Dr. G. Davidson (see acknowledgments) from Kisumu, Kenya. Those of each female were reared separately

and the progeny identified by crossing with reference colonies. This showed that both species A and B were present, and separate colonies of each were then established, early in July 1968, by pooling the progeny from several females.

Specimens used in the present work were as follows. Progeny of three females from the colony of A and of three from the colony of B reared in September and October 1968, each adult with its last larval and pupal skin mounted on a slide. These totalled only 16 A and 31 B, so in July 1973 further pupal specimens of A were obtained from the original colony (Yan), plus a small sample from another colony of A from Kisumu (Kis) founded in May 1968. Unfortunately the colony of B (Yin) no longer existed. These 1973-samples were in bulk, not with individually matched larval skins and adults, though bulk larvae and adults were obtained at the same time.

Results

Setal differences

For both species A and B samples of 10 or more of 25 different pairs and combinations of pairs of setae (characters) were examined and the number of branches counted and summed.

Using the original sample of 16 A and 31 B, two of the combinations employed by Coluzzi (1964, p. 213) were examined. His combination of the sum of the branches on both setae 1 on abdominal segments III and IV, which he found the most promising for distinguishing between A and B, was applied to 15 pupal skins of A and 28 of B. Out of the total of 43 examined 23, or 54 per cent, had sums falling in the numerical zone where the ranges for A and B overlapped (hereafter called the overlap zone) and so were not identified. This compares with respectively 85 and 79 per cent in overlap when the single pairs 1,III or 1,IV were separately employed, and shows the improvement obtained in this instance by combining the sum of the branches on more than one pair of setae. However, other combinations gave lower degrees of overlap than 54 per cent. (Table 1) and so this character was not pursued further, but it should not be forgotten.

By contrast, when Coluzzi's character of the sum of the branches on both setae 2 on the four abdominal segments IV-VII, was used on samples of 10 each of A and B and compared with the sums for the individual segments, a rather different result was obtained. With the sums for seta 2 on the four segments added together, 55 per cent of the specimens were in the overlap zone. With the individual segments the percentages in overlap were as follows: segment IV 60 per cent, V 100, VI 85, VII 32 per cent. Clearly segment VII alone gave a much better result than any of the other three segments and better than the combination of all four. The same was true for pupae of A and B from Kaduna, West Africa; with all four segments 14/20 or 70 per cent were in overlap compared to 18/40 or 45 per cent with segment VII alone.

Out of the 25 characters examined, nine were used in identifying the specimens in the sample. Details of these nine are given in Table I below, which is based on the full sample of 31 B and 46 A (16 A from 1968, 30 from 1973).

TABLE I

Showing the range, mean number of branches, overlap zone and proportion of specimens in overlap, for 9 pairs or combinations of pairs of setae on pupae of species A and B originating from Kisumu, Kenya.

Setal pairs and combinations	No. exam.	A		No. exan.	B		Overlap zone	No. in overlap		Total A+B in overlap Proportion	%
		Range	Mean		Range	Mean		A	B		
1. 1, II	41	6-11	8.8	29	8-13	10.7	8-11	36	22	58/70	83
2. 2, II	46	6-16	11.2	31	10-16	12.6	10-16	33	31	64/77	83
3. 4, II	43	8-16	11.2	31	5-10	8.1	8-10	15	23	38/74	51
4. 3, III	44	2-5	2.5	27	2-7	4.3	2-5	44	23	67/71	94
5. 2, VII	46	2-8	5.8	31	6-9	7.0	6-8	35	30	65/77	84
6. 9, VIII	40	12-32	24.5	28	25-35	29.2	25-32	19	24	43/68	63
7. 4, II - 2, VII	43	3-9	5.5	31	-2 to +4	1.1	3-4	13	5	18/74	24
8. 9, VIII - 4, II	39	0-22	13.2	28	17-28	21.0	17-22	9	20	29/67	43
9. 1, II + 2, II	41	13-26	19.7	29	20-29	23.3	20-26	24	28	52/70	74

From Table I it will be seen that for single pairs of setae (characters 1 to 6 in the first column) the range and mean number of branches are higher in B than in A, except for seta 4, II where they are lower. This is the reverse of what was found with the larval setae in samples originating from Kaduna (Reid, 1973), in which B had a lower number of branches than A on several posterior abdominal setae, but a higher number on prothoracic seta 1. As with the Kaduna larvae, this fact can be used to amplify the differences between the Kisumu pupae of A and B (characters 7 and 8). The following key, which uses characters 7 and 4, can be applied to all the 77 specimens in the sample; that is all specimens happened to have the necessary pairs of hairs complete with countable branches for one or both of the characters.

Provisional key to the pupae of species
A and B from Kisumu, East Africa

Sum of the branches on both setae 4, II minus sum of the branches on both setae 2, VII is 5 or more; and/or both setae 3, III commonly simple, neither with more than 2 branches species A

This sum is 2 or less; and/or both setae 3, III commonly with 2 or more branches species B

This key identifies 70/77 or 91 per cent of the pupae. Character 7 (4, II - 2, VII) identifies 76 per cent of the 74 specimens to which it can be applied, compared to 66 per cent of 71 specimens for character 4, and for this reason is placed first in the key. Also character 4 by itself gives some misidentifications.

From Table I it might be thought that character 4 would be of little use, as 94 per cent of the specimens to which it can be applied fall in the overlap zone. But the means are different, and where 28/44 (64%) of A have both 3, III simple, the figures for B are 3/27 (11%), giving a ratio of 5.8:1 in favour of A. That is, specimens with both setae 3, III simple are nearly six times more likely to be A than B. Similarly, specimens having 2 or more branches on both setae 3, III are in a ratio of 3.9 B to 1 A, that is such specimens are nearly four times more likely to be B than A.

Of the seven specimens (5A, 2B) not identified by the key, 4A can be identified by one or more of the other characters in Table 1. This gives a total of 74/77 (96%) of the pupae identifiable by characters of the setal branching. This is an encouraging result, especially as the sample of A was composed of two parts (p. 2), the second reared more than four years after the first by which time some character-drift seems to have occurred in the colony (see discussion). This accounts for the overlap with seta 2, VII in Table I being 84 per cent compared to 32 with the first part of the sample (p. 2).

Further encouragement is given by applying the key to a small sample of 20 pupae from the other colony of A from Kisumu (Kis). The key identifies 18 as A and one more is identified by character 6 (Table I), giving 19/20 (95%) identified.

Male genital lobes

Inspection, confirmed by measurements, shows that there is usually a difference in the shape of the tips of the male genital lobes. In species A the points of the lobes (these points are present in subgenus *Cellia*, absent in subgenus *Anopheles*; Reid, 1968) are seldom as wide apart as in species B, and the depth of the emargination between them tends to be greater (Fig. 1). Measurements of the width, w, and the depth, d, were made on 15 male pupae each of A and B as indicated in the figure. With specimens in which the lobes were not pressed together, the width of the gap between them at the base of the emargination was subtracted from the total width between the points to get the correct figure for w. The depth, d, was measured separately for the left and right lobes of each specimen and the two figures

averaged. The results of these measurements are summarised in Table II.

Table II
Measurements of the tips of the male genital lobes in microns, for 15 specimens each of species A and B

Species	Width between the points, w.		Depth of the emargination, d.		Ratio, w/d.	
	Range	Mean	Range	Mean	Range	Mean
A	34-69	60	8-37	26	1.80-4.00	2.43
B	69-101	83	15-24	20	2.96-6.60	4.23

Table II shows that in the samples measured the difference between the two species is greatest in the width between the points, and secondly in the ratio width/depth. There is very little overlap in the width ranges and only three specimens (2A, 1B) fall in the overlap zone of 69 microns; that is 3/30 (10%) in overlap. The difference between the means of the ratios, w/d, is greater ($B/A = 4.23/2.43 = 1.74$) than that between the means of the widths ($B/A = 83/60 = 1.38$). But there is more overlap in the ratios with six specimens, or 20 per cent (2A, 4B) in the overlap zone of 2.96-4.00.

A small sample of five male pupae from the other colony of A(Kis), agreed quite well with the figures for A in Table II, thus: width 52-60 microns, depth 27-33, w/d 1.79-2.16.

Among the three pupae out of 77 not identified by any setal character (p. 4), one B is a male and the genital lobe measurements clearly place it as this species. The width between the points is 92 microns and the ratio, w/d, 5.00, both being well beyond the upper limits for A in Table II.

The shape of the tip of the male genital lobes seems to vary geographically, as do other characters. In a small sample of five each of A and B from Kaduna, West Africa, the average width between the points and the ratio width to depth were greater in A (w 71, w/d 2.71) than in B (w 56, w/d 2.00), instead of smaller as in the East African (Kisumu) samples.

In the Kisumu specimens another difference was noted in the shape of the male genital lobes, more variable than that of the points. This is a tendency for the outer margins of the lobes to be more parallel or slightly emarginate in A than in B; in B they more often tend to converge towards the apex. These differences show slightly in figure 1 and are more pronounced in other specimens, but a few are intermediate or resemble the other species.

Discussion

The key plus other setal characters together identify 96 per cent of the specimens in the main samples as A or B. Although the samples are small and that of A is composed of two parts drawn from the same colony (Yan) with an interval of more than four years between, the characters used seem fairly reliable, as they work well on another sample of A from Kisumu from a different colony (Kis).

The two parts of the sample of A from colony Yan show some differences. For example, the range and mean number of branches for the setal pairs 2, VII and 4, II are higher in the specimens of 1973 than in those of 1968. For 2, VII the ranges and means are as follows: 1968, range 2-6, mean 4.7; 1973, range 5-8, mean 6.4. For 4, II: 1968, range 8-14, mean 10.0; 1973, range 9-16, mean 11.8. The effect of this on the use of these pairs of setae for distinguishing A from B is that the difference between A and B for 2, VII is reduced, because the figures for A have risen nearer to those for B (Table I). But with the pair 4, II, where the figures for B are lower than those for A, the increase in the figures for A increases the difference from B. Consequently the combination of these pairs used in the key is only slightly affected and retains its value.

Seta 4, II is a small one and an oil immersion objective is sometimes needed to count the branches. However, as few workers would want to identify living pupae, but would examine the skins after the adults had emerged, this is no great disadvantage.

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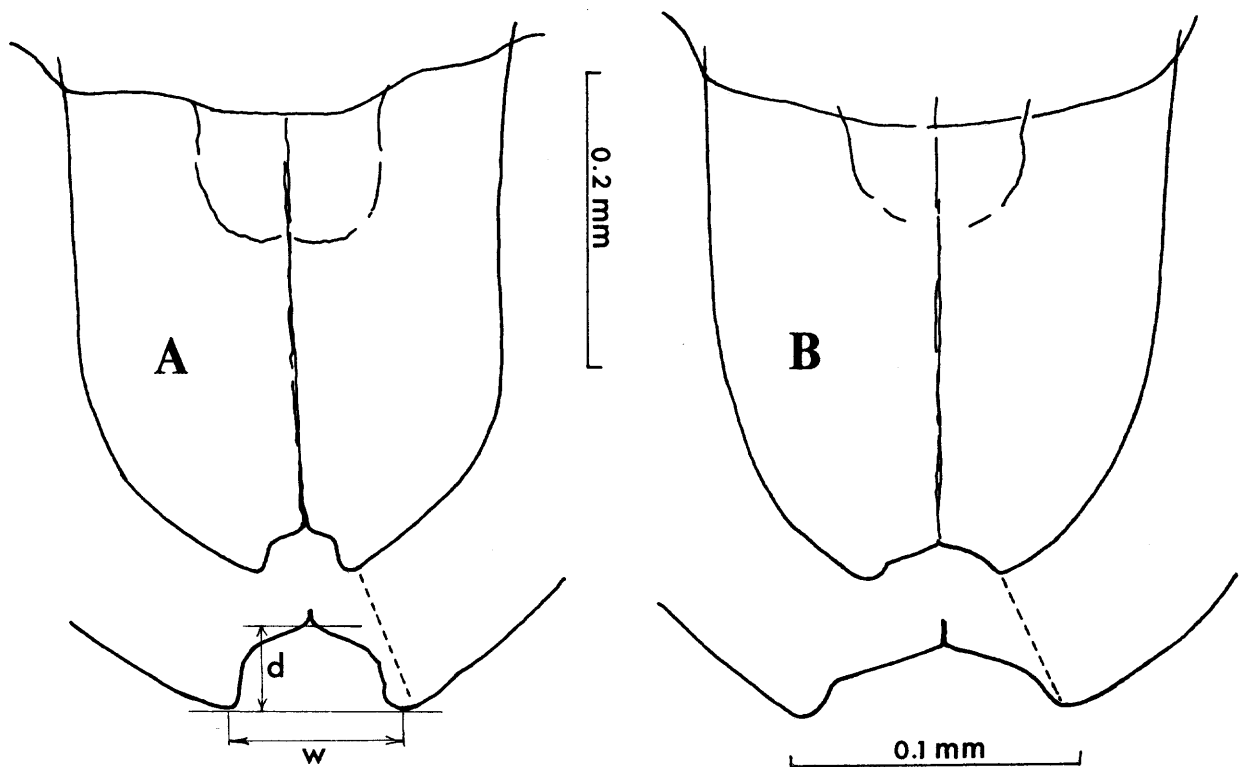


Fig. 1 To show the shape of the male pupal genital lobes in species A and B from Kisumu, Kenya, East Africa. The specimens figured are near the averages for the samples measured (Table I). For A the width, w , is 62 microns and the ratio width/depth, w/d , is 2.35; for B, w is 88 and w/d 4.06.