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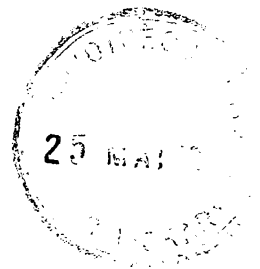
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ON THE STATUS OF THE EAST AFRICAN SALT WATER-BREEDING
VARIANT OF ANOPHELES GAMBIAE GILES¹

by

H. E. Paterson
The South African Institute for Medical Research,
Johannesburg



Introduction

Records of a form of Anopheles gambiae breeding in East African estuarine waters at salinities above the normal tolerance limits of the typical form have appeared from time to time over the last 30 years (Mackay, 1935; Muirhead-Thomson, 1951; de Meillon, 1947; and others). Records apparently referring to this form range from St Lucia, Natal (de Meillon, 1947) to Somalia (Maffi, 1960), and on Mauritius a similar or identical form occurs (Jepson, et al. 1947; Halcrow, 1957). It has been demonstrated that this East Coast form is not identical with A. melas Theo. sensu Muirhead-Thomson (1948) which occupies a similar niche on the West Coast of Africa (de Meillon, 1947; Muirhead-Thomson, 1951). There are apparently no clear-cut morphological characters separating the form from A. gambiae s.str.

This communication reports the results of some crossing experiments made as an attempt to elucidate the status of the salt water-breeding form in nature.

Procedure and Results

The A. gambiae s.str. used in these experiments were from: (a) the colony maintained at Amani which was started by Shute (Shute, 1956) with mosquitos which came, originally, from Kisumu (K); (b) a colony started with the eggs of wild caught females from Muheza, a village lying some 25 miles inland from Tanga

¹ This investigation was carried out during the tenure of a WHO short-term consultantship in the laboratories of the East African Institute of Malaria and Vector Borne Diseases at Amani, Tanganyika

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on the railway to Arusha (M). The mosquitos of the salt water-breeding form were from a colony maintained at Amani and which was started by Kuhlou. The founder mosquitos were from Tanga (T).

The following crosses and reciprocals were set up in cubic cages measuring one foot along a side: K ♂♂ x T ♀♀ and M ♂♂ x T ♀♀. The cages were kept in a normally lit room without any control of humidity or temperature.

The F₁ eggs were hatched in fresh water, and the method used, ultimately, for feeding the larvae was that described by Shute (1956).

All four crosses yielded F₁ generations. Table 1 presents the results in each cross.

TABLE 1. RESULTS OF CROSSINGS

Cross (F ₁)	No. of eggs laid	% hatch	% first stage larvae reaching maturity
TK	4276	30.7	8.7
KT	2486	40.7	14.7
TM	1056	21.9	14.8
MT	541	42.9	22.8

Note: TK = Tanga ♂♂ x Kisumu ♀♀

KT = Kisumu ♂♂ x Tanga ♀♀

TM = Tanga ♂♂ x Muheza ♀♀

MT = Muheza ♂♂ x Tanga ♀♀

Although the larval feeding regimen used yielded healthy adults when applied to the parental forms, the hybrid adults obtained were mostly very feeble, many being incapable of flight, despite the fact that the pupae from which they emerged were large and active. Fewer hybrid eggs hatched than of the parental forms. The sex ratios of the F₁ generations deviated from the expected 1:1 as shown in Table 2.

TABLE 2. SEX RATIOS IN HYBRID F₁ GENERATIONS

Cross (F ₁)	Males	Females	χ^2 (2)	P
TK	26	82	29.04	< 0.001
KT	103	14	67.70	< 0.001
TM	8	23	7.29	< 0.010
MT	31	22	1.53	0.25 > P > 0.20*

* = Not significant

It should be noted that although the deviation in the cross M oo x T ^{oo} ₊₊ might have arisen by chance, it is in the same direction as the corresponding cross, K oo x T ^{oo} ₊₊.

It was found that the males examined from the hybrid generation resulting from the crosses T ^{oo} ₊₊ x M ^{oo} ₊₊ and T ^{oo} ₊₊ x K ^{oo} ₊₊ (i.e. those yielding a deficiency in males), had degenerate testes. These were so reduced as to be little thicker than the vasa deferentia, and were clearly not capable of spermatogenesis (c.f. crosses made by Muirhead-Thomson (1948) between A. melas and A. gambiae s.str.). The testes of F₁ males of the reciprocal crosses were apparently of normal size and spermatogenesis was noted in them.

Ideally, back-crosses should have been made between the hybrid males in order to demonstrate whether the hybrids were fertile or not. However, owing to the general weakness of the hybrids it was possible to attempt this only in the case of F₁ females of the cross T oo x K ^{oo} ₊₊ of which eight fairly robust specimens were available; these were back-crossed to Kisumu males.

Three of these females died within the first nine days. The remainder appeared gravid at this time. When one was sacrificed and dissected it was found to have been fertilized, and its ovaries held eggs which were judged to be at Christophers' late stage IV or early stage V (Christophers, 1911). The other females produced no eggs in the next seven days when the attempt terminated with their accidental deaths.

The ovaries of the newly-hatched hybrids seemed small but normal, as far as could be judged without special studies.

Because of Holstein's (1957) report of sterility being detected in the F_1 offspring of crosses between colonies of fresh water-breeding A. gambiae from different parts of Tanganyika, it was decided to cross the Kisumu and Muheza colonies. Tables 3 and 4 present the results of the cross $M \text{ ♂} \times K \text{ ♀}$.

TABLE 3. RESULTS OF CROSS $M \text{ ♂} \times K \text{ ♀}$

Experiment	No. of eggs laid	% hatch	% 1st stage larvae reaching maturity
1	131	71.8	67.0
2	136	30.1	-*
Totals	267	50.6	-

* Not completed through lack of time

TABLE 4. SEX RATIO IN F_1 GENERATION OF CROSS $M \text{ ♂} \times K \text{ ♀}$

Males	Females	χ^2 (1)	P
24	39	1.36	$0.25 > P > 0.1$ *

* Not a significant deviation from 1:1 ratio

The testes of F_1 males of this cross were examined and found to be apparently normal.

Although little time was devoted to the search for morphological characters, which would help separate the two forms, a study was made of their eggs. It was found that the eggs were very similar in morphology but that the mean length of the Tanga colony sample was significantly greater than the means of the samples from the colonies of A. gambiae s.str. Table 5 summarizes these differences.

TABLE 5. EGG LENGTHS

	n	\bar{x} (mm)	S	S \bar{x}	C (%)	Range (mm)
K	100	0.481	0.0196	0.00196	4.1	0.429 to 0.525
T	100	0.575	0.0172	0.00172	3.0	0.525 to 0.627
M	100	0.490	0.0252	0.00252	5.1	0.397 to 0.531
KT*	100	0.545	0.0234	0.00234	4.3	0.435 to 0.589
TK*	50	0.488	0.0179	0.00253	3.7	0.454 to 0.531

* Eggs of the P₁^{oo}₊₊

It will be noted that the mean egg lengths of the K, M and T samples are all significantly different from each other ($P < 0.01$), and that the means of the F₁ egg samples of the crosses K ♂^{oo} x T ♀⁺⁺ and T ♂^{oo} x K ♀⁺⁺ are significantly different from the means of the K and T samples ($P < 0.05$). This is somewhat unexpected since egg characters are generally believed to be characters of the mother. It is possible that the differences are due to sampling errors, despite efforts which were made to minimize these. It is also noteworthy that the means of the two samples of eggs of A. gambiae s.str. should be significantly different. The smallness of the eggs from the Kisumu colony may be exceptional, and may be the result of some selective influence acting during the many years since the colony started.

Preliminary work on obtaining LC₅₀ values for the salinity tolerance of the parental and hybrid populations indicated that the hybrids were intermediate in their tolerances of high salinities.

Discussion

Strictly, the problem can be resolved only when it is known whether there is a flow of genes between the gene-pool of A. gambiae s.str. and the gene-pool of the salt water-breeding form at a locality where they coexist, and where, therefore, the opportunity is present for hybridization to occur. In other words, we need to know whether the two forms hybridize, with the production of fertile offspring, when the opportunity exists for them to do so in nature.

This demonstration is seldom practicable and we must attempt to arrive at an answer by less direct methods.

Thus, we can say at once that we are not dealing with two sub-species, for the two forms coexist at several localities. At Dar-es-Salaam we know that they have coexisted without loss of their integrities (as judged by the fact that the two forms remained recognizably distinct) for at least 14 years (Mackay, 1935; Muirhead-Thomson, 1951), despite the fact that there was clearly opportunity for hybridization. Taking into account the relative abundance of the two forms, we are left with two possible alternatives: that the forms are polymorphs of a single species, or, that they are two separate species.

The demonstration of at least partial sterility in the F_1 generations of crosses between the two forms, and the relative inviability of these hybrids, makes it very unlikely that we are dealing with a single polymorphic species. This evidence, taken together with the other evidence of genetic differences between the forms, indicates that they represent two separate biological species. This conclusion is not contradicted by the data which has accrued from field studies (Muirhead-Thomson, 1951; Iyengar, 1961), which indicates that we are dealing with two separate populations with different behaviour patterns. These behavioural differences are summed up by the differences between the forms in their potential as vectors of malaria and in their different responses to spraying with dieldrin at Pemba.

At this point it might be as well to say a few words about the very similar situation which exists on the West Coast of tropical Africa. The available evidence (Muirhead-Thomson, 1948; Bruce-Chwatt, 1950; and others) makes it quite clear that two forms, well defined by their differing responses during the immature stages to saline waters occur, one breeding in fresh water and the other in brackish estuarine waters. Detailed crossing experiments have not been made but the work of Muirhead-Thomson (1948) and Bruce-Chwatt (1950), although differing in detail, does demonstrate that the F_1 hybrids of the two forms are at least partially sterile, and does not contradict the possibility that they are completely sterile. Fox (see Marchal, 1959) states, without elaboration, that crossing experiments which he has performed between the two forms produced fully fertile F_1 and F_2 generations. This statement cannot be accepted without confirmation

since it contradicts the work of Muirhead-Thomson and Bruce-Chwatt quoted above. It contains no experimental details, and there are aspects of his brief statement which are open to query (e.g. he states the F_1 eggs are intermediate in morphology and he makes no reference to recombinant forms in the F_2 generation when he deals with the character of the larval pecten).

Accepting the following significant evidence: (1) the forms are often sympatric with the opportunity for hybridization occurring; (2) the F_1 hybrids resulting from crosses between the forms are at least partially sterile; (3) there are morphological and physiological differences between the forms; and applying a similar argument to the one used above for the East Coast forms, the conclusion reached is that the forms represent two separate species.

Thus, there is evidence to support the sub-division of "A. gambiae" into at least three sibling species, and the possibility that other sibling species of the complex may be unmasked in time should be borne in mind. Many anomalous observations made during eradication campaigns can be accounted for on an hypothesis that "A. gambiae" breeding in fresh water represents, in fact, a pair of sibling species which differ from each other in behaviour.

Finally, I should like to support Mattingly (1955) in his plea for the use of the terms "species" and "sub-species" in their strict biological sense, as defined in modern works on evolution and systematics theory such as Mayr et al. (1952) or Cain (1954).

Addendum

The recent work by Davidson & Jackson (1962) makes it very likely that the fresh water-breeding form of A. gambiae, in turn, represents two sibling species, since their group A and group B, which are partially isolated by a partial sterility barrier, coexist in at least one locality (Diggi, N. Nigeria).

It is unlikely that this will affect the arguments developed above, but clearly the crosses between the salt water-breeding species and fresh water-breeding "A. gambiae" should be repeated with members of both fresh water-breeding forms. Davidson & Jackson consider the Kisumu colony as group A (although our material appears to be group B anatomically). This demonstrates that the Muheza colony

used for the experiments reported above was also of group A. Since Muheza is only some 25 miles inland from Tanga it is likely that group A, at least, occurs at the Tanganyikan coast. Nevertheless, it will be necessary to repeat the crossing experiments using the Tanga colony and some group B colony, preferably from near the Tanganyikan coast.

Work done independently at Amani by Kuhlow (1961) has also been reported in outline. This agrees well with the work reported here and adds some further information. He finds that some hybrid females from the cross Tanga ♂ x Muheza ♀, at least, were fertile. He also found characters which are likely to make the separation of larvae of the two species possible.

Summary

1. On the East Coast of Africa and on Mauritius "A. gambiae" has been recorded breeding in estuarine waters of high salinity.
2. Crossing experiments are described in which the salt water-tolerant form was crossed with typical fresh water-breeding A. gambiae.
3. At least partial sterility was demonstrated, as well as reduced viability, among the F₁ hybrids.
4. The argument, based on the modern biological species concept, that the two forms represent separate species, is outlined.
5. Brief consideration is given to the comparable situation involving A. gambiae and A. melas on the West Coast of Africa.

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