I

INCIPIENT SPECIATION IN ANOPHELES GAMBIAE, GILES

by

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In the course of studies on the mode of inheritance of dieldrin-resistance in Anopheles gambiae, Giles, it was found that crosses between the first resistant and susceptible strains used, produced sterile F_1 males (Davidson, 1956). On dissection these males were found to have atrophied testes, usually completely devoid of spermatozoa. The females of this F_1 generation, on the other hand, were apparently normal reproductively and could be back-crossed to the males of either parent strain.

It was first thought that this sterility was associated with resistance and not until further strains of A. gambiae were acquired was this shown not to be so.

A total of 15 strains of this species have now been used in crossing experiments. These are:

1. Lagos — An insecticide-susceptible strain from Lagos, Nigeria.

2. Maidahini) 3. Diggi) — Two insecticide-susceptible strains from the Western Sokoto Region of Northern Nigeria, obtained in 1956, from outside the dieldrin-sprayed area.

4. Ambursa — A homozygous, dieldrin-resistant strain from within the dieldrin-sprayed area, Western Sokoto, Northern Nigeria.

5. Tungan Buzu — An apparently insecticide-susceptible strain acquired in 1961 from near Ambursa, four years after dieldrin had been replaced by DDT.

6. Kano RR. — A homozygous dieldrin-resistant strain selected from a mixed population from Kano, Northern Nigeria.

7. Kano SS — An insecticide-susceptible strain from Kano, Northern Nigeria, selected from the same mixed population from which the Kano RR strain originated.

8. Bobo — A homozygous, dieldrin-resistant strain selected from a population from Bobo Dioulasso, Upper Volta.

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9. Liberia — A mixed population containing dieldrin-resistant individuals and susceptible ones, from Kpain, South of Gonta, Central Province, Liberia.

10. Ruzizi — An insecticide-susceptible strain from the Ruzizi Valley, Kivu Province, Congo Republic.

11. Kisumu — An insecticide-susceptible strain from Kisumu, Kenya, on the eastern shore of Lake Victoria.

12. Taveta — An insecticide-susceptible strain from Taveta, Kenya.

13. Pare — An insecticide-susceptible strain from the dieldrin-sprayed Pare area of Tanganyika, between Muheza and Moshi.

14. Muheza — An insecticide-susceptible strain from Muheza, Tanganyika, near Tanga.

15. Somalia — An insecticide-susceptible strain from Somalia.

The result of performing 64 of the possible 210 reciprocal crosses between these 15 strains shows the existence of two groupings as follows:

Group A		Group B	
1.	LAGOS	4.	AMBUSA
2.	MAIDAHINI	6.	KANO RR
3.	DIGGI	7.	KANO SS
5.	TUNGAN BUZU	8.	BOBO
9.	LIBERIA	10.	RUZIZI
11.	KISUMU	13.	PARE
12.	TAVETA	15.	SOMALIA
14.	MUHEZA		

The members of Group A are interfertile as are the members of Group B, but any member of Group A crossed with any member of Group B produces sterile F_1 males. Both groups include dieldrin-resistant strains. Group A ranges in distribution from Nigeria and Liberia on the west side of Africa to Kenya and Tanganyika on the east side. Group B ranges from Nigeria and Upper Volta on the west side, through the Congo Republic to Tanganyika and Somalia on the east side. There seems no obvious relation between this grouping of strains and geographical or climatological distribution.

Of particular note is that the resistant Ambursa strain and the susceptible Maidahini and Diggi strains belong to different groups even though they originate from places very close to one another. The susceptible Tungan Buzu strain, which might be taken as having replaced the Ambursa strain after a change from dieldrin to DDT belongs to the opposite group to the Ambursa strain. A similar mixture of the two groups is to be found in the Taveta-Pare-Muheza area where distances are not great and where all the strains are insecticide-susceptible.

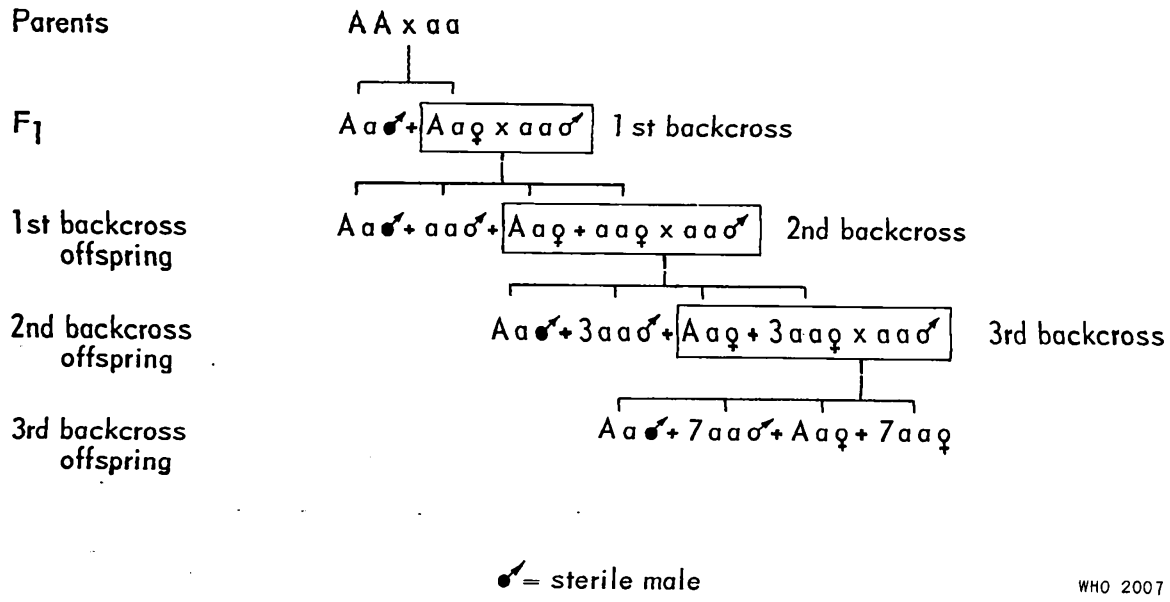
The mode of inheritance of the sterility has been worked out by crossing members of one group with members of the other group and backcrossing the female hybrid to males of one of the parents. The female offspring of this backcross have then been crossed again to the same parent this process being repeated for several generations. The males have been examined for sterility in each generation. Examination involved both gross examination under the binocular dissecting microscope and detailed examination under the compound microscope after staining with aceto-carmine. Normal testes appear as brown bulbous bodies in which spermatozoa can be distinguished and in which concentrations of sperm heads can be seen distally in the vasa deferentia just before these join in the common genital tract. Abnormal testes appear as narrow, transparent, tapering bodies almost devoid of contents and no such concentration of sperm heads is apparent in the vasa deferentia.

Using the Diggi and Pare strains, crossing the male of the former with the female of the latter and backcrossing the female hybrid to the Diggi male in each generation, the following percentages of sterile males were recorded (the actual numbers of males examined are given in the brackets):

Generation	Percentage of sterile males	
F_1	100	(100)
Backcross 1	52	(100)
" 2	23	(100)
" 3	12	(100)
" 4	6	(200)

These results fit almost exactly the proportions expected where a single, autosomal, sex-limited (expressed only in the male) factor is involved, according to the following scheme:

INHERITANCE OF STERILITY IN *A. gambiae*



That this sterility is in no way associated with the factor for dieldrin-resistance was shown when males of the Lagos strain were crossed with females of the Kano HR strain. The offspring of the first backcross to the Lagos strain were exposed to the dosage of dieldrin known to kill susceptibles but not hybrids (Davidson, 1958) and the knocked-down and surviving males examined for sterility. Normal and abnormal males were found in both those succumbing to the insecticide and in those surviving it.

These observations, then, indicate a divergence within the species A. gambiae which, perhaps, may be occurring independently in different parts of Africa. It would be of great interest to observe in those areas where the two forms of the species occur together, whether there is any relation between this divergence and the bionomical differences in adult behaviour, host preference, breeding habits etc. which have been described (De Meillon, 1951; Holstein, 1954; Gillies, 1956).

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II

MORPHOLOGICAL VARIATION IN ANOPHELES GAMBIAE, GILES

by

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Investigations by Davidson and Jackson (Paper I) involving the crossing of several strains of A. gambiae from different parts of Africa have shown that some of these crosses produce sterile hybrid males. Of 15 strains examined, eight fell into one group (A) and seven into another group (B). Crossing members within a group produces normal fertile hybrids but crossing any member of group A with any member of group B produces sterile hybrid males.

A search has now been made for morphological differences between these two groups and common to all the members of each group. Two such differences have been found:

1. A difference in the width of the sector spot, the pale interruption between the first and second main dark areas of the wing costa.

2. A difference in the shape and arrangement of the spines of the pupal paddle.

Sector Spot

Group A members normally show a narrow sector spot (Fig. 1(c)), sometimes almost completely absent (Fig. 1(d)). Group B members normally show a wide sector spot, which is never absent. It may (Fig. 1(a)) or may not (Fig. 1(b)) be accompanied by an interruption of dark scales on the white area of vein 1 below the sector spot, i.e. an accessory sector spot may be present or absent.

Detailed measurements of the width of the sector spot have been made on the following strains:

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Group A

Lagos, S. Nigeria
Maidahini, Diggi) Western Sokoto, N. Nigeria

Group B

Ambursa, Western Sokoto
Kano, N. Nigeria
Bobo Dioulasso, Upper Volta
Somalia
Pare, Tanganyika

The mean measurements and their standard deviations are given in Table I. Group A measurements range from 0 to 147 microns and Group B from 118 to 294 microns. Though the difference between the two means is great, and it is statistically very significant (more than 40 times its standard error), the wide variation about the mean in both cases causes some overlap between members of the two groups. A specimen in which the spot exceeds 143 microns can be assigned with reasonable assurance to Group B, the probability being about 0.977, and one over 158 microns with strong assurance (P=0.994). Similarly, one with a spot less than 117 microns can be reasonably, and one less than 101 microns confidently, attributed to Group A. For ordinary working purposes it will be sufficient to draw a dividing line at 132 microns which would lead to the correct identification of 95 per cent. of specimens.

TABLE I. ANALYSIS OF MEASUREMENTS OF WIDTHS OF WING SECTOR SPOTS IN GROUP A AND GROUP B TYPES OF A. GAMBIAE

Group	Number of mosquitos examined	Mean width of sector spot in microns	Standard deviation
A	300	82	31
B	500	182	32

Pupal Paddle Spines

Group A members show spines tapering towards the tip, curved and with their bases lying close together (the width between them being the same as or less than the width of the base of the spine). Fig. 2(b).

Group B members show stout, rather blunt, spines, not curved and widely separated (the distance between their bases being about twice the width of the spine base). Fig. 2(a)

In addition to the strains mentioned, which were studied in detail, the following strains have been examined and classified:

Group A		Group B
Kpain, Liberia		Diggi, Western Sokoto, N. Nigeria (a second colony recently acquired)
Muheza, Tanganyika		
Taveta, Kenya		
Tungan Buzu, Riga Fulani, Tarassa, Suru	Western Sokoto, Northern Nigeria	

This classification agrees with the classification derived from the crossing of the strains, as far as this had been done. Of interest is the fact that the first and second colonies from Diggi belong to the two different groups indicating that the two types of the species may be mixed in one and the same area. An examination of males in this area should then reveal sterility.

Examination of specimens of A. gambiae held in the British Museum of Natural History shows the Group B type to be the type form. The examination of specimens (mainly adults) from 63 localities showed 53 to belong to the type form (Group B) and 10 to Group A.

The Group A type possessing the variation described might, therefore, after more intensive study, be considered for rank as a new species.

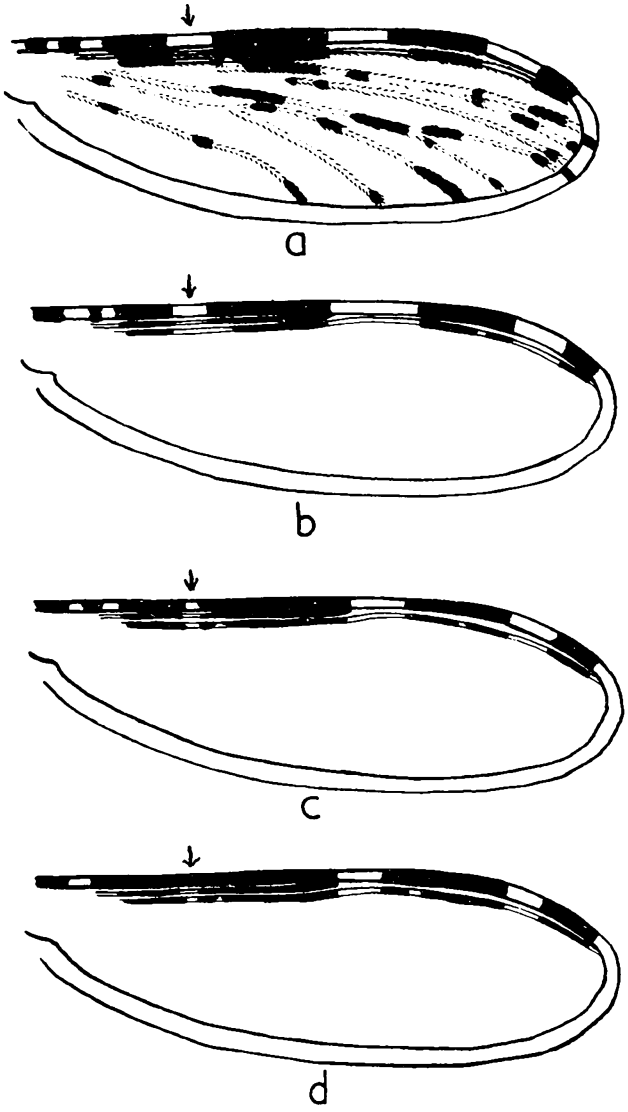

FIG. 1 Semi-diagrammatic wing-forms of *A.gambiae*.

a. Type-form (Group B).

b. Group B form without dark-scale interruption on pale area on vein 1 below sector spot.

c. Group A form with narrow sector spot.

d. Group B form without sector spot.

(The arrow points to the position of the sector spot).

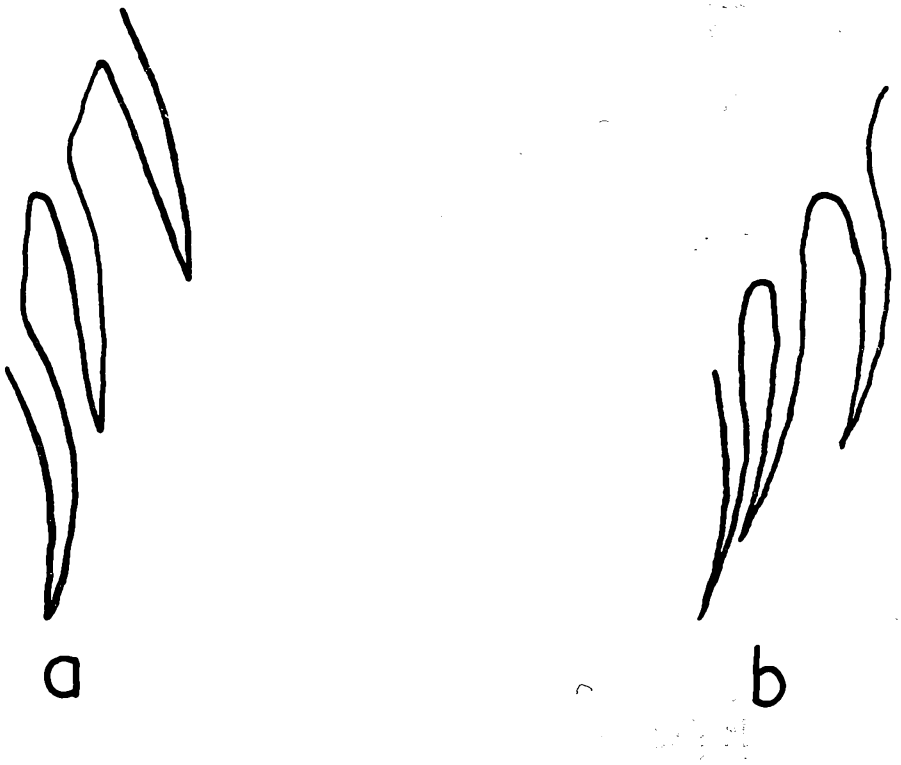

FIG. 2 Camera lucida drawings of the spines of the pupal paddle of *A.gambiae*

a. Group B - type - form

b. Group A form