WHO/Mal/354
3 August 1962

ORIGINAL: ENGLISH

THE PROBLEM OF BEHAVIOUR CHANGES IN ANOPHELES GAMBIAE GILES[1]

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It is no longer seriously doubted that residual insecticides, as currently used, are capable of exerting a selective effect on mosquito species populations and thereby altering their genetical constitution. It has been clearly shown, in a number of cases, that the presence of dieldrin on the inner walls of houses has led to the appearance, in greater frequency, of individuals resistant to dieldrin in populations of A. gambiae exposed to this insecticide (see Hamon & Garrett-Jones, 1962, for a recent review).

The behavioural counterpart of this effect would be the appearance of more numerous individuals with behavioural idiosyncracies causing them to avoid contact with the insecticide (Mattingly, 1962). While there is some evidence for a change of this kind in Anopheles albimanus in Panama, there is no conclusive evidence for such a change in A. gambiae anywhere in Africa. As to how far this lack of evidence is indicative of the actual situation it is difficult to be sure. Adequate pre-eradication data are at present available only from the Pare-Taveta area (Wilson, 1960).

There does not seem to have been any evidence, in Pare-Taveta, for marked behaviour changes of the kind claimed to have been observed in Panama. On the contrary, the evidence suggests that feeding and resting habits remained much the same after spraying as before. The conclusion of those responsible was that, in Pare-Taveta, A. gambiae is promiscuous in its feeding and resting habits, i.e. that such plasticity as it shows is phenotypic rather than genotypic, in the sense

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[1] Prepared as a working paper for the WHO Third African Malaria Conference (3-13 July 1962) at Yaoundé, Cameroun

that it depends on variation within the behaviour of the individual rather than on the existence of genetically distinct elements in the population.

In so far as there was no selective destruction of endophilic elements this conclusion seems inescapable. It should, however, be noted that behavioural plasticity may in itself be adaptive or contra-adaptive according to circumstances. At the same time its genetic basis may vary and it may be more strongly buffered against some types of selection than others. This is a complex subject which has been little studied (see review by Caspari, 1958).

In comparing the Pare-Taveta results with those reported from other areas attention must be paid both to the possibility of genetic heterogeneity in A. gambiae and to the nature of the selection to which the population was subjected. Thus, aside from intrinsic differences between A. gambiae and A. albimanus, there were differences in the insecticide employed (DDT in Panama, dieldrin in Pare-Taveta), differences in the times during which the two populations were subjected to insecticide (20 sprayings in Panama, 6 in Pare-Taveta) and, perhaps, differences in availability of alternative hosts and outside resting places.

Two other kinds of situation need to be distinguished. Both may produce an appearance of behaviour change and they may, indeed, involve changes which, to the malariologist, are the equivalent of behaviour changes, though of a different order from those discussed above. The first is the replacement of an endophilic by a predominantly exophilic species in consequence of the reduction or eradication of the former. An example is the replacement of A. funestus Giles by A. rivulorum Leeson in the Pare-Taveta area (Gillies & Smith, 1960). Such a species pair could well resemble one another so closely as to be, for practical purposes, inseparable, in which case only a genetical analysis would reveal the true nature of the situation.

The second situation involves the destruction of an endophilic element in the species population without replacement by another species or by any compensatory increase in the exophilic element in the population. This may have happened in Pemba where it is believed that fresh water A. gambiae may have been eliminated as a result of spraying with dieldrin (Iyengar, 1961). There does not yet seem to have been any replacement of this form by salt water gambiae, which are very abundant in Pemba and seem always to have predominated there. This could well be a very interesting test case in relation to the taxonomic status of the salt water form which is at present in doubt.

Similar arguments apply to the reverse process, i.e. reversion of an exophilic population to endophilic habits on cessation of spraying. This needs to be distinguished from reinfestation of a cleared area by incursion of endophilic elements from neighbouring untreated areas. The latter has been held to account for a resurgence of malaria in Swaziland where the *A. gambiae* population had become wholly or largely exophilic and transmission had been interrupted. Subsequently a considerable outbreak occurred in the vicinity of the Mozambique border, apparently as a result of incursions from unsprayed areas in the latter territory.

Another area in which *A. gambiae* is now highly exophilic is the Mazoe Valley area of Southern Rhodesia (Muirhead-Thomson, 1960, Hadjinicolaou, this symposium). Here there is evidence to the effect that *A. gambiae* was relatively abundant in houses prior to spraying. The data are, however, insufficient to reveal how this change has come about. In Mauritius the situation is different. Here it is claimed that *A. gambiae*, although they enter houses freely, do not remain there but fly out shortly after feeding. In view of the relative abundance of outside resting places, particularly in dry stone walls and the like, and the very close proximity of cattle sheds to houses this situation may well have prevailed before spraying. In this case a change may be suspected but cannot be proved.

If we wish to evaluate situations of this kind, it is essential that the extent to which *A. gambiae* rests in houses and the extent to which it feeds on man should be adequately assessed during the preparatory stages of future campaigns, before spraying begins. The Pare-Taveta scheme would make an excellent model, to be adapted to local conditions. In the present state of our ignorance every pre-eradication assessment would be a piece of research and it should be treated as such. The entomologist should be prepared at all times to question his own preconceived ideas and assumptions. Since his target was shifted from the early stages of the mosquito and their aquatic environment to the relatively unfamiliar adult stage and the human domestic milieu, the malaria entomologist has been learning as he went along. Progress has been remarkable, and nowhere more so than in Africa, but the gaps in our knowledge are still immense.

At the same time, operational research of this kind needs constantly to be supplemented by fundamental research divorced from, but continually informed by, work carried out in the field. In so far as it relates to the causes and nature of behavioural change such research needs to be carried out under three heads, Taxonomy, Genetics and Behaviour. These will be discussed to some extent separately but it will be found that they overlap considerably. They are in fact artificial categories erected by us for our own convenience and the true measure of progress lies in the extent to which they can be synthesized.

Taxonomy and Genetics

It has been realized for some time that *A. gambiae* exhibits considerable behavioural and morphological diversity and might, therefore, repay more detailed taxonomic study. Such studies as have been made have, however, been fragmentary and inconclusive. It is proposed to undertake a more comprehensive study in which all possible collaboration from workers in the field and from those in a position to carry out genetical studies will be welcome. Two problems at present call for investigation. They are those of the relationship between fresh and salt water *A. gambiae* and of the status of partially intersterile fresh water forms (Davidson et al., 1962).

The problem of salt water *A. gambiae*

Two main groups of salt water forms can be distinguished, a West African group and an East African. The former extends from Senegal at least to Nigeria and the evidence of egg and pecten studies suggests that it is relatively homogeneous. The latter has been less studied and might be more diverse. It includes forms extending from Somalia to Natal. A form occurring at the mouth of the Congo (Wanson, 1935) probably belongs to the West African group but very little is known about it. Forms from Mauritius and Aldabra probably belong to the East African group but a comparative study is needed. The West African group appears to be distinguishable by relatively constant morphological characters of the egg and larval pecten but no crosses have as yet been made between the West African and East African groups. Their status vis-à-vis one another has, therefore, yet to be determined.

The West African forms have been equated with a melanic form from Gambia described by Theobald (1903), under the name "var. melas", from a unique female now in the British Museum. The main distinguishing feature noted by Theobald is a reduction of the pale costal spots. A very similar reduction occurs in a fresh water form from Diggi in northern Nigeria (Davidson et al., 1962, Fig. 1d). Theobald also notes the presence of a dark interruption in the apical pale bend of the palp but this occurs frequently in fresh water forms from many inland parts of Africa. I bred out a highly melanic form, with this character, at Pong Tamale in the extreme north of Ghana. Since no biological data are attached to Theobald's type its identity remains very doubtful and it will need to be compared in detail with other material. It is unfortunate that this was not done before the name melas was validated (Ribbands, 1944) since it may now have to be attached to a fresh water form.

Crossing experiments between salt and fresh water forms have given good grounds for believing that there may be more than one species in the A. gambiae complex. They are, however, difficult to evaluate since in none of the published cases (Muirhead-Thomson, 1948, Burgess, 1961, Paterson, 1961, Kuhlow, 1961) is it known which of the two mating types of fresh water A. gambiae was employed. Very recent work has shown that crosses between the Tanganyika salt water form and fresh water forms of groups A and B both produce sterile F_1 males (Davidson, personal communication). The extent to which this limits gene flow cannot be assessed until back crosses have been made. The East African also requires to be crossed with the West African salt water form, as already noted.

With the advent of a method of artificial mating (McDaniel & Horsfall, 1957), which has been brought to a high pitch of perfection at the Ross Institute and shown to be perfectly applicable to A. gambiae, there seems to be no further obstacle to a genuinely objective assessment of the status and affinities of the four forms of A. gambiae at present known to exhibit marked genetic discontinuity. Suitable nomenclature will no doubt follow when it has been possible to establish the identity of the type specimens of forms, such as A. melas, A. merus Donitz and A. gracilis Donitz, known or believed to be synonymous with A. gambiae. The types of the two latter species are in East Berlin and, it appears, accessible. This is, however, only a beginning. The recognition and characterization of reproductively isolated or partially isolated forms implies no more than a clearing of the ground in order to make possible more significant and fundamental studies. If we wish to study behavioural changes in <u>A. gambiae</u>, resulting from the use of insecticides, we must contrive to associate these, at least statistically, with measurable physiological changes, such as irritability, on the one hand and with palpable morphological characters on the other (see Mattingly, in press). A number of characters of this kind have been shown to exhibit marked variation as between different populations, e.g. four-banded palp, reduction of tarsal bands, reduction of sector spot. These should be regarded as valuable markers and not merely discarded because they have proved unsuitable for certain crude analyses carried out in the past. We need to know how they vary with season, with breeding place, with hosts, with resting places. Where a population is found to be polymorphic with respect to easily scored morphological characters this situation should be exploited to the fullest possible extent.

In any study of this kind it will, of course, be essential to distinguish between genotypic and merely phenotypic variation (Evans, 1931, 1938) discussed the effect of larval environment on melanism in <u>A. gambiae</u> and drew the conclusion that "it would appear . . . that the melanic condition is largely (if not entirely) brought about by environmental factors". Subsequent work has shown that she seriously confused the generalized integumentary melanism association with certain types of breeding place with more restricted melanic characters, such as additional banding of the palps and reduction of pale wing or leg markings which are almost certainly under genetical control. It is, however, essential to bear in mind that the expression of a particular gene is likely to be controlled by the environment in which its effects are manifested. Absurdities such as the attempt to attach taxonomic significance to variations in wing length without studying the effects on this of temperature and larval diet are a case in point. The notorious case of the maxillary index is another. It is essential that the genetical study of any morphological character should be accompanied by a study of its phenotypic variation in a controlled environment. It is extraordinary how little experimental taxonomy of this kind has been carried out in such a well studied group as the mosquitos. An honourable exception is the work of Marks (1954) on the <u>Aëdes scutellaris</u> complex.

Nor has our approach to physiology been much more enlightened. All that has been shown about the "salt water" West African A. gambiae is that it has no preference for laying its eggs in salt water (Muirhead-Thomson, 1951), that it can be found in nature in water with a salinity of less than 0.1 per cent. sea water (Muirhead-Thomson, 1945) and that it thrives in the laboratory in distilled water (Fox, 1958). It has even been claimed (Fox, 1958) that it loses its tolerance for salt water after a few generations in the laboratory. It seems evident that we are here dealing with a character which is only of secondary importance to the mosquito, rendering available to it breeding places with other (unknown) characteristics of primary importance. One of these may be the nature of the bottom deposits which are quite different as between fresh and brackish breeding places (Barber & Olinger, 1931, Evans, 1931). The elucidation of this problem may well be necessary, not only from an immediate, practical point of view, but for a truly rational taxonomy. How else are we to make proper allowance for such phenomena as the production of typical "Anopheles melas" eggs by females taken far inland in Nigeria (Bruce-Chwatt & Service, 1957).

Mating types in fresh water A. gambiae

The discovery of two distinct mating types of fresh water A. gambiae (Davidson et al., 1962) is of the first importance. Every attempt should clearly be made to ascertain their geographical distribution and to try to discover what, if any, are the biological differences between them. It must, however, be emphasized that at the present time this can only be done by means of mating tests. The morphological characters suggested as possibly diagnostic by Coronel are now known to be unreliable. Although diagnostic of the strains listed by her, they have since been found to be unreliable for others. Thus the Kisumu strain, which is of Group A mating type, has Group B morphological characters. This has been confirmed by myself, Gillies, Paterson and Davidson. The latter also informs me that two other strains of Group A mating type, from Taveta and from Man, in Liberia, respectively, have Group B morphological characters.

It follows from this that the distribution of the two forms can only be judged from those strains which have been tested and possibly from those which show reduction of the sector spot sufficient to place them outside the known limits of Group B.

This is not to say that the character of the sector spot is useless. On the contrary, it may well be a valuable marker for a variety of purposes. It is, however, desirable that it should be expressed in the form of a ratio and not, as hitherto, in terms of absolute length since variation in over-all wing length in A. gambiae is too great for any character, expressed in this way, to be meaningful. It is also very much to be hoped that further details will be given of the variations within individual strains.

Behaviour

Very little is known regarding the genetics of behaviour. The general principles are summarized by Caspari (1958). Much of the available information has been gained from the study of genes affecting morphological characters and with pleiotropic effects on behaviour. The association between morphological and behavioural characters in mosquitos deserves more attention than it has received. The association between house haunting and coloration in Aëdes aegypti seems to be well established (Mattingly, 1957) and there is believed to be an association between pale coloration and phototropism in Culex pipiens var. molestus (Mattingly, 1951). These may be extreme cases but it seems possible that, for example, the pronounced melanism of some strains of A. gambiae may have behavioural associations.

Sufficient progress has recently been made with the analysis of behaviour changes associated with irritability to give hope that this phenomenon can be studied genetically (Coluzzi, in press). Our ultimate concern is, however, with the whole behaviour of the mosquito and this cannot be adequately comprehended from the study of its component elements. An attempt is therefore being made to devise an exploratory research programme concerned with the feasibility of applying audio-frequency recording to the study of mosquito behaviour. Such a technique would permit continuous recording under all conditions, including complete darkness. It would obviate a great deal of tedium and it would permit the complete withdrawal of the observer from the experimental environment and the entry of the mosquito into this environment "at will". It has previously been applied with some success on a small scale. It remains to be seen whether modern electronic equipment will permit it to be developed further. Present indications seem to be favourable. Not only would such a technique permit us to study behaviour as a whole rather than by trying to reconstruct it from piecemeal observation of limited aspects but it might well afford evidences of aspects of behaviour whose significance is at present unsuspected.

Summary

1. A number of alleged cases of behaviour change in A. gambiae have been recorded from Africa since the introduction of residual insecticides. These appear to be associated with the reduction of pre-existing endophilic elements in the population rather than with any change in the behaviour of such elements. The evidence, however, is for the most part very exiguous. It is highly desirable that more comprehensive, quantitative, pre-eradication data should be provided in relation to any future eradication projects.

2. Such behaviour changes as have been at all clearly demonstrated elsewhere seem to be associated with heightened irritability and, specifically, with the use of DDT. Although this type of change may have a relatively simple genetical and physiological basis, its effects may be dramatic and easily observed. A promising technique has now been evolved whereby this phenomenon can be studied in the laboratory. It is not, however, suitable for general use.

3. Other changes may well be less easy to demonstrate. They may also have a more complex genetical and ecological basis and may, therefore, take longer to become apparent.

4. The detection of such changes demands a more refined technique for the study of behaviour in the laboratory. It is suggested that audio-frequency recording might provide the basis for such a technique. Thorough preliminary study of this method would, however, be required and it would be foolish to squander resources on it until its possibilities have been further explored.

5. A. gambiae is highly polymorphic with respect both to morphology and to behaviour. Both forms of polymorphism require further study from the genetical, behavioural and ecological point of view. In many cases the simple scoring of easily observed characters would provide valuable data.

6. A part of this polymorphism can be definitely associated with elements in the population which are wholly or partly reproductively isolated by intrinsic genetical factors. Four such forms are at present known. They are now being studied genetically and taxonomically with a view to the provision of a sound nomenclature.

7. When this work is completed there will remain a large residuum of variation. The analysis of this variation may well enable us to restrict our target and improve our aim. It is also an essential prerequisite for the objective study of behaviour change.

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