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The Secretary of the Expert Committee on Malaria has the honour to communicate hereunder the following note:

ON THE POSSIBILITY OF SPECIFIC DIFFERENCES IN RESISTANCE-DEVELOPMENT-POTENTIAL IN ANOPHELES

by

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I. Dr. Mary HARRISON (1952) has summarized the evidence for arthropod resistance to chemical insecticides. The species in which this has occurred were, prior to the discovery of the insecticidal effects of the chlorinated hydrocarbons, all of agricultural and not of medical importance.

II. With the first general use of DDT in Italy from 1945, resistance to this compound soon manifested itself in Musca domestica and Culex pipiens autogenieus (C.molestus). In 1949 WHITNAL et al reported that the already arsenic-resistant blue-tick of South Africa (Booponus decoloratus) had developed resistance to gamma-BHC.

III. It is only with regard to DDT that the nature of resistance in arthropods appears to have been investigated. It is stated to consist of a capacity to metabolize DDT to DDE by the double-bond oxidation of the ethane radicle, the metabolite being inert. (References in HARRISON's paper, sup.)

IV. Resistance is a genetic phenomenon. HARRISON (loc.cit) showed that resistance to "knock-down" is due to a single recessive gene, but that resistance to an applied dose appears to be controlled by multiple factors. Certain types of resistance in insects appear to die out after a number of generations (six apud FAY, BAKER and GRAINGER, 1949). This raises the question whether such types of resistance may not be due to plasmagenes ("Dauer-Modifikationen") which in insects can be induced by heat or chemicals. DARLINGTON and MATHER (1949, p.185) showed the disappearances of "Dauer-Modifikationen" in seven generations in the bean Phaseolus vulgaris treated with chloral hydrate. Obviously the genetics of resistance is a barely explored subject. Among the Diptera, adequate chromosome maps on which such a study would need to be founded only exist for species in the genus Drosophila.

Regarding the genetics of the Culicidae I have only been able to find the following references:* STEVENS (1910): SUTTON (1942): GILCHRIST and HALDANE (1947): PAL (1947): FRIZZI (1947): PERRY (1951).

These papers contain contradictory statements regarding the presence or absence of sex chromosomes in the Family. An entomologist can only draw attention to our lack of knowledge, which could and should, in the light of the practical implications involved, be remedied by studies using stenogamic species like A.atroparvus and A.stephensi.

V. Subsequent to the appearance of resistance in C.molestus, this has become a serious problem in at least three species of Nearctic Aedes, all of which are exophagic in their habits. In these cases resistance appears to have been induced by anti-larval operations. KING (1950) stated that A.pseudo-punctipennis was apparently not acquiring resistance in the USA.

FAY, BAKER and GRAINGER (1949) discussed, without reaching a conclusion, the evidence for resistance in A.quadrimaculatus. I have later been informed that such has now definitely been recognized in the TVA area. Even as late as HARRISON's paper (loc.cit) she conjectures whether anophelines possess the necessary qualities for the development of resistance. The first well-documented evidence for resistance in an anopheline is the recent paper by LIVADAS and GEORGOPOULOS (1953) on A.sacharovi, which removes all doubt as to

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*RUSSELL, P.F. WHO/Mal/83, 27-2-53, gives references to 5 additional papers.

the development of resistance in at least this species. Resistance may therefore be expected with time to reveal itself in other species of the genus.

VI. The purpose for which the Secretary has asked me to prepare this discussion-note is the consideration of the extent to which species of varying habits are likely to be exposed to contact insecticides, on the assumption that genetically conferred resistance is the result of the ordinary process of selected genetic change. This is a strictly entomological matter, in the discussion of which most of the Expert Committee will be on more familiar grounds than those of biochemistry or genetics.

Broadly, the adult habits of anophelines may be divided into four groups: (see Annex 1)

(i) Endophily, i.e. remaining in man-made structures throughout the gonotrophic cycle, with the exception of the generally necessary out-door journey for oviposition.

(ii) Exophily, i.e. living out-of-doors. Man-made structures are not entered.

(iii) Endophagy, the blood-meal is sought in a man-made structure whether this be a house or a stable.

(iv) Exophagy, the blood-meal is sought out-of-doors.

A pair of each of the four thus contrasted attributes applies to every species. The divisions are not, of course, absolute, each is variable in its degree of fixation.

Category A. The supreme instance of endophily and endophagy (i-iii) is A.atroparvus during its season of gonotrophic discordance. SWELLENGREBEL and de BUCK (1938) have shown the extreme infection rates which this mode of life can engender in that part of the atroparvus population which is fixed in houses. Of species which do not undergo gonotrophic discordance but come into this category a good instance is A.fluviatilis in the hills of South India where, with suitable breeding water within easy flight range and the human blood supply confined to well separated villages, the house is continually haunted for at least the first 24 hours of the gonotrophic cycle.

During the remainder of the cycle, fluviatilis is exophilic up to oviposition.

I have shown (1945) by means of stained recaptures, the extremely restricted activity-range that ensues, the recapture rate being nearly 37 per cent.

Category B. According to the recent work of HOLSTEIN (1951) A.gambiae furnishes a startling instance of exophily combined with endophagy (ii-iii). I use the word startling, inasmuch as until recently it was believed that this species fell in category i-iii. HOLSTEIN (1952) has shown that the anthropophilic part of the population falls into category ii-iii. DOWLING (1950) has shown an apparently similar habit for the same species in Mauritius but, whether its local behaviour pattern is natural, or induced by the island-wide DDT campaign, appears uncertain. Over-hasty use of the chlorinated hydro-carbons by enthusiastic public health workers is giving glimpses of many supremely important matters of anopheline biology only after it has become too late to give them proper study. Such haste, in my opinion, is only justified by conditions of war or the carrying out of a major engineering project. Thus information which may be of the utmost scientific and practical value is lost. The supreme instance of this was the campaign against A.gambiae in Brazil. In that case no one would deny the paramount importance of haste, but opportunities for every kind of biological study were perforce neglected. (SENIOR-WHITE 1948, p.22.)

Another important finding by HOLSTEIN is that the zoophilic part of the West African gambiae population falls in category i-iii. There is apparently no bar to inter breeding of the anthropophilic and zoophilic populations, and it is difficult to understand how the two populations segregate themselves. However their maxillary indices show that they do.

A.maculatus is another vector which comes into this category, ii-iii.

Category C. Species which exhibit both exophily and exophagy (ii-iv) must usually have a breadth of contact with man so tenuous as to render them innocuous in respect of malaria transmission. The best instance to the contrary is A.bellator in Trinidad. This is a potent vector that feeds entirely out-of-doors (though open verandas are entered) and which is never found resting within man-made structures. As tropical man is, in great part, only in the house to feed and sleep, to be under these circumstances an effective vector involves day-time feeding, and these umbriphilous Kerteszziae seem unique among the anophelines in this respect.

Category D. I am indebted to Dr. DOWNS of the Rockefeller Foundation for what is perhaps the sole species in category i-iv: endophily with exophagy. This occurs in A.pseudo-punctipennis in the desert State of Sinaloa in North West Mexico, where there are now areas of irrigated rice-land. The general terrain is quite unsuited to out-door resting. A small proportion of the day resting house population remains in-doors, but the vast majority leaves to feed at dusk. In the absence of precipitin data, what they feed on, out-of-doors, is unknown. There is no malaria in the area.

The strictly limited categories defined above include exophilous species which are endophagic by night. Striking instances of such exceptions are minimus flavirostris, aquasalis and leucosphyrus (s.s.) including var. balabacensis.

Let us now consider how these various classes of habits may induce the development of resistance, assuming the man-made structure is being treated with some chemical which acts as a selective agent inducing genetic change. A.darlingi in coastal British Guiana is strictly a species falling into category A (category i-iii). The attack upon it in this region was made in overwhelming and prolonged strength, as GIGLIOLI feared the development of resistance if less intense measures were pursued. In these wind-swept coast lands darlingi finds no other feeding or resting place than a man-made structure. As a result, the species has apparently been locally eradicated, and now only the riverine corridors via which the coastal belt could be repopulated from the vast breeding grounds of the interior are treated. In less inhospitable terrain, or where communication with an untreated area is less restricted, the control of darlingi appears to rest on a much less sure foundation. The species is not an almost obligatory anthropophile, but in coastal British Guiana alternative sources of blood seem to be restricted to a few stabulated cattle, the shelters of which are apparently also treated. Thus engorged darlingi, save when ovipositing in the rice-lands adjacent to the settlements, finds no alternative to the treated shelter and has suffered the fate already described. Under these circumstances a lesser degree of exposure was unlikely, and, so long as the invasion corridors are held, the development of resistance on the coast is improbable. In other parts of its distribution area, where there are vast roaming herds of cattle, development of resistance may be feared.

The funestus group, funestus in Africa, fluviatilis in India, minimus in the foothills of South East Asia generally are other species in category A (i-iii) though I have shown (SENIOR-WHITE 1947) that even if the flimsy cattle sheds of the Orient were treated, a considerable proportion of the human, house-fed population would complete digestion in the open, unaffected by insecticides other than by such, possibly sub-lethal doses, as they would have picked up in the feeding act, that is immediately before or after feeding. With these species there have been some striking successes, in the case of Mauritius supposedly amounting to eradiction, with DDT. Success must have hinged on intensity of attack. Half measures, leaving house contents untreated, will induce to escapes and resistance.

The position of sacharovi in Greece, under the circumstances set forth for the Peloponesus by LIVADAS and GEORGOPOULOS (loc.cit) merits special consideration. The species is no more than a facultative anthropophile (HACKETT 1937). Open-sided cattle sheds in the Peloponesus, shown me by BALFOUR in 1934, contained, in the thatch of the roof, a large day-resting population of sacharovi. LIVADAS and GEORGOPOULOS make no reference to the treatment of animal shelters, but* Professor LIVADAS has elicited that "animal shelters were indeed treated during the campaign in Greece". Under these circumstances, it is difficult to explain how the resistance reported has developed, unless it be that in sacharovi there is some degree of exophily.

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* Upon request from WHO Malaria Section. Note of Editor.

It is noteworthy that there appear to be no studies on this aspect of anopheline behaviour in the Palearctic Region. It seems then to be accepted that the vector species all fall into category i-iii.

A.culicifacies, though only a facultative anthropophile, is largely both a house-rester and house-feeder, category A, i-iii. But there is a considerable population out-of-doors by day (SENIOR-WHITE 1947). Precipitin results, however, show that in the main these specimens have fed by night in man-made structures. They now will, to the extent (negligible I believe in India) to which cattle sheds are treated, have been exposed before or after feeding (a.c. or p.c.) to insecticides. No word of resistance in culicifacies has, so far as I know, been published. Whether such is to be apprehended will largely rest on studies such as are described below for aquasalis. Its habits in Ceylon where cattle remain all night in the open, require detailed study.

A.aquasalis is not found to any appreciable extent in any type of man-made structure by day, no more than 1% of a night's entrance remains indoors after daylight. It certainly does much house entrance by night, otherwise the results which the Trinidad Malaria Division has obtained with DDT could not have been obtained. But it is largely a crepuscular feeder, either out-of-doors or in open verandas as the AI of up to 36.4 found in out-door day catches in a treated area clearly indicates. Under these trophic conditions, it has no contact with DDT and unless anti-larval measures supplement house spraying, as in the Island of Tobago, there is no hope of eradication, in fact no hope of anything better than a reduced measure of malaria incidence. The species is not, therefore, easily locatable in any of my categories. Part of the population is in group ii-iii, a larger part in group ii-iv. A gene-selection or mutation tending to resistance in that part which is in group ii-iii will be swamped, unless dominant by the population in group ii-iv.

The average length of exposure of night entrants to DDT has not yet been assessed. It was hoped to do this by a DDT treated window-trapped hut, but of 2430 trap captures in such, 84.4% had not fed at all, though a human blood supply was continuously present. The six months experiment is now being repeated with ox-bait. However, there must have been a large measure of immediate irritation and ensuent departure, as in the untreated comparison hut, 63% of 5315 entrants had not fed, a difference which is highly significant. In the treated hut there was immediate mortality, as judged by morning "dead on floor" collections. This amounted to 45.2% of the total entrance, as compared with 0.5% in the comparison hut: specimens suffering immediate death have no chance of developing resistance and bearing resistant progeny. In the first daylight period after the window traps were removed, entrants died to the extent of 19.6% in the comparison against 52.2% in the DDT sprayed hut. By the morning of the second day, the figures were 47.6% and 79.6% respectively. After 24 hours, however, mortality in these largely unfed entrants was so high that it is doubted if the figures have any significance for insecticidal effect, they were however, 72.1% and 96.1%. But the figures show that there is a quite high percentage of survival up to 24 hours, within which, normally, hungry survivors would have re-fed, and thus if a sub-lethal dose is inducing resistance, the production of resistant progeny should be well under way.

The length of a.c. stay near a bait has been assessed in the open, when the population has no contact with insecticides. Unirritated rest before movement to attack showed periods varying from 10 seconds to 38 minutes, average 7' 25" (SENIOR-WHITE, in press). These results will be useful for comparison with behaviour in the presence of an insecticide the first effect of which is irritation and movement. P.c. resting, both in untreated and treated huts, has not yet been assessed.

For three years I have carried out monthly outdoor catches in a DDT sprayed and a comparison area. The effect of the insecticide is shown by the male percentage of the treated area catch being very much higher, over 70%, than in an untreated area, 40-45%. But the three years' figures show a disconcerting effect on female numbers, the density per man hour of catching being 1.8 in 1950, 2.4 in 1951 and 3.9 in 1952, and this in the face of breeding within half-a-mile of the treated area having been vigorously controlled since 1951-52. These figures will require many years of regular collections to assess the phenomenon, but they are definitely suggestive of incipient resistance. The population of this particular treated area, is, save at one point of contact, somewhat isolated from the general dense coastal population.

There remains for consideration species in category C, group ii-iv. The only vector species strictly conforming to this category are the Kerteszìae of Trinidad, and no attempt has been made to use contact insecticides in their control. But A.leucosphyrus (s.s.) of Borneo appears also to come into this class. So far as its feeding habits have been unravelled, it enters human dwellings only in the early hours of the morning, and there "dive-bombs" its victims, departing forthwith to the out-of-doors. McARTHUR (1949) apparently did not consider it all susceptible to contact insecticides, and worked out a method of larval control based on his discovery of its very specialized breeding places. Before accepting these results, however, WHO has in progress an independent investigation into its bionimics; meanwhile one must await ZULUETA's report.

Probably the vast majority of the non-vectorial species of anopheles come into this category.

It is obvious from these notes that the whole subject of mosquito, and in particular, of anopheline resistance calls for further investigation, commencing at the genetic level. In the first place one requires chromosome maps of various species in the natural condition, and for this only stenogamic species can be used. Having prepared these, the next step is to induce resistance in a cage colony, by both larval and imaginal application of insecticides, and to study the chromosome maps of the resistant forms. During this work it should be possible to study the inheritance of resistance, whether this is a mutation, or a multiple condition; whether plasmagenes are involved; whether resistance, once developed, becomes a permanent feature of the genome, and to what extent constant intermingling of the normal and the resistant forms, which must occur in all species except those strictly in class

A (i-iii) and D (i-iv) will revert the phenotype to the natural susceptible condition.

On the entomological side far more detailed studies than have so far been made are required regarding specific nocturnal behaviour in both untreated and treated structures. The length of non-fatal contact required to induce genome changes is unknown. Similar long-term catch analyses to those already in progress with aquasalis for any other species in which it may be suspected that resistance is developing should be instituted, exophilic gambiae being particularly well worth study.

Meanwhile, it is hoped that the classification of anopheline habits proposed in this note will be found useful. This note could not have been prepared without the advice of Mr. N.W. SIMMONDS, Banana-geneticist at ICTA, to whom my thanks are hereby tendered.

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ANNEX I

	iii. Endophagic	iv. Exophagic
i. Endophilic	i-iii = Category A A. atroparvus (during its hibernation in the Netherlands) A. funestus Africa A. fluviatilis (at least during the first 24 hours of its gonotrophic cycle in South India) A. minimus (South East Asia zoophilic A. gambiae West Africa) (acc. to Holstein) A. darlingi British Guiana A. culicifacies	i-iv = Category D A. pseudo-punctipennis N.W. Mexico (acc. to Downs)
ii. Exophilic	ii-iii = Category B anthropophilic A. gambiae West Africa A. maculatus A. aquasalis A. minimus flavirostris A. leucosphyrus A. leucosphyrus balabacensis	ii-iv = Category C A. ballator Trinidad A. leucosphyrus (ss) Borneo (acc. to McArthur)