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Problems Facing Anopheline Vector Control

Vector Ecology and Behavior Before, During, and After Application of Control Measures

J. Hamon,[1] J. Mouchet,[2] J. Brengues,[1] and G. Chauvet[3]
Office de la Recherche Scientifique et Technique, Outre-Mer (O.R.S.T.O.M.), Paris, France

ABSTRACT

The responses of anopheline vector populations to malaria-control operations were studied. Most of the emphasis was placed on their response to DDT, dieldrin, and lindane residual house spraying and on their implication for malaria control and eradication programs. The investigation deals mainly with *Anopheles labranchiae* Falleroni, *A. sergentii* (Theobald), *A. stephensi* Liston, *A. funestus* Giles, *A. gambiae* Giles, s.l., *A. pseudopunctipennis* Theobald, *A. albimanus* Wiedemann, *A. darlingi* Root, *A. nuneztovari* Gabaldon, *A. aquasalis* Curry, *A. culicifacies* Giles, *A. m. minimus* Theobald, *A. m. flavirostris* (Ludlow), *A. maculatus* Theobald, *A. sundaicus* (Rodenwaldt), *A. b. balabacensis* Baisas, *A. leucosphyrus* Dönitz, and the *A. punctulatus* Dönitz group.

The intrinsic vector ecology and behavior are of importance in explaining the response of any vector population to control measures, but the most important clues to this response are the relationships between ecology, behavior, and the environment. Exophilic and exophagic tendencies have no protective value in an insecticide-house-sprayed area if there are no available outside shelters or convenient alternative hosts spending the night outside treated premises. In some instances major differences in response to insecticide treatment within a vector species in various areas of its distribution cannot be explained by changes in the environment. This may be attributed to less genetic plasticity of the species in some areas, restricting its adaptability.

It is stated that even where extensive prespraying data on vector ecology and behavior were available, the predictions about the vector population response to treatment have often been misleading, indicating that the investigations may have been incomplete or biased by sampling difficulties.

At times vector control has been surprisingly easy and has led to malaria eradication without difficulty. On the other hand, there have been many continuous control operations which have been unsuccessful in tropical areas. Quite often, a weakness in control operations or a change in the environment allows the vector to resume malaria transmission at the same or higher levels.

Since the end of World War II, malaria control and eradication programs have relied mostly on the destruction of adult vectors by indoor deposits of residual insecticides, sometimes supplemented by breeding place oiling, space fogging, or mass distribution of drugs. Only in rare instances has malaria eradication been attempted without any residual spraying operation.

Apart from administrative and logistic difficulties, obstacles to malaria control and eradication have arisen mostly either from unexpected vector ecology and behavior or from insecticide and/or drug resistance. On the reverse, the fair success of some insecticide spraying operations was unexpected, preliminary investigations having concluded that the local vector could not be controlled easily by residual insecticides (Pampana 1951; Anonymous/WHO 1967, 1968).

As the problem of insecticide resistance is discussed in another document presented before this conference (Schoof 1970), and that of drug resistance is outside the scope of this meeting, we shall examine the situation of malaria control and eradication in some selected areas of the main biogeographical regions and then discuss the situation for each facet of vector ecology and behavior.

We are afraid our report will be biased in favor of the Ethiopian region, because we have more detailed information on this part of the world than the Americas and Asia. Furthermore, a large number of very valuable contributions written by WHO Staff are either available only as WHO/Mal and WHO/Vector Control mimeographed documents which usually cannot be quoted, or which remain in the files of WHO Regional Offices and Headquarters, thus preventing the presentation of an up-to-date picture of the situation in many areas.

MALARIA CONTROL AND ERADICATION PROBLEMS IN SELECTED AREAS

Mediterranean Area and Near East

Anopheles labranchiae Falleroni.—In 1946 a large campaign began on Sardinia Island, Italy, to eradicate the main malaria vector, *A. labranchiae.* It was based both on DDT residual spraying of all houses, animal shelters, and buildings in which adult anophelines could overwinter, and on weekly treatment of all potential larval breeding sites with DDT and Triton in fuel oil and later with paris green. In late 1949 malaria transmission had entirely ceased, but *A. labranchiae* still occurred in some sections of the island (Logan 1950). After the general eradication campaign was concluded, larviciding and house spraying were continued for the following 2 years where the vector was still present in very restricted areas. Upon the discontinuance of the treatments *A. labran-*

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[1] O.R.S.T.O.M. Team, B.P. 171, Dobo Dioulasso, Upper Volta.
[2] O.R.S.T.O.M. Central Laboratories, Bondy, France.
[3] O.R.S.T.O.M. Center, Tananarive, Madagascar.

chiae increased in numbers very slowly. Most of its usual breeding sites were invaded by *A. algeriensis* Theobald and in 1 area by *A. hispaniola* Theobald. It was not clearly established if the repopulation of the island by *A. labranchiae* was delayed mostly by the competition of other species with a shorter life cycle or longer flight range or by its greater susceptibility to the intense DDT contamination of the larval breeding and adult resting places. Despite great efforts and large amounts of manpower and money, *A. labranchiae* could not be eradicated. However, residual malaria was at such a low level that the slow reappearance of the major vector was apparently not followed by malaria transmission (Trapido and Aitken 1953, Aitken et al. 1954, Loddo et al. 1956).

A malaria-control program was undertaken on Sicily Island, Italy, from 1946 to 1953 based on DDT house spraying. Some areas of the island were regularly treated until 1955. At the end of the campaign there was only a slight reduction in the number of larval breeding sites of the main vector, *A. labranchiae*. During the years of treatment, adults were commonly found resting and even hibernating outside in rock holes, caves, small slits, and other natural shelters. Malaria transmission was considerably reduced but not entirely interrupted (D'Alessandro et al. 1954, Mariani and Cefalu 1954). In some areas the interruption of spraying was followed by small malaria outbursts, and DDT treatment had to be resumed for several years. About 3 years after the last DDT application, an investigation in 1960 showed that *A. labranchiae* had reappeared in large numbers inside houses and animal shelters. Although always common out-of-doors, especially in caves, its trophic preferences were about the same as before spraying had started. A capture, marking, and release experiment showed that the distribution of house- and cave-collected adults was at random, not indicating the selection of an endophilic or exophilic biological race (Cefalu and Giulotta 1959, Cefalu et al. 1961).

In rice-growing areas of Morocco, DDT residual house spraying has controlled transmission by *A. labranchiae* with good results (Houel 1954). However, field and laboratory investigations have shown that females of this species may enter sprayed premises, bite, and leave unharmed in large numbers because of their high level of irritability to DDT deposits. The level of irritability appeared to be the same in treated and untreated areas. Similarly, selective action of the treatment was not apparent in laboratory tests (Sacca and Guy 1960). These observations support those made in Sicily; DDT treatment has only reduced the man–mosquito contact and induced a greater exophily. The low killing action could not induce any selection, and the pretreatment situation reappeared soon after the interruption of the spraying.

Anopheles sergentii (Theobald).—In the Jordan Valley the elimination of the major vectors, *A. superpictus* Grassi and *A. sacharovi* Favre, through DDT house spraying supplied evidence of the vector importance of *A. sergentii*. This anopheline, biting as well inside as outside houses and tents, was mostly exophilic there. It preferred to rest in caves, rock cracks, and similar natural shelters even rather than in unsprayed tents and houses.

The DDT treatment of dwellings could interrupt malaria transmission in areas where natural shelters were scarce or absent, whereas it was inefficient in calcareous hilly areas with numerous caves and rock cracks. In these areas the only effective control was to supplement house spraying with the oiling of larval breeding sites (Farid 1954, 1956).

Anopheles stephensi Liston.—The first malaria-control operations in Iraq, Iran, and Saudi Arabia against *A. stephensi* were based upon DDT residual house spraying. Although they were fairly successful in interrupting transmission before the appearance of DDT-resistant populations, this vector was never eradicated. However, when DDT was replaced by dieldrin, *A. stephensi* was apparently eradicated from large areas of southern Iraq and Iran and from some oases of Saudi Arabia but maintained itself on the slopes of the Zagros Mountains in Iran (Daggy 1959, Al-Amin 1961, Hendow 1963, Hamon and Garrett-Jones 1963). Basic behavior of *A. stephensi* was probably the same over all the treated area. Insecticide pressure was stronger with dieldrin than with DDT. The more favorable environments on the Zagros slopes and the Iranian hinterlands favored survival by exophily, while the hot tropical climate of Saudi Arabian oases and the coastal plains of Iran and Iraq did not permit this type of behavior.

Anopheles gambiae Giles.—This species entered Egypt from the Sudan before March 1942 and caused dramatic outbreaks of malaria between the Sudan border and Assiut in 1942 and 1943. One eradication campaign, based almost entirely on paris green larviciding, started in February 1944 and was stopped in August 1945, 6 months after the last specimen of *A. gambiae* was caught. Further checks after the discontinuance of control operations found no *A. gambiae*. A similar successful campaign was carried out at the same time in the Wadi Halfa area of northern Sudan. The other anopheline species occurring in the treated area were not eradicated despite the intensity of larviciding (Shousa 1948, Lewis 1949).

It may be assumed from the presently known distribution of the *A. gambiae* complex that the species eradicated from Egypt and the northern Sudan was *A. gambiae* species B. Such a specific eradication, in a very short period, infers that *A. gambiae* species B had very restricted breeding places and was ecologically less plastic than it usually is in tropical Africa.

Ethiopian Region

Anopheles funestus Giles.—This is one of the major malaria vectors of tropical Africa, and as such has been subjected to various insecticide pressures in a great variety of ecological environments.

It was eradicated or disappeared completely after 1 or 2 rounds of DDT, benzene hexachloride (BHC) or dieldrin house spraying in vast areas of East Africa, the highlands of Central Africa, and the forested zone of West Africa. The eradication was especially striking in Mauritius Island (Dowling 1951, 1953). Similar observations were made in Rwanda highlands (Jadin 1951, 1952), Madagascar highlands (Lacan 1953, Bernard 1954), southern Nigeria (Bruce-Chwatt et al. 1955), Swaziland (Mastbaum 1957a, b), Transvaal (Brink 1958), the Pare-Taveta Scheme of Kenya and Tanzania (Anonymous/EAHC 1960), Liberia (Hamon et al. 1963), and the Uganda highlands (De Zulueta et al. 1964). In the Pare-Taveta Scheme the *A. funestus* disappearance was followed by a sharp increase in numbers of other members of the *A. funestus* complex, mainly *A. rivulorum* Leeson (Gillies and Smith 1960). *A. rivulorum* is not normally found in houses, and no factor other than competition inside a narrow ecological niche in the larval breeding site was suspected of being responsible for this change (Gillies 1960). When spraying was discontinued the reappearance of *A. funestus* in previously treated areas of Kenya and Tanzania was delayed for several years. Then its reappearance was very rapid, perhaps following the introduction of females from untreated areas. The new *A. funestus* population apparently had the same ecological characteristics as the eradicated one (Smith 1966).

Several malaria control and/or eradication schemes based on DDT or dieldrin house spraying were organized between 1953 and 1960 in the savannas of West Africa from northern Cameroons to Senegal. Despite an unusually good insecticide coverage obtained during several consecutive years, *A. funestus* was never eradicated in northern Cameroon, northern Nigeria, and Upper Volta. It was even found in several instances inside the treated areas in large numbers infected with sporozoites. The main observations have been made in northwestern Nigeria (Bruce-Chwatt and Haworth 1956, Bruce-Chwatt et al. 1958), Upper Volta (Choumara et al. 1959, Escudie et al. 1962), and in northern Cameroons (Cavalie and Mouchet 1961); whereas some conflicting data are available from Senegal (Escudie and Abonnenc 1958, Lacan[4]). More recently a large-scale application of dichlorvos dispensers in Katsina Province, northern Nigeria, was no more successful than the previous DDT and dieldrin treatments for eradicating *A. funestus* (Foll et al. 1965, Foll and Pant 1966).

A detailed taxonomic study of this species in Nigeria did not demonstrate distinct morphological races in the southern and northern parts of the country.

Basic ecological and behavioral data on *A. funestus* populations from unsprayed areas of East Africa (Gillies 1954a, b; Draper and Smith 1957; Smith and Draper 1959a, b; Chauvet et al. 1964) and West African savannas (Hamon et al. 1956, 1964; Choumara et al. 1959; Escudie et al. 1962; Hanney 1960; Service 1963, 1965; Bruce-Chwatt et al. 1960) appear to be very homogeneous. The behavioral response of *A. funestus* females to DDT house spraying as observed in experimental trap huts is basically the same in East Africa (Wilkinson 1951; Davidson 1951, 1953a, b), in highlands of Central Africa (Cullen and De Zulueta 1964), and in West African savannas (Kuhlow 1959, Service 1964, Coz et al. 1965). Furthermore, the general environmental conditions of the savannas of West Africa and of those of Taveta-Pare, Kenya, and Tanzania are similar with their permanent *A. funestus* larval breeding sites, the abundance of either game or cattle, or both, and the apparent scarcity of natural resting places for exophilic adults, etc.

So there is probably no simple explanation of the dramatic differences in response to insecticide house spraying observed in *A. funestus* populations in different parts of tropical Africa. In some areas, eradication may have been caused by the use of BHC or dieldrin which do not have the strong irritant properties of DDT. In other areas the eradication may be attributed to the cold nights outside huts or lack of convenient alternative hosts for females living in exophily. Lack of genetic plasticity of the species in some parts parts of its range could also be the main reason for disappearance from insecticide-treated areas.

Anopheles gambiae Giles.—The very well-known name *A. gambiae* includes 5 species which cannot accurately be identified at all stages of their development and do not have the same importance as malaria vectors. Two of these species, *A. melas* Theobald and *A. merus* Dönitz, are restricted to coastal brackish water habitats, while the 3 others, known only as species A, B, and C, occur mostly in inland fresh and sometimes slightly brackish waters. In many areas 2 or even 3 species belonging to the *A. gambiae* complex coexist side by side, thus complicating the interpretation of observations (Davidson 1964, Paterson 1964, Chauvet et al. 1968).

In the experimental malaria eradication schemes of southern Cameroons and Liberia, *A. gambiae* species A, which was apparently the only species of the complex present, almost completely disappeared after the 1st rounds of DDT spraying (Livadas et al. 1958, Chastang 1959, Hamon et al. 1963, Guttosa[5]). This phenomenon is very interesting, because initial investigations had shown that this anopheline was highly exophilic and would be difficult to control with residual insecticides in southern Cameroons (Rageau et al. 1953, Mouchet and Gariou 1957). The 1st evaluation of residual DDT in trap huts in Africa had been carried out in the same biogeographical

[4] A. Lacan. 1962. Le secteur antipaludique de Thiès dans la République du Sénégal. AFRO/MAL/9/40, 13 p. (Unpublished.)

[5] C. Guttosa. 1962. Le project antipaludique du Rpain (Liberia). AFRO/MAL/9/43, 8 pp. (Unpublished.)

area on this species with very discouraging results (Muirhead-Thomson 1947).

In other parts of tropical Africa the situation is very complex, but on the whole insecticide house spraying has neither eradicated *A. gambiae* s.l. nor entirely interrupted *gambiae*-transmitted malaria. The only exception could be 2 areas in the highlands of Uganda, where malathion and DDT have been house sprayed every 4 months (Shidrawi,[6] De Zulueta et al. 1961).

In Southern Rhodesia BHC house spraying was said to have selected a zoophilic and exophilic population of *A. gambiae* s.l. which was no longer transmitting malaria (Hadjinicolaou[7]). More recent observations point to the mass destruction of the anthropophilic species of the complex, species A and/or B, whereas the zoophilic and exophilic species C have been left undisturbed (Ramsdale[8]). However the situation cannot be settled so easily, because malaria transmission still occurs at a low level (Wolfe 1964). A similar situation could exist in the countries farther south which have also been treated with BHC and where *A. gambiae* s.l. is said to be mostly zoophilic and exophilic (Brink 1958, Mastbaum 1957a).

In Mauritius, Reunion Islands, Madagascar, Pemba and Zanzibar Islands, and the Taveta-Pare Scheme of Kenya and Tanzania, DDT or dieldrin house spraying have considerably reduced malaria transmission without seriously disturbing *A. gambiae* s.l. Any weakness in the organization of the scheme was sufficient for increasing transmission (Garrett-Jones[9]). Since inhabitants of these countries spend most of the night indoors there is no reason for suspecting that outdoor malaria transmission has occurred to any great extent. When and where spraying was discontinued, malaria transmission resumed more or less quickly, according to the level of residual malaria and to the length of efficacy of the last spraying (see references concerning *A. funestus* in East Africa; also Hamon and Dufour 1954; Smith and Draper 1959a, b; Smith 1962 a, b; Chauvet et al. 1964). During the period of treatment the level of malaria transmission was so low that it could not be detected by entomological means, and it was even said on Mauritius that *A. gambiae* s.l. was not an important vector (Halcrow 1956). This assertion was disproved some years later when severe malaria outbursts occurred (Verdrager 1964). It has been published that house spraying has eradicated the fresh-water species of the *A. gambiae* complex on Pemba Island, but there is a lack of prespray data (Iyengar 1962). In Madagascar species A is definitely more anthropophilic than species B, and the former has a lower reproductive rate than the latter (Chauvet et al. 1968). It is probable that the years of house spraying have favored species B, which now has a very widespread distribution on the island, whereas species A has a restricted patchy distribution. Here also prespray data are missing.

Because of the rapid development of dieldrin resistance in the savannas of West Africa, DDT has been the main insecticide used. Malaria transmission inside sprayed areas has always been more important than in East Africa (Hamon et al. 1963). The reasons for this are not very clear, as the behavior of *A. gambiae* species A and/or B in DDT-treated huts was on the whole the same in East Africa (Muirhead-Thomson 1949, 1960; Hadaway 1950, Wilkinson 1951, Davidson 1953b), in Central Africa (Cullen and De Zulueta 1964), and in the savannas of West Africa (Kuhlow 1962, Service 1964, Coz et al. 1965). One difference might be attributed to the human host. Sleeping out-of-doors for part of the night is apparently much commoner in the savannas of West Africa than in East Africa (Dodge[10]). The presence in West Africa of numerous nomadic Fulani, who sometimes use very primitive mat shelters, may have also faciliated contact between *A. gambiae* s.l. and human hosts in sprayed areas of the West African savannas (Prothero 1961, Dodge[10]).

The difference in response to DDT house spraying between *A. gambiae* species A populations in southern Cameroons and Liberia and apparently similar anopheline populations in Upper Volta and northern Nigeria may be due to the total absence of alternative hosts spending nights outdoors in the forested area and to the abundance of cattle and/or game in the northern savannas. In the 1st area humans sleeping inside were the only available hosts, thus inducing a close contact between the vector and the sprayed premises, whereas in the 2nd area the contact between the treated surfaces and *A. gambiae* s.l. was facultative and did not decrease the average longevity of the species.

Neotropical Region

Anopheles pseudopunctipennis Theobald. — This species has a very wide distribution in the Americas, where it is an important vector in most areas of its range. It has been especially studied in Mexico, where the 1st investigations gave promise of controlling malaria transmitted by this vector (Gahan et al. 1949, Bordas et al. 1951). Although malaria transmission was interrupted in more than 75% of the originally malarious areas, it persisted after several years of complete DDT or dieldrin coverage. *A. pseudopunctipennis* remained the major vector in the DDT-house-sprayed areas of the State of Oaxaca on the Pacific slopes of Mexico. Investigations have shown that females of this species enter sprayed houses, bite, and soon escape unharmed by the DDT deposits. This behavior is especially noticeable in

[6] G. R. Shidrawi. Personal communication.

[7] J. Hadjinicolaou. 1962. Reaction des vecteurs aux insecticides, evitement de l'insecticide. AFRO/MAL/9/20, 7 p. (Unpublished.)

[8] C. D. Ramsdale. 1965. The effect of residual hut spraying with HCH on mixed populations of the *Anopheles gambiae* complex in Rhodesia. WHO/Mal/508.65, 24 p. (Unpublished.)

[9] C. Garrett-Jones. Personal communication of observations made in 1963 in Pemba and Zanzibar Islands.

[10] J. S. Dodge. 1962. La campagne antipaludique du Nigeria septentrional et ses rapports avec le programme de prééradication. AFRO/MAL/9/9, 18 pp. (Unpublished.)

small hamlets with houses built of less durable materials, but has been recorded also in the most modern types of buildings (Roman y Carrillo et al. 1965, De Zulueta and Garrett-Jones 1965). A special trap hut experiment attempted to duplicate conditions present in 1950 before there was general spraying of the country. It was concluded there was an ethological change in *A. pseudopunctipennis* after prolonged use of DDT (Martinez-Palacios and De Zulueta 1964). However, the differences in behavior inside a DDT-treated hut recorded between the 1950 and the 1963 observations were of a small magnitude and may be attributed to unavoidable differences in the hut plastering and spraying and in the general handling of the experiment. Our interpretation would be that the behavior of *A. pseudopunctipennis* entering a DDT-sprayed house has not changed at all despite about 10 years of DDT pressure. Furthermore, it has been proved by crossing experiments between 5 *A. pseudopunctipennis* populations from different parts of Mexico that they all were a single species (Martinez-Palacios and Davidson 1967). The main difference between this species from the Oaxaca problem area and other parts of Mexico could not be behavior but in probability of survival until it attains an epidemiologically dangerous age. This difference is not intrinsic to the anopheline population but extrinsic, caused by the environment.

Anopheles albimanus Wiedemann.—This species was an important vector in Central America and in some of the Caribbean Islands. Although well controlled by residual insecticides in most countries it is still the major vector in some problem areas. It has been suspected of developing behavioristic resistance following DDT pressure in 1 area of Panama.

In the Chagres River area of Panama, DDT was sprayed inside houses every 4 months from 1944 to 1952. A comparison of 1945, 1946, and 1952 observations showed increased density and especially increased survival rate of the females feeding inside sprayed houses in 1952 (Trapido 1952). Laboratory and field investigations were carried out on several *A. albimanus* populations of Panama differing in their previous exposures to DDT and an old established colony of this species. Brown (1958), using the WHO irritability test method, concluded the Chagres River strain had a slightly significant increase in irritability as compared with a strain of *A. albimanus* from an untreated area. Using other techniques, Duret (1961, 1964) concluded that the Chagres River *A. albimanus* was no more irritable to DDT than other field-collected populations from unsprayed or very recently sprayed areas, but that all of them were much more irritable to DDT than the old laboratory colony.

In El Salvador as in Oaxaca, Mexico, *A. albimanus* is transmitting malaria in DDT-sprayed problem areas. Transmission occurs in El Salvador with both DDT-susceptible and -resistant vector populations. Detailed investigations concluded that the failure to interrupt transmission was due to the readiness of *A. albimanus* to bite humans outside in the early night hours and to the ease with which this species can enter and leave the typical houses of the problem areas without picking up a lethal dose of DDT (Rachou et al. 1965, De Zulueta and Garrett-Jones 1965). These characteristics of *A. albimanus* were known before any use of DDT and have been described in detail by Muirhead-Thomson and Mercier (1952a, b), who concluded their study by stressing that, according to the method of population sampling used, this species, which is mostly exophilic and exophagic and largely zoophilic, may appear to be endophilic and endophagic.

The species is prone to very sharp increases in population size when ecological conditions become favorable and as such constitute a threat for any malaria eradication program as long as carriers are present. This fact was recently demonstrated in Haiti (Mason and Cavalie 1965).

Anopheles darlingi Root.—This species is a major malaria vector of northeastern South America. In the northern part of its range *A. darlingi* was practically eradicated after the 1st house spraying as observed in Venezuela (Gabaldon 1949, Gabaldon and Berti 1954) and in the Guianas (Charles 1953, Giglioli 1954, Floch 1954). A similar situation has been reported also in the southern parts of its range. In the Amazon Basin and in surrounding heavily forested areas this species seems less anthropophilic and more exophilic than on the periphery of its distribution and cannot be suppressed by house spraying (Deane et al. 1948, De Bustamente 1959). After 4 years of DDT spraying the species was not eradicated from the Magdalena Valley area of Colombia. This area could serve as a secondary center of dispersion of the species (Vincke and Pant[11]). Sylvatic populations of *A. darlingi* have been observed in southern Venezuela and the Macarena forested area of Colombia where control was not possible (Gabaldon 1965, Renjifo and De Zulueta 1952).

From the densely forested areas *A. darlingi* may invade far-away territories at apparently a quinquennial periodicity. In Brazil a 300-km dispersion along valleys was reported in 1 year (Rachou et al. 1954b). A similar dispersion for a shorter range has been recorded also in Guiana (Giglioli and Charles 1954).

The behavioral difference of *A. darlingi* in the center and the periphery of its distribution may explain its different responses to insecticide treatments, but the reasons for these differences have not been investigated. This species is the most exophilic and the least anthropophic only in dense rain forests.

Anopheles nuneztovari Gabaldon.—This anopheline is apparently an important malaria vector only along the Venezuela-Colombia border, where it was first found infected 20 years ago (Rey and Renjifo

[11] I. H. Vincke and C. P. Pant. Personal communication on 1962 investigation on the Venezuela-Colombia border.

1950). Fifteen years of house spraying (performed every 3 months during recent years) and supplemented by peridomestic insecticide fogging and drug distribution have not succeeded in interrupting malaria transmission (Gabaldon et al. 1963, 1965; Gabaldon 1965; Vincke and Pant[11]). The importance of *A. nuneztovari* depends largely on the amount and density of vegetation in the immediate vicinity of houses. Residual insecticides are inefficient where houses are surrounded by dense bush. Vector density is reduced in areas where vegetation has been cleared around houses (Gabaldon et al. 1963). These views have been largely supported by observations on the parous ratio of *A. nuneztovari* populations carried out in 1962 by Vincke and Pant.[11] They found this species was largely exophagic when outside baits were available, and that the proportion of parous females was the highest in densely forested areas (0.64–0.72), where it was the lowest in partly deforested areas (0.31–0.53). Both investigators stressed that other anopheline species of the area with the same ecology and behavior as *A. nuneztovari* should be fully investigated before attributing residual malaria transmission to this species alone.

Anopheles aquasalis Curry.—This species is chiefly exophilic and zoophilic and has acted in the past as a malaria vector because of its high density (Senior-White 1951). As a consequence this anopheline is hardly affected by residual house spraying, which only decreases the already very weak link between inhabited houses and *A. aquasalis* females (Giglioli 1949). When cattle are scarce, female *A. aquasalis* bite humans to a greater extent and are largely protected from insecticide application by their exophilic habits. Under such conditions this species has played a role in residual malaria transmission in sprayed areas of eastern Venezuela (Gabaldon 1965) and the Guianas (Floch 1956, Giglioli 1959). In one of the Guianas the replacement of cattle by tractors has induced *A. aquasalis* mainly to bite humans. In the absence of insecticide spraying a malaria outburst occurred in a restricted area (Giglioli 1963).

In all these instances the main factor appears to be an environmental change rather than a basic change in the anopheline ecology.

Kerteszia anophelines.—Anophelines of the subgenus *Kerteszia,* especially *A. (K.) bellator* Dyar & Knab and *A. (K.) cruzii* Dyar & Knab, were formerly vectors in Trinidad and are at present of some importance in certain coastal regions of Brazil and probably Guiana. Females may bite both by day and by night near the margin of forested areas. They can enter houses and rest inside after biting but are also found extensively resting outside. In such conditions residual spraying has proved of low efficacy in reducing *Kerteszia*-borne malaria (Rachou and Azambuja 1949, Rachou et al. 1954a, Ferreira and Azambuja 1955, Charles 1959). The only effective way of controlling these vectors is by the mechanical or chemical destruction of the bromeliads which constitute their larval breeding sites (Veloso 1958, Anonymous/T.G. 1959).

Oriental and Indomalayan Regions

Anopheles culicifacies Giles.—This is mostly a zoophilic species, playing a role in malaria transmission either when very abundant or when cattle are scarce. In was a major vector in peninsular India, West Pakistan, and Ceylon (Covell 1962). It has usually been very well controlled by dieldrin or DDT house spraying, even after the appearance of insecticide-resistant populations, but has never disappeared from treated areas (Hamon and Garrett-Jones 1963). In Ceylon it has even been shown that *A. culicifacies* could form purely sylvatic populations far from inhabited areas (Rajendram et al. 1950).

A. culicifacies may increase in numbers very rapidly when ecological conditions are favorable, and it has been prone in the past to cause cyclic malaria epidemics of great severity (Macdonald 1957). While spraying operations have mostly acted by decreasing contact between man and the vector, closer relationships have been reestablished with the interruption of the campaigns. Despite the very low level of residual malaria this phenomenon has resulted in several urban malaria epidemics in India (Pal[12]) and in a very large outbreak of malaria in Ceylon with about one million human cases (Lartigue[13]).

Anopheles minimus minimus Theobald. — Almost everywhere in its range *A. m. minmus* was anthropophilic, endophilic, and a major malaria vector. Although the 1st trap-hut evaluations of insecticide against it gave conflicting results (Bertram 1950, Macdonald 1950), *A. m. minimus* has been very susceptible to DDT, BHC, and dieldrin house spraying. It has either been eradicated or has almost disappeared in treated areas in India (Sharma 1958, Chakrabarti and Singh 1957, Soma Sundera Rao et al. 1961). southern continental China and Hainan Island (Ho Ch'i and Feng Lan-Chou 1958, Ho Ch'i 1965), northern Malaysia (Sandosham et al. 1963) and Nepal (Shrestha 1966).

However, in Taiwan *A. m. minimus* was not entirely eradicated by several years of DDT spraying. It persisted in some foothill valleys populated by an impoverished community inhabiting widely scattered houses where some residual transmission occurred (Hsieh and Liang 1956, Anonymous/T.P.M.R.I. 1958). *A. m. minimus* also persisted in some DDT-treated areas in North Vietnam and was sporadically transmitting malaria (Tomaszunas 1966).

Very precise investigations in North Vietnam have shown that the longevity of *A. m. minimus* varies widely not only seasonally but also with the environment. Longevity is greatest in the mountain-river zone, where epidemiologically dangerous females have been observed 8 months of the year (Zalutskaya 1959, Lysenko and Dang Van Ngu 1965). Such areas

[12] R. Pal. Personal communication, 1968.
[13] J. J. Lartigue. Personal communication, 1968.

should be especially favorable for ensuring the survival of vector populations in treated environments.

Anopheles minimus flavirostris (Ludlow).—This species is the major malaria vector of the Philippine Islands. It is mostly exophilic and zoophilic, and the possibility of controlling it with residual insecticides first appeared very remote (Smith and Dy 1949, Ejercito 1955). However, it has been controlled by dieldrin spraying (Anonymous/WHO 1956), and after the appearance of resistance, DDT has been used. The safety margin is very narrow, and malaria transmission can be controlled only as long as good DDT coverage is achieved. Chow (1968) showed that the vector survives in large numbers in treated areas. Recent observations showed that malaria transmission is not yet interrupted (Catangui et al. 1969).

Anopheles maculatus Theobald.—This species has a wide distribution in the Oriental Region but has been reported as a major vector only in Malaysia, where it is less zoophilic than in other parts of its range (Bruce-Chwatt et al. 1966) and where favorable larval breeding places were created by the agricultural development of the hilly region (Sandosham 1962, Davidson and Ganapathipillai 1956, Scanlon et al. 1968). It is largely exophilic but enters houses to feed on humans. It has also been suspected to be an important vector in the highlands of Java (Chow et al. 1960).

In Malaysia small-scale experimental schemes based on DDT, BHC, and dieldrin house spraying showed that *A. maculatus*-transmitted malaria could be controlled by disrupting the contact between the vector and humans but without any noticeable change in anopheline vector density (Reid and Wharton 1956, Edeson et al. 1957). A large-scale malaria-eradication project confirmed these observations. After 8 applications of DDT in 4 years, malaria persisted in residual foci among the aborigines where the malaria incidence was the highest of the treated areas at the beginning of the project (Moorhouse 1965).

In the South Vietnam highlands, where *A. maculatus* had never been previously recorded as a malaria vector, the concentration of the population in restricted areas and the destruction of cattle and game by the war have increased the contacts between *A. maculatus* and humans. This anopheline is now considered there as a potential malaria vector (Holway et al. 1967). The human-blood ratio varies widely for this plastic species according to the frequency and range of vertebrate hosts available.

Anopheles sundaicus (Rodenwaldt).—An interesting change in behavior following several years of DDT spraying has been reported for this species from the southern coast of Java. After entering dieldrin-sprayed houses female *A. sundaicus* were seen avoiding contact with treated surfaces (Sundaraman et al. 1957, Sundaraman 1968). The dieldrin application was sprayed on walls treated several times previously with DDT, and the irritant effect of this compound was probably not masked by the dieldrin deposit. The high level of irritability of *A. sundaicus* to DDT was noticed soon after the 1st applications of this compound and was an inate characteristic of this anopheline. It appears that the behavioristic response observed by Sundaraman was acquired through selection.

Anopheles b. balabacensis Baisas.—This species now appears to be one of the most important malaria vectors of the forested areas of Southeast Asia from Assam to Hainan Island and southern China, through northern Malaysia, Thailand, Cambodia, and parts of South Vietnam (Holway et al. 1967, Scanlon et al. 1968). It is an important vector also in North Borneo (McArthur 1949; Colless 1956b, 1957).

The most accurate investigations on this vector have been carried out in North Borneo, Thailand, and Cambodia (Colless 1956 a, Eyles et al. 1964, Scanlon and Sandhinand 1965). These have shown that *A. b. balabacensis* is mainly an exophilic and exophagic mosquito, biting as well at the ground level as at the forest canopy level. It is very fond of primate blood, either human or monkey, and is furthermore a very effective natural vector of simian malaria (Cheong et al. 1965). Although exophagic, it may bite in large numbers inside houses. When this occurs it stays for some time in the immediate vicinity of houses, on grass, then on outside surfaces of the walls. It spends only a very short time inside, immediately before and after biting. The natural resting places of this species are not known but are probably in the forest. Similar observations have been made on a more restricted scale in Vietnam (Wiel 1968) and Hainan (Sheng et al. 1963).

A preliminary trap hut investigation in North Borneo suggested that *A. b. balabacensis* would probably be difficult to control with DDT spraying (Colless 1953). Large-scale investigations have confirmed this observation over almost all the range of the species. Residual insecticide applications had the lowest efficacy where housing was poor and adjacent to the forest. Malaria transmission was not interrupted under these conditions (Ho Ch'i and Feng Lan-Chou 1958, Ho Ch'i 1965, Scanlon and Sandhinand 1965, Sandosham et al. 1963, Cheng 1968).

In North Borneo the longevity and infection rates of *A. b. balabacensis* were considerably affected by climatic conditions (Colless 1952). As this situation very much resembles that of *A. nuneztovari*-borne malaria, the same control measures might be used; i.e., frequent treatments, clearing vegetation around houses, insecticide fogging of vegetation, etc.

Anopheles l. leucosphyrus Dönitz.—This species is an important malaria vector in Sarawak where it has been extensively studied by Colless (1956a). Its bionomics are very similar to those described for *A. b. balabacensis* but must differ in some particulars, as the malaria transmission has apparently been successfully interrupted there by insecticide treatments

(De Zulueta and LaChance 1956, Colbourne 1962, Wharton 1962).

Australasian Region

The *Anopheles punctulatus group*.—This group at the present time includes *A. punctulatus* Dönitz, *A. farauti* Larveran, and *A. koliensis* Owen, but it could constitute a complex of more or less than 3 species (Peters 1965). The nature of this complex is under investigation at the Ross Institute, London (Chow 1968). The 3 species have a good longevity, are readily exophagic and exophilic, and bite man to a great extent. They are important malaria vectors in most of their range. The group has a wide distribution in the South Pacific. Most of the recent investigations have been carried out on the island of New Guinea (Papua, New Guinea, and West Irian), where the 3 species coexist in coastal areas.

In West Irian the 1st trap hut experiments gave conflicting results. Swellengrebel and Stack (1949) stressed that *A. punctulatus* bites humans mostly outdoors and even during the the day. Van Thiel and Metselaar (1954, 1955) and Metselaar (1957) showed that most of the anophelines biting inside were entering unsprayed huts during the 1st half of the night and were not leaving until dawn, and that in DDT- and dieldrin-sprayed huts only a very small proportion of the entering anophelines was escaping the lethal effect of the insecticides.

Semiannual DDT spraying was started in a pilot area in West Irian in 1954 and was gradually extended to an area inhabited by 180,000 persons in early 1960 and was extended to a population of 260,000 in 1963. Early in the campaign dieldrin was used in 2 areas with disappointing results and was replaced by DDT. Spraying alone produced a reduction in transmission (Metselaar 1957). It was supplemented in 1958 and 1959 by distribution of chloroquine and pyrimethamine 2 or 3 times yearly. Then malaria transmission was almost interrupted in moderately endemic areas, and eradication appeared possible even in holoendemic areas (Metselaar 1961). From the end of 1959 the DDT spraying was supplemented by mass distribution of pyrimethaminized salt, then by chloroquinized salt. Malaria transmission again decreased without attaining complete cessation. The results were most successful in areas with a certain level of economic development (Meuwissen 1963). A very fine analysis of the entomological reasons for the practical failure of the pilot project was made by Slooff (1964), who concluded that the 3 species of the *punctulatus* group were exophilous, and that none of them showed a tendency to remain very long indoors. The frequency with which a house was visited depended upon the habits of the human population, as suitable hosts were bitten irrespective of whether they happened to be indoors or outdoors. In DDT-sprayed huts the entry of anophelines was decreased as was the proportion of entering females actually biting, and the mosquitoes proceeded more rapidly both to attack the host and to fly outside after trying to bite. The average mortality of naturally entering females produced by DDT treatment was not sufficient, according to Macdonald's formula (1957), to interrupt the transmission of malaria.

The observations carried out in Papua very closely corroborate the observations made in West Irian both on the ecology and behavior of the vector and on the probability of interrupting malaria transmission by DDT or dieldrin spraying (Peters and Standfast 1960; Peters 1960 a, b, 1962, 1965).

DISCUSSION

The behavior of anopheline vectors in the presence of insecticide treatment has been discussed by many entomologists during the past 20 years (Gabaldon 1953, Gillies 1956, Hamon 1958, Muirhead-Thomson 1960, Mouchet,[14] De Zulueta and Cullen 1963, Garrett-Jones 1964, Busvine 1964, De Zulueta 1964) and was one of the very important topics discussed during the meeting of the Eleventh WHO Expert Committee on Malaria (Anonymous/WHO 1964).

Three major aspects may be taken into consideration: the possibilities of vector survival in exophily; the relationship between the vector, the *Plasmodium* parasite, and the human host; and the selection of ecological or behavioristic changes in vector populations.

Vector Survival in Exophily.—It is well known that DDT deposits exert an irritant effect on adult mosquitoes (Kennedy 1947). The precise response of each mosquito varies with the species, physiological condition, environment, and nature of the DDT deposit. Later it was shown that such irritant properties are shared by other insecticides to a variable extent, and that DDT and dieldrin may have a deterrent effect, preventing some anophelines from entering treated houses (De Zulueta and Cullen 1963). This deterrent effect has been recorded for several organophosphate and carbamate insecticides.

Prolonged contact with insecticide deposits is lethal for normally susceptible anophelines. In house-sprayed areas the individual vector will either not enter treated houses; or will enter and leave before acquiring a lethal dose of the toxicant; or will enter, stay, and die. For the vector population as a whole the only chance of survival is exophily as stressed by Sautet (1953). If the environment is favorable, with alternative hosts outside, convenient natural shelters, and permissible ranges of relative humidity and temperature, the vector population will probably survive in "imposed exophily"; if not, the vector will be eradicated. One of the determinant factors will also be the irritant or deterrent properties of the insecticide used for the local vector.

Relationship Between the Vector, the Plasmodium *Parasite and the Human Host*.—If the environmental

[14] J. Mouchet, 1962. Les cas de persistance de la transmission palustre imputables au comportement des vecteurs. Mal/Exp. Com. 9/WP/12, 26 p. (Unpublished.)

conditions are not very favorable, the longevity of the vector will be reduced and very few females will reach an epidemiologically dangerous age. However, if conditions are good, the average longevity of the vector population will be normal, permitting malaria transmission dependent on the relationship between the vector and the human host.

When humans spend part of the night sleeping outside, or if the vector feeds outdoors during the day, or if houses are scattered in the bush and/or are loosely constructed, contact between the vector and man will be virtually normal despite insecticide spraying, and malaria transmission will not be interrupted.

If humans spend all the night inside, with a vector biting only in the middle of the night, or if houses are compact with few openings, the vector will either not bite humans, or the fraction of the population biting man will mainly die. Malaria transmission may be interrupted, depending upon the normal blood-feeding habits and survival rate of the vector as well as the residual properties of the insecticide.

In all cases, any decrease in the quality of the insecticide coverage and any increase in outdoor sleeping of humans will facilitate malaria transmission. When spraying operations are interrupted the vector population is there, ready to resume malaria transmission if there is a gametocyte carrier left in the vicinity. The speed for resuming transmission may then be easily estimated from Macdonald's formula (1957).

Selected Ecological and Behavioristic Changes in Vector Populations. — Theoretically, the possibility exists that insecticide pressure may induce ethological changes in vectors as it has selected insecticide resistant populations. Evidence is missing, either because our investigative methods are too inaccurate or too poorly used, or because no such selection has occurred in the field. When the species under attack is not sufficiently adaptable it disappears, and when it is very adaptable it usually has too few contacts with the insecticide to be selected in any noticeable direction unless the selected character is advantageous to survival, such as insecticide resistance. Even then we cannot ignore the fact that in many areas the insecticide-resistant populations have been selected as larvae rather than as adults through contamination of the larval breeding sites.

A recent laboratory experiment with *A. atroparvus* Van Thiel on the acquired ability to escape through a small hole demonstrates that minute differences in behavior may have an important survival value (Gerold 1968). Furthermore, field and laboratory investigations should be conducted on selected ethological changes, taking into consideration features other than irritability.

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Discussion of Paper by Dr. J. Hamon et al.

DR. DE MEILLON.—I'd like to have Dr. Hamon's opinion on 2 things. First, what does he think of the importance of investigating the possible existence of siblings among species that show differences in behavior in different parts of their distribution. Second, I should like to have his opinion on the value of gathering base-line pre-spray or pre-operational entomological data.

DR. HAMON.—Well, Mr. Chairman, I think that it will be worthwhile to investigate the possible occurrence of siblings in species which are very widely distributed or which exhibit very variable behavior in different parts of their range. I'm not sure that it will give any more information, but if we don't carry out these investigations we shall always be in a position of suspecting that there are differences, because we are dealing with different species. For the 2nd question, I'm sure that it is of very great importance to assess all basic data about the vector before undertaking antimosquito measures. For if we do not know the behavior and the relative importance of the vector before beginning the control program, it will be much more difficult to understand the changes of behavior and the relative importance of this vector later. If we want to assess the total program by mathematical means we must also check all our sampling methods; we must very accurately assess every detail of our mathematical model without interfering with the environment by insecticide.

DR. KITZMILLER.—I think it might be worthwhile to speak on this sibling-species problem just a little bit. Certainly, when we have distinct species that are morphologically separable and which also exhibit different physiological reactions, such as the ability to carry malaria, we are in very good shape. When we have a situation one step down from that, when we have sibling species that are still perfectly good, distinct, and valid species, even though they are a little difficult to tell apart, then we are still dealing with the same situation. However, I'm afraid that in many situations we are dealing not with good species, either sibling or otherwise, but with different Mendelian populations which differ in gene frequencies, which are semi-isolated, and which probably differ in their ability to transmit malaria and to do a lot of other things. For example, all of the behavioristic differences that Dr. Hamon was talking about, the multiplicity of events that must determine the ecological preferences and the niche selection, must be under the control of numerous genes in which populations certainly differ. Now, with *pseudopunctipennis,* for example, these populations are still interfertile, they can still exhibit gene flow, but this doesn't mean that they aren't necessarily different in their ecological preferences and their behavior. Certainly this is true with *pseudopunctipennis* which we have collected from many places in Central America, and it is true of the populations of *albimanus* that we've looked at. Unfortunately there are differences that we may or may not be able to recognize, but they surely are real differences that do exist in the Mendelian populations. Sometimes we may be able to find inversions that are characteristic of some populations and not of other populations. I think in summary that we should have to say that these differences, not only in physiology, but also in ethology are ultimately genetically based. I don't think there is any question that these populations differ whether or not we can recognize the differences.

MR. GARRETT-JONES.—Dr. Kitzmiller has properly stressed just now the genetical basis which must underlie many observed differences of behavior of anophelines at different places and time. On the other hand, in his presentation Dr. Hamon laid a healthy emphasis on differences according to circumstances in the environment, which to my mind need not be genetically based. After all, any animal is bound to have a certain amount of plastic adaptability to the circumstances in which it finds itself. In the course of Dr. Hamon's paper he mentions the investigation we carried out in Mexico 6 years ago. That investigation was designed to unravel some of the factors which underlay the low-level restabilization of malaria transmission, by vectors which had not developed any physiological resistance to the DDT, which was being adequately and regularly applied. The investigation was limited in time and as to the area that could be covered. Even before we got into the field, we had the idea that a primitive type of sociological survey could be carried out. We prepared a form to be filled out in the field, describing house by house the malaria positivity, the situation of the house, its size, the number in the family, the sleeping habits, and other factors which could vary even within the small area we studied. My purpose, sir, in bringing this up now is to ask the meeting to consider whether studies of this type should not become more frequent as a part of field studies of malaria transmission. Though these studies are not specifically entomological, very often the entomologist is the only man well placed for carrying them out. So this is a plea for some elementary sociological training to be given to malaria entomologists going into the field. At any rate, these men should be made aware of the fact that the mosquitoes they will be studying are, like any other animal, capable of adapting themselves to circumstances which may vary in every single village.

Now if I may please turn to one other point. Later in his paper, Dr. Hamon drew attention to the fact that various insecticide-induced changes of behavior in malaria vectors have been postulated, but there is little concrete evidence showing that such changes have actually occurred within a given vector population. I think all of us who have been in any way concerned with organizing or guiding operations of malaria eradication are somewhat to blame for neglect of this aspect. We have been content sometimes to accept, sometimes merely to criticize, those observations which have claimed to show a change of behavior under insecticide pressure; for example, consider the well-known observations of Trapido, followed by those of A. W. A. Brown in Panama. What we have not done is to make any effort to develop standard test methods which would at least identify whether a change of behavior has occurred. With regard to Mexico, for example, it is very difficult to be sure whether *A. pseudopunctipennis* really has changed its behavior over the years with regard to huts sprayed with DDT. Surely it should not be beyond our wits to devise some kind of test method which could be carried to the field and repeated according to a standardized procedure. Now this of course would not be observing a mosquito under its natural conditions; the mosquitoes would be caught and submitted to frankly artificial conditions, and perhaps this is why this has not gone forward. Certainly the mosquitoes won't behave as in nature, but I still think that portable devices carried into the field could provide evidence as to whether the mosquito has undergone selected and genetically controlled changes of behavior under pressure of the insecticide. I should also, of course, like to have Dr. Hamon's opinion and to hear others on this question.

DR. HAMON.—I appreciate the comments of Mr. Garrett-Jones. I am perfectly sure that such sociological differences play an important role in the difference of response of the vector to malaria control activities. Recently a large WHO project apparently has been partly spoiled because 30% of the human population in 1 area moved in and out in a matter of 1 year, making it very difficult to analyze the data which have been accumulated during the 6- or 7-year period. As no sufficient notice had been taken about this population movement a lot of the data cannot be used. I am sure a minimum sociological and ethnological background must be valuable, not only for the entomologist but for all people staffing malaria-control or malaria-eradication programs.

For the 2nd point, I'm very well aware that we lack any standardized method for studying the behavior of mosquitoes, and we have discussed this matter with Mr. Garrett-Jones. I know that he is working to develop such a method. We do not have it yet, but I hope that we will have it 1 day. It shall be a very fine advance to have such a method, but there are many difficulties in developing a laboratory method which could reproduce in any way what occurs in field conditions.

DR. PLETSCH.—I wish to speak to the same point that has been well covered by Mr. Garrett-Jones, but I'd rather press perhaps a little harder on what Dr. Hamon and Mr. Garrett-Jones mentioned were the alleged instances of behavioristic resistance. In your opinion, Dr. Hamon, do you believe there are any valid reported situations to justify this term?

DR. HAMON.—It is my opinion that we do not have any proof at the present time of behavioristic resistance in mosquito populations. We have a population which does not respond to the insecticide spraying, but it is impossible to prove that this response has been acquired through insecticide pressure. In the past I conducted quite a number of tests with the WHO irritability test kit, and there are so many variables that it is very difficult to reach conclusions. Some minutes ago Dr. Fay was telling me that most of our investigations are done by day, and we are dealing with vectors which are normally active at night. Recent investigations performed in Savannah show that exactly the same caged population has a very different type of response in the morning than in the night. So there are many factors which interfere with our observations, and we are probably underestimating some very important factors. In such a situation I cannot think we may prove or disapprove that insecticide pressure caused a permanent change. All the alleged changes in behavior that I have seen published cannot be proved.

DR. FAY.—In relation to what Dr. Hamon has just mentioned, to save ourselves time in evaluating light traps we have found it advantageous to entrain the mosquitoes so that they think the sundown is at various times of the day. When we tested normal mosquitoes (*A. albimanus*) at 10 AM, we caught about 30% in our light trap, but with mosquitoes entrained so that 10 AM is sundown, we caught 95% under the same conditions. In evaluating residual deposits, the entrained mosquitoes are giving a different mortality response than those on the normal day–night cycle. And I think that if you are going to evaluate such things as irritability or any other factor that involves the adults, you are going to have to take into consideration what time of day you are making these tests in relation to the normal activity cycle of the species involved.

MR. JOHNSON.—There are some interesting developments in the Jordan Valley. In the past 6 years or so, apparently there have been no *A. sacharovi* found in the Jordan Valley. Recently *A. sacharovi* is again showing up in the collections there. I'm not sure to what this may be attributed, but during the 1967 Arab-Israeli war, the spraying program was interrupted in the Jordan Valley and house spraying is almost nonexistent in that area now. In the absence of house spraying, *A. sacharovi* has returned. Apparently it has come from Syria in the north.

DR. GILLIES.—Mr. Chairman, I wish to make 2 points. One is to express my dismay that at this stage of malaria eradication it would be necessary even to question the necessity for adequate prespray investigation of any scheme. It seems to me deplorable that it isn't universally accepted at this stage that we must have adequate investigation for any control undertaken. Second point, I wanted to ask Dr. Hamon or Dr. Davidson whether they had any more recent information on the very interesting and important apparent change in behavior of *A. sundaicus* in Java in unsprayed houses, with an apparent alteration in resting site?

DR. DAVIDSON.—No more recent information than the information we had from the entomologist in Java in 1955, who remarked on this behavior change. I'd like to mention that a student of mine studied 1 aspect of this irritation by DDT, using an acoustic apparatus in which a single mosquito was confined in a small space over a sensitive microphone. He recorded the number of flights in conditions where external changes of lights etc. didn't affect activity, with automatic recording over 24 hr. He showed that species differ in their response to DDT and one of the most irritated species was *A. pseudopunctipennis*. Included in this investigation were *pseudopunctipennis* taken from Acatlipa. Using 1st-generation mosquitoes reared in London, he was not able to show any major difference between this and a population which hadn't experienced very much DDT.

I'd like to elaborate on the question of sibling species complexes which was brought up earlier. I think we're getting a complex about complexes, and I'm particularly involved in this because I get populations of many species sent to me in London to investigate whether they are the same species, and it involves a lot of work. We have already investigated populations of *stephensi, sacharovi, pseudopunctipennis, sundaicus, albimanus* and *funestus* from different parts of the areas of distribution—areas where the entomologist on the spot is convinced that he has behavior differences, etc. We've never been able to show sibling species complexes with sterility barriers in any thing but *punctulatus* and *gambiae*. This is not to say that we shouldn't go on investigating, but I think we have to realize that the same species can show very varying behaviors in different parts of its range.

DR. HAMON.—Mr. Chairman, I agree entirely with the point raised by Dr. Davidson. But I think that when there is any doubt about the existence of a single species with a large distribution, there is a possibility of the occurrence of sibling species, and we must investigate it. I know it is a very time-consuming work, but it must be done when there is reason to think we could be dealing with different species. About *sundaicus* in Java, the entomologist in fact was not dealing with houses which had been sprayed with DDT a long time ago. It is a matter of experience when houses have been DDT sprayed during several years the behavior of the mosquitoes entering these houses is altered, because the quantity of DDT which exists in surfaces of walls is not sufficient to kill the mosquitoes, but is quite sufficient to change the behavior. I am almost sure that it was exactly this which occurred in the Java areas as investigated by Mr. Sundaraman. We studied houses very closely in the Upper Volta 3 years after the last spraying, and we saw the house-resting habits of *A. gambiae* and *funestus* resumed only very, very slowly. There was almost no mortality induced by the treatment, but the mosquitoes were leaving the houses in the hours following their entrance.

DR. COLUZZI.—I should like to make some additional comments on the importance of geographical variability in behavior especially in connection with DDT irritability in *Anopheles*. Quite consistent evidence is now available both from the laboratory and from the field that house spraying with DDT may sometimes essentially prevent long resting of the *Anopheles* vector in the house. Accordingly, it must be expected that the effect of the insecticide on the vector would depend on how important the man-made environment is in the biology of the vector population. *A. labranchiae* provides an interesting example of a gradient of domesticity which is mostly facultative in this species in the southern part of the Mediterranean region, becoming almost obligatory in the northern part of its range. In fact, we have observed in Italy that DDT house spraying had a dramatic effect on the *labranchiae* populations of Tuscany and Latium (central Italy), while it only slightly reduced the density of the species in South Italy and Sicily. When considering the almost complete eradication of *labranchiae* in central Italy and slight repopulation of this zone as well as Sardinia, we must take into account the environmental changes made by land reclamation schemes and insecticides which are continually used in agriculture. However, there is scarcely any doubt that *labranchiae* responded in a very different way to DDT pressure in central Italy and in Sicily. In central Italy, the success achieved in species control is presumably related to the fact that *labranchiae* was strictly house inhabiting there.

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