

MOSQUITO BEHAVIOR AND VECTOR CONTROL

Helen Pates and Christopher Curtis

*London School of Hygiene & Tropical Medicine, London WC1E 7HT,
United Kingdom; email: helenjamet@yahoo.co.uk; chris.curtis@lshtm.ac.uk*

Key Words endophily, biting time, oviposition site choice, mosquito dispersal, competitive mating, monogamy

■ **Abstract** Effective indoor residual spraying against malaria vectors depends on whether mosquitoes rest indoors (i.e., endophilic behavior). This varies among species and is affected by insecticidal irritancy. Exophilic behavior has evolved in certain populations exposed to prolonged spraying programs. Optimum effectiveness of insecticide-treated nets presumably depends on vectors biting at hours when most people are in bed. Time of biting varies among different malaria vector species, but so far there is inconclusive evidence for these evolving so as to avoid bednets. Use of an untreated net diverts extra biting to someone in the same room who is without a net. Understanding choice of oviposition sites and dispersal behavior is important for the design of successful larval control programs including those using predatory mosquito larvae. Prospects for genetic control by sterile males or genes rendering mosquitoes harmless to humans will depend on competitive mating behavior. These methods are hampered by the immigration of monogamous, already-mated females.

RESTING BEHAVIOR IN RELATION TO HOUSE SPRAYING

The spraying of the walls and ceilings of houses with residual insecticides such as DDT reduces the survival prospects of indoor resting *Anopheles* mosquitoes sufficiently to greatly reduce the chance of malaria transmission (47); this was the key method by which malaria was eradicated in the temperate zones and in reducing malaria incidence in India from 75 million cases per year in the 1930s to 110,000 per year in the 1960s (99.8% reduction). There are now serious problems of physiological resistance to DDT in some Indian vector species (79). In addition, behavioral resistance in vectors in some countries has arisen in response to prolonged spraying programs. This can have an impact on a control effort and may result from an immediate response to the irritant insecticides (DDT or pyrethroids), or it may be a genetic trait evolved under selection from the presence of insecticides in houses. Insecticide irritancy can be demonstrated by a strong stimulation to take off and fly, a high proportion of mosquitoes exiting from a treated house, or both.

The irritating effect of DDT was demonstrated in verandah trap huts (82), where mosquitoes inside sprayed huts were irritated and exited huts sooner than those in unsprayed huts. This “bite and run” behavior has been shown in populations of *Anopheles gambiae sensu stricto* in the Tanga region of Tanzania (31), where high proportions leaving the sprayed house were fully engorged and displayed a high flight activity. Similarly, a 94% exit rate of *An. gambiae* and *An. funestus* from pyrethroid-treated huts was observed in Burkina Faso (23). A change in the place of egress for *A. gambiae s.s.* mosquitoes, from the windows to the eaves, has also been recorded in huts sprayed with tetramethrin (81).

In Tanzania, Mnzava et al. (55) found that a higher number of fed *Anopheles arabiensis* were exiting DDT-sprayed houses than lambda-cyhalothrin-sprayed houses, from which most exiting mosquitoes were unfed. This difference was considered to be partly due to the excitorepellent properties of DDT, although host availability also influenced the response of *An. arabiensis* to the two chemicals. In the DDT-sprayed areas, most cows were kept indoors, and mosquitoes therefore had to go inside to take a blood meal and were then driven out by the effects of the DDT. Fewer cows were kept indoors in the lambda-cyhalothrin-treated area, and this insecticide had a higher impact on malaria transmission than did DDT because it acted rapidly by either deterring mosquitoes from feeding or killing them.

Irritancy may not always be counterproductive. DDT was reintroduced in the 1980s in India, where *Anopheles culicifacies* is DDT resistant. Some reduction in malaria transmission was achieved because the irritant effect of DDT lasts much longer than the toxic effect (92). A comparison of DDT and bendiocarb in Mexico found that both insecticides had a similar impact on the incidence of malaria (45), but the mode of action of the two insecticides on the local vector, *Anopheles pseudopunctipennis*, was different (DDT is a much more irritant insecticide than bendiocarb; 26). DDT caused a decrease in the indoor resting densities and a tendency to avoid biting humans (46), whereas bendiocarb resulted in high mortality but no significant reduction in the number of indoor biting mosquitoes.

Naturally Endophilic *Anopheles* Species

The term endophily refers to the preference of a female mosquito to rest indoors during the period between the end of feeding and the onset of the search for an oviposition site. Residual house spraying is likely to be effective only if the mosquito species concerned is endophilic or at least partially endophilic, because the mosquito needs to rest on the insecticide-treated walls for a sufficient time if it is to pick up a lethal dose. Naturally endophilic species include *An. gambiae s.s.* and *An. funestus* in Africa, *An. culicifacies* in India, and *An. minimus* in East and Southeast Asia. DDT house-spraying programs in the 1940s and 1950s in Venezuela and Guyana (32) were successful in the coastal areas because houses provided the only safe resting place for *Anopheles darlingi* mosquitoes. However, eradication was impossible in forested areas because of the existence of “wild” populations outside human settlements. In Suriname and Colombia, *An. darlingi*

appears to have developed a shorter indoor resting period owing to insecticide pressure (76).

In Greece in the 1950s, DDT spraying programs led to exophilic behavior in the originally endophilic local vector, *Anopheles sacharovi*. It was considered that the survival of this species was due to a high level of irritability and physiological resistance (24). However, it is also possible that *An. sacharovi* became more zoophilic during the spraying campaign (4). Similarly, malaria transmission by *Anopheles sundaicus* in southern Java was not controlled by DDT house spraying, because of increased exophily (84). A decrease in the number of *An. sundaicus* resting on sprayed walls at night was observed, yet large numbers were still recovered from human bait.

In India, malaria control is based on indoor spraying of insecticides. A major resurgence of malaria occurred after the slackening of the eradication program in the 1970s (2) and increased problems of physiological resistance (79). This was exacerbated by the development of irrigation schemes and the movement of susceptible human populations into risk areas as well as the existence of outdoor resting populations of the major malaria vector, *An. culicifacies*. DDT spraying in Karnataka state began in 1945, and in one district in the 1970s, *Anopheles fluviatilis* (naturally endophilic) was found resting largely outdoors and only entering houses to take blood. Similarly, in the state of Tamil Nadu, outdoor resting populations of *An. culicifacies* with DDT resistance were found. Malathion spraying in 1978 replaced DDT spraying, which successfully reduced the indoor biting density. However, owing to the high gametocyte load in the community and the presence of outdoor transmission, a low-grade transmission in the area was maintained.

In Hainan Island, China, residual house spraying, launched in 1959, eliminated the main malaria vector, *An. minimus*, but recent malaria outbreaks have incriminated this vector again. Entomological surveys have shown a complete change in the behavior of *An. minimus*, which is now exophagic (i.e., it enters human dwellings to take its blood meal) exophilic and has an equal preference for humans and cattle (41a).

Endophagic-Exophilic *Anopheles* Species

Control of exophilic vectors by residual house spraying is usually ineffective. However, if the vector species is endophagic, then there is still a good chance that house spraying will be at least partially effective if a suitable chemical is used, such as fenitrothion, which has a fumigant effect.

Important successes have been achieved by using impregnated bednets against another endophagic but exophilic vector, *Anopheles dirus*, in Hainan Province, China, where the malaria incidence was high despite DDT residual spraying until the introduction of deltamethrin-treated bednets (17).

Predominantly endophilic mosquito populations may include varieties that exhibit exophilic tendencies. This tendency may be selected for by the use of insecticides or other human interventions such as deforestation; it was considered

the reason for the failure of a World Health Organization (WHO) house spraying control program in the district of Garki, Nigeria, to interrupt malaria transmission as had been the declared aim of the project (56). It was estimated that before spraying began in Garki, only about half the blood meals were followed by indoor resting (57). Populations composed of some consistently endophilic individuals and other consistently exophilic ones apparently led to nonuniform exposure of the mosquito population to the insecticide; the exposed fraction of the population may be so much affected that it nearly disappears. However, the unexposed fraction of the population persisted to ensure that sufficient transmission continued, even though major reductions in malaria mortality were noted.

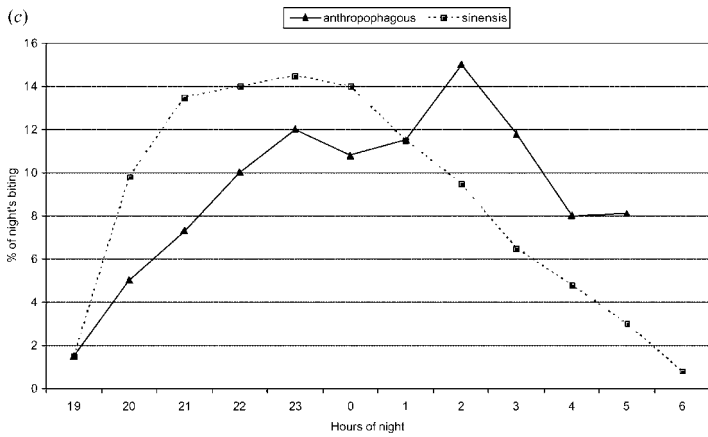
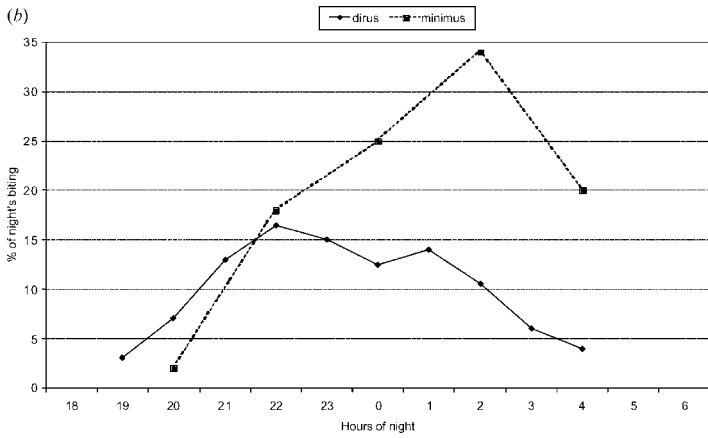
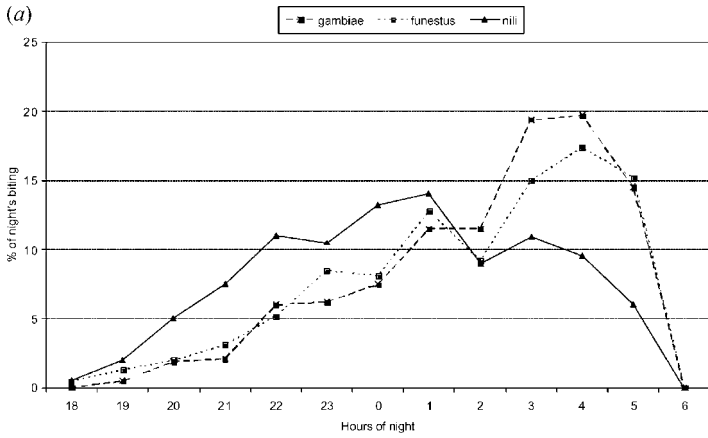
Culex and *Aedes* Mosquitoes

Residual house spraying is now rarely used as a control method against *Culex* or *Aedes* mosquitoes. This is partly because many vector species have outdoor resting habits or rest indoors on unsprayed objects such as clothes or curtains. *Culex* mosquitoes exhibit an innate tolerance to residual insecticide deposits, possibly owing to the pulvilli beneath *Culex* tarsi (6).

MOSQUITO BEHAVIOR IN RELATION TO INSECTICIDE-TREATED NETS

Insecticide-treated bednets or curtains have a remarkable impact on the incidence of malaria in several Asian countries and on African child mortality (41, 63). In 1910 Ross (75) had recommended bednets as a protection against malaria because of the late-night biting tendency of most anophelines. For most efficient anthropophilic vector species such as *An. gambiae* (35), *An. funestus* (35), and *An. nili* (35) (Figure 1a) in Africa; *An. dirus* (74) and *An. minimus* (38) (Figure 1b) in Southeast Asia; and *An. anthropophagus* (69; Xu Bozhao, unpublished data) in China

Figure 1 Percentage of the night's biting by the anopheline vector species in various parts of the world that occurred starting at the times indicated on the *x* axis. Data based on human landing catches outside houses except in the case of *An. anthropophagus*, in which the catches were inside, and *An. albimanus*, in which indoor and outdoor catches are compared. (a) Africa (Bobo-Dioulasso, Burkina Faso) (35). (b) Southeast Asia [*An. dirus* in Thailand (74); *An. minimus* in Assam, India (38)]. (c) China [data on *An. anthropophagus* from Anhui, Hubei, Zeijiang, Guizhou, and Yunnan Provinces (69) and on *An. sinensis* from Hubei and Hunan Provinces (Xu Bozhao, unpublished data)]. (d) Indian subcontinent (*An. culicifacies* species A and *An. stephensi* in Punjab, Pakistan; cold months: November–March; hot months: April–October) (66). (e) South America [*An. darlingi* in southern Brazil (29) and Suriname (36); *An. nuneztovari* in Venezuela (77)]. (f) Central America (*An. albimanus*: indoor and outdoor biting in Guatemala, Honduras, Nicaragua, Costa Rica, and Panama) (60).



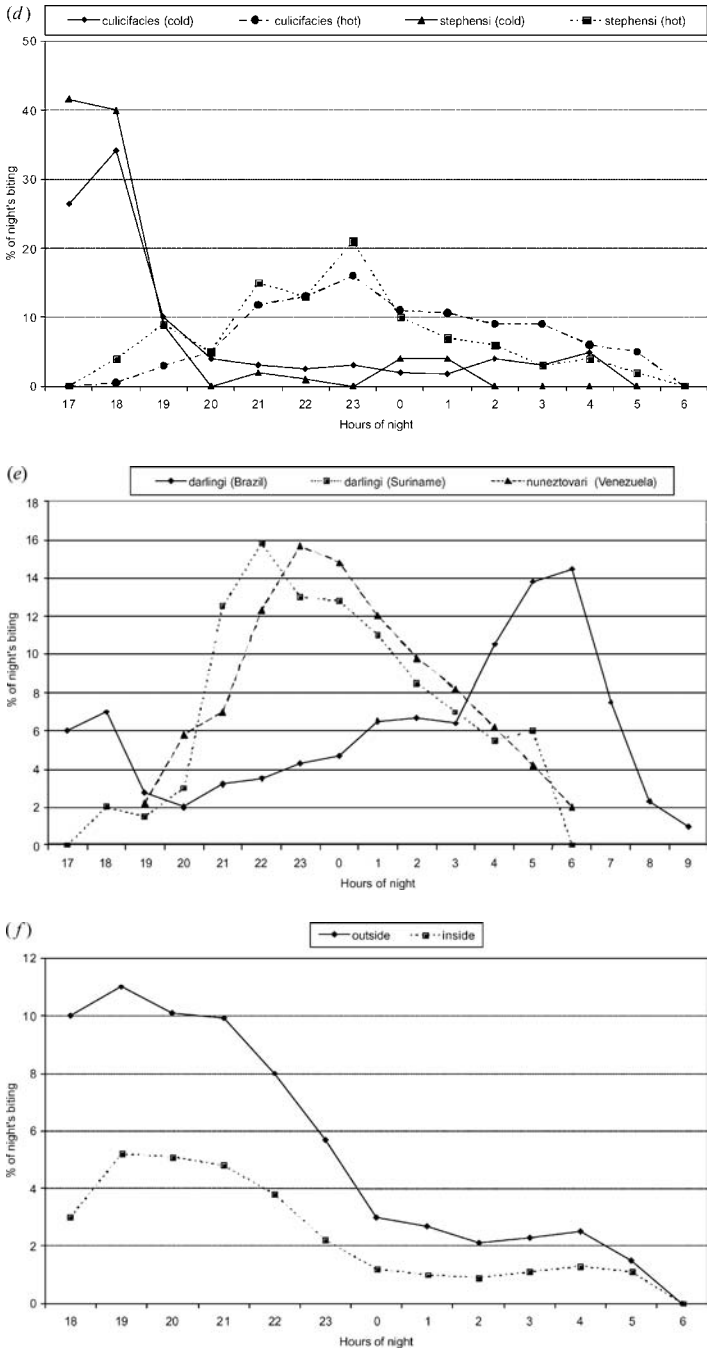


Figure 1 (Continued)

(Figure 1c), the biting rhythm and the times when most people go to bed and get up (assumed to be 2200 and 0500 h in rural areas) should ensure that more than 75% of anopheline bites would be prevented by mosquito-proof bednets. The same applies to *An. culicifacies* (66) in Pakistan in hot weather, but in cold weather almost all biting is completed before 2000 h, presumably before low temperatures inactivate the mosquitoes (Figure 1d). In South America (Figure 1e) more than 75% of the biting of *Anopheles nuneztovari* (77) is between 2200 and 0500 h, but *An. darlingi* populations have different biting rhythms in different areas (29, 36). In Suriname the rhythm is similar to that of *An. nuneztovari*, but in southern Brazil much of the biting occurs before 2200 h and after 0500 h.

In Central America, much of the biting by *An. albimanus* occurs outdoors and before 2200 h (60) (Figure 1f). There is a strong tendency for older, parous *An. albimanus* (which are the only ones that can carry infective sporozoites) to bite earlier than the rest of the population (60). The mean parity rate was 49% before 2200 h, 30% from then until 0500 h, and 17% thereafter. The early high parity rate would further reduce the protection from malaria transmitted by this species that one would expect to be achievable with bednets. Surprisingly, however, a considerable effect of nets on malaria transmitted by *An. albimanus* has been reported (71).

Tendencies for significantly higher parity or sporozoite rates among earlier biters have been recorded in several studies on *An. gambiae* and *Anopheles farauti* (see tabulation in Reference 53). The proportion of the night's sporozoite positive bites by *An. gambiae s.s.* and *An. funestus* occurring between 2200 and 0500 h was about 88% (53).

It has been feared that the effectiveness of nets may be reduced if the biting rhythm changes in a community where net use is widespread (40). Charlwood & Graves (12) found a marked shift toward earlier biting by *An. farauti* when nets were introduced in Papua New Guinea. They attributed this not to a genetic change in the population but to the fact that mosquitoes returning to a netted village from egg laying during the night would have difficulty in obtaining a blood meal before dawn. Thus many would remain hungry during the day and would attempt to find a meal as soon as dusk fell.

For *An. gambiae s.l.* two studies comparing treated and untreated villages have reported little or no difference in biting rhythm (49, 64), but two other studies (54, 58) reported a marked shift in biting time in those houses.

The above-mentioned studies were carried out soon after net introduction, when selection for genetic changes in behavior could hardly be expected. However, biting times of *An. funestus* have recently been recorded (59), nearly three years after introduction of treated nets into a village, which might be expected to be long enough for genetic changes to occur. The same percentage of the night's biting occurred between 2300 and 0500 h in this village and in a village without nets. Furthermore, studies conducted six years after net introduction (5) showed that the mean biting time remained unchanged but that there were indications of a wider range of biting times after dusk and before dawn.

In comparison with untreated nets, pyrethroid-treated nets are much more effective in reducing malaria incidence (38, 41, 52) presumably because of one or a combination of the following effects of pyrethroids on mosquitoes. (a) Contact with the nets may drive mosquitoes away before they have had a chance to penetrate the nets and bite (43), thus enhancing the physical protection provided by the net. This effect is due to the excito-repellency and rapid knockdown effect of pyrethroids. Such diversion away from treated nets may lead to more blood feeds taken from nonhuman hosts (12) from which the *Plasmodium* species that infect humans could not be acquired. However, highly anthropophilic species refuse to be diverted from humans, and the human blood index remains high even when almost everyone in a village is using a treated net (49). (b) Mosquitoes attracted to the odor of sleeping humans may be killed in sufficient numbers so that widespread use of nets in a community leads to a decline in vector population density and survival, which results in few mosquitoes surviving long enough for malarial sporozoites to mature inside them. In village-scale studies in which almost everyone was provided with a treated net, major reductions in vectorial capacity of the village mosquito populations have been observed in Tanzania (18, 49–52), Burkina Faso (10), D.R. Congo (39), Guinea Bissau (37), and Assam, India (38). However, in Sierra Leone the results were equivocal (48), and in The Gambia (42, 65) and Thailand (83) such effects have not been found. The explanation of the latter results may be that the mosquito populations of treated and untreated control villages shared the same breeding places, thus leading to continual mixing of the populations so that the effects of mass mosquito killing may be obscured in the trial results (65).

A study in Tanzania in which one person in a room was provided with a treated net showed that this markedly reduced the amount of biting by *An. arabiensis* (a member of the *An. gambiae* complex) on another person in the same room without a net, compared with when neither had nets (43). This is presumably because many mosquitoes, which would have attacked the unprotected person in the absence of nets, rested on the treated net long enough to be killed or repelled from the hut. However, when a person with an untreated net sleeps in the same hut as someone without a net, more biting is diverted to the latter person than would occur if neither person had a net (43). There are indications (C.A. Maxwell, unpublished data) that current efforts in northeast Tanzania to increase net usage by social marketing are leading to a situation in which a considerable proportion of adults buy nets for their own use, but seldom buy nets for their children and few of the nets are treated with insecticide. Nets used in this way are expected to lead to an *increase* in the malaria risk for vulnerable children. This contrasts with programs of organized free provision that can ensure that all age groups are provided with effective insecticidal nets (19).

BEHAVIOR IN RELATION TO LARVAL CONTROL

Identification of breeding sites is necessary for control measures such as source reduction, commonly used for *Aedes aegypti* control. Knowledge of oviposition behavior can help identify breeding sites and monitor populations. When using

chemicals for control of immatures, choosing the correct larvicide and larvicide formulation is critical. For example, oviposition traps containing a high concentration of emulsifiable chlorpyrifos were repellent to ovipositing *Aedes triseriatus* (3). Because granular formulations of chlorpyrifos and temephos are not repellent, it may be that the additives and/or solvents in the emulsifiable concentrate were repellent, rather than the insecticidally active ingredient. Ovitrap traps can help monitor oviposition activity (68) and are also useful for collecting samples of adults for the detection of arboviruses (13).

Choice of Oviposition Sites

The distribution of larvae is generally determined by the oviposition site selected by the female. For example, mosquitoes may selectively avoid oviposition in water containing notonectids, fish, and tadpoles (72). A release-and-recapture study with *Ae. aegypti* in Puerto Rico (25) showed significantly higher rates of recapture in houses where suitable breeding containers had been added than in houses where containers had been removed. This is taken as indirect evidence that dispersal of *Ae. aegypti* is driven by the search for oviposition sites. This has important implications because vector control efforts with this species center on source reduction, i.e., removal or destruction of breeding sites. Increased adult dispersal caused by females searching for a suitable breeding site may increase the spread of viruses such as dengue (67).

An. dirus, the main malaria vector in parts of Southeast Asia, is generally exophilic and lives in forests, where it feeds on primates. Movement of people into forested areas creates ideal transmission conditions because they provide a ready food supply and create many of the small, stagnant transitory pools used as breeding sites by this mosquito (73). It is extremely difficult to find and destroy all these breeding sites. In Bangladesh, larvae appear to exhibit a particular behavior (73) to aid survival under harsh conditions. Immatures appeared to be adapted to and possibly dependent on small temporary pools. Females were observed depositing eggs at the water line, where they embryonate and can remain viable for up to two weeks. Heavy rain results in a synchronous hatch that leads to complete development in 5–6 days and hence waves of biting females. During dry periods, larvae were observed leaving a draining pool before it became completely dry and crawling as far as 53 cm, sometimes successfully finding another pool. Larvae were also recovered alive from drained pools, up to 94 h after being stranded. Many of the metazoan cohabitants that would have preyed on them or competed with them for food were eliminated in the same pool as it dried up. Fourth instars were also observed cannibalizing the first or second instars of the same species.

Larvivoracious Fish and Arthropods

Much effort has been devoted to optimizing biological control by metazoan predators such as fish, *Toxorhynchites*, and *Mesocyclops*. However, larvivoracious marsh fish are often ineffective in controlling synchronous mosquito broods during periods of heavy rainfall or tidal intrusion because of the immediate hatching of large

numbers of mosquito eggs, the dilution of predatory fish densities due to increased water levels, and a delayed increase in fish numbers (9). *Ae. taeniorhynchus* normally oviposits on exposed salt marsh and mangrove soil and has been observed avoiding ovipositing near water with a high fish concentration (72). It is possible that in such areas prolonged wet weather can result in smaller mosquito populations, because there is a decrease in the number of sites free of fish when the water table rises (9).

Toxorhynchites brevipalpis is a tree-hole-breeding mosquito widely distributed in tropical Africa (89) that feeds on both surface prey (small animals sharing the habitat or other animals on the water surface or trapped in the air-water interface) and subsurface prey (mosquito larvae and other small animals) (44). On detection of surface prey, *T. brevipalpis* assesses both the angle and the distance to its prey and uses this information to optimize the approach path (44). The effectiveness of this predatory behavior seems dependent on temperature, because in the laboratory it has been found that one *T. brevipalpis* destroys an average of 154 *Ae. aegypti* larvae when reared at 26°C and up to 359 larvae at 32°C (9). *Toxorhynchites* spp. exhibit “killing behavior” both in the field and in the laboratory (86). This behavior is characterized by the killing but not consumption of prey larvae and conspecifics. One proposed explanation of this behavior is the vulnerable pupa hypothesis, whereby larvae are protecting their own interests before pupation by slaughtering as many of their competitors as possible, because the onset of killing begins when the larva reaches a critical weight in the fourth instar, which also permits pupation (14). Killing of prey larvae also deprives earlier instars of food, slowing development and increasing the chance of cannibalism (14). *Toxorhynchites* spp. appear to have a level of efficacy similar to that obtained with larvivorous fish and they would probably be good biological control agents in thick rain forests or peridomestic habitats (89). Studies on the oviposition behavior of *T. rutilus rutilus* in Louisiana, United States, indicated that this species has little value as a biocontrol agent of *Ae. aegypti* and *Culex quinquefasciatus* in the urban environment because the primary larval habitat of the latter two species is artificial containers, whereas *T. rutilus rutilus* preferentially breeds in tree-holes (27). However, a 45% reduction in *Ae. aegypti* densities in New Orleans, Louisiana, was achieved with weekly releases of *Toxorhynchites ambionensis* (Doleschall) (28). The degree of control achieved did not increase by increasing the number of *T. ambionensis* adults released each week and little movement of *T. ambionensis* between each study site was observed. The results from this study indicate that use of *T. ambionensis* as a biocontrol agent against container-breeding mosquitoes in urban situations may be feasible.

GENETIC CONTROL

The term genetic control covers all methods by which a mechanism for pest or vector control is introduced into a wild population through mating. These include (a) the sterile insect release method or sterile insect technique (SIT), in which

males are sterilized by irradiation or other means and released to mate with wild females, causing them to lay sterile eggs; and (b) introduction of genetic factors into wild populations that render pests harmless to humans.

Sterile Insect Technique

Much research was carried out about 30 years ago, especially in India and El Salvador, on the application of SIT to mosquitoes, but this research virtually stopped in the mid-1970s, not because the method was a technical failure, but because of political problems in India (91) and the intensifying civil wars in Central America. There is now a revival of interest, especially in the use of transgenesis to improve sex separation so that only nonbiting males are released and to ensure that their female progeny die without the need for radiation or chemosterilization (1, 87).

Three issues of mosquito behavior are especially relevant to SIT: (a) mating competitiveness of artificially reared, sterilized males; (b) dispersal/migration; and (c) female monogamy/polygamy.

MATING COMPETITIVENESS Doubts about whether sterilization or artificial rearing might lead to behavioral abnormalities not detectable in cages led to field tests in which fluorescently marked males were released and the population was then sampled to ascertain the sterile:fertile male ratio for comparison with the sterile:fertile mating ratio, as shown by the fertility of the eggs laid by captured females. To ensure that the females tested were not immigrants but had mated in the presence of the sterile males, virgin females of wild origin were released into the area, given time to mate, and then as many as possible were collected and allowed to oviposit. This procedure was used with (a) chemosterilized *Anopheles quadrimaculatus* (22), (b) chemosterilized *An. albimanus* carrying a genetic sex separating system (21), (c) chemosterilized or cytoplasmically incompatible *Cx. quinquefasciatus* (33), and (d) chemosterilized or chromosomally translocated and sex ratio-distorting *Ae. aegypti* (34, 78). All showed moderately good mating competitiveness. Thus, adequately large releases well mixed with isolated wild populations might have been expected to yield high levels of egg sterility. However, in practice they frequently did not do so. This has been attributed to an influx of already-mated females from outside the sterile-male release area.

The first cage tests of the fitness of transgenic *Anopheles* and *Aedes* yielded poor results (11, 36a) possibly because of inbreeding depression. Whether transgenic mosquitoes with adequate competitiveness can be selected is now a key research question.

DISPERSAL/MIGRATION Dispersal ability is a major concern for the SIT (a) to ensure that sterile males are released near enough to all emerging wild females so that they have a fair chance of mating with them; and (b) to assess whether there are appreciable numbers of immigrant females that have already had fertile matings and will lay fertile eggs and nullify the effect of the sterile releases.

Ae. aegypti was considered a poorly dispersing species and sterile males would need to be released at intervals of about 50 m along urban streets to find all the local females (70, 80). However, more recent studies with rubidium labeling found labeled eggs from the same group of released females scattered over more than an 800-m radius (67). With *Cx. quinquefasciatus* a 3-km-wide barrier zone around release villages was expected to be adequate to isolate them, but fluorescently marked sterile males were frequently found 7 km from their release point and egg rafts continued to be laid throughout the barrier zone, within which great efforts had been made using larvicides to eliminate all emergence of adults (93). For *An. albimanus*, release of competitive sterile males into a 20 km² area protected by a barrier zone in which breeding was prevented by methoprene treatment of potential breeding sites had a major suppressing effect on the population compared with the upward seasonal trend seen in an untreated area. However, even though the barrier was 4 km wide, it seemed insufficient to ensure isolation (21). Thus, there seems to be a tendency to consider immigration negligible until the indigenous population is eliminated by sterile releases, after which immigrants make their presence felt. Clarification of the true proportion of immigrants in a population is important not only for the SIT, but also for assessing whether local efforts at larval control with insecticides or environmental management could have a worthwhile impact on the adult vector population or whether they are likely to be swamped by immigration.

FEMALE MONOGAMY/POLYGAMY Most laymen and some scientists believe that successful application of SIT is limited to species in which the females are monogamous. This belief is based on an incorrect assumption that radiation or chemical sterilization involves elimination of sperm so that a female mated to a sterile and a fertile male would be fully fertile. However, dominant lethal mutations in Diptera do not inactivate sperm but kill after zygote formation, so that some eggs of a multiply-mated female are sterilized (90). Thus, female polygamy is no barrier to the successful application of SIT. On the contrary, it would be a great advantage because after arrival in the release area, immigrant inseminated females would be willing to accept sterilizing matings. However, unfortunately, for mosquitoes monogamy is the rule.

In *Ae. aegypti*, monogamy appears to result from the action of a peptide, given the name *matrone* (15, 30), that is carried in the seminal fluid produced in the male accessory glands and inhibits the reception of sperm from second and subsequent matings. However, Klowden (39a) has recently shown that in *An. gambiae* and *An. albimanus*, in contrast to *Ae. aegypti*, implantation of male accessory glands into females did not prevent them from receiving sperm when they were mated. Bryan (7, 8) studied the effects of mating females of the *An. gambiae* complex to sterile aspermic hybrids that are produced by laboratory crosses between different members of the complex. The females that had been mated to aspermic hybrids were subsequently mated to fertile males, and these females were tested for ability to lay fertile eggs. When the hybrid males used in these experiments had *An. melas* as their female parent, these hybrids could inhibit their mates from being fertilized

in their subsequent matings. However, when the hybrid males had *An. melas* as their male parent, the hybrids failed to inhibit fertilization in the subsequent mating. Bryan (8) explained these contrasting results with the different hybrids as being due to the underdeveloped male accessory glands observed in the latter type of hybrid but not the former, and she assumed that the latter type could not produce matrone but the former type could do so. However, in view of the above-mentioned recent evidence for the apparent irrelevance of male accessory glands (39a) to monogamy in *Anopheles*, the interpretation of the data of Bryan (7, 8) needs to be reconsidered, especially if hybrids in the *An. gambiae* complex are ever to be tried again as a means of genetic control (23a).

Introduction of Genetic Factors into Wild Populations

It is the strong tendency toward monogamy of mosquitoes that renders local efforts at genetic control futile, unless one can either (a) guarantee isolation of the population, as may be the case with urban populations that are surrounded by rural populations of a different species; (b) afford a huge rolling program of release, as with the screwworm program, so that immigrant females mostly receive sterile matings before they can reach the area in which the eradication effort is currently in progress; or (c) replace SIT with the use of insects genetically engineered to carry a genetic driving system tightly linked to a gene that renders the insect harmless to humans (16). The twin aims of the latter concept are that the desirable gene will be driven to spread from a modest initial "seeding" and that the driving action will counteract the effect of immigration. Among the challenges facing such a scheme are behavioral barriers to free cross-mating in the field, such as those between the Mopti, Savannah, Bamako, and other chromosomally recognizable forms of *An. gambiae s.s.* in West Africa (88). Clearly, one would have to ensure that the released mosquitoes belong to the same form as the target population. Further considerations of behavioral-ecological issues surrounding this method are discussed in a recent book (85).

The genetics of effective driving factors and genes for physiological nonsusceptibility to malaria, or other pathogens, are beyond the scope of this review. It must be recognized that such genes would place strong selection on the pathogen to evolve evasion mechanisms. This problem could be avoided by using a genetic factor that renders a mosquito harmless by making it willing to bite only nonhuman animals (i.e., zoophily). Marked differences in the degree of zoophily/anthropophily exist between *An. gambiae* and *An. quadriannulatus* (61, 62), which can be cross-mated in the laboratory with fertile female progeny, so that crossing genes causing zoophily into the genetic background of *An. gambiae* may be possible.

ACKNOWLEDGMENTS

We are grateful to colleagues who provided us with unpublished or inaccessible information. Present address for Dr. Helen Pates is Ubwari Research Centre of the Tanzanian National Institute for Medical Research, Box 81, Muheza, Tanga, Tanzania.

The *Annual Review of Entomology* is online at <http://ento.annualreviews.org>

LITERATURE CITED

1. Alphey L, Andreasen M. 2002. Dominant lethality and insect population control. *Mol. Biochem. Parasitol.* 121:173–78
2. Barai D, Hyma B, Ramesh A. 1982. The scope and limitations of insecticide spraying in rural vector control programmes in the states of Karnataka and Tamil Nadu in India. *Ecol. Dis.* 1:243–55
3. Beehler JW, Mulla MS. 1993. Effect of the insect growth regulator methoprene on the ovipositional behavior of *Aedes aegypti* and *Culex quinquefasciatus*. *J. Am. Mosq. Control Assoc.* 9:13–16
4. Boreham PFL, Garrett-Jones C. 1973. Prevalence of mixed blood meals and double feeding in a malaria vector (*Anopheles sacharovi* Favre) *Bull. World Health Organ.* 48:605–14
5. Braimah N, Maxwell CA. 2003. Assessment of the effect of long term use of treated bednets on biting time of mosquitoes using bednets and light traps. *Trans. R. Soc. Trop. Med. Hyg.* 97:263
6. Brown AWA, Pal R. 1971. Insecticide resistance in arthropods. *W.H.O. Monogr.* 38
7. Bryan JH. 1968. Results of consecutive matings of female *Anopheles gambiae* species B with fertile and sterile males. *Nature* 218:489
8. Bryan JH. 1972. Further studies on consecutive matings in the *Anopheles gambiae* complex. *Nature* 239:519–20
9. Carlson DB, Vigliano RR. 1985. The effects of two different water management regimes on flooding and mosquito production in a salt marsh impoundment *J. Am. Mosq. Control Assoc.* 1:203–11
10. Carnevale P, Robert V, Boudin C, Halna JM, Pazart L, et al. 1988. Control of malaria using mosquito nets impregnated with pyrethroids in Burkina Faso. *Bull. Soc. Pathol. Exot.* 81:832–46
11. Catteruccia F, Godfray HC, Crisanti A. 2003. Impact of genetic manipulation on the fitness of *Anopheles stephensi* mosquitoes. *Science* 299:1225–27
12. Charlwood JD, Graves PM. 1987. The effect of permethrin-impregnated bednets on a population of *Anopheles farauti* in coastal Papua New Guinea. *Med. Vet. Entomol.* 1:319–27
13. Clark CG, Crabbs CL, Elias BT. 1986. Absence of La Crosse virus in the presence of *Aedes triseriatus* on the Delmarva Peninsula. *J. Am. Mosq. Control Assoc.* 2:33–37
14. Corbett PS, Griffiths A. 1963. Observations on the aquatic stages of two species of *Toxorhynchites* in Uganda. *Proc. R. Entomol. Soc. London* 38:125–35
15. Craig GB. 1967. Mosquitoes: female monogamy induced by male accessory gland substance. *Science* 156:1499–501
16. Curtis CF. 1994. The case for malaria control by genetic manipulation of its vectors. *Parasitol. Today* 10:371–74
17. Curtis CF, Lines JD, Carnevale P, Robert V, Boudin C, et al. 1990. Impregnated nets and curtains against malaria mosquitoes. In *Appropriate Technology in Vector Control*, ed. CF Curtis, pp. 5–46. Boca Raton, FL: CRC Press
18. Curtis CF, Maxwell CA, Finch RT, Njunwa KJ. 1998. A comparison of use of a pyrethroid either for house spraying or for bednet treatment against malaria vectors. *Trop. Med. Int. Health* 3:619–31
19. Curtis CF, Maxwell C, Lemnge M, Kilama WL, Steketee RW, et al. 2003. Scaling-up coverage with insecticide-treated nets against malaria in Africa: Who should pay? *Lancet Infect. Dis.* 3:304–7
20. Deleted in proof
21. Dame DA, Lowe RE, Williamson DL. 1980. Assessment of released *Anopheles albimanus* and *Glossina morsitans*. In *Cytogenetics and Genetics of Vectors*, (*Proc. XVI Int. Cong. Entomol., Kyoto*), ed. R Pal, JB Kitzmiller, T Kanda, pp. 231–47.

- Amsterdam: Kodansha, Tokyo, and Elsevier Biomedical Press
22. Dame DA, Woodard DB, Ford HR, Wedhaas DE. 1964. Field behavior of sexually sterile *Anopheles quadrimaculatus* males. *Mosq. News* 24:6–14
 23. Darriet F. 1991. Field trial of the efficacy of three pyrethroids in the control of malaria vectors. *Parassitologia* 33:111–19
 - 23a. Davidson G, Odetoyinbo JA, Colussa B, Cox J. 1970. A field trial to assess the mating competitiveness of sterile males produced by crossing two members of the *Anopheles gambiae* complex. *Bull. World Health Organ.* 42:55–67
 24. de Zulueta J. 1959. Insecticide resistance in *Anopheles sacharovi*. *Bull. World Health Organ.* 20:797–822
 25. Edman JD, Scott TW, Costero A, Morrison AC, Harrington LC, Clark GG. 1998. *Aedes aegypti* (Diptera: Culicidae) movement influenced by availability of oviposition sites. *J. Med. Entomol.* 35:578–83
 26. Evans RG. 1993. Laboratory evaluation of the irritancy of bendiocarb, lambda-cyhalothrin and DDT to *Anopheles gambiae*. *J. Am. Mosq. Control Assoc.* 9:285–93
 27. Focks DA, Sackett SR, Dame DA, Bailey DL. 1983. *Toxorhynchites rutilus rutilus* (Diptera: Culicidae): field studies on dispersal and oviposition in the context of the biocontrol of urban container-breeding mosquitoes. *J. Med. Entomol.* 20:383–90
 28. Focks DA, Sackett SR, Dame DA, Bailey DL. 1985. Effect of weekly releases of *Toxorhynchitesamboinensis* (Dobsonflies) on *Aedes aegypti* (L.) (Diptera: Culicidae) in New Orleans, Louisiana. *J. Econ. Entomol.* 78:622–26
 29. Forattini O. 1987. Comportamento exofilo de *Anopheles darlingi* Root, em região meridional do Brasil. *Rev. Saúde Públ. São Paulo* 21:291–304
 30. Fuchs MS, Craig GB, Despommier DD. 1969. The protein nature of the substance inducing female monogamy in *Aedes aegypti*. *J. Insect Physiol.* 15:701–9
 31. Gerold JL. 1977. Evaluation of some parameters of house-leaving behaviour of *Anopheles gambiae s.l.* *Acta Leiden.* 45:79–90
 32. Giglioli G. 1956. Biological variations in *Anopheles darlingi* and *Anopheles gambiae*. Their effect on practical malaria control in the Neotropical Region. *Bull. World Health Org.* 15:461–71
 33. Grover KK, Curtis CF, Sharma VP, Singh KRP, Dietz K, et al. 1976. Competitiveness of chemosterilized males and cytoplasmically incompatible-translocated (IS31B) males of *Culex pipiens fatigans* in the field. *Bull. Entomol. Res.* 66:469–80
 34. Grover KK, Suguna SG, Uppal DK, Singh KRP, Ansari MA, et al. 1976. Field experiments on the competitiveness of males carrying genetic control systems for *Aedes aegypti*. *Entomol. Exp. Appl.* 20:8–18
 35. Hamon J. 1963. Les moustiques anthropophiles de la région de Bobo Dioulasso (République de Haute-Volta). Cycles d'agressivité et variations saisonnières. *Ann. Soc. Entomol. France* 132:85–144
 36. Hudson JE. 1984. *Anopheles darlingi* Root (Diptera: Culicidae) in the Suriname rain forest. *Bull. Entomol. Res.* 74:129–42
 - 36a. Irvin N, Hoddle MS, O'Brochta DA, Carey B, Atkinson PW. 2004. Assessing fitness costs for transgenic *Aedes aegypti* expressing GFP marker and transposase genes. *Proc. Natl. Acad. Sci. USA* 101:891–96
 37. Jaenson TG, Gomes MJ, Barreto dos Santos RC, Petrarca V, Fortini D, et al. 1994. Control of endophagic *Anopheles* mosquitoes and human malaria in Guinea Bissau, West Africa, by permethrin-treated bed nets. *Trans. R. Soc. Trop. Med. Hyg.* 88:620–26
 38. Jana-Kara BR, Wajihullah, Shahi B, Dev V, Curtis CF, Sharma VP. 1995. Deltamethrin impregnated bednets against *Anopheles minimus* transmitted

- malaria in Assam, India. *J. Trop. Med. Hyg.* 98:73–83
39. Karch S, Garin B, Asidi N, Manzambi Z, Salaun JJ, Mouchet J. 1993. Mosquito nets impregnated against malaria in Zaire. *Ann. Soc. Belge Méd. Trop.* 73:37–53
 - 39a. Klownden MJ. 2001. Sexual receptivity in *Anopheles gambiae* mosquitoes: absence of control by male accessory gland substances. *J. Insect Physiol.* 47:661–66
 40. Knols BGJ, Takken W. 1998. The wide-scale use of impregnated bednets for malaria control in Africa: impact on mosquitoes. *Proc. Exp. Appl. Entomol.* 8: 15–20
 41. Lengeler C. 1998. Insecticide treated bednets and curtains for malaria control. In *The Cochrane Library, Issue 3*. Oxford, UK: Update Software
 - 41a. Li M, Liang LT, Zhang HS, Chen TY. 1983. The bionomics of *Anopheles minimus* in the Zhongsha area of Hainan Island. *Ann. Bull. Parasit. Soc. Guangdong Prov.* pp. 180–83. Curtis CF, Zhang K. 1988. (Abstr.) *Trop. Dis. Bull.* 85:662–63
 42. Lindsay SW, Alonso P, Armstrong-Schellenberg JRM, Hemingway J, Adiamak JH, et al. 1993. A malaria control trial using insecticide-treated bed nets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa. *Trans. R. Soc. Trop. Med. Hyg.* 87(Suppl.):45–52
 43. Lines JD, Myamba J, Curtis CF. 1987. Experimental hut trials of permethrin-impregnated mosquito net and curtains against malaria vectors in Tanzania. *Med. Vet. Entomol.* 1:37–51
 44. Linley J. 1995. Behavior on approach to surface prey by larvae of *Toxorhynchites amboinensis* and *T. brevipalpis* (Diptera: Culicidae). *J. Med. Entomol.* 32:53–65
 45. Loyola EG, Rodriguez MH, Gonzalez L, Arredondo JI, Bown DN, Vaca MA. 1990. Effect of indoor residual spraying of DDT and Bendiocarb on the feeding patterns of *Anopheles pseudopunctipennis* in Mexico. *J. Am. Mosq. Control Assoc.* 6:635–40
 46. Loyola EG, Vaca MA, Bown DN, Perez E, Rodriguez MH. 1991. Comparative use of bendiocarb and DDT to control *Anopheles pseudopunctipennis* in a malarious area of Mexico. *Med. Vet. Entomol.* 5:233–42
 47. MacDonald G. 1957. *The Epidemiology and Control of Malaria*. London: Oxford Univ. Press
 48. Magbity E, Marbiah N, Maude G, Curtis C, Bradley D, et al. 1997. Effects of community-wide use of lambda-cyhalothrin-impregnated bednets on malaria vectors in rural Sierra Leone. *Med. Vet. Entomol.* 11:79–86
 49. Magesa SM, Wilkes TJ, Mnzava AEP, Njunwa KJ, Myamba J, et al. 1991. Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria. Part 2. Effects on the malaria vector population. *Acta Trop.* 49:97–108
 50. Maxwell CA, Chambo W, Mwaimu M, Magogo F, Carneiro IA, et al. 2003. Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. *Malaria J.* 2:28. <http://www.malariajournal.com/>
 51. Maxwell CA, Msuya E, Sudi M, Njunwa KJ, Carneiro IA, et al. 2002. Effect of community-wide use of insecticide-treated nets for 3–4 years on malarial morbidity in Tanzania. *Trop. Med. Int. Health* 7:1003–8
 52. Maxwell CA, Myamba J, Njunwa KJ, Greenwood B, Curtis CF. 1999. Comparison of bednets impregnated with different pyrethroids for their impact on mosquitoes and on re-infection with malaria after clearance of pre-existing infections with chlorproguanil-dapsone. *Trans. R. Soc. Trop. Med. Hyg.* 93:4–11
 53. Maxwell CA, Wakibara J, Tho S, Curtis CF. 1998. Malaria-infective biting at different hours of the night. *Med. Vet. Entomol.* 12:325–27
 54. Mbogo CNM, Baya NM, Ofula AVO, Githure JI, Snow RW. 1996. The impact of permethrin-impregnated bednets

- on malaria vectors of the Kenyan coast. *Med. Vet. Entomol.* 10:251–59
55. Mnzava AE, Rwegoshora RT, Wilkes TJ, Tanner M, Curtis CF. 1995. *Anopheles arabiensis* and *An. gambiae* chromosomal inversion polymorphism, feeding and resting behaviour in relation to insecticide house-spraying in Tanzania. *Med. Vet. Entomol.* 9:316–24
56. Molineaux L, Grammiccia G. 1980. *The Garki project: Research on the Epidemiology and Control of Malaria in the Sudan Savanna of West Africa*. Geneva: World Health Organization
57. Molineaux L, Shidrawi GR, Clarke JL, Boulzaguet JR, Ashkar TR. 1979. Assessment of insecticidal impact on the malaria mosquito's vectorial capacity, from data on the man-biting rate and age-composition. *Bull. World Health Organ.* 57:265–74
58. Njau RJA, Moshia FW, Nguma JFM. 1993. Field trials of pyrethroid impregnated bednets in northern Tanzania. 1. Effects on malaria transmission *Insect Sci. Appl.* 5: 575–84
59. Paine T, Maxwell CA, Curtis CF. 1999. Does prolonged use of insecticide-treated bednets lead to a change in the biting-time of malaria vectors? *Trans. R. Soc. Trop. Med. Hyg.* 93:120
60. Pan American Health Organization. 1996. Biology and ecology of *Anopheles albimanus* in Central America. *Tech. Pap.* No. 43
61. Pates HV, Takken W, Curtis CF. 2004. Laboratory studies on the olfactory behaviour of *Anopheles quadriannulatus*. *Entomol. Exp. Appl.* In press
62. Pates HV, Takken W, Suik K, Curtis CF. 2001. Differential behaviour of *Anopheles gambiae sensu stricto* to cow and human odours in the laboratory. *Bull. Entomol. Res.* 91:289–96
63. Phillips-Howard PA, Nahlen BL, Kolczak MS, Hightower AW, Ter Kuile FO, et al. 2003. Efficacy of permethrin-treated bed nets in the prevention of mortality in young children in an area of high perennial malaria transmission in Western Kenya. *Am. J. Trop. Med. Hyg.* 68(Suppl. 4):23–29
64. Quiñones M, Lines J, Thomson M, Jawara M, Greenwood B. 1998. Permethrin-treated bed nets do not have a 'mass-killing effect' on village populations of *Anopheles gambiae s. l.* in The Gambia. *Trans. R. Soc. Trop. Med. Hyg.* 92:373–78
65. Quiñones M, Lines J, Thomson M, Jawara M, Morris J, et al. 1997. *Anopheles gambiae* gonotrophic cycle duration, biting and exiting behaviour unaffected by permethrin-impregnated bednets in The Gambia. *Med. Vet. Entomol.* 11:71–78
66. Reisen WK, Aslamkhan M. 1978. Biting rhythms of some Pakistani mosquitoes (Diptera: Culicidae). *Bull. Entomol. Res.* 68:313–30
67. Reiter P, Amador MA, Anderson RA, Clark GC. 1995. Short report: dispersal of *Aedes aegypti* in an urban area after blood feeding as demonstrated by rubidium-marked eggs. *Am. J. Trop. Med. Hyg.* 52:177–79
68. Reiter P, Amador MA, Colon N. 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. *J. Am. Mosq. Control Assoc.* 7:52–55
69. Ren Min Health Publication. 1991. *Research and Control of Malaria in China*.
70. Reuben R, Rahman K, Panicker P, Das P, Brooks G. 1975. The development of a strategy for large-scale releases of sterile males of *Aedes aegypti*. *J. Commun. Dis.* 7:313–26
71. Richards FO Jr, Klein RE, Flores RZ, Weller S, Gatica M, et al. 1993. Permethrin-impregnated bed nets for malaria control in northern Guatemala: epidemiologic impact and community acceptance. *Am. J. Trop. Med. Hyg.* 49:410–18
72. Ritchie SA, Laidlaw-Bell C. 1994. Do fish repel oviposition by *Aedes*

- taeniorhynchus?* *J. Am. Mosq. Control Assoc.* 10:380–84
73. Rosenberg R. 1982. Forest malaria in Bangladesh. III. Breeding habits of *Anopheles dirus*. *Am. J. Trop. Med. Hyg.* 31:192–201
 74. Rosenberg R, Andre RG, Somchit L. 1990. Highly efficient dry season transmission of malaria in Thailand. *Trans. R. Soc. Med. Hyg.* 84:22–28
 75. Ross R. 1910. *The Prevention of Malaria*. London: Murray
 76. Rozendaal JA, Van Hoof JPM, Voorham J, Oostburg BFJ. 1989. Behavioral responses of *Anopheles darlingi* in Suriname to DDT residues on house walls. *J. Am. Mosq. Control Assoc.* 5:339–50
 77. Rubio-Palis Y, Curtis CF. 1992. Biting and resting behaviour of anophelines in western Venezuela and implications for control of malaria transmission. *Med. Vet. Entomol.* 6:325–34
 78. Seawright JA, Kaiser PE, Dame DA. 1977. Mating competitiveness of chemosterilized hybrid males of *Aedes aegypti* (L.) in field tests. *Mosq. News* 37:615–19
 79. Sharma VP. 2003. DDT: the fallen angel. *Curr. Sci.* 85:1532–37
 80. Singh KRP, Brooks GD. 1975. Semi-automatic release system for distribution of mosquitoes during genetic control operations. *J. Commun. Dis.* 7:288–93
 81. Smith A, Chabeda PI. 1969. A verandah-trap hut for studying the house-frequenting habits of mosquitoes and for assessing insecticides. IV. The effect of tetramethrin on the behaviour and mortality of *Anopheles gambiae* Giles. *Bull. Entomol. Res.* 59:457–63
 82. Smith A, Webley DJ. 1968. A verandah-trap hut for studying the house-frequenting habits of mosquitoes and for assessing insecticides. III. The effect of DDT on the behaviour and mortality. *Bull. Entomol. Res.* 59:33–46
 83. Somboon P, Lines J, Aramrattana A, Chitprarop U, Prajakwong S, et al. 1995. Entomological evaluation of community-wide use of lambda-cyhalothrin-impregnated bed nets against malaria in a border area of north-west Thailand. *Trans. R. Soc. Trop. Med. Hyg.* 89:248–54
 84. Sundavaraman S. 1958. The behaviour of *A. sudaicus* Rodenwaldt in relation to the application of residual insecticides in Tjilatjap, Indonesia. *Ind. J. Malariol.* 12:129–56
 85. Takken W, Scott T. 2003. *Ecological Aspects for Application of Genetically Modified Mosquitoes*. UR Frontis Ser., Vol. 2. Dordrecht: Kluwer
 86. Taylor DS. 1989. Preliminary field observations on the killing behavior of *Toxorhynchites amboinensis* larvae. *J. Am. Mosq. Control Assoc.* 5:444–45
 87. Thomas DD, Donnelly CA, Wood RJ, Alphey LS. 2000. Insect population control using a dominant, repressible, lethal genetic system. *Science* 287:2474–76
 88. Touré Y, Petrarca V, Traoré SF, Coulibaly A, Maiga HM, et al. 1998. The distribution and inversion polymorphism of chromosomally recognized taxa of the *Anopheles gambiae* complex in Mali, West Africa. *Parassitologia* 40:477–511
 89. Trpis M. 1973. Interaction between the predator *Toxorhynchites brevipalpis* and its prey *Aedes aegypti*. *Bull. World Health Organ.* 49:359–65
 90. von Borstel RC. 1960. Population control by release of irradiated males. *Science* 131:878–82
 91. World Health Organisation. 1976. WHO-supported collaborative research in India: the facts. *WHO Chron.* 30:131–39
 92. World Health Organisation. 1986. Resistance of vectors, and reservoirs of disease to pesticides. *World Health Organ. Tech. Rep. Ser.* 737
 93. Yasuno M, Macdonald WW, Curtis CF, Grover KK, Rajagopalan PK, et al. 1978. A control experiment with chemosterilised male *Culex pipiens fatigans* in a village near Delhi surrounded by a breeding-free zone. *Jpn. J. Sanit. Zool.* 29:325–43

Copyright of Annual Review of Entomology is the property of Annual Reviews Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.