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Field measurement of the effective dominance of an insecticide resistance in anopheline mosquitos*

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Anopheles culicifacies *that were susceptible, heterozygous, or homozygous resistant to HCH and dieldrin were differentially marked with fluorescent dusts and released twice weekly into village huts in Pakistan that had been sprayed with four different dosages of HCH to see which of the genotypes died and which survived. The three highest dosages killed all three genotypes in the first four weeks, and heterozygotes and susceptibles for at least 12 weeks. The lowest dosage killed all the susceptibles throughout the period, and all but 0.07% of the heterzygotes. Thus the resistance is effectively recessive at the higher dosages and unlikely to be selected rapidly, as long as the gene frequency is low to start with and the houses are sprayed regularly. Similar releases of partially and completely resistant* A. stephensi, *and completely resistant* A. subpictus, *showed greater survival rates on exposure to the high HCH dosages than the same genotypes of* A. culicifacies.

The effectiveness of the selection of any genetic factor depends mainly on the original gene frequency, its dominance, and the selection pressure. When an allele is rare, it is likely to occur only in the heterozygous state. Thus a change in the frequency of that allele requires the survival of the heterozygotes until the gene frequency has risen to a level at which increasing numbers of homozygotes are produced.

Insecticide resistance in mosquitos usually depends on relatively simple genetic mechanisms. The dose/mortality relationships for the susceptibility of homozygotes and heterozygotes are usually such that the heterozygote is intermediate between the two homozygotes though it may resemble one homozygote more than the other, and in many cases there is no specific dose that can conclusively discriminate heterozygotes from homozygotes in the laboratory. It is not easy to relate the results of laboratory tests (in which mosquitos are confined on impregnated papers for a standard time) to conditions in the field where sprayed surfaces are irregular, and the mosquito is free to either rest or not rest on them. It is therefore difficult to judge from laboratory tests whether the dose of insecticide received by mosquitos in the field is above or below the lethal dose for the heterozygote, i.e., whether resistance is effectively recessive or dominant. Also with residual insecticides, the effective dominance may change as the chemical breaks down. The effective dominance under field conditions, and the extent of the increased probability of survival of the resistant homozygotes over the heterozygotes, and of the heterozygotes over susceptible homozygotes, would be expected to have a dramatic effect on the rate of evolution of resistance (*1, 2*) and on the feasibility of delaying it (*3–8*).[a,b]

The relationship between the rate of evolution of resistance and the level of resistance is exemplified by the slow increase of DDT resistance in *Anopheles sundaicus* (*9, 10*), *A. albimanus* (*11*), and *A. quadrimaculatus* (*14*) because the level of resistance in the homozygote was low (10–50 times increase in the LD_{50}) and the gene was virtually recessive. Only when a high level of resistance is attained with a resistance gene in the heterozygous state does the evolution of DDT resistance proceed rapidly—as seen in *A. stephensi* in Iraq (*14*). Similarly, the rapid evolution of dieldrin resistance in many anopheline species can be attributed to the high levels of resistance in the heterozygote. In *A. arabiensis*, for example, the

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[a] MUIR, D. A. *Genetic aspects of developing insecticide resistance of malaria vectors. Part I. Selection pressure.* Unpublished WHO document, VBC/75.571, 1975.

[b] MUIR, D. A. *Genetic aspects of developing insecticide resistance of malaria vectors. Part II. Gene flow and control patterns.* Unpublished WHO document, VBC/77.659, 1977.

heterozygotes proved to be 30 times more resistant, whereas, the homozygotes were 800 times more resistant than the susceptible homozygote (*14*). Although there is cross-resistance from dieldrin to other cyclodienes and gamma HCH, the resistance factors for HCH are only 8 and 30 times higher for the heterozygote and homozygote, respectively. Therefore one would predict that the increase in the frequency of this resistance under HCH pressure would be slower than under dieldrin pressure.

Davidson & Pollard (*15*) attempted to measure the effective life of dieldrin and HCH against the three genotypes. Mosquitos from a laboratory colony of *A. arabiensis* of standard age were confined for various times on mud surfaces that had been sprayed with 250–500 mg/m² of gamma HCH or with 500 mg/m² of dieldrin. The fresh deposits of both insecticides killed all exposed heterozygotes and susceptible homozygotes, but only HCH killed the resistant homozygotes. Eight weeks later, the lower dosage of HCH caused 100% mortality in the heterozygotes after 4 hours' exposure, the higher one gave the same result after only 3 hours, but even after 7 hours' exposure to dieldrin only 32% mortality was recorded.

This laboratory experiment was, however, unrealistic in the sense that the mosquitos were confined to treated surfaces in plastic containers and were not allowed to move between different types of treated surface as they would in sprayed huts. Within such dwellings, movement could take place between sprayed and unsprayed surfaces and between surfaces having different insecticide levels. Even disregarding any irritant effect, such as that found with DDT, mosquitos often rest in niches, which may, for operational reasons, not receive any spray. However, the tendency for the highly endophilic species to spend more than 7 hours (the maximum test time used by Davidson & Pollard) resting indoors will tend to counteract this problem. Those mosquitos that are inside houses at dawn or shortly thereafter, will probably remain inside until the following dusk—a period of 12 hours or more (*16*).

Ideally, effective dominance should be studied by controlled releases of differentially marked members of the genotypes obtained from the wild that are subsequently recaptured in biting-catches in the vicinity of sprayed structures. However, it is not possible to identify non-destructively the genotypes from the wild and therefore we released laboratory reared, differentially marked mosquitos of the three genotypes. Very low recapture rates are often recorded when mosquitos are released freely into the open air; therefore we introduced the mosquitos into closed, sprayed huts fitted with window-traps. This reduced the variation between individual mosquitos in the resting time, since the releases were made at a set time each day. Movement into and out of the huts was limited to the window-traps. Within the huts the mosquitos had complete freedom of movement and were therefore free to choose their exit-time. This was the closest approximation to "natural" conditions that we could achieve.

MATERIALS AND METHODS

The study site was Shahzada, a small village 27 km south of Lahore, Pakistan. Five rooms were hired, each about 3 m long, 2 m wide, and 2 m high (approximately 26 m² of sprayable walls and ceilings). The walls were made of mud bricks, and were lined on both sides by mud plaster. The flat brushwood roof was also covered externally with mud plaster. Therefore each room only admitted natural light through the door and through the windows (2–4 per room) that varied in size from 7.5 x 10 cm to 30 x 30 cm. The rooms were made almost light-tight by fitting a matting door externally and an internal curtain over the doorway, and by blocking all but two of the windows with bricks and mud. These two windows then became the only obvious source of light and route of exit. The traps were made from 3.8-litre cylindrical, cardboard ice-cream cartons, according to a design of Dr W. K. Reisen. One end of each cylinder was covered with netting and into the other was inserted a plastic cone with a hole at the apex 2.5 cm in diameter. Each trap was fitted into a wooden frame that was larger than the opening, and the frames were permanently fixed over the outside of the window. The traps could easily be removed from this frame and replaced. As a protection against rain, a piece of projecting metal plate was fixed to the outside wall above the trap. The temperature inside the rooms varied from 21 to 32 °C and the relative humidity varied from 60 to 82% during the experimental period (August–November 1979). This period coincided with the seasonal transmission of malaria in the Punjab when *A. culicifacies* and *A. stephensi* (the two principle vectors) are naturally abundant (*17*).

Four of the five rooms were sprayed with HCH, using a locally available wettable powder containing 13.2% of the gamma isomer. The fifth room was left as an unsprayed control. The spraying machine was a Hudson Expert, of the type usually used in malaria control operations, operating at a pressure of 27.5 Pa (2.8 kgf/cm²). Calculations were made of the weights of powder required per litre of water to give four dosages between 250 and 1000 mg of HCH/m² on the assumption that the rate of application would be 4.5 litres/100 m² of the surface. The volume left in the machine after spraying each room was measured, and the dosage estimates corrected accordingly. They

were found to be 270, 530, 700, and 880 mg of HCH/m². In accordance with normal house spraying practice, only the walls, roof, and door were sprayed. At the same time, a mud block (made of the same material as the wall surface) was sprayed for subsequent laboratory exposures of mosquitos under plastic cones (the conventional World Health Organization bioassay test).

The main test species was *A. culicifacies*[c] and the three genotypes used were: homozygous susceptible (++), heterozygous (R+), and homozygous (RR) HCH–dieldrin resistant (*18*). The ++ and RR homozygotes had been isolated from strains colonized from several locations in Pakistan (*19*) and the heterozygotes were the F_1 generation produced by crossing the homozygous stocks. In addition, colonies of local *A. stephensi* were tested—one was homozygous for HCH–dieldrin resistance, and another was unselected but known to contain R genes. Finally, wild-caught homozygous, resistant *A. subpictus* that were caught in unsprayed rooms at Shahzada (the experimental village) were tested. *A. culicifacies* and *A. stephensi* were reared in the laboratory in well water, at 28 °C, with liver powder as food for the larvae. Adults were maintained at the same temperature, and at a relative humidity of between 70 and 80%, with mice as a blood source. Each day a sample of newly emerged *A. culicifacies* adults from each rearing pan was tested (using the standard WHO dieldrin test) to confirm that there was no contamination of the genotype. Only two instances of such contamination were discovered throughout the experiment and these batches were discarded before the release date. Releases of *A. culicifacies* and *A. stephensi* contained only blood-fed females (fed during the previous night) 2–4 days old. Males were removed, as far as possible, from the cages of newly emerged mosquitos before release. Wild-caught *A. subpictus* were mainly females, which varied in physiological state (unfed, freshly-fed, half-gravid, and gravid), but there was a sizeable proportion of males in some releases.

Preparation of mosquitos for release was done in the laboratory. Mosquitos were aspirated out of their cages, anaesthetized with CO_2, and counted out on a glass plate across which a stream of CO_2 was directed. They were then transferred to 0.475-litre cartons; after revival, they were dusted with different, readily distinguishable fluorescent powders, by the method previously described by Curtis & Rawlings (*20*). Resting wild mosquitos were then collected using mouth aspirators and a motorized sweeper (*21*). Males and females were counted in the aspirator and transferred to the holding cartons for marking. Just over half-way through the experiment, the collection of wild-caught mosquitos was abandoned, as the ratio of *A. subpictus* to *A. stephensi* had fallen to such a low level. All laboratory reared mosquitos were transported to Shahzada in polystyrene insulated boxes, each carton being covered with a damp cotton wool pad. Usually the releases were made between 08h30 and 09h30. Once the traps had been put in place and the curtain drawn, the mosquitos were released by removing the cotton wool pad, the carton collar, and the netting. Gentle tapping of the sides of the carton usually induced most of the mosquitos to fly out, except in the case of homozygous resistant *A. culicifacies*, which were reluctant to leave the carton. Some injury inevitably occurred during handling and dusting, and the mosquitos that were unable to fly out of the carton were kept, counted on return to the laboratory, and subtracted from the totals released.

Exit-traps were removed about 45–60 min after dusk, and replaced by empty ones that were then left in place overnight. The following morning at 06h00 the replacement traps were removed. Later the same morning, some 15 hours after removal of the evening window traps and 4 hours after removal of the morning ones, their contents and the mosquitos found on the floor were classified as live or dead, and identified by species and genotype—the latter being indicated by the fluorescent marking observed with the aid of an ultraviolet lamp.

In the early part of the experiment, the exit-traps soon became contaminated with HCH as a result of its volatility. This contamination was detected by leaving heterozygous *A. culicifacies* in them overnight. Contaminated traps were washed with soap and water and left in the sun to dry. Subsequently, little or no mortality occurred even with homozygous susceptible *A. culicifacies* held in the traps overnight. Releases were made on Wednesday and Sunday of each week for 13 weeks. Simultaneously with the releases into the huts, standard WHO bioassay cone tests were carried out on the hut walls as well as on sprayed mud blocks similar to those described by Davidson & Pollard (*15*).

RESULTS

Experiments with A. culicifacies *in huts*

The proportions of mosquitos recovered alive from the twice weekly releases of *A. culicifacies* have been combined and are presented in Table 1. Fig. 1 shows fortnightly averages for each of the sprayed huts as a percentage of the control-hut recoveries. Over the 13 weeks, no homozygous susceptible (++) *A. culicifacies* and only 3 heterozygotes (R+) were recovered alive from the sprayed huts. In the huts given the three highest doses, almost all the mosquitos were killed during the first 4–6 weeks, then the recoveries of live

[c] *The taxon Anopheles culcifacies* is now recognized as comprising at least two sibling species and all references to it in this paper refer to species A of Green & Miles (*22*).

Table 1. Percentage of released mosquitos recovered alive from sprayed and unsprayed huts[a]

Genotype	Dosage of HCH (mg/m²)	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13	Average	Average corrected for control
RR	880	0.5	0.2	0	0	0	0	0.7	0.8	1.6	3.6	1.8	1.5	2.7	1.03	5.0
RR	700	0.4	0.3	0	0	0.7	1.2	1.9	2.1	2.8	2.6	1.1	0.9	3.0	1.30	6.3
RR	530	—	0.2	0.3	0	0.9	1.1	1.0	0.8	0.8	2.5	2.8	0.6	2.0[b]	1.00	4.9
RR	270	0	5.2	6.3	2.8	3.5	11.0	7.9[b]	7.6	8.8	17.8[b]	23.1	13.4	10.1	9.07	44.1
RR	0	—	27.9	17.4	21.7	23.2	19.4	15.8	24.8	18.1	22.4	29.1	11.9	13.9	20.4	—
R+	0	—	33.2	39.0	48.6	51.7	63.8	36.8[b]	38.0	36.0	29.4[b]	33.5	19.6	31.8[b]	38.4	—
++	0	—	24.0	15.0	34.4	43.7	37.8	46.5	38.2	24.2	35.8	26.2	9.6	25.6	30.1	—

[a] Mean numbers of blood-fed female *A. culicifacies* released were: RR 179.0 (SD 62.8); R+ 279.2 (SD 52.0); ++ 173.0 (SD 64.0).

[b] On these weeks, one heterozygote (0.3% of those released) was recovered live in the 270 mg of HCH/m² (weeks 7 and 10) and 530 mg of HCH/m² (week 13) sprayed huts.

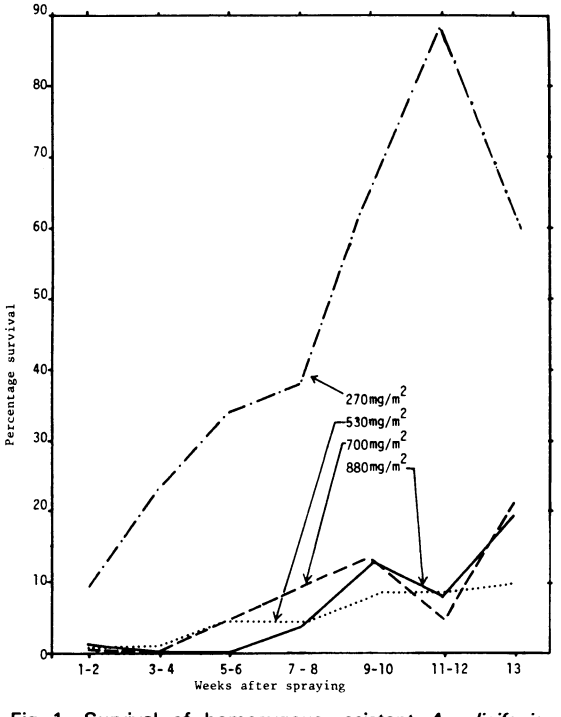

Fig. 1. Survival of homozygous resistant *A. culicifacies* (RR) in four sprayed huts, corrected for control mortality, and grouped into fortnightly averages. One heterozygote survived in weeks 7–8 and one in weeks 9–10 in the hut sprayed with 270 mg of HCH/m², and one heterozygote survived in week 13 in the hut sprayed with 530 mg/m². None of the ++ homozygotes survived in any of the sprayed huts.

RR in the window-traps increased steadily until the end of the experiment. However, even after 3 months, only small proportions of the RR survived the natural resting period (which shortened during the experiment as winter approached) in the three huts with the higher dosages. There was little difference in the rate of recovery of live RR between these three huts except that both the 530 mg of HCH/m² (the recommended WHO target dose) and the 700 mg of HCH/m² huts began to yield live recoveries 2 weeks before the hut with the highest dose.

The hut with the lowest dose (270 mg of HCH/m²), however, quickly began to yield *A. culicifacies* RR survivors. After week 9, the HCH dosage had decayed to such an extent that it was virtually ineffective against the homozygous resistants but still exerted complete control over the homozygous susceptibles and almost complete control of heterozygotes. In fact, only two live recoveries were made from this hut out of the 7392 mosquitos that had been released inside it.

Table 1 shows that *A. culicifacies* RR recoveries in the control hut were considerably lower than those for the other two genotypes. This is understandable in view of the observation that at the time of release the RR adults were reluctant to leave the holding cartons. Therefore, the relatively small catches in the window-traps of the control hut were probably due to the low mobility that may be either a characteristic of the RR genotype itself or due to other genes that happen to be carried by the RR stocks. Recoveries of R+ in the control hut were generally higher than recoveries of ++ (higher in 10, lower in 2, and equal in 1 week of the 13-week experimental period). This might have

been due to undetected low-level insecticidal contamination of the control hut, which would affect the very sensitive + + strain, or might have been the result of hybrid vigour of the R+, which was the F_1 from crosses between two inbred homozygous strains.

Simulations based on the experiments with A. culicifacies *in sprayed huts*

The data on *A. culicifacies* in two of the huts was used to try to assess the probable rates of evolution of resistance if the observed mortalities of the different genotypes were repeated on a population-wide scale. The average mortality of RR mosquitos exposed to each dosage was calculated over the whole period (Table 1) and corrected for the average control mortality. The data for the 700 and 270 mg doses of HCH/m² were selected for further study. There was no heterozygote survival at the former dose, and only 2 out of 7392 survived on exposure to the lower dose of HCH—0.027% or 0.07% when corrected for the survival of the heterozygote unsprayed controls. This estimate has an extremely high standard error, since it is based on only two survivors. These data on the probability of surviving a day in a sprayed house are taken as quantitative measurements of the relative fitness of the genotype to survive in a village in which all the houses are sprayed with the specified dose every three months. This procedure can be justified as an approximation because with an endophilic species such as *A. culicifacies*, at least one day of house resting occurs between emergence and egg laying. Table 2 shows the estimates of relative fitness, for the cases where all the houses are sprayed and where 1% of the houses are unsprayed, that is where there are "*refugia*" in the sense used by Georghiou & Taylor (*4*) and Wood & Mani (*8*). Table 2 also shows a case where there are several different categories of house, which is intended to represent a village where high-dosage spraying has been used, but in which the residents have allowed the residue to decay without renewal for more than 3 months in 10% of the houses.

Table 2. Percentage probability of surviving one day in a hut sprayed with HCH, averaged over a 3-month spraying cycle

HCH dosage (mg/m²)	RR	R+	++
All houses sprayed			
(1) 700	6.3	0	0
(2) 270	44.1	0.07	0
1% "refugia"			
(3) 700	7.2	1.0	1.0
(4) 270	45.6	1.07	1.0
(5) 90% of (3) + 10% of (4)	11.0	1.007	1.0

The values in Table 2 were substituted in the equation:

$$p_1 = \tfrac{1}{2}\{(ap_0^2 + bp_0q_0)/(ap_0^2 + 2bp_0q_0 + cq_0^2) + p_0\}$$

where p and q are the frequencies of the resistance and susceptibility genes and the subscripts 0 and 1 refer to the generations; a, b, and c are the relative fitness values from Table 2. The equation assumes that males do not enter houses and are therefore not exposed to selection (hence the last term p_0 is unmodified by any of the fitness parameters). The equation also makes the approximation that there is a Hardy Weinberg ratio in the female population despite the strong selective pressures.

Fig. 2 shows the result of iterative solutions to the equation using the 5 sets of fitness parameters in Table 2. Also, if there are no *refugia*, the population would evolve a high level of resistance from an assumed initial frequency of the resistance gene of 0.1% in one or two generations. However, as soon as a few *refugia* are assumed the situation is transformed, so that even with the high-dosage treatment (line 3) the appearance of a high level of resistance is delayed for over 300 generations (i.e., about 25 years). This is because in the sprayed houses only a minute number of RR homozygotes survive, and these are overshadowed in the breeding population by the females that survived by resting in the *refugia*. Even the small degree of

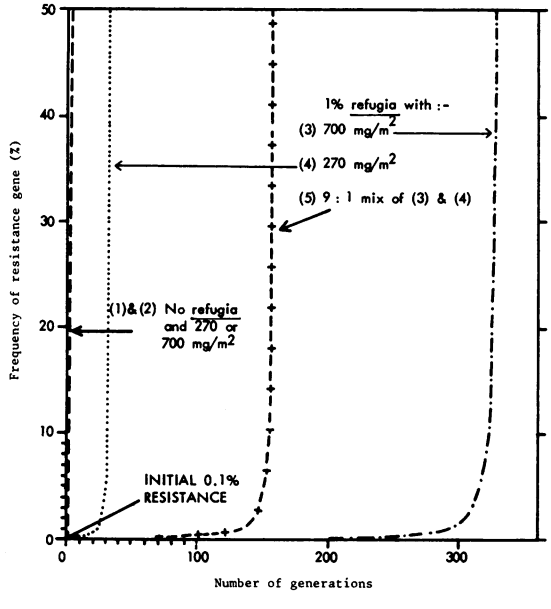

Fig. 2. Predictions regarding changes in the frequency of the resistance gene under different spraying conditions, with and without *refugia*.

Table 3. Percentage of released mosquitos recovered alive[a] from huts sprayed with HCH

Week	HCH dosage (mg/m²)											
	880			700			530			270		
	Wild	StUn	StSl	Wild	StUn	StSl	Wild	StUn	StSl	Wild	StUn	StSl
1	7.3	–	–	3.9	–	–	1.1	–	–	32.6	–	
2	41.3	0	–	18.7	0	–	11.5	4.8	–	75.3	0	
3	25.9	–	–	17.6	–	–	7.4	–	–	7.4	–	
4	–	0	3.5	–	–	2.5	–	0	0	–	0	19.0
5	24.5	0	0	18.0	0	0	38.5	0	0	44.6	0	6.1
6	11.5	0	0	65.3	0	7.9	36.4	0	0	67.3	0	16.8
7	–	–	–	–	–	–	–	0	–	–	0	–
8	–	–	–	–	–	–	–	0	12.5	–	0	8.5
11	–	9.6	–	–	4.5	–	–	1.2	–	–	9.2	–
12	–	0	–	–	3.9	–	–	7.1	–	–	18.1	–
13	–	0	–	–	14.4	–	–	8.9	–	–	18.5	–

[a] Corrected for control mortality.

Wild = wild-caught mosquitos (mostly *A. subpictus*).
StUn = reared *A. stephensi* (unselected strain).
StSl = reared *A. stephensi* (selected resistant strain).

advantage of the heterozygotes over the susceptible homozygotes found with the low-dosage spraying greatly speeds up the evolution of resistance, which then appears in about three years. If it is intended to spray regularly with the high dosage, but 10% of previously sprayed houses are not re-sprayed and their residues decay to the equivalent of a lower-dosage spray, the efficacy of spraying with a high dosage to delay the development of resistance is undermined and resistance appears in about 13 years.

Experiments with A. stephensi *and* A. subpictus *in sprayed huts*

The percentage of live recoveries of reared *A. stephensi* (Table 3), both unselected and selected, generally followed those for *A. culicifacies*. During the first 11 weeks, only a few of the selected RR strain of *A. stephensi* were recovered from the huts that had been sprayed with the three higher dosages, whereas after only 4 weeks, the first release of this strain of *A. stephensi* into the hut sprayed with 270 mg of HCH/m² yielded many survivors, as did subsequent releases. In samples of the unselected stock, which contained a small proportion (less than 10%) of RR, there was almost 100% mortality until after week 8 (no releases were made during weeks 9 and 10). Even 2 months after spraying, this group of mosquitos, which is probably representative of a natural population evolving resistance, yielded only a small number of live recoveries from any of the treated huts.

The series of releases of wild mosquitos (mostly RR *A. subpictus*) produced live recoveries from all 4 huts even in the first week. The survival rate increased in successive weeks until the density of the *A. subpictus* naturally declined at about week 5. Further releases were not possible owing to lack of material.

Cone bioassays

Simultaneously with releases into the huts to simulate natural resting conditions, WHO bioassay cone-tests were carried out. Blood-fed *A. culicifacies* of the three genotypes (differentially colour-marked) were transported to the study site, and exposed for 30 min inside the cones on the hut walls. These tests were started during week 5, and almost zero mortality was found in all tests among the homozygous resistant mosquitos. Half-hour exposures on the sprayed walls in week 5 did not kill all the heterozygotes (even in the hut with the highest dosage), and from week 8 to week 11 only the hut sprayed with 700 mg of HCH/m² caused a high mortality in the heterozygotes (Table 4).

The first homozygous susceptible survivors from the bioassay cones occurred during week 9 in the hut with the lowest dosage. By week 13, all but the hut sprayed with 700 mg of HCH/m² yielded ++ survivors after a half-hour exposure.

Table 4. Number of blood-fed female *A. culicifacies* killed, and number tested, in cone bioassay tests carried out on the hut walls, in sprayed and unsprayed huts[a]

HCH dosage (mg/m²)	Week												
	5	6	8	9[b]		10		11		12		13	
	R+	R+	R+	R+	++	R+	++	R+	++	R+	++	R+	++
880	17/19	9/12	1/25	2/23	21/21	0/22	21/21	0/18	22/22	0/20	24/24	3/18	22/23
700	14/15	11/11	12/23	20/23	18/18	13/20	15/15	13/20	24/24	0/22	24/24	0/20	23/23
530	14/21	6/12	1/19	1/22	20/20	1/28	21/21	0/19	24/24	0/19	23/23	3/19	1/12
270	7/19	1/12	2/23	0/22	19/20	0/ 9	17/17	0/24	24/24	0/20	16/20	1/18	9/20
0	2/20	1/12	1/23	1/22	5/21	0/26	0/17	1/21	2/19	0/20	0/22	0/20	1/25

[a] In all tests the RR mortality was similar to that in the unsprayed hut.
[b] Before week 9, the ++ mortality was 100% in all the sprayed huts.

Three sets of half-hour exposures in the plastic bioassay cones were made on mud blocks that had been sprayed at the same time as the huts. The first week after spraying, all but one of the blood-fed female *A. culicifacies* exposed were killed, the one RR survivor being from the block with the lowest dosage (Table 5). By week 4, virtually no RR or R+ were killed after a half-hour exposure (no ++ exposed), and there were incomplete kills of ++ by week 7.

DISCUSSION

This study, using the technique of releasing mosquitos of known genotype into sprayed huts and observing their mortality over approximately the natural resting period, has provided previously unavailable information on the actual selective impact of HCH residues at different initial dosages and at different ages of the residues.

The continued, complete kill of homozygous susceptible female *A. culicifacies* and the almost complete kill of those heterozygous for HCH–dieldrin resistance in all the sprayed huts, indicates that this resistance is virtually recessive for three months after the application of insecticide. In addition, considerable control was exerted over homozygous resistants both in the huts treated with the three higher doses, and, initially, in the hut treated with the lowest dosage. Therefore, a high target dosage, such as 880 mg of HCH/m², would be expected to give effective control of the mosquito population for almost two months, irrespective of the presence of strains resistant to HCH–dieldrin before spraying commenced. However, the decay of the HCH on the sprayed walls would eventually allow survival of RR homozygotes; and, after considerable further decay, of the heterozygotes as well.

Our simulations used the simplifying approximation that the population was in Hardy Weinberg

Table 5. Number of blood-fed female *A. culicifacies* killed, and number tested, in cone bioassay tests carried out on mud blocks in the laboratory

HCH dosage on the block (mg/m²)	Week								
	1			4			7		
	RR	*R+*	++	*RR*	*R+*		*RR*	*R+*	++
880	17/17	17/17	21/21	0/29	2/16		1/22	1/15	13/15
700	16/16	15/15	10/10	0/16	1/16		0/29	2/13	13/15
530	–	–	–	2/27	0/13		0/21	1/14	5/12
270	11/12	11/11	20/20	0/15	0/15		1/22	0/19	7/12
0	0/10	0/9	1/12	0/19	1/12		0/19	1/15	2/14

equilibrium. More recent computer simulations by Dr G. S. Mani (personal communication), which did not require this simplification, showed a reduction of about 10% in the times required for evolution of resistance. However, the three conditions represented by curves 3, 4, and 5 in Fig. 2 showed the same relationship with respect to each other. Even these improved simulations do not provide accurate predictions about the evolution of resistance because of unknown factors—such as the initial frequency of resistance genes; whether there is any appreciable selection on males; the exact proportion of heterozygous survival at the lower dosage; the proportion of the population in *refugia*; and the effect of cumulative exposure of females on repeated visits to sprayed houses. However, we consider that the simulations give substance to the theoretical conclusions of Comins (*3*), Curtis (*7*), and Wood & Mani (*8*), since the present simulations are based on fitness parameters that are derived from observations made under realistic conditions. The conclusions that are underlined by the present work are the desirability of maintaining residues at a level known to be high enough to kill all heterozygotes. Even a very small deviation from complete recessiveness of resistance has a disastrous effect, and this is likely to arise if a small proportion of previously sprayed houses are not resprayed regularly. From the point of view of the evolution of resistance, totally unsprayed houses (e.g., houses built since the last spraying cycle) are beneficial in providing *refugia*. However, too many houses in this condition would, of course, be harmful from the point of view of effective malaria control.

Though we have shown that effective recessive resistance can be achieved for HCH, unfortunately there are few areas in the world where there is not an already high level of resistance within the malaria vector population to this cheap, safe, and effective insecticide. However, Sri Lanka is one example of an area where HCH could be used effectively for the control of *A. culicifacies*. We consider it highly desirable that similar experiments should be carried out to investigate resistance to organophosphorus compounds. These can only be done in areas where resistance has already evolved. However, the information that they would provide could only be of use to other (more fortunate) areas in which there is as yet no appreciable resistance. In the case of new insecticides to which there is no known resistance, there can be no certainty about how to ensure the recessiveness of the new resistance mechanisms that would evolve. However, it seems unwise to use dosages and treatment schedules that are only barely sufficient to kill all the existing susceptible population.

The data from the bioassay cone-tests showed that a half-hour exposure 13 weeks after spraying did not kill all ++ *A. culicifacies* females, and that many R+ survived these tests after only 5 weeks. When ++ females were used, the test suggested that HCH was effective only against a susceptible population for the first 11 weeks, whereas the hut releases that allowed a longer resting period inside the huts showed that even after 13 weeks virtually no R+, let alone ++, females could be recovered live in the window exit-traps. The use of half-hour exposures (in plastic cones) to sprayed walls, therefore gives a very misleading impression of the real lifespan of the insecticide against any type of population, even if it is homozygous susceptible. The small amount of data from similar exposures on sprayed mud blocks reinforced this finding. The HCH seemed to decay very quickly on these mud blocks and this information in isolation would have indicated that the toxicity of the HCH was much too short-lived to make its use an economic proposition.

Against the body of data from the release of laboratory reared mosquitos stands the high levels of live recoveries of wild-caught adults. Even though these releases were not continued throughout the experiment and the specific composition changed slightly each week—as the density of *A. subpictus* dropped and that of *A. stephensi* rose—the low mortality caused by HCH to these wild populations is disturbing. The resting periods in the huts of the wild and laboratory reared mosquitos must have been the same and therefore the differences were possibly due to differences in the size of adults, since *A. subpictus* is twice the size of *A. culicifacies*. However, variation in the body size of *A. culicifacies* does affect the dose–response curve to dieldrin (*23*). Such discrepancies between wild and laboratory reared releases, again highlight the need for field techniques that use or closely simulate natural conditions.

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RÉSUMÉ

MESURE SUR LE TERRAIN DE LA DOMINANCE EFFECTIVE D'UNE RÉSISTANCE AUX INSECTICIDES CHEZ LES ANOPHÈLES

Des *Anopheles culicifacies* des trois génotypes de résistance/sensibilité aux HCH-dieldrine à transmission monofactorielle ont été marqués de façon différente avec des poudres fluorescentes et lâchés dans 5 huttes expérimentales. Différentes doses de HCH ont été pulvérisées dans 4 des huttes, la cinquième non traitée servant de témoin. Les anophèles ont été lâchés le matin, deux fois par semaine pendant 13 semaines, et les survivants ont été récoltés le soir même et le matin suivant à la sortie dans des pièges de fenêtre. Les 3 huttes contenant les doses les plus élevées ont tué les 3 génotypes pendant 4 semaines, et les hétérozygotes et homozygotes sensibles pendant plus de 12 semaines. La hutte contenant la dose la plus faible a rapidement produit sélectivement des survivants homozygotes résistants, tout en tuant effectivement tous les homozygotes sensibles, et tous les hétérozygotes sauf environ 0,7%.

Les simulations, fondées sur les mortalités observées chez les 3 génotypes, font ressortir la nécessité d'utiliser des doses d'insecticide relativement élevées et de les appliquer régulièrement dans toutes les habitations. Dans ces conditions, et si seule une faible proportion de la population d'anophèles échappe complètement au contact avec l'insecticide, on peut escompter que l'évolution de la résistance sera ralentie pendant des décennies. Toutefois, l'existence d'un certain nombre de dépôts anciens dégradés sur lesquels les hétérozygotes pourraient gagner même un très petit avantage par rapport aux homozygotes sensibles pourrait grandement accélérer l'evolution de la résistance.

Les expériences de lâchage de moustiques dans les huttes ont également été pratiquées avec des colonies d'*A. stephensi* partiellement ou totalement résistantes et avec des souches homozygotes résistantes d'*A. subpictus* capturées dans la nature. Ces deux espèces ont survécu sur des dépots de teneur en HCH beaucoup plus élevée que dans le cas de *A. culicifacies*. Ont été également entrepris sur les murs des huttes et sur des mottes de pisé traitées des essais biologiques comparatifs au moyen de chambres d'exposition coniques. Les résultats ont montré une très faible corrélation avec les expériences beaucoup plus réalistes de lâcher de moustiques dans les huttes.

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