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Modifications of pyrethroid effects associated with *kdr* mutation in *Anopheles gambiae*

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Abstract. Effects of knockdown resistance (*kdr*) were investigated in three pyrethroid-resistant (RR) strains of the Afrotropical mosquito *Anopheles gambiae* Giles (Diptera: Culicidae): Kou from Burkina Faso, Tola and Yao from Côte d'Ivoire; compared with a standard susceptible (SS) strain from Kisumu, Kenya. The *kdr* factor was incompletely recessive, conferring 43-fold resistance ratio at LD_{50} level and 29-fold at LD_{95} level, as determined by topical application tests with Kou strain. When adult mosquitoes were exposed to 0.25% permethrin-impregnated papers, the 50% and 95% knockdown times (KdT) were 23 and 42 min for SS females, compared with 40 and 62 min for RS (F_1 Kou × Kisumu) females. On 1% permethrin the KdT_{50} and KdT_{95} were 11 and 21 min for SS compared with 18 and 33 min for RS females. Following 1 h exposure to permethrin (0.25% or 1%), no significant knockdown of Kou RR females occurred within 24 h.

Permethrin irritancy to *An. gambiae* was assessed by comparing 'time to first take-off' (TO) for females. The standard TO_{50} and TO_{95} values for Kisumu SS on untreated paper were 58 and 1044 s, respectively, vs. 3.7 and 16.5 s on 1% permethrin. For Kou RR females the comparable values were 27.3 s for TO_{50} and 294 s for TO_{95}, with intermediate RS values of 10.1 s for TO_{50} and 71.9 s for TO_{95}. Thus, TO values for RS were 2.7–4.4 times more than for SS, and those for RR were 7–18 times longer than for SS.

Experiments with pyrethroid-impregnated nets were designed to induce hungry female mosquitoes to pass through holes cut in the netting. Laboratory 'tunnel tests' used a bait guinea-pig to attract mosquitoes through circular holes (5 × 1 cm) in a net screen. With untreated netting, 75–83% of laboratory-reared females passed through the holes overnight, 63–69% blood-fed successfully and 9–17% died, with no significant differences between SS and RR genotypes. When the netting was treated with permethrin 250 mg ai/m^2 the proportions that passed through the holes overnight were only 10% of SS vs. 40–46% of RR (Tola & Kou); mortality rates were 100% of SS compared with 59–82% of RR; bloodmeals were obtained by 9% of Kou RR and 17% of Tola RR, but none of the Kisumu SS females. When the net was treated with deltamethrin 25 mg ai/m^2 the proportions of *An. gambiae* that went through the holes and blood-fed successfully were 3.9% of Kisumu SS and 3.5% of Yaokoffikro field population (94% R). Mortality rates were 97% of Kisumu SS vs. 47% of Yaokoffikro R. Evidently this deltamethrin treatment was sufficient to kill nearly all SS and half of the Yaokoffikro R *An. gambiae* population despite its high *kdr* frequency.

Experimental huts at Yaokoffikro were used for overnight evaluation of bednets against *An. gambiae* females. The huts were sealed to prevent egress of

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mosquitoes released at 20.00 hours and collected at 05.00 hours. Each net was perforated with 225 square holes (2 × 2 cm). A man slept under the net as bait. With untreated nets, only 4–6% of mosquitoes died overnight and bloodmeals were taken by 17% of SS vs. 29% of Yaokoffikro R ($P < 0.05$). Nets treated with permethrin 500 mg/m^2 caused mortality rates of 95% Kisumu SS and 45% Yao R ($P < 0.001$) and blood-feeding rates were reduced to 1.3% of SS vs. 8.1% of Yao R ($P < 0.05$). Nets treated with deltamethrin 25 mg/m^2 caused mortality rates of 91% Kisumu SS and 54% Yao R ($P < 0.001$) and reduced blood-feeding rates to zero for SS vs. 2.5% for Yao R ($P > 0.05$).

Pyrethroid-impregnated bednets in experimental huts and 'tunnel tests' gave equivalent results, showing that nets impregnated with permethrin or deltamethrin provided good levels of protection against *kdr* homozygous strains of *An. gambiae* (Kou and Tola), and against the field population at Yaokoffikro with 94% *kdr* frequency. The explanation seems to be that (a) high proportions of *kdr* females are killed by prolonged contact with pyrethroids through diminished sensitivity to the usual irritant and repellent effects, and (b) relatively few *kdr* females take advantage of this prolonged contact to ingest a bloodmeal.

Key words. *Anopheles gambiae*, bednets, deltamethrin, excito-repellency, irritancy, *kdr*, knockdown resistance, impregnated nets, malaria control, permethrin, pyrethroid resistance, Côte d'Ivoire, West Africa.

Introduction

For the prevention of human malaria, vector control is an important component of the global strategy (WHO, 1993). Vector control aims to prevent the transmission of malaria parasites by methods such as house-spraying with residual insecticides (Pampana, 1969) or personal protection by the use of pyrethroid-impregnated bednets (Lengeler *et al.*, 1996).

Mosquitoes of the *Anopheles gambiae* complex are major vectors of malaria in tropical Africa, where ~90% of the world's malaria cases occur. Resistance of *An. gambiae* Giles *sensu stricto* to pyrethroid insecticide was first observed by Elissa *et al.* (1993) in Côte d'Ivoire, West Africa, with significantly reduced mortality after exposure to permethrin and lower knockdown effects from deltamethrin and lambda-cyhalothrin. More recently, knockdown resistance (*kdr*) to pyrethroids and DDT was observed by Chandre *et al.* (1999a) in *An. gambiae sensu lato* from several West African countries. Investigations on the target site of action for pyrethroids and DDT showed that *kdr* was associated with a point mutation of the gene coding for the sodium channel structure of the nerve, resulting in one amino-acid change (Martinez-Torres *et al.*, 1998). As expected from their common mode of action on the sodium channel, this mutation conferred cross-resistance to a wide range of pyrethroids and DDT (Chandre *et al.*, 1999b).

Pyrethroids have the advantages of acting very rapidly as insecticides, with both knockdown and lethal effects at dosages very well below the threshold of mammalian toxicity (Chavasse & Yap, 1997). They also produce excito-repellent effects on mosquito behaviour. When used for impregnation of bednets or curtains, the overall effect of pyrethroids is as much excito-repellent as lethal. For personal protection against mosquitoes, the excito-repellent and rapid knockdown effects of pyrethroids are even more important than mortality. Therefore, we investigated whether these different effects could be modified by pyrethroid resistance due to *kdr*, and what consequences it may have on the protective efficacy of impregnated netting. Behaviour of *kdr*-type resistant mosquitoes exposed to impregnated netting was studied in cages with simulated field conditions and using experimental huts in the field.

Material and Methods

Mosquitoes

Four strains of *Anopheles gambiae* Giles *s.s.* (identified by the PCR method of Scott *et al.*, 1993) were assessed for *kdr* frequency by the method of Martinez-Torres *et al.* (1998) and used for bioassays and genetic crosses.

– Kisumu: a susceptible (SS) reference strain from Kenya originated in 1953.

– Kou and Tola, populations from Burkina Faso and Côte d'Ivoire, respectively, resistant to pyrethroids and DDT. Both strains were selected with permethrin until becoming homozygous resistant (RR) for *kdr* in the laboratory and maintained under selection pressure. To evaluate the phenotypic expression of *kdr* in heterozygotes (RS), F_1 progeny were produced from crossing Kou males (RR) with Kisumu females (SS).

– Yao: a field population from Yaokoffikro, a suburb of Bouaké, Côte d'Ivoire, with *kdr* allelic frequency of 94.4% (91.7% RR, 5.5% RS, 2.8% SS). Laboratory tests were made on adult females obtained from field-collected larvae.

Mosquitoes were maintained by standard methods in an insectary at 27 ± 2°C, ≈ 80% relative humidity. Investigations on each strain are summarized in Table 1.

Table 1. Summary of investigations on *Anopheles gambiae* strains: see text for details.

	Test				
				Net hole pass	
Strain	Topical application (LD)	Susceptibility by WHO test (KdT, Mortality)	Irritancy (TO)	Lab tunnel	Field bednet
Kisumu (SS)	permethrin	permethrin	untreated	untreated	untreated
				permethrin	
			permethrin	permethrin	
				deltamethrin	deltamethrin
Kou × Kisumu (F1 RS)	–	permethrin	permethrin	–	–
Kou (RR)	permethrin	permethrin	permethrin	permethrin	–
Tola (RR)	–	–	–	permethrin	–
Yaokoffikro (S:R ~ 6:94)	–	–	–	untreated	untreated
				permethrin	permethrin
				deltamethrin	deltamethrin

Pyrethroid insecticides

Most tests were performed with permethrin (25 : 75 cis : trans isomeric ratio) of 93.8% technical grade quality. Formulations of deltamethrin (K-Othrine® 2.5% SC) and permethrin (Peripel® 10% EC) were also used for impregnation of netting and bednets. All insecticides were obtained from Agrevo, Berkhamsted, U.K.

Topical applications

To determine precisely the resistance level conferred by *kdr* on adults of *An. gambiae*, permethrin (serial dilution in acetone) was topically applied to individual mosquito adults. Unfed female mosquitoes (3–5 days old) were briefly anaesthetized (~30 s) with carbon dioxide and placed on a cold glass surface (1–2°C). A droplet of 0.1 µl of insecticide solution was applied to the female thorax using a glass micropipette (Microcaps®). Six concentrations providing a range of 0–100% of mortality were used on 25–30 females per concentration. Tests were replicated three times. For each replicate, a batch of 25–30 females was weighed and the lethal doses (LD_{50}, LD_{95}) were calculated in nanograms per milligram of mosquito body weight. Control treatments with 0.1 µl of acetone alone gave control mortality rates of < 10%. After treatment, mosquitoes were kept in plastic cups and supplied with 10% honey solution for 24 h before counting mortality. Temperature and humidity were maintained at 26°C and 80% r.h.

Bioassays

These were carried out with WHO (1970) susceptibility test-kits for adult mosquitoes, using papers impregnated with 0.25% or 1% permethrin in Dow Corning 556 silicon oil, prepared according to Chandre *et al.* (1999a). Tests employed *An. gambiae* batches of 20–25 unfed females (3–5 day old) in test-kit tubes, with four replicates per bioassay. Mosquitoes were exposed for 60 min to impregnated papers in tubes maintained in the normal vertical position (see item 4.2.2.2 in WHO, 1998). During exposure, knockdown rates were checked periodically. After exposure, mosquitoes were supplied with 10% honey solution during 24 h before counting mortality. Temperature and humidity during observation time were maintained at 26°C and 80% r.h. Knockdown rates were expressed as KD_{50} and KD_{95} times using log-probit analysis.

Irritant effect of permethrin

This was assessed from the time until first take-off using the protocol of Mouchet & Cavalie (1961). The test involves measuring the time spent by mosquitoes on an insecticide-treated surface before their first flight. An individual unfed female (3–5 days old) was introduced under a plastic cone (11 cm diameter) on a filter paper impregnated with 1% permethrin, inclined at 45°. After a settling period of 60 s, the time until first take-off (TO) was measured, i.e. time between the first landing and the following take-off. Each test involved 30 females and was replicated three times. Paper impregnated with carrier alone (silicone oil) was tested for controls. Mosquitoes were clustered by classes of take-off time and cumulative frequencies of different classes were analysed upon a log time-probit model (Mouchet & Cavalie, 1961).

Effect of pyrethroid-impregnated netting

Polyester 100 denier multifilament netting was tested to determine if *An. gambiae* females (RR, RS & SS genotypes) were able to pass through holes of 1 cm diameter in sheets of treated or untreated netting, and whether the mosquitoes would take a bloodmeal thereafter.

Laboratory tests were performed in a square glass tunnel (height 25 cm, width 21 cm, length 60 cm) with cage ends, as described by Elissa & Curtis (1995), subdivided by a changeable piece netting with 5×1 cm holes (1% of total area) inserted on a cardboard frame across the tunnel. Netting was previously impregnated with insecticide formulation diluted in water (35 ml/m^2) to give a predetermined treatment rate (mg ai/m^2). After drying, the impregnated piece of net was stored at room temperature and used for testing one week post-impregnation. In one end of the tunnel a guinea-pig was placed as bait, held in a small metallic cage to prevent contact with the netting. In the other end of the tunnel, ≈100 unfed female mosquitoes (5–8 days old) were introduced at 18.00 hours and the apparatus was left overnight in a dark room maintained at 28°C and 80% r.h. The next morning, at 08.00 hours, the numbers of mosquitoes in both compartments were counted and their mortality and blood-feeding rates were scored. Tests were replicated two or three times for each net treatment, i.e. SC deltamethrin 25 mg/m^2, EC permethrin 250 and 500 mg/m^2, and control untreated netting.

Field tests in Côte d'Ivoire used polyester multifilament bednets (11 m^2) impregnated with EC permethrin 500 mg/m^2 (two nets), or SC deltamethrin 25 mg/m^2 (two nets). From each treated pair, one net was used for samples tested in the tunnel cage apparatus (see above). Each of the other treated bednets had two rows of 225 holes of 4 cm^2 (2×2 cm) cut along lines 20 and 30 cm from the lower edge. Each was hung in an experimental hut (Darriet *et al.*, 1999) and used every night by a sleeper from 20.00 to 05.00 hours. About 50–100 females were released in these closed huts, left overnight, and collected the next morning, when mortality and blood-feeding rates were recorded. Tests in tunnel cages and experimental huts were done in parallel, using mosquitoes from the same batches, in order to compare results obtained with these two methods. Tests were done with both the susceptible strain (Kisumu) and the local field resistant population (Yaokoffikro), using adults emerged from field-collected larvae. Each test was replicated three or four times during the 6 months following impregnation.

Data analysis

Data were analysed using log-probit software (Raymond *et al.*, 1993), based on Finney (1971). Mosquito mortality and blood-feeding rates were compared between the samples by χ^2-tests. The level of significance of each test was adjusted to take into account the other tests using the sequential procedure of Bonferroni (Rice, 1989).

Results

Topical applications

LD_{50} and LD_{95} of susceptible mosquitoes (Kisumu strain) were, respectively, 1.0 and 4.4 ng of permethrin per mg of mosquito (Table 2). The mean weight of adult females ranged from 1.1 to 1.5 mg. Resistance ratios for the Kou strain were 43-fold at LD_{50} and 28.5-fold at LD_{95} levels.

Mortality and knockdown in WHO test tubes

With both concentrations of permethrin (0.25% or 1%), less than 5% mortality was observed with Kou strain vs. almost 100% with the Kisumu susceptible strain (Table 3). High levels of mortality (80–84%) were recorded among heterozygotes (RS) obtained by crossing these two strains (F_1 progeny). Mixed batches of susceptible homozygotes (SS) and resistant heterozygotes (RS) in various proportions, exposed to 1% permethrin, showed reduced mortality in comparison with the susceptible strain only when the proportion of RS was 60% or more.

Knockdown time (KdT) was significantly more for hybrids (RS) compared to the susceptible strain, whereas RR individuals all survived without knockdown for 24 h following 1 h exposure to permethrin at both dosages (0.25% or 1%). In tests where SS and RS were mixed, a strong correlation ($P < 0.001$) was observed between KdT and the proportion of heterozygotes. However, as the variation in KdT between SS and RS was about two-fold, a significant increase of KdT was not observed before the proportion of RS reached 40%. The observed KdT_{100} increased significantly for mixed samples when the proportion of RS was at least 60%.

Irritancy of permethrin

Irritant effects of permethrin were investigated on the Kisumu (susceptible), Kou (resistant) strains and their hybrids

Table 2. Log dose-probit mortality data for permethrin topical applications on *An. gambiae* adults

Strain	*n*	LD_{50} (ng/mg)	LD_{95} (ng/mg)	RR_{50}	RR_{95}
Kisumu	480	1.024 (0.876–1.184)	4.447 (3.050–6.540)	–	–
Kou	559	43.988 (39.563–48.267)	126.730 (109.161–154.853)	43.0	28.5

LD_{50} and LD_{95} are, respectively, 50% and 95% lethal doses in nanograms per milligram of mosquito, with 95% confidence intervals in brackets.
RR_{50} and RR_{95} represent the resistance ratios for Kou/Kisumu strains at LD_{50} and LD_{95} levels, respectively.
n, number of mosquitoes tested.

Table 3. Relationship between time (min) for knockdown (KdT) and mortality rate in susceptible (SS), resistant homozygous (RR), and heterozygous (RS) strains for *kdr*. *n*, total number of mosquitoes.

Treatment	*n*	KdT50	KdT95	KdT100 obs.	Mortality %
Permethrin 0.25%					
Kisumu (SS)	100	23.3 (22.1–24.4)	42.0 (39.2–45.9)	80	98.6
Kisumu × Kou (RS)	75	40.1 (38.8–41.5)	62.2 (58.3–67.6)	>80	84.0
Kou (RR)	100	None*	None*	None*	0
Permethrin 1%					
Kisumu (SS)	200	11.4 (10.9–11.9)	18.2 (16.9–20.4)	25	100
95% SS+5% RS	80	9.6 (8.4–10.5)	19.1 (17.3–22.1)	30	99.0
90% SS+10% RS	80	11.6 (10.8–12.2)	19.5 (17.4–23.6)	20	100
80% SS+20% RS	80	12.0 (11.2–12.7)	21.4 (19.6–24.1)	30	100
60% SS+40% RS	79	14.4 (13.7–15.2)	26.3 (24.2–29.5)	35	100
40% SS+60% RS	80	16.6 (15.8–17.4)	29.3 (27.1–32.3)	50	91.3
Kisumu × Kou (RS)	102	20.7 (19.9–21.5)	32.5 (30.7–35.0)	60	80.0
Kou (RR)	100	No Kd	No Kd	No Kd	5.0

*Less than 5% knockdown after 1 h exposure.

(Fig. 1). The relationship between log time until first take-off and probit of cumulative frequency for different time classes was linear ($P>0.05$). With untreated paper, the mean times for first take-off of 50% and 95% of mosquitoes (TO_{50}/TO_{95}) were, respectively, 58.5 s and 1044 s for the susceptible Kisumu strain. On paper impregnated with 1% permethrin, TO_{50} and TO_{95} were 3.7 s and 16.5 s, respectively, indicating that susceptible mosquitoes were strongly irritated by permethrin.

For the resistant Kou strain, the TO_{50} and TO_{95} were 27.3 s and 294 s, respectively, 7.4 and 17.8 times higher than for the susceptible strain. Hybrids (RS) were exactly intermediate between those of SS and RR individuals, with values of 10.1 s for TO_{50} and 71.9 s for TO_{95}.

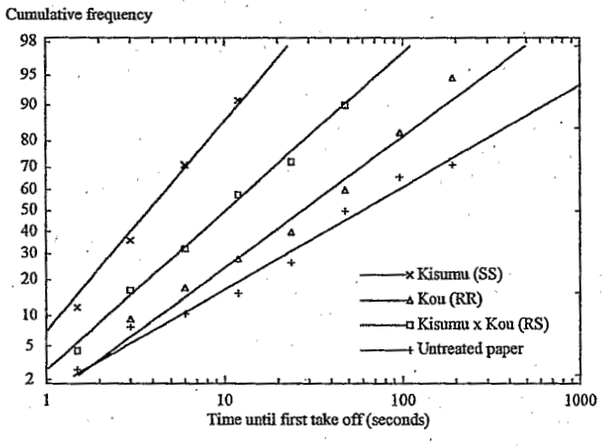

Fig. 1. Time (in seconds) until first take-off of *An. gambiae s.s.* females (2–5 days old, non-blood-fed) exposed to permethrin 1% impregnated paper.

Effects of impregnated netting

For three strains of *An. gambiae* (Kisumu, Tola, Kou) tested in the tunnel cage (Table 4), mortality rates ranged from 9% to 17% with non-impregnated netting (attributed mainly to presence of the guinea-pig and its excreta). The proportions of mosquitoes which succeeded in passing through holes in the net (75–83%) and taking a bloodmeal (63–69%) were very similar for all three strains. When exposed to permethrin-impregnated netting, all susceptible mosquitoes were killed, only 10% of them passed through the net holes, and none of them was able to blood-feed (Table 4). For both resistant strains (Tola, Kou), a significant proportion of mosquitoes survived (41%, 18%), some 40–46% passed through the holed nets, and 17% (Tola) and 9% (Kou) blood-fed successfully.

When mosquitoes of the same strain were released either in tunnel cages or experimental huts in Côte d'Ivoire, similar results were obtained for mortality and blood-feeding rates ($P>0.05$). For the susceptible reference strain (Table 5) exposed to permethrin 500 mg/m^2 and deltamethrin

Table 4. Effect of netting impregnated with permethrin 250 mg/m^2 in tunnel cage on *An. gambiae* susceptible strain (Kisumu) and *kdr* homozygous resistant strains (Tola, Kou). *n*, number tested.

Strains		*n*	Mortality %	Passed through net %	Engorged %
Kisumu (SS)	Control	200	16.0	82.0	69.0
	Permethrin	214	100	10.1	0
Tola (RR)	Control	282	9.2	83.0	69.5
	Permethrin	310	58.7	45.8	16.8
Kou (RR)	Control	328	17.0	75.0	62.8
	Permethrin	389	81.7	39.9	8.7

Table 5. Effect of bednets impregnated with permethrin 500 mg/m^2 or deltamethrin 25 mg/m^2 in tunnel cage and experimental huts on *An. gambiae* susceptible strain (Kisumu) and field resistant population (Yaokoffikro).

		Tunnel cage			Experimental hut		
Mosquitoes	Insecticide	*n*	Mortality %	Blood fed %	*n*	Mortality %	Blood fed %
Kisumu	Control	265	14.3	60.4*	126	6.3	16.7
	Permethrin	237	100*	0.4	148	94.6	1.3
	Deltamethrin	232	97.4	3.9	107	90.7	0.0
Yaokoffikro	Control	291	11.0*	20.2	247	4.1	29.2
	Permethrin	282	36.5	6.7	210	45.4	8.1
	Deltamethrin	289	46.9	3.5	198	53.9	2.5

*significant differences ($P < 0.05$) between values for tunnel and experimental huts.

25 mg/m^2, the rates of mortality were very high (97–100%) and rates of blood-feeding were only 0.4–3.9%.

Using resistant mosquitoes from the field (Yaokoffikro) the proportion blood-feeding in the tunnel cage (20.3%) was much reduced in comparison with experimental huts (60.4%). This may reflect the intrinsic affinity of field-collected *An. gambiae s.s.* for humans in preference to guinea-pigs. Mosquito mortality rates in experimental huts with treated bednets were slightly higher ($P > 0.05$) than in the tunnel with treated netting, perhaps because mosquitoes were more attracted to man in huts than to guinea-pig in the tunnel – affecting the time they spent in contact with treated netting. However, the mosquito blood-feeding rates were not significantly different between these two test procedures. With both permethrin and deltamethrin-treated bednets, *An. gambiae* mortality in huts was high (45.4% and 53.9%, respectively) and blood-feeding rates were low (8.1% and 2.5%, respectively).

Discussion

Our pyrethroid-resistant strains of *An. gambiae s.s.* were laboratory-selected with permethrin until homozygosity for the *kdr* gene (Darriet *et al.*, 1997; Martinez-Torres *et al.*, 1998). It seems that *kdr* was the only resistance mechanism involved. Bioassays using synergists and biochemical tests failed to demonstrate any involvement of metabolic detoxification due to oxidases, esterases or glutathion-S-transferases.

Insecticide resistance is commonly assessed by exposing adults to a diagnostic dosage (concentration × time), which normally induces 100% mortality of susceptible individuals (WHO, 1998). This approach is more suited to scoring resistance frequencies in populations. To determine precisely the permethrin resistance levels of individual mosquitoes, we used topical application; this precludes variable uptake of pyrethroids from treated substances in consequence of rapid knockdown and irritant effects (Hemingway, 1980; Magesa *et al.*, 1994). By topical application tests with *An. gambiae*, the Kou *kdr* strain showed permethrin resistance ratios of 30 × (RR_{95}) to 40 × (RR_{50}), within the range induced by *kdr* in other insect species (Soderlund & Bloomquist, 1990; Williamson *et al.*, 1996). The permethrin resistance ratio observed for larvae of the same strain was significantly higher, 100–300 × (Darriet *et al.*, 1997).

The very high degree of permethrin resistance shown by the Kou strain was confirmed by exposure to 1% permethrin-impregnated papers for 1 h, giving little knockdown and only 5% mortality.

As observed for *Culex quinquefasciatus* mosquitoes (Halliday & Georghiou, 1985) and other insect species (see Bourguet & Raymond, 1998 for review), inheritance of *kdr* in *An. gambiae* was incompletely recessive, with a high mortality rate of heterozygotes (80%) exposed to the diagnostic concentration. Resistance to the knockdown effect was functionally also recessive because the mean knockdown time (KdT) of heterozygotes (RS) was only two-fold more than for susceptible homozygotes (SS), with almost no knockdown of resistant homozygotes (RR). In a previous survey of DDT and pyrethroid resistance in *An. gambiae s.l.* from several African countries (Chandre *et al.*, 1999a), KdT was found to be a good

indicator for early detection of resistance, as it increased significantly before any change in mortality rates was observed in populations.

By testing samples of RS and SS mixed in various proportions, a strong correlation ($P < 0.001$) was observed for *kdr* between the RS frequency and KdT increase. This difference was detected before any change in mortality, which became evident only when the proportion of RS was > 60%. This raises the value of using KdT as an indicator for early detection of *kdr* in the field.

Kdr was associated with reduced irritant effects of permethrin, allowing mosquitoes longer contact with the impregnated substrate before taking off. This was also shown for a pyrethroid-resistant strain of *An. stephensi* (Hodjati & Curtis, 1997). Therefore, resistant mosquitoes apparently acquired more insecticide from longer duration of tarsal contact with the impregnated substrate. Attempts were made to test this hypothesis using tunnel cages, then experimental huts in the field. Preliminary tunnel experiments made in Montpellier showed that all susceptible mosquitoes died on permethrin 250 mg/m^2, whereas 20–40% of resistants survived. More interestingly, 40% passed though holes in the treated netting, vs. 10% for the susceptibles, indicating that resistant mosquitoes more effectively find a way to reach the bait, consistent with longer exposure to treated netting. Some of the resistants were also able to take a bloodmeal (9–17%, vs. zero for susceptible ones). These results indicated that *kdr* reduced the efficacy of impregnated bednets, although a high proportion of homozygous *kdr* resistant individuals were effectively killed and did not succeed to pass through netting holes. Similar conclusions were reached in Côte d'Ivoire with field resistant populations (92% *kdr* homozygotes), using both tunnel cages and experimental huts: mortality was significantly reduced (but remained high: 45.4–53.9%), while blood-feeding rates were limited to 2.5–8.1%. These paradoxical results can be partly explained by the effects of *kdr* on pyrethroid toxicity, whereby *kdr* mosquitoes can withstand more prolonged contact with a pyrethroid-impregnated net. Hence, when searching longer to feed or pass through a bednet, *kdr* individuals tend to pick up more insecticide than susceptible mosquitoes, which are sooner irritated. Thus, *kdr* individuals tend to acquire a higher dose of pyrethroid, resulting in relatively good efficacy of pyrethroid-impregnated bednets in killing resistant mosquitoes and preventing them from blood-feeding. Moreover, *kdr*-type resistance to irritant effect appeared to be dominant, whereas resistance to mortality and knockdown were only semi-dominant. Therefore, impregnated bednets are expected to have considerable efficacy against *kdr* heterozygotes. This would delay the evolution of resistance problems in populations of mosquitoes where *kdr* is rare and usually occurs in the heterozygous state.

Reduction of mosquitoes entering houses, reduction of blood-feeding rates and increase of exophily are the main factors providing the efficacy of permethrin-impregnated bednets (Darriet *et al.*, 1984). The two latter functions are consequences of the excito-repellent effect of permethrin (Hodjati & Curtis, 1997). Different insecticides show various degrees of irritancy and killing ability against mosquitoes when used for net impregnation (Miller *et al.*, 1991; Curtis *et al.*, 1992, 1998) and more detailed field investigations are necessary to evaluate these differential effects on mosquito responses. The multiple effects of *kdr* mutation probably allow relatively good efficacy of pyrethroid-impregnated bednets for personal protection in places where anopheline populations carry this type of pyrethroid-resistance. For example, in Kafine area of Côte d'Ivoire, where *An. gambiae* has high frequencies of *kdr*, one year after the distribution of permethrin-impregnated bednets malaria morbidity of children was reduced by more than 50% (Doannio *et al.*, 1997), which is similar to results obtained in areas where mosquitoes are susceptible (Choi *et al.*, 1995). We are now investigating how pyrethroid resistance evolves where multiple mechanisms occur together, as suggested for *An. gambiae* populations in Côte d'Ivoire (Chandre *et al.*, 1999b).

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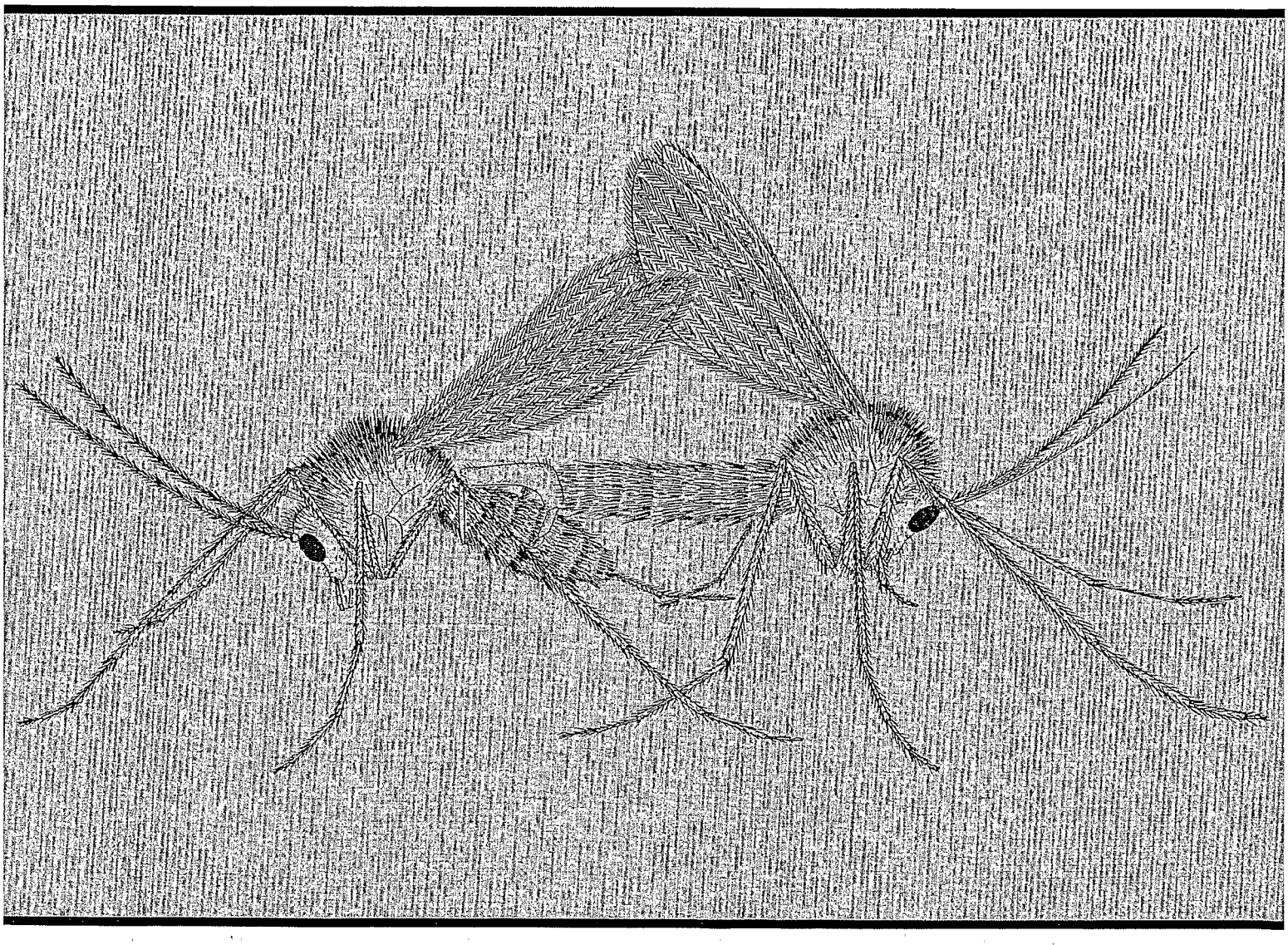

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