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TEST FOR ASSOCIATION OF DDT RESISTANCE WITH INVERSION POLYMORPHISM IN *ANOPHELES ARABIENSIS* FROM ETHIOPIA

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ABSTRACT. Association of DDT resistance levels with chromosome inversion polymorphism was investigated in *Anopheles arabiensis* samples collected from southwestern Ethiopia. The frequencies of the 2Ra, 2Rd, and 3Ra inversions in 1988 and 1990 between the DDT survivors pooled from the 3 times of exposure and unexposed controls did not differ significantly. However, for 2Rb a significant association was observed (Mantel-Haenszel χ^2, stratified for year of collection = 10.4, $P < 0.001$). The inversion frequency was 56% among unexposed individuals, but it was 64–92% among those surviving exposure.

There is much information on inversion polymorphism (Coluzzi and Sabatini 1967, Coluzzi 1968) and DDT resistance (Davidson and Zahar 1973) in anopheline mosquitoes. In the 1950s there were several attempts to show an association between these genetic variants in Italian populations on *Anopheles atroparvus* Van Thiel and *Anopheles labranchiae* Falleroni (D'Alessandro et al. 1957, Mariani et al. 1959).

Ethiopia is one of the few parts of tropical Africa with a concerted program of DDT spraying against malaria vectors (Mekuria and Wolde-Tsadik 1970). This had the unfortunate consequence of selecting for DDT resistance in the vector species, *Anopheles arabiensis* (Patton), a member of the *Anopheles gambiae* complex (Nigatu et al. 1994).

The present study was carried out in one of the areas with DDT spraying and resistance—Gambella region (8°N, 35°W) in southwestern Ethiopia. For an investigation of the possible association between DDT resistance and inversion frequencies bloodfed *An. arabiensis* were collected by aspiration from among those resting in sprayed houses in Itang, Gambella, during the peak of the malaria transmission seasons (September–October) of 1988 and 1990. Most were exposed to 4% DDT-impregnated papers in World Health Organization test kits (World Health Organization 1981) for 1, 2, or 4 h. The remainder were exposed to noninsecticidal papers only. Eight hours after exposure, delayed mortality due to the DDT had reached completion and survivors were placed in Carnoy's fixative. The ovaries were dissected out, stained in Orcein, washed with propionic acid and squashed under a cover slip (Hunt 1973). The polytene chromosomes of the ovarian nurse cells were examined.

Table 1 shows the mortality from DDT exposure and the numbers of homozygotes and heterozygotes for the 2Ra, 2Rb, 2Rd, and 3Ra inversions systems in the survivors for each of the times of DDT exposure and in controls not exposed to DDT. There was a higher level of DDT resistance in 1990 than 1988. However, the upward trend was not steady—1989 data shown elsewhere (Nigatu et al. 1994), unaccompanied by cytogenetic observations, showed no mortality from DDT exposure in that year.

Table 1 shows that the frequencies of the 2Ra, 2Rd, and 3Ra inversions were low and did not differ markedly between survivors of any of the times of DDT exposure and unexposed controls. However, for 2Rb in 1988 and 1990 the inversion frequency was markedly higher in DDT survivors (64–92%) compared with controls (56%).

As a basis for testing the significance of this apparent association, the number of haploid chromosome sets observed to be carrying, or not carrying, the 2Rb inversions were calculated by counting 2 for homozygotes and 1 for heterozygotes for DDT-exposed and unexposed mosquitoes (Table 2). Comparison of the 3 times of exposure by χ^2 for trend showed no significant increase of inversion frequency among survivors of successively higher times of exposure, in either year or both years combined (Table 2). Pooling the 3 times of exposure and comparing with the unexposed mosquitoes showed a significantly higher inversion frequency in the survivors of DDT treatment than in the general population (stratifying for year, Mantel-Haenszel $\chi^2 = 10.4$, $P < 0.001$). Because the 2Rb inversion is thus associated with survival of DDT, one might have expected a rise in 2Rb frequency between 1988 and 1990 as DDT resistance level rose between those years (Nigatu et al. 1994). However, in fact, the observed 2Rb frequencies in exposed and unexposed mosquitoes declined slightly, but nonsignificantly, between those years.

The cause of the association of 2Rb with DDT resistance seen each year is presumably that a resistance gene is included in the 2Rb inverted

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Table 1. Mortality following exposure to 4% DDT of *Anopheles arabiensis* and numbers among DDT survivors of homozygotes and heterozygotes for 4 inversion systems.[1]

Hours DDT exposure	% mortality (no. tested)	No. examined cytogenetically	2Ra			2Rb			2Rd			3Ra		
			+/a	a/a	%a	+/b	b/b	%b	+/d	d/d	%d	+a/a	a/a	%a
1988														
0	0 (159)	31	5	1	11.3	15	10	56.5	1	0	1.6	1	0	3.2
1	21.2 (99)	39	10	1	15.5	18	19	71.7	4	0	5.1	4	0	5.1
2	45.0 (40)	13	1	0	3.8	4	9	84.6	0	0	0	3	0	11.5
4	50.0 (60)	6	2	0	16.7	1	5	91.7	0	0	0	1	0	8.3
1990														
0	0 (180)	25	9	1	22.0	10	9	56.0	6	0	12.0	4	0	8.0
1	10.0 (140)	7	2	0	14.3	3	3	64.3	0	0	0	0	0	0
2	11.0 (100)	23	7	2	39.1	9	14	80.4	4	0	8.7	0	0	0
4	15.0 (160)	31	15	0	24.2	17	12	66.1	6	0	9.7	5	0	8.1

[1] Number of +/+ homozygotes may be obtained by subtraction of inversion homozygotes from total number examined cytogenetically.

chromosome segment, from which it cannot recombine into the noninverted chromosome because of the cross-over suppressing property of inversions.

Table 2. Number of haploid chromosome sets observed with the 2Rb inversion (b) and without it (+) in *Anopheles arabiensis* exposed to DDT for zero, 1, 2, or 4 h in 1988 and 1990.

Hours DDT exposure[1,2]	+	b	Total	% b
1988				
0	27	35	62	56.4
1	22	56	78	71.7
2	4	22	26	84.6
4	1	11	12	91.7
1–4	27	89	116	76.7
1990				
0	22	28	50	56.0
1	5	9	14	64.2
2	9	37	46	80.4
4	21	41	62	66.1
1–4	35	87	122	71.3

[1] For 1, 2, and 4-h exposure, χ^2 for trend: 1988: 3.36 (n.s.); 1990: 0.42 (n.s.); 2 years combined: 0.55 (n.s.).

[2] For unexposed mosquitoes vs. those exposed for any of the 3 times of exposure: Mantel-Haenszel χ^2, stratified for year = 10.4, $P < 0.001$.

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