Insecticide susceptibility in the South African malaria mosquito *Anopheles arabiensis* (Diptera: Culicidae)

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Despite a century of insecticide use in agriculture and public health in South Africa, no insecticide susceptibility data exist for the malaria mosquito *Anopheles arabiensis* in South Africa. Biochemical assays and insecticide susceptibility tests were used to provide baseline data on DDT, deltamethrin, fenitrothion and propoxur susceptibility in field populations of *An. arabiensis* sampled from three areas, Mamfene, Thomo and Malahlapanga, South Africa, from March to May 1996. Mamfene and Thomo had been exposed for decades to heavy insecticide use for agricultural purposes and for malaria vector control, respectively. Malahlapanga had not been exposed to insecticide use and acted as a control. Mosquitoes from the three areas demonstrated complete susceptibility on standard testing, except for those from Thomo, which showed 75.1% mortality on one-hour exposure to 0.1% propoxur. The results of the biochemical assays showed no elevation of enzyme levels/activity for acetylcholinesterase, non-specific esterases or glutathion-s-transferase. Sensitivity to all the insecticides suggested no heterogeneity within the mosquito populations. This study provides the first insecticide susceptibility and biochemical data for *An. arabiensis* in South Africa and should serve as the baseline against which to compare chemical resistance when choosing insecticides for malaria vector control.

Introduction

The growing resistance of mosquitoes to insecticides is a potentially serious challenge to malaria vector control. Following the discovery and general use of DDT and dieldrin for mosquito control in the 1940s, reports of resistance to these insecticides in target insects began to appear. Dieldrin, BHC and chlordane resistance in *Anopheles gambiae* from Nigeria was reported by Davidson.[1] Further discovery of resistance to dieldrin and DDT in both *An. gambiae* and *An. arabiensis* was reported in Nigeria.[2–4] The banning of DDT during the 1970s resulted in the use of other groups of insecticides such as pyrethroids, carbamates and organophosphates for malaria vector control and agricultural purposes in Africa. The first account of pyrethroid resistance in *An. gambiae* due to reduced target site sensitivity, arising from a single point mutation in the sodium channel gene and increased levels of detoxifying enzymes, came from West and East Africa.[5–10] Recently, *Anopheles funestus*, that was eradicated from South Africa as a result of the widespread use of DDT for indoor house spraying, re-appeared and was found to be pyrethroid resistant.[11]

The emergence and spread of insecticide resistance in malaria vectors threatens its control[12,13] and has necessitated resistance detection techniques such as insecticide susceptibility tests, biochemical microplate assays, and molecular methods for monitoring resistance.[14–17] Conventional dose-mortality (bioassay) tests of resistance are based on insecticide susceptibility. However, such susceptibility tests on their own do not indicate underlying insecticide resistance mechanisms.[18] These are probed using biochemical and molecular assays in association with susceptibility results.[18] Despite decades of insecticide use in South Africa, both for agricultural and public health purposes, there is no information on possible resistance in *An. arabiensis*, the main malaria vector in South Africa, to insecticides used for malaria control. We compared biochemical assays and susceptibility tests to provide baseline data on *An. arabiensis* that may be used when monitoring mosquito populations for insecticide resistance, and to complement the use of effective agents for malaria vector control in future.

Methods and materials

Study area. Mosquitoes were sampled from three areas, Mamfene (KwaZulu-Natal), Thomo (Limpopo, formerly Northern Province) and Malahlapanga (Kruger National Park). The three areas represented sites where insecticides were never used (Malahlapanga), where they were employed for both agricultural purposes and malaria control (Mamfene), and where insecticides were used only for malaria control (Thomo). Malahlapanga, a geothermal spring in the central Kruger National Park, supports a pure perennial colony of *An. arabiensis*.[19] The site is not accessible to tourists and the nearest human dwelling is about 12 km distant. Thomo is a rural area where DDT has been used against mosquitoes since 1946. In Mamfene, insecticides are liberally used for agricultural and malaria control purposes.

Mosquito collection. Mosquitoes were sampled from the three study areas using direct landing catches, baited net traps and larval collection from March to May 1996, inclusive.[20] Female adult mosquitoes belonging to the *An. gambiae* complex were blood-fed and individually placed in breeding tubes, four days post-feeding, to lay eggs. Their progeny were kept separately and the parents were identified to species level using gel electrophoresis.[21–23] Adult *An. arabiensis* mosquitoes that emerged (F_1 generation) were used in the assays.

Susceptibility tests. Four insecticides, DDT, deltamethrin, fenitrothion and propoxur, were investigated. Insecticide-impregnated papers and insecticide susceptibility kits from the WHO were used, and standard adult bioassay procedures were followed with minor modifications to the mosquito exposure period of one hour for fenitrothion assays.[24,25] Sugar-fed, 2–5-day-old male and female *An. arabiensis* mosquitoes were exposed by tarsal contact to filter papers impregnated with 4% DDT, 0.025% deltamethrin, 1% fenitrothion and 0.1% propoxur inside exposure tubes for one hour. One-hour and 24-hour post-exposure mortality counts were recorded. Each bioassay experiment comprised more than two replicates. Controls were exposed to untreated papers.

Biochemical assays. Homogenates from individual mosquitoes were screened for non-specific esterase (NSE) and glutathione-s-transferase (G-S-T) enzyme activity, and to test the presence of altered acetylcholinesterase (AChE) in individual mosquitoes. Levels of mixed function oxidases were not assayed in this study. Single mosquitoes were homogenized in Eppendorf microtubes after the removal of the abdomen (containing sugar water meal) with 100 μl of 0.05 M potassium phosphate buffer (pH 7.0) using a glass rod for each mosquito. Each homogenate was diluted to a final volume of 500 μl with the same buffer, transferred into

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Table 1. F_1 progeny from families of *Anopheles arabiensis* mosquitoes reared from wild-caught females from Thomo, Mamfene and Malahlapanga.

Area	F_1 mosquitoes		Total
	Male	Female	
Thomo	393	498	891
Mamfene	449	502	951
Malahlapanga	385	842	1227

microcentrifuge tubes and centrifuged at 8000 rpm for 10 minutes. Aliquots of 25 μl of the clear homogenate were then transferred by pipette into a microplate well. Reagents were added using a transfer plate that loaded all wells at once. Esterase activity was measured, as described by Brogdon and Dickson,[15] in 25-μl replicates of mosquito supernatant with the substrate β-naphthyl acetate. Glutathione-s-transferase activity, that may confer DDT resistance when elevated, was measured, as described by Brogdon and Barber,[26] in 20-μl replicates of supernatant. Acetylcholinesterase activity, that confers propoxur and fenitrothion resistance, was measured as described by Brogdon and Barber.[27] The intensity of the chromophores was scanned by microphotometer at 550 nm (NSE), 340 nm (G-S-T) and at 414 nm (AChE).

Results

In total, 106, 178 and 500 *An. gambiae s.l.* female adult mosquitoes were collected from Mamfene, Thomo and Malahlapanga, respectively. Using electrophoresis, 79.2% (84/106) and 97.8% (174/178) of the mosquitoes from Mamfene and Thomo respectively, were identified as *An. arabiensis*. *Anopheles quadriannulatus* constituted the remainder of the *An. gambiae s.l.* mosquitoes caught from these two areas. All the mosquitoes of the *An. gambiae* complex from Malahlapanga were identified as *An. arabiensis*, thus confirming the findings of Braack *et al.*,[19] who reported a pure colony of *An. arabiensis* at the site. The family groups of *An. arabiensis* were reared in the insectary at 25°C and 80% relative humidity. The eggs from wild parents did not hatch in synchrony, resulting in small numbers of F_1 adults of different family groups emerging each day.

Table 1 shows the number of adult mosquitoes tested for insecticide resistance; 891, 951 and 1227 F_1 *An. arabiensis* adult mosquitoes were produced by females reared from wild-caught females from Thomo, Mamfene and Malahlapanga, respectively. Table 2 shows the susceptibility of adult mosquitoes to the four insecticides. The one-hour mortality of insects from the three areas exposed to 4% DDT and 0.025% deltamethrin was more than 90%, but less than 80% on 1% fenitrothion and on 0.1% propoxur in mosquitoes from Mamfene and Thomo. Tests with 0.1% propoxur on subjects from Malahlapanga demonstrated a one-hour mortality of more than 90%. Fenitrothion exhibited slow insecticide activity as evidenced by the absence of mortality after one hour. There were no mosquito survivors from all three areas at the WHO discriminating dosage of DDT, deltamethrin and fenitrothion, 24 hours after exposure. There were also no survivors from Malahlapanga and Mamfene on 0.1% propoxur. However, mosquitoes from Thomo exhibited some degree of resistance/tolerance to propoxur; 24-hour mortality in control mosquitoes was 6%.

Figure 1 shows G-S-T, AChE and NSE enzyme levels of *An. arabiensis* mosquitoes from all three areas. The resistance threshold absorbance values for Guatemalan *Anopheles albimanus*, reported by Brogdon *et al.*,[28] are shown in the same figure. Glutathione-s-transferases are involved in DDT resistance in mosquitoes, NSE in deltamethrin, and AChE and NSE in fenitrothion and propoxur resistance.[29] The maximum AChE, NSE and G-S-T absorbance values in mosquitoes from Mamfene were 0.1 (at 414 nm), 0.25 (at 550 nm) and 0.03 (at 340 nm); 0.12 (at 414 nm), 0.3 (at 550 nm) and 0.02 (at 350 nm) from Thomo; and 0.18 (at 414 nm), 0.3 (at 550 nm) 0.02 (at 320 nm) from Malahlapanga, respectively. The general enzyme activity levels were consistently low, with no significant difference in the activity spectra between areas (Kruskal Wallis test, $P = 0.368$).

Discussion

Malaria transmission is seasonal from October to May in Mpumalanga, Limpopo and KwaZulu-Natal.[30] The main malaria vector in the provinces at the time these tests were carried out was *An. arabiensis*, a member of the *An. gambiae* complex.[31] Large-scale use of DDT for malaria vector control began in 1946 and was superseded by pyrethroids in 1995, 1996 and 1998 in KwaZulu-Natal, Mpumalanga and Limpopo, respectively.[32] Pyrethroids and other insecticides belonging to the two insecticide classes, organophosphates and carbamates, have been extensively used in agriculture for many decades in the three provinces. Widespread use of insecticides for agricultural purposes as well as antimalarial house spraying has been

Table 2. Insecticide susceptibility of *Anopheles arabiensis* from Thomo, Mamfene and Malahlapanga and mortality rates following exposure to 4% DDT, 0.025% deltamethrin, 1% fenitrothion and 0.1% propoxur for one hour. Overall mortality recorded 1-h and 24-h post exposure. Each mortality rate represents a mean value of three or more replicates of 10 or more mosquitoes per test.

Area	Insecticide	Replicates	Number exposed		Mortality after 1-h exposure (%)		Mortality after 24-h exposure (%)		Total mortality (%)	
			Female	Male	Female	Male	Female	Male	1 h	24 h
Thomo	4% DDT	3	34	33	90.7	100	100	100	95.9	100
	0.025% del	3	20	12	95.0	100	100	100	96.2	100
	1% fenitro	5	59	32	0	0	100	100	0	100
	0.1% prop	5	49	44	23.8	35.0	77.2	72.9	29.4	75.1
	Control	3	46	34	0	0	2.1	8.6	2.1	6.1
Mamfene	4% DDT	8	120	84	95.9	96.3	100	100	96.1	100
	0.025% del	4	46	37	95.7	95.8	100	100	95.8	100
	1% fenitro	6	55	49	0	0	100	100	0	100
	0.1% prop	5	63	41	87.3	68.3	100	100	79.8	100
	Control	3	41	39	NR	NR	4.9	6.8	3.1	6.4
Malahlap	4% DDT	6	99	28	100	100	100	100	100	100
	0.025% del	3	20	20	100	100	100	100	100	100
	1% fenitro	5	89	32	0	0	100	100	0	100
	0.1% prop	5	50	86	NR	NR	100	100	94	100
	Control	11	45	35	NR	NR	6.2	10.4	2.0	6.0

Malahlap = Malahlapanga, del = deltamethrin, fenitro = fenitrothion, prop = propoxur, NR = not recorded.

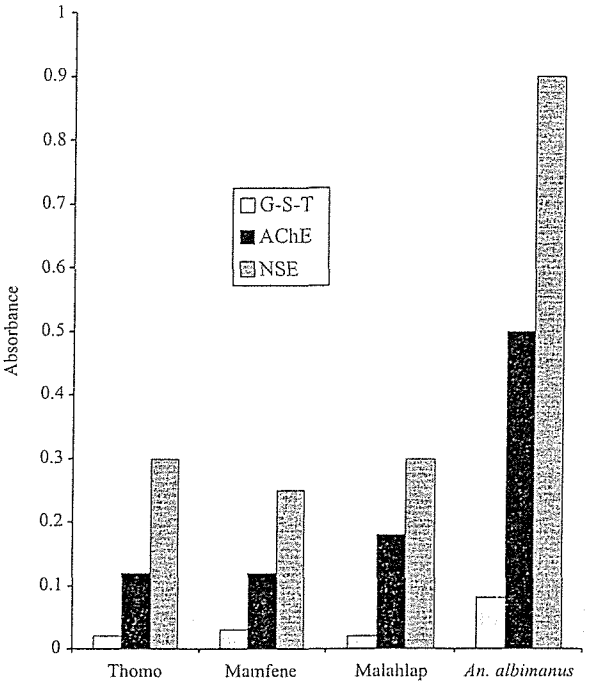

Fig. 1. Enzyme assays of field *Anopheles arabiensis* adult mosquitoes from Thomo, Mamfene and Malahlapanga (Malahlap), as estimated by biochemical methods, March–May 1996.

reported to result in the emergence of resistant strains of malaria vector mosquitoes.[12,33]

The WHO bioassay kit is used to determine resistance in mosquitoes, but no information on resistance mechanisms is provided.[18] Biochemical tests are required to supplement and complement bioassay results. Hemmingway and Smith[34] reported that it is possible to detect the presence of an altered AChE type organosphosphate and carbamate resistance in individual insects using AChE assay. Brogdon *et al.*[28] detected the presence of elevated non-specific esterase and insensitive AChE type of organophosphate and carbamate resistance in Guatemalan *An. albimanus* and determined that the threshold absorbance values for *An. albimanus* were 0.5 (AChE), 0.9 (NSE), and 0.08 (G-S-T). Mixed function oxidases may be involved in the detoxification of almost all insecticides but are most notably associated with pyrethroid resistance.[35,36] In the present study the absorbance values from the three collection areas were low and there was no significant difference between the three areas, suggesting little or no heterogeneity within the mosquito populations. The absorbance values for *An. arabiensis* were lower than the threshold values for *An. albimanus* but there are no supporting data to confirm that the threshold absorbance values for *An. albimanus* are also applicable to *An. arabiensis*.

The lack of survivors at the WHO discriminating dosage of DDT in mixed-age field collections of *An. arabiensis* mosquitoes must be interpreted with caution. Lines and Nassor[37] concluded that significant mortalities of DDT-resistant *An. gambiae* adults occur at the discriminating dosage when they are more than five days old. These authors showed that, in 12–14-day-old DDT-resistant *An. gambiae* adults, mortality with 4% DDT was 92%. The samples of *An. arabiensis* adults used in our study were mixtures of different ages due to non-synchrony in their emergence but were less than five days old. DDT resistance can be due to increased metabolism by a range of G-S-T values,[29] and declines with increasing adult age in all insects.[37] Biochemical tests with altered AChE and elevated NSE were not evident in our study, thus these insecticide resistance mechanisms appear to be absent, suggesting that *An. arabiensis* in the three areas was susceptible to organophosphorus compunds, synthetic pyrethroids and carbamates. Mosquitoes from Thomo, however, showed tolerance/resistance to propoxur by bioassay test and further investigations are required to elucidate the mechanism involved. Other carbamates such as bendiocarb, which is used in malaria vector control, should also be tested on the Thomo mosquito population. These first baseline data indicated no evidence of DDT, deltamethrin and fenitrothion resistance in *An. arabiensis* in three localities, and no resistance to propoxur in two areas.

The monitoring of insecticide resistance is crucial in programmes against malaria mosquitoes. As a result, this study is being extended to other southern African countries using new techniques and assaying for other enzyme systems such as mono-oxygenases. This programme, supported by the Multilateral Research Initiative on Malaria, aims to document the distribution and prevalence of insecticide resistance, and to explore underlying biochemical mechanisms in the malaria vectors where these might occur.

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A common thread of humankind

Humanity from African Naissance to Coming Millennia. Edited by P.V. Tobias, M.A. Raath, J. Moggi-Cecchi and G.A. Doyle. Pp. 409. Firenze University Press and Witwatersrand University Press: 2001. R280.

This book is the product of a conference on human origins and human biology held in Sun City, South Africa, in late June and early July of 1998, with delegates from 70 countries. This 'Dual Congress' brought together two professional bodies: the International Association for the Study of Human Palaeontology and the International Association of Human Biologists.

In the Foreword, C.K. Brain (honorary president of the Dual Congress) writes, 'In all respects, the Dual Congress reflected in this volume was a milestone occasion for South Africa and for international science at large'. In the Preface, Phillip Tobias states further, in phrasing that gives the volume its title: 'This book goes out to the world of human biology and palaeoanthropology from the continent which gave birth to its humanity, its African Naissance, at the turn of the millennium, after five thousand millennia of humanity as we stand poised before many Coming Millennia'.

More than 50 contributors cover topics from each of 18 colloquia, relating to human biology, the emergence and evolution of *Homo*, early modern humans, dating, taxonomy, palaeodiet and the brain. The contributors include scientists from the Gondwanan regions of Africa (Cape Town, Pretoria, Johannesburg), South America (Santiago and Sao Paulo), Australia (Canberra and Melbourne) and New Zealand (Palmerston North); and from the northern components of Pangaea, including Europe (Ferrara, Florence, Hamburg, London, Moscow, Madrid, Palma de Mallorca, Paris, Poitiers, Poznan, Rome, Warsaw, Zurich), the United States (Arkansas, Atlanta, Baltimore, Detroit, Florida, New York, Michigan, Philadelphia and Washington DC), Canada (Ontario), Mexico (Merida) and Asia (Bandung in Indonesia, Nanjing in China, Pune in India, and Hayama Kanagawa in Japan).

Introducing the volume is the 8th Robert Broom Memorial lecture entitled 'Conversion in palaeoanthropology: The role of Robert Broom, Sterkfontein and other factors in australopithecine acceptance' by Tobias. 'Conversion' in this instance relates to Broom's role in converting scepticism into acceptance of *Australopithecus africanus,* first described by Raymond Dart as a primate 'on the way' to becoming human.

Topics in the field of human biology include adaptation of human populations and 'their niches in time and space' (Wolanski), 'anthropo-ecological' research in Asia (Alexeeva), human ecology and culture (Piontek), relationships between fertility, mortality and subsistence (Sellen), and family structure and development (Siniarska and Wolanski).

Papers in the field of palaeoanthropology include those concerning new discoveries of an australopithecine from Chad (Brunet), dental development in *Australopithecus africanus* (Moggi-Cecchi), the dispersal of early *Homo* (Anton, Aziz, Zaim), the diversity of this genus (Grine, Manzi, Wood and Collard), the origin(s) of early humans (Brauer, Chase, H.J. Deacon, Misra and Rightmire), catastrophic mortality of hominins from Atapuerca and Krapina (Bocquet-Appel and Arsuaga), and other analyses of middle or late Pleistocene samples from Eurasia (Jelinek, Kennedy and Peretto).

Developments in dating techniques, and the application of these techniques to address problems relating to the chronology of human evolution, are discussed in papers by Roberts, Rhys Jones, Schwartz, Shen and Vogel. Issues concerning taxonomy and systematics (Cela-Conde, Goodman, Czelusniak, Page, Meireles, Groves, Takahaa, Watson, Easteal and Penny) reflect the diversity of opinions expressed by anthropologists working in the separate but closely related fields of palaeoanthropology and human genetics.

Palaeodiets of Plio-Pleistocene hominins are discussed in the context of chemical analyses (Lee-Thorp and Sillen), with special reference to stable carbon isotopes and strontium-calcium ratios. Parkington emphasizes the importance of the consumption of long-chain polyunsaturated fatty acids as a factor contributing to the expansion of the brain in human evolution, with special reference to the exploitation of marine food resources. Ungar and Teaford discuss dental features of extant pongids and Plio-Pleistocene hominids, focusing on micro-habitat variability rather than global cooling as a key factor contributing to palaeodietary changes.

This volume is a useful addition to the shelves of researchers with interests in the spectrum of subjects that inform palaeoanthropology and human biology. It is the tangible outcome of a meeting which, thanks to the efforts of Henry de Lumley (president of the International Association for the Study of Human Palaeontology) and Tobias (president of the International Association of Human Biologists), gave a much-needed opportunity for palaeoanthropologists (with special interests in a diversity of Plio-Pleistocene hominin species) and biologists (with special interests in the species *Homo sapiens*) to convene in South Africa, in circumstances that allowed scholars of all colours and creeds to talk together, work together, and even (on occasion) to sing.

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