Captures of mosquitoes of the *Anopheles gambiae* complex (Diptera: Culicidae) in the Lowveld Region of Mpumalanga Province, South Africa

J. Govere[1,2], D.N. Durrheim[1], M. Coetzee[2], R.H. Hunt[2] & J.J. la Grange[1]

[1]*Mpumalanga Department of Health, Private Bag X11285, Nelspruit, 1200 South Africa*
[2]*Medical Entomology, Department of Tropical Diseases, School of Pathology of the South African Institute for Medical Research and the University of the Witwatersrand, P.O. Box 1038, Johannesburg, 0001 South Africa*

Monthly collections of the *Anopheles gambiae* complex mosquitoes were made on human bait at seven fixed sites in the Lowveld Region of Mpumalanga Province, South Africa, between August 1997 and May 1998 to contribute to the evaluation and planning of the malaria vector control programme. Members of the *An. gambiae* complex were distinguished from other anopheline species using morphological keys and were subsequently specifically identified by polymerase chain reaction (PCR). A total of 5084 anophelines were collected during the survey, of which 2837 (55.8 %) were *Anopheles coustani* Laveran, 1418 (27.9 %) were members of the *Anopheles funestus* group, 435 (8.6 %) were members of the *An. gambiae* complex, 264 (5.2 %) were *Anopheles pretoriensis* Theobald, and 130 (2.6 %) comprised nine other anopheline species. From a total of 425 PCR identifications of adult females of the *An. gambiae* complex, 238 (56.0 %) were *Anopheles merus* Donitz, 129 (30.4 %) *Anopheles quadriannulatus* Theobald and 58 (13.6 %) were *Anopheles arabiensis* Patton. No circumsporozoite antigen for *Plasmodium falciparum* was detected in any of the female *An. gambiae* complex mosquitoes. Monthly *An. gambiae s.l.* captures were significantly correlated with rainfall but there was no correlation between mosquito captures and monthly malaria notifications. Malaria notifications were, however, strongly associated with mean daily temperatures. The peak in malaria incidence paralleled the peak in rainfall with a time lag of 2–3 months. This study provides updated information on the distribution of the *An. gambiae* complex in Mpumalanga Province's Lowveld Region, notably the incidence of mosquitoes biting humans outside sprayed houses between 18:00 and 22:00. The study also provides the first documented evidence of large numbers of *An. merus* feeding on humans in Mpumalanga. Further analysis of rainfall and temperature patterns may facilitate the prediction of malaria epidemics with sufficient lead-time to enable the Provincial Malaria Control Programme to launch pre-emptive control measures.

Key words: *Anopheles gambiae* complex, indoor house spraying, feeding behaviour, anopheline mosquitoes, malaria vectors, Mpumalanga Province.

INTRODUCTION

Indoor application of residual insecticides has been the main form of malaria vector control in South Africa since 1946 (Gear *et al.* 1981). Exposure to insecticide has multiple effects on mosquito populations and behaviour, including a mass killing effect (Mnzava *et al.* 1993), deterrency (Miller *et al.* 1991), avoidance (Coluzzi *et al.* 1979; Sharp *et al.* 1984; Lines *et al.* 1987; Sharp & Le Sueur 1991; Njau *et al.* 1993), diversion (Charlwood & Graves 1987), biting pattern (Njau *et al.* 1993) and altered gonotrophic cycle length (Hii *et al.* 1995). *Anopheles gambiae s.s.* Giles and *An. funestus s.s.* Giles, which manifest anthropophilic and endophilic tendencies (Service 1970; White *et al.* 1972), have apparently been eradicated from Mpumalanga Province by indoor house-spraying with insecticides (Gear *et al.* 1981). House spraying with insecticides is also believed to have caused behavioural changes in *An. arabiensis* Patton (Sharp & le Sueur 1991). This species has been described as displaying a large degree of ecophenotypic plasticity (White 1974; Coluzzi *et al.* 1979).

The earliest studies and reviews of the bionomics of *An. gambiae s.l.* in Mpumalanga Province were initiated in the late 1920s (Ingram & De Meillon 1927, 1929; De Meillon 1931). These studies preceded the replacement of DDT with deltamethrin for indoor house-spraying that occurred in 1994, and were conducted at a time

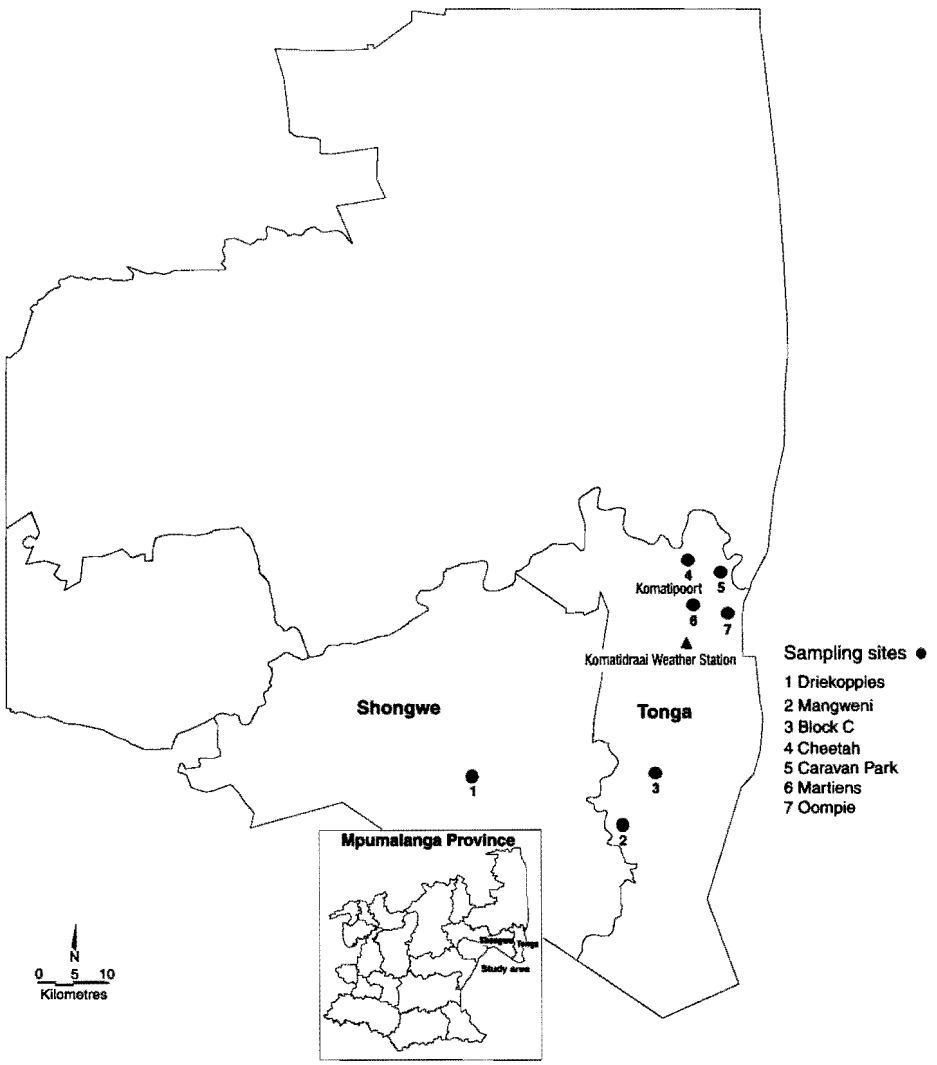

Fig. 1. Mosquito collection sites in the Lowveld region of Mpumalanga Province (August 1997 to May 1998).

when agricultural activities in the area were limited. Intensive agricultural development has since altered the environment, replacing forests with sugarcane and banana plantations and other fruit and vegetable crops. The present diversity and ecology of the *An. gambiae* group of species in the Province is consequently unclear. We conducted a study to update information on *An. gambiae s.l.* abundance, composition and distribution in the *malarial* areas of Mpumalanga with specific reference to the exophagic and anthropophagic portion of the *An. gambiae s.l.* population that is active during the early hours of the night (18:00–22:00). This time selection incorporates the period when people are active outdoors before retiring indoors to sleep. We also analysed the incidence of notified malaria cases and determined its association with meteorological features and mosquito captures in the Lowveld region during the study period.

MATERIAL AND METHODS

Study area

An entomological survey was carried out between August 1997 and May 1998 in the Lowveld region of Mpumalanga Province, South Africa (Fig. 1). Summer seasonal malaria transmission

occurs in the region with an annual average incidence of approximately 3200 notified cases and a case-mortality ratio of 0.5 %. *Anopheles arabiensis* is considered to be the primary malaria vector in the area (Gear *et al.* 1981). Annual indoor house-spraying with deltamethrin at 20 mg/m^2 application is the cornerstone of malaria control, and houses are sprayed between September and December. The regional climate is characterized by a distinct dry season from June to September and a wet season from October to May. Seasonal malaria transmission usually begins in October and peaks during January, February and March. During the study period the area received sporadic rainfall from August to April. People in the area tend to spend the early hours of the night outside their sprayed houses, thereby exposing themselves to the bites of exophilic *An. gambiae s.l.* mosquitoes (Govere *et al.* unpubl.). Sampling was carried out at seven fixed sites; Martiens, Caravan Park, Cheetah, Oompie (Komatipoort), Mangweni, Block C and Driekoppies, situated in the centre of the malaria area where intensive commercial sugarcane and banana farming is practised (Fig. 1).

Mosquito collections

Field staff comprising two four-person teams performed mosquito-landing catches (females captured before biting) from 18:00–22:00 for four consecutive nights at each site per month using flashlights and aspirators (Service 1993). The teams rotated through the seven sites during different months with each team sampling three sites per month. All mosquito specimens were identified using morphological characteristics (Gillies & De Meillon 1968; Gillies & Coetzee 1987). Females of the *An. gambiae* complex were individually stored in numbered vials with silica gel desiccant for laboratory processing.

Attempts to capture indoor-resting anopheline mosquitoes and mosquitoes exiting huts were made at five sites, Martiens, Oompie, Driekoppies, Block C and Mangweni, during January and February 1998 using indoor pyrethrum-spray knockdowns in tandem with exit-window-traps (Service 1993). Window traps were fitted in four houses in each village for four consecutive nights, after which the houses were sprayed with 0.2 % pyrethrum in kerosene (paraffin) for collection of indoor-resting mosquitoes. In each village a single unsprayed house was used as a control.

Laboratory procedures

Mosquitoes were identified by polymerase chain reaction (PCR) (Scott *et al.* 1993) and enzyme-linked immunosorbent assay (ELISA), used to detect circumsporozoite antigen (Burkot *et al.* 1984). PCR identification of members of the *An. gambiae* complex was carried out at the Mpumalanga Provincial Entomology Laboratory. The Medical Entomology Department of the South African Institute for Medical Research (SAIMR) in Johannesburg, South Africa, carried out PCR quality control and ELISA tests. The association between *An. gambiae s.l.* monthly densities, prevailing meteorological conditions and malaria notifications was established using linear regression with two-tailed significance testing. To achieve linearity, dependent and independent variables were log transformed. Rainfall and temperature recordings from Komatidraai Weather Station (Fig. 1) were used to represent the study area.

RESULTS AND DISCUSSION

Monthly anopheline catches during the ten-month period are summarized in Table 1. Owing to logistical constraints, including harsh weather and mosquito collectors taking leave during public holidays, catches were interrupted during December, March, April and May. As a result, the relative proportional capture at the seven sites could not be accurately determined. A total of 5084 anopheline mosquitoes comprising nine species were captured. *Anopheles coustani* was the most abundant species, comprising 55.8 % of the total catch. Members of the *An. funestus* group formed 27.9 % of the total catch and ranked second in abundance. The exophilic behaviour of *An. coustani* and members of the *An. funestus* group may account for their abundance in the area. These species feed and rest outdoors and are therefore not susceptible to indoor house-spraying. Furthermore, the two species are not seasonal and since they breed in permanent freshwater swamps and vegetated streams their densities remain relatively stable throughout the year (De Meillon 1951; Gillies & Coetzee 1987). *Anopheles gambiae s.l.* ranked third in abundance, accounting for 8.6 % of the total catch. *Anopheles pretoriensis* was also found in large numbers while other species, including *Anopheles demeilloni* Evans, *Anopheles rufipes* Gough, *Anopheles squamosus* Theobald, *Anopheles longipalpis* Theobald, *Anophe-*

Table 1. Monthly anopheline captures from human mosquito-landing catches between 18:00 and 22:00 at seven sites in the Lowveld region, Mpumalanga Province, South Africa.

Month	Sampling days (%)	Anopheline species				
		An. gambiae s.l.	*An. funestus* group	*An. coustani*	*An. pretoriensis*	Others
August 1997	15 (12.0)	6	579	809	20	9
September	17 (13.6)	8	321	650	9	1
October	20 (16.0)	63	227	518	5	18
November	17 (13.6)	95	212	291	14	7
December	8 (6.4)	53	0	142	35	15
January 1998	14 (11.2)	117	12	105	17	6
February	15 (12.0)	77	23	251	60	13
March	7 (5.6)	3	6	55	44	7
April	4 (3.2)	4	6	14	39	14
May	8 (6.4)	9	32	2	21	40
Total (%)	125 (100.0)	435 (8.6)	1418 (27.9)	2837 (55.8)	264 (5.2)	130 (2.6)

les marshallii Theobald and *Anopheles maculipalpis* Giles, were uncommon. Abundance of these species appeared to be independent of season.

Table 2 records catches of three species of the *An. gambiae* complex and their distribution among the collection sites. A total of 435 female members of the *An. gambiae* complex were captured and the majority of mosquitoes were recorded at the Martiens, Block C and Driekoppies sites. Of the 435 members of the *An. gambiae* complex, 425 were identified by PCR as *An. merus* Donitz (56.0 %), *An. quadriannulatus* Theobald (30.4 %) and *An. arabiensis* (13.6 %). All three species occur sympatrically in Mpumalanga Province. All specimens were females and tested negative for the *Plasmodium falciparum* circumsporozoite antigen. *Anopheles gambiae s.s.* and *An. funestus s.s.* were not recovered during this study, probably as a result of the indoor residual insecticide spraying programme and the endophilic and anthropophilic tendencies of both species (Service 1970; White *et al.* 1972; White 1974). Previous attempts by malaria surveillance teams to capture *An. arabiensis* produced disappointing results, despite locally occurring malaria cases fuelling speculation that members of the *An. funestus* group could be involved in malaria transmission in these areas. A study of the role of members of the *An. funestus* group in malaria transmission failed, however, to incriminate them (Speare *et al.* 1999). Failure to capture malaria vectors despite reported malaria cases has also been documented in the adjoining Northern Province of South Africa (La Grange & Coetzee 1998). In this survey, both pyrethrum spray-catches and exit-window traps failed to capture *An. gambiae s.l.* Absence of vectors within sprayed structures suggests that the current vector control programme using indoor deltamethrin application is effective, supporting evidence from bioassays (Govere *et al.* unpubl.).

Table 2. Captures of species of the *Anopheles gambiae* group from human mosquito-landing catches between 18:00 and 22:00 at seven sites from August 1997 to May 1998 in the Lowveld Region, Mpumalanga Province, South Africa.

Site	Sampling days (%)	PCR identifications			
		An. merus	*An. arabiensis*	*An. quadriannulatus*	Total (%)
Martiens	27 (21.6)	223	20	115	358 (84.2)
Block C	9 (7.2)	10	16	10	36 (8.5)
Driekoppies	36 (28.8)	0	19	0	19 (4.8)
Oompie	13 (10.4)	4	1	2	7 (1.6)
Mangweni	18 (14.4)	1	2	2	5 (1.2)
Caravan Park	12 (9.6)	0	0	0	0 (0)
Cheetah	10 (8.0)	0	0	0	0 (0)
Total (%)	125 (100.0)	238 (56.0)	58 (13.6)	129 (30.4)	425 (100)

Table 3. Monthly malaria notifications from the Lowveld Region, Mpumalanga Province, South Africa, in relation to monthly rainfall and temperature records for Komatidraai Weather Station and mosquito catches at Martiens (August 1997 to May 1998).

Month	Malaria notifications	Total rainfall (mm)	Mean daily temperature (°C)	Mosquito captures
August	85	13	22.8	0
September	153	36	22.8	8
October	154	25	23.1	61
November	148	194	23.6	93
December	171	118	25.0	53
January	943	186	25.5	106
February	1094	30	27.0	33
March	1690	56	28.1	—
April	797	10	27.6	—
May	586	0	24.3	5

However, absence of vectors in unsprayed structures may conversely suggest that vectors have adopted a highly exophilic behaviour to avoid the sprays.

In the present survey, the most abundant species of the *An. gambiae s.l.* mosquitoes was *An. merus*. This is the first documented case of large numbers of *An. merus* being collected in Mpumalanga Province. *Anopheles merus* is a saltwater breeder (De Meillon 1951; Muirhead-Thomson 1951; Gillies & De Meillon 1968; White 1974; Le Sueur & Sharp 1988; Coetzee & le Sueur 1988; Coetzee *et al.* 1993) and a vector of malaria and filariasis in eastern Africa (Bushrod, 1981; Temu *et al.* 1998). This species is highly exophilic and exophagic (White 1974). Recent work on the role of four anopheline species in the transmission of malaria in coastal Tanzania indicates that *An. merus* plays an unexpectedly important role in malaria transmission in that area, with a *P. falciparum* circumsporozoite antigen rate of 9.8 % compared to 8.4 %, 7.3 % and 6.1 %, for *An. gambiae s.s.*, *An. arabiensis* and *An. funestus s.s.* respectively (Temu *et al.* 1998). Although no recent data incriminate *An. merus* as a malaria vector in Mpumalanga, its abundance in the area warrants further investigation.

The distribution of *An. merus* within the Afrotropical Region has been described (Gillies & De Meillon 1968; White 1974; Coetzee *et al.* 1993). *Anopheles merus* is known to adapt readily to freshwater and the first record of *An. merus* breeding in freshwater in South Africa was in 1993 (Coetzee *et al.* 1993). Larvae of *An. arabiensis*, *An. quadriannulatus* and *An. merus* have also been collected from single breeding sites in Swaziland (La Grange 1995) and Mpumalanga Province (La Grange, pers. comm.). Current knowledge of *An. merus* distribution in southern Africa is based largely on chromosome identification using the X chromosome banding sequence (Coluzzi & Sabatini 1968, 1969; Hunt & Coetzee 1986), a feature which is shared by *An. gambiae s.s.* At the time when these earlier surveys were performed, the extent of the inland distribution of *An. merus* was not determined and many workers simply relied on X chromosome arrangement to identify species from areas remote from a saltwater environment. Owing to the extensive inland distribution of *An. merus* recently demonstrated in the Lowveld region, a review of the distribution of *An. gambiae* complex species in southern Africa using modern diagnostic technology is essential.

To investigate monthly mosquito captures in relation to environmental factors and malaria notifications, we used the entomological data collected from Martiens only because sampling at the site was most complete (Table 3). The most important environmental factors known to influence malaria are rainfall, temperature and humidity. Rainfall provides temporary pools necessary for breeding of vector mosquitoes. Temperature influences the development period of mosquitoes from egg to adult and the development period of sporozoites in the infected mosquito, with *An. gambiae s.l.* taking 65 days to develop at 12 °C and only 7.3 days at 31 °C. In addition, *P. falciparum* sporogony ceases below 20 °C and above 33 °C (Gilles & Warrell 1993). Relative humidity (RH) influences the life span and activity of vector mosquitoes. Malaria transmission ceases below

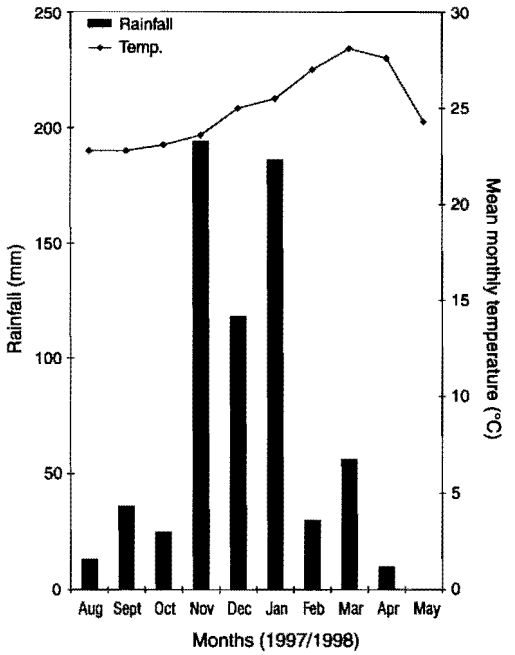

Fig. 2. Monthly rainfall at the Komatidraai Weather Station (August 1997 to May 1998) and mean monthly temperature.

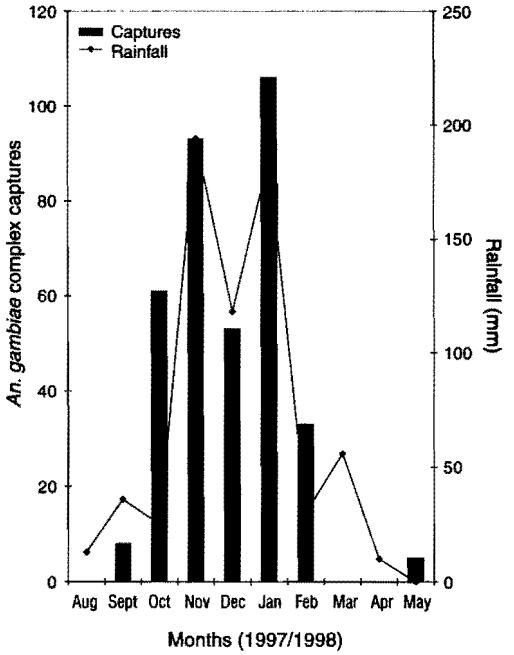

Fig. 3. Monthly mosquito captures of *Anopheles gambiae* complex at Martiens between 18:00 and 22:00 in relation to rainfall at the Komatidraai Weather Station.

60 % RH (Gilles & Warrell 1993).

Rainfall between August and April was bimodal with peaks in November and January, while March was the warmest month (Fig. 2). No mosquito catches were possible during March and April and rainfall, temperature and notification data from these months were excluded from the analysis of association between mosquito catches and these three factors. During the study period the numbers of *An. gambiae s.l.* increased during the warm rainy season and were strongly correlated with rainfall ($R^2 = 0.777$; $P = 0.009$) (Fig. 3). However, no association was found between total mosquito catches and malaria notifications ($R^2 = 0.243$; $P = 0.261$) (Fig. 4), although this might reflect the absence of mosquito capture data during March and April as these are the most important months during which peak malaria transmission occurs. Rainfall increases the amount of surface water resulting in greater breeding activity of *An. gambiae* complex mosquitoes (Gillies & De Meillon 1968; White 1974; Mpofu 1985; Gillies & Coetzee 1987; Thompson *et al.* 1997; Robert *et al.* 1998), thereby contributing to the seasonality of malaria transmission in the area. Excessive rainfall may have a negative effect on

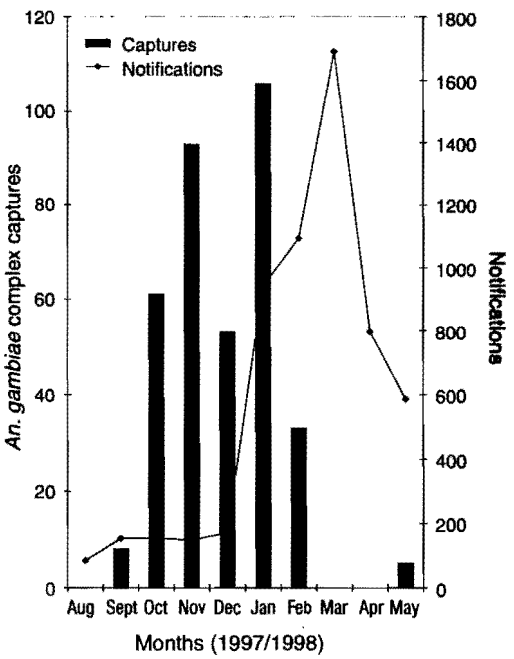

Fig. 4. Comparison of mosquito captures of *Anopheles gambiae* complex from human landing catches at Martiens with malaria notifications from the Lowveld Region, Mpumalanga Province, South Africa.

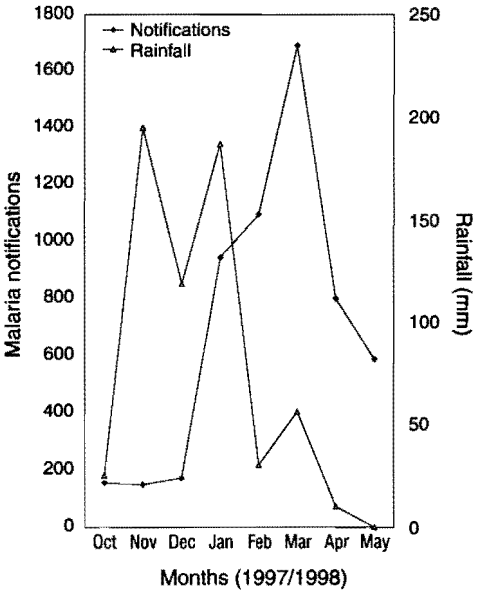

Fig. 5. Monthly comparison of rainfall at the Komatidraai Weather Station (August 1997 to May 1998) with monthly malaria notifications from the Lowveld region, Mpumalanga Province, South Africa.

mosquito breeding by flooding breeding sites, killing larvae and pupae or creating sites that are unsuitable for breeding. This may explain the time lag of 2–3 months between peak rainfall and the peak of malaria incidence (Fig. 5), rainfall peaking between November and January while malaria incidence peaked between January and April.

Malaria incidence was closely related to temperature with the highest number of notifications being recorded in March when temperature was at a maximum and rainfall almost at a minimum ($R^2 = 0.753$; $P = 0.001$) (Table 3). High temperatures increase RH in the area, thus positively affecting vector longevity and duration of the sporogonic cycle (Garret-Jones 1964; Dye 1986). Based on these findings it may be possible to design an early warning system derived from the rainfall and temperature patterns to predict malaria epidemics and prevent their negative effects. Logistical constraints prevented mosquito catches during March and April (see Fig. 4) which would have provided better insight into the relationship between mosquito captures and malaria notifications and this will need to be addressed by future field work to substantiate current information.

This study provides a description of the current distribution and composition of *An. gambiae* complex species in Mpumalanga Province using PCR technology. Further studies are necessary to delineate more clearly the distribution and ecology of *An. merus* and its role in malaria transmission in Mpumalanga. Outdoor catches between 18:00–22:00 hours provided information on a presumably representative sample of mosquitoes responsible for malaria transmission outside sprayed houses. These findings indicate that further reduction in the burden of malaria in the Province may require measures to supplement indoor spraying that will protect people from outdoor mosquito bites between dusk and the time that they retire to sleep inside sprayed houses. However, a detailed appraisal of catches throughout the night is necessary to provide information on patterns of biting density of female mosquitoes of the *An. gambiae* complex in Mpumalanga Province. Analysis of rainfall and temperature patterns may serve to predict malaria epidemics and facilitate pre-emptive control measures.

ACKNOWLEDGEMENTS

Financial support for this study was provided by the Mpumalanga Department of Health. Ethical approval for this study was granted by Mpumalanga Research Ethics Committee and the Witwatersrand University Committee for Research on Human Subjects Clearance.

REFERENCES

BURKOT, T.R., WILLIAMS, J.L. & SCHNEIDER, I. 1984. Identification of *Plasmodium falciparum* infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. *American Journal of Tropical Medicine and Hygiene* **33**: 783–788.

BUSHROD, F.M. 1981. The *Anopheles gambiae* Giles complex and bancroftian filariasis transmission in a Tanzania coastal village. *Annals of Tropical Medicine and Parasitology* **75**: 93–100.

CHARLWOOD, J.D. & GRAVES, P.M. 1987. The effect of permethrin-impregnated bednets on a population of *Anopheles farauti* in coastal Papua New Guinea. *Medical and Veterinary Entomology* **1**: 319–327.

COETZEE, M. & LE SUEUR, D. 1988. Effects of salinity on the larvae of some Afro-tropical anopheline mosquitoes. *Medical and Veterinary Entomology* **2**: 385–390.

COETZEE, M., HUNT, R.H., BRAACK, L.E.O. & DAVIDSON, G. 1993. Distribution of mosquitoes belonging to the *Anopheles gambiae* complex, including malaria vectors, south of latitude 15 °S. *South African Journal of Science* **89**: 227–231.

COLUZZI, M. & SABATINI, A. 1968. Cytogenetic observations on species C of the *Anopheles gambiae* complex. *Parasitologia* **10**: 155–166.

COLUZZI, M. & SABATINI, A. 1969. Cytogenetic observations on the salt water species *Anopheles merus* and *Anopheles melas*, of the *gambiae* complex. *Parasitologia* **11**: 177–187.

COLUZZI, M., SABATINI, A., PETRARCA, V. & DI DECO, M.A. 1979. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **73**: 483–497.

DE MEILLON, B. 1931. Illustrated keys to the full-grown larvae and adults of South African anopheline mosquitoes. *Publication of South African Institute for Medical Research* **4**: 275.

DE MEILLON, B. 1951. Species and varieties of malaria vectors in Africa and their bionomics. *Bulletin of the World Health Organization* **4**: 419–441.

DYE, C. 1986. Vectorial capacity: must we measure all its components? *Parasitology Today* **2**: 203–209.

GARRET-JONES, C. 1964. Prognosis for the interruption of malaria transmission through assessment of the mosquito's vectorial capacity. *Nature* **204**: 1173–1175.

GEAR, J.H.S., HANSFORD, C.F. & PITCHFORD, R.J. 1981. *Malaria in South Africa.* Publication of the Department of Health, Welfare and Pensions, Pretoria.

GILLES, H.M. & WARRELL, D.A. 1993. *Bruce-Chwatt's Essential Malariology.* 3rd Edition. Edward Arnold, London.

GILLIES, M.T. & COETZEE, M. 1987. A supplement to the Anophelinae of Africa south of the Sahara. *Publication of South African Institute for Medical Research* No. 55.

GILLIES, M.T. & DE MEILLON, B. 1968. The Anophelinae of Africa south of the Sahara. *Publication of South African Institute for Medical Research* No. 54.

GOVERE, J., DURRHEIM, D.N., LA GRANGE, J.J., MABUZA, A. & BOOMAN, M. (In press) Community knowledge and perceptions about malaria, and practices influencing malaria control in Mpumalanga Province, South Africa. *South African Medical Journal.*

HII, J.L.K., BIRLEY, M.H., KANAI, L., FOLIGELI, A. & WAGNER, J. 1995. Comparative effects of permethrin-impregnated bed nets and DDT house spraying on survival rates and oviposition interval of *Anopheles farauti* No.1 (Diptera: Culicidae) in Solomon Island. *Annals of Tropical Medicine and Parasitology* **89**: 521–529.

HUNT, R.H. & COETZEE, M. 1986. Field sampling of *Anopheles* mosquitoes for correlated cytogenetic, electrophoretic and morphological studies. *Bulletin of the World Health Organization* **64**: 897–900.

INGRAM, A. & DE MEILLON, B. 1927. A mosquito survey of certain parts of South Africa, with special reference to the carriers of malaria and their control. Part I. *Publications of the South African Institute for Medical Research* **4**: 1–80.

INGRAM, A. & DE MEILLON, B. 1929. A mosquito survey of certain parts of South Africa, with special reference to the carriers of malaria and their control. Part II. *Publications of the South African Institute for Medical Research* **4**: 83–170.

LA GRANGE, J.J.P. 1995. Survey of anopheline mosquitoes (Diptera: Culicidae) in a malarious area of Swaziland. *African Entomology* **2**: 217–219.

LA GRANGE, J.J.P. & COETZEE, M. 1998. A mosquito survey of Thomo village, Northern Province, South Africa, with special reference to the bionomics of exophilic members of the *Anopheles funestus* group (Diptera: Culicidae). *African Entomology* **5**: 295–299.

LE SUEUR, D. & SHARP, B.L. 1988. The breeding requirements of three members of the *Anopheles gambiae* Giles complex (Diptera: Culicidae) in the endemic malaria area of Natal, South Africa. *Bulletin of Entomological Research* **78**: 549–560.

LINES, J.D., MYAMBA, J. & CURTIS, C.F. 1987. Experimental hut trials of permethrin-impregnated mosquito nets and eave curtains against malaria vectors in Tanzania. *Medical and Veterinary Entomology* **1**: 37–51.

MILLER, J.E., LINDSAY, S.W. & ARMSTRONG, J.R.M. 1991. Experimental hut trials of bednets impregnated with synthetic pyrethroid or organophosphate insecticides for mosquito control in The Gambia. *Medical and Veterinary Entomology* **5**: 465–476.

MNZAVA, A.E.P., RWEGOSHORA, R.T., TANNER, M., MSUYA, F.H., CURTIS, C.F. & IRARE, S.G. 1993. The effects of house spraying with DDT or lambdacyhalothrin against *Anopheles arabiensis* on measures of malarial morbidity in children in Tanzania. *Acta Tropica* **54**: 141–151.

MPOFU, S.M. 1985. Seasonal vector density and disease incidence patterns of malaria in an area of Zimbabwe. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**: 169–175.

MUIRHEAD-THOMSON, R.C. 1951. Studies on salt-water and fresh-water *Anopheles gambiae* on the East African coast. *Bulletin of Entomological Research* **41**: 487.

NJAU, R.J.A., MOSHA, F.W. & NGUMA, J.F.M. 1993. Field trials of pyrethroid impregnated bednets in Northern Tanzania. 1. Effect on malaria transmission. *Insect Science and its Applications* **14**: 575–584.

ROBERT, V., DIENG, H., LOCHOUARN, L., TRAORE, S.F., TRAPE, J.F., SIMONDON, F. & FONTENILLE, D. 1998. The transmission of malaria in Niakhar area, Senegal. *Tropical Medicine and International Health* **3**: 667–677.

SCOTT, J.A., BROGDON, W.G. & COLLINS, F.H. 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* **49**: 520–529.

SERVICE, M.W. 1970. Ecological notes on species A and B of *An. gambiae* complex in the Kisumu area of Kenya. *Bulletin of the World Health Organization* **60**: 105–108.

SERVICE, M.W. 1993. *Mosquito Ecology: Field Sampling Methods.* 2nd Edition. Elsevier Applied Science, London and New York.

SHARP, B.L. & LE SUEUR, D. 1991. Behavioural variation

of *Anopheles arabiensis* (Diptera Culicidae) populations in Natal, South Africa. *Bulletin of Entomological Research* **81**: 107–110.

SHARP, B.L., QUICKE, F.C. & JANSEN, E.J. 1984. Aspects of the behaviour of five anopheline species in the endemic malaria area of Natal. *Journal of the Entomological Society of Southern Africa* **47**: 251–258.

SPEARE, R., GOVERE, J., DURRHEIM, D.N. & MNGOMEZULU, N. 1999. Malaria control in South Africa: symposium in the wilderness. *Journal of Travel Medicine* **6**: 6–7.

TEMU, E.A., MINJAS, J.N., COETZEE, M., HUNT, R.H. & SHIFF, C.J. 1998. The role of four anopheline species (Diptera: Culicidae) in malaria transmission in coastal Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**: 152–158.

THOMPSON, R., BEGTRUP, K., CUAMBA, N., DGEDGE, M, MENDIS, C., GAMAGE-ENDIS, A., ENOSSE, S.M., BARRETO, J., SIDEN, R.E. & HOGH, B. 1997. The Matola malaria project: a temporal and spatial study of malaria transmission and disease in a suburban area of Maputo, Mozambique. *American Journal of Tropical Medicine and Hygiene* **57**: 550–559.

WHITE, G.B., MAGAYUKA, S.A. & BOREHAM, P.F.L. 1972. Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Diptera: Culicidae): bionomics and vectorial capacity of species A and B at Segera, Tanzania. *Bulletin of Entomological Research* **62**: 295–317.

WHITE, G.B. 1974. *Anopheles gambiae* complex and disease transmission in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **68**: 278–301.

Accepted 31 January 2000