THE POTENTIAL FOR DENGUE IN SOUTH AFRICA: MORPHOLOGY AND THE TAXONOMIC STATUS OF *AEDES AEGYPTI* POPULATIONS

PETER G. JUPP[1], ALAN KEMP[1] AND CHRIS FRANGOS[2]

ABSTRACT. Some 1,881 *Aedes aegypti* (L.) progeny were reared as sibling samples taken from 196 families representing populations from 18 localities in South Africa, including anthropophilic and non-anthropophilic populations. The number of white scales on tergite I (T_1) and in the basal band on tergite II (T_2) were counted. Study of family samples showed that 60.2% of families were heterogeneous, containing both the type and *formosus* forms. Hence the division into nominate (type) and *formosus* subspecies is considered invalid. Multivariate statistical analysis of variance in the population samples in respect of T_1 and T_2 together showed that each population was significantly different from all the others. However, statistical analysis of T_1 and T_2 alone showed that although some populations differed significantly, there was no consistent difference between anthropophilic and non-anthropophilic populations. It is concluded that in South Africa *Ae. aegypti* is a single polymorphic species displaying plasticity in its man-biting behavior.

INTRODUCTION

Dengue fever occurred in epidemic form in South Africa in Durban in 1926–1927 (Edington 1927). Since then it has not been reported until 1985 when dengue 1 virus was isolated from one person and dengue antibodies detected in two other people, all of whom were Durban residents recently returned from trips to India (Blackburn and Rawat 1987, Blackburn et al. 1987). This development and the recent outbreaks of dengue in nearby countries, including Moçambique (Anonymous 1985), the Seychelles (Metselaar et al. 1980) and Kenya (Johnson et al. 1982) has caused concern that the virus might be reintroduced into South Africa. Because of this, it was decided that a study should be undertaken of *Aedes aegypti* (Linnaeus), and other mosquitoes, particularly *Aedes* (*Stegomyia*) species, occurring mainly along the eastern coast (Natal) to evaluate their candidature as potential vectors which could participate in epidemic transmission. The overall study has several aspects. The first is a morphological study of *Ae. aegypti* populations to elucidate their taxonomic status, which is reported in this paper. Other aspects to be reported will deal with isozymes and the taxonomy of *Ae. aegypti* the ecology of *Ae. aegypti* and other mosquitoes, and the results of experiments undertaken to assess the vector competence of *Ae. aegypti* and selected mosquitoes with dengue viruses.

Populations of *Ae. aegypti* may vary in their morphology, ecology, physiology and genetics (e.g., Machado-Allison and Craig 1972, McClelland 1974, Trpis and Hausermann 1975, Tabachnick et al. 1979). Domestic and sylvan forms of the mosquito have been recorded in coastal Kenya (Van Someren et al. 1955, Trpis and Hausermann 1975) and apparently in inland Uganda (Haddow 1945). The usually paler domestic form breeds in domestic containers and exists in close association with man, while the usually darker sylvan form breeds in tree holes in rural areas away from houses. In 1957, Mattingly divided the species into two subspecies *viz. Ae. aegypti aegypti*, the type form with pale scaling on the first abdominal tergite and/or a distinctly paler or browner body than the African subspecies *Ae. aegypti formosus* (Walker). *Aedes aegypti formosus*, which never has any pale scales on the first tergite, has a markedly blackish appearance and is confined to Africa

[1] Arbovirus Unit, National Institute for Virology, Department of Virology, University of the Witwatersrand, Private Bag X4, Sandringham 2131, South Africa.
[2] Department of Statistics, University of the Witwatersrand, P O WITS, 2050, South Africa.

south of the Sahara. *Aedes aegypti* var. *queenslandensis* (Theobald) has increased white scaling on the abdominal tergites beyond the first tergite and/or has a lighter mesonotal color. Van de Hey et al. (1978) and Tabachnick et al. (1979), respectively, reported extensive morphological and genetic studies and agreed with Mattingly (1957) that *Ae. aegypti* was a polytypic species. In contrast, McClelland (1974), who studied the morphology of populations worldwide, concluded that there were probably two species or incipient species, *viz. Ae. aegypti* and *Ae. formosus*. In these studies these workers did not analyze morphological variation within families to look for intraspecific variation, i.e., polymorphism.

In 1960, McClelland obtained both the type and *formosus* forms in the progeny from matings between *formosus* phenotypes, although he appears to have pooled several families of progeny together upon which to make his morphological analysis. Similar heterogeneous progeny resulted from matings between phenotypes of the type form. Hartberg (1969) appears to be the only worker who has reared separate families from field-collected females, but his important results were only published in a mimeographed document by the World Health Organization. He also found variation in abdominal tergal scale pattern within single families from eggs laid by single *formosus* or type form females.

In South Africa, little has been reported on *Ae. aegypti*. Earlier work by Muspratt (1956) indicated the presence of populations in South Africa with differing ecologies. More recent work by our unit at two rural localities, Ndumu in northern Natal-Kwa Zulu (McIntosh et al. 1972, Kemp and Jupp 1991) and Mica in the northeastern Transvaal (Jupp and McIntosh 1990) has shown the presence there of non-anthropophilic (non-man-biting) populations. At both these localities, mosquito collecting over several years has shown that although *Ae. aegypti* is abundant in these areas as judged by its prevalence in tree holes and bamboo pots, the mosquito is rarely attracted to man and taken off human bait. We have also just completed field studies at Skukuza and in the Magaliesburg Mountains, both inland in Transvaal (Kemp and Jupp 1991), where similar but more limited observations indicated that the same situation probably prevails at these further two rural localities. We have therefore designated these sylvan populations "probably non-anthropophilic" (Fig. 1). In this same project (Kemp and Jupp 1991), collections off human bait have, however, shown that anthropophilic populations are present at domestic or peridomestic sites along the whole Natal coast line and at Grahamstown near the coast in the eastern Cape Province (Fig. 1).

We report here a quantitative examination of the scaling of the first two segments of the abdominal tergite in families reared from anthropophilic and non-anthropophilic population samples of *Ae. aegypti* collected at various localities in South Africa. We wished to determine whether distinct type (nominate) and *formosus* forms were present in South Africa, and if they were present, whether they bred true and could be related to geographically and ecologically different populations of *Ae. aegypti*.

MATERIALS AND METHODS

Populations of *Ae. aegypti* were sampled at 18 localities: one in the Cape Province, 12 in Natal Province and five in the Transvaal Province (Table 1). The localities are shown on the map (Fig. 1), which also indicates where anthropophilic and non-anthropophilic populations occurred. Mosquitoes were collected either as eggs deposited in bamboo pots exposed in woodland, as larvae removed from artificial containers or as adults taken in landing/biting catches. Eggs and larvae were reared into adults, and the female mosquitoes thus obtained or females collected directly in the field were fed on hamsters in the laboratory. The gravid females were placed singly in tubes containing moist cotton wool and filter paper for oviposition. Subsequently, the different egg batches were reared separately and a group of 10 or more adult female siblings representing each family was pinned. These specimens were examined under a binocular dissecting microscope at 60–80 times magni-

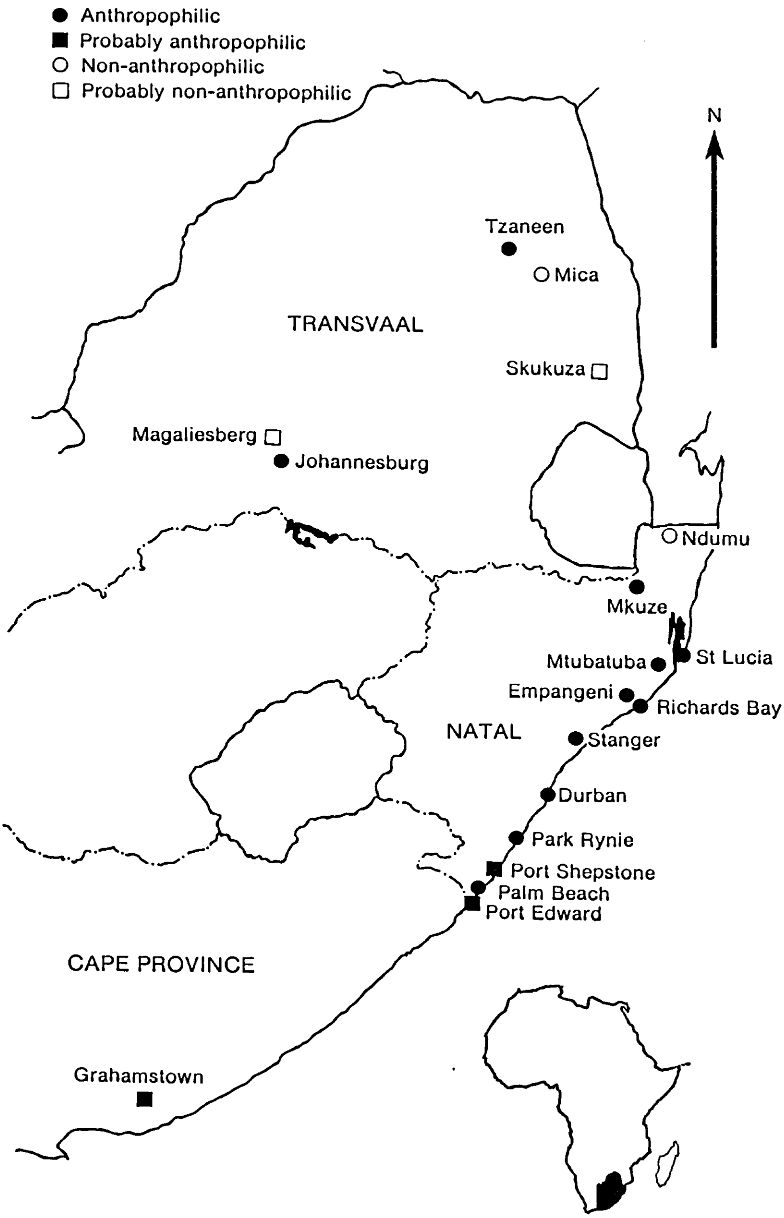

Fig. 1. Localities in South Africa where *Ae. aegypti* populations were sampled.

fication, using a blue filter on incident light. The number of white scales on the first abdominal tergite (T_1) and the number in the basal band on the second tergite (T_2) were counted. The second tergite was examined in addition to the first because, like McClelland (1960), we had noticed variation; the degree of white scaling at its base varied from complete absence to a well marked band.

The 95% confidence limits were calculated for the mean number of white scales on T_1 and T_2, respectively, for each of the 18 populations. The data were subjected to further statistical analysis using the general linear models procedure of a SAS statistical package implemented on an IBM mainframe computer. The means of both tergal characters (T_1 and T_2) together were compared on the 18 population samples by multivariate analysis of variance using the following four statis-

Table 1. Localities in South Africa where *Ae. aegypti* populations were sampled.

Locality of origin	Stage collected
CAPE	
Grahamstown	Larvae
NATAL	
Port Edward	Larvae
Palm Beach	Engorged females
Port Shepstone	Larvae
Park Rynie	Engorged females
Durban	Engorged females
Stanger	Engorged females
Empangeni	Engorged females
Richards Bay	Engorged females
Mtubatuba	Engorged females
St Lucia Estuary	Larvae
Mkuze	Larvae
Ndumu	Eggs (bamboo pots)
TRANSVAAL	
Johannesburg	Engorged females
Magaliesberg	Eggs (pots)
Skukuza	Larvae
Mica	Eggs and larvae (pots)
Tzaneen	Engorged females

tics: Wilks' Lambda (Wilks 1932), Pillai's Trace (Pillai 1954), Hotelling-Lawley Trace (Hotelling 1951) and Roy's Greatest Root (Roy 1957). Furthermore, the population means for T_1 and T_2, respectively, were grouped using the Waller-Duncan K-Ratio t-test (Waller and Duncan 1969) to find which means differed significantly. Conclusions could then be drawn as to the morphological similarity and variability between the 18 populations.

If a mosquito specimen possessed an entirely black T_1, it was classified as *formosus* form, while specimens with one or more white scales on this tergite were classified as the nominate or type form. The nature of T_2 was not taken into account when assigning a form classification to a specimen. In this manner the sibling groups representing the different families were classified as "homogeneous type form," or "homogeneous *formosus* form" or "heterogeneous with both forms." In a homogeneous family every specimen in the sample belonged to the same form, whereas a heterogeneous family consisted of a mixture of both forms in which one or more specimens did not match the remainder.

RESULTS

Figure 2 gives the results for three of the families examined. It shows T_1 and T_2 of three field-collected adult females (mothers) taken biting at Park Rynie in Natal, together with the tergites of 10 of the siblings in their respective F_1 families. It can be seen that although the mothers were *formosus* (1 M and 2 M) and type form (8 M) phenotypes, both forms appeared in their progeny with a wide range in the degree of white scaling on both tergites. Some of the specimens from some of the other localities displayed a heavier degree of white scaling than shown in Fig. 2. The types of families identified in the samples of F_1 families representing each locality are shown in Table 2. Out of 196 families, only three families (1.5%) were *formosus* form, 75 (38.3%) were type form and 118 (60.2%) were heterogeneous. When families were classified in a similar way according to the presence of white scaling on T_2, it was found that, out of all the families, 55.6% were heterogeneous and 44.4% were homogeneous for presence of white scaling.

In Table 3, all the specimens representing a locality, usually belonging to 10–13 families, were treated as one sample. For each of the 18 samples, the range of values for T_1 and T_2 is given together with the respective means and standard deviations. All the ranges of values overlap for both T_1 and T_2. These results were used to calculate the $(1-\alpha)$ 100% large sample confidence limits, were $\alpha = 0.05$, for T_1 and T_2, respectively. Figure 3 gives these limits as histograms and shows that the confidence limits for T_1 and T_2 on the various populations differ in length and position, although some populations were very close to one another. When, however, the means of the populations were compared by the Waller-Duncan test in respect of T_1 alone, it was found that none of the four non-anthropophilic or probable non-anthropophilic populations, Ndumu, Mica, Magaliesberg and Skukuza, differed consistently from the remain-

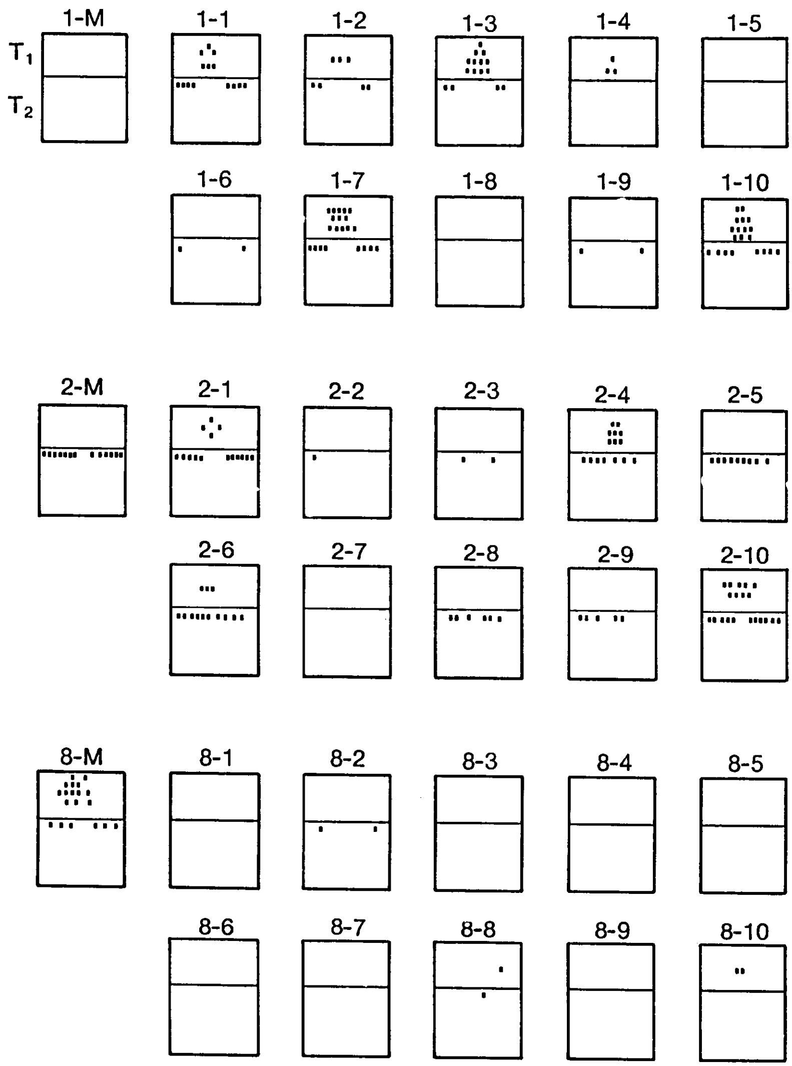

Fig. 2. Distribution of white scales on the first (T_1) and second (T_2) tergites in three *Ae. aegypti* mothers (M) and samples of their respective F_1 progeny.

ing anthropophilic populations $P > 0.05$). The same analysis done for T_2 alone indicated some similarity between these four populations in their having more white scales than most anthropophilic populations ($P < 0.0001$). Ndumu differed from all other populations except Mica. Mica differed from all others except Ndumu and Skukuza. Skukuza was similar to Mica and the anthropophilic populations of Port Edward and Johannesburg. Magaliesberg was similar to Richards Bay, Empangeni, Palm Beach and Mkuzi, all anthropophilic populations. Grahamstown differed from all other populations by having a considerable number of white scales.

When both sample means (T_1 and T_2) were compared together by multivariate analysis, all 18 populations were found to be significantly different from one another ($P < 0.0001$) using the four chosen statistics.

DISCUSSION

Hartberg et al. (1986) showed that abdominal tergal scale pattern in *Ae. aegypti* appeared to be controlled by one major poly-

Table 2. Classification of F_1 families of *Ae. aegypti* from various localities into those which are homogeneous for either the typical or *formosus* forms and those which are heterogeneous.

Locality	Total	No. families Homogeneous: typical form	Homogeneous: *formosus* form	Heterogeneous: both forms
Grahamstown	11	5	0	6
Port Edward	11	4	0	7
Palm Beach	12	3	0	9
Port Shepstone	12	4	0	8
Park Rynie	12	0	1	11
Durban	10	5	0	5
Stanger	10	4	0	6
Empangeni	9	4	0	5
Richards Bay	11	5	0	6
Mtubatuba	8	3	0	5
St Lucia Estuary	11	9	0	2
Mkuze	10	1	0	9
Ndumu	11	4	0	7
Johannesburg	11	4	0	7
Magaliesberg	15	3	2	10
Skukuza	11	8	0	3
Mica	13	4	0	9
Tzaneen	8	5	0	3
	196	75	3	118
		38.3%	1.5%	60.2%

genic system with modifiers. Hence he considered that it would be better to use differences in ecology, physiology and behavior when attempting to divide *Ae. aegypti* into different species or subspecies. He concluded that while abdominal tergal scale pattern may be useful for distinguishing populations, it may not be valid as the exclusive taxonomic basis for classification. In earlier work, Hartberg (1969) showed that the offspring of 35 female *formosus* and typical forms collected at Dar-es-Salaam, Tanzania, nearly all failed to breed true, indicating that the populations were simply polymorphic for abdominal tergal scaling. In the present study, about two-thirds of the 196 families examined from 18 localities in South Africa similarly failed to breed true, but gave offspring that were heterogeneous for the presence or absence of white scales on the first tergite. This finding shows that the basis for separating *Ae. aegypti* into nominate and *formosus* subspecies proposed by Mattingly (1957) should be regarded as invalid. It would be interesting to study the variety *queenslandensis* in a similar way.

Using two characters, the degree of scaling on both the first and second tergites, we found that all 18 populations of *Ae. aegypti* differed significantly regardless of their degree of anthropophilism or geographic location. This supports the view that *Ae. aegypti* is a single polymorphic species in South Africa with a variable preference for man. Other studies by us using isozyme electrophoresis to analyze genetic difference between South African populations of *Ae. aegypti* (Kemp and Jupp, unpublished observations) also failed to reveal any evidence for the existence of more than

Table 3. Number of white scales on first tergite (T_1) and in basal band on second tergite (T_2) in F_1 female mosquitoes from various localities.

Locality	n	T_1 Range	T_1 x	T_1 s	T_2 Range	T_2 x	T_2 s
Grahamstown	104	0–45	7.58	9.54	4–35	18.60	6.01
Port Edward	112	0–40	5.63	7.51	0–18	7.54	4.50
Palm Beach	110	0–30	5.73	7.06	0–25	4.55	4.63
Port Shepstone	104	0–34	5.98	7.38	0–11	3.13	2.96
Park Rynie	110	0–24	1.95	4.38	0–15	4.15	4.44
Durban	102	0–35	8.77	9.95	0–22	5.75	5.44
Stanger	100	0–60	13.08	15.11	0–25	6.01	5.34
Empangeni	94	0–30	6.62	7.12	0–23	4.84	5.02
Richards Bay	109	0–40	7.15	7.47	0–16	5.40	4.33
Mtubatuba	81	0–24	5.80	5.47	0–17	7.09	4.66
St Lucia Estuary	100	0–40	11.91	8.89	0–19	3.88	4.02
Mkuze	96	0–15	2.76	3.37	0–21	4.40	4.00
Ndumu	125	0–19	4.50	4.07	0–27	9.72	5.88
Johannesburg	104	0–26	5.02	6.54	0–22	7.41	5.10
Magaliesberg	129	0–25	3.42	5.98	0–20	4.37	3.97
Skukuza	108	0–31	8.81	7.39	0–26	8.39	6.41
Mica	130	0–30	5.38	5.83	0–20	9.29	4.96
Tzaneen	63	0–30	8.05	8.34	0–16	5.68	4.95
	1,881						

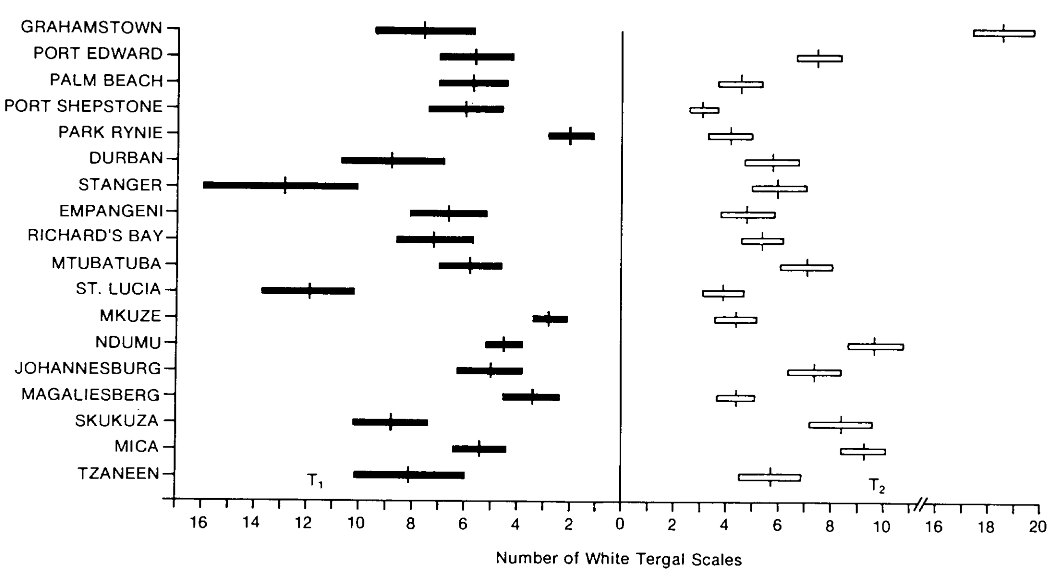

Fig. 3. Sample means and 95% confidence limits for the number of white scales on T_1 and in the basal band on T_2 in *Ae. aegypti* populations. Fourteen populations were anthropophilic, two non-anthropophilic (Ndumu and Mica) and two were probably non-anthropophilic (Skukuza and Magaliesberg).

one species. Some of the populations were significantly different in respect of the T_1 value alone by the Waller Duncan test, although all the ranges of values for T_1 overlapped. The ranges of values also overlapped for T_2 in all of the populations, although some samples also differed statistically in respect of this character. Nevertheless, our results failed to show that non-anthropophilic populations invariably exhibited a larger number of white scales on T_2 than anthropophilic populations. Apart from the work done on *Ae. aegypti* from East African countries, morphological variation has been recorded for Nigeria (Summers-Connal 1927), the Philippines (Mogi et al. 1984) and northern Thailand (Mogi et al. 1989). The presence of such variation needs to be analyzed within families.

It is concluded that in South Africa, *Ae. aegypti* is a single polymorphic species showing a considerable amount of variation in the degree of white scaling on the first two abdominal tergites. The presence of some rural sylvan non-anthropophilic populations in our country (Kemp and Jupp 1991) might, however, indicate incipient speciation.

ACKNOWLEDGMENTS

We wish to thank the Directors of the KwaZulu Bureau of Natural Resources, the Natal Parks Board and the National Parks Board as well as the head of the Department of Community Services of Richards Bay Municipality for permission to collect mosquitoes in areas under their jurisdiction. Special thanks are due to Mr. Graham Wiltshire (Ndumu reserve), Mr. Tommy Wessels (Richards Bay) and Dr. Leo Braak (Kruger Park) for their kind assistance. Mr. Edward Chauke and Mr. Obed Matlala rendered technical assistance in the laboratory, which is gratefully acknowledged. Finally, one of us (P.G. Jupp) is indebted to the South African Medical Research Council for a grant toward the project.

REFERENCES CITED

Anonymous. 1985. Dengue fever. Weekly Epidemiol. Rec. 60:242–243.

Blackburn, N.K. and R. Rawat. 1987. Dengue fever imported from India. South Afr. Med. J. 71:386–387.

Blackburn, N.K., G. Meenehan and N. Aldridge. 1987. The status of dengue fever virus in South Africa—serological studies and diagnosis of a case of dengue fever. Trans. R. Soc. Trop. Med. Hyg. 81:690–692.

Edington, A.D. 1927. "Dengue" as seen in the recent epidemic in Durban. J. Med. Assoc. South Afr. 1:446–448.

Haddow, A.J. 1945. On the mosquitoes of Bwamba County, Uganda: I. Description of Bwamba, with special reference to mosquito ecology. Proc. Zool. Soc. Lond. 115:1–13.

Hartberg, W.K. 1969. Genetical assessment of taxonomic characters of *Aedes aegypti* (L) in Tanzania. WHO/VBC/69.152. Mimeographed document, World Health Organization, Geneva.

Hartberg, W.K., C.K. Meeks and K.R. Williams. 1986. A model for polygenic inheritance of abdominal tergal scale pattern in *Aedes aegypti*. J. Am. Mosq. Control Assoc. 2:490–502.

Hotelling, H. 1951. A generalized t-test and measure of multivariate dispersion. Proceedings of the 2nd Berkeley symposium on mathematical statistics and probability. pp. 33–41. University of California Press, Berkeley.

Johnson, B.K., D. Ocheng, A. Gichogo, M. Okiro, D. Libondo, P. Kinyanjui and P.M. Tukei. 1982. Epidemic dengue fever caused by dengue type 2 virus in Kenya: preliminary results of human virological and serological studies. East Afr. Med. J. 59:781–784.

Jupp, P.G. and B.M. McIntosh. 1990. *Aedes furcifer* and other mosquitoes as vectors of chikungunya virus at Mica, northeastern Transvaal, South Africa. J. Am. Mosq. Control. Assoc. 6:415–420.

Kemp, A. and P. G. Jupp. 1991. Potential for dengue in South Africa: mosquito ecology with particular reference to *Aedes aegypti*. J. Am. Mosq. Control Assoc. 7:574–583.

Machado-Allison, C.E. and G.B. Craig. 1972. Geographic variation in resistance to desiccation in *Aedes aegypti* and *A. atropalpus* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 65:542–547.

Mattingly, P.F. 1957. Genetical aspects of the *Aedes aegypti* problem, I. Taxonomy and bionomics. Ann. Trop. Med. Parasitol. 51:392–408.

McClelland, G.A.H. 1960. A preliminary study of the genetics of abdominal colour variations in *Aedes aegypti* (L.) (Diptera: Culicidae). Ann. Trop. Med. Parasitol. 54:305–320.

McClelland, G. A. H. 1974. A worldwide survey in variation in scale pattern of the abdominal tergum of *Aedes aegypti* (L.) (Diptera: Culicidae). Trans. R. Entomol. Soc. Lond. 126:239–259.

McIntosh, B.M., P.G. Jupp and J. De Sousa. 1972. Mosquitoes feeding at two horizontal levels in gallery forest in Natal, South Africa, with reference to possible vectors of chikungunya virus. J. Entomol. Soc. South. Afr. 35:81–90.

Metselaar, D., C.R. Grainger, K.G. Oei, D.G. Reynolds, M. Pudney, C.J. Leake, P.M. Tukei, R.M. D'Offay and D.I.H. Simpson. 1980. An outbreak of type 2 dengue fever in the Seychelles, probably transmitted by *Aedes albopictus* (Skuse). Bull. WHO 58:937–943.

Mogi, M., T. Okazawa, I. Miyogi and L.A. de las Llagas. 1984. Variation in abdominal color pattern in eight populations of *Aedes aegypti* from the Philippines. Mosq. News 44:60–65.

Mogi, M, W. Choochote, T. Okazawa, C. Khamboonruang and P. Suwanpanit. 1989. Scale pattern variations of *Aedes aegypti* in Chiang Mai, northern Thailand. J. Am. Mosq. Control Assoc. 5:529–533.

Muspratt, J. 1956. The *Stegomyia* mosquitoes of South Africa and some neighbouring territories. Mem. Entomol. Soc. South. Afr. 4:1–138.

Pillai, K.C.S. 1954. On some distribution problems in multivariate analysis. Mimeographed series No. 88, Chapel Hill Institute of Statistics, University of North Carolina.

Roy, S.N. 1957. Some aspects of multivariate analysis. John Wiley & Sons, New York.

Summers-Connal, S. 1927. On the variations occurring in *Aedes argenticus* Poiret, in Lagos, Nigeria. Bull. Entomol. Res. 18:5–11.

Tabachnick, W.J., L.E. Munstermann and J.R. Powell. 1979. Genetic distinctness of sympatric forms of *Aedes aegypti* in East Africa. Evolution 33:287–295.

Trpis, M. and W. Hausermann. 1975. Demonstration of differential domesticity of *Aedes aegypti* (L.) (Diptera: Culicidae) in Africa by mark-release-recapture. Bull. Entomol. Res. 65:199–208.

Van de Hey, R.C., M.G. Leachy and K.S. Booth. 1978. Analysis of colour variations in feral, peridomestic and domestic populations of *Aedes aegypti* (L) (Diptera: Culicidae). Bull. Entomol. Res. 68:443–453.

Van Someren, E.C.C., C. Teesdale and M. Furlong. 1955. The mosquitoes of the Kenya coast; records of occurrence, behaviour and habitat. Bull. Entomol. Res. 46:463–493.

Waller, R.A. and D.B. Duncan. 1969. A Bayes Rule for the symmetric multiple comparisons problem. J. Am. Stat. Assoc. 64:1484–1503.

Wilks, S.S. 1932. Certain generalizations in the analysis of variance. Biometrika 24:471–494.