Anopheles marshallii (Theobald) is a Complex of Species

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ABSTRACT. Cytogenetic analysis of ovarian nurse cell polytene chromosomes has revealed the existence of three biological species within the taxonomic species *Anopheles marshallii*. Composite photographic maps of the sex chromosomes of the three species are presented detailing the fixed rearrangements which have accompanied the evolution of the group. Details of the sites of collection of the new species are also included. Two of the species have been recorded from Salisbury, Zimbabwe, the type locality of *A. marshallii*.

INTRODUCTION

The taxon *Anopheles marshallii* has a wide distribution in the Ethiopian region, extending from South Africa to Ethiopia with "an atypical form" recorded from West Africa. Evans (1938), De Meillon (1947) and Gillies and De Meillon (1968), report large amounts of variation in both adult and larval morphology together with some variation in biting behaviour. This variation suggested a possible species complex within the taxonomic species. Because previous studies of *Anopheles*, approached from a genetical viewpoint, have revealed a number of species within taxonomic species, it was decided to investigate *A. marshallii* using ovarian polytene chromosomes.

Since the original description of *marshallii* by Theobald in 1903 a number of species, e.g. *A. gibbinsi* Evans, *hargreavesi* Evans and *mousinhoi* De Meillon and Pereira have been separated from *marshallii* after having been described as varieties by various authors. This possibly reflects an awareness on the part of alpha taxonomists that often only subtle morphological differences have accompanied speciation events in *Anopheles*.

This study was initiated in order to define genetically the taxonomic species *Anopheles marshallii* found in South Africa and Zimbabwe, with the intention of then redefining any genetical species morphologically. The advent of these genetical techniques has produced the need to bring into alignment, in every case, the taxonomic and genetical species.

MATERIALS AND METHODS

Collections of female mosquitoes for ovarian polytene chromosome analysis have been made from a number of sites in South Africa including many localities within the area surrounding Makonde (Northern Transvaal), Kwa-Nkuzane

(Northern Transvaal), Rustenburg (Magaliesberg), Johannesburg and Komatipoort together with Kanyemba in Zimbabwe. A number of collections have also been made from the Salisbury area because this is the type-locality of *Anopheles marshallii*. Females were collected in the wild and analysed for ovarian nurse cell polytene chromosomes using the method of Hunt (1973). Routine slide analysis was facilitated by the use of a camera lucida for the examination of banding patterns (as first used in *Drosophila* by Carson, 1970) and photomaps mounted on a black background to reduce glare.

RESULTS

The chromosome analysis has revealed the presence of three species which differ chromosomally and which have been temporarily named species A, B and C. Analysis of 1358 animals, including 942 species A, 322 species B and 94 species C, from the localities mentioned has not revealed the presence of any hybrids between the species. Figure 1 shows the sex chromosomes of the three species and, using species B as a standard sequence, shows the rearrangement of blocks of chromosomes. The figure also shows the direction of each block. A segment which is facing in the opposite direction in either species A or C compared to the standard species B pattern is represented by an inverted letter. Symmetrical letters such as A and H have been given arrows which indicate which way the letter, and hence the chromosome block that it represents, is facing.

In the Makonde area the three species have been found to be sympatric. Species A has not been recorded from any area in the highveld but has been found in high numbers in a number of lowveld areas. The collections from Salisbury have revealed the presence of species B and C from that locality. Table 1 shows the sites of collection and the species recorded from those localities.

DISCUSSION

This preliminary report provides unequivocal evidence of the existence of a number of genetically distinct species within the taxonomic species *A. marshallii*. Figure 1 shows the large number of fixed inversion differences between the sex chromosomes of the species, for example there are 7 break-points which mark the differences between species A and B. The autosomes of the three species differ also in a large number of fixed sequence changes. The details of these fixed differences together with phylogenetic relationships among the species will be reported later. The importance of these large differences in sex chromosome banding patterns is that firstly this provides a method of identification of these morphologically cryptic species. Secondly these differences, together with the large number of autosomal rearrangements, mean that a hybrid between any two of these species would show a very large number of heterozygous inversions. Any such individual would be unmistakably identified using ovarian polytene chromosome analysis.

To this date 1358 wild caught females of the complex have been examined from the 8 areas shown in table 1. No hybrid individuals have been found.

This is unequivocal evidence for the existence of complete positive assortative mating and hence of the specific status of the forms described here. If mating between these forms was at random, we would expect to have found many hundreds of animals with heterozygous inversions. These results then would have been inexplicable.

Some differences in the biologies of the species have emerged. Species A exclusively, is often caught biting man even in the Makonde area where all three species are caught biting cattle. It has been suggested[1] that *"A. marshallii"* may be a low-level malaria carrier and may be responsible for the sporadic outbreaks of malaria which are common in the northern areas of South Africa. The facts presented here, of the high incidence of man-biting by *A. marshallii* species A, together with its presence in large numbers in lowveld areas, suggest that efforts at determining the status of *"A. marshallii"* as a malaria carrier should concentrate on species A. Species B and C are less likely to be responsible, based on the evidence that they rarely bite man.

A discriminant function analysis is presently being employed in order to provide a method, using adult female morphological characters, which is capable of separating these cryptic species. Measurements of the types of *A. marshallii* as well as those species currently recognised as synonyms will then be entered into the programme as unknowns in order to determine whether any of these available names should be assigned to the species uncovered in this study. Preliminary evidence indicates that, the species used as a chromosomal standard, species B, is *A. marshallii sensu strictu* with a 99.9% probability. A study of egg and larval stages of the species is also underway to further help in the clarification of these taxa.

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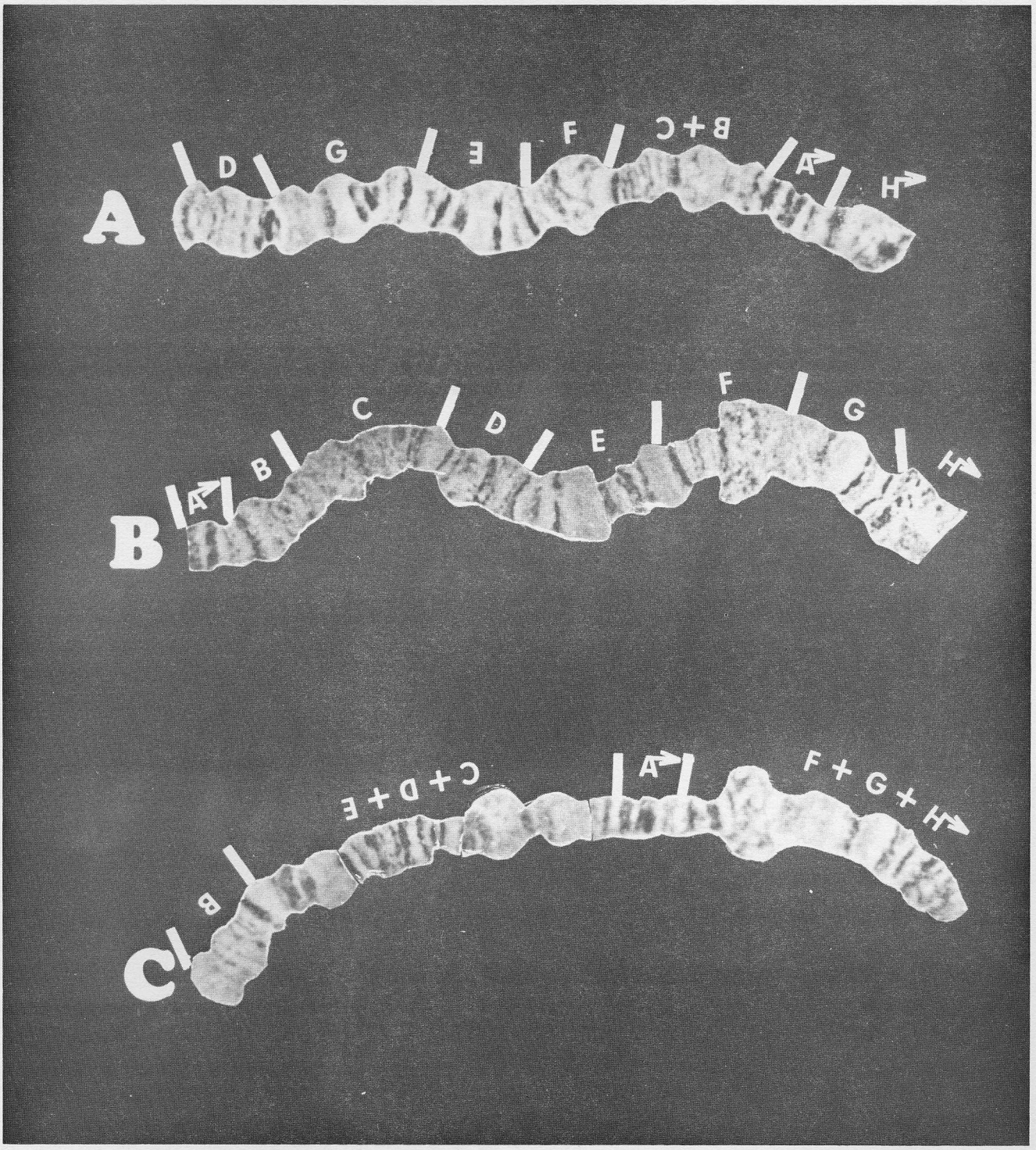
Figure 1. Sex chromosomes of species A, B and C of the *A. marshallii* complex showing sequence homologies. Breakpoints are marked and rearranged blocks are indicated by letters of the alphabet. Inverted letters indicate that the block represented faces the opposite direction to that of the standard species B sequence.

Table 1

Sites of collection of *A. marshallii* complex species

Collection Site	Species and numbers recorded
Makonde (Northern Transvaal) 22° 45'S, 30° 35'E	A - 824 B - 222 C - 79
Rustenberg (Magaliesberg, South Africa)	B - 2
Johannesburg (South Africa)	B - 1
Komatipoort (Transvaal, South Africa)	A -104
Salisbury (Zimbabwe)	B - 97 C - 15
Kanyemba (Zimbabwe) 15° 40'S, 30° 20'E	A - 1
Kwa-Nkuzane (Northern Transvaal) 23° 15'S, 30° 20'E	A - 7
Tzaneen (Northern Transvaal)	A - 6


A
D
G
E
F
B+C
A
H
B
A
B
C
D
E
F
G
H
C
B
C+D+E
A
F+G+H