CYTOGENETIC EVIDENCE FOR A NEW SPECIES WITHIN THE TAXON *ANOPHELES (CELLIA) MARSHALLII* (THEOBALD) (DIPTERA: CULICIDAE)

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ABSTRACT. Ovarian polytene chromosome studies of *Anopheles marshallii* (Theobald) have revealed a possible new species within this taxon. A fixed inversion difference on autosome arm five and a polymorphic inversion on arm three were recorded from populations collected in the Transvaal, South Africa. A chromosome map is provided.

INTRODUCTION

Anopheles (Cellia) marshallii (Theobald) consists of at least four cytogenetically defined species (A, B, C and E of Lambert 1979a, 1981), subsequently named *An. letabensis*, *An. marshallii*, *An. hughi* (Lambert and Coetzee 1982) and *An. kosiensis* (Coetzee et al. 1987), respectively. One of these, *An. letabensis*, readily bites man in the eastern Transvaal district of South Africa (Lambert and Coetzee 1982). Its status as a vector of malaria parasites, however, is unknown. Bafort (1985) working in West Africa found significant numbers of "*An. marshallii*" with sporozoites in their salivary glands and suggested that the species concerned may be *An. letabensis* because of its man-biting behavior. Unfortunately, none of the specimens were chromosomally identified to support this conjecture. The other species of the complex have been collected mainly in cattle enclosures or resting outdoors in natural and artificial shelters (Lambert 1979b, Lambert and Coetzee 1982, Coetzee et al. 1987).

Lambert (1979a, 1981) provides photographs of the X chromosomes for identification of the four species. Morphological characters for identification were described by Lambert and Coetzee (1982) and Coetzee et al. (1987). Recent studies of the chromosomes of the *An. marshallii* group of mosquitoes revealed differences indicating a new species apparently morphologically identical to *An. marshallii* and possessing the same X chromosome.

MATERIALS AND METHODS

Chromosomes were obtained from the progeny of five wild female mosquitoes resting in a cattle enclosure near Lydenburg (25°07′S 30°27′E) and two individuals from a CO_2 baited net trap at Onderstepoort (25°34′S 28°10′E). Onderstepoort is just north of Pretoria while Lydenburg is approximately 270 km east. The wild females were induced to lay eggs in the laboratory and their progeny reared to adults. The F_1 females were offered blood meals and when half gravid, the ovaries dissected and stored in Carnoy's fixative. Chromosomes were prepared using the method described by Hunt and Coetzee (1986). Photographs of chromosomes from seven females from the type locality, Harare, Zimbabwe, (17°48′S 31°04′E) collected on Ardmore Farm in a cattle enclosure were provided by C.A. Green. Inversion notations follow on from Green (1982), Subbarao et al. (1983) and S.K. Subbarao (personal communication) for the Myzomyia group.

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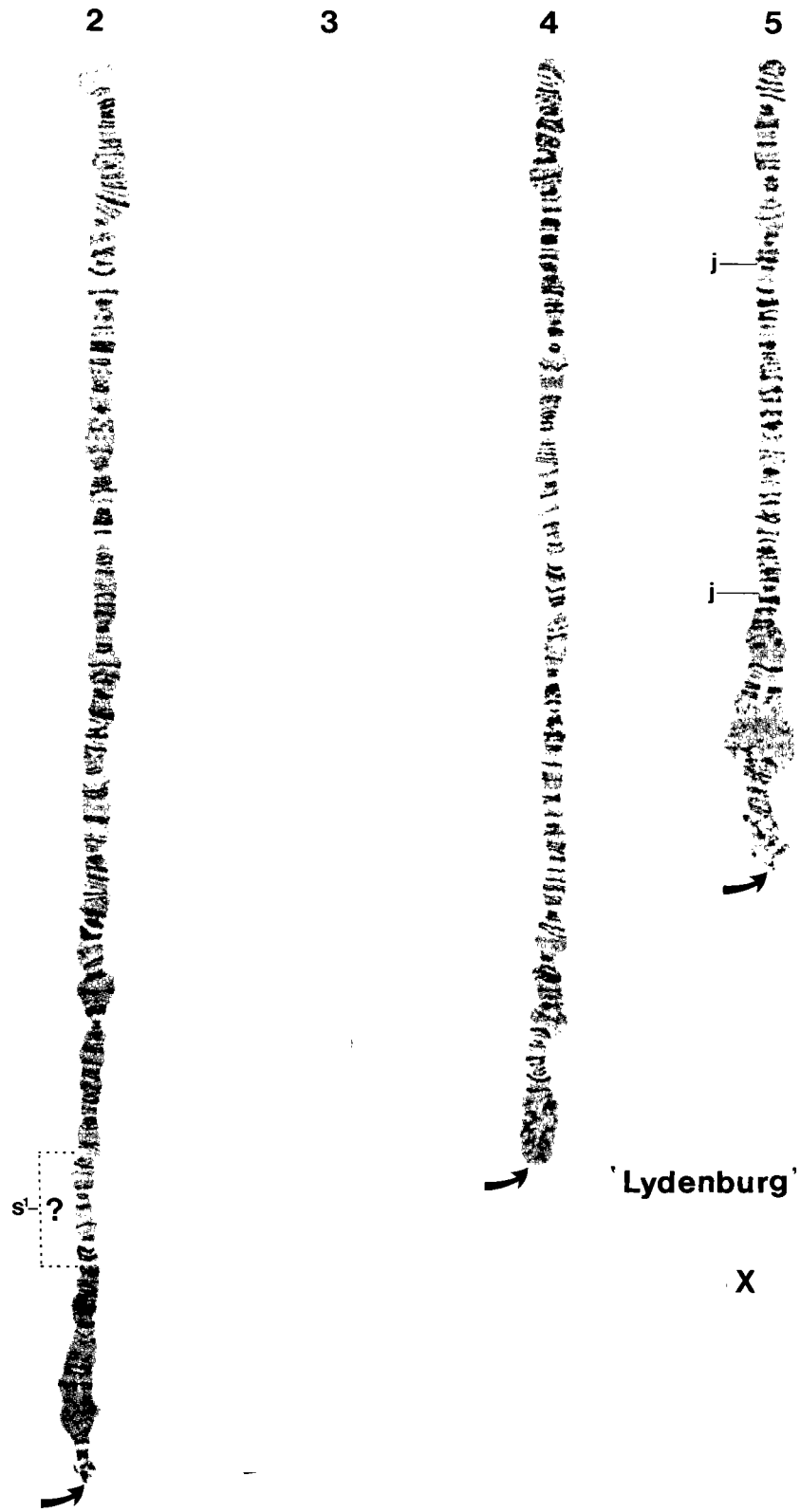
2
3
4
5
j
j
s
?
'Lydenburg'
X

RESULTS AND DISCUSSION

Lambert (1979b) examined 322 specimens of *An. marshallii* from four widely separated localities (Harare 97, Makonde, N. Transvaal 222, Johannesburg 1, Rustenburg 2) and reported no polymorphic inversions. The chromosomes obtained from the type locality and used in the present study were identical to the full karyotype map of *An. marshallii* of Lambert (1979b). These chromosomes were then compared with the Lydenburg and Onderstepoort samples.

A map of the ovarian polytene chromosomes of *An. marshallii* "Lydenburg" is given in Fig. 1. Comparisons with *An. marshallii* of Lambert (1979a, 1979b) revealed that the X chromosomes were homologous. However, a fixed inversion difference occurred on arm 5 ("j", Fig. 1) (n = 27). Two polymorphic inversions were seen. One, "s" on arm 3, was present as a heterozygote in progeny from five of the seven families. Progeny from the other two were homozygous for the standard *An. marshallii* arrangement. The other inversion, "s^1" involved a small segment of arm 2 seen in two offspring from the same family. The precise breakpoints of this inversion were not determined, as only heterozygotes of rather poor quality were seen. The remaining families had the normal *An. marshallii* arrangement for arm 2. The region in which this inversion occurred is marked on the map with dotted lines. The bands at the centromere end of the X chromosome were often obscured by a large puff. The two expressions of the puff are shown on the map.

The autosomal arm associations for *An. marshallii* were 2+3 and 4+5, which differs from all other chromosomally typed species in the series Myzomyia (Green and Hunt 1980, Green 1982). The associations 2+5 and 3+4 have been recorded in the Asian species *An. fluviatilis* James and *An. culicifacies* Giles as well as the African species *An. leesoni* Evans, *An. rivulorum* Leeson, *An. fuscivenosus* Leeson, *An. demeilloni* Evans, *An. theileri* Edwards and *An. wellcomei* Theobald. The third combination, 2+4 and 3+5, is found in the African species *An. funestus* Giles, *An. parensis* Gillies and *An. vaneedeni* Gillies and Coetzee. Thus, all three possible arm association combinations have now been recorded in African species of Myzomyia.

Considering Lambert's (1979b) large sample of monomorphic *An. marshallii* from South Africa and Zimbabwe, the fixed inversion difference on arm 5 strongly suggests that what is now known as *An. marshallii* consists of two separate species. This is supported by the polymorphic 3s inversion in both the Lydenburg and Onderstepoort populations. Cryptic species often share the same X chromosome but differ by fixed inversions on the autosomes. For example, in the *Anopheles gambiae* Giles group, the six named species share only three different X chromosome arrangements, i.e., *An. gambiae* and *An. merus* Dönitz share the same X while *An. quadriannulatus* Theobald, *An. melas* Theobald and *An. bwambae* White share an X differing from the first by a single inversion. *Anopheles arabiensis* Patton has a unique X chromosome (Coluzzi et al. 1979).

The external morphology of the two *An. marshallii* forms showed no apparent differences, and both conformed to the morphological descriptions of *An. marshallii* (Gillies and De Meillon 1968, Lambert and Coetzee 1982).

The limited material available prevents determination of the specific status of these two populations. However, the map presented here will facilitate future studies on this group.

Fig. 1. Ovarian polytene chromosome map of *Anopheles marshallii* "Lydenburg." Inversion differences between it and *An. marshallii* are indicated with letters. The breakpoints of the arm 2 inversion were not determined accurately (only two heterozygotes were seen) and are delimited with dotted lines. The bands at the centromere end of the X chromosome are usually obscured by extensive puffing. An example of each expression of the puff is shown. Centromere ends are marked with arrows. Arm nomenclature follows that of Green and Hunt (1980). Arm associations are X, 2+3 and 4+5.

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