

Observations On the Mitotic Chromosomes of
the Mosquito *Toxorhynchites amboinensis* (Doleschall)

Robert D. Hunter, Jr. and W. Keith Hartberg
Institute of Arthropodology and Parasitology
Department of Biology
Georgia Southern College
Statesboro, Georgia 30460-8042

ABSTRACT. The karyotype of *Toxorhynchites amboinensis* consists of 1 metacentric and 2 slightly submetacentric pairs ($2n=6$). The average lengths of the metaphase chromosomes are: I=6.68 μ m, II=7.83 μ m, and III=8.55 μ m. Average arm ratios are: I=1.000, II=1.034, and III=1.017. Somatic pairing is exhibited in all three pairs of mitotic chromosomes.

INTRODUCTION

Of the more than 60 described species of *Toxorhynchites*, only a few have been karyotyped. These include *Tx. rutilus septentrionalis* (Breland 1961), *Tx. yamadai* (Kanda 1968), *Tx. splendens* (Bhat 1976), and *Tx. brevipalpis* and *Tx. amboinensis* (Hemingway and White 1980).

Hemingway and White (1980) reported in a correspondence on a laboratory meeting of the London School of Hygiene and Tropical Medicine that they had prepared and examined mitotic and polytene chromosomes of *Tx. amboinensis* and *Tx. brevipalpis*; however, they did not include in their note figures or give measurements of the chromosomes. The present study was undertaken to confirm their observations and to provide and record measurements and figures for the mitotic chromosomes in *Tx. amboinensis*.

MATERIALS AND METHODS

The larval specimens of *Tx. amboinensis* used in this study were randomly selected from the colony maintained by Dr. R. Novak at the San Juan Laboratories, CDC, San Juan, Puerto Rico. The third and fourth instar larvae selected were fixed in a solution of 6 parts methanol, 3 parts chloroform, and 2 parts propionic acid (Pienaar 1955), and the brains were also dissected from the larvae in this solution. The chromosomal slide preparation was essentially the same as that described by Breland (1961) except that the brains were stained for 10-15 minutes in a 1% lacto-aceto-orcein stain. The preparation was then gently squashed using coverslips and slides treated with Sigmacote and then sealed with fingernail polish. All measurements were made by using a micrometer disc and

slide micrometer. Each measurement given in Table 1 is based on an arithmetical mean of two separate measurements from fourteen separate chromosome figures (minimum of 28 measurements/chromosome) judged to be in metaphase.

All photographs of dividing cells were taken under phase at 1000x magnification with an Olympus PM-7 camera mounted on an Olympus FHA microscope. Kodak Plus-X Pan film was used.

RESULTS AND DISCUSSION

The diploid number of *Tx.amboinensis* is 6 ($2n=6$). The karyotype consists of 3 pairs of homomorphic chromosomes, with two slightly submetacentric pairs and one, the smallest, metacentric pair (Table 1). Hemingway and White (1980) classed all three pairs as metacentric, but did report one pair as smaller than the other two. The chromosomes have arbitrarily been numbered according to the system of Rai (1963) in which the shortest chromosome is designated as chromosome I and the longest is III. Assignment of chromosome numbers on the basis of length is tentative, with a final number assignment needing to be based on the correlation of linkage groups and chromosomes as was done by McDonald and Rai (1970).

During prophase somatic pairing of the homologous chromosomes is evident. The homologs separate by metaphase, but remain closely associated in the region of their centromeres.

The karyotype of *Tx.amboinensis* can be described as one each of a small, medium, and large pair of chromosomes (Fig. 3, 4; Table 1). The overall dimensions of the chromosomes (Table 1) are fairly large, but they compare favorably with measurements from other mosquitoes (Rai 1963, Asman 1974, Hartberg and Faircloth 1983, Hartberg et al. 1985). The ratio of length of chromosome I to chromosomes II + III is 0.408.

Measurements for the smallest chromosome (I) ranged from 4 to 10.5 mm with an average of 6.68 mm. With the next in size, chromosome II, measurements ranged from 5.5 to 13.00 mm with an average of 7.83 mm. The largest chromosome (III) had measurements ranging from 5.5 to 14.00 mm with an average of 8.55 mm. Bhat (1976) reports measurements for the chromosomes of *Tx.splendens* of 9.6, 13.4, and 14 mm respectively. His measurements for *Tx.splendens* generally lie within the ranges derived for *Tx.amboinensis* in this study. To date, Bhat's measurements for *Tx.splendens* chromosomes are the only ones that have been published. The other authors (Breland 1961), Kanda 1968, Hemingway and White 1980) did not report actual measurements for the species they examined.

Breland (1961) noted that in *Tx.rutilus septentrionalis* each of the pair of chromosome has one arm that is somewhat larger and longer than its mate. In effect he is stating that all three pairs are submetacentric. In examining the figure Breland has of the chromosomes, it is obvious that the two larger pairs are submetacentric. The smaller pair appears to be metacentric which would make this karyotype very much like what we find in *Tx.amboinensis*. Examination of

the figure in Bhat's (1976) paper of chromosomes of *Tx. splendens* clearly shows that at least the two larger pairs are submetacentric and the smallest essentially metacentric, even though he states in the paper that all three pairs are metacentric. In short, the karyotype is very similar to what we see in *Tx.amboinensis*. No direct comparisons between our findings and those of Kanda (1968) and Hemingway and White (1980) can be made since no figures were published in those papers.

As has been noted by many workers (Breland 1961, Rai 1963, Asman 1974, Hartberg et al. 1985), measurements of metaphase chromosomes must be regarded as relative values rather than absolute ones since a wide range is found. The degree of contraction of the chromosomes can vary greatly between early and late metaphase, thus affecting measurements.

All of the *Toxorhynchites* examined to date have very similar mitotic karyotypes. It would be interesting to use various techniques, such as the Giemsa C-Banding used by Motara and Rai (1978) with *Stegomyia* mosquitoes, to see if more precise similarities or differences between the karyotypes of different *Toxorhynchites* species could be determined.

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Table. 1. Average measurements of mitotic chromosomes in *Toxorhynchitesamboinensis*.

Chromosome Number	Centromere Locaton	Length in mm		Total Length	Arm Ratio*
		Arm a	Arm b		
I	metacentric	3.34	3.34	6.68	1.000
II	submetacentric	3.98	3.85	7.83	1.034
III	submetacentric	4.31	4.24	8.55	1.017

* Arm ratio = length of longer arm/length of shorter arm.

Plate 1. Mitotic division in *Toxorhynchites amboinensis*.

- Fig. 1 - Early prophase
- Fig. 2 - Late prophase
- Fig. 3 - Metaphase
- Fig. 4 - Metaphase
- Fig. 5 - Anaphase
- Fig. 6 - Telophase

