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Meiotic chromosomes and sex determination mechanism in Thailand and Hawaii isolates of *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae)

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Abstract. *Angiostrongylus cantonensis*, the nematode lungworm of rats, has a XX/X0 sex-determination mechanism. The chromosome constitution consists of 10 autosomes, with 2n = 12, XX in the female and 2n = 11, X0 in the male. Meiosis-I shows five bivalents and one univalent for the male worm, and six bivalents for the female worm. The chromosome constitution of the Thailand and Hawaii isolates of *A. cantonensis* is similar to those reported for the taxa from Japan, Egypt and mainland China.

INTRODUCTION

The rat lungworm *Angiostrongylus cantonensis* now referred to as *Parastrongylus cantonensis* (Figure 1) is a food-borne zoonotic parasite of considerable public health concern in Thailand and many countries in the tropics and subtropics of both the Old and New Worlds. Human is a non-permissive host. The parasite is the primary cause of eosinophilic meningitis or meningoencephalitic meningitis (for review, see Eamsobhana, 2006).

To-date the karyotype of *A. cantonensis* nematode has only been reported for the taxa from Japan (Sakaguchi & Nojima, 1980), Egypt (Ashour *et al*., 1993) and mainland China (Sheng & Ding, 1996). Meiotic chromosomes were reported for the male Japan taxon (Sakaguchi & Nojima, 1980) and both the male and female Egypt taxon (Ashour *et al*., 1993).

This study was initiated to investigate the chromosome constitution of Thailand and Hawaii isolates of *A. cantonensis*. We report here the meiotic chromosomes and sex-determination mechanism in these isolates.

MATERIALS AND METHODS

Parasites of the Thailand and Hawaii isolates of *A. cantonensis* were maintained

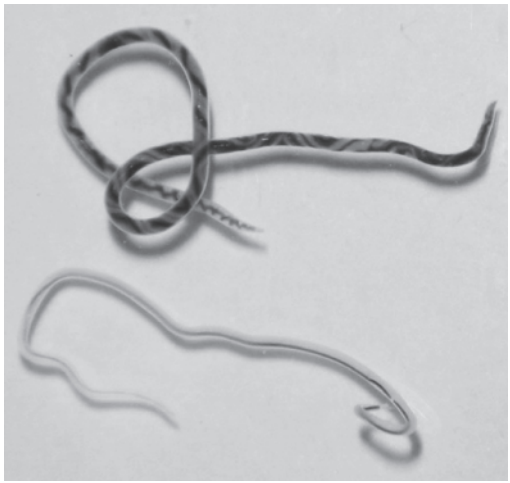

Figure 1. *Angiostrongylus cantonensis* adult worms – top: female; bottom: male.

in Wistar albino rats at the Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok.

Adult worms (Thailand isolate – 3 males, 2 females; Hawaii isolate – 4 males, 3 females) were treated with 0.01% (w/v) colchicine in RPMI for 2 h. Reproductive tissues (testis, ovary) of treated worms were used for chromosome preparation by the air-drying method (Yong & Dhaliwal, 1972). Briefly, small pieces of the colchicine-treated tissues were treated with 0.5% KCl solution for 30 min, and then fixed in 3:1 ethanol: acetic acid preservative (three changes). The tissues could be stored or processed immediately. For air-drying preparation, the tissue was immersed in 60% acetic acid for 1-2 min before dispersing the cells on a microscopic slide over a table lamp or on a hot plate. Meiotic chromosomes were stained with 2% Giemsa, examined under oil immersion and photographed for analysis.

RESULTS

Both the Thailand and Hawaii isolates of *A. cantonensis* possessed similar chromosome constitution. The male worms had a diploid number of 11 chromosomes, comprising 5 bivalents (autosomes) and 1 univalent (X-chromosome) at diakinesis and metaphase-I (Figures 2, 3). On the other hand, the female worms had 12 chromosomes (10 autosomes, 2 X-chromosomes), with 6 bivalents in meiotic-I cells (Figure 4). The difference in chromosome number was due to the sex chromosomes – a single X in the male but two (XX) in the female worm. No variation in chromosome number was observed in both the Thailand and Hawaii isolates.

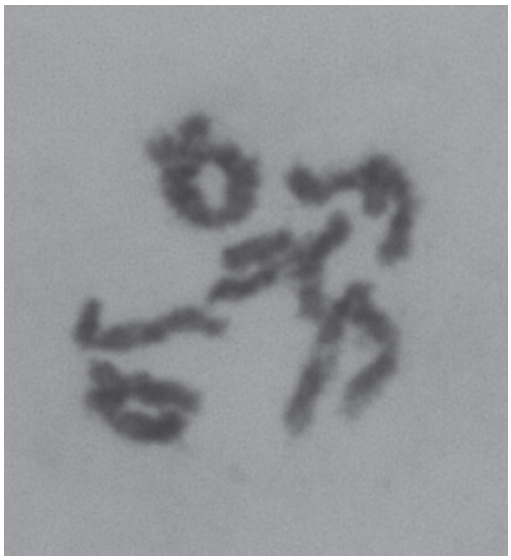

Figure 2. Diakinesis of a male *Angiostrongylus cantonensis* of Hawaii isolate, with 5 bivalents and a univalent X-chromosome, indicating 2n = 11.

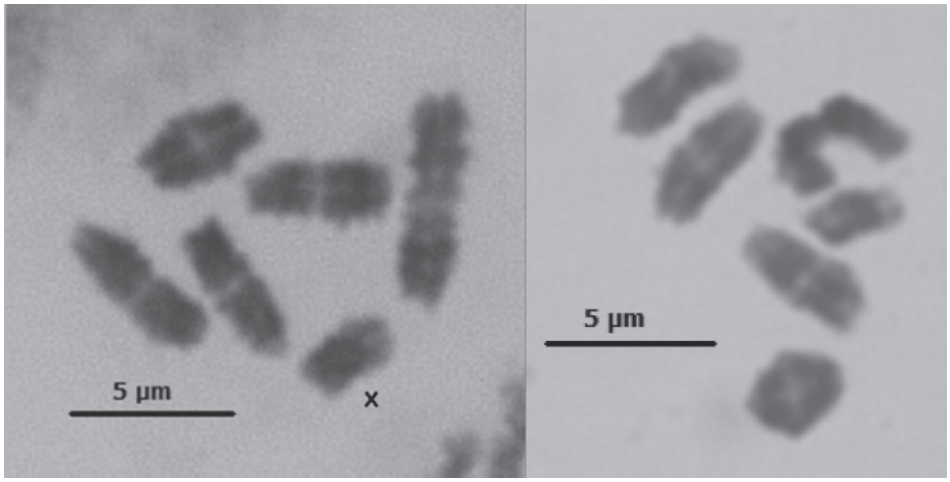

Figure 3. Metaphase-I chromosomes of male *Angiostrongylus cantonensis* of Hawaii isolate (left) and Thailand isolate prepared by conventional air-drying technique (right), with 5 bivalents and a univalent X-chromosome, indicating 2n = 11.

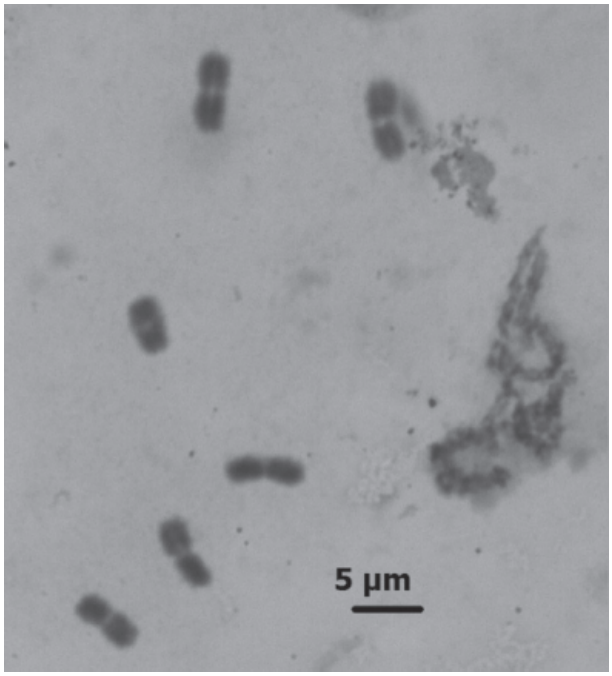

Figure 4. Metaphase-I chromosomes of a female *Angiostrongylus cantonensis* of Hawaii isolate, with 6 bivalents indicating XX sex-chromosome constitution and 2n = 12. The smallest pair may be the X-chromosomes.

DISCUSSION

Cytogenetics is a hybrid science of cytology and genetics. It encompasses the study of the structure, number, function, and movement of chromosomes as they relate to the transmission, recombination and expression of genes. It also deals with non-chromosomal factors.

Cytogenetic studies on nematode parasites have centered mainly on karyotype analysis. There are few studies on the meiotic chromosomes (Yong & Mak, 1988).

The rat lungworms *A. cantonensis* from Japan, Egypt and mainland China possess similar chromosome constitution. The male worm has 11 chromosomes, the female 12 chromosomes (Sakaguchi & Nojima 1980; Ashour *et al.*, 1993; Sheng & Ding, 1996). There appear to be some morphological differences in the mitotic chromosomes of the Japan and mainland China taxa (Sheng & Ding, 1996). The occurrence of chromosome polymorphism and geographical variation remains to be ascertained.

The difference in chromosome number between the male and female worms is due to the presence of a single X-chromosome in the male worm and two sex chromosomes (XX) in the female. Our findings in the Thailand and Hawaii taxa concur with the chromosome number and XX/XO sex-determination mechanism reported for the taxa from Japan, Egypt and mainland China.

Many, if not most, nematodes have a XX/XO sex-determination mechanism. For example, in four species of *Trichinella*, the females are homogametic with 2n = 6 and the males are heterogametic with 2n = 5 (Mutafova *et al.*, 1982). Examples with an XX/XY sex-determination mechanism include *Bayliscaris transfuga*, *Brugia* spp, *Contracaecum incurvum*, *Onchocerca volvulus* and *Trichuris* spp.

Meiotic figures of male and female *A. cantonensis* have been reported for the Egypt taxon (Ashour *et al.*, 1993) and of the male for the Japan taxon (Sakaguchi & Nojima 1980). The univalent is the smallest in size. In the present study, the X-

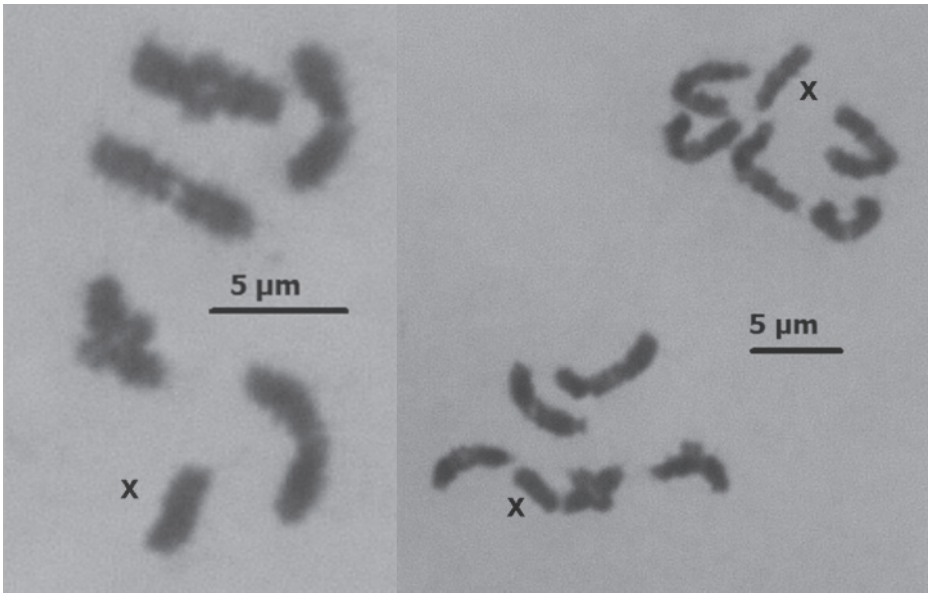

Figure 5. Meiosis-I chromosomes of male *Angiostrongylus cantonensis* of Hawaii isolate, with 5 bivalents and a univalent X-chromosome, indicating 2n = 11. The X-chromosome does not always appear to be the smallest element.

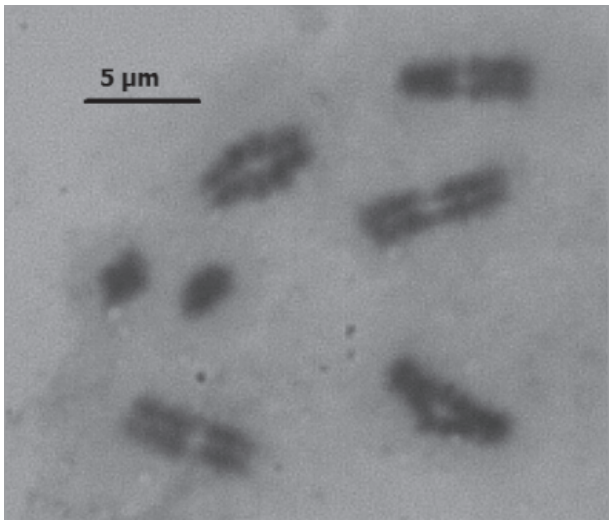

Figure 6. Meiosis-I chromosomes of a female *Angiostrongylus cantonensis* of Hawaii isolate. The separated pair is presumed to be the X-chromosomes.

chromosome did not always appear to be the smallest (Figure 5). At metaphase-I, depending on the stage of condensation, it measures about 2.7-3.8 ìm. Its size in relation to the autosomes needs to be confirmed by mitotic metaphase chromosomes. In the present study, the sex-chromosomes in the female appear to separate earlier than the autosomes (Figure 6).

As reported for the Egypt taxon (Ashour *et al.*, 1993), mitotic metaphase (Figure 7) was rarely observed in the present study. Furthermore the morphology of the chromosomes was not well defined.

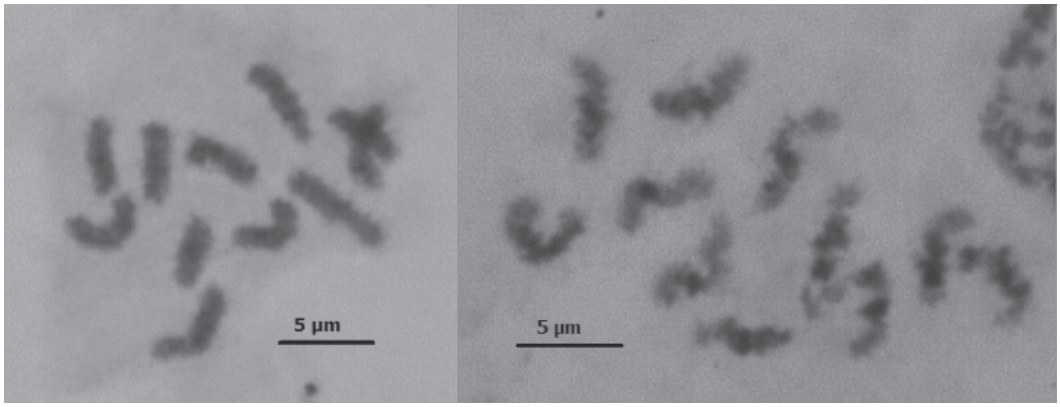

Figure 7. Metaphases of male *Angiostrongylus cantonensis* of Hawaii isolate with 11 chromosomes.

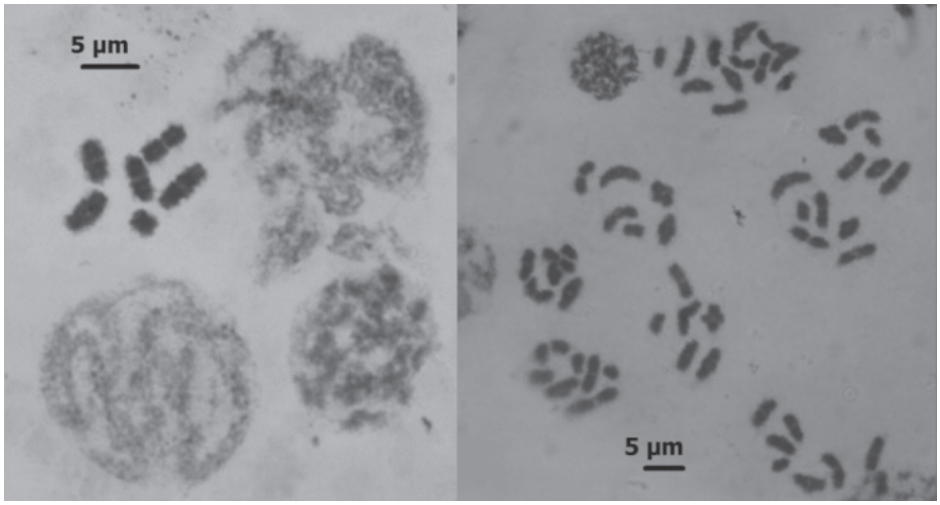

Figure 8. Meiosis-I chromosomes of male *Angiostrongylus cantonensis*.

Two methods were used in the present study to prepare the chromosomes. The conventional air-drying technique involving dispersing the cells before hypotonic treatment and fixation was less satisfactory as much fewer dividing cells were obtained. The use of 60% acetic acid to disperse the cells after hypotonic treatment and fixation of colchicine-treated tissue was superior, producing many good quality meiotic chromosomes (Figure 8). In addition, the use of methanol in place of ethanol as fixative was equally satisfactory.

Our on-going studies will examine the mitotic metaphase chromosomes with various banding techniques to determine the morphology of the autosomes and X-chromosome.

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