[Jap. J. Parasit., Vol. 29, No. 5, 377-381, 1980]

The Chromosomes and Gametogenesis of *Dirofilaria immitis*

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(Received for publication; January 8, 1980)

Filarial worms comprise various species which cause serious disease widely in medical and veterinary fields. However, only a few cytogenetic studies have been done on this group of parasites, most of them dealing with sectioned material and/or smear specimens. Hence, our knowledge is still meager and obscure.

This paper deals with the results of observation on the chromosomes and gametogenesis in *Dirofilaria immitis*, by means of an air-drying method (Takagi, 1971) and a squashing procedure (Snow, 1963).

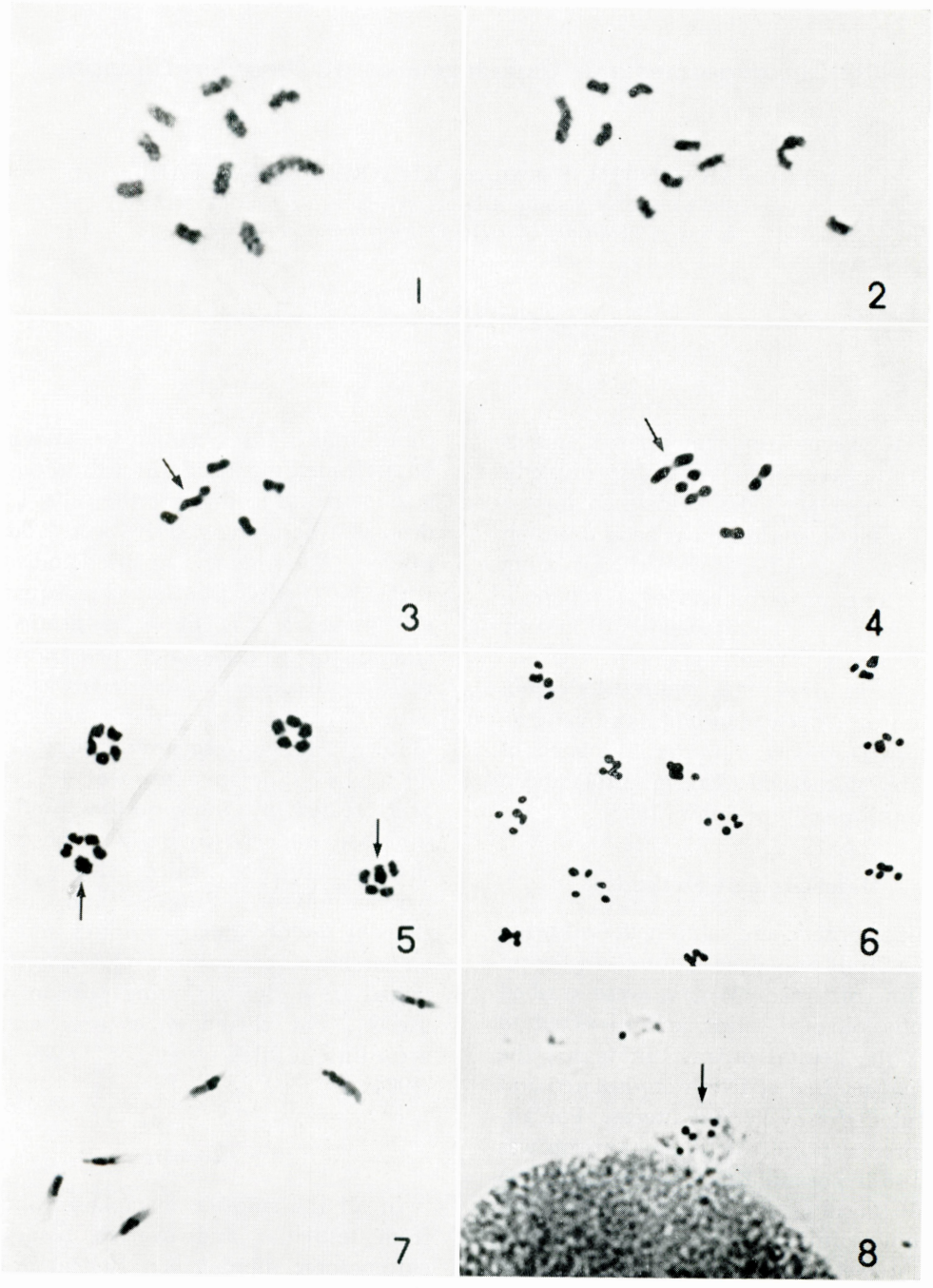
Materials and Methods

Adult worms were obtained following necropsy from stray dogs captured in Beppu city, Oita province. Worms were washed with physiological saline and dissected to remove the genital organs. In males, the testes and seminal vesicles were isolated and in females, the ovary and uterus. For air-dried preparations, the gonadal tissue was dipped in hypotonic KCl solution (0.075 M) for 5–10 minutes and fixed in acetic acid-methanol solution (1:3) for 2–3 hours. Then the specimen was put into a 3:1 acetic-lactic acid solution to loosen intercellular connections. The specimen was then placed on a cleaned glass slide in a drop of acetic acid-methanol to disperse the cells; 5 to 10 drops of the above fixative were added as needed. The slide was air dried and stained with 5% Giemsa solution. For squashing, the material was fixed in acetic acid-ethanol for 24 hours and then transferred to 70% ethanol. The specimen was stained with alcoholic hydrochloric acid-carmine (Snow, 1963), cut into several pieces, placed on a glass slide in a drop of 45% acetic acid, teased by insect-needles, and then squashed under a coverslip. The edge of the coverslip was sealed with a balsam-paraffin mixture. Supplementary observations by use of Nomarsky optics were made on unfixed spermatozoa and oocytes obtained from the beginning portion of the uterus. The chromosomes were classified according to the system of Levan *et al.* (1964).

This study was supported by a Scientific Research Grant (No. 467070) from the Ministry of Education, Japan

Results

In all the suitable metaphases obtained from testicular and ovarian tissues, the chromosome number was 10 (2n) or 5 (n) (Figs. 1–4). The diploid metaphase plate in ovarian tissue consisted of 2 large sub-metacentrics and 8 small meta- or sub-metacentrics, while in the testicular speci-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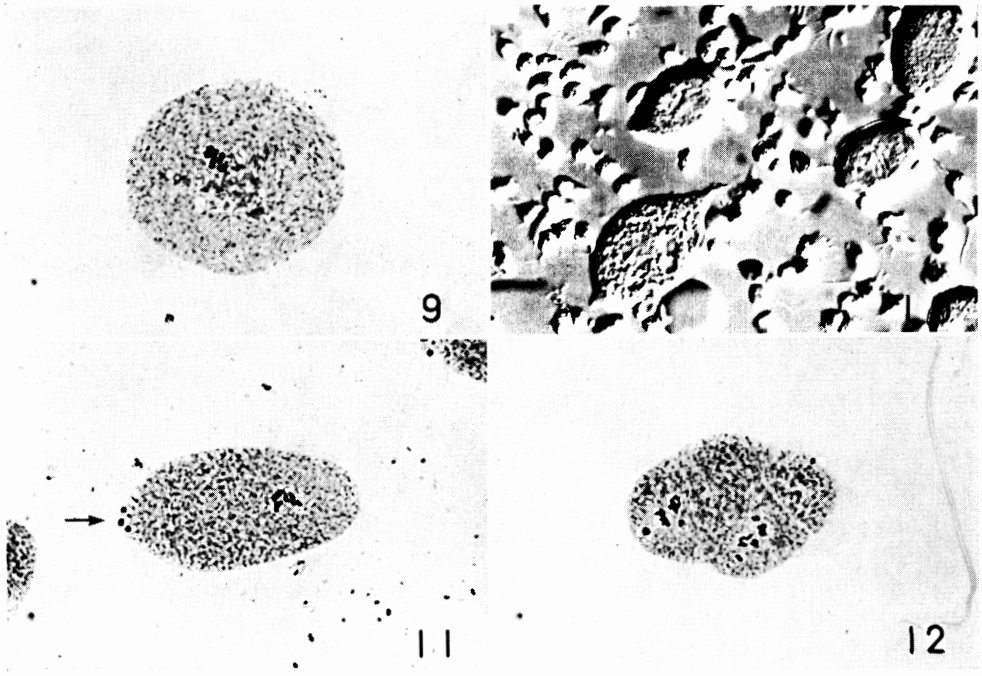

Explanation of Figures

Figs. 1, 2 Spermatogonial (1) and oogonial (2) metaphases of *D. immitis*, showing 10 chromosomes in each. Ca ×1,700.

Figs. 3, 4 Diakinesis with 5 bivalents in spermatocyte (3) and oocyte (4). Arrow indicates the sex pair. Ca ×1,700.

Fig. 5 Four primary spermatocytes at metaphase each composed of 5 bivalents. Arrows indicate the possible sex pair. Ca ×1,700.

Fig. 6 Secondary spermatocytes at metaphase each composed of 5 univalents. Ca ×3,200.

Fig. 7 Spermatid. Ca ×1,700.

Fig. 8 A spermatozoa attached to a primary oocyte at the beginning portion of uterus. Ca ×3,200.

Fig. 9 Primary oocyte at diakinesis, showing 5 bivalents. Ca ×1,700.

Fig. 10 Primary oocyte and surrounding spermatozoa in the beginning portion of uterus by Nomarsky optics. Ca ×1,700.

Fig. 11 A zygote with 10 univalents and 3 polar bodies (arrow). Ca ×1,700.

Fig. 12 Cleavage divisions at 4-cell stage. Ca ×1,770.

mens it contained only one large submetacentric and 9 small meta- or submetacentrics. The primary spermatocyte had 5 bivalents including one sex-bivalent in which the X and Y were attached end to end at diakinesis (Fig. 3). The primary oocyte had 5 bivalents including one large sex-bivalent which was probably the XX pair (Fig. 4). The primary spermatocytes (Fig. 5) developed into the secondary spermatocytes after a maturation division (Fig. 6), spermatids (Fig. 7) and finally produced many spermatozoa (Fig. 8). Both the spermatid and sperm were provided with 5 chromatids.

The chromosomes of primary oocytes

which appeared in the beginning portions of the uteri were composed of 5 bivalents (Fig. 9) and the oocytes were surrounded by sperms (Fig. 10). Thus a zygote, finally produced, contained 10 univalents and 3 polar bodies (Fig. 11). In fertilized eggs, cleavages occurred through mitosis (Fig. 12) and shell formation was seen around the individual egg. The embryo commenced to organize as morula and then the tadpole stage and finally transformed into a microfilaria in the uterus.

Discussion

So far as the chromosomes and gametogenesis of *D. immitis* are concerned, the most valuable study to date was that of Taylor (1960). She suggested the XO-XX type sex mechanism for this species on the basis that spermatozoa had either 4 or 5 chromosomes. The present study, however, shows that the chromosome number is n=5 and 2n=10, equally for both female and male specimens. Further, karyotype analysis reveals the presence of a heteromorphic pair in males, while in females all the 5 pairs of chromosomes are homomorphic. We propose that the sex mechanism of this species is the XY-XX type, but not the XO-XX one.

In other species of filarial worms, the following chromosome numbers have been reported: n=4,5 in *Litomosoides carinii* (Taylor, 1960); n=5,6 in *Dipetalonema witei* (Terry et al., 1961); n=2 and 2n=4 (Mallén *et al.*, 1962) or n=5 (Miller, 1966) in *Onchocerca volvulus* and n=5 in *Wuchereria bancrofti* (Miller, 1966). No detailed descriptions were presented on the sex mechanisms in these reports. In *Setaria digitata*, we recently found n=6, 2n=12 in females and n=6, 2n=11 in males, suggesting the XO-XX type sex mechanism (Sakaguchi *et al.*, unpublished). This may indicate the presence of at least two different sex mechanisms in the filarial parasites. Further systematic studies might clarify the taxonomic relationships of individual filarial parasites by using cytological procedures such as in this paper.

Observations on gametogenesis demonstrated that both the sperm and ovum showed haploidy following two successive meiotic divisions. Further, the ovum, fertilized with sperm in the uterus, commenced rapid division showing diploidy and forming an embryo. The above process indicates that this parasite produces microfilariae through ordinary sexual reproduction as is seen in most nematodes.

Summary

A study was made of the chromosomes and gametogenesis of *Dirofilaria immitis* by means of an air-drying method and/or a squashing procedure. The haploid and diploid numbers were 5 and 10, respectively, in both sexes. Meiotic analyses disclosed that sex-determining mechanism of this species was the XY-XX type, where the X chromosome was the largest of the complement standing out from both the autosomes and the Y chromosome. Observation on the process of fertilization and early cleavage division revealed that microfilariae were produced by ordinary sexual reproduction.

Acknowledgements

The authors are grateful to Dr. M. Sasaki of Hokkaido University and to Dr. L. R. Ash of UCLA for their kind revision of the manuscript and encouragement.

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犬糸状虫の染色体と配偶子形成

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犬糸状虫の染色体と配偶子形成過程をエアードライ法と押しつぶし法によって観察した．得られた成績は次の通りである．

1) 犬糸状虫の染色体数は雌雄共 $n=5$, $2n=10$ である．

2) しかしその倍数染色体の構成は雌雄間で異なり，一対の異形染色体が存在する．

3) 性染色体は XY—XX 型で，雄で XY，雌では XX である．

4) 本種は正常な有性生殖によって，ミクロフィラリアを産生している．