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Chromosomes of *Onchocerca volvulus* and *O. gutturosa*

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Onchocerca volvulus (Spirurida: Onchocercidae) is the only human parasite in this genus, which is distributed mainly in East and West Equatorial Africa and Central and South America. *O. gutturosa*, known from West Africa, Europe and South America, is a common parasite of domestic bovine in Guatemala (Hashiguchi et al. 1981). These two species have often been compared morphologically as adult worms (Beaver et al. 1974; Bain 1981) and as microfilariae (Gibson 1952; Tada et al. 1981). It may also be possibile that histochemical enzyme-stainings of microfilariae, such as given by Omar (1978) show differences between these two species. Such information would help the diagnosis of human infection (Beaver et al. 1974) with zoonotic *Onchocerca* sp. The *O. gutturosa* antigen was also found to be sensitive in immunological diagnosis of onchocerciasis (Mueller et al. 1973; Korenaga et al. 1983). Therefore it is very important to clarify the biological differences between *O. volvulus* and the several other species found in animals. In this paper we report the chromosome number, morphology of autosomes and sex chromosomes of *O. volvulus* and *O. gutturosa.*

Adult worms of *O. volvulus* were isolated from nodules which were extirpated surgically from patients suffering from onchocerciasis in Finca Florencia, Departamento de Chimaltenango, Guatemala. Adult *O. gutturosa* were picked out from nuchal ligaments of bovine obtained in a slaughter house in Siquinala, Departamento de Escuintla, Guatemala. Chromosome preparations were made using testes and ovaries or the basal part of the uterus by applying an air-drying technique (Imai et al. 1977; Hirai et al. 1981). The chromosome number of *O. volvulus* was counted using 30 cells of five female adults and 28 cells of seven males. In the same manner, 36 cells of four females were counted in *O. gutturosa,* but no appropriate specimens were obtained in males.

Two chromosomal types were observed in the fertilized eggs obtained from the basal part of the uterus of *O. volvulus*: the first one had a pair

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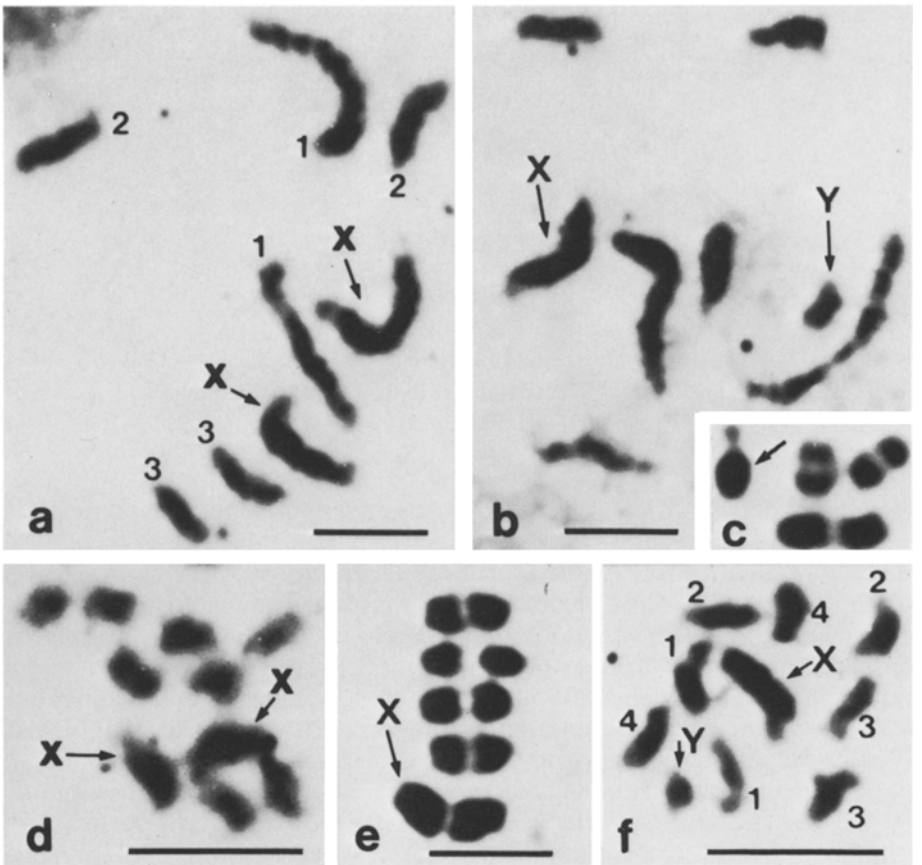

Fig. 1 a–f. Chromosomes of *Onchocerca volvulus* and *O. gutturosa.* **a–c** A mitotic metaphase in a fertilized female egg, a mitotic metaphase in a fertilized male egg and four bivalents at metaphase I in the testis, respectively, of *O. volvulus.* **d–f** A mitotic metaphase in a fertilized female egg, five bivalents at metaphase I in an egg at the growth zone of the uterus and a mitotic metaphase in a fertilized male egg, respectively, of *O. gutturosa. Arrows* indicate sex chromosomes. *Bars* represent 3.3 μm

of large, a pair of medium and two pairs of small chromosomes (Fig. 1 a), while the other possessed one pair of large, two pairs of small, one medium and one small chromosome in metaphase (Fig. 1 b). As is shown in Fig. 1 b, the medium-sized and small chromosomes (arrows) are considered to be heteromorphic homologous chromosomes, because a heteromorphic bivalent was observed at metaphase I (Fig. 1 c, arrow). This heteromorphic bivalent probably consists of the sex chromosomes, X and Y. It was found that a diploid cell of female *O. volvulus* consisted of six autosomes (a large pair, denoted as no. 1, and two small pairs, nos. 2 and 3) and a pair of X chromosomes, i.e., $2n=6+XX$ (Fig. 1 a). Likewise, the male had six autosomes composed of a large pair (no. 1) and two small pairs (nos. 2 and 3), and a medium-sized X and a small Y chromosome, $2n=6+XY$ (Fig. 1 b).

O. gutturosa also had two chromosomal types in fertilized eggs. The first type had a pair of large and four pairs of medium chromosomes (Fig. 1d), and the second, four pairs of medium-sized chromosomes, and one large and one small unpaired chromosome (Fig. 1f). The fertilized eggs, characterized by heteromorphic homologous chromosomes (Fig. 1f, arrows), would have developed into male microfilaria, though the meiotic figures were not observed. Chromosome complements at the first meiotic metaphase, obtained in an egg located at the growth zone of the ovary, had no heteromorphic bivalents (Fig. 1e). Thus we conclude that the diploid of female *O. gutturosa* consists of four pairs of medium size (nos. 1, 2, 3 and 4) and two large X chromosomes, i.e., $2n=8+XX$. The male has eight medium-sized chromosomes, one large X and one small Y chromosome, $2n=8+XY$.

We conclude that *O. volvulus* ($2n=8$) and *O. gutturosa* ($2n=10$) are karyotypically distinctive. Recently, Agatsuma revealed that isozyme patterns of lactic dehydrogenase, malic dehydrogenase, glucose phosphate isomerase and phosphoglucomutase are remarkably different in *O. volvulus* and *O. gutturosa* in Guatemala (personal communication). Bain (1981) morphologically examined the phylogeny of 24 species of the genus *Onchocerca*. She suggested that *O. volvulus* and *O. gutturosa*, in terms of morphology, are located on considerably different phylogenetic lines in the genus. Thus, biochemical and morphological data are consistent with our present conclusion which is based on cytogenetic data.

However, intraspecific differences may also exist. Salazar et al. (1962) reported that $n=2$ in a Mexican *O. volvulus*, whereas Miller (1966) reported $n=5$ in an African strain; these are in contrast to our findings of $n=4$ for Guatemalan *O. volvulus*. Salazar et al. may have reached an incorrect conclusion because of the technical limits of the smear method. It would be very interesting to know if the *O. volvulus* of Africa and Guatemala are indeed chromosomally different. A re-examination of the chromosomes of African *O. volvulus* by the air-drying technique is needed in order to clarify this possible discrepancy. Also, it has been speculated that African and American *O. volvulus* might be different strains on morphological grounds, although no distinctive morphological differences have ever been found (Sandground 1934; Franz 1980). In addition, there are differences in the clinical manifestations (Browne 1961; Woodruff et al. 1966; Oomen 1969; Tada et al. 1974) and the susceptibility of the vector blackflies to the parasite of different continents (De Leon and Duke 1966; Duke et al. 1967; Omar and Garms 1975; Garms and Ochoa 1979; Garms 1983). Most recently, Basáñez et al. (1983) reported that Venezuelan *O. volvulus* has the chromosome number $n=4$ or 5 ($2n=8$ or 10). Although they showed fairly detailed pictures of bivalents by a squash method, their conclusion may be inaccurate. In their Fig. 1c, for example, three bivalents and two univalents are recognizable, as stated in the legend. If the two univalents are in fact X and Y, then their results are identical with ours ($2n=6+XY=8$). Chromosome observations on the fertilized eggs, as reported here, would have solved this problem.

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