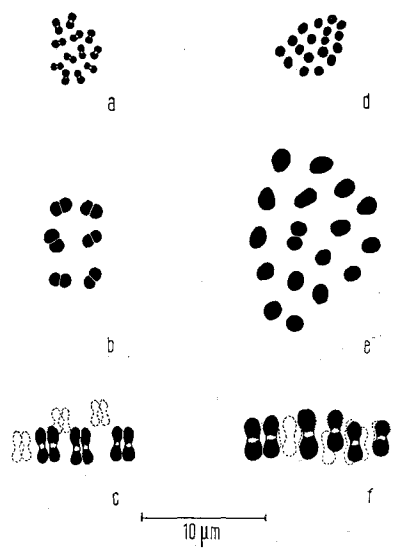
and *M. richtersi* found near Modena. These data and the cytological work on the population of *M. richtersi* from Modena show the presence of a geographic parthenogenesis. As a matter of fact the bisexual population of *M. richtersi* from Pisa has 12 chromosomes in the gonial cells (Figure a) and in the midgut cells of both males and females; the observation of the first metaphase of the oocytes shows 6 chromatic bodies (Figure b); every chromatic body in the meiotic spindle clearly appears to be formed by 4 chromatides, 2 of which are over and 2 under the equatorial plane (Figure c); thus there are 6 bivalents. Also 6 clustered chromatic bodies are observed in the first metaphase of the spermatocytes, but in this case it is not possible to see whether they are bivalents. The unisexual population of *M. richtersi* from Modena shows 18 chromosomes both in the somatic and gonial mitoses (Figure d): i.e. a triploid number. In the prometaphases and metaphases of the oocytes (Figure e–f), there are 18 chromatic bodies without longitudinal cleft, which are to be interpreted as univalents. So the maturative division in *M. richtersi* from Modena is similar to a mitosis and, any reduction of the chromosome number being absent, a constant parthenogenetic behaviour is to be inferred. The parthenogenesis in this Tardigrade is ameiotic as in some other invertebrates: the triploid pattern in the invertebrates was connected with an ameiotic parthenogenesis every time where the ripening eggs were studied by a caryological point of view[11]. Besides it must be remarked that in another Tardigrade, *H. dujardini*, the parthenogenesis is diploid and meiotic: the chromosome number is duplicated by endomitosis in the oocytes before the second maturative division[9].

Species	Location	♂♂	Total
M. areolatus Murray	Appennin (Modena)	13	38
M. hufelandii Schultze	Appennin (Modena)	128	371
M. hufelandii Schultze	Coastal plain (Ravenna)	0	41
M. intermedius Plate	Appennin (Modena)	5	16
M. richtersi Murray	Coastal plain (Pisa)	54	122
M. richtersi Murray	Appennin (Modena)	0	80



Ripening eggs in bisexual (a-c) and parthenogenetic (d-f) *M. richtersi* (Lactic-acetic-orcein. From microphotography).

Riassunto. In *Macrobiotus* esistono popolazioni bisessuate con un rapporto-sessi di circa 1:1. Due specie (*M. richtersi* e *M. hufelandii*), che in alcune località sono bisessuate, in altre presentano popolazioni prive di maschi. L'esame cariologico di *M. richtersi* mostra che la popolazione partenogenetica è triploide e che le uova maturano in assenza di meiosi.

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Multiple *W* Chromosome in a Sea Snake, *Enhydrina schistosa* Daudin

The first case of a multiple sex chromosome complex in vertebrate species with female heterogamety was recorded by Singh et al.[1], when they described the $Z_1Z_1Z_2Z_2$♂Z_1Z_2 W♀ sex chromosome complex in the common Indian krait, *Bungarus caeruleus*. We are presenting in this paper another type of multiple sex chromosome constitution in the sea snake, *Enhydrina schistosa* of the highly evolved family Hydrophiidae.

The specimens under study, 12 females and 7 males, were collected from the coast of the Bay of Bengal at Digha in West Bengal, India, where this species is abundant. The heart was exposed from the ventral side in living condition and blood was drawn out directyl from the heart with the help of heparinized syringe for leucocyte culture. Colcemid (0.25 ml/kg body wt.) was injected immediately afterwards for chromosome preparations from the marrow of ribs and from spleen. The leucocyte culture was made according to the procedure described by Singh et al.[1].

For the study of *W* chromatin (Ray-Chaudhuri et al.[2]) brain, kidney and leucocyte culture were directly fixed in

[1] L. Singh, T. Sharma and S.P. Ray-Chaudhuri, Chromosoma *31*, 386 (1970).

aceto-alcohol (1:3) and slides were prepared by air-drying procedure. Staining was done in carbol-fuchsin and microphotographs were taken in Carl-Zeiss Photomicroscope. Chromosomes have been classified according to the system proposed by LEVAN et al.[3].

Nearly 1200 good metaphases were scored from spleen, marrow of ribs and leucocyte culture of all the 19 individuals used in this study. The chromosome analysis has revealed 32 as the diploid number in the males and 33 in the females, invariably. There is a sharp bimodality between macro- and microchromosomes (Figure 1). The number of microchromosomes is 18 in the males and 19 in the females whereas 14 macrochromosomes are present in both the sexes (Figures 2 and 3). According to the centrometric positions, the macrochromosomes can be classified into 3 different groups. Group A consists of 4 pairs of chromosomes having their centromeres in the median region (m). There is a secondary constriction in 1 of the arms of both the chromosomes of pair No. 1 (Figure 1). Occasionally the secondary constriction is revealed more clearly in 1 of the chromosomes only (Figure 4). All the 4 pairs of chromosomes of this group are separately identifiable by their size and centromeric positions. The chromosomes of pair No. 5, having their centromere in the submedian region (sm), alone constitute group B.

If we examine the karyotype prepared from metaphase plates from females (Figure 2), we find a heteromorphic pair of chromosomes of which the larger one, because of its submedian centromere (sm), belongs to group B. In the male plates (Figure 3) we have 2 such chromosomes and they are the Z chromosomes. The latter therefore also falls in the B group to which pair No. 5 belongs. The other member of the heteromorphic pair has its centromere at the terminal point and is the smallest one among the macrochromosomes. Since this chromosome is restricted to the female sex only, it must be the W_1 chromosome. Group C consists of pair No. 6 having their centromere in subterminal region (st). All the macrochromosomes can be considered as marker chromosomes in this species. The single extra microchromosome which is found only in the females of all the metaphase spreads must be the W_2, although it cannot be identified definitely.

The interphase nuclei of brain, kidney and leucocyte culture of females directly fixed in aceto-alcohol (1:3) exhibit a darkly stained body (Figure 5) in approximately 20%, 30% and 35% of the nuclei respectively, which we have termed as W chromatin (RAY-CHAUDHURI et al.[2]).

It is difficult to suggest the mechanism of the origin of the multiple complex in *E. schistosa* without prior study of the chromosomes of other sea snakes with simple sex

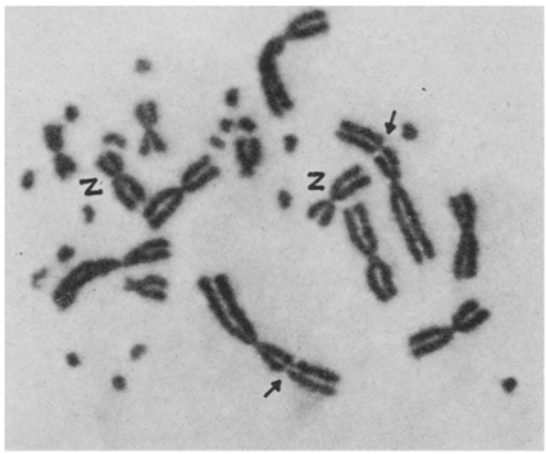

Fig. 1. Metaphase plate of somatic cell from leucocyte culture of *E. schistosa* male showing secondary constriction in both the chromosomes of the largest pair. ×2000.

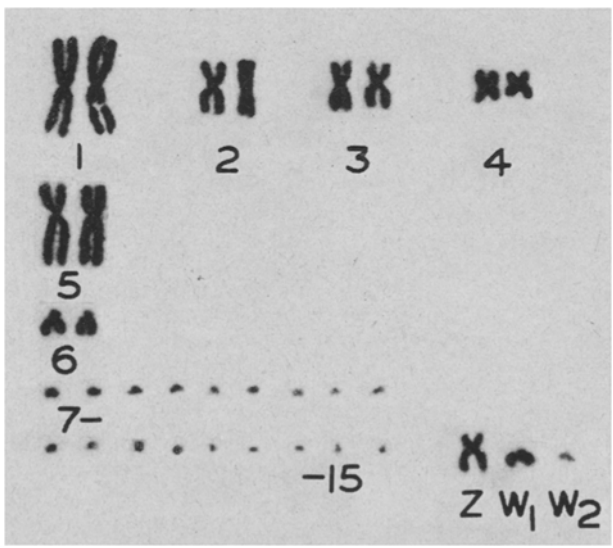

Fig. 2. Female karyotype of *E. schistosa* from marrow of ribs showing multiple sex chromosomes ($Z\ W_1W_2$). ×1555.

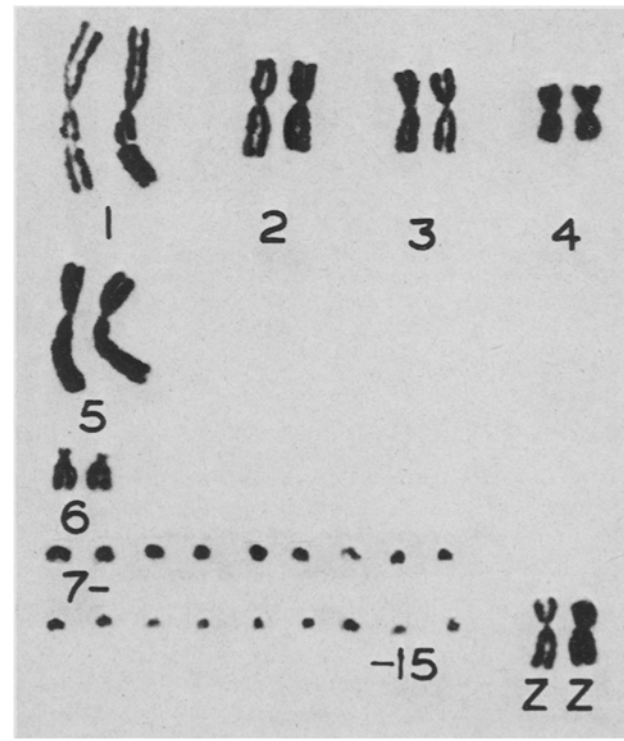

Fig. 3. Male karyotype of *E. schistosa* showing homomorphic sex chromosomes (ZZ). ×2000.

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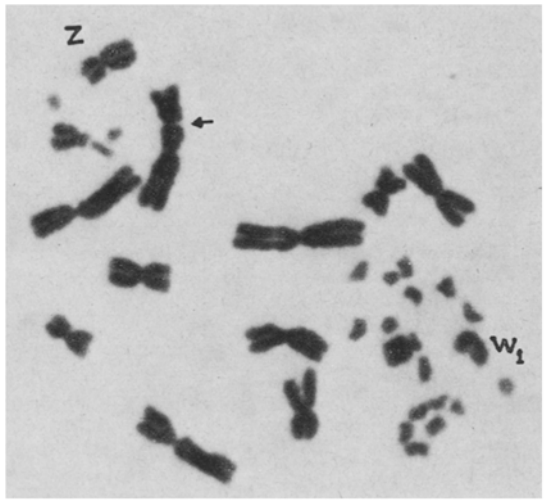

Fig. 4. Metaphase plate of somatic cell from spleen of *E. schistosa* female showing secondary constriction in 1 of the chromosomes of pair 1. ×2000.

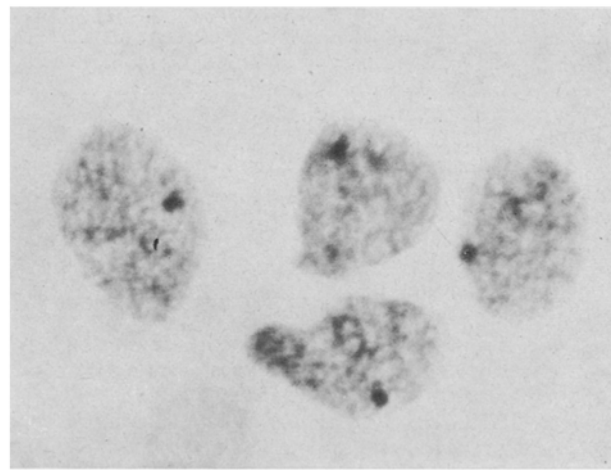

Fig. 5. Interphase nuclei of *E. schistosa* female from kidney showing single *W* chromatin body. ×2000.

chromosome mechanism. The only publication on the chromosomes of sea snakes is that of NAKAMURA[4], who studied only the males of *Laticauda semifasciata* with the help of classical techniques and reported 38 as the diploid number in that species. A number of species of sea snakes have now been collected by us and our preliminary observations reveal that multiple sex chromosome complex of the kind described here is quite widespread in Hydrophiidae, specially in the genus *Hydrophis*. A fuller account of the chromosomes of all the species, together with the probable mechanism of the origin of multiple mechanism, will be the subject of a future communication.

The *W* chromosome in snakes, whenever it is distinguishable from the *Z* either by its morphology and/or by its allocycly in its DNA replication, forms a heteropycnotic body in cells at the interphase stage in various somatic tissues which has been termed *W* chromatin[3]. Therefore the *W* chromosomes in *E. schistosa* should show in the interphase nuclei two *W* chromatin bodies, one bigger and one smaller corresponding in size to W_1 and W_2 respectively. Further, our unpublished observations on the chromosomes of various species of snakes of the genus *Hydrophis* reveal that there is an exact correspondence between the number of *W* chromosomes at metaphase and the *W* chromatin bodies in the interphase nuclei. Contrary to our expectation, only 1 *W* chromatin body has been detected in the interphase nuclei of brain, kidney and leucocyte culture. Perhaps the very minute size of the W_2 prevents the detection of the second *W* chromatin body.

Résumé. Un serpent marin (*Enhydrina chistosa* Daudin, Hydrophiidae) a 32 chromosomes chez le ♂, 33 chez la ♀, cette dernière ayant un *W*-chromosome supplémentaire. La digamétie serait: ♂-*ZZ*; ♀ *Z* W_1W_2.

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[5] The author is indebted to Prof. S.P. RAY CHAUDHURI for guidance and the University Grants Commission, India, for financial assistance.

Discrepancies between the Sites of Replication and Cytocidal Action of Encephalitogenic Viruses

It is generally taken for granted that in such viral encephalomyelitides as result in a severe and widespread nerve cell destruction, the virus is replicated mainly by the neurons. How erroneous this assumption is became evident in the course of comparative studies on the ultrastructural pathomorphology of several experimental viral infections of the simian and murine central nervous system.

In the first part of our experiments, adult cynomolgus monkeys were inoculated i.m. or s.c. with a highly virulent strain of type 3 poliovirus. Beginning with the 4th or 5th post-inoculation day, the animals developed a rapidly progressive paralysis of the limbs often terminating in a total tetraplegia. Electron microscopically, numerous spinal motoneurons of the paralytic monkeys exhibited unequivocal signs of incipient or advanced necrobiosis. Rather frequently, nerve cells in the stage of complete cytolysis and/or undergoing actual neuronophagia were observed. However, no one ultrastructural finding could be observed in the more or less damaged neurons which would have been directly indicative of the intracellular presence of poliovirus. On the other hand, many mononuclear elements of the inflammatory infil-