seemed to move around their cages more slowly than control littermates of the genotypes  $W^xw$ ,  $W^vw$  and ww. Initially we felt that the difference in movement was due to a variation in body weight between the control and tumor-bearing animals. However, we calculated no significant differences in body weight between the genotypes. The present work shows that genetic ovarian tumorigenesis of the genotype  $W^xW^v$  mice is associated with inhibited wheelrunning activity.

Materials and methods. Adult female mice of the C3B6F1-W series were obtained from crosses of strains  $C3H/HeJ-W^x/$  + with  $C57BL-W^v/$  + which gave rise to C3B6F1 hybrids segregating only for W-alleles. The animals were then divided into 4 genotypes:  $W^xW^{v_-}$  black eyed whites (ovarian tumor bearers);  $W^{x}w$ -agoute, white tip tail; Wvx-agoute, all white tail; and ww-agoute, all black tail. The agoute animals served as controls, while the black eyed whites were the experimental genetically developed ovarian tumor-bearing animals. We weighed the animals and placed each mouse in a tread wheel for 2 h. The number of revolutions per h was recorded and averaged for each genotype. We calculated the standard error for each mean by using the formula, S.E. =  $\sqrt{\Sigma d_2 N(N-1)}$  and the probability values (P) were determined by Student's t-test5.

Results. The  $W^xW^v$  ovarian tumor-bearing mice had mean running activity significantly less than the running activity of any control agoute genotype. Animals with ovarian tumors ran 212.5  $\pm$  13.6 revolutions per h. This running activity was significantly slower than the slowest running genotype  $W^vw$  which had an activity of 271.7  $\pm$  12.6 revolutions per h (P < 0.05). On the other hand, the fastest running activity was recorded by  $W^xw$  mice.

Discussion. Ovarian tumorigenesis as well as genetics appear to be contributing factors in the slower running of ovarian tumor-bearing mice. The spontaneous motor activity of the 3 control groups appear to be genetically determined. The adrenals of mice with ovarian tumors commonly contained cells simulating luteal cells, and we interpreted their presence as a stimulating effect from the

Spontaneous running activity of C3B6F-W series adult female mice 7–9 months of age

Genotype	No, of mice	Body wt.	P	Revolutions per h	P
$W^xW^v$	45	$29.7 \pm 0.5$		212.5 + 13.6	
$W^vw$	23	$30.3 \pm 0.7$	> 0.5	$271.7 \pm 21.6$	< 0.0
ายาย	30	$30.3 \pm 0.5$	> 0.1	$289.6 \pm 24.2$	< 0.0
$W^{x}w$	19	31.3 + 1.0	> 0.1	308.8 + 26.2	< 0.0

 $P = \text{Probability value}; \pm = \text{standard error}.$ 

ovarian tumors. The remarkable modification of the adrenal glands may in turn produce an altered hormonal secretion which along with decreased estrogen secretion and overproduction of gonadotropin may be responsible for the inhibited running of the ovarian tumorigenic mice. In rats, spontaneous motor activity was greater during estrus. at a time when sexual receptivity and estrogen levels reached the highest point suggesting that running behavior depends upon sex-related hormone action. Since the uteri of ovarian tumor-bearing animals manifested atrophy, a decrease in endometrial glands and subtle metaplasia of the normal stroma. we feel that low estrogen levels occur in genotype  $W^xW^v$  mice.

The faster running control animals all showed signs of a more mature and stimulated exterior vaginal orifice in contrast to experimental ovarian tumor-bearing animals <sup>6</sup>. The ovaries and uteri of control animals were of normal size. The uteri of control animals were red, vascular, had a normal myometrium, and commonly exhibited cystic hyperplasia of the endometrial glands. Individual differences in running activity of control genotypes may be due to genetic differences in the ability to utilize or secrete estrogen. Future studies will be needed to clarify this point.

Résumé. Les ovaires de souris C3B6F1, génotype  $W^xW^v$  développent des adénomes tubulaires à l'âge de 7 mois. Dans l'essai du «tread wheel» l'activité spontanée de ces animaux était plus petite que celle des contrôles.

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## Sex Ratio and Geographic Parthenogenesis in Macrobiotus (Tardigrada)

The cell number in the Tardigrades is not absolutely fixed, because mitoses can be detected in various tissues. But a constant number of cells being secundarily attained, it must be deduced that some cells have a short lifetime. Previous observations have also given interesting hints about the sex ratio and the sexual dimorphism.

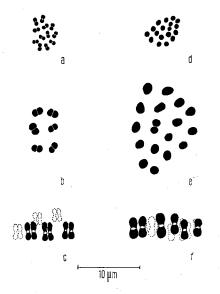
It is well known that in the Tardigrades the males are generally very few or absent<sup>3,4</sup>; sometimes they are

frequent in springtime but they are always absent in the other seasons  $^{5-7}$ . This suggested a parthenogenetic behaviour which was afterward proved by cytological observations on *Hypsibius dujardini*  $^{8,9}$  and by culture methods on *Milnesium tardigradum*  $^{10}$ .

A sex ratio near to 1:1 is observed in collections of Tardigrades belonging to the genus *Macrobiotus* (Table); populations without males are observed in other territories, as in the case of *M. hufelandii* found near Ravenna

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

Species	Location	<b>33</b>	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).

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.

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.<sup>1</sup>, 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

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