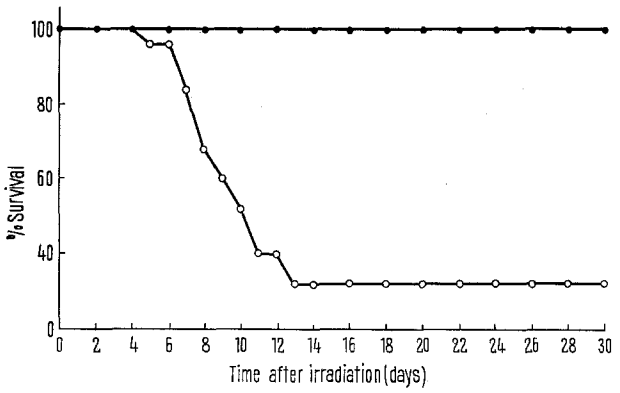

Fig. 1. Survival of X-irradiated mice which have received 10 mg of carbon particles 24 h before irradiation. ○—○, control (600 R-irradiated); ●—●, irradiated after carbon injection.

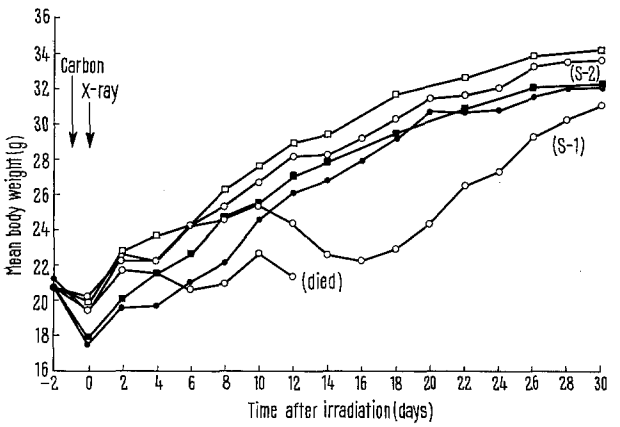

Fig. 2. Changes in the body weight of mice which have received carbon particles 24 h before irradiation. □—□, normal; ■—■, carbon-injected control; ○—○, X-irradiated; ●—●, X-irradiated after carbon injection. S-1, wasted but survived; S-2, survived without loss of body weight.

cause RES to overlook the cells which were somewhat injured but still maintain their nearly normal function.

Another possibility, however, should be taken into account: that carbon particles facilitate the recovery of hematopoietic system. This can be inferred from the observation of the radioprotective effect of carbon particles for the antibody response in rats[13], and that of the carbon-induced hyperplasia of lymphoreticular tissue[14,15]. In this respect, our using rapidly growing young adult mice might be of advantage, since KOJIMA et al.[16] have observed, in a similar experiment, a favorable but not clearcut result as to the effect of carbon particles employing 8- to 9-week-old mice[17].

Zusammenfassung. Die i.v. Injektion von Kohlepartikeln an Mäusen, 24 h vor einer Bestrahlung mit 600 R, bietet einen bemerkenswerten Strahlenschutz. Die Problematik der biologischen Schutzfunktion durch die Reticulo-histiocytäre Systemblockade wird diskutiert.

K. J. MORI and S. NAKAMURA

Department of Industrial Health, Osaka Prefectural Institute of Public Health, Higashinariku, Osaka (Japan), 10 June 1970.

[13] H. S. STENDER, D. STRAUCH and H. WINTER, in *The Effects of Ionizing Radiations on Immune Processes* (Ed. C. A. LEONE; Gordon and Breach, New York 1962), p. 315.
[14] D. STRAUCH, H. S. STENDER and H. WINTER, J. Immun. *82*, 298 (1959).
[15] K. J. MORI and S. NAKAMURA, Proc. Osaka Prefect. Inst. Public Health and Industrial Health *6*, 10 (1968).
[16] E. KOJIMA, W. NAKAMURA and H. ETO, at the 12th Assembly of Japan Radiat. Res. Soc. (1969).
[17] We gratefully acknowledge Dr. S. MURAMATSU of Laboratory of Radiation Biology, Faculty of Science, Kyoto University for his suggestions in preparing the manuscript.

A New Interpretation of the Sex Determining Mechanism of the European Earwig, *Forficula auricularia*

It has long been known that some populations of *Forficula auricularia* L. are polymorphic for the number of male sex chromosomes. All previous authors have interpreted males with 24 chromosomes as $(22+X_1Y)$ and males with 25 chromosomes as $(22+X_1X_2Y)$. It follows that females in such populations should display a varying number of sex chromosomes corresponding to chromosome numbers of 24 $(22+X_1X_1)$, 25 $(22+X_1X_1X_2)$ and 26 $(22+X_1X_1X_2X_2)$. Early reports of chromosome counts from females were unreliable, usually in the expressed opinions of the authors[1–3]. These results were the basis of the above interpretation of the sex chromosomes by some reviewers[4], and by recent investigators[5,6] who themselves found only 24 chromosomes in females. To clarify the issue both sexes of a suitable local population were investigated (the species has been introduced into Australia and is often common in gardens and parks).

Materials and methods. 25% of the males in a small area of the grounds of Melbourne University were found to have 25 chromosomes in 1964[7]. A quite exhaustive collection at this locality in December (early summer) 1968 yielded 188 males and 146 females from which material for this study was selected at random.

Testes from young adult males were hand-squashed in acetic-orcein, sealed and stored in the deep freeze.

Females were injected with 0.2 µl of a 0.05% solution of colcemid (CIBA) per mg of body weight, 12–16 h later the ovaries were dissected into hypotonic 1.0% sodium citrate for 5–10 min. Subsequent preparation was similar to that described for males except that the material was squashed in a hydraulic press at 600 to 800 lb in^{-2}.

Results and discussion. 11 females did not have the 10 or more scoreable divisions considered necessary for this study but the results in them were consistent with the results shown in Table I. The results in this table

[1] F. BRAUNS, Naturf. Ges. Rostock, Sitzungsbericht *4*, 99 (1912).
[2] W. P. MORGAN, J. Morph. *46*, 241 (1928).
[3] F. PAYNE, J. Morph. *25*, 559 (1914).
[4] K. R. LEWIS and B. JOHN, Int. Rev. Cytol. *23*, 277 (1968).
[5] H. G. CALLAN, J. Genetics *49*, 209 (1949).
[6] E. ORTIZ, *Chromosomes Today* (Oliver and Boyd, Edinburgh 1969), vol. 2.
[7] J. WONG, unpublished (1964).

Table I. Chromosome counts of C mitoses in ovarian follicle cells of *Forficula auricularia* from Melbourne University

No.	Chromosome counts												Discarded cells	
	12–18	19	20	21	22	23	24	24 + fragment	25	26	27–40	41–48	2N	4N
1	7	8	5	4	12	26	60		2		4	6	14	0
2			1	1	2	4	104					7	90	13
3	2	2	1	4	6	25	81		1				55	0
4		1	1	1	6	8	25				1	1	39	1
5		1		1	4	17	112	6		1		1	63	0
6				3	2	11	41					1	48	8
7		1	1		3	11	55		1				35	0
8				1	3	4	13						28	2
9	2			3	3	5	29	3	1			2	74	0
10	1			1			12		1				9	6
11					2	2	22						18	1
12		2			1	1	8						1	4
13		2			2	2	9						8	6
14		1		2	3	4	31			1		1	43	4
15				1	2	5	25						50	0
16					3	6	16						15	0
17	2				1	6	12						11	1
18	1		1		4	5	14						63	0
19	2				2	7	20						44	0
20	2	1	1	1	6	5	23		1	1			36	2
21	2		2	3	5	9	46			1			5	1
22					1	5	14						43	1
23[a]					5	5	10						12	0
24[a]			1	2	2	4	4					1	8	0
25[a]			1	1	1	2	5						11	0
26[a]				2	1	4	6						7	0

[a] Counts from autoradiographs, 4–6 h colcemid.

are also consistent with female cell counts made in 1964[7] and in 1965 and 1970 by the first author.

The decision to score each mitotic cell was made before counting and the number of discarded cells is shown. The female chromosome set is quite uniform in size (Figure 1a) and the counts shown were obtained by counting individual separated elements within the mitotic cell. Counts lower than 24 were almost certainly caused by breakage of cells or by overlaps in poorly spread cells and fragments of chromosomes were not recorded from such cells as they would not have been obvious. Cells with 24 chromosomes plus a fragment (Figure 1b) or 25 chromosomes were quite rare and in the latter case the count could often be attributed to the counting of separated chromatids as two chromosomes.

The results appear to be entirely consistent with a 24 ($22 + XX$) diploid chromosome set in females of *Forficula auricularia*. Therefore it must be considered that the polymorphism in males is limited to the male line and is caused by variation in chromosomes which behave as Y chromosomes.

Details of the behaviour of chromosomes during spermatogenesis including accurate measurements of chromosome size, behaviour during meiosis and results from autoradiographic studies of late replication during spermatogonial divisions will be published shortly. 3 types of sex chromosomes can be distinguished in males: 1. The X chromosome, which is as large as the largest autosomes and only rarely shows a bilobed condition at first metaphase. 2. A sex chromosome which has the size of the smallest autosomes and is interpreted as a Y_1 chromosome. 3. Another Y chromosome, designated Y_2, which is a little larger and appears more frequently bilobed than the X chromosome.

Different combinations of these 3 sex chromosomes produce the 4 types of males which can be distinguished at the Melbourne University locality and published photographs indicate that they occur at other localities in the cosmopolitan, cool climate, range of the species[5,6,8].

The frequency of the different types of males is shown in Table II and cells from them are illustrated in Figures 2a–d. A mosaic individual in which 24 ($22 + XY_1$) are associated with 25 ($22 + XY_2Y_2$) cells has not been found. In some individuals with the Y_2 chromosome, cysts of cells with up to 6 associated sex chromosomes per cell have been observed. The cells of one testis of a male from the 1969/70 population at the locality were all 27 ($22 + XY_2Y_2Y_2Y_2$); the other testis contained 25 ($22 + XY_2Y_2$) cells.

A distribution of chromosome counts indicating a 24 ($22 + XX$) chromosome set in 22 females was obtained

Table II. Distribution of types of males of *Forficula auricularia* at Melbourne University

Interpretation of karyotype	No.	%	[a]	%
a) 24 ($22 + XY_1$)	23	44.2	24-type	73.1
b) 24 ($22 + XY_2$)	15	28.8		
c) 24 ($22 + XY_2$)/25 ($22 + XY_2Y_2$)	5	9.6	25-type	26.9
d) 25 ($22 + XY_2Y_2$)	9	17.3		

[a] Chromosome type of previous authors.

from a collection made in 1965 at an isolated single tree 700 metres away from the above locality. 24 males of the next generation were found to be 24 ($22+XY_1$) and 1 was 24 ($22+XY_2$). A locality at which 25-chromosome males did not occur was found by CALLAN[5] at Merton (England).

Evidence from our second locality and from mosaic individuals indicates that it is the Y_2 chromosome which accumulates in the cells of the line of males in which it occurs. It is not known if it can be transmitted in multiple Y_2 sperm or if it is accumulated in post-zygotic development.

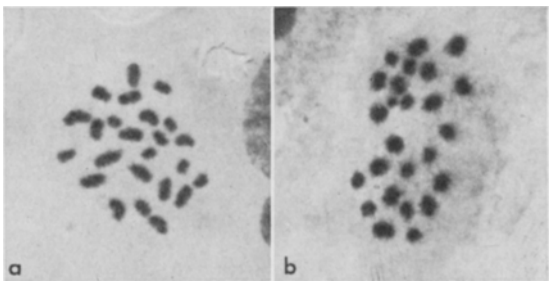

Fig. 1. Chromosomes in C mitosis in ovarian follicle cells of *Forficula auricularia*. a) 24 chromosomes; b) 24 chromosomes plus one fragment. Both ×1330.

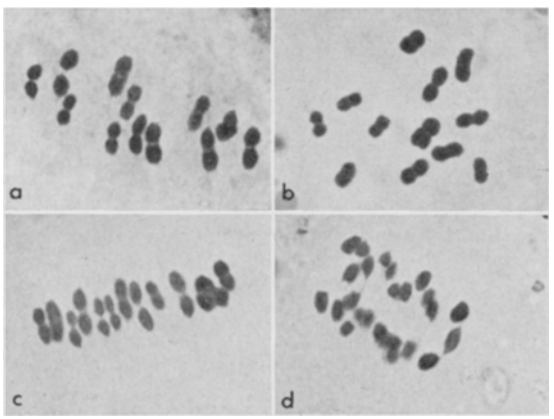

Fig. 2. Meiotic chromosomes of *Forficula auricularia* males. a) First metaphase, side view, karyotype 24 ($22+XY_1$), sex bivalent second from right. b) First metaphase, polar view, karyotype 24($22+XY_2$), one chromosome of the sex bivalent bilobed. c) First metaphase, side view, karyotype 25 ($22+XY_2Y_2$), sex trivalent second from right. d) Early first anaphase, side view, karyotype 25 ($22+XY_2Y_2$), apparent meiotic error with the Y_2 chromosomes migrating to each pole, X chromosome not committed. All ×1200.

A small but significant excess of males (56.3%) was found in the Melbourne University collection. This fact does not support a hypothesis of LEWIS and JOHN[4] based on X_1X_2Y sex chromosomes in 25-chromosome males. These authors explained the excess of females found in many populations of *Forficula*[9] as being due to meiotic errors producing an excess of female-determining sperm in these males. Meiotic errors were noted in 25-chromosome males of the present study but on the new interpretation they would lead to an excess of Y_2 bearing sperm (Figure 2d).

The apparent necessity for a Y chromosome for male determination in the cytologically known species of Dermaptera[6] has influenced our designation of the Y_2 chromosome. Otherwise it might be considered to be a supernumerary chromosome which segregates from the X during meiosis[10,11].

HENDERSON[12] has recently published a detailed study of males of a population of *Forficula auricularia* at Cambridge, interpreting 25 chromosome males as XXY. He interpreted variation in the size of the sex chromosomes as a complex polymorphism of both the X and Y. We have made similar observations but interpret the situation as due to increases in the size of some of the Y_2 chromosomes.

Riassunto. Osservazioni su femmine di *Forficula auricularia* L. indicano che esse possiedono un solo cariotipo formato da 22 autosomi e da 2 cromosomi X. Sulla base di questo studio, suggeriamo che i maschi con 3 cromosomi sessuali sono del tipo XYY, e non XXY come precedentemente ritenuto da varii autori. Ci sono maschi XY_1, XY_2, XY_2Y_2 e mosaici XY_2/XY_2Y_2 nella popolazione. Inoltre viene dimostrato che solamente il più grande dei due cromosomi Y, che caratterizzano questa specie, viene accumulato nei maschi con un meccanismo multiplo di cromosomi sessuali.

G. C. WEBB and M. J. D. WHITE[13]

Genetics Department, University of Melbourne, Parkville 3052, Victoria (Australia), 10 August 1970.

[8] B. JOHN and K. R. LEWIS, *Protoplasmatologia 6*, F1 (Springer, Wien and New York 1965).
[9] H. H. BRINDLEY, Proc. Camb. Phil. Soc. *19*, 167 (1912).
[10] W. D. JACKSON and D. S. M. CHEUNG, Chromosoma *23*, 24 (1967).
[11] M. J. D. WHITE, *Animal Cytology and Evolution*, 2nd edn (Cambridge University Press 1954).
[12] S. A. HENDERSON, Chromosoma *31*, 139 (1970).
[13] Supported by a postgraduate award from the Commonwealth of Australia to G.C.W. and by Public Health Service Grant No. GM-07212 from the Division of General Medical Sciences, U.S. National Institutes of Health and by a grant from the Australian Research Grants Committee.

Amino Acid Excretion Patterns in the Offspring of a 'Doubly-Heterozygous' Cystine Stone Former

The genes regulating the expression of the different biochemical types of cystinuria[1] may be allelic, as more than one form of the disease has recently been found within the pedigree[2]. 4 such families are on record, 2 carrying Types I and III cystinuria[2,3], 1 with Types I and II[2], and 1 with Types II and III[2]. The cystinuria in at least 3 other families, described before the 3 types were defined[4-6], may also be heterogeneous. Stone formers in

[1] H. HARRIS, U. MITTWOCH, E. B. ROBSON and F. L. WARREN, Ann. Human Genet. *20*, 57 (1955).
[2] L. E. ROSENBERG, Science *154*, 1341 (1966).
[3] S. KELLY and W. COPELAND, J. med. Genetics *5*, 281 (1968).
[4] H. BOSTROM and K. TOTTIE, Acta Paediat. *48*, 345 (1959).
[5] W. H. FLEMING, G. B. AVERY, R. I. MORGAN and T. E. CONE JR Pediatrics *32*, 358 (1963).
[6] L. HAMBRAEUS, J. Clin. Lab. Invest. *13*, 74 (1961).