

longitudinal section of the membrane bone of pike (Fig. 1), the curve of decrease in intensity along the long axis of scatter showed a pronounced shoulder which suggested the superimposition of a definite diffraction peak on the low angle scatter corresponding to the particle diameters. When this was treated to the first approximation as a Bragg diffraction, a spacing of 65 Å to 70 Å was obtained. In the direction of the short axis of scatter, the low angle reflections usually attributed to collagen appeared to be of exceptionally high intensity. This has been observed to a less marked degree in previous patterns, and suspected to reflect a reinforcing of the collagen diffraction by associated apatite particles. In the case of the pike bone, the specimen was refluxed with ethylene diamine for 24 h to remove the collagen, and the complete extraction was confirmed by chemical analysis of the residual tissue. The diffraction pattern subsequently obtained from the extracted specimen is shown in Figure 2. The meridional low angle diffractions remain intense and well defined, and must be associated with the precise spacing of the apatite particles in the direction of the longitudinal axis of the bone. The main reflections gave Bragg spacings of 650 Å, 218 Å, and 130 Å, which coincide approximately with the first, third, and fifth order diffractions of collagen. As compared with the collagen diffraction, the 218 Å reflection of the apatite system appeared to have an unusually high intensity relative to the other reflections.

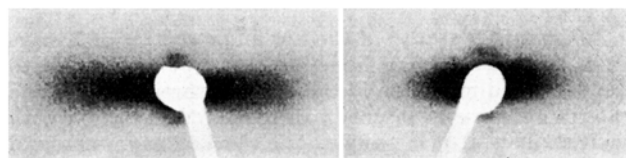


Fig. 1.

Fig. 2.

The fundamental repeating unit in the apatite structure thus appears to coincide with that of collagen, precise reflecting planes occurring only at intervals of 650 Å, but the outstanding intensity of the third order reflection suggests that this spacing of 218 Å may be related to the apatite particle length of about 210 Å deduced previously from continuous particle scatter. One can readily imagine a system of particles with lengths varying slightly about an average of 210 Å, which are aligned along the collagen fibres in such a way as to produce precise crystallographic reflecting planes only where they coincide with the collagen period. Such a picture would appear to be capable of explaining the observed diffraction effects. After extraction of collagen, the scattering curve corresponding to the short axis of the particles no longer shows the shoulder which in intact bone seemed to suggest a superimposed Bragg reflection. Instead, there is a regular decrease in intensity of scatter with increase in scattering angle which can be treated as independent particle scatter, and from which a particle diameter of about 65 Å has been calculated. This value agrees well with the Bragg spacing found in the pattern of intact bone, and both the continuous low angle scatter from the somewhat disordered systems of particles usually encountered and the well-defined reflections associated with the organisation in the membrane bone of pike, can be interpreted in terms of systems of particles whose basic dimensions are 65 Å and 215 Å. It is assumed that the removal of the collagen from the pike bone introduces sufficient disorder into the system to remove the diffuse reflection at 65 Å.

The basic structural components in all types of bone appear to be collagen and apatite. The collagen has the same essential characteristics in all systems, and it would appear that the apatite particles are of approximately uniform size, the length in particular being remarkably constant in all types of bone studied. The probable length of the apatite particle corresponds almost exactly to one third of the fundamental repeating unit in the structure of collagen, and X-ray diffraction and electron microscope studies have indicated that this may be an important repeating distance in the structure of collagen itself. There can be little doubt of the close relationship between the form of crystallization of the apatite component during the development of bone and the detailed structure along the collagen fibre, and it can be suggested that the structure is basically the same in all bone tissues, the observed differences in diffraction effects being associated with slight differences in degree of order among structural components and in their relative proportions.

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#### *Zusammenfassung*

Es konnten Kleinwinkel-Röntgenbeugungen an Diffraktionsbildern von dekolagenisiertem Fischknochen beobachtet werden. Diese Beugungen können vollständig zu der Partikelgrösse in der kontinuierlichen Kleinwinkelbeugung weniger geordneter Systeme in Beziehung gesetzt werden. Die Ergebnisse betonen die nahe Verwandtschaft zwischen der Grösse der Apatit-Partikel und der periodischen Struktur des Kollagens.

### **Cytological Demonstration of Female Heterogamety in Isopods**

The nature of the sex-determining system in bisexual species of Isopods has repeatedly been discussed by several authors. VANDEL<sup>1</sup> assumed the presence of female heterogamety and postulated directed segregation of the sex chromosomes in female meiosis caused by cytoplasmic influence to account for the occurrence of unisexual (thelygenic and arrhenogenic) broods in many species of terrestrial Isopods. DE LATTIN<sup>2</sup>, however, interprets his own and VANDEL's results as due to a system of multiple sex-determining factors distributed over several chromosomes and partly acting through female predetermination. Neither in male<sup>3</sup>, nor in female meiosis of many species of Isopods, studied by VANDEL<sup>4</sup> and others, have sex chromosomes ever conclusively been shown to occur. On the whole, the sex-determining system of Isopods seems to be highly unstable, as it is indicated by the frequent occurrence of intersexuality, parthenogenesis, hermaphroditism, and unisexuality.

The first observations of sex chromosomes in Isopods have recently been made in 4 closely related marine

<sup>1</sup> A. VANDEL, *Bull. biol. France Belg.* 72, 147 (1938); 75, 316 (1941).

<sup>2</sup> G. DE LATTIN, *Z. Vererbungslehre* 84, 1 (1951); 84, 536 (1952).

<sup>3</sup> The statement of J. DWORAK (*Fol. Morph.* 5, 209, 1935) about the finding of an XO mechanism in males of *Asellus aquaticus* is probably erroneous; cf. the paper of A. VANDEL, footnote 4.

<sup>4</sup> A. VANDEL, *Bull. biol. France Belg.* 81, 154 (1947); review of earlier papers in this work.

species of the superspecies *Jaera marina* (*Janiridae*, *Asellota*)<sup>1</sup> in the region of Roscoff (Brittany, France). All of these 4 species show different chromosome numbers. The following account deals mainly with *Jaera marina forsmanni*. In female meiosis (Figs. 1, 3) it contains 8 bivalents and 1 trivalent configuration in first metaphase. The trivalent, present in all females, is formed by the pairing of each of the two arms of a metacentric chromosome with an acrocentric element. The two acrocentric chromosomes, which are of different size, have always been found to be co-oriented at metaphase. The metacentric chromosome is connected with them by terminal chiasmata, as in most bivalents.



Fig. 1.—*J. m. forsmanni*, female 1<sup>st</sup> metaphase. 8 bivalents and sex trivalent. 3100 ×.

The significance of the trivalent in female meiosis is made clear by the chromosomal constitution found in males. Male diakinesis (Fig. 2) shows 9 bivalents without any sign of structural heterozygosity. Most of the bivalents are rod-shaped with terminal or interstitial chiasmata. Some are ring-bivalents, among which one is of outstanding size. The trivalent configuration in females therefore represents a sex trivalent similar to that found in male meiosis of several species of animals (e.g. Mantids<sup>2</sup>, Marsupials<sup>3</sup>, Eutherians)<sup>4</sup>.

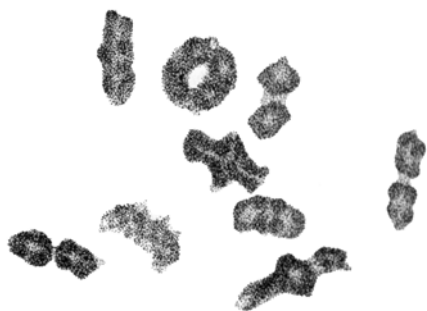


Fig. 2.—*J. m. forsmanni*, male diakinesis. 9 bivalents. 4200 ×.

Homozygosity for 9 chromosomes in males proves that the metacentric chromosome involved in the formation of the female trivalent is present as a bivalent in male meiosis. The sex chromosome system of *Jaera marina forsmanni* is therefore of the  $XY_1Y_2$  type, the females being the heterozygous sex. Males are homozygous for the X chromosome. The XX bivalent is probably represented by the large ring bivalent.

The sex trivalent has been found in all 4 members of

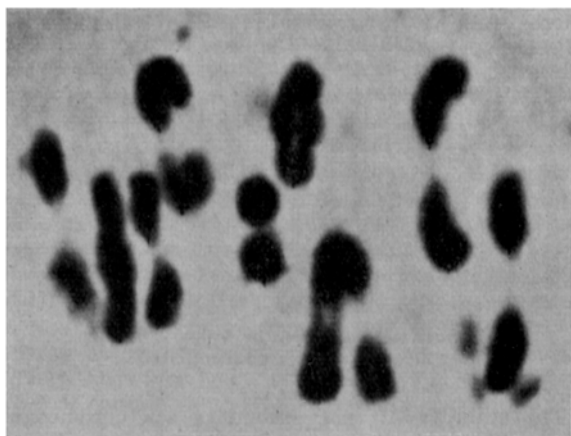


Fig. 3.—*J. m. forsmanni*, photograph of female 1<sup>st</sup> metaphase. 4000 ×.

the *Jaera marina* superspecies so far studied. Several facts indicate that the different chromosome numbers (ranging from 8 to 12 autosomes) are connected by a Robertsonian relation. Thus, *Jaera marina ischiosetosa* (Fig. 4) with 12 bivalents and a sex trivalent in female meiosis, shows considerably fewer metacentric, but more acrocentric chromosomes than *forsmanni* with its lower chromosome number, as it is seen from the structure of bivalents and their fainter staining in *ischiosetosa*. Since no sex chromosomes are found in other species of the family *Janiridae*, the original situation must have been a system of morphologically undifferentiated chromosomal carriers of sex-determining factors. This conclusion is justified, too, by the normal (euchromatic) condensation of the  $XY_1Y_2$  chromosomes in metaphase. The Robertsonian change, if involving carriers of sex-determining factors, may alter indistinguishable sex chromosomes into a differentiated multiple system as that found in the *Jaera marina* species group. Its underlying chromosomal mutation process<sup>1</sup> is not known with certainty. There are two possibilities, resulting in a  $XY_1Y_2$  system, of this evolutionary change: either a fusion of a primarily acrocentric X with an acrocentric autosome, converting the corresponding unfused autosome into a second Y chromosome (Fig. 5); or a fragmentation of a metacentric Y, giving rise to two acrocentric Y chromosomes. No decision is possible at present between these two modes in the *Jaera* case, but the former seems to be more probable on general grounds, since the change from metacentric chromosomes to acrocentric pairs has never been conclusively demonstrated to occur. As far as the origin is concerned, the *Jaera* system differs from most known multiple sex chromosome systems which have evolved either from an XO mechanism or from differentiated, partly heterochromatic XY chromosomes, presumably by reciprocal translocation with an autosome. The "principle of homologous change", formulated by WHITE<sup>2</sup>, gives a useful descriptive base for the chromosome relation within the *Jaera marina* superspecies, applying to both autosomes and sex-determining chromosomes. The *Jaera* case, in this respect, closely resembles the  $XY_1Y_2$  system of *Drosophila americana*<sup>3</sup> in the *virilis* group

<sup>1</sup> The taxonomic status of these species with relation to ecology and population structure has recently been investigated by CH. BOCQUET, Arch. zool. exp. gén. 90, 187 (1953).

<sup>2</sup> M. J. D. WHITE, J. Genet. 42, 143 (1941). – S. HUGHES-SCHRAMMER, Chromosoma 4, 1 (1950).

<sup>3</sup> W. E. AGAR, Quart. J. Micr. Sci. 67, 183 (1923). – G. B. SHARMAN et al., Nature 166, 996 (1950); Heredity 6, 345 (1952).

<sup>4</sup> R. BOVEY, Arch. Julius-Klaus-Stiftg. 23, 506 (1948); R. suisse Zool. 56, 371 (1949). Review of all cases in Mammals by R. MATTHEY, Les chromosomes des vertébrés (Lausanne, 1949).

<sup>1</sup> Cf. M. J. D. WHITE, Animal cytology and evolution (Cambridge, 1945). – R. MATTHEY, Les chromosomes des vertébrés (Lausanne, 1949).

<sup>2</sup> M. J. D. WHITE, Animal cytology and evolution (Cambridge, 1945).

<sup>3</sup> J. T. PATTERSON, W. S. STONE, and A. B. GRIFFEN, Univ. Texas Publ. 1940, No. 4032, 218; 1942, No. 4228, 162.

where, too, Robertsonian relations exist between different species.

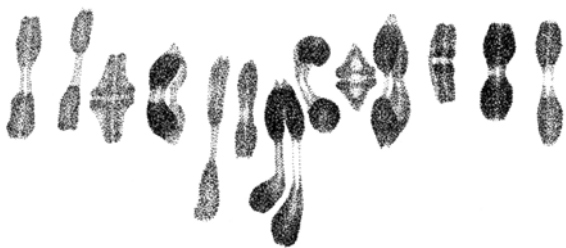


Fig. 4.—*Jaera marina ischiosetosa*, female 1<sup>st</sup> metaphase. 12 bivalents and sex trivalent. 3100 ×.

The stability of such secondary sex chromosomes necessarily demands that a change of position from X to Y or *vice versa* of the sex-determining factors by crossing over must be excluded. Crossing over presumably occurs in both sexes of *Jaera*, as seen by the presence of chiasmata in male and female meiosis. Stability, then, can be attained by a lack of crossing over in the proximal region near the centromere. A small proximal differential segment, preventing homozygosity for the structurally different chromosomes in one of the two sexes, may thereby become established.

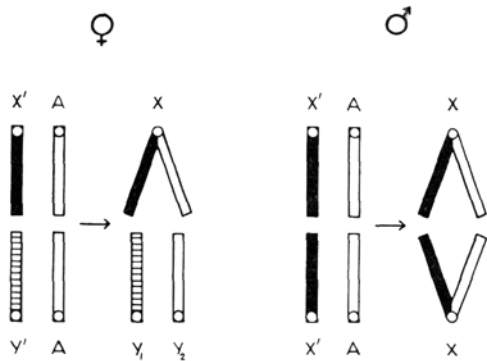


Fig. 5.—Probable mode of origin of the  $XY_1Y_2\text{♀}$ — $XX\text{♂}$  system of *Jaera* by a fusion  $X'-A$ .  $X'$ ,  $Y'$  = original (undifferentiated) sex-determining chromosomes;  $A$  = a pair of acrocentric autosomes.

The results obtained in the *Jaera marina* superspecies support VANDEL's assumption on female heterogamety in Isopods, but without justifying his further views. Contrary to the conclusions of DE LATTIN, they show that the sex-determining system may be represented by one single pair of chromosomes. Generalizations, however, as to the structure of the sex-determining system in other Isopod species cannot be drawn from the *Jaera* results. It is well-known that in groups with a primitive sex-determining mechanism, as fishes, changes may occur from male to female heterogamety and *vice versa* even within the lowest taxonomic units.

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Résumé

Des chromosomes sexuels, inconnus jusqu'à présent chez les Isopodes, ont été trouvés dans 4 espèces de la

superespèce *Jaera marina* (*Janiridae*, *Asellota*). Il s'agit d'un système multiple de chromosomes sexuels, ce qui aboutit à la formation d'un trivalent sexuel dans la méiose femelle. Les femelles – hétérogamétiques – ont la constitution  $XY_1Y_2$ , les mâles étant  $XX$ . D'après les données chromosomiques présentées par les 4 espèces étudiées, la formation du système multiple sexuel est due à des processus cytologiques évolutifs d'ordre Robertsonien.

Nouvelles données sur les formules chromosomiques des *Muridae*

J'ai publié récemment un premier travail d'ensemble sur les chromosomes des *Muridae*<sup>1</sup>. Ce travail portait sur 15 espèces et une sous-espèces qui n'avaient jamais été l'objet d'investigations cytologiques. D'autre part, il renfermait une révision critique des données relatives à 14 autres espèces qui avaient déjà été étudiées par moi-même ou par d'autres auteurs.

Dans cette note, je présente brièvement les résultats obtenus chez 18 *Muridae* d'entre lesquels il n'y a que deux espèces ayant été chromosomiquement analysées. Voici un tableau récapitulant mes observations.

Sous-famille	Espèce	Nombre diploïde
Murinae . . . .	<i>Acomys russatus</i> WAGNER	38
	<i>Cricetomys gambianus</i> WATERH.	78
	<i>Rhabdomys pumilio</i> SPARR.	48
Cricetinae . . . .	<i>Mastomys coucha</i> SMITH	36
	<i>Mystromys albicaudatus</i> SMITH	32
Microtinae . . . .	<i>Ondatra zibethica</i> L.	54
	<i>Microtus californicus</i> KELLOG	54
	<i>Microtus irani</i> THOMAS	54
	<i>Microtus oeconomus</i> PALLAS	30
	<i>Pitymys subterraneus</i> SELYS	54
	<i>Ellobius lutescens</i> THOMAS	17
	<i>Steatomys pratensis</i> PETERS	68
	<i>Gerbillinae</i> . . . .	44
	<i>Tatera brantsii</i> draco WROUGHTON	44
	<i>Tatera afra</i> GRAY	42
	<i>Tatera schinzi</i> NOACK	54
	<i>Gerbillus garamantis</i> LAT.	44
	<i>Meriones vinogradovi</i> HEPTNER (?)	52
	<i>Desmodillus auricularis</i> SMITH	

**Chromosomes sexuels.** Toutes ces espèces, à l'exception d'*Ellobius lutescens*, ont une paire X-Y se disjoignant préréductionnellement. L'X et l'Y varient beaucoup d'une espèce à l'autre, soit par leur forme, soit par leurs dimensions. Le cas d'*Ellobius lutescens* est traité ici même dans une autre note.

**Espèces ayant fait l'objet d'investigations antérieures.** Chez *Microtus oeconomus*, MULDAL<sup>2</sup> a compté 46 chromosomes et décrit des hétérochromosomes géants de type *agrestis*. Mais, MAKINO<sup>3</sup> ayant compté 30 chromo-

<sup>1</sup> Aided by a grant from the Janggen Pöhn Foundation (St. Gallen, Switzerland) and supported by the Swiss Commission for the Biological Station of Roscoff.

<sup>2</sup> R. MATTHEY, Rev. suisse Zool. 60, 225 (1953).

<sup>3</sup> S. MULDAL, Ann. Rep. J. Innes Hort. Inst. 40, 19 (1949).

<sup>3</sup> S. MAKINO, Annot. Zool. Jap. 23, 63 (1950).