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STUDIES ON ENCHYTRAEIDAE IV.
PRELIMINARY REPORT ON CHROMOSOME NUMBERS
OF 7 DANISH GENERA.

By

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With 4 figures in the text.

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In connection with an ecological study on the occurrence and abundance of *Enchytraeidae* in various Danish habitats certain taxonomical difficulties arose.

As a possible way of negotiating them chromosome counts suggested themselves. However, the taxonomical problems proved still more complicated than assumed at first and in abeyance of a detailed analysis it was decided to publish this preliminary account.

Material and methods.

The greater part of the material was obtained from the grounds of the Mols Laboratory [and in most cases from the permanent stations described by NIELSEN (1955), to which reference is made]. The station numbers given here are identical with those used in the paper mentioned.

The *Enchytraeidae* were extracted from soil samples by means of the technique described by NIELSEN (1952—1953) or, in the case of samples obtained outside the grounds of the Mols Laboratory, by hand-sorting, and as a rule the worms were examined cytologically without delay.

In the present study spermatogonial mitoses and spermatocyte-MI were used. Therefore, only a description of the male reproductive system will be given. Unfortunately, no eggs in the process of division were found, thus further studies on the reproductive biology are required, in particular the oocyte-MI of the presumably parthenogenetic species is badly needed.

The *Enchytraeidae* are monoecious (hermaphrodites) with both male and female sex organs ventrally placed in each individual. The male reproductive system includes (1) a pair of minute testes (ordinarily in somite 11), (2) a varying number of paired seminal vesicles (somites 10—11) on either side connected to a vas deferens consisting of a wide ental part with a ciliated opening and a narrow ectal part of varying length which leads to the male pores (somite 12) immediately behind the ovaries.

Immature sperm cells separate from the testes to complete their differentiation in the vesicles. The multiplying spermatogonial cell forms

a rounded body which, in *Fridericia galba*, at MI contains 64 cells the divisions of which are completely synchronized. The mature sperm is discharged through the ducts during copulation.

As the multiplying cells are closely packed counting is facilitated by an artificial contraction of the chromosomes. The live animals were therefore treated with 0.001 M 8-oxychinoline for two hours at 29° C. As a fixative Carnoy's fluid (3:1) was used (15 minutes), maceration and hydrolysis in 1 N HCl (6 min.), staining in Feulgen's fuchsin (30 min.). Following this treatment the animals were dissected in 20% acetic acid under a binocular microscope. (During the dissection, damage to the alimentary canal should be carefully avoided as sand grains are present in the gut contents).

The preparations were made permanent by deep freezing (carbon dioxide snow). The cover slip was removed and together with the slide carried through abs. alcohol, abs. alcohol-xylo (1:1) to Canada balsam.

The chromosomes are rather small and with a few exceptions very uniform in size. No detailed morphological description of chromosomes will therefore be given. The meiotic divisions proceed in a regular manner except in the species with a very high number of chromosomes, where the MI show complete asynapsis. These species are probably parthenogenetic. Nothing conclusively can be said, however, before the oocytes have been examined.

Taxonomical notes.

The sequence of genera and species follows UDE (1929) which was also used as the standard key supplemented by other papers.

1. Genus *Henlea* MICHAELSEN 1889.

ČERNOSVITOV (1934) suggests that the members of this genus having intestinal pouches be separated into *Michaelсениella* and *Henlea* (s. s.) the former genus comprising the species with one pair of intestinal pouches and in which the origin of the dorsal vessel is between the pouches while species with two pairs of pouches should be referred to *Henlea* (s. s.). The proposed further subdivision of the genus need not be considered here as all individuals examined belong to the two subdivisions (genera) mentioned.

UDE (l. c.) lists one species belonging to either of these genera, viz. *nasuta* (EISEN) and *ventriculosa* (UDEKEM) respectively.

Because of the confusing chromosome counts obtained much labour has been spent on obtaining a precise characterization of the species. However, no constant deviations from published descriptions were found. From the chromosome and morphological point of view the material of *M. nasuta* was perfectly homogeneous while indications are at hand that the species *H. ventriculosa* as conceived by us (and UDE) is heterogeneous. In some cases lymphocyte and body size characters seemed to be correlated with certain chromosome numbers. However, until extended field and experimental studies have confirmed this we prefer to leave the question open.

Origin of material: Mols Laboratory, st. 28. Raw humus under pure stand of *Juniperus communis* (*M. nasuta* and *H. ventriculosa*); st. 20 Grass field (*H. ventriculosa*).

2. Genus *Bryodrilus* UDE.

One species examined: *B. ehlersi* UDE. All characters in complete agreement with description by ČERNOSVITOV (1928) although, as BACKLUND (1947) points out,

some variation is found in certain characters (e. g. shape of brain, shape of oesophageal enlargement).

Origin of material: Mols Laboratory, under bark of decaying alder stump in alder swamp (st. 31).

3. Genus *Mesenchytraeus* EISEN 1878.

One species examined: *M. beumeri* (MICH.). All characters agree with UDE's description except that the lymphocytes are distinctly oval.

Origin of material: Same as preceding species.

4. Genus *Pachydriilus* CLAPARÈDE 1861.

Three species examined: *P. lineatus* (MÜLLER), *P. pagenstecheri* (RATZEL), *P. helgolandicus* (MICH.) and *P. sp.* In the three former species agreement with UDE's description was found while the fourth species could not be referred to any species described.

Origin: Wrack bed at Øresund, Sofienberg, N. of Rungsted (Zealand).

5. Genus *Enchytraeoides* ROULE 1888.

Two species examined: *E. sphagnetorum* (VEJD.), and *E. sp.* A description of the latter species will be given in connection with a detailed analysis of the reproductive biology.

Origin: Mols Laboratory, small water course leading from spring at st. 31.

6. Genus *Fridericia* MICHAELSEN 1889.

Five species examined: *F. callosa* (EISEN), *F. leydigi* (VEJD.), *F. bisetosa* (LEVINSEN), *F. galba* (HOFFM.) and *F. ratzeli* (EISEN). All species appear to be well defined and the individuals agree with the description.

Origin: *F. leydigi*, *F. bisetosa*, pasture sts. 1 and 4. *F. galba*, raw humus and pure stand of juniper st. 28. *F. ratzeli*, meadow near st. 31. *F. callosa*, earth and debris on alder stump near st. 3.

7. Genus *Enchytraeus* HENLE 1837.

One species examined: *E. albidus* HENLE. Conforms to description.

Origin: Same as *Pachydriilus*.

Cytological observations.

Genus *Henlea*.

The difficult taxonomic problems within this genus are clearly reflected in the chromosome numbers.

H. nasuta, n: 17, spermatocyte MI (fig. 2a).

H. ventriculosa (figs. 3b and 1d). In this apparently well defined species three chromosome numbers are found, n: 17 in spermatocyte MI and spermatogonial mitosis; n: 34 in spermatocyte MI; 2n: 120—140 were counted at MI, in a third sample; no bivalents were observed. The last count may refer to a hepta- or octoploid, but the whole problem will be discussed in greater detail in a later publication.

Genus *Bryodrilus*.

B. ehlersi, n: 15, spermatocyte MI (fig. 2d). Only few individuals were available, and no mitoses were found.

Genus *Mesenchytraeus*.

M. beumeri, n: 16, spermatocyte MI (fig. 2c). VEJDOVSKY (1907) who counted the chromosomes of *M. flavus* (LEVIN.) and *M. setosus* (MICH.)

found in both species $n: 16$. In VEJDOVSKY'S paper there are observations on the oogenesis.

Genus *Pachydrius*.

Here we find not only the lowest number but also the most differentiated chromosomes (10 large V-shaped, 12 small and 6 very small).

P. pagenstecheri, $n: 14$, spermatocyte MI and spermatogonial mitosis (figs. 3f and 1a).

P. helgolandicus, $n: 14$, spermatocyte MI (fig. 2b).

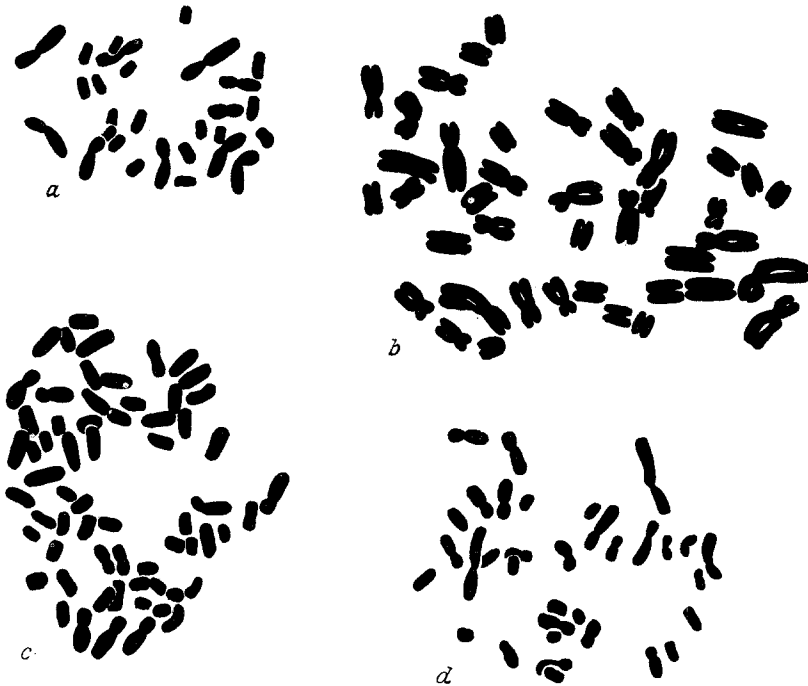


Fig. 1a—d. Mitosis, spermatogonial divisions, $\times 2000$. Camera lucida drawings. a *Pachydrius pagenstecheri*; b *Enchytraeus albidus*; c *Fridericia bisetosa*; d *Henlea ventriculosa* ($2n: 34$).

P. lineatus, $n: 13$, spermatocyte MI and spermatogonial mitosis (fig. 3e).

P. sp., $n: 14$.

Genus *Enchytraeoides*.

E. sphagnetorum $2n: 180-200$ (fig. 3c). This very high-numbered species is probably parthenogenetic judging from the total absence of bivalents during MI.

An undescribed *E. sp.* has also a very high chromosome number.

Genus *Fridericia*.

The chromosomes of all the species belonging to this genus are small and of equal length. Our findings were as follows:

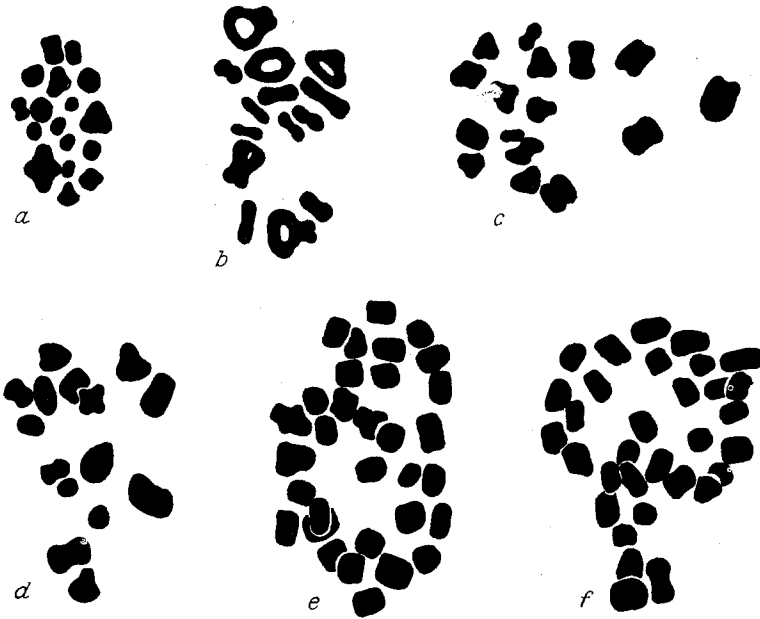


Fig. 2 a—f. Meiosis, spermatocyte M I. $\times 2000$. Camera lucida drawings. a *Henlea nasuta*; b *Pachydrilus helgolandicus*; c *Mesenchytraeus beumeri*; d *Bryodrillus ehlersi*; e *Fridericia galba*; f *Fridericia ratzei*.

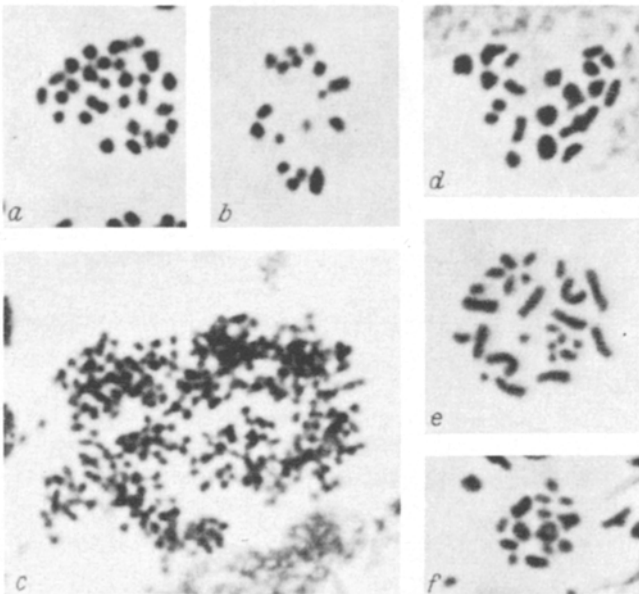


Fig. 3 a—f. Microfotos, $\times 1250$. a *Fridericia bisetosa*, meiosis, spermatocyte M I.; b *Henlea ventriculosa*, meiosis, spermatocyte M I.; c *Enchytraeoides sphagnetorum*, testis; d *Enchytraeus albidus*, meiosis, spermatocyte M I.; e *Pachydrilus lineatus*, spermatogonial division; f *Pachydrilus pagenstecheri*, meiosis, spermatocyte M I.

F. bisetosus, n: 32, spermatocyte MI and spermatogonial mitosis (figs. 3a and 1c).

F. ratzeli, n: 32, spermatocyte MI (fig. 2f).

F. galba, n: 32, spermatocyte MI (fig. 2e).

VEJDOVSKY (1907) reports n: 32 in *F. hegemon* (VEJD.).

F. callosa and *F. leydigi* have also been examined but no sperm was found although the animals were sexually mature with well developed female reproductive organs (vas deferens also well developed). A somatic mitosis showed the diploid chromosome number in *F. callosa* to be $2n: 64$. If the absence of spermatozoa in this species can be taken as an indication of parthenogenetic reproduction, we have in this genus, sexual as well as parthenogenetic species with $2n: 64$.

Genus *Enchytraeus*.

E. albidus, n: 21, spermatocyte MI and spermatogonial mitosis (figs. 3d and 1b).

VEJDOVSKY (1907) found that *E. adriaticus* (VEJD.) has a haploid number of 12 and *E. humicultor* (VEJD.) n: 16. According to MICHAELSEN (1900) *E. humicultor* is a synonym of *E. albidus* (HENLE). Therefore, either MICHAELSEN is wrong or the situation is as complicated as in the genus *Henlea* (s. l.).

Parthenogenesis in *Enchytraeidae*.

Parthenogenesis has hitherto not been described in *Enchytraeidae*. Although our evidence for the occurrence of parthenogenetic reproduction cannot be considered conclusive until the oogenesis has been investigated, the cytological facts suggest parthenogenetic reproduction in the genus *Enchytraeoides*, where both species examined show asynapsis. On the same evidence at least one *Henlea ventriculosa* type should be parthenogenetic, but this genus also includes sexual species. In all these species mature sperm was observed. In *F. callosa* on the other hand, the absence of spermatozoa in sexually mature species may likewise be taken as an indication of parthenogenetic reproduction.

If these observations are confirmed by further cytological studies and by breeding experiments we have a situation quite similar to that recently described by MULDAL in *Lumöricidae*; parthenogenesis was also unknown in the earthworms until MULDAL undertook a cytological investigation, which in his case was carried further than the present one because he was able to make a careful investigation of the oogenesis.

MULDAL has shown that seminal vesicles are present even in the parthenogenetic earthworms, but he does not mention whether spermatozoa are present or not.

Finally it should be mentioned that the *Enchytraeidae* in general seem to tolerate a much higher chromosome number without a conse-

quent breakdown of sexual reproduction than do the *Lumbricidae*. In the earthworms the highest number found in sexual species is $2n: 38$ whereas in *Enchytraeidae* species with $2n: 64$ and 42 chromosomes show no indication of parthenogenetic reproduction. On the 64 chromosome level, however, both sexual and parthenogenetic species seem to occur. Correspondingly in *Lumbricidae* the lowest number found in a parthenogenetic species is $2n: 38$, a figure also found in sexual species. In both families the breakdown of sexual systems seems to be associated with polyploidy.

Discussion.

The use of chromosome investigations as a tool to overcome taxonomical difficulties, like all other scientific methods, is subject to certain limitations. The modern genetic species concept requires identity between chromosome number and species. If a „species“ is found to contain different chromosome numbers it is most likely that it is too broadly defined. In most such cases closer morphological examination shows that the different chromosomal types are morphologically differentiated to such a degree that they should be given specific rank (taxonomical studies in the higher plants over the last 30 years have given ample support to the soundness of this principle). The reason why the modern species concept requires identity between chromosome number and species is, of course, that types with different chromosome numbers are separated by a sterility barrier, hence they cannot belong to the same species. On the other hand identical chromosome numbers are non-informative *per se*. In such cases however, an examination of chromosome morphology may throw further light on the taxonomical relationships. Furthermore it has already been mentioned that cytological observations on the chromosome behaviour may tell us something about the mode of reproduction, and in certain families a high chromosome number *per se* may be taken as an indication of asexual reproduction.

Judging from our preliminary counts the cytological investigations seem to offer a promising approach which may help a good deal in clearing up the very confused taxonomical situation in this family, since considerable variation in the chromosome numbers has been found. Already at least one clear case of „intraspecific“ chromosome variation has been encountered (*Henlea ventriculosa*) suggesting a taxonomical subdivision of the species. Moreover different genera have different chromosome numbers. In certain genera two or more diploid numbers have been found within the genus and although it would be dangerous to postulate that species belonging to the same genus should always have the same diploid number, this variation may nevertheless throw light upon the whole problem of chromosome evolution in the genera and the family.

In certain genera there is an indication of polyploid evolution and also the intergeneric variation in chromosome numbers show evidence of polyploid evolution.

The same trend was observed by MULDAL in the earthworms and he discusses the problem at some length. He found the diploid numbers $2n$: 32, 34, 36, and 38 in sexual species and parthenogenetic tri-, tetra- and decaploids and assumes that the diploid sexuals are really "tetraploids" derived from the "original haploid numbers" n : 8, 9, 10, 11 (*Branchio-*

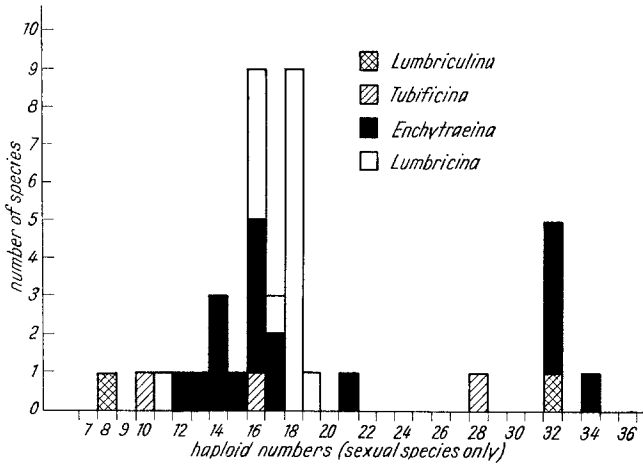


Fig. 4. Haploid chromosome numbers of Oligochaeta. Based upon MAKINO, MULDAL and own numbers. Systematics after MICHAELSEN (Handbuch der Zoologie, Bd. II).

bdella astaci n : 8, *Tubifex bavaricus* n : 10 and *Eisenia foetida* n : 11). Only the numbers 8, 10—12, 16—19, 28 and 32 were known to MULDAL (the diagram in fig. 4), and probably the absence of the numbers 13—15 was partly responsible for the formulation of his hypothesis (there is no systematic evidence). Even this preliminary investigation bridges to some extent the gap between the two groups n : 8, 10, 11 and n : 16—19, and thus the basis for MULDAL'S hypotheses as to the polyploid origin of the *Lumbricidae* has been considerably weakened, at any rate the diagram in fig. 8 in his paper is too simple. On the other hand it cannot be doubted that several cases of true polyploidy occur among the *Oligochaeta* but as yet it seems premature to discuss the evolution of the family on this basis as long as the chromosome numbers are known only for a small fraction of the species.

It will appear from fig. 4 that there exists an interesting difference in the variation of the chromosome numbers within the two families *Enchytraeidae* and *Lumbricidae*. *Lumbricidae* are one of the youngest families, and only 5 haploid numbers are reported in sexual species from

8 genera. In contrast, in the more primitive family *Enchytraeidae* 9 different haploid numbers are found representing 6 different genera. It may be more than a coincidence, that the intergeneric demarcations of the latter family is very clearcut, whereas the taxonomical demarcations of the genera of earthworms is much less clearly defined.

Summary.

1. Chromosome numbers from 12 species of *Enchytraeidae* representing 7 genera are reported. The following numbers were found n : 13, 14, 15, 16, 17, 21, 32 and $2n$: 120—140 and 180—200.

2. Several cases of intraspecific chromosome variation are found, and both intra- and interspecific polyploidy has been observed.

3. The meiotic behaviour of the chromosomes in several polyploid species, and the absence of spermatozoa in one sexually mature species suggest parthenogenetic reproduction. Breakdown of sexual reproduction seems to be associated with polyploidy.

4. The taxonomical implications of the results are discussed in relation to MULDAL's investigations of the *Lumbricidae* and his hypothesis as to the evolution of chromosome numbers in *Oligochaeta*.

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References.

- BACKLUND, H. O.: Swedish *Enchytraeidea*. Kgl. fysiogr. Sällsk. Lund. Handl., N. F. 2 58, 1—31 (1947). — ČERNOSVITOW, L.: Die *Oligochaetenfauna* der Karpathen. Zool. Jb., Abt. System., Ökol. u. Geogr. 55, 1—28 (1928). — Zur Kenntnis der *Enchytraeiden*. II. Zool. Anz. 105, 295—304 (1934). — MAKINO, S.: Chromosome numbers in animals. 1951. — MICHAELSEN, W.: *Oligochaeta*. Das Tierreich, Liefg 10. 1900. — *Oligochaeta*. Handbuch der Zoologie, Bd. II 8. 1928—1934. — MULDAL, S.: The chromosomes of the earthworms. Heredity 6, 55—76 (1952). — NIELSEN, C. O.: Studies on *Enchytraeidae*. I. A technique for extracting *Enchytraeidae* from soil samples. Oikos 4, 187—196 (1952/53). — Studies on *Enchytraeidae*. II. Field studies. Natura Jutlandica 4, 1—58 (1955). — UDE, H.: *Oligochaeta*. Die Tierwelt Deutschlands, Teil 15. 1929. — VEJDOVSKY, F.: Neue Untersuchungen über die Reifung und Befruchtung. Prag 1907.

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