The Chromosome Numbers and Nuclear Cytology of some Common British Serpulids

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With two plates (figs. 1 and 2)

Summary

The chromosome numbers of *Hydroides norvegica*, *Mercierella enigmatica*, and *Pomatoceros triqueter* were determined from squashes of somatic cells in young embryos obtained by artificial fertilization, and stained with iron-alum/aceto-carmine. All had a diploid count of $2n = 26$ chromosomes. Mitotic and meiotic divisions in the 5 species of *Spirorbis* examined, and in *Filograna implexa*, all revealed a diploid chromosome number of $2n = 20$. A diploid chromosome number of 14 is suggested for the ancestral serpulid.

Introduction

INVESTIGATIONS dealing with the nuclear cytology and chromosome numbers in the polychaete worms have been neglected in the past. This group has both hermaphrodite and bisexual forms and includes a wealth of species with diverse modes of life. It seemed that investigations of chromosome number and nuclear cytology in Polychaeta, along similar lines to the work on lumbricids (Muldal, 1949), might yield results of taxonomic value and indicate probable lines along which the evolution of the group has taken place.

While chromosome numbers vary considerably in different families of Polychaeta, there may also be diverse counts within one family. *Amphitrite* sp. has a diploid count of $2n = 22$ (Scott, 1906), while *Lanice conchilega* Malgreu, which belongs to the same family, the Terebellidae, has a count of $2n = 6$ (Dehorne, 1911). Polyploidy may have played a part in the evolution of the group; the phenomenon seems widespread in hermaphrodite annelids (White, 1940). On the whole, however, higher counts seem predominant among sedentary polychaetes, e.g. *Aricia* sp. with $2n = 18$ (Kostanecki, 1909); *Chaetopterus pergamentaceus* Cuvier with $2n = 18$ (Mead, 1898), and *Serpula crater* Claparède with $2n = 14$ (Soulier, 1906).

Methods

Mitotic and meiotic divisions were studied from preparations made by an iron-alum/aceto-carmine squash technique (Belling, 1926) involving the use of a separate bath of iron-alum mordant (Godward, 1948; Austin, 1959).

In the sub-family Serpulinae, the preparations were made from developing embryos obtained by means of artificial fertilization. Male and female worms

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were taken out of their tubes and placed in a dish containing sea-water. The sex cells were seen streaming out of the body and were allowed to mix together for fertilization to take place. Føyn and Gjøen (1954) described artificial fertilization in *P. triqueter* L. and we found it to be equally successful in *H. norvegica* and *M. enigmatica*. At 18° to 20° C the first cleavage in these 3 species occurred between 1½ and 1¾ h after fertilization. The best preparations were obtained with embryos between 2 and 4 h old, when active cell-division was taking place to form a many-celled blastula. Later stages were less suitable as the rate of mitosis slowed down. Cell-division almost stopped after the trochophore stage.

In the sub-family Spirorbinae both embryos and mature worms were examined. *Spirorbis borealis* shows a tidal periodicity in liberation of its larvae (Garbarini, 1933). They are liberated during neap tides, so that the ideal time for collection of material both for maturation division in adult worms and for mitotic activity in the embryos occurs a few days before spring tides, i.e. between the last batch of liberated larvae and the next spring tide.

In *F. implexa* mitotic divisions in somatic cells of young worms were examined. Table 1 (see Appendix, p. 400) gives the sources of material of the species studied.

OBSERVATIONS

The cytological observations made on the species under study are summarized in table 2 (see appendix). Both in mitosis and in meiosis the prometaphase and metaphase presented the clearest figures from which counts could be made.

The species *H. norvegica* (Gunnerus), *P. triqueter* L., and *M. enigmatica* Fauvel were found to possess a chromosome number of $2n = 26$. The centromere occurs in a terminal or sub-median position on the chromosomes of all three species (fig. 1, A, B, D, E). The chromosomes are all of similar size and form. At metaphase the chromosomes arrange themselves around a central spindle element (fig. 1, B) and anaphase separation is normal (fig. 1, C). The only available material of *Serpula crater* Claparède yielded only a single countable nucleus; this confirmed Soulier's count of $2n = 14$.

In the Spirorbinae, 5 species of *Spirorbis* were investigated. Both mitotic divisions in somatic cells and meiotic divisions during spermatogenesis were studied. All 5 species showed a uniform count of $2n = 20$ (fig. 2, A–F). In general, the chromosomes of this sub-family have terminal and sub-terminal centromeres. At meiotic metaphase I, 10 bivalents are discernible which assume characteristic shapes, there being no central element in the spindle.

FIG. 1 (plate). A, early mitotic prophase of *H. norvegica*: chromosomes in extended state.
B, mitotic metaphase of *H. norvegica*, showing the 26 chromosomes in one plane. Central elements are present in the spindle.
C, late mitotic anaphase in *H. norvegica*.
D, mitotic prophase in *M. enigmatica*: the 26 chromosomes can be counted.
E, late mitotic prophase or prometaphase in *P. triqueter*: the 26 chromosomes can be counted.
F, polar view of metaphase plate of *F. implexa*.

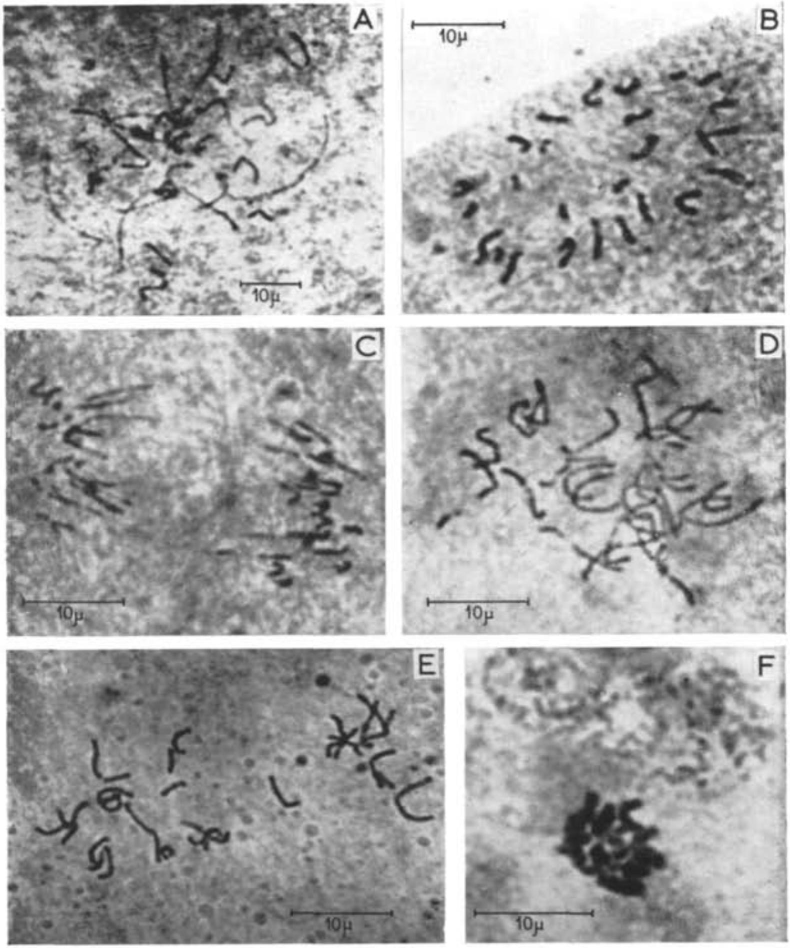

FIG. 1

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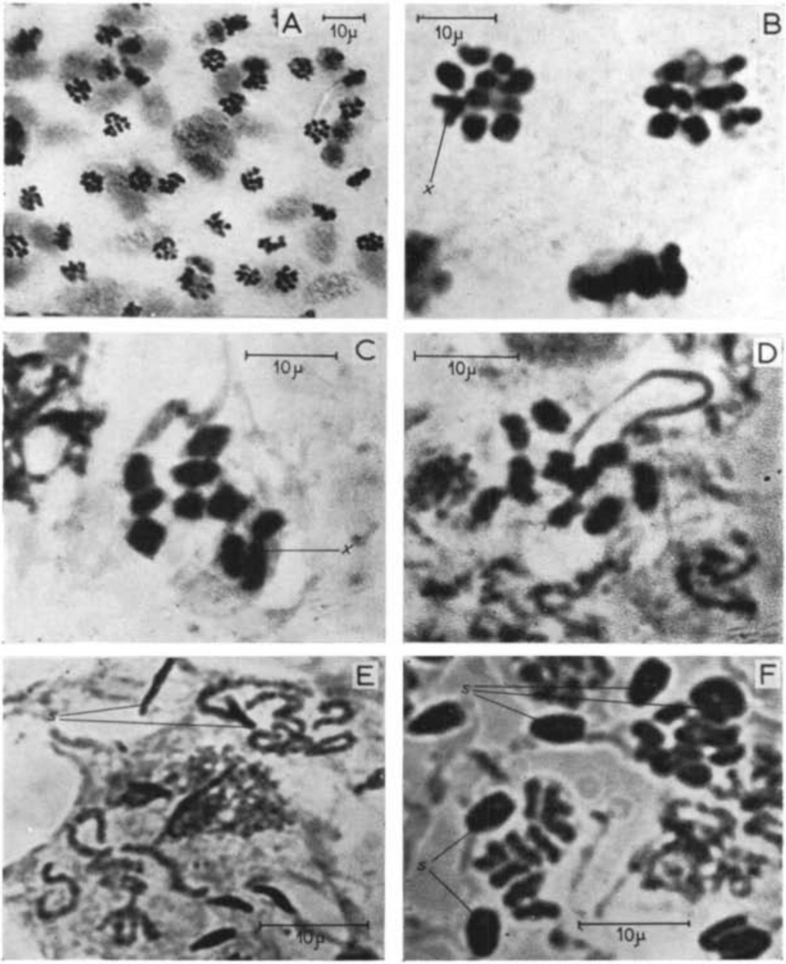

Fig. 2
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A possible hybrid between *S. borealis* and *S. corallinae* has been reported by de Silva (1960).

At metaphase II the chromosomes are highly contracted and dot-like. In *S. borealis* (fig. 2, B) and *S. corallinae* (fig. 2, C) one of the chromosomes (marked with an *X*) is larger than the rest, while in the other species they are all of about the same size (fig. 2, D–F). The nucleolus could be demonstrated only in *S. tridentatus*. It disappeared in early prophase (fig. 2, E).

In *F. implexa* (figs. 1, F; 3) the chromosome number is 20. Ten distinct bivalents can be seen during meiosis in both spermatogenesis and oogenesis. Chromosome size and position of centromere are similar to those found in the Spirorbinae.

Discussion

The family Serpulidae has three sub-families: the Serpulinae, Spirorbinae, and Filograninae. While the Spirorbinae and Filograninae are hermaphrodites, *P. triqueter* (Føyn and Gjøen, 1954) and *H. norvegica* (Ranzoli, 1954) of the Serpulinae are protandrous hermaphrodites. The chromosome numbers in this family are as follows:

(*a*) Serpulinae

H. norvegica (Gunnerus)	$2n = 26$
M. enigmatica Fauvel	$2n = 26$
P. triqueter L.	$2n = 26$
Serpula crater Claparède	$2n = 14$

(*b*) Spirorbinae

Spirorbis borealis Daudin	$2n = 20$
S. corallinae de Silva (1960)	$2n = 20$
S. tridentatus Levinsen	$2n = 20$
S. pagenstecheri Quatrefages	$2n = 20$
S. spirillum L.	$2n = 20$

(*c*) Filograninae

F. implexa (Berkeley)	$2n = 20$

The occurrence of $2n = 14$ in *Serpula crater* and $2n = 26$ in the other three species suggests that polyploidy may have played a part in the evolution of some of the species of the Serpulinae (fig. 4). If in this way the original

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FIG. 2 (plate). A, synchronous division (meiotic metaphase II) of groups of nuclei in testes of *Spirorbis borealis*.

B, meiotic metaphase II in testes of *S. borealis*: 10 bivalents can be counted, including one dot and one large chromosome (marked *X*).

C, D, E, meiotic metaphase I of *S. corallinae*, *S. pagenstecheri*, and *S. tridentatus*, to show 10 bivalents in each case. In *S. corallinae* (C) the large chromosome is marked *X*. Many sperm heads can be seen in E. *s*, sperm heads.

F, meiotic prophase I of *S. spirillum*. The chromosomes are extended and the developing sperm can be seen. *s*, sperm heads.

FIG. 3. Mitotic metaphase in *F. implexa*, showing 20 chromosomes of considerably differing sizes.

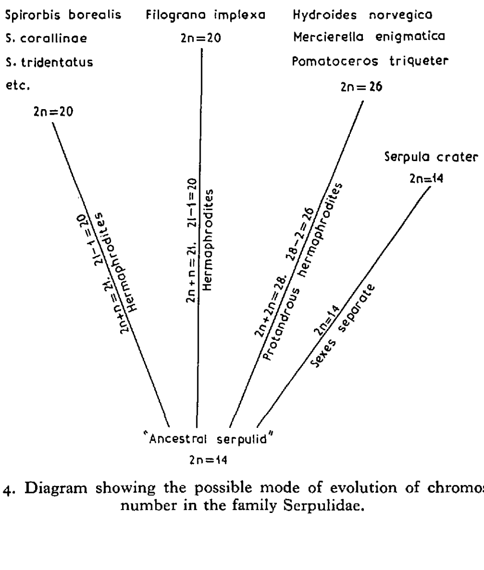

FIG. 4. Diagram showing the possible mode of evolution of chromosome number in the family Serpulidae.

number $2n = 14$ was doubled to $2n = 28$, then, by the loss of a pair of chromosomes in the course of evolution, the count found in *Hydroides*, *Mercierella*, and *Pomatoceros* of $2n = 26$ could have been arrived at. If we take a form with $2n = 14$ as the 'ancestral serpulid', the families Filograninae and Spirorbinae could be considered as $2n+n-1 = 20$, with the loss of a single chromosome taking place in the course of evolution of the group. The assumption that one chromosome from the haploid constituent is lost is supported in the sub-group Serpulinae, where two may have been lost (see fig. 4).

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APPENDIX

TABLE 1. *Sources of material*

Species	*Breeding season*	*Usual habitat*	*Collecting-ground*
H. norvegica (Gunnerus)	Aug.	Stones and shells	Swansea, Queen's Dock
P. triqueter L.	Mar.–Apr.	Stones, rock	Menai Straits
M. enigmatica Fauvel	Summer	Stones, rock	Swansea Dock and Milford Dock
Spirorbis borealis Daudin	Apr.–Nov.	*Fucus serratus*	Menai Straits
S. corallinae de Silva (1960)	Summer	*Corallina officinalis*	Menai Straits and Rhosneigr
S. tridentatus Levinsen	Summer	Pebbles and stones	Menai Straits and Holyhead harbour
S. pagenstecheri Quatrefages	Summer	Boulders and rock	Menai Straits and Holyhead harbour
S. spirillum L.	Summer	Bases of hydroids	Dredged off Puffin Island
F. implexa Berkeley	July, Aug., Sept.	Stones and shells	Dredged off eastern Anglesey

TABLE 2. *Cytological details of the nuclei of members of the Serpulinae, Spirorbinae, and Filograninae investigated*

					Prophase Average size in μ			*Prometaphase Average size in μ*		
						Chromosome bivalent, length			*Chromosome bivalent, length*	
Species	*Haploid chromosome number (n)*	*Diploid chromosome number (2n)*	*Diameter (in μ) of resting nucleus*	*Diameter of nucleolus (in μ)*	*Nucleus diameter*	*largest*	*smallest*	*Nucleus diameter*	*largest*	*smallest*
Serpulinae:										
H. norvegica	13	26	15·6	..	..	25·0	6·0	..	9·5	3·0
M. enigmatica	13	26	16·7	..	..	..	..	14·25	..	..
P. triqueter	13	26	..	..	..	..	..	13·5	..	..
Spirorbinae:										
S. borealis	10	20	15·15	..	16·5	13·5	4·12	14·85	7·25	3·0
S. corallinae	10	20	18·15	..	22·5	15·0	4·5	20·62	7·5	3·0
S. tridentatus	10	20	12·0	2·25	14·7	12·5	4·5	12·6	6·0	2·0
S. pagenstecheri	10	20	9·6	..	14·1	13·5	4·5	..	7·5	3·0
S. spirillum	10	20	9·75	..	13·2	14·25	3·0	..	..	..
Filograninae:										
F. implexa	10	20	14·10	..	..	..	13·5	..	..	..