THE CHROMOSOMES OF THE EARTHWORMS
I. THE EVOLUTION OF POLYPLOIDY

SYLFEST MULDAL

John Innes Horticultural Institution, Hertford, Herts.

CONTENTS

1. INTRODUCTION

CYTOLOGY has been an important aid for taxonomists in plants, where breeding work on many economically important crops is now largely based on chromosome studies. But it has not been applied to animal taxonomy to anything like the same extent. The present work originated as an investigation of the breeding systems in earthworms. This group shows many remarkable reproductive features which make the results rather special and does not perhaps call for wide generalisation. The work has shown, however, that chromosome study can serve to remove systematic confusion in animals much as it has done in plants.

2. GEOGRAPHICAL DISTRIBUTION AND SYSTEMATIC POSITION

The *Lumbricidae* comprise 220 species. Of these 19 are cosmopolitan, introduced by man to almost all parts of the world ; they are very robust species which often compete successfully with the local earthworm fauna. The remaining 201 are endemic ; they are found only in restricted areas and are believed to be of local origin.

The family is one of the youngest in the *Oligochaeta*, and has great powers of adaptation. Their introduction into new territory often causes the disappearance of the native worms. Michaelson (1903) notes that the only genus which successfully can oppose them is *Pheretima*, the youngest shoot of the *Megascolicidae*. *Pheretima* and *Lumbricidae* share the domination of Eastern Eurasia, while the European territory belongs to the *Lumbricidae* with only one genus of another family represented—*Hormogaster*.

The distribution of the endemic species is shown on the map (fig. 1). In the eastern part of the U.S.A. a few endemic " species " are found, but it seems as if there were no really old species there, and that these forms are recently introduced and rapidly changed from their European progenitors.

The southern distribution is limited by water and dry areas. The northern line cannot be explained on present geographical conditions but it follows the edge of the ice cap during glacial periods.

After the retreat of the ice the northern area was again invaded by the more adaptable (non-endemic) species. This can clearly be
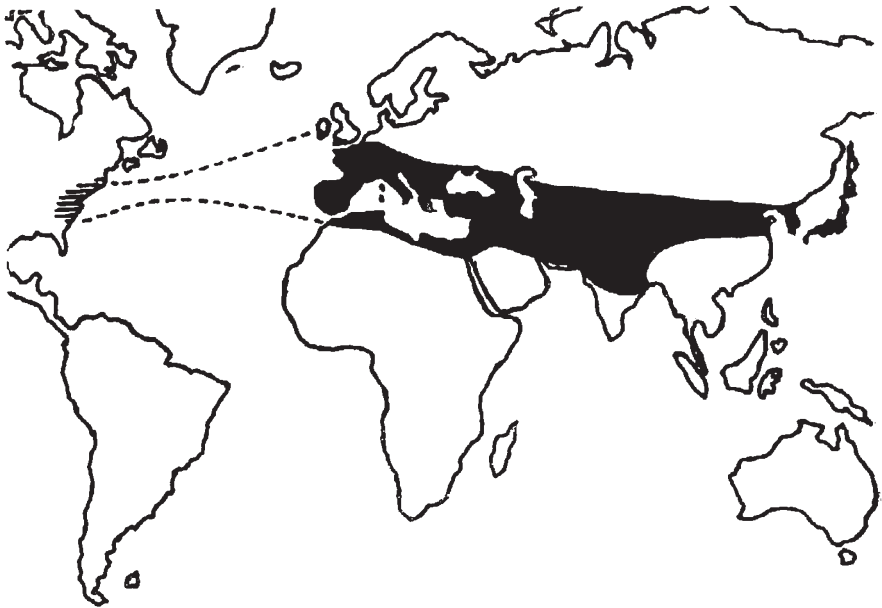

FIG. 1.

shown by the distribution of endemic species in an area which was only partly covered by the ice :—

	Endemic species	Total number of species
Germany	5	24
Czechoslovakia . . .	20	37
Italy	40	57

It is interesting that Switzerland, although covered by the ice, has just as many endemic species (20 of a total of 38) as the ice-free Czechoslovakia. Bretscher (1903) thinks that these Swiss species should be considered as very recently developed. Michaelson (1903)

gives a different explanation. He assumes that oasis-like areas have remained ice-free between the glaciers, and that the pre-glacial endemic forms were able to maintain themselves in such places. Southern (1909) gives a similar explanation for the occurrence of *Lumbricus friendi* in Southern Ireland and elsewhere only in the Pyrenees and the Alps.

The main systematic characters of the *Lumbricidae* are the structure and position of the sexual organs, which makes it sometimes impossible to identify immature specimens. Their classification is one of the difficult problems in *Oligochaeta*. In *Das Tierreich* (1900) Michaelson divided the family into 5 genera : *Lumbricus, Octolasium, Eisenia, Eiseniella* and the super-genus *Helodrilus*, which was split into the subgenera *Allolobophora, Dendrobaena, Helodrilus* and *Bimastus* :—

With Testes Sacs :—
    Paired .   .   .   . *Octolasium*
    Unpaired   .   .   .   .   .   . *Lumbricus*
No Testes Sacs :—
    Spermathecal pores—
        Dorsal : Gizzard long   .   .   .   . *Eisenia*
                    Gizzard short   .   .   . *Eiseniella*
        Ventral : Genus *Helodrilus*—
                Seminal vesicles—
                    4 pairs, setæ closely paired   .   *Allolobophora*
                    3 pairs, setæ widely paired   .   . *Dendrobaena*
                    2 pairs, clitellum ending on—
                      segments 32/33 at the most .   . *Bimastus*
                      segments 32/33 at least .   .   . *Helodrilus*
                                                    (now *Eophila*)

In addition to these generic characters come the position of the spermathecal pores relative to the setæ, general size and pigmentation.

There are several difficulties in applying such a clear-cut system. The reader is referred to the discussion in Michaelson's paper of 1910, which will briefly be considered here :—

(1) *Allolobophora* and *Dendrobaena* show many intermediate forms. There are species with D-arrangement of seminal vesicles and closely paired setæ (A-character). Other species have A-arrangement of seminal vesicles, but with pigmented integument and widely paired or distant setæ.

(2) A long series of intermediate forms between *Dendrobaena* and *Eisenia* with regard to position of spermathecal pores, is given by Michaelson. The close relation here cannot be doubted.

(3) *Eisenia* and *Eiseniella*, Michaelson thinks, are so closely allied that even their complete fusion could be justified. The only distinction between them is the short gizzard of *Eiseniella* (confined to segment 17). There are, however, *Eisenia* species which have gizzards occupying only half of 18 in addition to 17, so even this character is questionable.

More recently Pool (1937) and Pop (1941) have investigated the structure of the muscle sacs in 35 species. According to their findings

there are great affinities between *Bimastus* and *Eophila* on the one hand, and between *Bimastus* and *Dendrobaena* on the other. Pop will even abolish the genera *Bimastus and Eophila* and divide these species on *Allolobophora* and *Dendrobaena*. They also claim that *Eiseniella* has no particular relationship to *Eisenia*.

We have then systematic evidence for a close relationship between the following genera :—

ALLOLOBOPHORA
|
EISENIELLA—EISENIA—DENDROBAENA—BIMASTUS—EOPHILA

It remains to be said that Svetlov (1924) by ignoring the genus *Helodrilus*, gave all the subgenera generic status. Cognetti (1927) followed and in the next year Michaelson accepted this classification which is the one used here. Since the present study only includes British species no attempt has been made to alter the systematics.

3. MATERIAL

Most of the material came from natural populations. Several samples have been received from Holland for comparison, but since no differences between these and the British types were found, they will not be considered separately. Samples from Switzerland were obtained through the British Museum of Natural History.

Unless especially stated the number of specimens examined have not been lower than 10. The cytological technique applied will be described elsewhere.

Owing to the small size of the chromosomes a proper study of meiosis in spermatogenesis cannot be carried out in all earthworm species.

Oogenesis, however, has been studied in nearly all species mentioned here. It appears that the whole family can be characterised by the complete absence of major spirals on the meiotic chromosomes in the eggs. But they are apparently present in spermatogenesis—hence the great size difference between the chromosomes in male and female meiosis.

It is impossible to make an accurate comparison of chiasma frequency between the various species, partly due to the impossible task of estimating variation within individuals (in the majority of species only one egg at MI can be recovered from each animal). But it is also made difficult by the long duration of MI with the pronounced stretching of the chromosomes often leading to the complete separation of the homologues.

Apart from *Eisenia foetida* Sav. the mitotic chromosomes of the British *Lumbricidae* are small. Because one only rarely finds plates with chromosomes showing marked centric constrictions it is difficult to make accurate observations on the relative length of the chromosome arms. We shall therefore only consider gross differences in morphology and number of chromosomes. In order to reduce the text the following abbreviations will be used :—

V = large v-shaped chromosome
J = large chromosome, arm ratio less than 0·5
I = medium sized rod
v = small v-shaped
i = small rod chromosome

The somatic counts were made from mitosis in embryo or testes stained with Feulgen or acetic orcein.

4. CHROMOSOME NUMBERS IN BRITISH SPECIES

The following species and forms have been studied cytologically :—

	Source of material	$2n$
Genus Lumbricus (Linné) :		
rubellus Hoffmeister	Merton	36
castaneus Savigny	Wisley, Kew and Merton	36
terrestris L.	Merton	36
friendi Cognetti	Basel, Switz.	36
festivus Savigny	Wisley and Merton	36
Genus Allolobophora (Eisen em Rosa) :		
caliginosa f. typica (Sav.) . . .	Merton	36
caliginosa f. trapezoides (Duges) . .	Stringstone, Somerset	36
nocturna Evans	Merton	36
terrestris f. typica (Savigny) . .	Groningen, Holland	36
terrestris f. longa (Ude) . . .	Merton	36
chlorotica (Sav.)	Merton	32
icterica f. typica (Sav.) . . .	Merton	32
icterica f. dicystes (Cernosvitov) . .	Merton	32
Genus Bimastus (Moore) :		
eiseni Levinsen	B.	32
tenuis Eisen	B., and Switz.	48
Genus Eisenia (Malm em Michaelson) :		
foetida (Savigny)	Merton and Kew	22
veneta f. typica (Rosa) . . .	Plymouth	36
veneta var. Hibernica f. typica (Friend) .	Kew and Merton	36
veneta var. Hortensis (Michaelson) .	Oxford Bot. Garden	36
rosea f. typica (Sav.) . . .	Merton and B.	54
rosea mut. Macedonica (Rosa) . .	East London	53
Genus Eiseniella (Michaelson) :		
terraedra f. typica (Sav.) . . .	Wisley and B.	72
terraedra mut. Hercynia (Michaelson) .	Tring, Herts.	72
Genus Dendrobaena (Eisen em Rosa) :		
mammalis (Savigny)	Merton and Kew	34
rubida (Savigny)	Wisley and Merton	68
subrubicunda (Eisen)	Wisley, Merton and Hertford	68
Genus Octolasium (Oerley) :		
lacteum (Oerley)	B., and Valais, Switz.	38
cyaneum (Sav.)	B., and Merton	ca 190
Genus Eophila (Rosa) :		
oculata (Hoffmeister)	Kew (Thames)	32

Note.—B., Bayfordbury near Hertford.

The different genera show great homologies with regard to chromosome shape and structure. Chromosomes with strictly terminal centromeres are not present in the examined forms, and even where a mitotic chromosome in metaphase appear to be a rod, it can be shown that during prophase a short arm is always visible. Consequently it has not been possible to apply the principle of splitting or " fusion " of the centromeres in order to explain the differences in basic numbers.

Lumbricus.—Five species of *Lumbricus* have been examined ; they are all diploids with $2n = 36$.

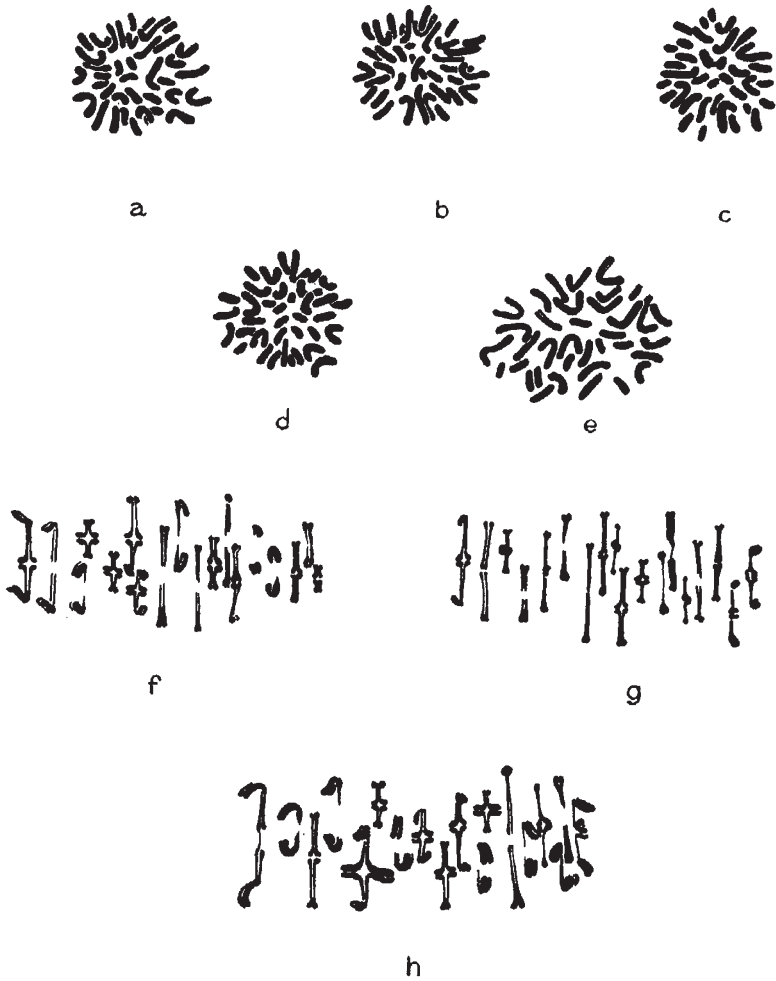|	V	I	i	$2n$
L. terrestris . .	16	6	14	
L. castaneus . .	16	4	16	
L. friendi . .	16	12	8	36
L. festivus . .	14	12	10	
L. rubellus . .	10	18	8	

FIG. 2.—Mitosis : *Lumbricus castaneus*, embryo. (b) *L. rubellus*, embryo. (c) *L. terrestris*, embryo. (d) *L. festivus*, embryo. (e) *L. friendi*, testes.
 Meiosis : MI plates from eggs of (f) *L. terrestris*. (g) *L. rubellus*. (h) *L. castaneus*. All plates × 2150.

There are no difficulties in classification in this genus.   Meiosis in male and female organs occur with great regularity.   The eggs are small as in the pigmented *Allolobophora* species.   The receptaculæ contain generally not more than one egg at MI, except in *L. rubellus* and *L. castaneus* where two metaphase eggs were frequently found. This means that they produce cocoons faster than the others, or their cocoons contain more than one young.   All the species examined are crossbreeders.

Allolobophora.—Seven forms of *Allolobophora* were examined.   *A. terrestris f. typica*, *A. t. longa* and *A. nocturna* are closely related.   They are the only *A*-species with dark pigmentation in England.

	J	V	I and *v*	*i*	2*n*
A. terrestris typica . .	2	2	18	14	
A. t. longa . . .	2	...	18	16	
A. nocturna . . .	2	2	22	10	} 36
A. caliginosa . . .	4	2	22	8	
A. chlorotica . . .	...	2	22	8	
A. icterica (two forms) . .	...	2	16	14	} 32

A. caliginosa has bigger cells and chromosomes than the first three species.

There is no cytological difference between the yellow and the green form of *A. chlorotica*.   The former is probably a gene mutation. (Dr Kalmus has crossed the two forms, but the results have not yet been published.)   *Chlorotica* and *icterica* are very similar cytologically. A specimen of *A. icterica* classified as *A. i. dicystes* could not be distinguished in chromosome shapes from the common type.

All the examined species of *Allolobophora* are obligatory cross-breeders.

Bimastus.—Two species have been examined :—

	V	J	I	*i*	2*n*
B. eiseni	10	4	4	14	32
B. tenuis	21 (V and J)			27 3*x* = 48	

The latter is a triploid.   The size of the chromosomes of *B. tenuis* makes it difficult to distinguish between the V and J chromosomes, but their number in the triploid agrees roughly with what one could expect if it was derived from a chromosome set similar to that of *B. eiseni*.   The two species are frequently found together in the same habitat and are fairly similar in appearance.   But *B. eiseni* has *closely paired setæ* and no spermathecæ.   Spermathecæ are usually absent in the triploid but specimens with one or two pairs are not uncommon.   This
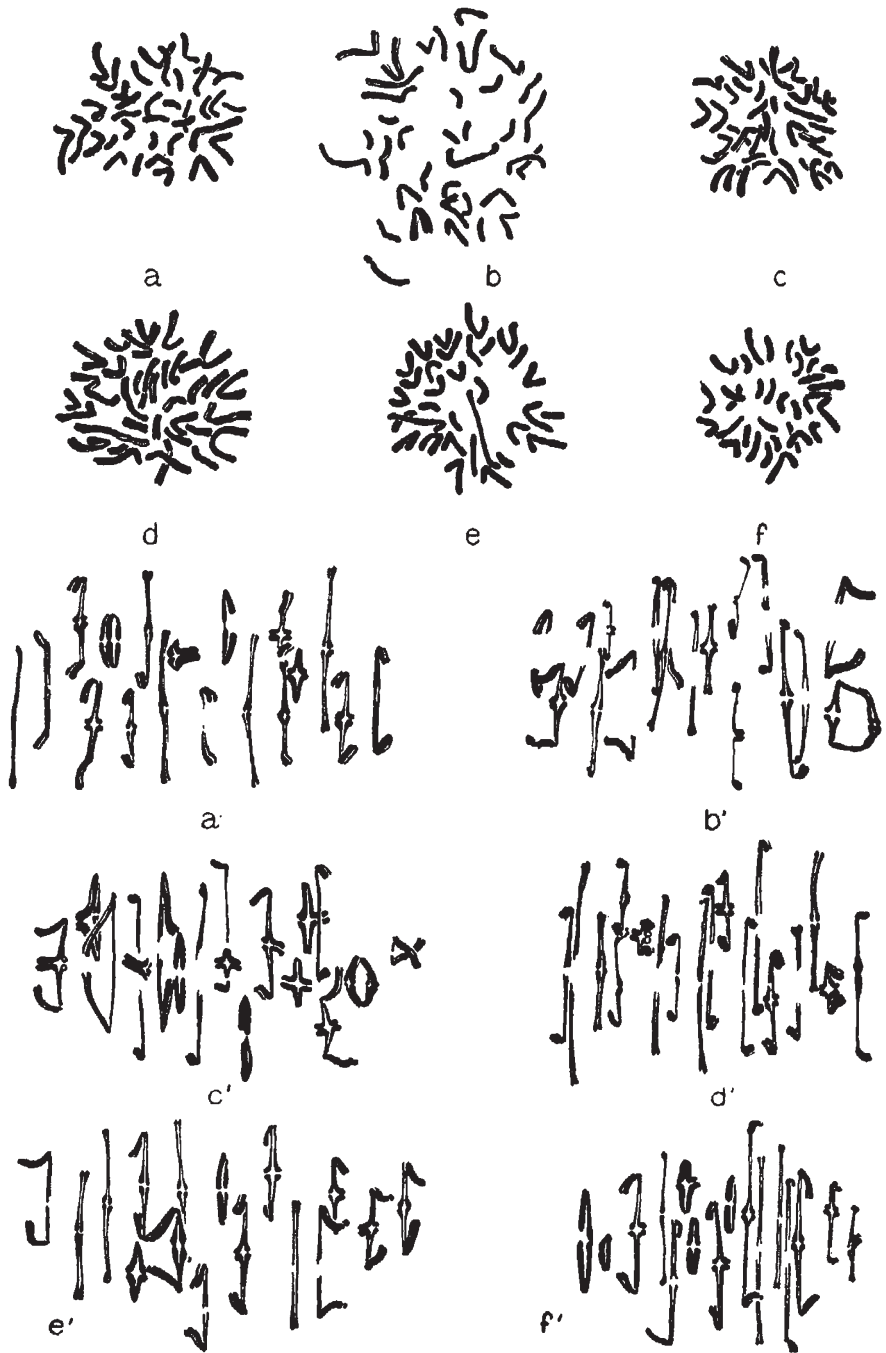
Fig. 3.—Mitoses : (a) *Allolobophora terrestris*, testes.   (b) *A. caliginosa*, embryo.   (c) *A. longa*, embryo.   (d) *A. nocturna*, testes.   (e) *A. chlorotica*, embryo.   (f) *A. icterica*, embryo. All mitotic plates × 2500.

Meiosis :   MI plates from eggs arranged in the same sequence as above (a'-f'). × 1750.

is surprising since the triploid *B. tenuis* has obligatory parthenogenetic reproduction.

Eisenia.—The genus *Eisenia* has two haploid numbers, $n = 11$ and 18. The low-numbered *Eisenia foetida* is the only species which allows a detailed examination of the somatic chromosomes. It has $n = 11$, as found by Foot (1896), probably the lowest number known in *Lumbricidae*.

E. foetida :—

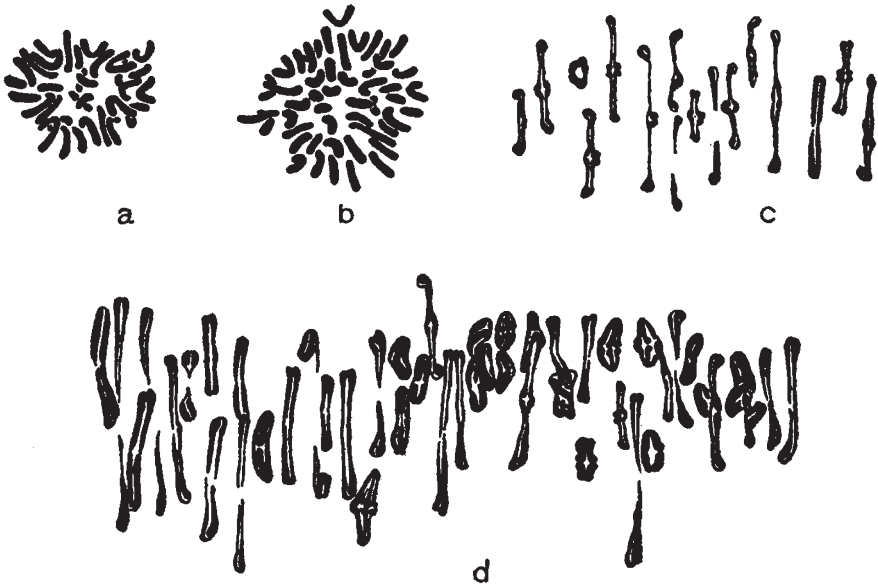| Shapes . .	V	J	V	J	*v*	*v*	*v*	*j*	*j*	*j*	*j*
Arm ratios . .	0·7	0·4	0·7	0·3	0·6	0·9	0·7	0·3	0·4	0·3	0·2
Lengths (μ) . .	7·5	7·0	5·5	5·0	4·5	4·0	3·5	3·5	3·0	3·0	2·3

The measurements refer to late prophase.

FIG. 4.—Mitosis : (a) *Bimastus eiseni*, embryo. (b) *B. tenuis*, embryo. × 2720.
Meiosis : MI plates from eggs of (c) *B. eiseni*, and (d) *B. tenuis*. × 1970.

Since this number deviates from the usual number in the genus, it may be relevant that according to Evans and Cernosvitov (1947), *E. foetida* appears to be the only British E-species with ornamented setæ. It also differs from other species by a considerably higher fertility and in some aspects of embryo development.

Cytologically, the two " varieties " of *E. veneta* differ but little from each other. There are great morphological differences, however,

and it is very doubtful that their relationship is as close as is generally assumed :—

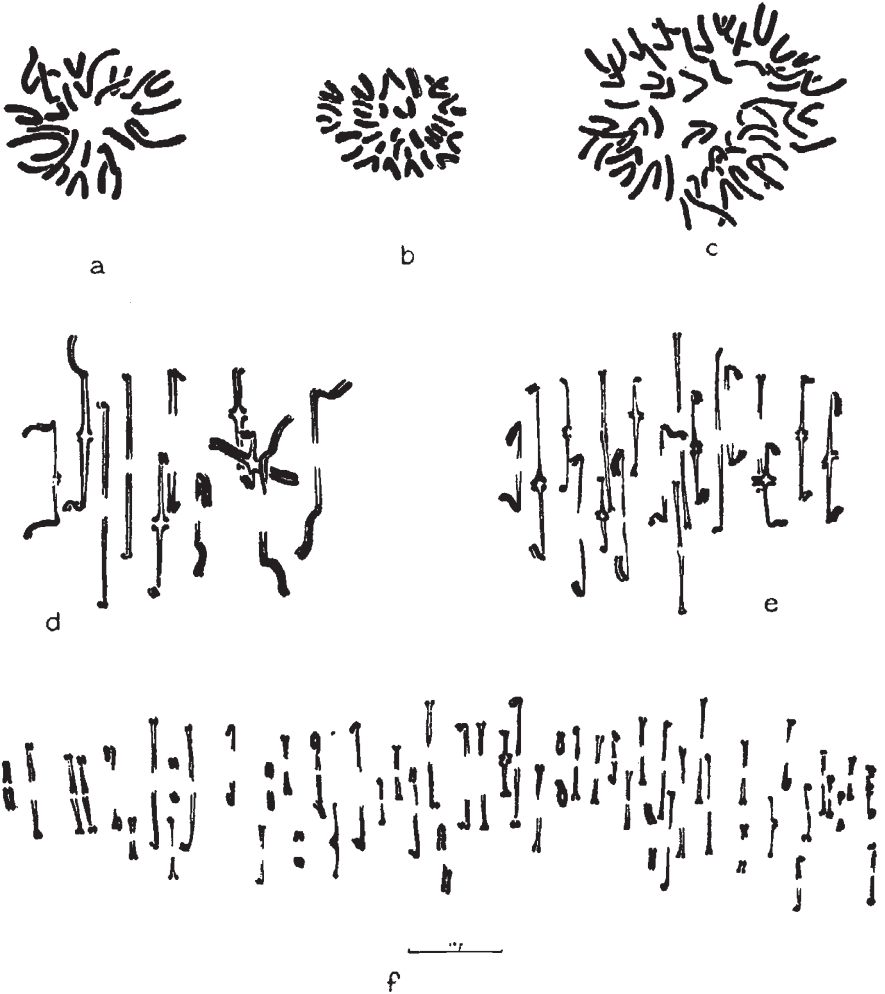|	J	V	v	j	i	$2n$
E. veneta typica . . .	2	2	4	2	26	} 36
E. v. hibernica . . .	8 (V and J)			2	26	

FIG. 5.—Mitosis : (a) *Eisenia foetida*, embryo.   (b) *E. venata typ.*, embryo.   ×1675.
    (c) *E. rosea*, embryo.   ×2310.
      Meiosis : MI plates from eggs of (d) *E. foetida*.   ×2310.   (e) *E. venata*.   ×2310.
    (f) *E. rosea*.   ×1170.

E. rosea has 54 chromosomes. It seems to be related to the small *Allolobophora* species, and to be a triploid with the basic number of 18.

The reproduction is obligatory parthenogenetic. The spermathecæ were never found to contain sperm and most of the specimens were practically male sterile.

The variety *E. r. Macedonica* (Rosa, 1893) has recently been found by Miss B. I. Roots in a garden in East London. Ten specimens examined by me had 53 chromosomes, $3x-1$. It seems quite likely that this loss of a chromosome is the cause of the presence of the lightly coloured glandular papillæ on the clitellum which distinguish the form from *typica* (5 specimens of *typica* collected in the same garden had the usual 54 chromosomes). This variety is previously reported from Chelsea, Kew and Wicken Fen, Cambridgeshire, and its presence in several places in England may be explained as the spreading of a clone. But it is also known from Yugoslavia (Macedonia), Switzerland and Manchoukuo, so that the chromosome loss may have occurred more than once.

Perhaps one could bridge the gap in basic numbers by examining some of the endemic species of *Eisenia*, for instance *E. alpina*. I hoped to obtain this species from Switzerland, but the three specimens received proved to be *E. rosea*, with the same chromosome number as the British type.

Eiseniella.—Two varieties of *E. tetraedra*, *E. t. typica* and *E. t. hercynia*, both had $2n = 72$, with obligatory parthenogenetic reproduction. The species is presumably tetraploid. It is polymorphic and there are only a few other species in the genus. They follow the *venata* group in distribution and may be considered as derived from the latter or a common ancestor. The generic differences have been discussed above, but one should draw attention to the frequent shift in clitellar position, which seems to be connected with limnic and aquatic habitat (Stephenson, 1930).

Dendrobaena.—Here the basic number is 17 :—

					2n
D. mammalis . . .	...	16 (V and J)	18 (I and *i*)	...	34
D. rubida . . .	2V	6*v*	10*j*	16*i*	34
D. subrubicunda . .	...	4 (V and J)	30 (*v* and *j*)	34*i*	68

The latter two are closely related. The tetraploid *subrubicunda* may be of recent origin since it has usually from 1 to 4 quadrivalents at MI in the eggs (Darlington, 1932). Some authors consider *subrubicunda* as a form of *rubida*. They are both widely distributed, often occurring together. *Subrubicunda* is the most common of the two.

Octolasium.—A diploid and a decaploid species of *Octolasium* have been examined :—

O. lacteum . . . 2J   14V   22*i* $2n = 38$

The chromosome shapes of *O. cyaneum* are not sufficiently clear to be analysed, but they are probably like those of *lacteum*. N = 19

is the highest basic number in the family, and the highest somatic number is also found in this genus : *O. cyaneum* with $2n =$ ca. 190. This number varies from egg to egg within 15-20 chromosomes. This variation may, of course, be due to errors in counting such high numbered plates, but considering the high frequency of abnormal divisions (micronuclei) found in somatic tissue, it seems more likely that there is a real variation.
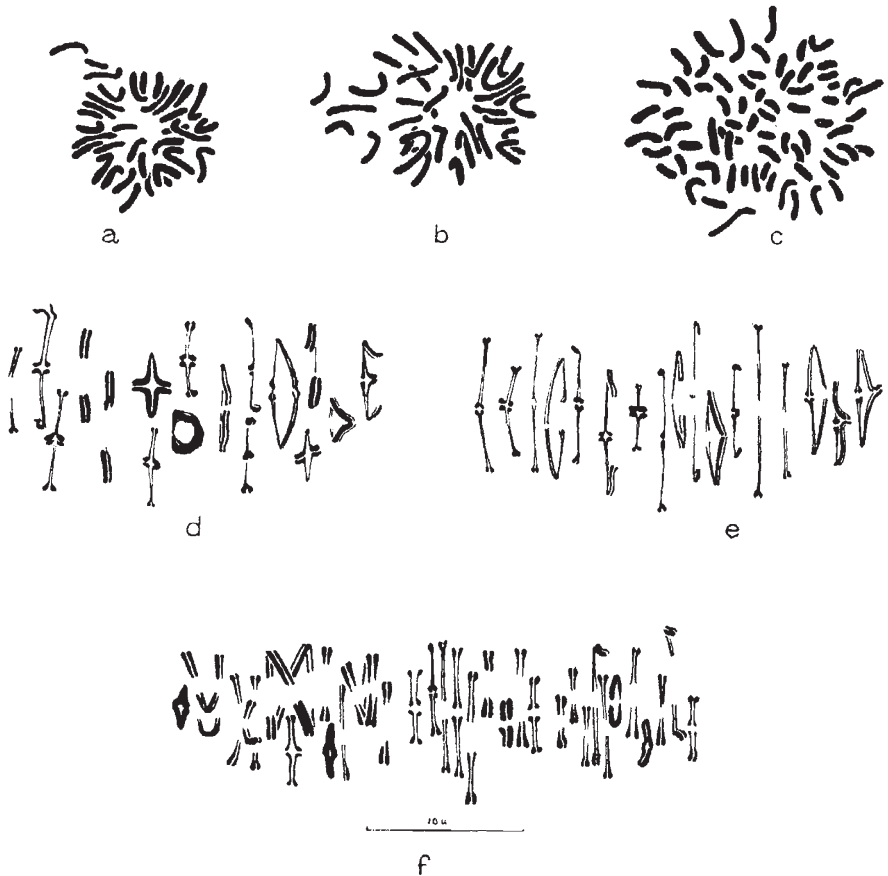

FIG. 6.—Mitosis : (a) *Dendrobaena mammalis*, testes. (b) *D. rubida*, embryo. (c) *D. subrubicunda*, embryo. × 1830.

Meiosis : MI plates from eggs of (d) *D. mammalis*. × 1830. (e) *D. rubida*. × 1830. (f) *D. subrubicunda*. × 1980.

The specific differences between *O. lacteum* and *O. cyaneum* are relatively slight. The latter should thus be considered as a decaploid. Some clones may have numbers nearer the octoploid. Both the examined species have obligatory parthenogenetic reproduction (apomictic) and reproduce with normal speed in isolation. The amount of sperm found in *O. cyaneum* is very small, but the diploid *O. lacteum* may have a fair amount of apparently normal sperm.
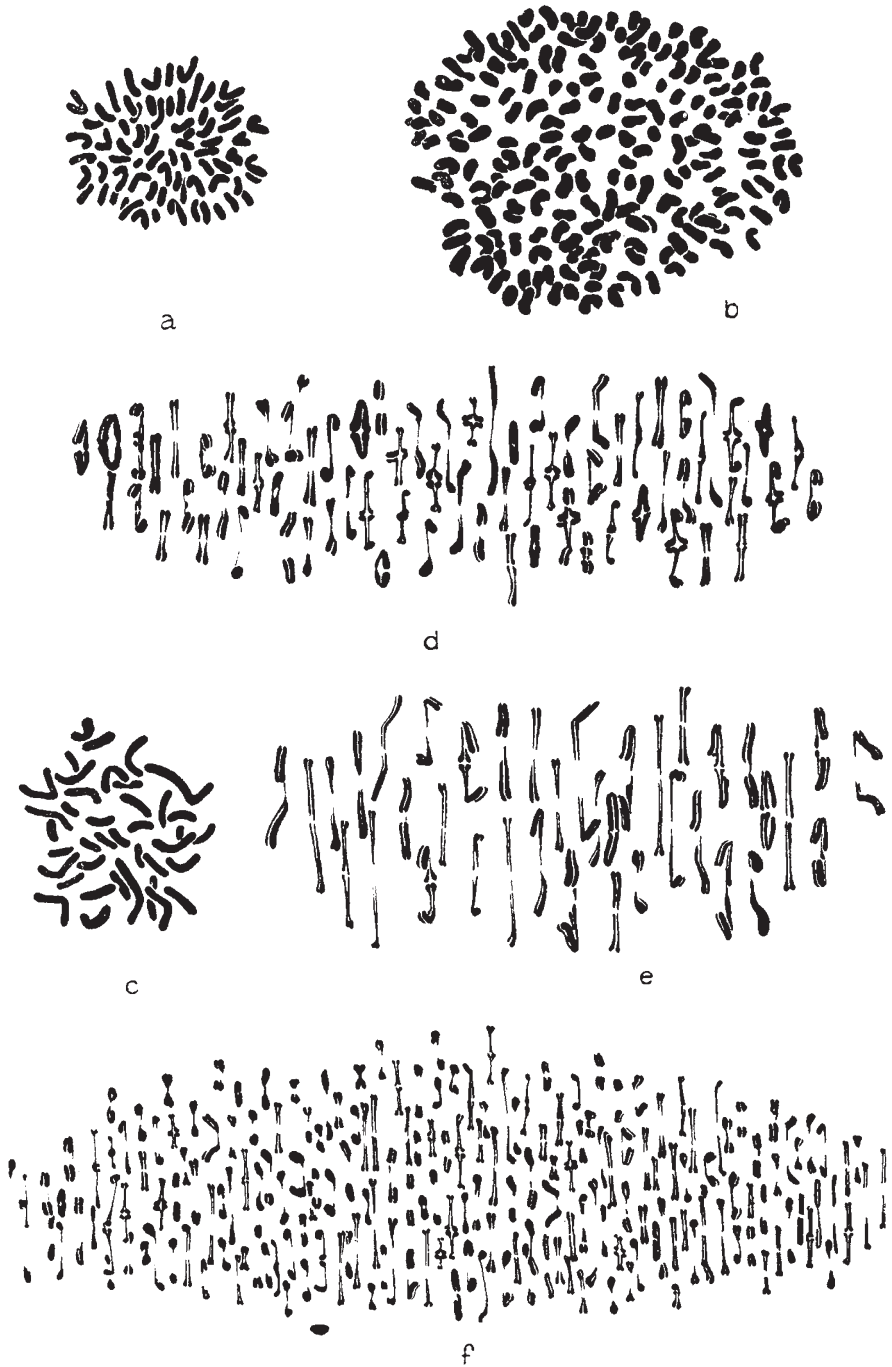
FIG. 7.—Mitosis : (a) *Eiseniella tetraedra typ.*, embryo. (b) *Octolasium cyaneum*, embryo. (c) *O. lacteum*, embryo. ×2500.
  Meiosis : MI plates from eggs of (d) *E. tetraeda*. ×2500. (e) *O. lacteum*. ×2500. (f) *O. cyaneum*. ×1260.

Although both species possess spermathecæ, these are invariably empty. A few specimens of *O. lacteum* received from Switzerland showed identical chromosome conditions to the British ones.

Eophila.—One damaged specimen of *E. oculata* gave no good plates, but the number seemed to be $2n = 32$.

5. SYSTEMATICS

Contrary to the *Megascolicidae* the family *Lumbricidae* may be distinguished cytologically by the absence of late meiotic prophase stages in the ovary and possibly also internal fertilisation. This is connected with the presence of a special receptaculum for the eggs. In analogy, meiosis in the male line takes place in the seminal vesicles. Species where these vesicles are reported to be totally absent need to be re-examined.

Since only one-tenth of the known species of *Lumbricidae* is included in this study, no attempt has been made to alter the commonly accepted classification. But the survey has revealed some cracks in this system and suggested some points of attack for the taxonomist.

Five haploid numbers are recorded in the British species : 11, 16, 17, 18 and 19. *Lumbricus* ($n = 18$) and *Octolasium* ($n = 19$) are both well defined genera. If we combine the haploid numbers from the other genera with the generic affinities discussed above, we get a certain agreement.

But in *Allolobophora* both the chromosome number and external morphology agree in singling out *A. chlorotica* and *A. icterica* as a distinct part of the genus. They have their first dorsal pore in the furrow 4/5, whereas its usual position in the others is further back, in 11/12 or 12/13. They are pigmentless and have three pairs of spermathecæ. Two pairs are the normal in the other species. The " active " part of the clitellum (segments with puberty tubercles) is considerably longer in the 32 than in the 36 group although there is a causal connection between length of clitellum and number of spermathecæ. Anyhow, the clitellum ends further back in *A. chlorotica* and *A. icterica* than in the others.

The close affinity between *Eophila* and *Bimastus* are reflected in their haploid numbers. But it is also remarkable that the examined species of *Eophila* shows affinities with the 32 group of *Allolobophora*. The unpigmented *E. oculata* has first dorsal pore at 4/5, 3 pairs of spermathecæ, closely paired setæ, long clitellum—but only two pairs of seminal vesicles. *A. icterica dicystes*, however, has also only two. In other words, there are no generic differences between them.

With regard to the two haploid numbers in *Eisenia* we have already mentioned that *E. foetida* ($n = 11$) differs considerably from other British *E*-species. The species *E. fasciata* Backlund is only a form of *E. foetida* and has the same chromosome number. The triploid apomict *E. rosea* has the same haploid number as the *veneta* group ($n = 18$),

but shows also great affinities to *Allolobophora caliginosa* ($n = 18$). Of particular importance is the intermediate position of the spermathecal pores, the thickening of the anterior septæ and pigmentation (see further Evans and Guild, 1947 ; Julin, 1949). A relationship between *Eisenia* and *Eiseniella* may find support in their basic numbers. It is suggested that some of the numerous forms of *Eiseniella tetraedra* may differ from each other in degree of ploidy or chromosome loss as in *E. rosea*.

Dendrobaena shows affinities with three genera : *Eisenia, Allolobophora* and *Bimastus*, and has the intermediate haploid number 17.

In order to understand how these haploid numbers have developed we have to consider some general points about the evolution of the *Oligochaeta*.

The chromosome numbers of 14 species of *Oligochaeta* have been reported by earlier workers. Of these only two are from *Lumbricidae*, *E. foetida* ($n = 11$), Foot (1896) and *L. terrestris* ($n = 16$), Calkins (1895). The latter is incorrect.

The *Oligochaeta* have presumably arisen from the aquatic and bisexual *Polychaeta*. The latter are not well studied, but 19 species have been examined, with the highest recorded haploid number of 14. A comparison between the two groups shows that the chromosome numbers in the *Oligochaeta* are on the average higher than in the present *Polychaeta* :—

Haploid numbers	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	—	—	—	—	28
Polychaeta	2	1	5	3		4		1	1		2												
Oligochaeta					1		2	1	1					9	3	11	2						1

It seems reasonable then to assume that there has been a general tendency towards increased chromosome numbers in the terrestrial annelids. The reason for this may be sought in the development of hermaphroditism with the subsequent release of variability in chromosome number following the loss of sex chromosomes.

The lowest numbers reported in the *Oligochaeta* are, apart from *E. foetida* ($n = 11$) :—

	n	
Branchiobdella astaci	8	Wendrowski (1928)
Tubifex bavaricus	10	Oschmann (1914)
T. rivulorum (10x)	$x = 10$	Gathy (1900)
Enchytraeus adriaticus	12	Vejdowsky (1907)

Considering the low number of species examined it seems reasonable to suppose that the haploid number 9 will also be found.

We may thus assume that our diploid species of *Lumbricidae* really are old tetraploids arisen from the basic numbers 8, 9, 10 and 11. The haploid number 11 (*E. foetida*) should then be the most primitive number known in the family, and 16 and 18 be derived by doubling

of 8 and 9.   Seventeen and 19 may have arisen by the loss or gain
of a centromere or from interspecific crosses of 8 and 9 and of 9 and
10, with subsequent doubling as known in many plants.

   If we combine the results of this survey we may attempt to
reconstruct the phylogeny of the genera by the diagram.

   If this be so, the two groups of *Allolobophora* and *Eisenia* may have
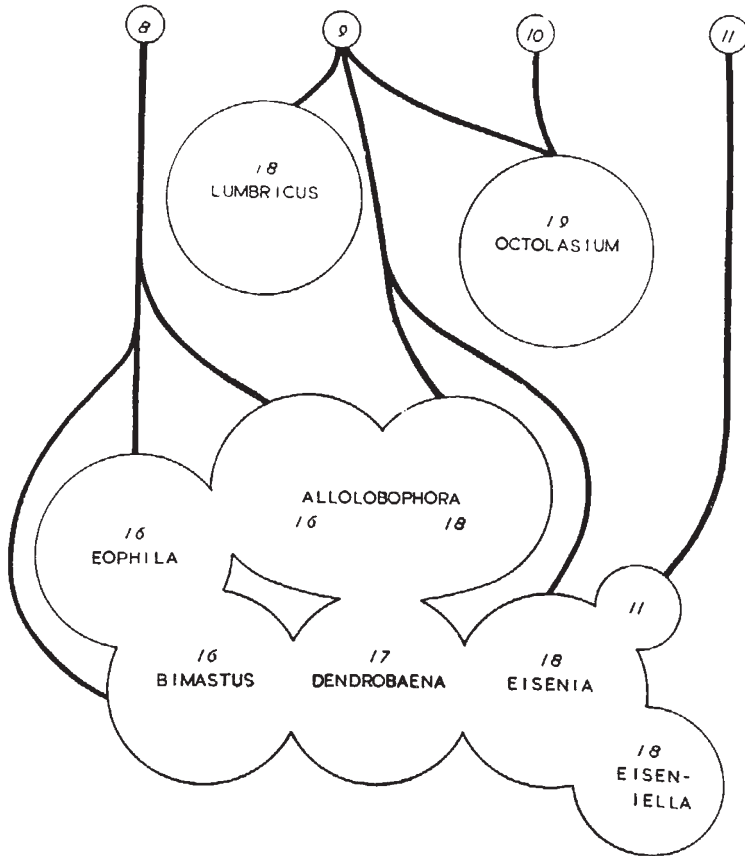evolved independently in more recent times and should thus constitute

FIG. 8.

examples of convergence, *i.e.* development of similar characteristics
irrespectively of ancestry, owing to lack of variety of habitat and
habits.   This phenomenon is regarded by systematists as common in
the *Oligochaeta* (Stephenson, 1930).

   From a practical point of view the present classification is perhaps
convenient, but if we are to take genera as units of common descent,
then *Allolobophora* and *Eisenia* ought to be divided into two separate
groups or new genera.   Only a more comprehensive study can decide
the question of regrouping of the species in " *Helodrilus.*"

6. POLYPLOIDY AND PARTHENOGENESIS IN *LUMBRICIDAE*

Of the 23 species investigated 6 are parthenogenetic and almost certainly polyploid :—

	$3x$	$4x$	$10x$	
Eisenia rosea (two forms) .	54	...	...	Non-sexual, Obligatory Parthenogenesis
Bimastus tenuis . . .	48	...	...	
Eiseniella tetraedra typica .	...	72	...	
Eiseniella tetraedra hercynia .	...	72	...	
Octolasium cyaneum . .	...	...	ca. 190	
Dendrobaena subrubicunda .	...	68	...	Sexual, Facultative Parthenogenesis

This clearly shows that polyploidy goes together with parthenogenetic reproduction in *Lumbricidae*.

Since there are about 220 species of earthworms described, the number of polyploids may be considerable. However, there is reason to believe that there are generic differences with regard to mode of reproduction (Evans and Guild, 1947), *viz.* cross breeding may be obligatory in *Allolobophora* and *Lumbricus*, so that polyploidy may be absent from these large genera.

Obligatory parthenogenetic reproduction found in *Lumbricidae* is apomictic. It differs, however, from the usual type of apomixis by the presence of pseudo-bivalents at MI in the eggs. These bivalents are quadrupartive in structure and show chiasma, although they consist of identical chromatids. This mode of reproduction is confined to polyploids, with the only exception of the diploid *Octolasium lacteum*.

Facultative parthenogenesis, which is also found in diploids, is automictic, *i.e.* the chromosomes pair to form the haploid number of bivalents, cross over and form chiasma. At the MI plate the centromeres divide precociously in parthenogenetic eggs, and upon division the diploid number is restored. Such eggs are frequent in unmated animals (isolated before sexual maturity) and are also common during the cold winter months. Consequently this automictic process differs from other types by the early signs of restitution.

The cytology of parthenogenesis in *Lumbricidae* will be described in detail elsewhere. In regard to morphology, there are some differences between the polyploids and their closest allies amongst the diploids. From Cernosvitov and Evans (1947) and Julin (1949) we get the following information (page 72).

Although the distribution within the limits given here in some cases certainly is left-skew (*Bimastus*), it seems to be a rule that polyploid earthworms are larger and have more segments than their allied diploid forms.

A very close affinity between the first two pairs of species may
be subject to doubt, but the near relationship between the last two
pairs cannot be questioned. In both these cases the species are similar
even in small details, in fact, the only difference apart from those
shown above is that polyploidy seems to have moved the clitellum

	Length (mm.)	Mean	Diam.	Segment no.	Mean
B. eiseni (2x) . . .	30-64	47	2-5	75-111	93
B. tenuis (3x) . .	20-85	52	3	90-120	105
E. venata hib. (2x) . .	23-45	34	3-4	90-120	102
E. rosea (3x) . . .	25-85	55	3-4	120-150	135
D. rubida (2x) . .	25-60	43	3·5	50-100	75
D. subrubicunda (4x) .	40-90	60	4	60-110	85
O. lacteum (2x) . .	35-160	97	4	87-165	126
O. cyaneum (10x) . .	65-180	122	7·5	104-150	127

forwards both in the case of D. subrubicunda and O. cyaneum. In the
latter species the polyploidy is sufficiently high to show an increase
in the diameter of the worm. In some cases polyploid partheno-
genetic species show greater variation in length and segment number
than sexual diploid species. This may be explained by the fact that
the parthenogenetic species mature earlier than the sexual, and the
sampling will thus be biased because of age differences.

The apomictic forms have all preserved their spermathecæ, which
supports the view that selection is frozen in parthenogenetic species.
This is neatly demonstrated in the genus Bimastus ; the triploid
apomict B. tenuis is the only British species with spermathecæ.
Ribaucourt (1896) made a study of variation of pigmentation of
earthworms throughout their vertical range in Switzerland. He
found it to be a general rule that pigmentation increased with
altitude. The only exceptions were the species O. cyaneum and
E. rosea, which were of the same colour throughout their range. This
is not surprising since they are both apomicts.

Vandel (1928) has called attention to the fact that if we have
a bisexual and a parthenogenetic form of a single species they will
usually show a deviating distribution. Usually the parthenogenetic
race is the more northern one (geographical parthenogenesis). In
the most typical cases the parthenogenetic form has been found to
be polyploid.

It is difficult to decide whether this variation in distribution
depends on parthenogenesis or polyploidy. It has been pointed out
(Suomalainen, 1940 ; Vandel, 1940) that the distribution of polyploid
parthenogenetic animals resembles that of polyploid plants. Since
both the animals and the plants occur in colder and drier areas than
the corresponding diploid forms, it seems that it is the polyploidy that

makes them hardier. Parthenogenesis is important as it makes the retention of polyploidy possible, and also favours the spread of the polyploid forms into new areas, since even a single parthenogenetic individual may establish a population there.

Like many diploid species, the polyploid earthworms are all peregrine, *i.e.* they are not restricted to a small area in their distribution. From Michaelson (1900) and Cernosvitov and Evans (1947) it can be seen that they are all successful and widespread species :—

Eisenia rosea typica ($3x$), cosmopolitan, the most common *Eisenia* species.

Bimastus tenuis ($3x$), the most widespread species in the genus.

Dendrobaena subrubicunda ($4x$), the most common species of its genus in Britain, but may be less common than *D. rubida* ($2x$) on the Continent. Recorded from more places all over the world than any other species.

Eiseniella tetraedra typica ($4x$) and *m. Hercynia* ($4x$) are the only representatives studied in the genus. Both are almost cosmopolitan, the latter may be less common ; it is only recorded from two localities in Britain.

Of all the recorded *Octolasium* species, *O. lacteum* ($2x$ apomict) and *O. cyaneum* ($10x$) have the widest distribution. They have been reported from all over the world.

On the whole it can be safely concluded that within their genera the polyploids are all the most successful and widespread species.

7. POLYPLOIDY IN HERMAPHRODITE ANIMALS

The present evidence of polyploidy can be taken to support Muller's arguments for the rarity of polyploidy in animals as opposed to plants. More than half of the higher plants are polyploids (Müntzing, 1936).

Based on the discovery of the X/autosome balance as a means of sex determination in *Drosophila*, Muller (1925) pointed out that in bisexual organisms polyploidy will inevitably upset the sex chromosome mechanism, since it automatically abolishes pairing of dissimilar chromosomes. Thus a tetraploid of an originally XY-XX form will have the composition XXYY-XXXX, and the gametes of the heterogametic sex should be XY due to the X's and Y's forming separate bivalents at meiosis. Pairing between two tetraploids would thus lead to only males and there would be no chance of starting a tetraploid race. Muller further considered that triploidy was a necessary step between diploid and tetraploid forms. In *Drosophila* $3n$ females are fertile and vigorous, and when mated to diploid males they produce, apart from diploids of both sexes, females like themselves and inviable or infertile unbalanced individuals. Thus the number of triploids will tend to decrease in each succeeding generation.

Muller concludes : " If the present theory is correct, it may receive two lines of support from comparative cytology. For amongst groups of plants the sporophytes of which are always dioecious and have the " XY " or " WZ " type of sex determination, there should be

the same lack of evidence of tetraploidy as amongst most animals.*
And amongst groups of animals like earthworms and freshwater snails,
which are normally hermaphroditic, tetraploidy or even higher forms
of polyploidy might occur as readily as amongst plants."

Now what is the evidence for polyploidy in hermaphrodite animals
in general ? White (1940) examined what was known of the Flat-
worms, *Oligochaeta*, Leeches and Pulmonate Molluscs and concluded
that the evidence was only slight and that polyploidy could not have
played anything like the important role it has in plants. This
statement, based on what is certainly very scrappy evidence, seems
safe enough as long as one assumes (i) that cross fertilisation is
obligatory in all species (in plants selfing is very common), and that
(ii) parthenogenesis is absent.

Male gonads do not usually offer good material for chromosome
determinations in the hermaphrodite animal groups, and never of
course give any indication of the mode of reproduction. In order
to answer these questions we must consider only reports describing
oogenesis, as follows :—

(1) In *Turbellaria* the number of species where oogenesis has been examined
is very small :—
Acoela : Only spermatogenesis examined.
Rhabdocoelida : As above, asynapsis reported.
Allocoela : *Bothrioplana semperi* Braun. Parth. : Reisinger (1940).
Tricladida : *Pylocelis nigra*. Parth. : Lepori (1948, 1949).
Dugesia gonocephala Parth., polyploidy (2x, 6x) : *D. benazzii* Lepori Parth.,
    polyploidy (3x, 4x) : Benazzi-Lentati and Nardi (1950).
Polycladida : *Notoplana humilis* Sexual : Kato (1940).
There seems to be no reason to doubt that parthenogenesis and polyploidy
are common in this group.

(2) In *Lumbricidae* cross breeding may be obligatory in the genus *Lumbricus*
and perhaps also in the bigger *Allolobophora* species (Evans and Guild, 1947). But
in other genera, such as *Bimastus*, in species lacking spermathecæ, self fertilisation
may occur unless parthenogenesis (with or without pseudofertilisation), is obligatory.
Facultative automixis and apomixis have been established in a number of species
(Muldal, 1949). It is unlikely that parthenogenesis and polyploidy should be
restricted to only one family of the *Oligochaeta*.

(3) In *Hirundinea* there are only 4 accounts of oogenesis (in Makino's list),
all apparently with sexual reproduction. Two species have $n = 8$, one has $n = 13$
and one $n = 16$. The latter may be a tetraploid, but the data are too meagre to
allow any conclusion.

(4) In Pulmonate Molluscs there are a lot of counts from spermatogenesis
(after 1930), but only in three instances has oogenesis been examined. These
reports give the haploid numbers 18, 27 and 31. In this group there are probably

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* However, there are some examples of natural polyploidy in dioecious plants :

Antennaria carpathica	. 3x	*Rumex dioica*	. . 6x	*Salix aurita* .	.	. 4x
Elodea canadensis .	. 4x	*Urtica dioica*	. . 4x	*Salix cinerea* .	.	. 4x
Fragaria elatior	. 6x	*Salix lucida* .	. . 4x	*Salix Andersoniana* .		. 6x

Westergaard (1940) produced a stable tetraploid strain of the dioecious *Melandrium
album*, and criticised Muller's theory. The diploid XY-XX system was transferred to the
stable XXYY-XXXX system. This is probably an example of strong male determining
power of the Y-chromosome.

great technical difficulties shown by the conflicting reports from spermatogenesis. The value of Perrot's work is greatly reduced by the absence of information on the modes of reproduction.   His report of sex chromosomes in 5 species seems doubtful on *a priori* grounds, and is best explained by the work of Husted and Burch (1946) who reported the presence of 1-4 extra chromosomes in different strains of *Triodopsis fraudulenta*.   Selfing or parthenogenesis is reported in very old literature, but new evidence must be put forward.   It is clear that a thorough survey of breeding systems is necessary for the whole group of Pulmonate Molluscs.

From this survey it must be quite clear that there is no reason for believing that (i) cross breeding is obligatory, and (ii) that parthenogenesis is rare in hermaphrodite animals.   White's conclusion that the evidence of polyploidy in hermaphroditic groups is only slight, does not seem to have a firm basis.

8. SUMMARY

1. The chromosome number of 30 species and forms of British *Lumbricidae* are given.

2. The basic numbers 11, 16, 17, 18 and 19 have been found in the family.   Four of these have not been reported previously.   These numbers confirm generally the systematic division of the group.

3. Polyploidy is reported from 5 genera, including triploidy, tetraploidy and decaploidy.

4. All the polyploids show parthenogenesis, a method of reproduction not previously shown in *Oligochaeta*.   In all but one the parthenogenesis is of the obligatory diploid type.   One diploid also had this mode of reproduction.

5. *Dendrobaena subrubicunda*, a tetraploid, like many diploid species, has facultative parthenogenesis, but is undoubtedly a case where polyploidy and sexual reproduction occur side by side.

6. All obligatorily parthenogenetic species are (i) polyploid, except one, (ii) peregrine, *i.e.* non-endemic, (iii) larger than the nearest diploids and (iv) relatively invariable.   It is suggested that these four characteristics are associated with parthenogenesis.

7. A survey of hermaphrodite animal groups indicates that parthenogenesis and polyploidy may be not infrequent.   Also that in them, as in dioecious groups, polyploidy is associated with parthenogenesis.

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