

CHROMOSOME NUMBERS OF SOME EQUISETUM SPECIES FROM THE NETHERLANDS

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EQUISETUM Linn. is the sole surviving genus of a "microphyllous" group—the *Equisetineae*—the members of which formed a conspicuous element in the vegetation of the earth during the Carboniferous period. Prior to MANTON's (1950) exhaustive work, exceedingly conflicting data were found in the literature with regard to the chromosome numbers of the various species. At present this genus is one of the few pteridophytic genera which have been thoroughly worked out cytologically. So far, out of a total of about 25 recognized species (at an earlier date BAKER (1887) described only 20 species) 18 species (see Table II) have been studied (cf. MANTON, 1950; NINAN, 1955 and MEHRA et BIR, 1959). Significant enough is the fact that all these species have identical chromosome number ($n = 108$), and the question that faces us at present is how this high number ($n = 108$) has been arisen. For this reason a critical and extensive study of representatives of this group obtained from various geographic regions of the world is highly desirable. The present paper is a sequel to a previous communication from this laboratory and deals with cytological observations made on four species occurring in the Netherlands.

MATERIAL AND METHOD

The Netherlands are very rich in "horsetails", and as many as 10 species are reported to occur in this country (cf. VAN OOSTSTROOM, 1948). Cytological material of only four of the latter, viz. *E. arvense* L., *E. palustre* L., *E. fluviatile* L. (sub-genus *Equisetum*) and *E. trachyodon* A. Br. (subgenus *Hippochaete*) was available for study. The distribution data with regard to these species are based on VAN OOSTSTROOM's (*loc. cit.*) account of the Pteridophytes in Flora Neerlandica I.

In all cases the young to medium-sized cones were fixed in 1:3 acetic alcohol and preserved in the latter. Table I gives exact sources of the cytological material.

The fixed material, along with pressed specimens from exactly the same localities, were kindly sent to me by Dr. K. U. Kramer. In the usual way aceto-carmines squashes were made at Amritsar in April, 1959, i.e. about nine to ten months after the fixation. The

¹⁾ The present study has been made possible through the kind help of Dr. K. U. Kramer (Utrecht), who supplied the fixed material as well as the herbarium specimens.

drawings have been made from temporary preparations, because many of the permanent proved to be distorted. The degree of fertility of the spores was estimated after the spores had been stained with acetocarmine and had been mounted in 50 % glycerine.

TABLE 1

Name of the species	Locality	Habitat	Date of Collection	Collector
<i>E. arvense</i> L.	De Bilt (Prov. Utrecht)	Grassy, sandy soil	22nd June, 1958	Dr. K. U. Kramer
<i>E. palustre</i> L.	Vechten, S. E. of Utrecht	Shallow boggy ditch, ca. 20 cm water	22nd Juni, 1958	Dr. K. U. Kramer
<i>E. fluviatile</i> L.	Veenendaal (Prov. Utrecht)	Swampy grassland	16th June, 1958	Dr. K. U. Kramer
<i>E. trachyodon</i> A.Br.	Near Asperen Neder-Betuwe (Prov. Gelderland)	Swampy spot, flooded in winter	13th May, 1958	Dr. J. C. Lindeman

OBSERVATIONS

E. arvense, *E. palustre* and *E. fluviatile* occur throughout the Netherlands, the last-mentioned species, however, is not common in the west and south-west. *E. arvense* is a weed of waste places and road sides, while the two other species prefer moist habitats.

All three species are generally distributed in the North Temperate and Arctic zones of both hemispheres, but *E. palustre* is rare in south Europe, and *E. fluviatile* flourishes only in ponds and lakes (cf. BAKER, *loc. cit.*). *E. arvense* is a polymorphic species, and from Michigan state (U.S.A.) alone six forms have been recognized (cf. BILLINGTON, 1952). *E. arvense* L. var. *boreale* (Bong) Ledeb. (= *E. arvense* L. ssp. *boreale* (Bong) Löve) is Arctic circumpolar in distribution.

As pointed out in an earlier communication (MEHRA et BIR, *loc. cit.*) the size and configuration of the bivalents is very variable. The chromosomes are slightly brittle and may break during squashing. The resulting fragments can easily be mistaken for small individual bivalents (cf. Fig. 2). Therefore, for exact counting, a large number of cells have been analysed in each case. In all three species, *E. arvense*, *E. fluviatile* and *E. palustre*, the haploid chromosome number is $n = 108$ (Figs. 1-2). In Fig. 2, which represents a spore mother-cell of *E. fluviatile*, in addition to the 108 bivalents two small, mechanically caused fragments are present. Such fragments usually lie near the respective bivalents and can easily be relegated to them. The spore mother-cell at prophase or diakinesis or even at metaphase usually shows 1-3 conspicuous nucleoli, one being large and generally vacuolated in the centre, while the remaining ones are small and almost equal in size. The latter are generally circular in outline.

The course of meiosis is perfectly normal, resulting in well developed spores. The mature spores contain numerous chloroplasts and fully

developed elaters. There are no traces of "plasmodial residue" intermixed with the spores.

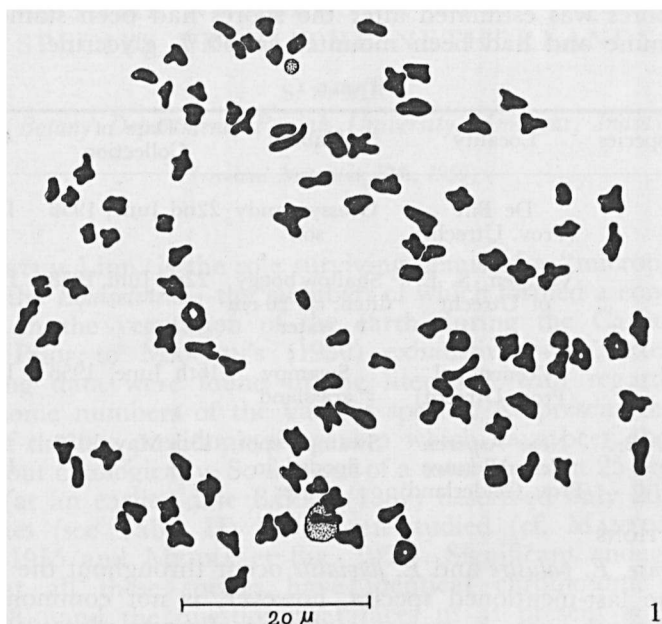


Fig. 1. A spore mother-cell of *E. arvense* showing $n = 108$.

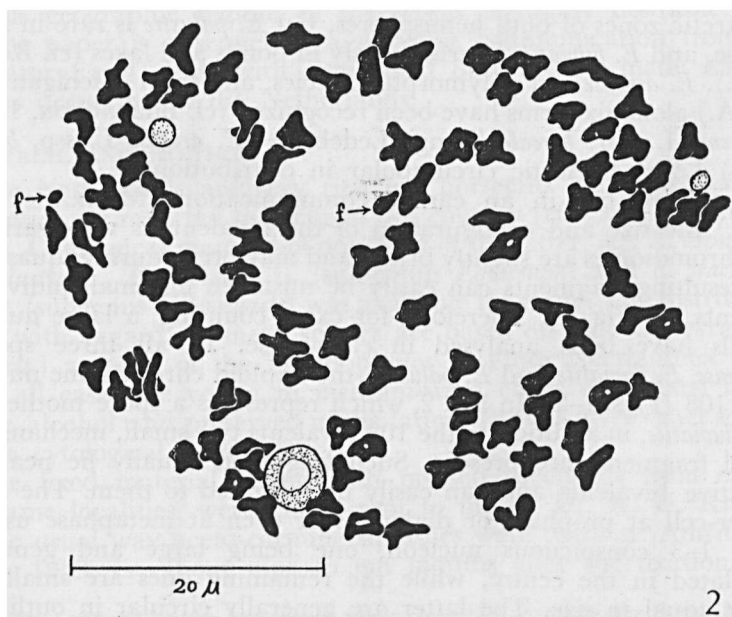


Fig. 2. Diakinesis in a spore mother-cell of *E. fluviatile* showing 108 bivalents and 2 mechanically caused fragments marked "f".

E. trachyodon A. Br.

This species has a comparatively restricted distribution; according to BAKER (*loc. cit.*) it is found in France, Germany, Ireland and Scotland. In the Netherlands it has recently been discovered, and is extremely rare (VAN OOSTSTROOM en REICHGELT, 1960). It was collected by Dr. J. C. Lindeman from a single station near Asperen,

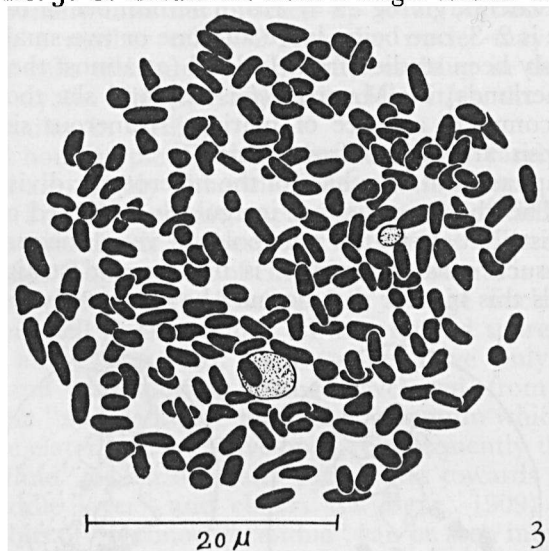


Fig. 3. A spore mother-cell of *E. trachyodon* showing 216 univalents.

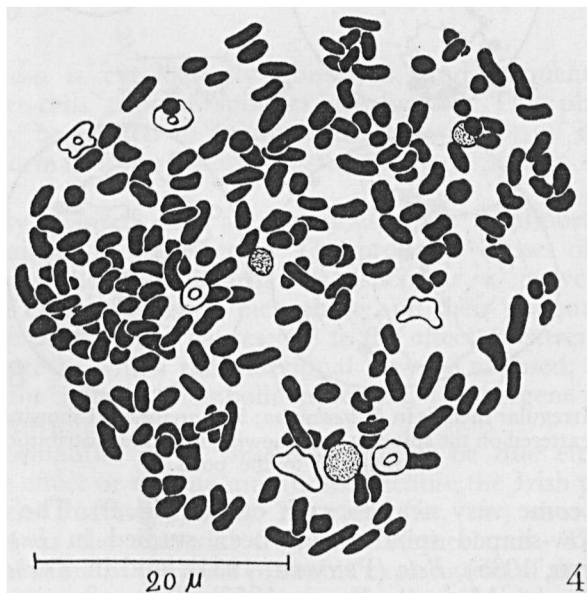
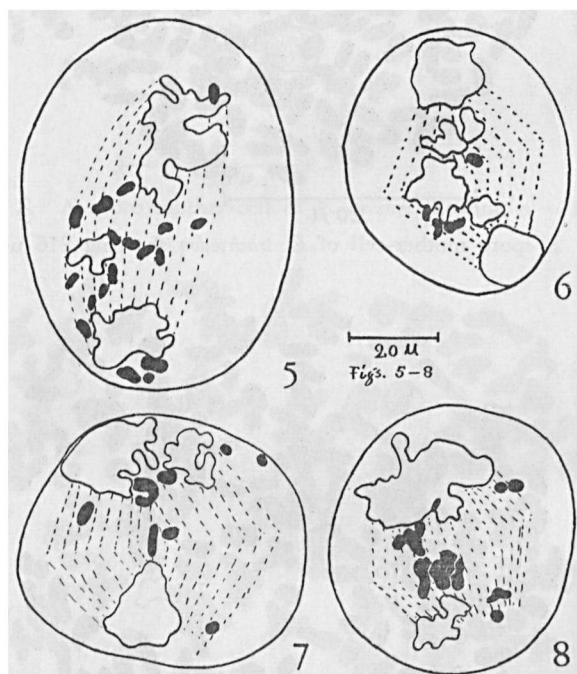


Fig. 4. Another cell of *E. trachyodon* with 204 univalents and 6 bivalents, thus giving $2n = 216$.

Neder-Betuwe (see Table I). It flourishes in swampy places which are usually flooded in winter.

Cytologically this species is rather interesting. Meiosis is exceedingly irregular, at diakinesis in the majority of the spore mother-cells there is a complete failure of pairing, and 216 univalents have been counted (Fig. 3). However, one spore mother-cell (Fig. 4) showed 6 bivalents and 204 univalents, giving $2n = 216$. The number of nucleoli present at this stage is 2-3, one being large and one or two small. This species has previously been studied from Ireland (at almost the same latitude as the Netherlands) by MANTON (*loc. cit.*) and she too has reported an almost complete absence of pairing. Numerous similar cases of total asynapsis are known from ferns.

The metaphase and anaphase of the heterotypic division are highly abnormal. The chromosomes are irregularly scattered on the spindle, and their distribution to the two poles is very unequal (Figs. 5-8). In most of such cells the spindle is bipolar and straight. However, in a few cells this bipolar spindle may be so strongly curved that the



Figs. 5-8. Irregular meiosis in *E. trachyodon*; 5-6, anaphase I showing chromosomes irregularly scattered on the spindle; 7-8, showing irregular distribution of chromatin material to the poles.

two poles come very near to each other (Fig. 9). The organization of such bow-shaped spindles has been studied in *Impatiens pallida* Nutt. (SMITH, 1935), *Beta* (PRYWER, 1931), and in *Trichomanes insignis* v. d. B. forma β (MEHRA et SINGH, 1957). Quite often in some mother cells a tripolar spindle is formed, and "spindle fibers" are developed.

between the three poles (Fig. 10). OKABE (1929) has also reported tripolar spindles in spore mother-cells of *Psilotum nudum*. But in certain cells the connecting fibers are not developed between each pair of nuclei. Therefore, while the chromosomes are distributed on two sides, no trace of them is evident on the third (Fig. 11). Spore mother-cells with this type of spindle are of common occurrence. Meiosis occasionally does not proceed up to or beyond anaphase I, and so within a mother cell 1-2 irregular nuclei may be formed. The daughter cells ultimately round off, resulting in the formation of monads or dyads. The interkinetic nuclei within the mother cells are rarely circular in outline.

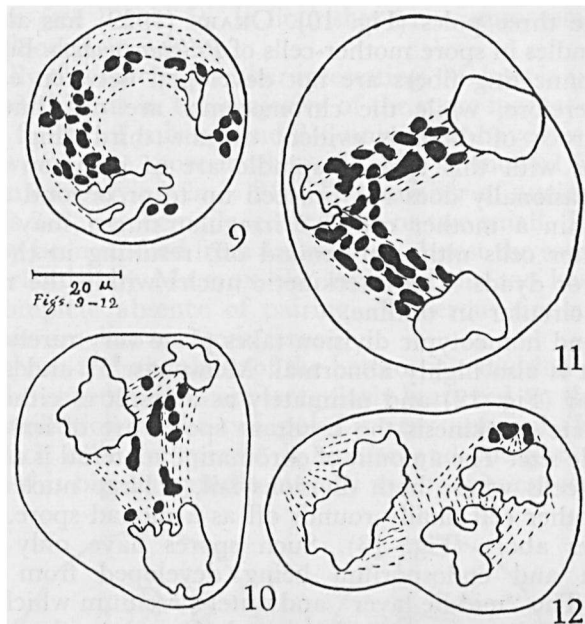
The second homeotypic division takes place very rarely, and when it occurs, it is also highly abnormal. Metaphase II and anaphase II are irregular (Fig. 12) and ultimately as a result of either failure of or incomplete cytokinesis the resultant spores are of irregular shape and variable size. The amount of chromatine material is also variable. In mother cells where both divisions fail, a large nucleus is found, and the mother cell finally rounds off as a monad spore. Ultimately these spores abort (Fig. 13). Such spores have only two coats, exosporium and endosporium being developed from the spore-protoplast. The "middle layer" and outer perenium which ultimately constitutes the elaters are not developed. Consequently the nucleated "plasmodial fluid" which normally contributes towards the development of "middle layer" and elaters (cf. BEER, 1909) is not fully utilized, and bits of "plasmodial residue" can be seen intermixed with sterile spores. In normally reproducing species of *Equisetum* no such "residue" is mixed up with the spores.

DISCUSSION

E. trachyodon is cytologically abnormal, and frequently in the spore mother-cells tripolar spindles are formed. This phenomenon may possibly be related to an abnormal constitution of the species, as in all normal forms characteristic bipolar spindles have been observed.

As already pointed out, at diakinesis there is almost complete failure of pairing in this species. The probable causes of asynapsis and the consequential disturbances (such as a curved spindle, scattering of chromosomes at metaphase and their unequal distribution) may be due to three causes; (1) to the effect of extreme changes of temperature to which the individual may be exposed, and which may upset the normal metabolic processes, (2) to gene mutations, or (3) to hybridity.

The abnormalities in *E. trachyodon* cannot be due either to the temperature effect or to gene mutations, because the Irish populations of this species investigated by MANTON (*loc. cit.*) showed similar irregularities as the material from the Netherlands. Therefore, a hybrid origin remains the most probable explanation of the abnormalities. The pairing at diakinesis is practically nil in the majority of the cells. This suggests that parents with different genomic constitution



Figs. 9-12. Irregular meiosis in *E. trachyodon*; 9. shows a bow-shaped spindle with some chromosomes scattered all over it; 10. tripolar spindle with fibres developed between the three poles; 11. tripolar spindle showing the absence of "spindle fibres" on one side; 12. homeotypic division, metaphase II and anaphase II.

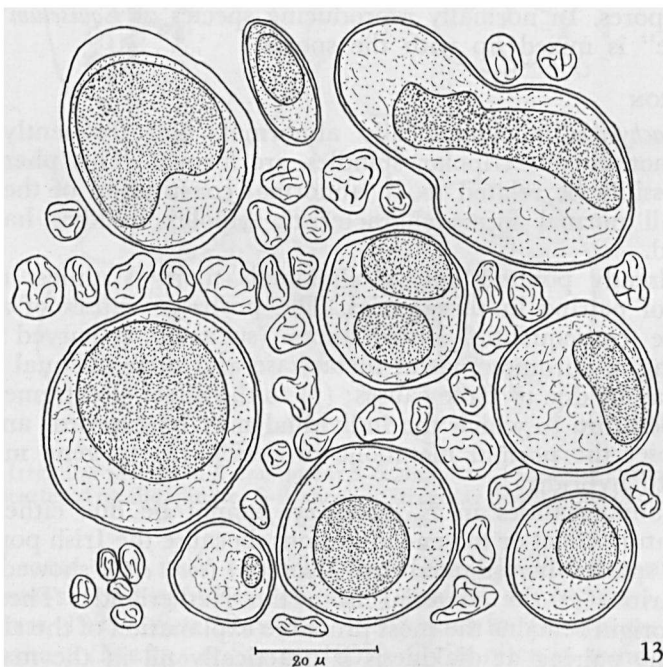


Fig. 13. Abnormal and abortive spores of *E. trachyodon*, fragments of "plasmodial residue" are seen intermixed with these.

may have been involved in the origin of this species, and that the chromosomal complements contributed by the two parents may have been totally non-homologous.

The hybrid nature of *E. trachyodon* has been suspected since long, and BAKER (*loc. cit.*) spoke of this species as "midway between *E. hyemale* and *E. variegatum*". According to MANTON (*loc. cit.*), a relationship of *E. trachyodon* with the south European *E. ramosissimum* has sometimes been suggested. In the opinion of the author the latter species differs morphologically so strongly from *E. trachyodon* that it cannot be regarded as one of the parents. From the distribution map given by MANTON (*loc. cit.* p. 227) it is clear that *E. trachyodon* is well distributed in Ireland, though in the rest of Europe it is rare. So far, this species has been cytologically investigated from two different sources, Ireland (MANTON, *loc. cit.*) and the Netherlands (present report), and from both the places the hybrid nature of the species is evident.

The question that arises next, is how this hybrid has become so widely distributed. It cannot be distributed by spores, because of the sterility of the latter. Only two possibilities suggest themselves, viz. (1) either the hybrids have arisen independently in each locality, or (2) the distribution from one common source has taken place in a remote past by vegetative means, *i.e.* by means of rhizomes. A study of distributional data (cf. BAKER, *loc. cit.* and MANTON, *loc. cit.*) of *E. hiemale*¹), *E. variegatum* and *E. trachyodon* shows that the areas of these species overlap. So it may have originated many times and at many places. It is also possible that once having arisen by hybridization it has propagated vegetatively through rhizomes which are known to be very hardy and resistant in the genus *Equisetum*.

Before discussing the evolutionary status of *Equisetum* in the light of cytological evidence, it would be proper to summarize the available data which are presented in Table II.

Löve (personal communication), who has worked out the somatic chromosome numbers of a large number of Arctic and N. American species of *Equisetum* (cf. Table II), has confirmed the earlier view of MANTON (*loc. cit.* p. 215-218) that there are some differences in the size of the chromosomes in the two sub-genera viz. *Equisetum* and *Hippochaete*. Originally the division of *Equisetum* into two sub-genera was proposed by MILDE (1867) because of the structure of the stomata, and since then this division has been followed. Recently ROTHMALER (1944) raised these quite distinct *Equisetum* sections to generic rank. The difference in relative chromosome size (meiotic and somatic) of two the sub-genera gives some support to ROTHMALER's (*loc. cit.*) proposal.

A perusal of Table II shows that thirteen species have been worked out from two or more geographically different regions and that all

¹) There is some difference in the spelling of the specific name. BAKER (*loc. cit.*) BILLINGTON (1952) and HAUKE (1958) write it as *hyemale*. The spellings used in this paper has been adopted from the "Index Londinensis" Vol. III, 1930. Oxford.

TABLE II
Resume of chromosome numbers in *Equisetum*¹⁾

Name of the species	Source	Chromosome number		Repro- duction	Author
		n	2n		
<i>sub-genus Eu-equisetum:</i>					
<i>E. arvense</i> L.	England	ca. 108	—	Normal	Manton (1950)
	Netherlands	108	—	Normal	Present report
<i>E. arvense</i> L.	Iceland	—	216	—	Löve (unpublished)
<i>ssp. boreale</i> (Bong) Löve	N. America	—	216	—	Löve (unpublished)
<i>E. maximum</i> Lam.	England	ca. 108	—	Normal	Manton (1950)
(= <i>E. telmateia</i> Ehrh.)	N. America	—	216	—	Löve (unpublished)
<i>E. sylvaticum</i> L.	England	ca. 108	—	Normal	Manton (1950)
	Iceland	—	216	—	Löve (unpublished)
	N. America	—	216	—	Löve (unpublished)
<i>E. pratense</i> Ehrh.	Hort.	ca. 108	—	Normal	Manton (1950)
	Iceland	—	216	—	Löve (unpublished)
	N. America	—	216	—	Löve (unpublished)
<i>E. palustre</i> L.	England	ca. 108	—	Normal	Manton (1950)
	Netherlands	108	—	Normal	Present report
	Iceland	—	216	—	Löve (unpublished)
	N. America	—	216	—	Löve (unpublished)
<i>E. limosum</i> L.	England	ca. 108	—	Normal	Manton (1950)
(= <i>E. fluviatile</i> L.)	Netherlands	108	—	Normal	Present report
	Iceland	—	216	—	Löve (unpublished)
	N. America	—	216	—	Löve (unpublished)
<i>E. diffusum</i> D. Don	Darjeeling,				
	E. Himalayas	108	—	Normal	Mehra et Bir (1959)
	Mussoorie,				
	W. Himalayas	108	—	Normal	Mehra et Bir (1959)
<i>E. litorale</i> Kuhn	Ireland	Irregular meiosis	—	Sterile hybrid	Manton (1950)
<i>sub-genus Hippochaete:</i>					
<i>E. robustum</i> A. Br.	Botanic Garden	ca. 108	—	Normal	Manton (1950)
<i>E. ramosissimum</i> Desf.	Italy	ca. 108	—	Normal	Manton (1950)
<i>E. ramosissimum</i> Desf.	Darjeeling,	108	—	Normal	Mehra et Bir (1959)
var. <i>altissimum</i> A. Br.	E. Himalayas				
	Mussoorie,	108	—	Normal	Mehra et Bir (1959)
	W. Himalayas				

¹⁾ The information about chromosome numbers of Icelandic and N. American species of the genus referred to here, has very kindly been supplied by Dr. Askel Löve (Montreal). However, nothing is known about the nature of spores and reproduction.

TABLE II (continued)

Name of the species	Source	Chromosome number		Reproduction	Author
		n	2n		
<i>E. hiemale</i> L. ¹⁾	England	ca. 108	—	Normal	Manton (1950)
	Iceland	—	216	—	Löve (unpublished)
	N. America	—	216	—	Löve (unpublished)
<i>E. scirpoides</i> ¹⁾ Michx.	Norway	ca. 108	—	Normal	Manton (1950)
	N. America	—	216	—	Löve (unpublished)
<i>E. variegatum</i> ¹⁾ Schleich	British Isles	ca. 108	—	Normal	Manton (1950)
	Iceland	—	216	—	Löve (unpublished)
	N. America	—	216	—	Löve (unpublished)
<i>E. trachyodon</i> A. Br.	Ireland	Irregular meiosis	—	Sterile hybrid	Manton (1950)
	Netherlands	216r	—	Sterile hybrid	Present report
<i>E. Moorei</i> Newm.	Ireland	Irregular meiosis	—	Sterile hybrid	Manton (1950)
<i>E. debile</i> Roxb.	South India	108	—	Normal	Ninan (1955)
	North India	108	—	Normal	Mehra et Bir (1959)
<i>E. laevigatum</i> A. Br.	Michigan, N. America	Irregular meiosis	—	Sterile hybrid	Hauke (1958)

¹⁾ Askel Löve (personal communication) following ROTHMALER (1944) prefers the names *Hippochaete hiemalis*, *H. scirpoides* and *H. variegata* respectively for these species.

possess identical chromosome numbers. Among 17 species worked out so far, 4 hybrids have been detected. This is a fairly high number, and it speaks for the evolutionary activity still present in this very ancient group. The presence of high chromosome numbers (which is decidedly not primitive) is probably an indication of high antiquity. *Equisetum* is surviving from the Carboniferous period to the present day with unbroken continuity, and it is therefore a relic of the past. It seems possible that during this process all the members with lower chromosome numbers have become extinct. From what basic number the present number may have been evolved, and what is the grade of ploidy in the genus *Equisetum*, are two important questions that still remain unanswered.

SUMMARY

The cytology of four Netherlandic species of *Equisetum* has been worked out. *E. arvense*, *E. fluviatile* and *E. palustre* are normal and show $n = 108$, while *E. trachyodon* is almost a totally asynaptic species with $2n = 216$. The results are in complete agreement with those obtained by Manton (1950) for these species. In *E. trachyodon* the course of meiosis has been critically examined, and it is concluded that this is a hybrid species as has already been suspected by earlier workers. The present-day evolutionary status of the genus has been discussed in the light of cumulative cytological evidence.

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* Not consulted in original.