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CHROMOSOME STUDIES IN THE GENUS SCIRPUS L., SECTION SCHOENOPLECTUS BENTH. ET HOOK., IN THE NETHERLANDS

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INTRODUCTION

As a part of a study of the specific delimitation within the section *Schoenoplectus* of the genus *Scirpus* the karyological characteristics are investigated. Owing to the special character of the karyological investigations the results are published here separately.

As is to be learned from the rather scarce literature with respect to the karyology of the genus *Scirpus*, the results of chromosome studies should not be overestimated for the specific delimitation. The absence of distinct series of polyploids makes even an interpretation of karyological results in terms of specific interrelationship within the whole family of *Cyperaceae* difficult and rather hazardous, as may be derived from the studies of DAVIES (1956), HEILBORN (1924) and LÖVE, LÖVE and RAYMOND (1957) in the genus *Carex*. On the other hand, these studies clearly illustrate the occurrence of many smaller differences in chromosome number in the *Cyperaceae*, which may often support taxonomical interpretations within this family.

The difficulties with regard to the karyology of the genus *Scirpus* are pronounced in the section *Schoenoplectus*, as is shown in a survey of the literature, summarized in Table I. It must be emphasized that the names from Table I may be used for different taxa in different parts of the world. In addition, it may happen that taxa which do not occur in Europe, in our opinion, should be placed under the section *Schoenoplectus*, though in literature they are considered to belong to other sections. Such taxa may be included in Table I.

The seven species, mentioned in Table I, can be divided into two groups based on chromosome counts, respectively with 40–42 and 76–78 somatic chromosomes. These numbers cannot be arranged into a series of polyploids. The variation within each group is very small and it is found that four species (*S. lacustris*, *S. tabernaemontani*, *S. validus* and *S. mucronatus*) have exactly the same number. The statement of HÅKANSSON (1928): "Wie man sieht, sind die Chromosomenzahlen der *Scirpus*-Arten sehr schwankend", has to be regarded as valid for the genus *Scirpus* in general. However, HÅKANSSON studied only two representatives of the section *Schoenoplectus* which he rightly mentioned "einander sehr nahestehend". Even when working from the conception of narrow specific interrelationship within this section, the slight

TABLE I

Chromosome numbers in the genus *Scirpus*, section *Schoenoplectus*, as recorded in literature.

Species	2n =	Authors	Origin of materials
S. lacustris L.	42	BAKKER 1954	Netherlands
	42	HÅKANSSON 1928	Sweden
	42	KOSTRIOUKOFF 1930	USSR (near Kiew)
	42	TANAKA 1938, 1939	Japan
S. tabernaemontani Gmel.	42	BAKKER 1954	Netherlands
	42	HÅKANSSON 1928	Sweden
S. validus Vahl	42	HICKS 1928	USA
S. triqueter L.	40	TANAKA 1942, 1948	Japan
S. mucronatus L.	42	MORINAGA and FUKUSHIMA 1931	Japan
	42	TANAKA 1937	Japan
S. americanus Pers.	76	HICKS 1928	USA
	± 80	WULFF 1937	Denmark
S. olneyi Gray	78	HICKS 1928	USA

variation in chromosome numbers of this geographically so widely distributed section, is rather surprising.

Furthermore, it is worth mentioning that HICKS (1928) found a putative hybrid of *S. americanus* and *S. olneyi*, two taxa with only a slight difference in chromosome number. In this case a great number of univalents were observed during the first meiotic division. The univalents segregated at anaphase I, but the daughter chromatids moved to the poles in a very irregular way. Aside from the interpretation of this phenomenon, this observation shows clearly that hybridizing between two species, which are only slightly different from a karyological point of view, may cause very striking cytological effects. It might not be excluded that in the Netherlands corresponding phenomenons occur in the numerous intermediates, considered as hybrids in this paper, of *S. lacustris*, *S. tabernaemontani* and *S. triqueter*. In this connection a karyological investigation of the Dutch material might be of great interest for the specific delimitation in this section.

As to the chromosome morphology, nearly all authors agree, with regard to the impossibility of dividing the *Scirpus*-chromosomes into size groups. The "compound chromosomes" in Japanese forms of *S. lacustris*, as described by TANAKA (1937, 1938, 1939), represent the only exception to this rule. Furthermore, it seemed that the different authors could not observe any constriction of the chromosomes. Before 1947 it was attributed to the smallness of the chromosomes and to imperfections of the fixation techniques, but after the investigations of CAMARA, DE CASTRO, MALHEIROS and GARDÉ (1947–1951) with

Luzula purpurea Link new light was thrown on this problem. They showed that a non-localized type of centromere, as described by HUGHES–SCHRADER and RIS (1942) in the chromosomes of coccids, also may occur in the higher plants. The chance that this type of centromere exists in the chromosomes of *Scirpus* is increased by the fact that HÅKANSSON (1954) showed non-localized centromeres in *Eleocharis*, a genus closely related with *Scirpus*.

The importance of these observations will be pointed out here shortly. Viz, if the kinetic activity is not localized in one region, all the chromosome fragments formed by transversal breakage may have kinetic activity. These fragments are comparable with normal chromosomes during the following divisions and are not lost, as happens with the acentric fragments of chromosomes with a localized centromere. This can be proved by studying X-ray treated material as has been done by HUGHES–SCHRADER and RIS (1942) for the first time.

The theory of the so-called diffuse centromere represents a special method of chromosome doubling by means of transversal breakage. MALHEIROS and GARDÉ called this agmatoploidy (= fragment-ploidy). However, agmatoploids cannot always be arranged in normal series of polyploids, e.g. the breakage may be restricted to a part of the set of chromosomes. This may be the explanation for the irregular variations in chromosome numbers within certain groups e.g. in *Luzula*, *Carex* and *Scirpus*.

Although the X-ray treatment must be considered as the only proof of the occurrence of diffuse centromeres, several observations are recorded in literature which are more or less in agreement with their existence. These observations deal mainly with the way of disjunction of the chromatids during anaphase, the nature of the meiosis (post- or pre-reductional) and the form of the spindle. Seeing that it was not the purpose of our investigation to study the behaviour of the *Scirpus*-chromosomes extensively this phenomenon was not included in this study. Moreover, in connection with the smallness of the chromosomes, it is not to be expected that such a study might contribute much to the discussion about these interesting problems.

Preliminary data concerning the taxonomic study are expressed in a paper of BAKKER (1954). In this paper the author concludes that *S. lacustris* L. and *S. tabernaemontani* Gmel. should not be considered as separate species. In the progress of the investigations new data has been obtained which speaks for a return to the old classification. In prospect of a publication about this subject by BAKKER, the above mentioned taxa will be regarded as separate species in this paper. In connection with this, the following taxonomical subdivision of the section *Schoenoplectus* is applied:

1. *S. lacustris* L.
2. *S. tabernaemontani* Gmel.
3. Hybrid swarm of *S. lacustris* and *S. tabernaemontani* (ssp. *flevensis* BAKKER (1954) included).
4. *S. triqueter* L.

5. Hybrid swarm of *S. lacustris* and *S. triqueter* (*S. x carinatus* Smith, BAKKER 1954).
6. Hybrid swarm of *S. tabernaemontani* and *S. triqueter* (*S. x scheuchzeri* BRÜGGER, BAKKER 1954).
7. *S. americanus* Pers.

METHODS AND MATERIALS

Nearly all the material studied has been collected from isolated clones. Some clones grew in their original habitat, whereas others had been transplanted to a shallow lake near Vollenhove and to a seepage region in the polder Eastern Flevoland. With the exception of clone 8 of *S. tabernaemontani*, herbarium specimens were collected from all clones and preserved in the "Herbarium IJsselmeerpolders" at Kampen.

Initial study was on the chromosomes of root tips. After fixing in acetic alcohol (1 : 3) the tips were treated with normal hydrochloric acid at 60° C to obtain maceration and hydrolisation, after which the material was stained with Feulgen's basic leucofuchsin. After staining, squashes were made in 45 % acetic acid. To obtain a greater spreading of the metaphase chromosomes, the tips were pretreated with a saturated aqueous solution of α-bromonaphtalene. Although this pre-treatment shortens the already short chromosomes still more, this is not a great difficulty, as the chromosomes do not show any visible differentiation. Moreover, the stronger contraction has the advantage of a sharper outlining of the separate chromosomes. However, the small size, in connection with the rather high number, usually made an exact determination of the chromosome numbers impossible. For this reason the study of the somatic chromosomes was abandoned. Hence, mainly haploid numbers were counted in the anthers.

For this purpose inflorescenses were submerged in acetic alcohol (1 : 3) immediately after collecting, and were stored in a refrigerator at — 2° C. Material stored in this way remained suitable for the making of good squashes for at least six months after collecting.

Squashes of the anthers were made in the following way:

a. dissecting the anthers.
b. tapping in 45 % acetic acid on a microscope slide, with the aid of the blunt end of a plastic needle holder.
c. pressing the covered slide between sheets of filter paper.

The preparations were viewed with phase-contrast. The slides were made permanent with Euparal in the usual way.

Microphotos were made with a Wild Phase-Fluotar oil-immersion objective (n.a. = 1.30) and a Wild Photographic eyepiece 10 ×, using a Wild Photomicrographic camera I. Light was supplied by a Wild low voltage lamp after Köhler, using a Wild 2 mm green filter. Adox R 14 panchromatic film was used and developed in Agfa Rodinal.

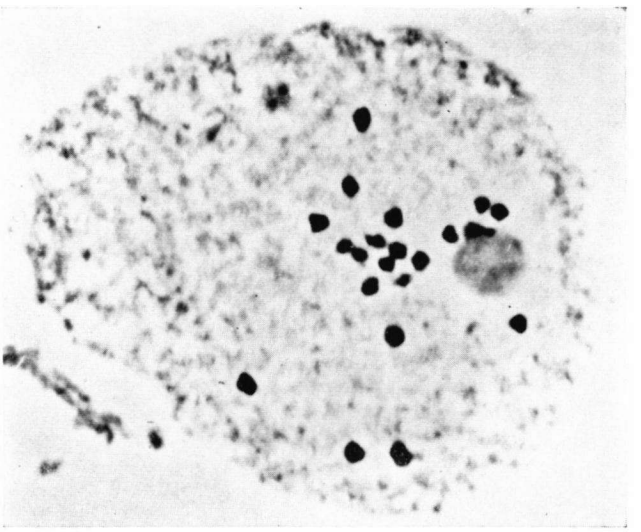

Fig. 1. *S. lacustris.* Diakinesis. 21 bivalents, one (connected with nucleolus) with a satellite. × 1700

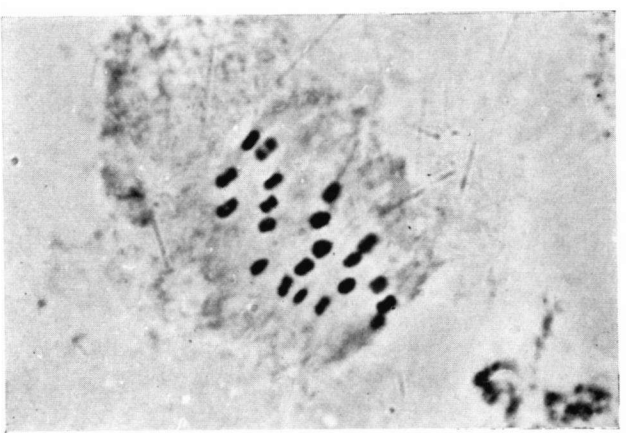

Fig. 2. *S. lacustris.* Metaphase I (side view), 21 bivalents. × 1700.

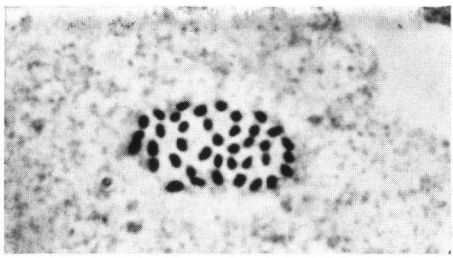

Fig. 3. *S. americanus*. Metaphase I (polar view), 39 bivalents. × 1700.

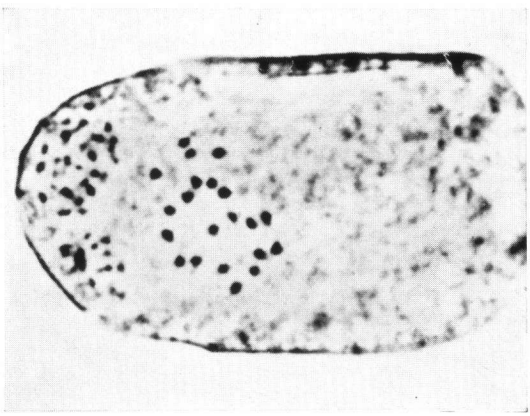

Fig. 4. *S. triqueter*. Prometaphase of first pollenmitosis, 21 chromosomes. × 1700.

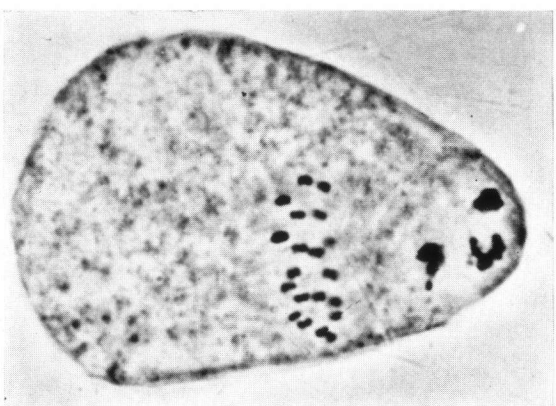

Fig. 5. *S. lacustris*. Metaphase of first pollenmitosis. Only 20 chromosomes visible (one chromosome probably overlapped). The three degenerating nuclei clearly visible in the top of the cell. × 1700.

Acta Botanica Neerlandica **11** (1962) *facing page* 40

D. OTZEN:

Chromosome studies in the genus Scirpus L., section Schoenoplectus Benth. et Hook., in the Netherlands.

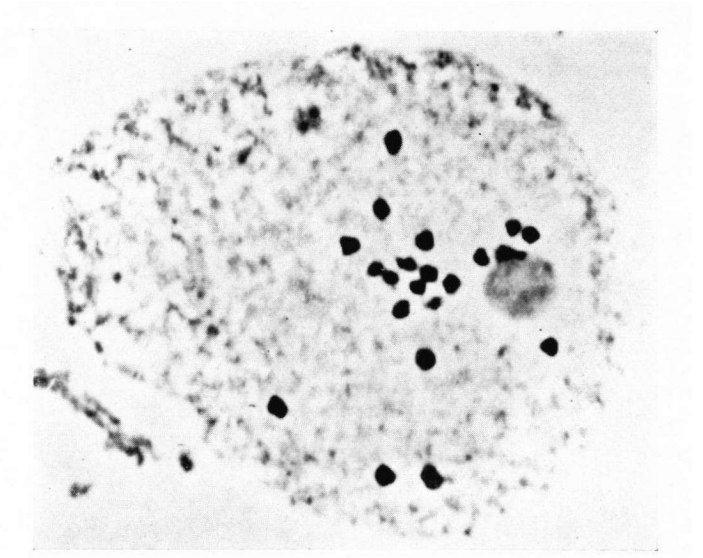

Fig. 1. *S. lacustris.* Diakinesis. 21 bivalents, one (connected with nucleolus) with a satellite. × 1700

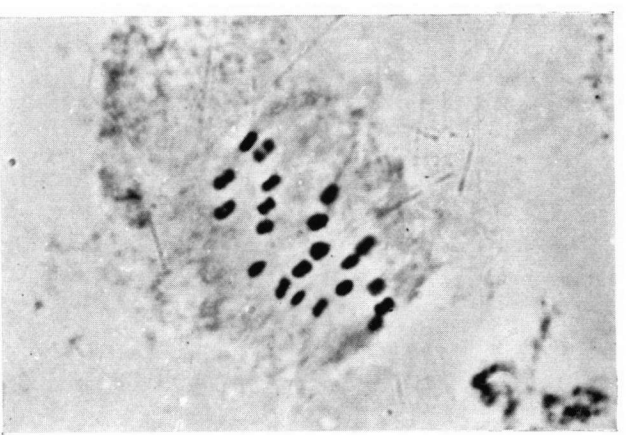

Fig. 2. *S. lacustris.* Metaphase I (side view), 21 bivalents. × 1700.

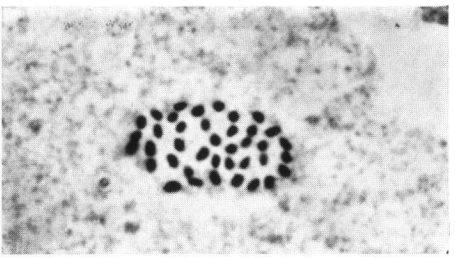

Fig. 3. *S. americanus.* Metaphase I (polar view), 39 bivalents. × 1700.

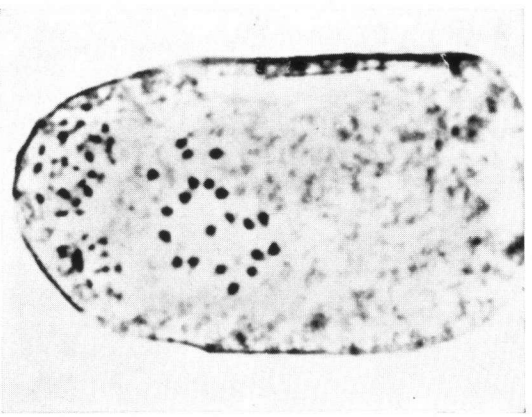

Fig. 4. *S. triqueter.* Prometaphase of first pollenmitosis, 21 chromosomes. × 1700.

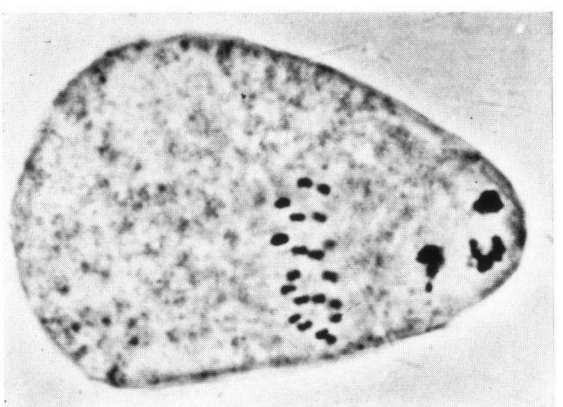

Fig. 5. *S. lacustris.* Metaphase of first pollenmitosis. Only 20 chromosomes visible (one chromosome probably overlapped). The three degenerating nuclei clearly visible in the top of the cell. × 1700.

As follows from the above, all the photographs in this paper are due to the phase-contrast images of fixed but unstained material.

Observations

The observations made, mainly refer to pollenmeiosis and pollenmitosis. There, it may be useful, to give a short description of the development of the pollen grains in the *Cyperaceae*. It is not a commonly known fact that their development differs principally from the state of affairs in other families (Tischler, 1951).

Strasburger (1884), based on observations of Elfving (1879) and Wille (1882), first described the formation of the pollen grains in the *Cyperaceae*. The outline of this description agrees very well with recent views and can be summarized as follows.

After meiosis three of the four nuclei of each tetrad are arranged in the narrow top of the ovate mother cell and the fourth is found in the central part. The latter develops into the primary pollen nucleus, whereas the other three remain small. Furthermore this primary nucleus passes into the first pollenmitosis (Fig. 5) and the other three degenerate after a fruitless attempt to divide in their narrow space.

The first pollenmeiosis (Figs. 2 and 3) and the first pollenmitosis (Figs. 4 and 5) of *Scirpus* are particularly suitable for karyological studies. Mostly the cytoplasm is still clear during these divisions and the cell wall is only a little thickened. These are two important advantages for phase-contrast microscopy of squash-preparations. Löve, Löve and Raymond (1957), investigating the chromosomes of *Carex*, rejected the use of anthers, "because of puzzling meiotic configurations and stickiness". With respect to *Scirpus* we cannot agree with these authors. In our opinion haploid numbers are of great advantage. Moreover, during the division of the primary pollen nuclei the size of the chromosomes was about twice that of the somatic chromosomes, in our preparations. This difference may be caused mainly by omitting the α-bromonaphtalene-treatment in our haploid material.

In accordance with the results of Häkansson (1928) and Hicks (1928) we were not able to find any point of connection with respect to the morphology of the chromosomes. Viz, they cannot be arranged in size groups, and the indistinct shape of the rather small chromosomes makes the observation of primary constrictions, if present, impossible. Sometimes, however, the connection of the nucleolus with a clearly visible constriction, was shown during diakinesis (Fig. 1). In later stages such constrictions could not be observed.

In all the investigated clones we could not find more than one nucleolus in the haploid and diploid nuclei.

As a result of the indistinct shape of the chromosome the author was not able to show if parallel disjunction of the chromatids during anaphase, as described by the various *Luzula*-investigators, takes place in *Scirpus*.

Irregularities during meiosis, e.g. the occurrence of univalents,

were only exceptionally observed. Generally the chromosomes proved to move very regularly to the poles during both the meiotic divisions. Chromosome-association, and the occurrence of chromatin bridges during the meiotic telophases, as recorded by TANAKA (1938, 1939) for Japanese paramorphs of *S. lacustris*, were not observed in the material studied.

RESULTS OF THE CHROMOSOME COUNTINGS

In Table II the obtained haploid chromosome numbers are summarized. The number of countings per clone mainly depends on the quality of the collected material. When more than twenty nuclei per clone could be counted, the mean number was calculated. In connection with the fundamental discontinuity of the counted values the means are given in round figures (M in Table II). Moreover the percentual frequency of M from Table II is considered as a measure for the variation within one clone. In other words, a high percentual frequency means a small variation in the counted chromosome numbers per clone.

With the exception of S. americanus (n = 39), it is clearly shown in Table 2 that all the studied clones have the same number, viz n = 21. The possible causes of variation within one clone will be discussed below.

In a few cases only, we succeeded in making good preparations during the first pollenmeiosis. For this reason we referred mainly to the first pollenmitosis. On the other hand, the smallness of the mitotic chromosomes of *S. americanus* made it necessary to spend much time in searching for good meiotic material of this species and to refer to the first pollenmeiosis only.

Although counting of the somatic chromosomes generally did not take place, in two plants the diploid number was counted in root tips. One of these plants was a dwarf form of *S. tabernaemontani*, which showed no flowering, even after transplantation in different habitats. The other plant belonged to a group of seedlings, grown in the greenhouse from seed of a typical representative of the hybrid swarm of *S. tabernaemontani* and *S. triqueter*. These seedlings belonged to this hybrid swarm as well, although they showed slight variation in morphological characteristics. In both plants the number of 2 n = 42 was found.

Owing to their small size, we did not succeed in counting the chromosomes during the second pollenmeiosis.

DISCUSSION

Although the cytological investigation of the section *Schoenoplectus* is still in progress, the conclusion may now be drawn, that *S. lacustris*, *S. tabernaemontani*, *S. triqueter* and the hybrid swarms usually do not show any difference in karyotype. However, further investigations are desirable with regard to small meiotic irregularities which occasionally occur. Differences in chromosome numbers within *S. lacustris* and

TABLE II

Comparative summary of the chromosome counts in the genus *Scirpus* L.

Clones studied [1]	Stage of division [2]	Total number of counts	Counted number of chromosomes								Mean number (M)	Percentual frequency of M
			n = 18	n = 19	n = 20	n = 21	n = 22	n = 23	n = 37	n = 38	n = 39	
S. lacustris												
clone 1	PG	37			6	27	4					21	73.0
„ 1	PMC	3				3						
„ 2	PG	77			12	64	1					21	83.1
S. tabernaemontani												
clone 3	PG	25			2	20	3					21	80.0
„ 4	PG	101			2	99						21	98.0
„ 5	PG	71			2	66	3					21	93.0
„ 6	PMC	3				3							
„ 7	PMC	1				1							
„ 8	PMC	1				1							
„ 9	PMC	2				2							
Hybrid swarm of *S. lacustris* and *S. tabernaemontani* (type 3)												
clone 10	PG	55	1	1	6	38	9					21	69.1
„ 10	PMC	19				14	5						
S. triqueter												
clone 11	PG	75			6	67	2					21	89.4
Hybrid swarm of *S. tabernaemontani* and *S. triqueter*												
clone 12	PG	36			1	35						21	97.2
„ 13	PG	32			4	28						21	87.5
„ 14	PG	1				1							
„ 14	PMC	1				1							
„ 15	PMC	9		1		7	1						
Hybrid swarm of *S. lacustris* and *S. triqueter*												
clone 16	PG	33		1	4	23	4	1				21	69.7
„ 17	PG	177		5	40	87	41	4				21	49.2
„ 17	PMC	4				4							
„ 18	PG	10				9	1						
„ 19	PG	104		3	24	70	6	1				21	67.3
S. americanus												
clone 20	PMC	24							1	1	22	39	91.7
„ 21	PMC	8								2	6		

[1] *Localities:*
a. Fresh areas; the following clones: 2 and 10 (Kampereiland), 5 (Lake Vollenhove), 8 and 20 (Eastern Flevoland), 4 (Oosterwolde) and 21 (Zwarte Meer).
b. Fresh tidal areas; the following clones: 1, 6, 7 and 14 (Nieuwe Merwede), 3, 11, 12 and 13 (Lek), 9, 17, 18 and 19 (Biesbosch), 16 (Maas) and 15 (Oude Maas).

[2] PG = Pollen Grain; first pollen mitosis.
PMC = Pollen Mother Cell; first meiotic division.

between *S. lacustris* and *S. triqueter*, as described by TANAKA in Japan, were not observed in the Netherlands. This means that, with the exception of *S. americanus*, the cytological data cannot give support to the subdivision of the section *Schoenoplectus* in the area studied. On the other hand, the rather frequent occurrence of swarms of fertile hybrids may be connected with the similarity in karyotype.

Owing to the regularity of meiosis, in all probability the high fertility of the hybrid swarms is not caused by apomixis. Embryological studies are necessary to confirm whether the seed formation in this section is sexual or not.

In many instances the number of counts within one clone permits the calculation of a reliable mean value. Nevertheless, the different chromosome numbers found within one clone may be the subject of closer considerations. It is obvious that the occurrence of counting errors may be the partial explanation of this phenomenon, presumably as a result of:

1. *insufficient distinction of chromosomes*

There is a rather considerable likelihood of making such errors with chromosomes of this type. For this reason the frequency of numbers below the mean value may in most cases be somewhat higher than those above. The short and rather irregularly shaped chromosomes cannot be distinguished from one another even in cases of partial overlapping.

2. *artefacts*

Typical artefacts are not observed.

3. *scattered chromosomes*

Squashing may damage the cells, by which their contents may scatter over the entire preparation. Optically those scattered chromosomes may not be distinguished from the chromosomes in an undamaged cell.

4. *premature separation of chromatids*

In chromosomes with a localized centromere the chromatids are held together by the functional undivided centromere and, depending on their length, by relational coiling. On the other hand, short chromosomes with a "diffuse" centromere do not exhibit these obstacles to separation. In this case, especially in squashes, already in prometaphase the chromatids might be diverged so far that they are counted as separate chromosomes. If "diffuse" centromeres are present in *Scirpus*, which has to be proved, this may be a cause of error.

It might be thought that the above-mentioned causes of error permit an explanation to be given for the variation of the chromosome numbers within a clone. But it does not explain why the distribution of the different chromosome numbers, as counted during pollen-mitosis, varies with the investigated species and hybrid swarms. Viz

the percentual frequency of M (see Table II) is smaller in the hybrid swarms with *S. lacustris* as one of the putative parents than in the other species or hybrid swarms. The smaller percentual frequency of M in the *S. lacustris* hybrids might be an indication of more meiotic irregularities.

With regard to the different chromosome number of *S. americanus* in the section *Schoenoplectus*, the results of this study corresponds with those of previous statements. The counted haploid number of 39 agrees very well with the number found by WULFF (1937) in Danish material ($2n = \pm 80$). In all probability the deviating chromosome number of *S. americanus* can explain the sporadic occurrence of hybrids with the other European species of the section.

On the basis of the performed investigation it is impossible to make a statement about the nature of the centromere in the chromosomes of *Scirpus*. The chromosomes are too small for an exact determination of their shape. Furthermore, squash techniques are not very suitable for a study of the spindle form and the chromosome movements, for they deform the spindle and dislocate the chromosomes.

SUMMARY

A karyological study was made of the genus *Scirpus* L., section *Schoenoplectus* Benth. et Hook., in the Netherlands. It was found that the species *S. lacustris* L., *S. tabernaemontani* Gmel. and *S. triqueter* L. all have the same chromosome number of $n = 21$.

S. americanus Pers. proved to have a different number ($n = 39$).

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REFERENCES

BAKKER, D. 1954. Acta bot. neerl. **3** (4): 425–445.
ELFVING, F. 1879. Jenaisch. Z. Naturw. **13**: 1–28.
DAVIES, E. W. 1956. Hereditas **42**: 349–365.
DE CASTRO, D., A. CAMARA and N. MALHEIROS. 1949. Genética Ibérica **1**: 49–54.
————. 1950. Genética Ibérica **2**: 201–209.
HÅKANSSON, A. 1928. Hereditas **10**: 277–291.
————. 1954. Hereditas **40**: 325–345.
HEILBORN, O. 1924. Hereditas **5**: 129–217.
HICKS, G. C. 1928. Bot. Gaz. **86**: 295–317.
HUGHES-SCHRADER, S. and H. RIS. 1941. J. Exp. Zool. **87**: 429–451.
KOSTRIOUKOFF, X. 1930. Bull. Jard. Bot. Kieff **11**: 10–14.
LÖVE, A., D. LÖVE and M. RAYMOND. 1957. Canad. J. Bot. **35**: 715–761.
MALHEIROS, N., D. DE CASTRO and A. CAMARA. 1947. Agron. Lusit. **9**: 51–71.
MALHEIROS-GARDÉ, N. and A. GARDÉ. 1951. Genética Ibérica **3**: 155–176.

STRASBURGER, E. 1884. Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen, als Grundlage für eine Theorie der Zeugung. Jena.
TANAKA, N. 1937. Cytologia; Fujii jub. vol.: 814–821.
———. 1938. Cytologia **8**: 515–520.
———. 1939. Cytologia **9**: 533–556.
———. 1942. Med. a. Biol. **2**: 91 ff. (original not seen).
———. 1948. Biol. Contr. Japan **2**: 425 ff. (original not seen).
TISCHLER, G. 1951. Allgemeine Pflanzenkaryologie, 2. Hälfte. Berlin.
WILLE, N. 1882. Christ. Vidensk-Selsk. Forh. **16**.
WULFF, H. D. 1937. Jahrb. Wissensch. Bot. **85**: 812–840.