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# Cytogenetic studies in the genus *Pentaschistis* (Poaceae: Arundinoideae)

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ABSTRACT

Cytogenetic studies of 45 specimens, representing 16 taxa of the genus *Pentaschistis* (Nees) Spach confirmed two basic chromosome numbers (x = 7, 13) for the genus. Chromosome numbers for five species and one subspecies are described for the first time, i.e. *P. airoides* subsp. *jugorum* (n = 2x = 14), *P. colorata* (n = 2x = 14), *P. densifolia* (n = x = 7), *P. lima* (n = 6x = ± 42), *P. rigidissima* (n = x = 7, n = 3x = 21) and *P. viscidula* (n = 3x = 21). Polyploidy occurs frequently and new ploidy levels are described in four of the species, namely *P. airoides* (Nees) Stapf subsp. *airoides* (n = 3x = 21), *P. cirrhulosa* (Thunb.) McClean (n = x = 7), *P. eriostoma* (Nees) Stapf (n = 3x = 39+0–4B) and *P. rupestris* (n = 4x = 28). The majority of species form young polyploid complexes. There seems to be no correlation between cytogenetic data and morphological groupings within *Pentaschistis*.

## INTRODUCTION

The genus *Pentaschistis* (Nees) Spach consists of 68 species (Linder & Ellis 1990) of which 57 are indigenous and 40 endemic to South Africa (Gibbs Russell *et al.* 1990). Cytogenetic studies reveal that *Pentaschistis* has basic chromosome numbers of 7 and 13 (De Wet 1954; Hedberg 1957; De Wet 1960; Hedberg & Hedberg 1977; Davidse *et al.* 1986; Spies & Du Plessis 1988; Du Plessis & Spies 1988, 1992; Spies *et al.* 1994).

The aim of this study was to determine whether the cytogenetic studies support the morphological groupings of Linder & Ellis (1990) and Ellis & Linder (1990); to determine the degree of polyploidy in the genus and to use ploidy levels to determine the age of the polyploid complexes in the genus.

## MATERIALS AND METHODS

The material used during this study was collected in the field. Voucher herbarium specimens are housed in the Geo Potts Herbarium, Bloemfontein (BLFU) and/or in the National Herbarium, Pretoria (PRE).

Young inflorescences were collected and fixed in Carnoy's fixative (Carnoy 1886). The fixative was replaced by 70% (v/v) ethanol 24–48 hours after fixation. Anthers were squashed in 2% (m/v) aceto-carmine (Darlington & La Cour 1976). Slides were permanently mounted by freezing them with liquid $CO_2$ (Bowen 1956), followed by dehydration in ethanol and mounting in Euparal. At least twenty cells per specimen were studied for each meiotic stage, except where otherwise indicated.

With the annotation of chromosome numbers we followed the example set by the series '*Index to plant chromosome numbers*' (Goldblatt 1981), where chromosome numbers derived from meiotic studies are presented as the gametic (n) number and chromosomes from mitotic studies as the somatic (2n) chromosome number. The average number of chiasmata per bivalent was considered to be the number of chiasmata per chromosome and were calculated by dividing the mathematical average of the chiasmata per cell by the haploid chromosome number of the plant. Chromosome configurations were calculated as the average number of each configuration per cell. The number of B-chromosomes is presented as the minimum and maximum number of B-chromosomes observed per cell. The numerical values of the chromosome abnormalities were obtained by calculating the average number of a certain abnormality per cell. Genomic relationships were calculated according to the models of Kimber & Alonso (1981).

## RESULTS AND DISCUSSION

Forty five specimens, representing the different groups in *Pentaschistis*, except Group 5, have been studied. The results are presented alphabetically in table form for each group (Table 1).

### *Number of specimens per ploidy level*

All published ploidy levels (Hedberg 1957; De Wet 1960; Tateoka 1965a & b; Davidse *et al.* 1986; Du Plessis & Spies 1988; Spies & Du Plessis 1988; Du Plessis & Spies 1992; Spies *et al.* 1994) were used to determine the number of specimens per ploidy level among the different species of the genus *Pentaschistis*. The frequency of specimens per ploidy level was also plotted for the two different basic chromosome numbers in *Pentaschistis* (Figure 4).

Meiotic analyses of the different *Pentaschistis* species revealed haploid chromosome numbers of seven, multiples of seven (Figures 1, 2), thirteen and multiples thereof (Figure 3). The low number of species with a basic chromosome number of thirteen can be attributed to the fact that only specimens from South Africa have been studied. All species with a basic chromosome number of thirteen, except *P. eriostoma*, grow in central Africa (Du Plessis & Spies

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TABLE 1.— List of *Pentaschistis* specimens studied with their respective gametic chromosome numbers, ploidy levels, figures where they are shown, localities and voucher specimens

| Group | Taxon | Gametic Chromosome no. | Ploidy | Figure | Locality and vouchers |
|---|---|---|---|---|---|
| 1 | *P. airoides* (Nees) Stapf subsp. *airoides* | n = 7 | Diploid | 1B | WESTERN CAPE.—3219 (Wuppertal): 108 km from Nieuwoudtville to Clanwilliam, (–AA), *Spies 5789*. |
| | | n = 21 | Hexaploid | 1A | WESTERN CAPE.—3119 (Calvinia): Uitkyk Pass at stream, (–CA), *Spies 4388*. |
| 1 | *P. airoides* subsp. *jugorum* (Stapf) Linder | n = 14 | Tetraploid | | EASTERN CAPE.—3027 (Lady Grey): 45 km from Barkly East to Rhodes, (–DD), *Spies 3990*; 40 km from Barkly East to Rhodes, (–DD), *Spies 4734*. |
| 1 | *P. capillaris* (Thunb.) McClean | n = 7 | Diploid | 1C | WESTERN CAPE.—3218 (Clanwilliam): 10 km from Clanwilliam in Nardauskloof, (–BB), *Spies 3810*; 23 km from Piketberg to Velddrift, (–DC), *Spies 5804*. |
| 1 | *P. cirrhulosa* (Nees) Linder | n = 7 | Diploid | 1D | WESTERN CAPE.—3421 (Riversdale): 3 km north of De Hoop Nature Reserve, (–AA), *Spies 4627*. |
| 1 | *P. densifolia* (Nees) Stapf | n = 7 | Diploid | 1E | WESTERN CAPE.—3319 (Worcester): Bainskloof Pass, (–CA), *Spies 3878a*. |
| 1 | *P. lima* (Nees) Stapf | n = ± 42 | Duodecaploid | | NORTHERN CAPE.—3018 (Kamiesberg): on top of Kamiesberg, (–AA), *Ellis 5422*. |
| 1 | *P. pallida* (Thunb.) Linder | n = 7+0–3B | Diploid | 1F | WESTERN CAPE.—3118 (Vanrhynsdorp): Uitkyk Pass in Cederberg, (–AC), *Spies 3840*. 3319 (Worcester): 11 km from Ceres in Mitchell's Pass, (–AD), *Spies 5393*; 11 km from bridge over Olifants River in Niewoudt's Pass via Algiers, (–BB), *Spies 5367*; 13 km from bridge over Olifants River in Niewoudt's Pass via Algiers, (–BB) *Spies 5368*; 3 km from Algeria to Citrusdal, (–CA), *Spies 4406*. 3421 (Riversdale): 41 km from Barrydale to Riversdale, (–AB), *Spies 5476*. |
| | | n = 14 | Tetraploid | | WESTERN CAPE.—3118 (Vanrhynsdorp): Uitkyk Pass in Cederberg, (–AC), *Spies 3828*; 33 km from Citrusdal in Middelberg Pass, (–CA), *Spies 5381*. 3319 (Worcester): 1 km south of old 'tolhuis' in Mitchell's Pass, (–AD), *Spies 3859*, *3860*; 11 km from bridge over Olifants River in Niewoudt's Pass via Algiers, (–BB), *Spies 5292*. |
| 1 | *P. rupestris* (Nees) Stapf | n = 28+0–1B | Octaploid | | WESTERN CAPE.—3219 (Wuppertal): 33 km from Clanwilliam to Cederberg in Uitkyk Pass, (–AC), *Spies 5796*. |
| 1 | *P. tomentella* Stapf | n = 7 | Diploid | 1G | NORTHERN CAPE.—2917 (Springbok): 17 km from Steinkopf to Port Nolloth via Aninaus' Pass, (–BA), *Spies 3773*. 3017 (Hondeklipbaai): 2 km from turnoff to Paternoster, (–BB), *Spies 3379*; 18 km from Kamieskroon to Leliefontein, (–CA), *Spies 4306*. |
| | | n = 14 | Tetraploid | 1H | NORTHERN CAPE.—3119 (Calvinia): at Willem's River, (–AC), *Spies 3782*. |
| | | | | | WESTERN CAPE.—3219 (Wuppertal): 91 km from Nieuwoudtville to Clanwilliam, (–AA), *Spies 5765*. 3319 (Worcester): Bainskloof Pass, (–CA), *Spies 3878b*. |
| 2 | *P. aristidoides* (Thunb.) Stapf | n = 7 | Diploid | 2A | WESTERN CAPE.—3219 (Wuppertal): 108 km from Nieuwoudtville to Clanwilliam in Pakhuis Pass, (–AC), *Spies 5320*. |
| 2 | *P. triseta* (Thunb.) Stapf | n = 7 | Diploid | 2B, C | WESTERN CAPE.—3218 (Clanwilliam): 26 km from Piketberg to Velddrift, (–DC), *Spies 4413*. |
| 2 | *P. viscidula* (Nees) Stapf | n = 21 | Hexaploid | | WESTERN CAPE.—3323 (Willowmore): 23 km from Knysna to Uniondale, (–CC), *Spies 3520*. |
| 3 | *P. colorata* (Steud.) Stapf | n = 14 | Tetraploid | | WESTERN CAPE.—3319 (Worcester): 7 km from Paarl to Franschoek in pass, (–CC), *Spies 5403*. |
| 3 | *P. rigidissima* Pilg. ex Linder | n = 7+0–2B | Diploid | 2D | WESTERN CAPE.—3320 (Montagu): 61 km from Montagu to Touwsrivier at FM tower, (–AD), *Spies 5431*. |
| | | n = 21 | Hexaploid | 2E | WESTERN CAPE.—3420 (Bredasdorp): 7 km from Bredasdorp to Waenhuiskrans on beach, (–CA), *Spies 5458*. |
| 4 | *P. curvifolia* (Schrad.) Stapf | n = 7 | Diploid | 2F | WESTERN CAPE.—3319 (Worcester): 34 km from Worcester to Paarl in Du Toit's Kloof Pass, (–CA), *Spies 3888*; Du Toit's Kloof Pass, (–CA), *Spies 4443*; 7 km from Paarl to Franschoek, (–CC), *Spies 5401*. |

TABLE 1.— List of *Pentaschistis* specimens studied with their respective gametic chromosome numbers, ploidy levels, figures where they are shown, localities and voucher specimens (cont.)

| Group | Taxon | Gametic Chromosome no. | Ploidy | Figure | Locality and vouchers |
|---|---|---|---|---|---|
| 6 | *P. eriostoma* (Nees) Stapf | n = 13+0–3B | Diploid | 3A, B | WESTERN CAPE.—3118 (Vanrhynsdorp): on top of Uitkyk Pass in Cederberg, (–AC), *Spies 5370*; 15 km from Citrusdal in pass, (–CA), *Spies 5372*. 3219 (Wuppertal): Uitkyk Pass, (–AC), *Spies 3850*. 3320 (Montagu): 10 km from Buffels Jagts River in Tradouw's Pass, (–BA), *Spies 5470*. |
| | | n = 26+0–2B | Tetraploid | 3C, D | WESTERN CAPE.—3118 (Vanrhynsdorp): on top of Gifberg Pass, (–DA), *Spies 5337*, *Spies 5339*. |
| | | n = 39+0–4B | Hexaploid | 3E | WESTERN CAPE.—3119 (Calvinia): 108 km from Nieuwoudtville to Clanwilliam in Pakhuis Pass, (–AA), *Spies 5319*. |
| ? | Unidentified specimens *Pentaschistis* sp. | n = 7+0–2B | Diploid | 2G, H | WESTERN CAPE.—3118 (Vanrhynsdorp): on top of Gifberg, (–DA), *Spies 5336*. 3219 (Wuppertal): 99 km from Nieuwoudtville to Clanwilliam, (–AA), *Spies 5768*. |

1992). The basic chromosome number of seven predominates in the southern African species, whereas x = 13 prevails in the remainder of Africa (Du Plessis & Spies 1992).

Polyploidy is common in the genus *Pentaschistis*. Nineteen of the 45 specimens studied were polyploid. Higher ploidy levels were detected in some of the cells of these specimens. This could be caused by cell fusion (Spies & Van Wyk 1995) which is not uncommon in the genus *Pentaschistis* (Figure 5).

*Pentaschistis airoides* subsp. *jugorum* and *P. colorata* were tetraploid. The genome homology of these two species was determined and the observed chromosome configurations corresponded best with the expected values for the 2:2 model (Table 2) of Kimber & Alonso (1981). It indicates that two sets of genomes are present and each set consists of two genomes. The relative similarity of the genomes within a set is 0.5 and the relative affinity between the sets is expressed by an x-value, which may vary between 0.5 (differences between sets are similar to differences within a set) and one (sets are totally different) (Kimber & Alonso 1981). The x-values for the specimens studied were one or tending towards one (Table 2), thus indicating little to no homology between the two sets of genomes (for example AABB). Based on the specimens used during this study, these species are alloploid. However, because of the occurrence of an occasional quadrivalent, *P. colorata* may be classified as a segmental allotetraploid. The occurrence of quadrivalents indicates that the genomes of this species correspond to some extent.

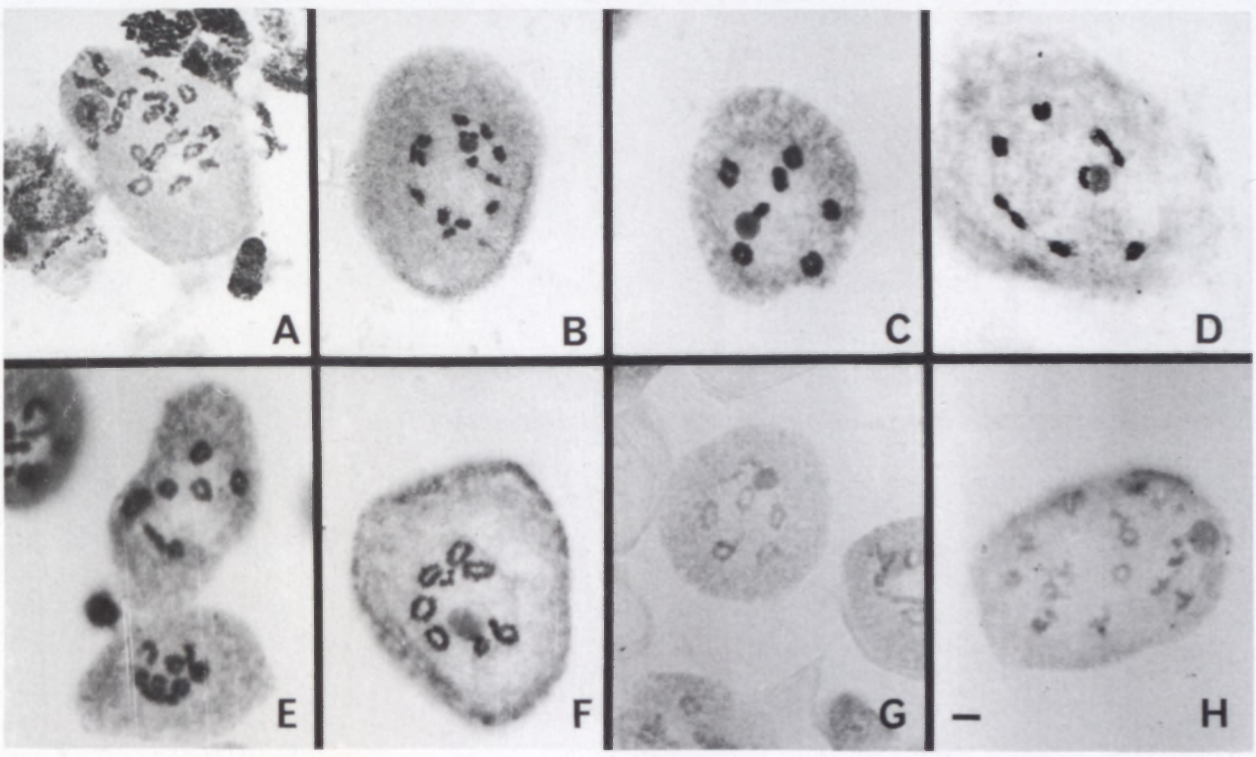


FIGURE 1.—Meiotic chromosomes in *Pentaschistis*. A, B, *P. airoides* subsp. *airoides*: A, *Spies 4388*, diakinesis, n = 21, $21_{II\,R}$; B, *Spies 4388*, diakinesis, n = 14, $10_{II\,R}$, $4_{II\,K}$. C, *P. capillaris*, *Spies 3810*, diakinesis, n = 7, $7_{II\,R}$; D, *P. cirrhulosa*, *Spies 4627*, diakinesis, n = 7, $5_{II\,R}$, $2_{II\,K}$; E, *P. densifolia*, *Spies 3878*a, diakinesis, n = 7, $6_{II\,R}$, $1_{II\,K}$; F, *P. pallida*, *Spies 5368*, diakinesis, n = 7+3B, $7_{II\,R}$, $1_{III}$. G, H, *P. tomentella*: G, *Spies 4306*, diakinesis, n = 7, $5_{II\,R}$, $2_{II\,K}$; H, *Spies 3782*, diakinesis, n = 14, $8_{II\,R}$, $6_{II\,K}$. Scale bar: 8.5 mm.

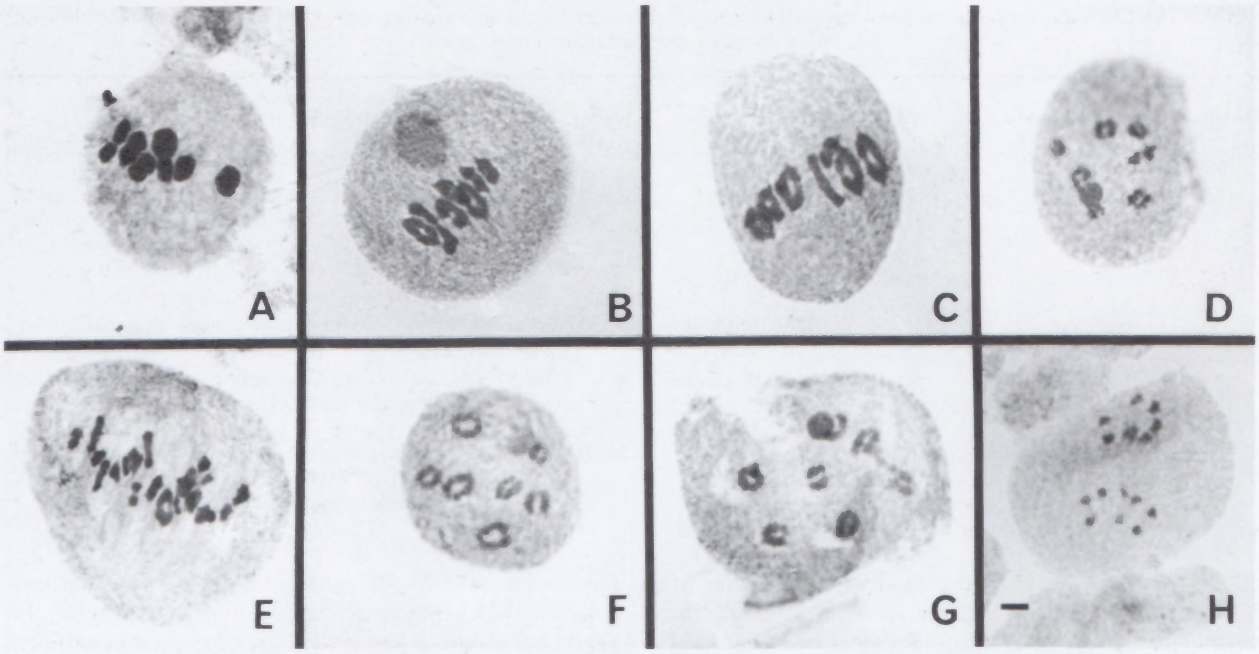


FIGURE 2.—Meiotic chromsomes in *Pentaschistis*. A, *P. aristidoides, Spies 5320*, metaphase I, n = 7, $6_{II\,R}$, $1_{II\,K}$; B, C, *P. triseta, Spies 4413*, metaphase I, n = 7, $6_{II\,R}$, $1_{II\,K}$. D, E, *P. rigidissima*: D, *Spies 5431*, diakinesis, n = 7+0–2B, $6_{II\,R}$, $1_{II\,K}$; E, *Spies 4388*, metaphase I, n = 21, $14_{II\,R}$, $5_{II\,K}$, $1_{IV\,R}$. F, *P. curvifolia, Spies 4443*, diakinesis, n = 7, $7_{II\,R}$. G, H, *Pentaschistis* spp: G, *Spies 5336*, diakinesis, n = 7+0–2B, $7_{II\,R}$, $1_{II\,K}$; H, *Spies 5768*, anaphase I, n = 7+2B. Scale bar: 8.5 mm.

The genome homology of the tetraploid specimens of *P. pallida* was determined. The observed and expected values corresponded best to the 2:2 model for two of the specimens, namely *Spies 3828* and *5292*, with x-values of one or tending towards one (Table 2). This indicates that little or no homology exists between the two sets of genomes, thus classifying these specimens as allotetraploids (for example AABB). However, the occurrence of quadrivalents indicates that the genomes of this species do correspond to some extent. Therefore, *Spies 3828* is classified as a segmental allotetraploid (AAA'A') with a x-value of 0.7 (Table 2).

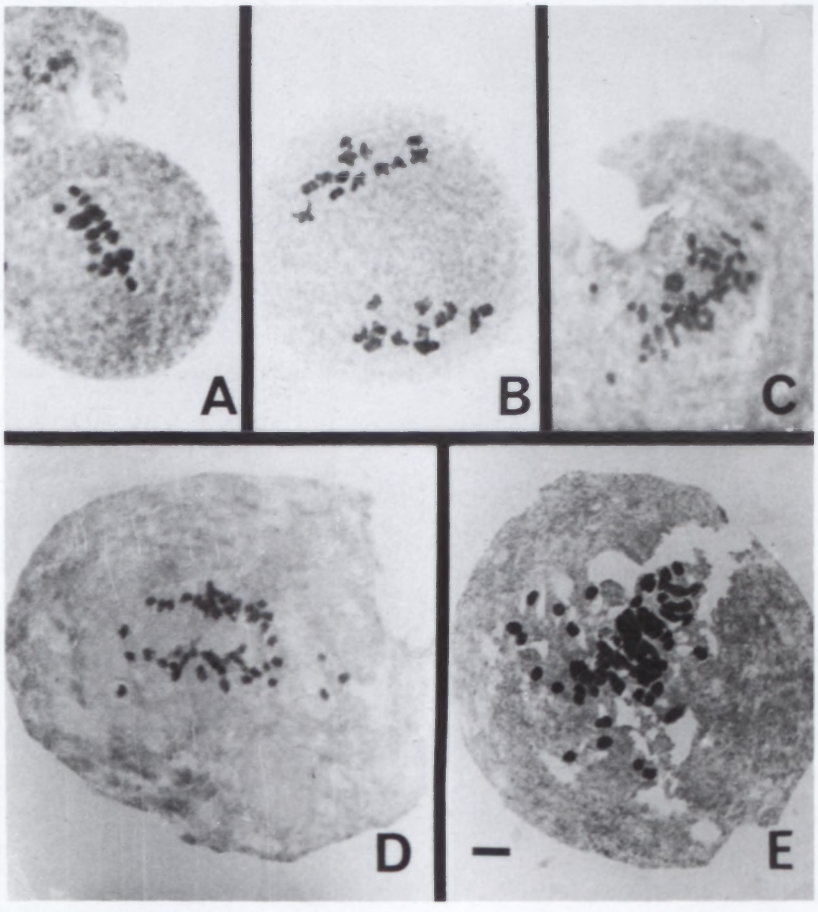


FIGURE 3.—Meiotic chromsomes in *P. eriostoma*. A, *Spies 5470*, metaphase I, n = 13+0–2B; B, *Spies 4390*, anaphase I, n = 13+0–3B. C, D, *Spies 5337*: C, metaphase I, n = 26; D, anaphase I, n = 26. E, *Spies 5319*, metaphase I, n = 39+0–4B. Scale bar: 8.5 mm.

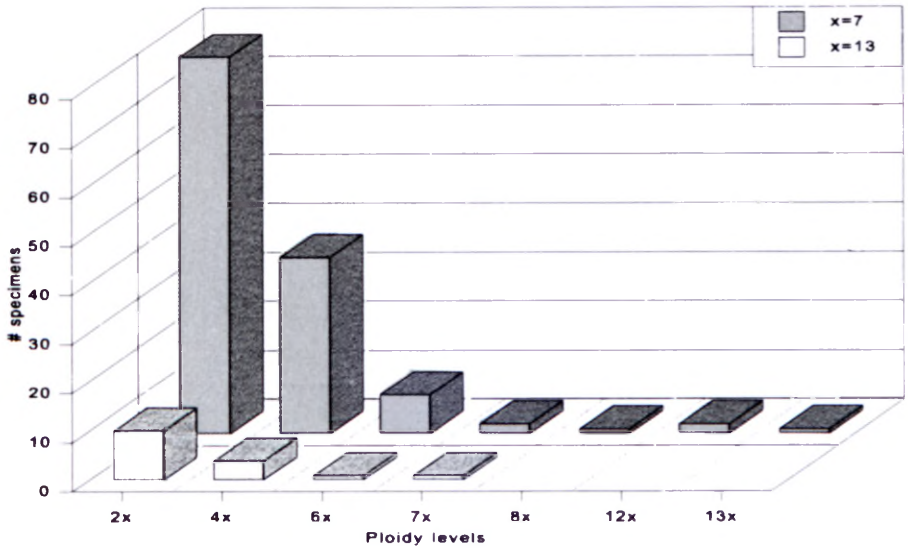


FIGURE 4.—Comparison of the number of specimens per ploidy level for the two existing basic chromosome numbers in *Pentaschistis* species.

The observed and expected values for the other two *P. pallida* specimens (*Spies 3859* and *5381*) corresponded best to the 2:1:1 model, with x-values of 0.8 and 1 respectively (Table 2). The 2:1:1 model indicates that three sets of genomes are present, one set consists of two genomes and the other two of one genome each. The x-values for the specimens studied were one or tending towards one (Table 2), indicating little or no homology among the three sets of genomes (for example AABC or AABB'). However, because of the occurrence of quadrivalents in these two specimens (*Spies 3859* and *5381*) they are classified as segmental allotetraploids (AAA'A"). Therefore, the specimens of *P. pallida* are either alloploids or segmental alloploids tending towards alloploidy (for example AABB, AABC, AABB' or AAA'A"). The two different genomic constitutions for this species may indicate that there is a high degree of variation within the species.

In *P. eriostoma* (Figure 3A–E) three different ploidy levels were detected, namely diploid, tetraploid and hexaploid, that correspond with the published literature (Spies & Du Plessis 1988; Du Plessis & Spies 1992). Heptaploidy was also observed by Du Plessis & Spies (1992). In the diploid (n = x = 13) specimens of *P eriostoma* 13 bivalents were observed. The genome homology of the tetraploid specimens of *P. eriostoma* was determined. The observed and expected values corresponded best to the 2:1:1 model with an x-value of 0.9 (Table 2), indicating that little homology exists among the three sets of genomes. This species is thus an allotetraploid species (for example AABC). However, a low frequency of quadrivalents occurred in the tetraploid, indicating that the genomes of this species do correspond to some extent. *Pentaschistis eriostoma* is, therefore, classified as a segmental allotetraploid (for example AAA'A").

All the existing chromosome data of *Pentaschistis* was used to determine the number of specimens per species per ploidy level. The number of specimens per ploidy level of the two different basic chromosome numbers was also determined (Figure 4). This was done to determine the degree of maturity (it is the ratio between diploid and polyploid specimens, as well as the level of polyploidy) in the polyploid complexes in the different species and in representatives of the two basic chromosome numbers.

The *Pentaschistis* species vary from young to old polyploid complexes. Most species studied (*P. airoides* subsp. *airoides*, *P. aristidoides*, *P. aurea* subsp. *aurea*, *P. borussica*, *P. capillaris*, *P. cirrhulosa*, *P. curvifolia*, *P. densifolia*, *P. eriostoma*, *P. natalensis*, *P. pallida*, *P. papillosa*, *P. patula*, *P. rigidissima*, *P. tomentella*,

TABLE 2.—Genomic relationships in tetraploid *Pentaschistis* specimens according to the models of Kimber & Alonso (1981). Values indicate sum of squares between observed and expected values for each model, whereas values in parentheses indicate x-values for each model

| Group | Taxon | Voucher specimen | Genomic relationships 4:0 | 3:1 | 2:2 | 2:1:1 |
|---|---|---|---|---|---|---|
| 1 | *P. airoides* subsp. *jugorum* | *Spies 3990* | 14.2 | 14.4 (1) | 0.02* (1) | 1.1 (1) |
| 1 | *P. pallida* | *Spies 3828* | 8.7 | 10 (1) | 0.7* (1) | 3.4 (1) |
| | | *Spies 3859* | 1.9 | 1.7 (0.7) | 1 (0.7) | 0.9* (0.8) |
| | | *Spies 5292* | 8.8 | 10 (1) | 0.5* (1) | 2.4 (1) |
| | | *Spies 5381* | 9 | 9 (0.8) | 1 (0.9) | 0.7* (1) |
| 1 | *P. tomentella* | *Spies 3782* | 10.9 | 11.3 (1) | 0.05* (1) | 0.3 (1) |
| | | *Spies 3878b* | 9 | 10 (1) | 0.3* (1) | 1.5 (1) |
| 3 | *P. colorata* | *Spies 5403* | 1.7 | 1.8 (1) | 0.4* (0.7) | 2.5 (0.9) |
| 6 | *P. eriostoma* | *Spies 5339* | 23.3 | 23.6 (0.9) | 2 (0.9) | 1.4* (0.9) |

* Accepted model

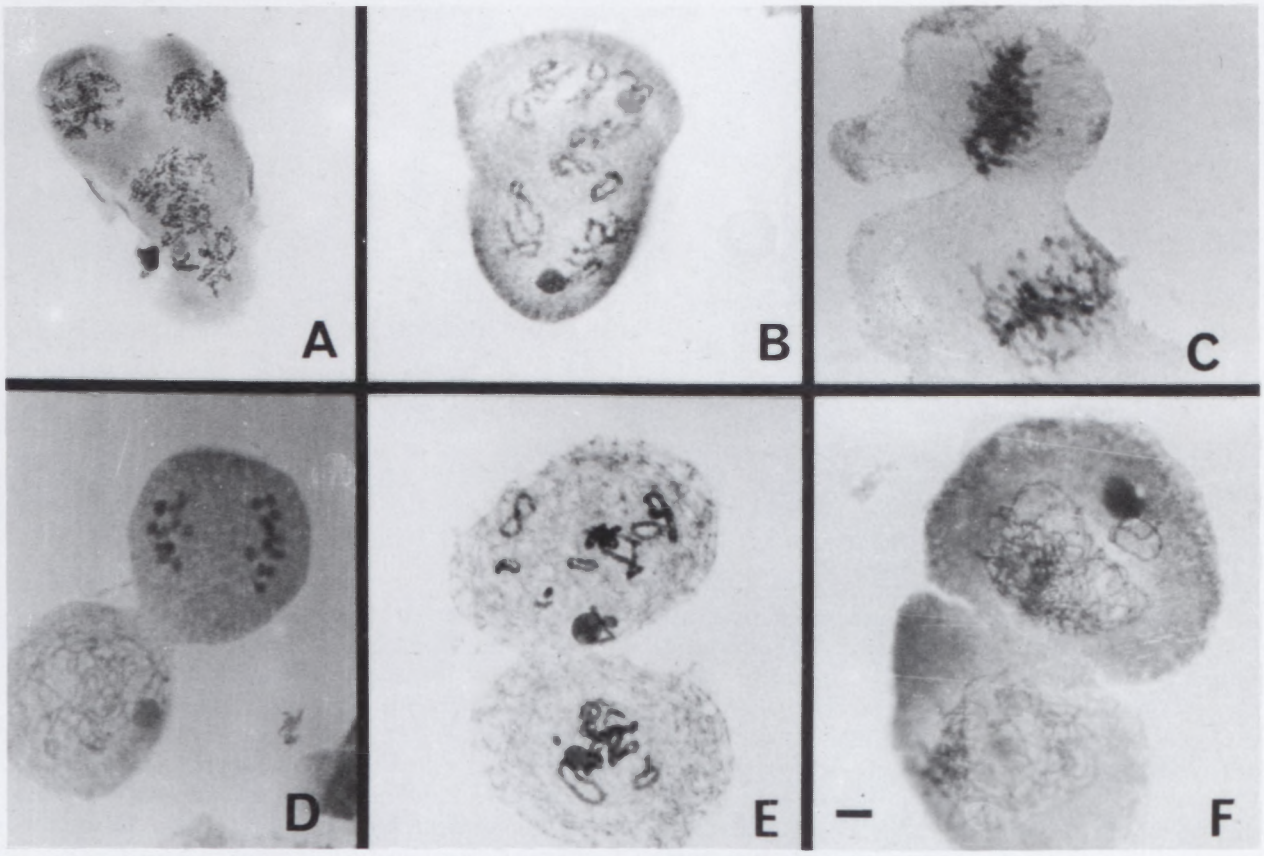


FIGURE 5.—Initial stages of cell fusion in *Pentaschistis*. A, *P. airoides* subsp. *airoides*, *Spies 4388*, n = 21; B, *P. cirrhulosa*, *Spies 4627*, n = 7; C, *P. lima*, *Ellis 5422*, n = ± 42. D, E, *P. pallida*: D, *Spies 3860*, n = 14; E, *Spies 5381*, n = 14. F, *P. eriostoma*, *Spies 3850*, n = 13+0–2B. Scale bar: 8.5 mm.

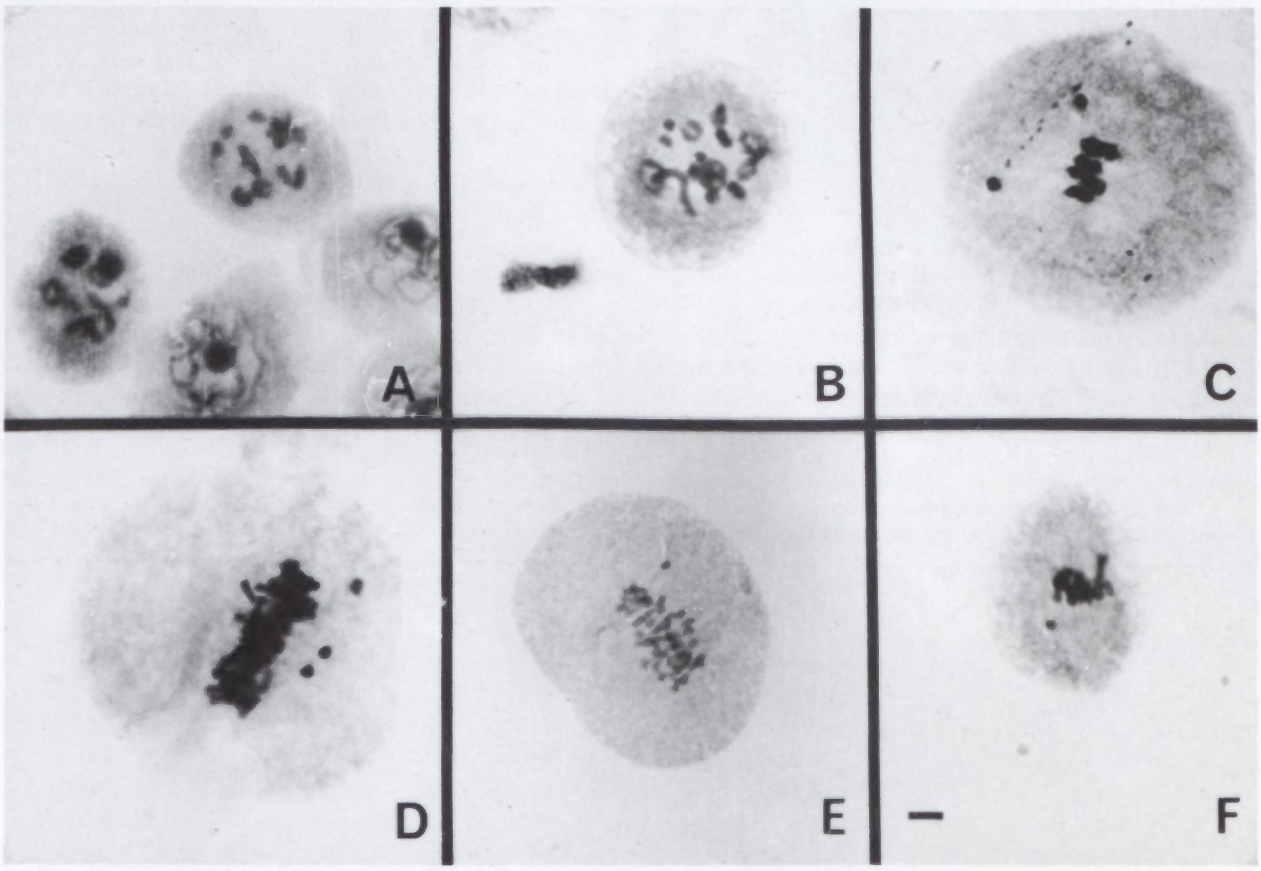


FIGURE 6.—Meiotic chromosomes in *Pentaschistis* with univalents. A, B, *P. pallida*: A, *Spies 4406*, diakinesis with 3 univalents, n = 7+0–1B; B, *Spies 5381*, diakinesis with 5 univalents, n = 7. C, *P. cirrhulosa*, *Spies 4627*, metaphase I with 2 univalents, n = 7. D, E, *P. eriostoma*: D, *Spies 5319*, metaphase I with 3 univalents, n = 39+0–4B; E, *Spies 5339*, metaphase I with 1 univalent, n = 26+0–2B. F, *Pentaschistis* spp, *Spies 5768*, metaphase I with 1 univalent, n = 7+0–2B. Scale bar: 8.5 mm.

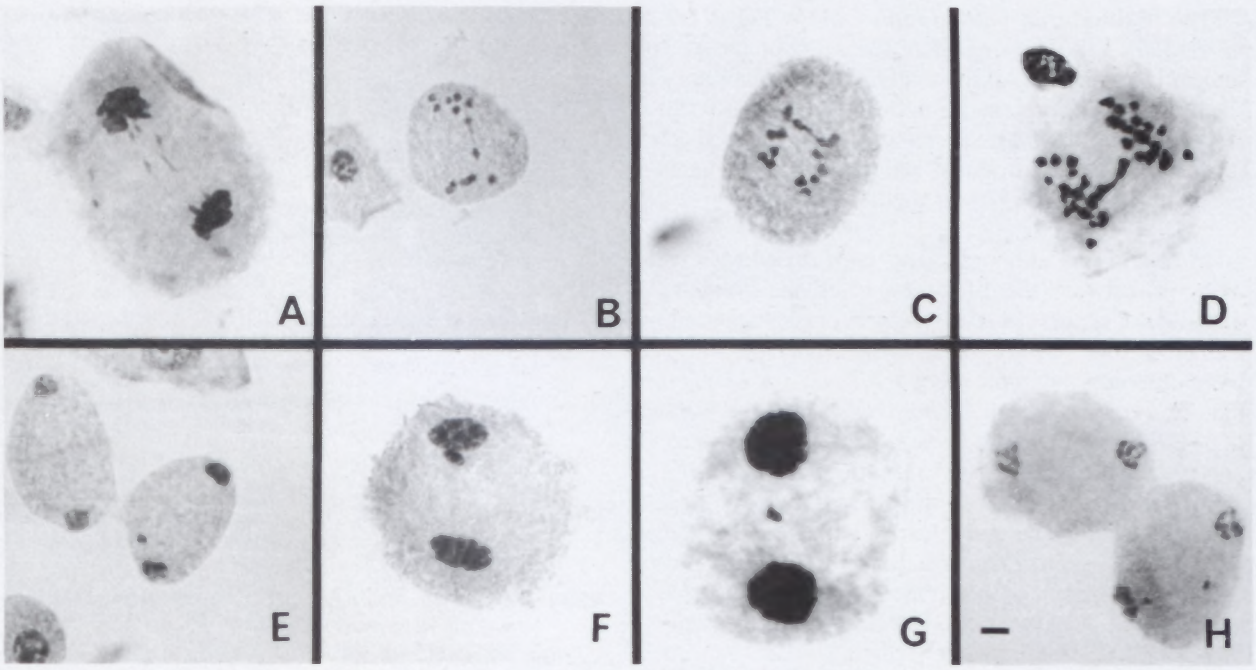


FIGURE 7.—Meiotic chromosomes in *Pentaschistis* specimens with abnormalities. A, G, *P. eriostoma, Spies 5319*: A, anaphase I cell with five lagging chromosomes; G, telophase I cells with micronuclei, n = 39+0–4B. B, *P. tomentella, Spies 3773*, anaphase I bridge, n = 7. C, D, *P. rigidissima*: C, *Spies 5431*, anaphase I bridge, n = 7+0–2B; D, *Spies 5458*, anaphase II bridge, n = 21. E, F, H, telophase I cells with micronuclei: E, *P pallida, Spies 5393*, n = 7; F, *P. triseta, Spies 4413*, n = 7; H, *Pentaschistis* spp., *Spies 5768*, n = 7+0–2B. Scale bar: 8.5 mm.

*P. triseta*, *P. tortuosa*, ) were young polyploid complexes. In a young polyploid complex diploidy prevails, but higher levels of ploidy do occur (Grant 1981). Polyploidy prevails in the mature polyploid complexes, but diploidy still occurs. No evidence of mature polyploidy was found.

In old polyploid complexes only polyploid levels are observed (Grant 1981). A few species (*P. airoides* subsp. *jugorum*; *P. argentea*; *P. aristifolia*; *P. barbata* subsp. *barbata*; *P. colorata*; *P. lima*; *P. malouinensis*; *P. mannii*; *P. minor*; *P.* aff. *patula*; *P. rupestris* & *P. viscidula*), were classified as old polyploid complexes. It is important to note that in almost all the old polyploid species, only one or a few specimens were examined. More specimens should be studied to verify these hypotheses. The classification of young polyploid complexes is better supported, since more specimens were examined. Representatives of both the basic chromosome numbers (x = 7; x = 13) were classified as young polyploid complexes, with similar frequency ratios (Figure 4). The ploidy levels obtained in this study, combined with existing data, suggest that the genus *Pentaschistis* is a young polyploid hybrid complex (Figure 4). Hybridisation could have resulted in the formation of a basic chromosome number of x = 13 for *P. eriostoma* and *P. borussica*. However, morphological and anatomical data (Linder & Ellis 1990; Linder *et al.* 1990; Ellis & Linder 1990) supports the inclusion of *P. borussica* in the genus *Pentaschistis*. It is thus more probable that polyploidisation and subsequent aneuploidy of a *Pentaschistis* species, or hybridisation between two different *Pentaschistis* species and subsequent aneuploidy, resulted in *P. borussica*. This is not true for *P. eriostoma*. Morphological and anatomical data placed *P. eriostoma* in group 6. According to Linder & Ellis (1990) and Ellis & Linder (1990) this is the group of species with uncertain affinity. It is more probable that *P. eriostoma* was formed by hybridisation of two unrelated species. Genomic *in situ* hybridisation (GISH) analysis, using various putative parental genomes as probes, should be used to determine the origin of the x = 13 basic chromosome number.

Univalents were observed in nearly all of the studied specimens (Figure 6A–F). Lagging chromosomes were observed during anaphase I in *P. aristidoides, P. curvifolia, P. eriostoma, P. lima, P. pallida, P. rigidissima, P. rupestris, P. tomentella, P. viscidula* and one of the two unnamed *Pentaschistis* species (Figure 7A). A small number of micronuclei (Figure 7E–H) was observed throughout the whole genus *Pentaschistis*. Anaphase I or II bridges (Figure 7B–D) were observed in five of the specimens studied. This is most probably the result of paracentric inversion. The frequency of these abnormalities was so low that it should not affect the fertility of the specimens.

In *P. pallida* (*Spies 4406*), a diploid (n = 7+0–1B), five bivalents and one quadrivalent were observed. In this diploid, seven bivalents are expected. A quadrivalent was also observed in a diploid specimen of *P. eriostoma* (*Spies 5370*). This phenomenon can be the result of a balanced translocation. If complete pairing between the two non-homologous chromosomes occurred, a quadrivalent would result. Three types of division are possible during anaphase I. If adjacent type I or II division occurred, there would be no fertile pollen. During adjacent type I division, the two centromeres belonging to non-homologous chromosome pairs move to the one pole and the other two to the other pole. During adjacent type II division, the two centromeres from a homologous chromosome pair move to each pole. If alternate type division occurred, all the pollen would be fertile. During alternate type division, the chiasmata of all the chromosome pairs lie on the metaphase plate and the centromeres of the non-homologous pairs move to the same pole (Schulz-Shaeffer 1980).

The fertility of *P. pallida* (*Spies 4406*) and *P. eriostoma* (*Spies 5370*) pollen is thus dependent on the type of division that occurs. All the material in our laboratory is fixed after collection, so no pollen germination fertility test could be done. More specimens of these two species must be collected to study whether this phenomenon occurs frequently or if it was a unique event.

In conclusion, although cytogenetic differences were observed between the different species, the cytogenetic data did not support or reject the groupings suggested by Linder & Ellis (1990) and Ellis & Linder (1990). The basic chromosome numbers of x = 7 and x = 13 suggest that *P. eriostoma* and *P. borussica* must be further removed from the other *Pentaschistis* species. Since *P. eriostoma* shows little cytogenetic, morphologic or anatomic similarity to the other species, we do suggest that *P. eriostoma* is not closely related to the other species of the genus *Pentaschistis*. The data also indicate that most *Pentaschistis* species form young polyploid complexes. Species classified as old polyploid complexes were usually inadequately studied.

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