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# PLOIDAL CHANGES IN CLONAL CULTURES OF SPIROGYRA COMMUNIS AND IMPLICATIONS FOR SPECIES DEFINITION[1]

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ABSTRACT

A clonal culture of *Spirogyra* filaments of initially uniform width produced filaments of three additional significantly different widths. Group I filaments of the original clone were 30.9 ± 0.7 μm wide (mean ± SD, $N = 50$). Group I filaments produced Group II filaments (22.0 ± 1.1 μm) through vegetative growth and sexual reproduction. Zygospores from homothallic Group I filaments produced germlings representative of Groups I and II; zygospores from homothallic Group II filaments produced germlings representative of Group II only. Germlings of Groups III (27.7 ± 1.0 μm) and IV (44.9 ± 0.8 μm) were produced in the cross of I × II. Viable zygospores from homothallic Group III filaments were obtained. Cells of Group IV filaments were initially binucleate and did not conjugate. Of the six intergroup crosses possible, four resulted in conjugation-tube formation only; two crosses yielded zygospores (I × II and III × IV). Germlings from the successful cross of Groups III and IV produced filaments of all four groups. Chromosome counts were: Group I (24), Group II (12), Group III (18), and Group IV (24, one nucleus). Relative nuclear fluorescence values of mithramycin-stained DNA were (mean ± SD, $N \geq 30$): Group I (11.1 ± 1.4), Group II (5.7 ± 0.7), Group III (8.8 ± 1.3), and Group IV (10.0 ± 0.9, one nucleus). Cytologically, Group II appears to be a diploid ($2x$), Group I a tetraploid ($4x$), and Group III a triploid ($3x$). Systematically, Groups I, II, and III key out to *Spirogyra singularis, S. communis,* and *S. fragilis,* respectively, using Transeau's mongraph of the family Zygnemataceae. These species are interpreted to represent a species complex of *S. communis* (whose name has priority) with the ancestral haploid ($x = 6$) missing.

POLYPLOIDY is an important process in the chromosomal evolution of higher plants (Stebbins, 1971), in which it promotes rapid speciation (Levin, 1983). In contrast, ploidal changes in algae have been little studied, although Nichols (1980) reported that polyploidy is known in most major algal divisions, especially in the green algae (Chlorophyta). Polyploids in algae occur as true hybrids, can be produced by laboratory treatment with chemicals or radiation, and are known to arise spontaneously in cultures through sexual or asexual processes. Cytological and ecological studies are needed in both the laboratory and field to elucidate the relationship of polyploidy in algae to chromosomal evolution and speciation.

The green algal family Zygnemataceae consists of 13 genera of unbranched filaments that reproduce sexually by conjugation (Hoshaw, 1968; Hoshaw, McCourt and Wang, 1986). Species descriptions in this family have been based on morphology, without consideration of the effects that ploidal changes might have on morphological and cytological characteristics. As a result, monographs on the Zygnemataceae (Transeau, 1951; Randhawa, 1959; Kadlubowska, 1972) have included ever-increasing numbers of species descriptions as more minor morphological variants were discovered. The genus *Spirogyra* now includes over 300 species, all described from field collections without reference to data from cultural studies of species complexes, polyploids, or hybrids. Although data from such cultural studies are limited, they should begin to play a role in taxonomic treatments of this family.

Filament width is a major character used in all species descriptions in the Zygnemataceae, but its use is complicated by the occurrence of species complexes containing filaments of very different widths. Species complexes are presumed to consist of the haploid and derived

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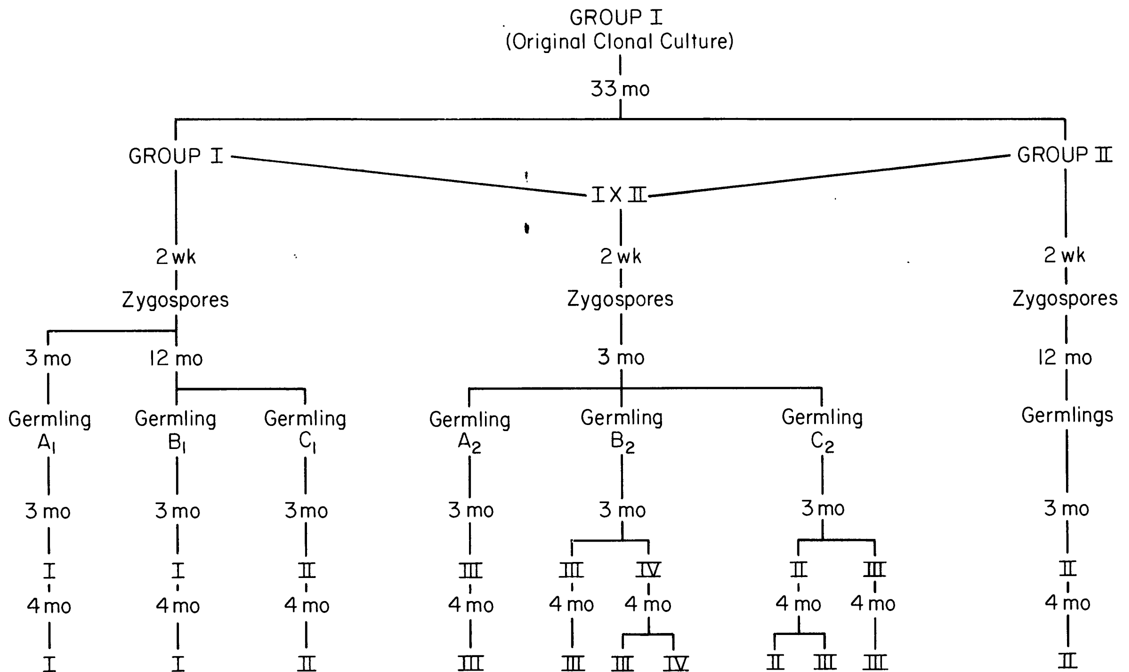


Fig. 1. Chronology of the occurrence of filament-width groups following the establishment of clonal cultures of *Spirogyra communis.* Filaments of Group II developed in cultures of Group I; filaments of Groups III and IV developed from the germination of zygospores produced by a cross between filaments of Groups I and II.

forms of higher ploidal levels. A higher ploidal level typically results in wider filaments with more chloroplasts and larger zygospores than the haploid. Allen (1958) reported a species complex for *Spirogyra pratensis* Transeau, which consisted of haploid, diploid, and tetraploid forms derived from a clonal culture. Variations in filament width were also found in *Zygnema circumcarinatum* Czurda by Miller and Hoshaw (1974), who proposed that these variations were caused by polygenes whose influence was additive.

The present paper describes increases and decreases in filament width that occurred in cultures of a *Spirogyra* species during vegetative and sexual reproductive phases. Changes in filament width were examined in relation to ploidal changes, which were determined by chromosome counts and measurement of nuclear DNA content. Filament-width changes are discussed in relation to their role in species definition in *Spirogyra.*

MATERIALS AND METHODS—A natural collection containing *Spirogyra* was obtained in June 1978 from Bear Creek near Sycamore

TABLE 1. *Summary of morphological and cytological observations on four filament-width groups of* Spirogyra *originating from a field collection of* S. singularis. *(N = 50, 10 cells in each of five filaments; for fluorescence data N ≧ 30 cells from five or more filaments)*

| | Filament group | | | |
|---|---|---|---|---|
| | I[a] | II | III | IV[b] |
| Mean cell width ± SD (μm) | 30.9 ± 0.7 | 22.0 ± 1.1 | 27.7 ± 1.0 | 44.9 ± 0.8 |
| Mean cell length ± SD (μm) | 120.7 ± 26.0 | 107.5 ± 20.2 | 153.7 ± 40.6 | 134.3 ± 28.8 |
| Mean nuclear width ± SD (μm) | 6.1 ± 0.9 | 6.6 ± 1.1 | 7.0 ± 1.1 | 7.9 ± 1.0[c] |
| Mean nuclear length ± SD (μm) | 12.0 ± 1.6 | 10.4 ± 1.9 | 12.1 ± 3.2 | 11.3 ± 2.1[c] |
| Chloroplast number | 1–2 | 1 | 1–2 | 2 |
| Chromosome number | 24 | 12 | 18 | 24[c] |
| Relative nuclear fluorescence | 11.1 ± 1.4 | 5.7 ± 0.7 | 8.8 ± 1.3 | 10.0 ± 0.9[c] |

[a] Original collection from field.
[b] Data for binucleate strain initially isolated.
[c] Data for one nucleus.

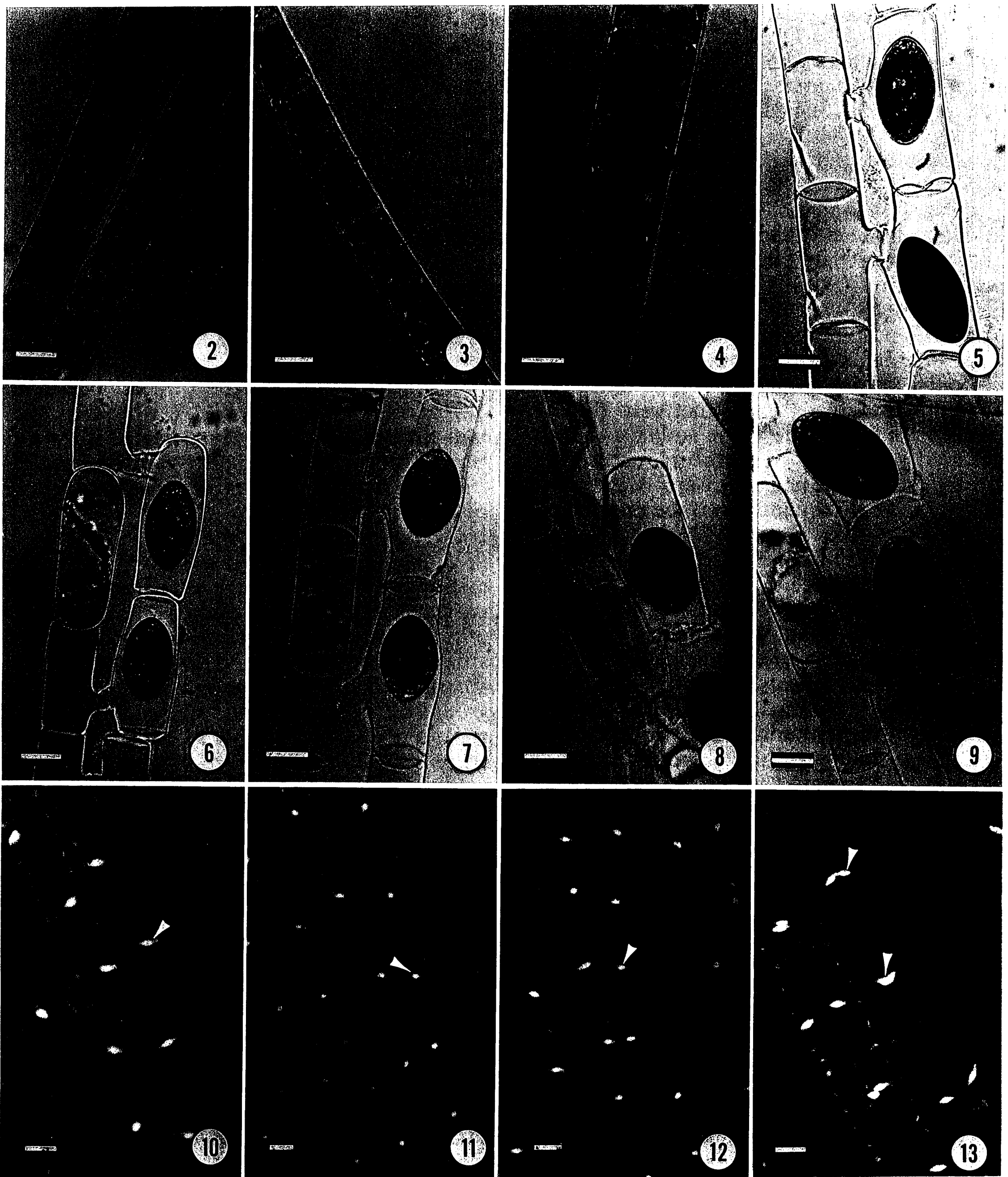


Fig. 2–13. Vegetative and reproductive filaments of *Spirogyra communis* showing characteristics of four filament-width groups (c, chloroplast; n, nucleus; p, pyrenoid). Scale bars = 20 $\mu$m. **2.** Filaments from original isolate: Group I (left), Group II (right). **3.** Filament of Group III from germling $A_2$ from a zygospore produced by a cross of Groups I and II. **4.** Filament of Group IV from germling $B_2$ from a zygospore produced by a cross of Groups I and II. **5.** Conjugation in Group I (homothallic) showing two zygospores. **6.** Conjugation in Group II (homothallic) showing two zygospores. **7.** Conjugation in Group III (homothallic) showing two zygospores. **8.** Conjugation between Groups I and II showing two zygospores. **9.** Conjugation between Groups III and IV showing two zygospores. **10–13.** Filaments showing nuclear fluorescence (arrows) following staining with mithramycin: Fig. 10, Group I; Fig. 11, Group II; Fig. 12, Group III; Fig. 13, Group IV (binucleate).

Spring Reservoir in the Santa Catalina Mountains northeast of Tucson, Arizona. The alga was found in abundance on the surface of the water as slimy masses of filaments. The alga was initially identified as *S. singularis* Nordstedt, based on filament width and zygospores produced by conjugating filaments in laboratory culture.

Pieces of filaments 3–5 cells long were isolated as clonal cultures in test tubes and later grown in one-half pint milk bottles of soil-water medium (Starr, 1978). These strains were grown under 40-w cool-white fluorescent lamps at 50 $\mu E \cdot m^{-2} \cdot s^{-1}$ on a 16:8 LD cycle at 20 ± 2 C. Filaments from cultures were observed periodically for changes in filament width. When filaments of a distinctively different width were found, they were isolated and subcultured separately from the parent culture. Isolates were assigned to groups based on filament width. Filaments from the original collection were called Group I. Groups II, III, and IV subsequently were named in chronological order of appearance. The following measurements were made for 50 cells of each group (10 cells from each of five filaments): cell width, cell length, nuclear width, nuclear length, and number of chloroplasts per cell. Small masses of filaments were transferred to new medium every 6 wk to maintain active growth.

Conjugation was induced by transferring 14-day-old filaments to the surface of 1.2% tapwater agar (pH 6.8) in 10-cm petri dishes. Filaments were checked after 14 days for the presence of maturing zygospores, which if present were then allowed to dry in covered petri dishes for at least 30 days. To induce zygospore germination, small pieces of dried agar with zygospores were transferred to 60-mm petri dishes and covered with soil-water medium. Germlings began to emerge in 3–4 days and were isolated as clonal cultures at the 5–6 cell stage.

Chromosome counts were made for a minimum of 25 cells from each group. Filaments were fixed routinely 1 hr after the onset of the dark period of the 16:8 LD cycle when cells were in late prophase or metaphase. Fixation was in 3 parts 95% ethanol : 1 part glacial acetic acid until all color was removed from chloroplasts. Staining was by the Feulgen technique (Hoshaw and Wells, 1982).

Measurement of nuclear DNA was made by the method described by Hull, Hoshaw and Wang (1982) for staining with the fluorochrome mithramycin. Cells were fixed for staining 2 hr after the beginning of the light period. Because cell division in these organisms occurs at a low rate and because we observed no extreme outliers in the readings for filament populations, all cells were assumed to be at approximately the same stage in the cell cycle. Fluorescence of stained DNA in nuclei was measured using a Leitz Ortholux microscope equipped with a Leitz MPV photometer head containing an EMI 6094 photomultiplier tube. Measurements of fluorescence (proportional to nuclear DNA content) were made using a Dana Digital Voltmeter, Model 5403. Photomicrographs of fluorescing nuclei were made with Ektachrome 400 Daylight film. DNA content was measured for a minimum of 30 nuclei for each of the four groups.

RESULTS—Although the original isolate of *Spirogyra* was identified as *S. singularis*, certain filaments that developed later from this isolate were characteristic of *S. communis* (Hassall) Kützing, a name we will use hereafter because it has priority.

Over a period of 45 mo the clonal culture of *S. communis* produced filaments of four distinctive widths through vegetative and sexual cycles of the alga in culture. The chronology of the occurrence and development of four filament-width groups is illustrated in Fig. 1. Filaments were clearly assignable to the four groups on the basis of width (Fig. 2–4). There was no gradient of filament width, and differences between groups were distinct (Table 1).

Filaments of Group IV contained a majority of binucleate cells when they were first subcultured. Data for this primarily binucleate strain are shown in Table 1. However, after a year or more in culture a majority of the cells in Group IV filaments were found to have reverted to a uninucleate condition and filament width had narrowed to a value similar to filaments of Group I.

Filaments of the original isolate (Group I) were transferred periodically, and after 33 mo filaments of Group II (Fig. 2) were found in a Group I culture. Because of the absence of any observable conjugation in the culture, these narrow filaments of Group II were apparently produced during growth by cell division. Group II filaments were subcultured separately as a second width group. Groups I and II were homothallic and produced many viable zygospores (Fig. 5, 6). Group III filaments were homothallic and produced viable zygospores (Fig. 7), whereas Group IV filaments produced conjugation tubes but no zygospores.

Cultures from germlings of Group II zygospores contained filaments representative of Group II throughout a 7-mo examination period (Fig. 1). Three germlings ($A_1$, $B_1$, and $C_1$) from Group I zygospores were isolated. During

a 7-mo period germlings $A_1$ and $B_1$ produced Group I filaments that remained stable in width; germling $C_1$ produced Group II filaments that remained stable in width (Fig. 1).

Of the six intergroup crosses possible, four resulted in conjugation-tube formation only, and two crosses yielded zygospores: I × II (Fig. 8) and III × IV (Fig. 9). The cross of Groups I and II produced numerous viable zygospores. The variation possible in offspring was demonstrated by three germlings ($A_2$, $B_2$, and $C_2$), which produced filaments of three width groups during a 7-mo examination period (Fig. 1). After 3 mo, germling $A_2$ had produced filaments of Group III, germling $B_2$ produced filaments of Groups III and IV, and germling $C_2$ produced filaments of Groups II and III. Filaments of each width group were subcultured and examined over an additional 4-mo period. Group III filaments from germlings $A_2$, $B_2$, and $C_2$ remained stable in width. In contrast to the stability of Group III filaments, Group IV filaments from germling $B_2$ produced filaments of Groups III and IV, and Group II filaments from germling $C_2$ produced filaments of Groups II and III.

Only a few zygospores were obtained from the III × IV cross, but they were viable. Two germlings from zygospores of the III × IV cross were followed for 15 wk. One produced filaments of Groups II and III and the other produced filaments of Groups I and IV.

All chromosomes observed were dotlike microchromosomes (less than 1.5 $\mu$m long). Chromosome counts revealed euploid differences in ploidy between groups (Table 1). Chromosome counts were consistent for groups regardless of their origin as shown in Fig. 1. Individual nuclei in the originally binucleate cells of Group IV had chromosome counts and DNA fluorescence readings similar to nuclei in Group I (Table 1). Nuclei in cells of the four width groups fluoresced a bright yellow-orange with mithramycin staining (Fig. 10–13), showing that cells of Groups I, II, and III were uninucleate and cells of Group IV were, at least initially, binucleate. Nuclear DNA fluorescence increased in proportion to increases in chromosome number and filament width (Table 1).

DISCUSSION—Our results show that changes in ploidy occurred during both the vegetative and reproductive phases of *Spirogyra communis*. These ploidal changes are significant for species definition because they resulted in changes in morphological traits used in species descriptions. The interconversion of groups through vegetative growth and sexual crosses was more complex than that reported by Allen (1958). The only apparently stable group was III, which, once formed, did not give rise to any others through vegetative growth. Group IV demonstrated that a change from uni- to binucleate condition (and back) can have effects similar to ploidal change. Crosses between groups seemed to increase variations in filament width, either by direct production of filaments that differed in width from either parent, or by subsequent changes in width through vegetative growth of progeny (Fig. 1). Width changes were apparently the direct result of ploidal change (Table 1).

The variability of filament width in *S. communis* differs from that in the series of *S. pratensis* studied by Allen (1958). Her original haploid clone gave rise to two groups of larger filaments through euploid steps to produce diploid and tetraploid strains. By contrast, in the first width change noted in *S. communis* a narrower filament-width group (II) of lower ploidy was derived from wider filaments of higher ploidy following repeated transfer over a 33-mo period. Ploidal changes involving the sexual process (zygospore formation and germination) occurred in the present study but were not studied in detail by Allen.

Observations of vegetative features of germlings of zygnematacean species at the time of zygospore germination are few. Conard (1936a, b) found that *S. majuscula* and *S. maxima* germlings produced mature filaments characteristic of parental filaments. Although Gerassimow (1905) reported that polyploids were produced in *Spirogyra* through zygospore germination, he failed to confirm this observation with cytological evidence. Allen (1958) reported a high degree of stability in filament width of diploid and tetraploid strains of the *S. pratensis* species complex through the sexual phase. However, inheritance of filament width in the haploid strain was variable. She suspected that ploidal changes were frequent during phases of the sexual cycle. In an examination of zygospore germination and germling cultures of *Zygnema circumcarinatum*, Miller and Hoshaw (1974) found that 3 of 63 clones established from zygospores contained filaments of significantly greater cell widths than those of the parental strains. The results of our investigation concur with those of Allen (1958) and Miller and Hoshaw (1974), who concluded that width variation between generations in the sexual cycle was greater than variation during vegetative growth. Regardless of how quantitative changes in structures occurred in *S. communis*, they were of such magnitude that the

resulting four filament-width groups fit more than one species description.

Conjugation between filament-width groups was expected because they are genetically similar, although they contain varying chromosome numbers and amounts of nuclear DNA. However, the conjugation response of the six crosses in *S. communis* was minimal compared to the crosses made by Allen (1958) for her three width groups of *S. pratensis*. She obtained zygotes in all three possible crosses of her haploid, diploid, and tetraploid strains, whereas zygotes were obtained in *S. communis* in only two of the six possible crosses (I × II and III × IV). Allen reported that gamete movement in two of her three crosses occurred in only one direction, from smaller to larger gametangia. Only the tetraploid gametangium was large enough to accommodate the zygospore in crosses of the tetraploid with smaller haploid or diploid filaments. She interpreted this condition as partial sexual compatibility between polyploid forms. Gamete movement in our intergroup crosses was also from small into the large gametangia.

Interpretation of nuclear cytology of the four groups of *S. communis* is complicated because of the presence of microchromosomes and the difficulty of identifying chromosomal features such as nucleolar-organizing chromosomes. Although Godward (1966) concluded that aneuploidy has been more often involved than euploidy in species formation in the Chlorophyceae, the most parsimonious explanations of ploidal changes in our study are based on euploid changes. If aneuploidy were occurring, we would expect to find more continuous variation in filament width, chromosome counts, and nuclear DNA content. Instead, we found four relatively discrete groups of filaments that differed in these measurements.

We offer two interpretations of ploidal levels. In one, Groups I and II with chromosome counts of 24 and 12, respectively, are considered diploid and haploid strains. This interpretation of events is also supported by the nuclear DNA fluorescence data (Table 1).

A second interpretation, and one we consider more likely, is that Group II is a diploid derived from a missing haploid and Group I is a tetraploid in the same series. If this is the case, a cross of I × II would be expected to yield a viable triploid, and Group III does have a chromosome count of 18 and relative nuclear DNA fluorescence intermediate between Groups I and II (Table 1). This interpretation is supported by the stability in width of Group III filaments, which, if triploid, might not readily change to either haploid or diploid forms except through the sexual cycle. In this interpretation, the ancestral haploid is missing from the polyploid series, but if it does exist in some other location, we predict that it would have a chromosome count of 6.

If polyploidy has been a frequent occurrence and an important factor in the evolution of *Spirogyra*, one would expect to find that chromosome numbers of species are multiples of one base number ($x$). There is a suggestion of such a series for *Spirogyra* with $x = 6$ in the compilation of Godward (1966). However, it is essential to correlate chromosome counts with nuclear DNA content in species of the Zygnemataceae because of the frequent occurrence of species with microchromosomes. Fragmentation (agmatoploidy) or fusion could result in changes in chromosome numbers that may or may not have acted in species formation while DNA content remained constant. Only by using chromosome counts and nuclear DNA content together can changes in chromosome number and size be differentiated from changes in actual genetic material.

We question the existence of 323 species of *Spirogyra* as compiled in the monograph of Kadlubowska (1972). Certainly it is common for a field collection of *Spirogyra* to contain filaments of several distinctive widths, which may represent several species as traditionally defined. We have identified as many as 10 distinctive filament widths from a single collection site (Hoshaw, 1982). But are these best described as separate species? In the present study, three of the four width groups that developed from an original clonal culture are identifiable as separate species using the key in Transeau's (1951) monograph. Groups I, II, and III key out to *S. singularis*, *S. communis*, and *S. fragilis* Jao, respectively (Fig. 14). The entire range of filament widths encompassed by Groups I, II, and III is too large to assign them to a single species. But the width ranges of individual groups do not overlap. The width range of each group falls clearly within the range of a described species. Had these three related strains been collected from nature rather than cultured in the laboratory, they would no doubt have been assigned to three separate species. Group IV produced no zygospores, but even so these filaments would not have been assigned to any of the other three species. Allen (1958) also used Transeau's key to identify her haploid strain as *S. pratensis*, her diploid strain as *S. parvula* (Transeau) Czurda, and her tetraploid strain as *S. catenaeformis* (Hassall) Kützing. She referred to her polyploid series of *S. pratensis* as a species complex, and *S. communis* in our study deserves a similar taxo-

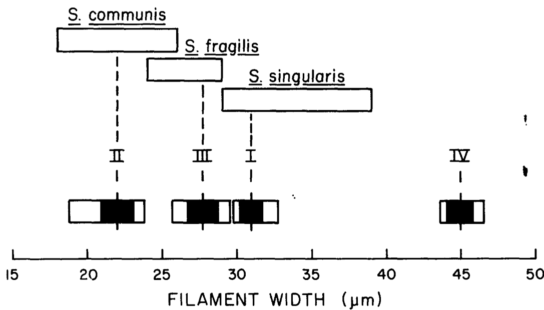


Fig. 14. Comparison of ranges in filament width for three species of *Spirogyra* as described by Transeau (1951) (upper bars) with filament widths of Groups I, II, and III of present study (lower bars). Open bar = range; vertical line = mean; shaded bar = 2 SD around mean.

nomic interpretation. Polyploidy and species complexes have not been recognized in monographs of the Zygnemataceae (Transeau, 1951; Randhawa, 1959; Kadlubowska, 1972). Given the importance of polyploidy in speciation of higher plants, we feel polyploidy should be incorporated into future systematic studies of *Spirogyra* and perhaps other algae.

The spontaneous changes in filament width, ploidal level, and nuclear DNA content that we have observed in the laboratory for *S. communis* after only a short time lead us to suspect that species complexes at any given site may be generated year after year or even more often. If this is so, gene flow between filament-width populations of different ploidal levels may be quite substantial, even in the absence of hybridization. Furthermore, filament-width populations of higher ploidal levels may be generated independently from a common ancestral species in species complexes at different sites. Clearly these filaments of higher ploidal levels would only be indirectly related to each other through descent from a filament-width population of lower ploidy. To assign filament-width populations of high ploidal level to a different species than those of a lower ploidal level would not only obscure the true phylogeny but also would ignore genetic relationships.

Evidence of ploidal changes in *S. communis* in laboratory cultures not only suggests the need for extensive cytological studies, but also arouses our curiosity about such changes in nature. If ploidal variation is common in field populations, it could be a ready source of phenotypic variability, which may have significant effects on the range of ecological conditions tolerable by *Spirogyra* populations. Studies are currently in progress on filament types collected from the original Bear Creek site that appear to represent naturally occurring strains of Groups I and II. We are investigating the potential for gene flow between filament groups collected from a single field site and between laboratory-derived and field-collected filament groups.

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