Chromosome Numbers for Species of Characeae from Southern Illinois

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ABSTRACT: Chromosome numbers were determined for five species of *Nitella* and five species of *Chara* representing 27 naturally occurring populations. Chromosome counts were as follows: *Nitella mirabilis*, $n=6$; *N. flexilis*, $n=12$; *N. acuminata*, $n=18$; *N. axillaris*, $n=18$; *N. oligospira*, $n=18$; *Chara braunii*, $n=14$; *C. contraria*, $n=42$; *C. globularis*, $n = 28$; *C. foliosa*, $n = 14$; and *C. haitensis*, $n = 42$. Relationships among these and similar forms reported from elsewhere in North America are discussed.

INTRODUCTION

Several authors have reported chromosome numbers for species of Characeae in North America (Hotchkiss, 1958, 1963; Tindall and Sawa, 1964; Griffin and Proctor, 1964; Tindall *et al.*, 1965; Sawa, 1965, 1966; Tindall, 1966, 1967, 1970; Proctor, 1970, 1971a, 1971b; Proctor and Wiman, 1971; Proctor *et al.*, 1971; Grant and Proctor, 1972). Reports of chromosome numbers for species of Characeae occurring in other countries are reviewed by Guerlesquin (1966) and Corillion and Guerlesquin (1972). The importance of polyploidy, intraspecific barriers to gene flow, minor ecological differences and geographical distribution in relation to speciation in the Characeae are stressed in several of the above reports. These studies have shown that knowledge of chromosome number is quite useful in resolving taxonomic problems and in clarifying distribution patterns for some species complexes. The present study was carried out to determine relationships between species occurring in southern Illinois and those previously reported from elsewhere in North America. Previous work on the taxonomy of the Characeae of Illinois was reviewed by Stotler (1968).

MATERIALS AND METHODS

Twenty-seven naturally occurring populations of Characeae in southern Illinois were examined. These populations represented five species of *Nitella* and five species of *Chara*. All collections consisted of cytological specimens and voucher specimens. Cytological material for *Nitella flexilis* was obtained from culture of the field collection.

Cytological material was fixed in an absolute ethanol-glacial acetic acid (3:1) solution for 24 h. Following fixation, cytological specimens were stored in 70% ethanol at 5 C until examined. Antheridial filaments were dissected from immature antheridia and were squashed and stained with aceto-carmine. No attempt was made to standardize the fixing and staining procedure for determining chromosome mor-

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phology, and no pretreatment of living material was undertaken. In all cases, polar metaphase configurations were used for counting, drawing and photographing. Illustrations were prepared from original drawings and photographs using a Kodak Kodalith reversal process.

RESULTS

Species of Characeae, respective chromosome numbers and specimens examined cytologically are included in Tables 1 and 2. A representative metaphase configuration is shown for each species (Figs. 1-10).

DISCUSSION

Nitella mirabilis.—A chromosome number of $n = 6$ is consistent with all previous reports for this species. Sawa (1965) reported $n = 6$ for *N. mirabilis*-like plants from Kentucky and Kansas. Tindall (1967) reported $n = 6$ for the species from Kansas, Oklahoma and Texas. Sarma and Khan (1964, 1965) reported $n = 6$ for the species

TABLE 1.—Chromosome numbers and localities for species of *Nitella* examined from southern Illinois. Collection numbers consist of collectors' initials followed by year, month, day and number of collection on that day

Species	Chromosome numbers (n)	Specimens examined collection no.	Locality
N. mirabilis Nordst. ex J. Gr.	6	DT 67-4-16-1	Roadside ditch, 2 miles S Junction Ill. Hwys. 149 and 3, Jackson Co.
N. flexilis (L.) Ag.	12	TH & DT 68-12-20-1	LaRue Spring, LaRue-Pine Hills Ecological Area, Union Co.
N. acuminata Br. ex Wall.	9	DT 67-8-2-1	Cambria Neck, Crab Orchard Lake, Williamson Co.
	9	TW & DT 68-7-8-1	Madison Pond, Williamson Co.
	9	TW & DT 68-7-17-1	Mermet Lake Conservation Area, Massac Co.
	9	TW & DT 69-8-7-1 & TW & DT 69-8-7-5	Little Grassy Lake, Williamson Co.
N. axillaris A. Br.	18	DT 70-7-2-1	Winter's Pond, LaRue-Pine Hills Ecological Area, Jackson Co.
	18	JY & RH 72-9-11-1[a]	Devil's Kitchen Lake, Williamson Co.
N. oligospira A. Br.	18	DT 70-7-2-2	Winter's Pond, as above.

[a] Collected by J. Yopp and R. Holst, Department of Botany, Southern Illinois University

from India. From a taxonomic point of view, *N. mirabilis* shows close affinity with *N. opaca* ($n = 6$; Sawa, 1965) and *N. missouriensis* ($n = 6$; Tindall, 1967). *N. mirabilis* is distinguished from these two species by consistent production of adventitious branchlets and gametangia at the bases of branchlet whorls. *N. mirabilis* from southern Illinois displays several morphological features similar to those described for *N. bastinii,* which is known only from a single collection from the type locality in northern Illinois (Allen, 1894).

N. flexilis.—Our counts of $n = 12$ confirm previous reports for the species from North America (Hotchkiss, 1963; Tindall and Sawa, 1964; Sawa, 1965). Imahori and Kato (1961) and Guerlesquin (1963) also reported $n = 12$ for the species from Japan and France,

TABLE 2.—Chromosome numbers and localities for species of *Chara* examined from southern Illinois

Species	Chromosome numbers (n)	Specimens examined collection no.	Locality
C. braunii Gm.	14	TW & DT 68-6-13-1	Lake Murphysboro, near boat dock, Jackson Co.
	14	TW & DT 68-7-8-4	Cambria Neck, Crab Orchard Lake, Williamson Co.
	14	TW & DT 68-7-15-1	Lake Murphysboro, near Archery Range, Jackson Co.
	14	TW 69-6-13-1	Crab Orchard Lake, Williamson Co.
C. contraria Kütz.	42	TW & DT 69-5-12-1	Stripmine pond, near DeSoto, Jackson Co.
C. globularis Thuil.	28	TW & DT 68-7-16-3	Lake Murphysboro, near Archery Range, Jackson Co.
	28	TW & DT 69-5-16-4	Lake Murphysboro, near boat dock, Jackson Co.
C. foliolosa Muhl. ex Willd.	14	TW & DT 68-6-21-1	Madison Pond, Williamson Co.
	14	TW & DT 68-6-21-2	Carterville City Lake, Williamson Co.
	14	TW 68-7-17-2	Mermet Lake Conservation Area, Massac Co.
	14	TW & DT 69-8-7-3	Little Grassy Lake, near boat dock, Williamson Co.
	14	TW & DT 69-8-7-6	Little Grassy Lake, near dam, Williamson Co.
	14	TW & DT 69-8-7-8	Devil's Kitchen Lake, Williamson Co.
	14	DT 69-8-18-3	Farm pond, Crab Orchard Wildlife Refuge, Williamson Co.
C. haitensis Turp.	42	TW 69-6-13-2	Stripmine pond, near Cambria, Williamson Co.
	42	TW & DT 69-9-16-2	Stripmine pond, near DeSoto, Jackson Co.

respectively. Sawa (1965, 1966) presented evidence that led him to suggest that the $n = 12$ genome of *N. flexilis* was comprised of $n = 6$ genomes from *N. opaca* and *N. mirabilis*-like plants. The hypothesis which can be drawn from these findings is that the monoecious *N. flexilis* represents an established allopolyploid which was derived

Figs. 1-10.—Polar view of metaphase configurations of species of *Nitella* and *Chara* from southern Illinois. 1. *N. mirabilis.* 2. *N. flexilis.* 3. *N. acuminata.* 4. *N. axillaris.* 5. *N. oligospira.* 6. *C. braunii.* 7. *C. contraria.* 8. *C. globularis.* 9. *C. foliolosa.* 10. *C. haitensis*

through hybridization of two ($n = 6$) dioecious species. Our observations lend support to this hypothesis in that we have shown further consistency in chromosome numbers of *N. mirabilis* and *N. flexilis* over a larger part of their geographical ranges in North America.

N. acuminata.—A chromosome number of $n = 18$ also has been reported for this species from Kentucky (Hotchkiss, 1958) and North Carolina (A.T. Hotchkiss, pers. comm.). Imahori and Kato (1961), Sarma and Khan (1965) and Hotchkiss (1965) reported $n = 18$ for the species from Japan, India, and the Fiji Islands, respectively.

In contrast, Tindall (1970) reported $n = 9$ for *N. acuminata* from Oklahoma, Texas and northeastern Mexico. The southern Illinois populations are of special interest because of their close geographical proximity to $n = 9$ populations. Examination of both forms revealed only minor morphological variations. A detailed analysis of physiology, morphology and ecology of the 18-chromosome form (from southern Illinois) has been completed (Yopp, 1968). Similar studies on the 9-chromosome form are in progress.

N. axilaris.—Chromosome numbers have not previously been reported for *N. axillaris* from North America. The report of $n = 18$ for specimens from India is the only previously published count for the species (Sarma and Khan, 1965). However, A. T. Hotchkiss (pers. comm.) has counted $n = 18$ chromosomes for *N. axillaris* from Boulware Spring, Florida. Specimens from the same locality were described by Wood (1965) as *N. translucens* var. *axillaris* f. *caroliniana.* The specimens examined during the present study appear to be most closely allied to *N. translucens* var. *axillaris* f. *axillaris* as interpreted by Wood (1965). The close relationship of *N. axillaris* to *N. translucens* is apparent from their treatment as a single species by Wood (1965). Lindenbein (1927) and Gillet (1959) reported $n = 18$ for *N. translucens* from Europe.

N. oligospira.—The present counts of $n = 18$ represent the first published numbers for this species from the United States. Tindall (1966) reported $n = 18$ for the species from San Luis Potosi, Mexico. Sarma and Kahn (1965) reported $n = 18$ for the species from India. Several authors have reported a chromosome number of $n = 18$ for species closely allied to *N. oligospira: N. mucronata* (Gillet, 1959), *N. microcarpa* ssp. *megacarpa* (Hotchkiss, 1963), *N. megacarpa* (Tindall and Sawa, 1964), *N. dictyosperma* (Sarma and Kahn, 1964), *N. furcata* var. *sieberi* (Hotchkiss, *in* Wood, 1963) and *N. furcata* (Hotchkiss, 1965). Contrasting chromosome numbers have been reported for some of these and related species from Japan (Imahori and Kato, 1961). All of the above species, including *N. oligospira* and some other species not yet examined cytologically, were relegated to minor taxonomic status in a large polytypic species which retained the name *N. furcata* (Wood, 1965).

Chara braunii.—A chromosome number of $n = 14$ for specimens from southern Illinois is consistent with previous reports from Massachusetts (Tindall and Sawa, 1964), Kentucky (Hotchkiss, 1963), Oklahoma, Texas, New Mexico, Arizona (Tindall, 1966) and

Florida (Proctor, 1970). Tindall (1966) also reported $n = 14$ for the species from northern Mexico. Other countries from which *C. braunii* has been reported with a chromosome number of $n = 14$ include Japan (Imahori and Kato, 1961; Sasaki, 1961; Proctor, 1970), India (Sarma and Khan, 1965), Israel, West Pakistan and Argentina (Proctor, 1970). There is but one conflicting report; Corillion, *et al.* (1959) reported $n = 12$ for *C. braunii* from France.

C. braunii is one of the most ubiquitous charophytes in North America. There are numerous taxonomic problems in this species complex, but it appears that investigations of chromosome numbers and chromosome morphology will offer little assistance in their resolution. However, Proctor (1970) clearly established the presence of reproductive barriers among specimens collected from several continents. Proctor suggested that some of these populations might be characterized as geographical races or incipient species.

C. contraria.—A single chromosome number of $n = 42$ was observed in the population examined from southern Illinois. Hotchkiss (1958) reported $n = 28$ and $n = 42$ for the same specimens from a single locality in Kentucky. Tindall (1966) reported $n = 28$ and $n = 42$ for separate specimens of typical *C. contraria* from southwestern United States and northern Mexico. Tindall (1966) also reported $n = 28$, 42 and 56 for a closely related form assignable to *C. inconnexa*. Grant and Proctor (1972) reported $n = 42$ for several specimens from the United States and one occurrence of $n = 14$ from one locality in New Mexico.

C. globularis.—Our counts of $n = 28$ for this species from Lake Murphysboro confirm an earlier report by Proctor (1971a) for specimens from the same locality. The same number has been reported for the species from Massachusetts, North Carolina, Indiana, Kentucky, Kansas, Missouri, Texas, New Mexico and Arizona (Hotchkiss, 1958; Tindall and Sawa, 1964; Tindall, 1966; Proctor, 1971a). Tindall (1966) and Proctor (1971a, 1971b) also reported $n = 42$ for specimens representing typical *C. globularis* from New Mexico. Both $n = 28$ and $n = 42$ have been reported for the species from other continents (Proctor, 1971a).

Wood (1965) synonymized *C. delicatula* ($n = 42$; Tindall and Sawa, 1964), *C. leptosperma* ($n = 28$; Tindall, 1966; Proctor, 1971a, as *C. globularis* var. *leptosperma*), *C. aspera* ($n = 14$; Gillet, 1959; Tindall, 1966; Proctor, 1971a, 1971b) and *C. connivens* ($n = 14$; Gillet, 1959; Proctor, 1971a, 1971b). These species form a complex species group, but all except *C. delicatula* are easily distinguished morphologically and/or cytologically. Proctor (1971a) has more conclusively demonstrated the distinctiveness of these forms by establishing the presence of reproductive barriers between the above species (excluding *C. delicatula*). It should be noted that Proctor (1971a) also observed similar barriers between a few apparently similar morphological types.

C. foliolosa.—The use of the name *C. foliolosa* follows the treatment of Proctor *et al.* (1971). Earlier accounts of this species will be

found under the name *C. sejuncta*. Our counts of $n = 14$ are in agreement with all counts previously reported for the species. Counts have been reported for specimens from Kentucky (Hotchkiss, 1958, 1963), Texas (Tindall, 1966; McCracken *et al.*, 1966) and Oklahoma (McCracken *et al.*, 1966). Imahori and Kato (1961) also reported $n = 14$ for the species from Japan.

Along with a consistent chromosome number, *C. foliolosa* displays consistent morphological features throughout its geographical range. Although the above consistencies are apparent, Wood (1965) reduced "*C. sejuncta*" to a minor form of *C. zeylanica* (*see C. haitensis* below).

C. haitensis.—This species was previously included in the large polytypic species, *C. zeylanica* (Wood, 1965; and others). Although all specimens examined from the two southern Illinois populations revealed a single chromosome number of $n = 42$, the species is known to have either $n = 42$ or 56 (Proctor *et al.*, 1971). Chromosome numbers of $n = 28$, 42, 56 and 70 have been reported for "*C. zeylanica*" from North America (Hotchkiss, 1963; Griffin and Proctor, 1964; McCracken *et al.*, 1966; Proctor and Wiman, 1971). Griffin and Proctor (1964) showed that definitive separation could be made between representatives of the *C. zeylanica* complex based upon antheridial morphology (four-scutate antheridia vs. eight-scutate antheridia). Proctor and Wiman (1971) demonstrated that eight-scutate and four-scutate plants from North America and several other continents were clearly reproductively isolated from one another and that there were no such barriers between clones displaying the same antheridial types. After examination of type specimens, Proctor *et al.* (1971) restored the name *C. haitensis* for plants with eight-scutate antheridia and retained the name *C. zeylanica* for four-scutate representatives of the species group. This systematic treatment results in some clarification of the problem of a wide range of chromosome numbers for a single species; however, there remains some overlap (*C. haitensis*, $n = 42$ or 56; *C. zeylanica*, $n = 28$ or 42). The one report of $n = 70$ for eight-scutate plants (Hotchkiss, 1963) extends the range of numbers of *C. haitensis*, but this number probably should be regarded as an anomaly until further study reveals its true significance.

C. haitensis and *C. foliolosa* are similar in general morphology and both display eight-scutate antheridia, but *C. haitensis* ($n = 42$ or 56) is monoecious with conjoined gametangia whereas *C. foliolosa* ($n = 14$) is monoecious with sejoined gametangia. Furthermore, Proctor *et al.* (1971) reported only negative results from numerous attempted crosses between these two species.

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