Pachytene Morphology of the Chromosome Complement in Cornus florida 'Sweetwater'¹,²

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Investigations of the forty or more species in the genus Cornus have been concerned primarily with taxonomy (Ferguson 1966a, b, Fairbrothers and Johnson 1964). In spite of the known polymorphism within this genus, cytological studies are limited to two major reports (Dermen 1932, Clay and Nath 1971). Dermen (1932) reported chromosome numbers for 23 species of Cornus based on meiotic and mitotic counts. He placed the species into four groups based on somatic chromosome numbers of 2n=18, 20, 22 and 44. On the basis of chromosome number and morphology, he suggested that nine pairs is the basic number in Cornus, and that the forms with 10, 11 or 22 pairs are alterations of the basic number resulting from fission of some larger chromosomes with median or submedian centromeres, or from duplication in the case of the single tetraploid species, C. canadensis. Thus, C. mas with 2n=2x=18 is considered to be the most primitive species, and C. florida with 2n=2x=22 is thought to be derived from it.

Clay and Nath (1971) confirm Dermen's chromosome counts in nine species; report 2n=2x=22 for an additional species, C. Nuttallii; and provide information on the meiotic behavior of five species of Cornus. These workers were unable to recognize some of the morphological features Dermen observed in the mitotic complement of C. florida and some other species. Karyotype analysis was attempted, but was dismissed as being "virtually impossible" because of the small chromosome size and the state of contraction (which precluded detection of many of the centromeres), and the lack of distinguishing morphological characteristics such as secondary constrictions and satellites. However, they did observe the lack of karyotypic differences among species of Cornus. The only karyotype analysis reported for this genus is that by Blair (1975) for Cornus alternifolia (2n=2x=20).

Pachytene analysis may provide an alternative approach to identifying chromosomes of a genome with small chromosomes, particularly if the chromosome number is low. Pachytene chromosomes are more extended, are paired (haploid number), and such criteria as relative length, position of the centromere, and distributional pattern of heterochromatin may be more readily applied to the identification of the chromosomes at this stage than is possible in the somatic complement.

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While studying the meiotic behavior of three species (*C. florida*, *C. kousa*, and *C. Nuttallii*) in a program of interspecific hybridization, we repeatedly found pachytene cells with several isolated, well stained bivalents in which the centromeres were discernible. Since the chromosome number in these three species is low \(2n=2x=22\), it seemed feasible to include pachytene analysis in our cytological studies.

This paper presents a morphological description of the 11 pachytene bivalents in *C. florida* 'Sweetwater' and is the first known report on pachytene analysis in this genus.

**Material and methods**

A single plant of *C. florida* 'Sweetwater' growing in the *Cornus* performance trials at the Ornamental Horticulture Research and Display Gardens at Rutgers University was utilized in this study. Inflorescences were collected during late July and early August, 1977. Bracts were removed prior to fixation. The methods described by Paris et al. (1978) for fixation, storage of buds, pectinase and cellulysin pre-treatments, and slide preparation were adapted for this material. The duration of incubation in the enzymes was variable. Thus, buds were checked periodically until optimal pre-treatment was obtained and, if necessary, the material was stored in the refrigerator in a very diluted pectinase solution for as long as one week, the solution being replenished daily to inhibit development of bacteria and yeast. A one percent staining solution (propionic carmine) was used and the staining time was reduced to two minutes. The materials and methods used for photography and chromosome measurements, as well as the optical equipment, were the same as used by Paris et al. (1978). The terminology recommended by Levan et al. (1965) was used for the localization of centromeres and other chromosome markers.

**Results**

I. **Morphological features of the *Cornus* genome at pachytene**

The following morphological features of the genome of *Cornus florida* 'Sweetwater' have been established. The complement consists of 11 bivalents which are regularly paired. However, there are unpaired terminal or subterminal regions in some bivalents. Morphologically, there are two kinds of bivalents: those which have unique markers and in some cases distinctive length, and consequently are easily recognized in each preparation; and those which are very similar in topology of the markers and in length. The former are described individually; for the latter, a tentative comparative description is presented.

Two microsporocytes with all 11 pachytene bivalents are shown in Figs. 1 and 2. Heterochromatin is abundant in this genome. It is distributed as large blocks and segments around the centromeres, with the short arms being entirely heterochromatic, and as chromomeres and small knobs, alternating with euchromatin in the interstitial and distal regions of the long arms.

With one exception, the centromere in each bivalent is located in a terminal...
Figs. 1–5. 1A, pachytene complement of Cornus florida 'Sweetwater' with the chromosomes rather extended and the nucleolus not stained. 1a, interpretive drawing. (Black bars at lower right corner of figures 1–5 represent 10 μm.) 2A, photomicrograph of a microsporocyte with contracted pachytene chromosomes. 2a, interpretive drawing. 3A, nucleolus-organizing bivalents (chromosomes 1 and 2) in juxtaposition (nucleolus faintly stained). Note the contracted, short arms. 3a interpretive drawing. 4A, the small nucleolus-like body attached to two bivalents (chromosomes 1 and probably 5). 4a, interpretive drawing. 5A, the small nucleolus-like body attached to chromosome 3. 5a, interpretive drawing.
region (t centromere). The single exception of the complement is a bivalent with the centromere in a submedian region (sm centromere). Thus, the karyotype of this species is notably asymmetric.

Typically, two bivalents are involved in organization of the nucleolus (Fig. 3). These chromosomes have been identified as chromosomes 1 and 11 on the basis of chromosome length. Occasionally, a third bivalent (either chromosome 5 or 6) appeared to participate in this function.

In addition to the nucleolus, a small nucleolus-like body was observed in most pachytene cells. It is attached in the interstitial region of the long arm to either chromosome 1 (one of the nucleolus organizing bivalents) (Fig. 6A, a), or to another chromosome, most often identified as 2, less often as 3 (Fig. 5A, a) and rarely as 5. Infrequently, this small nucleolus-like body appeared to be attached to both chro-

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<tr>
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<td>11</td>
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Table 1. Relative length of pachytene chromosomes of *Cornus florida* 'Sweetwater'

II. Description of the chromosomes

a. Individual chromosomes: The isolated bivalents are shown in Fig. 6. The total length in microns of each chromosome is presented in Table 1. These data are based on a total of 12 cells in which all chromosomes were measured. In addition, the average length of each of the five most easily identified and isolated chromosomes is given in the second part of the table based on a total of 25 cells, which include most of the 12 cells in which all chromosomes were identified and measured.

*Chromosome 1* (Fig. 6A, a). This is the longest chromosome (43.8 microns)
of the complement. The most distinguishing features are the regular attachment to the nucleolus and the frequent presence of the small nucleolus-like body in the euchromatic submedian region of the long arm. The short arm is typically elongated and narrow, and two segments are recognizable, if not too contracted. The proximal heterochromatin of the long arm consists of several square segments, and after a long interspersed region, this chromosome is mainly euchromatic with a few isolated chromomeres and a frequently unpaired terminal region. The nucleolus organizing region is located in the short arm.

**Chromosome 5** (Fig. 6E, e). This chromosome is identified by the size and shape of the short arm. The short arm appears to be the largest in diameter in most cells (except for the counterparts of the nucleolus organizing bivalents) and is pear shaped, with a peculiar distal attenuation in some cells. Two important features in the long arm are a distinct knob and a chromomere adjacent to the main heterochromatic region, but isolated by light euchromatin. The euchromatin of this chromosome stains conspicuously lighter distally than proximally. The terminal region is regularly unpaired.

**Chromosome 7** (Fig. 6G, g). It is characterized by a slender short arm, unpaired in some cells, and having an abundance of heterochromatin distributed over the entire length of the chromosome with euchromatic interruptions of variable length. The proximal heterochromatin region of the long arm terminates with three, round segments. Major identifying characteristics are: the two-stranded heterochromatin segment (also seen as two knobs) located in the interstitial region and flanked on both sides by a constriction; the three isolated chromomeres which are located in the subterminal and terminal regions; and the darkly stained distal ends. Occasionally, the terminal region containing the chromomeres is unpaired.

**Chromosome 8** (Fig. 6H, h). This chromosome is unique in that it is the only chromosome with a sm centromere and with small, but noticeable, zones of euchromatin in the short arm. The mean length of the long and short arms based on data from the 12 cells in which all chromosomes were measured and on a total of 25 cells was $13.5 \pm 1.53$ and $6.8 \pm 1.15$, and $13.5 \pm 1.89$ and $6.8 \pm 1.18$, respectively. In both instances, the arm ratio was $2.0 \pm 0.18$. The heterochromatin is distributed asymmetrically around the centromere. Other important markers for this chromosome are the large round segment flanking the centromere in the long arm and two small proximal segments of equal size in the short arm. The short arm has terminal chromomeres.

**Chromosome 9** (Figs. 6I, i; 6I', i'). There are two important markers which distinguish this chromosome from the others. The first is the short arm, which is the smallest of the chromosome arms and which gives the chromosome an almost telocentric appearance. The second is the proximal heterochromatin segment which is adjacent to the centromere in the long arm and is large, oval shaped, and isolated from the adjacent heterochromatin by a short euchromatic zone. The proximal heterochromatin occupies a long portion of the long arm. There are two distinct chromomeres located in the subterminal euchromatic region. The short arm or the entire proximal heterochromatic region was seen unpaired occasionally (Fig. 6I, i). When contracted, this chromosome appears almost all heterochromatic.
Chromosome 10 (Fig. 6J, j). This bivalent appeared similar to chromosome 9 in some cells. However, the short arm is longer than that of chromosome 9 and appears larger in diameter; the heterochromatic segment adjacent to the centromere is smaller and typically square. Important features are two knobs which are distinctively darker staining than the neighboring heterochromatin; the larger one is located adjacent to the main (proximal) heterochromatic region, whereas the smaller one is in the euchromatic submedian region. The chromosome, when contracted, appears strongly heterochromatic.

Chromosome 11 (Fig. 6K, k). This is the shortest chromosome (16.8 microns) of the genome. The most distinguishing feature of this bivalent is the regularity with which it is attached to the nucleolus (Figs. 2A, a; 3A, a). The short arm appears large in diameter in most cells. However, occasionally it was observed to be elongated and more narrow, and two segments could be recognized (Fig. 6K, k). The proximal region of the long arm is subdivided into three segments; a small euchromatic zone separates the third segment from the proximal ones. After a short interspersed region, the remaining portion of the long arm is mostly euchromatic. A darkly stained chromomere, mostly unpaired, is an important and consistent marker of the terminal region. The distal segments may be seen unpaired. The nucleolar organizing region is located in the short arm.

b. Group of similar chromosomes: Four chromosomes showed similarity of length and morphology in many cells. However, major distinguishing characteristics were identified for each of these chromosomes which are tentatively classified as 2, 3, 4 and 6 (Figs. 6B, b; 6C, c; 6D, d; 6F, f). These characteristics are the relative size of the short arms, and the proportional size within each chromosome of the short arm and the proximal heterochromatic segment in the long arm. Chromosome 3 (Fig. 6C, c) has the largest, and chromosome 6 (Fig. 6F, f) the smallest, short arm; the short arm appears to be of equal size in chromosomes 2 and 4 (Figs. 6B, b; 6D, d). The proximal heterochromatic segment adjacent to the centromere in the long arm is larger than the short arm in chromosome 2 (Fig. 6B, b), is of equal size in chromosome 3 (Fig. 6C, c), is smaller in chromosome 4 (Fig. 6D, d), and is of conspicuously larger size in chromosome 6 (Fig. 6F, f). In addition, minor distinguishing features were useful in identifying chromosomes within this group. Chromosome 2, though marked by a distinct interspersed region, appears to be the most euchromatic one (Fig. 6B, b); chromosome 3 has several chromomeres in the interstitial region of the long arm and is regularly seen unpaired in its entire subterminal region (Fig. 6C, c). There is a single, mostly unpaired chromomere in the long arm at the median point in chromosome 4 (Fig. 6D, d). Chromosome 6 has a characteristic chromomere pattern in the sub-medial region of the long arm, consisting of a proximal, mostly paired chromomere and a distal, mostly unpaired (double-dotted) one (Fig. 6F, f). This triangular-appearing landmark served as a consistent aid in the identification of this chromosome. There is a similar triangular chromomere pattern in chromosome 3, but it is located closer to the median point and the proximal, rather than the distal, chromomere is unpaired (Fig. 6C, c). Chromosomes 2 and 3 have been identified as the bivalents with the small nucleolus-like body attached to the interstitial region of the long arm.
Discussion

The results presented herein are in agreement with the chromosome numbers reported previously (Dermen 1932, Clay and Nath 1971). However, there are a few morphological aspects of this genome which warrant further consideration as there is some disagreement regarding identification and characterization of individual chromosomes, and new information is presented herein.

The differences noted in morphological detail may result from the fact that much higher resolution was possible in this work with pachytene chromosomes as compared with the earlier studies which involved somatic metaphase chromosomes. The presence of a small chromosome with the centromere in sub-median region (chromosome 8) was not mentioned by Dermen (1932) or Clay and Nath (1971) but it would not have been readily observed in contracted metaphase cells. Preliminary results obtained for other cultivars of this species clearly revealed the presence of such a small chromosome with the centromere in sub-median region. It also was observed in C. Nuttallii (authors' unpublished data), and in C. alternifolia (Blair 1975), and thus seems to be typical for other species of this genus as well.

The remarkable size difference between the longest and the shortest chromosome, as well as the predominantly sub-terminal centromeric positions in C. florida (previously reported by Dermen 1932) is supported by the present results. Such a karyotype is well known in other plant species, and has been termed asymmetric karyotype (Levitsky 1931, after Stebbins 1971). The evolutionary significance of asymmetric vs symmetric karyotype has been debated for the past four decades. It is generally considered that asymmetry is the derived condition, and hence represents the more advanced karyotype (Stebbins 1971). However, Jones (1978) reviewed this subject more recently and pointed out numerous cases in plant evolution where symmetry is more advanced than asymmetry. Further studies of this genus may shed more light on the origin of karyotypic asymmetry in this genus.

Although the chromosomes are considerably larger at pachytene than in somatic cells, comparative analysis for identification of individual chromosomes of this complement proved to be a formidable task. The main reasons are that about fifty percent of the chromosomes are of relatively uniform length and the distributional pattern of heterochromatin is very similar among the chromosomes. Furthermore, the telomeric positioning of the centromere in nearly all of the chromosomes is a major problem. This limits considerably the numerical parameters for characterization of the individual chromosomes. The variability in the condensation and stretching among cells in the preparations obscures small differences in length among the chromosomes. Nevertheless, half of the chromosome complement in this species is useful for experimental cytogenetics. Since Cornus encompasses numerous species (Ferguson 1966a, b) and numerous interspecific hybrids are readily obtainable, this material provides an opportunity to study the phylogenetic relationship of the species and their derivatives. For instance, studies of pachytene in the species with 2n=20 and 22 chromosomes may reveal whether or not these species originated by fission of chromosomes in the species with 2n=18. It is possible that an increase in chromosome number as a result of fission may account for some
of the difficulty encountered in distinguishing individual chromosomes in this study, since fission would be expected to involve metacentric chromosomes and yield chromosomes of similar length with terminal or nearly terminal centromeres.

An unambiguous identification of the remaining chromosomes is difficult. Further refinement of the staining procedure, and the use of specific stains known to be useful in banding procedures, may prove helpful in the identification and characterization of the entire genome.

The nucleolus is organized by at least two bivalents (chromosome 1 and 11). This has been reported for several other plant species (Hyde 1953, Gruber 1947, Jelenkovic and Harrington 1972, Paris et al. 1978). It is well documented that the nucleolus organizing region contains the DNA template producing ribosomal RNA (r'RNA) (Birnstiel et al. 1971). The genetic and evolutionary importance of having r'RNA in two pairs of the complement is not clear at present. Morphologically, it is interesting that no satellites were observed in any PMC studied. However, occasionally the short arm of both nucleolus organizing chromosomes appeared as a heterochromatic double structure, and in some cells, a small euchromatic zone separating the two heterochromatic segments could be recognized in chromosome 1. With the absence of satellites, the precise localization of the r'RNA cistrons in the short arm of chromosome 1 and 11 is uncertain; however, they seem to be in the heterochromatic region. Dermen (1932) reported one medium large chromosome pair with satellites, and a double constriction for the largest chromosome in C. florida. Neither Clay and Nath (1971) nor this present report confirm these morphological observations.

An interesting aspect of this genome is the presence of a small nucleolus-like body on either chromosome 1 or another bivalent during pachytene. Though it is better stained than the main nucleolus, the stainability suggests similarities to the latter one. The appearance of small nucleolus-like bodies during different meiotic stages has been reported by other investigators (Gall 1968, Jelenkovic and Harrington 1972, Jones 1957, Snoad 1956). However, such nucleoli vary greatly in size, number and position on the chromosomes. Hence, the case of this single small nucleolus-like body is somewhat different from those previously reported. Because its stainability is similar to that of the main nucleolus, and because of the consistency with which it appears, it is plausible to consider it as a secondary site of the r'RNA genes. Should further experiments (hybridization in situ) prove that this is indeed a ribosomal RNA site of production, it would be the first case in which two r'RNA tandons are separately located in the same chromosome.

The presence of an abundance of heterochromatin in this genome was not reported previously. This is not surprising, since the delineation of hetero- and euchromatin is far more difficult to recognize in somatic than in meiotic pachytene chromosomes. However, the distribution of heterochromatin in this genome is of considerable interest. Most plant species exhibit one or the other of two distributional patterns: either proximal as in tomato (Barton 1950) and in castor bean (Paris et al. 1978), or interspersed as in rye (Lima-de-Faria, 1952) and in barley (Sarvella et al. 1958). The bivalents of this genome display both patterns.

The partial asynapsis observed in some bivalents is not readily explainable in
this material, especially since some cells showed complete pairing for all bivalents.

Abstract

Morphology of the pachytene chromosomes was studied in *Cornus florida* L. Of the 11 pairs of chromosomes, seven were morphologically identified. The major features by which the chromosomes were distinguished are chromosome length, arm length, position of the centromere and distributional pattern of the heterochromatic chromomeres. Identification of the remaining four chromosomes was difficult, due to their similar length and lack of differential morphological markers. A general feature of the genome is an abundance of heterochromatic material which is distributed mainly around the centromere and the distal portion of the short arm. The genome is karyotypically asymmetric. Two pairs of chromosomes participate in the organization of a single nucleolus. In addition, a small nucleolus-like body associated with an interstitial segment of a bivalent was observed in most pachytene cells.

References


