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Chromosome Identification in the S and H Genomes of *E. trachycaulus* and *E. canadensis* and Several Possible Diploid Progenitors.

BY

© **CHAYAPORN WATTANASIRI**

A thesis submitted to the Faculty of Graduates Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

IN

PLANT BREEDING

DEPARTMENT OF PLANT SCIENCE

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Dedication

This dissertation is dedicated respectfully to the memory of my father,

Pol. Col. Chayant Chumvisoot,

who inspired me to pursue the doctoral degree.

Abstract

The objectives of this study were to identify individual chromosomes of the S and H genomes and to determine genome relationships among the two tetraploid *Elymus* species- *E. canadensis, E. trachycaulus* and their possible diploid progenitor species including- *P. spicata*- S-genome diploid species and *C. bogdanii, C. californicum*- H-genome diploid species. The techniques used were mainly standard (acetocarmine) staining, C- and/or N-banding. The S and H genome heterochromatin can be described as C^+N^- and C^+N^+, respectively. The karyotypes of *E. canadensis, E. trachycaulus* and their possible diploid progenitor species were established on the basis of chromosome length and banding patterns to facilitate comparisons among these closely related species. The sequential acetocarmine staining/N-banding techniques permit the identification of different chromosome pairs in the karyotype especially for satellited chromosomes that are difficult to identify using banded chromosome preparations. The assignment of *Elymus* chromosomes to the S and H genomes was made using comparisons of detailed observations on chromosome morphology as well as the N-banding patterns. The karyotype of *P. spicata* shows two pairs of SAT-chromosomes (1S and 5S). The karyotypes of *C. bogdanii* and *C. californicum* differ in satellite location (1H in *C. bogdanii* and 3H in *C. californicum*). The karyotypes of *E. canadensis* and *E. trachycaulus* are similar with two SAT-chromosomes (1S and 5S) in the S genome and one SAT-chromosome (4H) in the H genome. The H genome chromosomes of *E. canadensis* and *E. trachycaulus* have a closer relationship to the chromosomes of *C. californicum* than to those of *C. bogdanii.* On the basis of chromosome morphologies and N-banding patterns, it can be concluded that the S genome donor in *E. canadensis* and *E. trachycaulus* is *P. spicata* and the H genome donor is most probably *C. californicum.* The karyotype comparisons support the conclusion that *E. canadensis* and *E. trachycaulus* may have evolved from the same origin.

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study, has been crucial to the completion of this study. My beloved children, Chakree and Chalita, brought us joy and happiness, and I am very proud of their understanding in facing unfamiliar circumstances. Without their presence, this research would have been very difficult.

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CHAPTER 1 INTRODUCTION

Triticeae, containing the temperate cereals wheat, barley, rye, triticale, and several forages, is one of the most important tribes in the plant kingdom. Of the 400 species in this tribe, about 250 are perennials including most of the forage grasses. The genus *Elymus*, which contains three genomes H, S, and Y and approximately 150 species, is the largest and most widely distributed genus in the *Triticeae* (Dewey 1984a). About 75% of the *Elymus* species are allotetraploid (2n=4x=28) (Dewey 1984b). *E. canadensis* and *E. trachycaulus* are allotetraploids from North America with the genomic formula SSHH. The S genome is derived from *Pseudoroegneria spicata* Löve (Pursh) (= *Agropyron spicatum* (Pursh) Scribner and Smith) and the H genome from an unidentified species of *Critesion* (= *Hordeum* pro parte) (Stebbins and Snyder 1956; Dewey 1971, 1974b, 1982, 1984a; Wang and Hsiao 1986).

A better understanding of the genetic constitution and evolutionary relationships of the *Triticeae* species and their genomes is essential if we are to utilize this rich source of genetic variation (McIntyre 1988). Disease resistance and wide adaptation are some characteristics that make *Elymus* a genus of interest to wheat, barley, and rye breeders (Dewey 1984a). Sharma *et al.* (1984) reported that several *Elymus* species possess genes for resistance to wheat streak mosaic virus (WSMV) and barley yellow dwarf virus (BYDV). *E. trachycaulus* has tolerance to alkalinity and resistance to stripe rust, leaf rust, and powdery mildew (Report of research activities at the Punjab Agricultural University (PAU) 1992; Walton 1983). In addition, *E. canadensis* has been used as a good source for improvement of cold tolerance in a forage project at the University of Alberta (Kumar and Walton 1992a).

These *Elymus* species are potential sources for improvement of other *Triticeae* species. They are also well suited for cytogenetic analysis because the genomes, buffered by polyploidy, can tolerate structurally altered chromosomes and changes in chromosome number for analysis throughout many generations. In the past several years, attempts to produce wheat-*Elymus* intergeneric hybrids have been made with the objective of gaining insight into the polyploid nature of genome evolution in the genus *Elymus* (Gill *et al.* 1988b). Before gene transfers from *Elymus* to cereal crops can be efficiently made, the construction of genetic and physical maps to locate desirable genes on chromosomes has to be accomplished. Cytogenetically based physical maps of chromosomes are prerequisite for effective introgression.

With the advent of banding techniques, rapid and direct identification of somatic chromosomes has become feasible (Gill 1990). A number of chromosome banding protocols exist. Some, for example Q- and R-banding procedures, identify euchromatic regions and others, like C- and N-banding methods, identify heterochromatic regions. The latter two methods are useful for plant chromosome studies (Gill 1987). Whereas C-banding is a general technique for staining constitutive heterochromatin, N-banding detects specialized heterochromatic regions (Funaki *et al.* 1975; Pimpinelli *et al.* 1976; Jewell 1981). Each technique results in unique banding patterns of individual chromosomes, and the combination of the two facilitates the assignment of specific chromosomes to genomes. Both staining techniques also provide an additional means of studying genome evolution among related diploid and polyploid species (Morris and Gill 1987). Moreover, C- and N-banding have clarified and further substantiated phylogenetic relationships based on the chromosome

pairing of interspecific and intergeneric hybrids (Chen and Gill 1983; Gill and Kimber 1974b; Hutchinson *et al.* 1982; Naranjo 1992).

In addition, the use of heterochromatic bands as cytological landmarks is valuable in the physical mapping of genes, DNA sequences, and foreign chromosome segments. A standard nomenclature was proposed by Gill *et al.* (1988a) for the designation and description of heterochromatic bands of metaphase chromosomes in bread wheat. Due to the use of numerous DNA clones as genetic markers and efficient techniques for physical mapping of genes, there is an urgent need for a standard karyotype and a nomenclature system for the description of chromosome bands of other *Triticeae* species. A standard banded karyotype requires information about chromosome size, centromere position, and arm ratio of individual chromosomes from the measurement of acetocarmine stained and banded chromosomes. The position, size, and intensity of individual bands are also important criteria in chromosome identification (Gill 1987). Chromosome analysis can be combined with molecular data to advance the understanding of the genomes and to facilitate genetic manipulation of cereals and grasses of the *Triticeae* (Heslop-Harrison 1992).

The physical mapping of individual chromosome and a standard nomenclature for the designation and description of chromosomes and bands is available for bread wheat (Gill *et al.* 1991). However, karyotype studies of most *Elymus* species and their possible diploid progenitor species are incomplete, and their standard banded karyotypes have not been constructed. The objective of this study is to provide this basic cytogenetic information for *P. spicata, C. bogdanii, C. californicum, E. canadensis* and *E. trachycaulus*. C- and N-banded karyotypes of *E. trachycaulus* and three possible diploid progenitor species were previously studied by Morris and Gill (1987). They assigned *E. trachycaulus*

chromosomes to S and H genomes by comparing the position and size of heterochromatic regions in this species and its putative progenitor species. The chromosome designations among different species did not imply homoeology. Due to differential contraction even among homologous chromosomes, and polymorphism of banding patterns, the information in karyotypes constructed only on the basis of either chromosome size, or banding patterns is limited. Therefore, for more accurate karyotyping, sequential acetocarmine staining/banding analysis should be employed (Endo and Gill 1984b; Gill and Sears 1988).

Since, there is a lack of homoeologous chromosome information, an effort was made in this study to establish karyotypes of *E. canadensis* and *E. trachycaulus* and their possible diploid progenitors on the basis of chromosome length and banding patterns from sequential acetocarmine staining/banding analysis. Analysis of the S genome and H genome diploid progenitor species by Morris and Gill (1987) indicated that H-genome heterochromatin is mostly C-banding positive and N-banding positive (C^+N^+) whereas S-genome heterochromatin is C-banding positive and N-banding negative (C^+N^-). In this study for the construction of *Elymus* karyotypes, the assignment of chromosomes to the S and H genome is facilitated by the use of sequential acetocarmine staining/N-banding procedures. The chromosomes in each genome are arranged in a standard manner (based on order of decreasing size and centromere position), to facilitate comparisons among all species. Sequential acetocarmine staining/banding analysis has not only been helpful in the correct identification of different chromosomes but has also provided additional information on the heterochromatin content of the chromosome (Gill and Sears 1988). It provides detailed observations on the morphology, especially of the satellited

chromosomes which are difficult to identify on banded chromosomes. This study also determined genome relationships between the two tetraploid *Elymus* species and their progenitors based on karyotypes, which were then compared with meiotic pairing and other related information.

Objectives of this study are as follows:

1. To establish the karyotype of *P. spicata* by using acetocarmine staining and sequential C-/N-banding.

2. To establish the karyotypes of *C. bogdanii*, *C. californicum*, *E. canadensis* and *E. trachycaulus* by using sequential acetocarmine staining/N-banding.

3. To construct the standard banded karyotypes and to identify chromosomes and genomes of the two *Elymus* species and their possible progenitor species.

4. To determine the relationships between the S and H genomes of polyploids and diploid progenitors using standard karyotyping and the nomenclature system of chromosome banding.

CHAPTER 2 LITERATURE REVIEW

1. Plant material and genome relationships

1.1 Plant material

1.1.1 Diploid species

Pseudoroegneria spicata (bluebunch wheatgrass), a common native of western Canadian rangeland, is caespitose, long-anthered, and highly self-sterile (Dewey 1984a). This long-lived, cool-season perennial bunchgrass is widely distributed, ranging from Alaska southward throughout the western United States (US). Bluebunch wheatgrass is an exceptionally drought tolerant species with excellent forage quality and is an apparent progenitor of several other associated grasses on the western range (Dewey 1966b). Report of research activities at the Punjab Agricultural University (PAU), Ludhiana, India (1992) showed that out of 4 accessions of *P. spicata* screened for stripe rust and powdery mildew resistance under natural conditions at Keylong (Himachal Pradesh), 3 were free from stripe rust and all were resistant to powdery mildew. Wang *et al.* (1993) also reported that *P. spicata* was immune to powdery mildew and resistant to highly resistant to barley yellow dwarf virus (BYDV).

Bluebunch wheatgrass is closely related to beardless wheatgrass [*P. spicata* ssp. *inermis* (Scrib.&Smith) Löve] (Dewey 1983). One apparent difference between bluebunch and beardless wheatgrass is the absence of lemma awns on beardless wheatgrass. *Whitmar* beardless wheatgrass and *Secar* bluebunch wheatgrass are the two improved cultivars that are presently available. *Whitmar* was released in 1946 by USDA-SCS and cooperating Agriculture Experiment Stations of Oregon, Idaho, and Washington. It is a diploid cultivar with good drought resistance and good forage production. *Secar* was released by USDA-

SCS and the Agriculture Experiment Stations in Washington, Oregon, Idaho, Montana, and Wyoming. It is more drought resistant than *Whitmar* and is an early maturing, persistent cultivar. It is recommended for seeding in mixtures with other grasses to establish native plant communities (Barker *et al.* 1985).

Pseudoroegneria consists of about 15 species with only one genome, designated S. The S genome is found in combination with other genomes in most species in three polyploid genera -- *Elymus*, *Elytrigia*, and *Pascopyrum*. There are intergeneric hybrids derived from crosses between *P. spicata* and polyploid genera that have the S genome, with the exception of the genus *Pascopyrum* (Dewey 1984a). Development of a nonrhizomatous strain of 'quackgrass' *Elytrigia repens* (L) Nevski [=*Agropyron repens* (L) P. Beauvois] with some adaptability to the semiarid range was accomplished through hybridization, followed by backcrossing, using *P. spicata* as a donor parent (Dewey 1967c, 1976). Characteristics of both parental species were represented in the population, and heritable genetic variation was evident for important agronomic characters including degree of rhizome development and seed yield. Two germplasm releases (RS-1 and RS-2) have been made of this intergeneric hybrid derivative (Asay and Dewey 1981).

Hybrids derived from crosses between diploid *P. spicata* (SS) and tetraploid species of *Elymus* (SSHH) have yielded SSH triploids. The amphiploid derivative of the diploid *P. spicata* x 'thickspike wheatgrass' *E. lanceolatus* (Scrib.&Smith) Gould hybrid is reasonably fertile and genetically stable after six generations of selection. This hybrid was evaluated in range trials for possible release (Barker *et al.* 1985). Crosses between diploid *P. spicata* (SS) and diploid *Critesion spp.* (HH) produce SH intergeneric hybrids. The doubling of the chromosome number of these hybrids produces SSHH amphidiploids. Intergeneric hybrids derived

from crosses between tetraploid *P. spicata* (SSSS) and *A. cristatum* (PP) (Dewey 1964), and *A. desertorum* (PPPP) (Dewey 1967d) were generated, but were sterile.

The genus *Critesion,* to which the species ***bogdanii*** and ***californicum*** belong, consists of about 30 species, all of which had previously been in the genus *Hordeum*. The separation of *Critesion* from *Hordeum* is generally consistent with cytogenetic data. There is little or no homology among the genome of the *H. vulgare* complex and the genome(s) of the remainder of *Hordeum* (Morrison and Rajhathy 1959; Rajhathy and Morrison 1959). *Hordeum* consists of the *H. vulgare* complex plus *H. bulbosum*, with a genome designation of I, whereas *Critesion* consists of the remainder of the species from the *Hordeum* genus with a genome designation of H (Dewey 1984a).

C. bogdanii is a caespitose perennial and has a versatile reproductive system but is mostly self-fertilizing with a high percentage of seed set. It occurs in Central Asia, western Iran, Afghanistan, northern and western Pakistan, northern India, southern Siberia, Mongolia, and northern China. *C. bogdanii* usually grows in saline areas, such as shores of lakes, streams and ponds, and in meadows. Apart from these habitats it occurs in wet places with fresh water, on limestone cliffs, and, rarely, as a weed (Bothmer 1979, Yang *et al.* 1987). *C. californicum* is also a caespitose perennial and mainly self-fertilizing. It is endemic to southwestern California, to the north of the bay area (Baum and Bailey 1989; Bothmer *et al.* 1991). From the report of PAU (1992), there was no incidence of leaf rust observed on two accessions of *C. californicum* screened at Ludhiana. *C. californicum* was found by Wang *et al.* (1993) to be immune to powdery mildew caused by *Erysiphe graminis.*

In addition to being the genetic foundation of *Critesion*, the H genome is a component of most of the approximately 150 species of *Elymus* (Dewey 1982). Thus, intergeneric hybrids derived from crosses between *Critesion* and *Elymus* are easily produced. Hybridization between *Critesion* and *Triticum* has generated considerable plant breeding interest. Kimber and Sallee (1976) produced hybrids derived from crosses between *C. bogdanii* (HH) and *T. timopheevi* (AAGG) which show extremely low levels of chromosome pairing. The prospects of gene exchange between *Critesion* and *Triticum* are remote, but production of amphiploids and addition lines offer a means of bringing whole or partial genomes together. Amphiploids have been produced from *C. bogdanii* x *T. timopheevi* (Kimber 1979), *C. chilense* x *T. turgidum* (Martin and Laguna 1982), and *C. chilense* x *T. aestivum* (Chapman and Miller 1978) hybrids. The *C. chilense* x *T. aestivum* hybrid has shown some promise as a new cereal crop. Addition lines for each of the seven *C. chilense* chromosomes have also been produced (Miller *et al.* 1982).

1.1.2 Tetraploid species

E. canadensis L. (Canada wildrye), a large, coarse, short-lived perennial, and self-fertile bunch grass, is widely distributed throughout the US and Canada, but is most common in the northern Great Plains and the Prairie Provinces (Bowden 1964; Walton 1983). It is a native cultivated species, well adapted to the cold winters in the north. Its palatability is fair and hay quality is best when cut at the boot stage (Richard and Hawk 1945). The seedlings are vigorous, establish quickly but are not highly competitive with other grasses in mixtures (Schwendiman and Hawk 1973). This species has been used extensively for wild bird habitats and for erosion control of critical areas. *Mandan* wildrye is the only

known cultivar and was released in 1946 by the USDA-ARS and North Dakota AES.

This species is important for forage production, soil conservation, and nutrition value, and as a gene pool for cereal improvement. Intergeneric hybridizations for the transfer of disease resistance, cold and drought tolerance have been performed (Aung and Walton 1990; Kumar and Walton 1989, 1990, 1991). *E. canadensis* was used as a parent and hybridized with the following *Triticeae* species: *T. aestivum* (Mujeeb-Kazi and Bernard 1982, 1985; Yen and Liu 1987); *H. vulgare* (Mujeeb-Kazi and Rodriguez 1982; Dahleen and Joppa 1992); *Secale cereale* (Hang and Frankowiak 1984); and other forage grasses (Dewey 1965b, 1966a, 1966b, 1967a, 1967b, 1968a, 1969a, 1970, 1971, 1974a, 1974b, 1977b; Wang and Hsiao 1986; Jensen *et al.* 1989; Aung and Walton 1990; Kumar and Walton 1989, 1990, 1991).

E. trachycaulus (slender wheatgrass) is a self-fertilizing, short-lived perennial bunchgrass. It was the first native grass to be generally used for seeding in the western US and Canada (Rogler 1973) and was formerly known as Canada western ryegrass. It is a valuable and nutritive rangeland grass, highly tolerant to shade, alkaline soil, and with adaptation to a wide range of soil types. It begins growth relatively early in the spring and produces an abundance of palatable forage liked by all classes of livestock. It is well suited for use in disturbed areas to control soil erosion (Walton 1983).

In Canada, the most widely used cultivars are *Revenue* and *Primar* which were developed by the Agriculture Canada Research Station at Saskatoon, Saskatchewan and the USDA-SCS in cooperation with the Agricultural Experiment Stations in Oregon, Washington, and Idaho, respectively. *Revenue*

has shown superior establishment, higher salinity tolerance, better forage quality, and higher yield compared to *Primar* (Walton 1983). *San Luis* is a recent release (1984) from the USDA-SCS, the Agriculture Experiment Stations of Colorado, Utah, and New Mexico and the USDA-ARS. It originated from a seed collection made in 1975 near Center, Colorado. *San Luis* has long seed heads and is tall relative to other native selections of slender wheatgrass. It has potential for quick erosion control at high elevations (Barker *et al.* 1985). All accessions of *E. trachycaulus* screened at Ludhiana and Keylong in India during the summer of 1992 were completely free from leaf rust, stripe rust, and powdery mildew (Report of PAU 1992).

A number of hybrids derived from crosses between these species and other forage grasses have been generated (Boyle and Holmgren 1955; Dewey 1968a, 1977b; Aung and Walton 1987b, 1990; Jensen *et al.* 1989; Kumar and Walton 1990, 1992a, 1992b). Octaploid, hexaploid, and hyperploid plants were generated from the tetraploid cultivar *Revenue* and were studied cytologically (Napier and Walton 1982; Aung and Walton 1987a; Kumar and Walton 1991).

All early attempts to hybridize *Elymus* and *Triticum* failed (Armstrong 1936; Smith 1943; Cauderon 1958). Only in recent years have such crosses been successful. They usually require post-pollination treatment of the florets with gibberellic acid and embryo culturing on artificial media. Sharma and Gill (1981a, 1981b, 1983) reported hybrids derived from crosses between *T. aestivum* (AABBDD) with *E. trachycaulus* (SSHH) [inadvertently identified as *Pascopyrum smithii* (Rydb.) Löve (=*Agropyron smithii* Rydberg)], that were produced by using the embryo rescue technique. Chromosome pairing was negligible (less than one bivalent/cell), indicating few if any homologies between SH genomes and ABD genomes. Mujeeb-Kazi and Bernard (1982) reported success in crosses

between *T. aestivum* and *E. trachycaulus*, but the meiotic data for these hybrids have not been published. Franke *et al.* (1992) produced hybrids derived from crosses between *T. aestivum* and *E. trachycaulus* that survived to maturity, had a perennial habit, but were sterile.

1.2 Genome relationships

The determination of evolutionary relationships on the basis of chromosome pairing in hybrids was first accomplished by Rosenberg (1909) in the genus *Drosera*. Observing chromosome pairing at meiosis of interspecific hybrids has been the major approach to genome analysis of the *Triticeae* for many years. It has been recognized as the most reliable method for studying phylogenetic relationships (Kimber 1983). In Appendix 1 chromosome pairing data of interspecific and intergeneric hybrids are presented, and the genome relationships among species having S and/or H genomes are summarized.

1.2.1 The relationships among species having the S genome

P. spicata and *Pseudoroegneria libanotica* contain highly homologous genomes (Dewey 1969b). *Agropyron ferganense* is an SS diploid and more closely related to *P. spicata* than to *P. libanotica* (Dewey 1981). *P. spicata* is more closely related to *P. libanotica* than to *A. ferganense*.

1.2.2 The relationships among species having the H genome

The H genome of *Critesion violaceum* is different from the one in *C. bogdanii* and is designated as H^v. The H genome of *Critesion bulbosum* is designated as H^b. The H^b genome differs from the H genome in the South American species *Critesion chilense*. The H genome of *C. bogdanii* is different from the one in *C. chilense* (Dewey 1971; Padilla and Martin 1983; Wang and Hsiao 1986). From the study of Bothmer *et al.* (1985), the eight species included in *Critesion* section

Anisolepsis s. lat., viz., *C. pusillum, C. intercedens, C. euclaston, C. flexuosum, C. muticum, C. cordobense, C. stenostachys*, and *C. chilense* were crossed in all combinations to study the relationships among them. The analysis of meiosis in the hybrids shows that all hybrids showed a high pairing frequency which was lower than that observed within each species. This meiotic behavior in the hybrids indicates a high degree of homology or homoeology between the parental genomes in all hybrids and all species having the same basic H genome, regardless of modifications. The only species showing a special crossability is *C. chilense*. The hybrids derived from two crosses viz. *C. flexuosum* x *C. chilense* and *C. pusillum* x *C. intercedens*, showed segregation in fertility, otherwise there were strict sterility barriers among the taxa (Bothmer *et al.* 1985).

Data in Appendix 1, concerning sterility barriers and meiotic pairing, show that the three annual species *C. pusillum, C. intercedens*, and *C. euclaston* are closely related. However, there are no indications that these taxa are cytogenetically closer to each other than to the perennial species. Neither do the North American species *C. pusillum* and *C. intercedens* appear more closely related to each other than they are to South American taxa.

1.2.3 The relationships among species having both S and H genomes (*Elymus spp.*)

Genomes of both *E. canadensis* and *E. lanceolatus* are closely related but distinguished by structural differences consisting of several inverted segments and a small reciprocal translocation (Dewey 1967a). Natural introgression between *E. lanceolatus* and *E. trachycaulus* is highly probable because the two species are sympatric and the F_1 hybrids are partially fertile (Dewey 1975). The genomes of *E. canadensis* are closely related to those of *E. trachycaulus* (Aung

and Walton 199[illegible]; Kumar and Walton 1990). *E. canadensis* has the closest relationship with *E. lanceolatus* and *E. trachycaulus*. The degree of relationship is respectively less with *E. arizonicus*, *E. caninus*, and *E. sibiricus* (Dewey 1968a, 1974a; Jensen *et al.* 1989). However, there is no record of hybridization among *E. canadensis* and any other species possessing the S and H genomes. Besides the close relationship with *E. canadensis* and *E. lanceolatus*, *E. trachycaulus* is more closely related to *E. tilcarensis* than to *E. vaillantianus* (Dewey 1977a; Jensen *et al.* 1989). Similarly, *E. lanceolatus* is more closely related to *E. tilcarensis* than to *E. mutabilis* and *E. patagonicus*, respectively (Dewey 1972, 1977a, 1979). *E. caninus* is more closely related to *E. mutabilis* than to *E. sibiricus* and *E. canadensis*, respectively (Dewey 1968a, 1974a, 1979). *E. sibiricus* has closer relationship to *E. caninus* than *to E. canadensis* (Dewey 1974b).

1.2.4 The relationships among species having both S and H genomes and species with the S genome

E. canadensis and *P. libanotica* share a common genome 'S' which is a modified *P. spicata-libanotica* genome (Dewey 1974b). *E. trachycaulus* and *E. lanceolatus* are more closely related to *P. spicata* than to *A. ferganense* and *P. libanotica,* respectively (Dewey 1965a, 1968b, 1981; Aung and Walton 1987a). *E. caninus* is more closely related to *A. ferganense* than to *P. libanotica* (Dewey 1969b, 1981). The S genome of *E. patagonicus* has more homology with the *P. spicata* genome than with the *P. libanotica* genome (Dewey 1972). The S genome of *P. spicata* is essentially homologous to the S genome of *E. vaillantianus* (Jensen *et al.* 1989). One of the *E. agropyroides* genomes appears to be partially homologous with the *P. libanotica* genome (Dewey 1970). *E. mutabilis* carries a modified version of the S genome in *P. libanotica* (Dewey 1979).

1.2.5 The relationships among species having both S and H genomes and species with the H genome

The H genome in *E. canadensis* is more closely related to the genome in *C. bogdanii* and *C. californicum* than in *C. bulbosum* (Dewey 1971; Wang and Hsiao 1986). There are no reports about this for *E. trachycaulus*. The H genome of *E. caninus* is very similar to that of *C. violaceum* and unlike that in *C. californicum*. The genome of *E. caninus* is designated SH^V (Gupta and Fedak 1985) to reflect this relationship.

2. Chromosome banding

When eukaryotic chromosomes are stained for banding, there is a variation in the staining pattern of bands along the length of nonhomologous chromosomes. This variation in staining properties is generally independent of any obvious structural variation. Although the polytene chromosomes of *Dipteran* insects and certain other organisms have a banded structure, these bands are not produced by chromosome banding techniques. The banding patterns are visible without any staining. Specific chromosome banding may be produced in a variety of ways on chromosomes that show little or no structural differentiation along their length, and which are uniformly stained with certain dyes. The longitudinal differentiation of chromosomes revealed by the banding techniques permits the identification of individual chromosomes as well as parts of chromosomes. This has proved invaluable in clinical cytogenetics, karyotypic evolutionary studies, and gene mapping (Sumner 1990).

In conventional cytogenetic and plant breeding studies, chromosome identifications that were not possible on the basis of chromosome morphology, were based on the use of aneuploid stocks and chromosome pairing data. The

genotypes to be analyzed were crossed with a series of tester stocks (translocation, telosomic, or various aneuploid stocks) and F_1 hybrids were analyzed for patterns of chromosome pairing to identify unknown chromosomes. The cytogenetical analysis was painstaking and chromosome identification was indirect. Banding techniques, for the first time, provided a rapid and direct method of chromosome identification and are now extensively used to support plant breeding (Gill 1990).

2.1 Origins and evolution of chromosome banding methods

In 1928, Heitz introduced the concept that chromatin consists of two components, euchromatin and heterochromatin. Using a simple staining technique, he showed that certain segments (the heterochromatic ones) of chromosomes remained visible after telophase, in contrast to euchromatin which decondensed and became indistinguishable. The specific staining of heterochromatin by a variety of methods had been attempted in many organisms (predominantly plants) (Levan 1945, 1946; Yamazaki 1959, 1971), but the methods were not universally applicable. An alternative method of demonstrating heterochromatin was nucleic acid starvation (Darlington and Lacour 1938; Darlington 1940). They demonstrated that certain segments of chromosomes of *Trillium* appeared thinner than others and took up less stain after cold treatment. These methods were the first attempt in chromosome banding whereby a banding pattern could be induced in chromosomes by treatment before fixation. However the mechanism proposed for the latter method of chromosome banding, that DNA synthesis was inhibited in the thinner segments by the cold, has been shown to be wrong. Woodard and Swift (1964) showed that no loss of DNA was induced by cold treatment, and that disappearance of cold-induced segments at room temperature did not involve DNA synthesis. An alternative

explanation for the appearance of cold-induced segments is that they represent regions of differential chromatin packing (Wilson and Boothroyd 1944; Boothroyd 1953; Braselton 1973; La Cour and Wells 1974; and Rudak and Callan 1976).

Caspersson *et al.* (1968, 1969a, 1969b) described the use of fluorochrome (quinacrine mustard) to stain chromosomes and produce fluorescent banding on chromosomes. The discovery of the fluorescence bands ushered in a new era of chromosome banding (Hsu 1979). Variations in the intensity of fluorescence along chromosomes produced by *quinacrine* derivatives has become known as Q-bands. When Pardue and Gall (1970) used *in situ* hybridization to locate satellite DNA in mouse chromosomes, they noticed that the centromeric regions were more strongly stained with Giemsa dye. This observation of selective centromeric staining is the origin of the technique known as C-banding which is extensively used to demonstrate *constitutive heterochromatin* in all organisms. C-banding techniques are the most widely used banding methods for plant chromosomes. At present several banding techniques [Q(Fluorescent bands with quinacrine), C(Heterochromatin staining), G(Giemsa-stained euchromatic bands), R(Reverse-Giemsa banding) (ISCN 1985); T(Predominantly terminal bands) (Dutrillaux 1973); N(Giemsa staining for nucleolar organizers) (Matsui and Sasaki 1973); D(Fluorescent bands with daunomycin or adriamycin) (Lin and van de Sande 1975); etc.] are available for chromosome identification. In higher vertebrates, G-, Q-, and R-banding techniques can give a detailed pattern of bands throughout the lengths of the chromosomes. At present only C-and N-banding techniques are commonly applicable in plant chromosome studies.

2.2 C-banding

Modern C-banding methods were introduced by Pardue and Gall (1970), who carried out *in situ* hybridization of satellite DNA to mouse chromosomes. The

essentials of this method were treatment of chromosome preparation with 0.2 N hydrochloric acid, digestion with RNase to remove any RNA attached to the chromosomes, denaturation of chromosomal DNA with 0.07 N sodium hydroxide, and incubation of the chromosome preparation with labeled complementary nucleic acid in saline-citrate (2xSSC, 0.3 N NaCl + 0.03 N tri-sodium citrate). Essentially, with the omission of the complementary nucleic acid step, this procedure was used for producing C-banding of human chromosomes (Arrighi and Hsu 1971; Chernay *et al.* 1971). There are a wide variety of methods that have been used to demonstrate C-bands on chromosomes, e.g. sodium hydroxide treatment (Gagné *et al.* 1971; Alfi and Menon 1973), alcoholic sodium hydroxide treatment (Hansen-Melander *et al.* 1974), the BSG (Barium hydroxide/Saline/Giemsa) technique (Sumner 1972). The BSG method has become, with only slight modifications, the standard method for producing C-bands on plant and animal chromosomes (Sumner 1990).

It has been confirmed in *Drosophila melanogaster* (Hsu 1971) and in the field vole (*Microtus agrestis*) (Arrighi *et al.* 1970) that the C-bands correspond to the heterochromatin observed in pre-banding cytogenetical studies. Balícek *et al.* (1977) showed that during prophase the C-band regions of human chromosomes contracted less than the euchromatic regions; this is consistent with the C-bands being heterochromatic and therefore having to undergo less condensation. Not all animals show complete correspondence between C-bands and heterochromatin (John and King 1977). In plants it has been widely accepted that certain regions of chromosomes induced by cold treatment are heterochromatic, but in *Adoxa moschatellina* there are cold-sensitive regions that are not C-band positive (Greilhuber 1979). It indicates that not all organisms have a complete correspondence between C-banding and constitutive

heterochromatin. It would be wrong to specify constitutive heterochromatin exclusively in terms of reactivity to C-banding methods.

A similarly complex situation appears with the DNA content of C-bands. Pardue and Gall (1970) found that the mouse C-band regions contain almost exclusively highly repetitive satellite DNA. Subsequently, highly repetitive DNA has been located in C-band regions in a wide variety of species (Sumner 1990). Although this assumption is frequently correct, there are several situations in which highly repetitive DNAs have not been found in C-band regions. In the Chinese hamster, the long arm of the X chromosome, the entire Y chromosome, and the centromeric regions of chromosome 10 are all C-band positive, but do not appear to contain a high proportion of repetitive DNA (Arrighi *et al.* 1974).

Miklos and John (1979) reported that as much as 20% of the human genome shows positive C-bands, while only 4% of human DNA is satellite. Due to the difficulties in making accurate measurements of both the amounts of C-band material and the amounts of satellite DNA, it is unlikely that any such comparison will give a definite result. Nevertheless, there is a much closer correspondence between the amount of C-band material and the amount of satellite DNA in the mouse. When Kuo and Hsu (1978) made RNA transcripts from C-banded chromosomes of *Peromyscus eremicus*, they found that non-repetitive sequences were covalently linked to repetitive ones, suggesting that the C-bands contained both classes of DNA. Moreover, Gosden *et al.* (1975) reported that certain human C-bands contained more than one kind of satellite DNA. It indicates that C-bands are not homogeneous and may be composed of non-repetitive DNA fractions and satellite DNAs.

Information on the protein composition of C-bands is much less comprehensive than that for DNA composition. Hsieh and Brutlag (1979), Will and Bautz (1980), Schmidt and Keyl (1981), James and Elgin (1986), and Viglianti and Blumenfeld (1986) have discovered various proteins in *Drosophila* species which bind preferentially to highly repetitive DNA, and which in some cases, have been located in heterochromatin. Strauss and Varshavsky (1984) have described an HMG (high mobility group)-like protein from the African green monkey that binds preferentially to the satellite DNA that may promote the formation of a particularly compact chromatin structure by its interactions with nucleosomes. Masumoto *et al.* (1989) have found that human alpha satellite DNA which contains a 17 base pair motif, binds to the centromeric protein (CENP-B). Other observations have shown particular histone subfractions or modifications in constitutive heterochromatin. Blumenfeld *et al.* (1978) found that in *Drosophila spp.*, those fractions of histone H1 most closely associated with satellite DNAs were phosphorylated. Halleck and Gurley (1980) in *Peromyscus spp.* and Holmgren *et al.* (1985) in *Drosophila spp.* both presented a correlation between compaction of heterochromatin and a high degree of phosphorylation of the H2A histone. Study of the proteins of C-bands is limited because they are more difficult to isolate and identify than satellite DNAs. However, there is evidence both for the presence of distinctive proteins in C-bands, and for specific types of modifications of histones.

The DNA of C-bands was found to replicate late in the S phase (Bostock *et al.* 1972; Citoler *et al.* 1972; Sperling and Rao 1974). This corresponds with the generally late replicating characteristic of heterochromatin (Schmid 1967; Lima-de-Faria and Jaworska 1968). However, that is not the absolute rule. Kakeda and Yamagata (1992) conducted immunological analysis of chromosome

replication in barley, rye, and durum wheat by using an anti-BrdU antibody. They reported that C-bands tended to replicate late in the S phase, but that the late replicating chromosomal regions did not always correspond to C-bands. In durum wheat, the late replicating pattern was remarkably different from the C-banding pattern. The distal to terminal regions of most chromosome arms corresponded to late-replicating regions, but only a few of these regions were C-banded. Thus, it was concluded that C-banded heterochromatin does not always replicate towards the end of S phase.

At first, it was believed that C-bands were produced by differential annealing of denatured DNA. Specifically highly repetitive DNA renatured rapidly, resulting in very strong staining. Mace *et al.* (1972) reported that after C-banding treatment the DNA was denatured except in the C-bands. Further evidence comes from observations that C-banding can be produced by reagents whose action is essentially on proteins, and not on DNA (McKay 1973; Merrick *et al.* 1973; Ray and Hamerton 1973). It was soon reported that C-banding involved the preferential removal of DNA from non-C-banded regions of chromosomes (Comings *et al.* 1973; Pathak and Arrighi 1973). However, Kongsuwan and Smyth (1978) claimed that C-bands were demonstrated in *Lilium* without differential extraction of DNA. Gendel and Fosket (1978) showed that C-bands could be demonstrated in *Allium cepa* after total extraction of DNA. Electron microscopy has shown varying degrees of chromatin extraction from non-C-band regions of chromosomes while C-band chromatin is highly resistant to extraction and remains condensed even when non-C-band chromatin is totally extracted (Burkholder 1975). These studies suggest that quantitative differences in DNA in C-band and non-C-band regions achieved by C-banding techniques result in the visualization of C-banding patterns after Giemsa staining.

Holmquist (1979) described the chemical processes responsible for loss of DNA during C-banding. The preliminary treatment with 0.2 N hydrochloric acid depurinates the DNA, leaving the deoxyribose residue in the aldehyde form. The alkali treatment leads to DNA chain breakage, as well as denaturing the DNA irreversibly. Extraction of the depurinated DNA occurs during the final stage of incubation in SSC. This process can be inhibited by reducing the free aldehydes at apurinic sites with borohydride, which also prevents C-banding.

There is evidence that DNA in condensed chromatin is depurinated more slowly than that in dispersed chromatin (Duijndam and van Duijn 1975). Moreover, selective annealing of DNA cannot explain the production of C-bands satisfactorily because annealing does not occur after alkali treatment (Kurnit 1974; Holmquist 1979). Burkholder and Weaver (1977) claimed that C-band positive regions were protected against digestion with DNase by the presence of non-histone proteins, which bound tightly to the DNA and restricted access of the enzyme. There is evidence for distinctive types of proteins in C-band regions; thus, it is possible that there might be distinctive interactions between DNA and proteins in C-band positive regions.

The investigation of protein involvement in C-banding was examined by electrophoresis of the proteins extracted at each step of the C-banding procedure as well as the residual proteins in the C-banded chromosomes (Burkholder and Duczek 1982). The HCl and $Ba(OH)_2$ treatments extract subsets of the histone and nonhistone proteins while little additional intact protein is found in the SSC extract. The residual chromosomal proteins consist of a few nonhistones and some of each of the five major histones. There was also evidence for the presence of degraded proteins in the residual chromosomes particularly after the alkali treatment. Although many of the

residual proteins may be located in constitutive heterochromatin, and could determine the resistance of this chromatin to extraction, these can not yet be distinguished from proteins associated with residual non-C-band chromatin. However, the most plausible explanation of C-banding seems to be DNA loss from C-band negative regions and the stronger binding of DNA to proteins in the C-band positive regions. There is evidence that alternative mechanisms might be involved in at least some cases.

C-banding is valuable for the identification of chromosomes in plants. The characteristics that aid identification are the size and position of the C-bands which may be located pericentromerically, interstitially, or terminally. Generally C-bands appear as a solid block of material occupying the whole width of the chromosome. However, in *Scilla mischtschenkoana* C-bands revealed as clusters of narrow bands or dots (Greilhuber and Speta 1978), while in *Triturus marmoratus* C-bands appeared only part of the width of a chromatid (Herrero and Gosálvez 1985). Chromosomes which completely lack C-bands are rare, and possibly may not occur in normal karyotypes.

2.3 N-banding

The original N-banding technique of Matsui and Sasaki (1973) involved extraction of nucleic acids and histones from chromosomes, followed by Giemsa staining. Their method included successive treatments with hot trichloracetic acid and hot hydrochloric acid. As a result, acidic proteins were left after the extraction procedures which were stainable with Giemsa. N-bands were reported at the sites of the nucleolar organizing regions (NORs) on the chromosomes of several mammalian species. However, in the mouse the N-banding technique stained centromeric heterochromatin. Faust and Vogel (1974) believed that the

N-bands corresponded to a specific kind of heterochromatin located adjacent to the NORs.

Funaki *et al.* (1975) improved the technique, using a 1 M sodium dihydrogen phosphate (pH 4.2) at 96 °C to extract nucleic acids and histones. They demonstrated that the modified technique exhibited a correspondence between N-bands and NORs for 27 species of animals and plants. A number of experiments on a variety of species were conducted using the Funaki *et al.* (1975) method with slight modification, and have revealed patterns of bands many of which do not correspond to NORs, and which in many cases are also different from C-bands. This includes studies of *Drosophila* species (Pimpinelli *et al.* 1976; Hägele 1977), and various species of cereals (Gerlach 1977; Jewell 1979; Endo and Gill 1984a; Schlegel and Gill 1984; Kakeda *et al.* 1991). It was demonstrated that different patterns of N-bands could be produced by altering the conditions of treatment with trichloracetic and hydrochloric acids (Hägele 1979), or with hot phosphate (Jewell 1981).

In addition, many of the chromosomes in the species studied have unique N-banding patterns that facilitate their identification. Jewell (1979) identified all 14 chromosomes of *Aegilops variabilis* by using a conventional N-banding technique. Gerlach (1977) and Jewell (1979) identified 9 of the 21 chromosomes of bread wheat using this technique. With a modified N-banding method, Endo and Gill (1984a) identified 16 of the 21 chromosomes of bread wheat, including 5 in the A genome. The N-banding technique has also been used to identify chromosomes in rye (Jewell 1981, Schlegel and Gill 1984), barley (Islam 1980; Singh and Tsuchiya 1982a; Kakeda *et al.* 1991); lentils (Mehra *et al.* 1986), in the genus *Aegilops* (Jewell and Driscoll 1983; Chen and Gill 1983), and in the genus *Elymus* (Morris and Gill 1987). It has also been used in attempts to

identify various types of aneuploids (Singh and Tsuchiya 1982b), alien addition and substitution lines (Islam 1980) in wheat.

N-banding can be employed to demonstrate a distinctive set of heterochromatic bands, as well as nucleolar organizers. The original methods suggested that N-bands were regions rich in acidic proteins that were resistant to extraction. However, it cannot yet be said that this is true of all bands revealed by N-banding methods. Matsui and Sasaki (1973); Matsui (1974); Funaki *et al.* (1975) verified that the Giemsa-stainable substances were non-histone proteins by cytochemical tests. Matsui (1974) showed that all the DNA was extracted from the nuclei, and all but 6% of the proteins. Subsequent studies have attempted to define these resistant non-histones in more detail (Buys and Osinga 1984; Matsui *et al.* 1986). There are nevertheless substantial discrepancies between the results of these two groups with a completely different set of Giemsa-stainable proteins. Matsui *et al.* (1986) found one non-histone component to have many properties appropriate to an N-banded protein: Giemsa staining, specific binding to rDNA, localization at NORs, and the ability of an antibody to the protein to suppress rDNA transcription. Some of these properties would not necessarily be required of a NOR protein, but would hardly be expected of a protein not associated with NORs. Typical characteristics of N-banding proteins are still to be determined. Nevertheless, these techniques have been helpful in identifying plant chromosomes.

3. Relationships among heterochromatin bands revealed by C- and N-banding

The present study was undertaken to identify the chromosomes by using C-banding and N-banding techniques. It is necessary to understand the

relationship between C-banded and N-banded heterochromatin. C-banding techniques were first reported on animal chromosomes (Pardue and Gall 1970; Hsu 1973), in which the bands were revealed to consist of constitutive heterochromatin. In cereal species, the first successful C-banding procedures were demonstrated in rye, triticale, and bread wheat (Merker 1973; Sarma and Natarajan 1973; Shchapova 1974, Gill and Kimber 1974a, 1974b, 1974c; Verma and Rees 1974; Vosa 1974; Zurabishvili *et al.* 1974, 1978). The study on rye clearly indicated that C-bands in somatic metaphase chromosomes were equivalent to constitutive heterochromatin found in pachytene chromosomes (Gill and Kimber 1974b; Shchapova 1974).

N-banding is a method that was developed for staining the NORs of chromosomes (Matsui and Sasaki 1973). Matsui (1974) and Funaki *et al.* (1975) demonstrated that N-bands corresponded to the known sites of NORs in several species, including the satellite stalks (secondary constrictions) in human chromosomes by using a modified technique. However, it has been shown in many plant species and some insects that N-banding procedures stain distinctive types of heterochromatin not associated with NORs.

The observations of Schlegel and Gill (1984) on sequential C- and N-banding revealed the heterogeneity of heterochromatin of rye chromosomes. N-banding does not always reveal new bands not observed by C-banding, although certain bands may stain more intensely. This indicates that at least two classes of heterochromatin exist in rye chromosomes. Thus, the heterochromatic regions marked by the N-bands as well as C-bands can be described as C- and N-banding positive (C^+N^+), while the others are C-banding positive and N-banding negative (C^+N^-). Similarly, bread wheat heterochromatin can also be described as C^+N^+ and C^+N^- (Endo and Gill 1984b).

Morris and Gill (1987) used these heterochromatin characteristics to allocate chromosomes of *Elymus* species into genomes. They found that seven chromosomes contained mostly C^+N^+ heterochromatin, and the remaining seven chromosomes contained mostly C^+N^- heterochromatin. C-banding patterns of all seven chromosomes of *P. spicata* showed localized staining in terminal regions and lack of N-banding. These chromosomes were characterized as C^+N^-. C- and N-banding analysis of *C. bogdanii* and *C. californicum* demonstrated the similarities of centromeric C^+N^+ heterochromatin among 5 corresponding chromosomes. It indicates a close phylogenetic relationship between these two *Critesion* species. In brief, S-genome heterochromatin is exclusively C^+N^- and H-genome heterochromatin is mostly C^+N^+.

Moreover, only C^+N^+ heterochromatin exclusively coincided with the localization of polypyrimidine DNA sequences (Dennis *et al.* 1980; Appels *et al.* 1978). So far only two kinds of heterochromatin, C^+N^+ and C^+N^- heterochromatin, have been reported in bread wheat (Gill 1987), rye (Schlegel and Gill 1984), and *Elymus* (Morris and Gill 1987). With respect to the relationship between the two classes of heterochromatin, Gill (1987) suggested that C-banding is a general technique for staining all classes of heterochromatin while N-banding reveals only some specialized heterochromatin containing polypyrimidine DNA sequences. However, Kakada *et al.* (1991) demonstrated that C-banding negative and N-banding positive (C^-N^+) heterochromatin is present at all centromeric sites of four barley cultivars examined. This finding makes the above explanation most likely applicable for the C^+N^+ heterochromatin in barley chromosomes (Dennis *et al.* 1980), but of course not applicable for the formation of C^-N^+ heterochromatin.

Burkholder (1988) hypothesized that one of major effects of the C-banding technique is the differential extraction of DNA and proteins from the C-band negative regions. The formation of C^- (N^+) heterochromatin of barley chromosomes at the centromeric regions could be the result of the extraction of large portions of DNA and proteins from the centromeric sites. Kakeda *et al.* (1991) showed that N-banding produced new centromeric bands even after the C-banding treatment. It is possible that at the centromeric sites some chromosomal material still remains after C-banding treatment and its structure is altered so as to be stainable by the subsequent N-banding treatment. They suggested that the differential alteration of chromosome structure rather than the differential extraction of chromatin may cause the formation of C^-N^+ heterochromatin to C- and N-banding techniques.

Although the C-banding technique allows identification of all chromosomes in wheat and other species in *Triticeae*, and the N-banding technique identifies fewer chromosomes, N-banding does offer some advantages over C-banding in chromosome identification. The N-banding procedure is rapid and reproducible, often stains some bands more intensely, and provides excellent resolution of bands (Endo and Gill 1984a). Moreover, C-banding and N-banding techniques reveal a biochemical heterogeneity of heterochromatin (Jewell 1981; Endo and Gill 1984a; Schlegel and Gill 1984).

4. Chromosome Band Nomenclature

Identification of chromosomes is an obligatory stage in any cytogenetic study. Based on standard staining procedures, individual chromosome identification of symmetric karyotypes is difficult because many chromosomes appear similar in size and arm ratio. Valid identification of somatic chromosomes became possible

with the application of chromosome banding. Comprehensive applications of both C- and N-banding techniques and the classification of C^+N^+ and C^+N^- heterochromatin are crucial in identifying individual chromosomes of plants such as in *Triticeae* tribe.

The report of the Paris Conference in 1971 was the first attempt to provide a system of designated chromosome bands in human chromosomes. Such a system is necessary for various purposes, for example, for identifying designated break points in rearranged or deleted chromosomes, and defining the location of genes on chromosomes. Subsequently the system of nomenclature proposed for human chromosomes has been extended to other species, for which standard karyotypes has been published. In earlier studies of chromosome identification by C-banding in *Triticeae*, Gill and Kimber (1974a), Lukaszewski and Gustafson (1983) and Sybenga (1983) used a standard nomenclature to designate the chromosomes but no attempt was made to develop a nomenclature system for the description of bands. Iordansky *et al.* (1978) proposed the Generalized Cytological Nomenclature for Cereal Chromosomes (GCNCC) after the Paris Conference (1971) on standardization of identification of human chromosomes. According to the GCNCC system, chromosomes were numbered on the basis of their lengths and centromere positions rather than on the existing genetic nomenclature. Van Niekerk and Pienaar (1983) and Gill (1987), took initial steps in combining the genetic and GCNCC nomenclatures and made proposals for a standard nomenclature system for the description of chromosome bands in *Triticum* species.

The chromosome banding nomenclature proposals were discussed at the first North American Wheat Cytogenetics Workshop held in Columbia, Missouri in 1986 (Gill 1986). Later at the 7th International Wheat Genetics Symposium

(IWGS), Cambridge, England, in 1988 an international chromosome banding nomenclature committee was formed. The committee agreed on a consensus for the nomenclature and designation of chromosome bands in *T. aestivum* L. cultivar "Chinese Spring". It was hoped that this nomenclature system would be widely adopted by wheat cytogeneticists, and that it should be applicable to all members of the *Triticeae* (Gill 1987; Gill *et al.* 1991).

The designation of chromosome bands is in part based on the recommendations of the 1971 Paris Conference on standardization of identification of human chromosomes. However, nomenclature in human chromosome banding was based on Q and G bands, whereas in wheat it is based on C- and N-bands. According to the 7th IWGS held at Cambridge, England, in 1988, each chromosome short arm is designated S and the long arm as L for wheat chromosomes. Each arm may or may not have "landmark" bands. A chromosome landmark is defined as a consistent and distinctive morphological feature that is an important diagnostic aid in the identification of a chromosome. Landmarks include the ends of chromosome arms, the centromere, and certain bands (Paris Conference 1971).

A region is defined as any area of a chromosome lying between two adjacent landmarks (Paris Conference 1971).

A band is defined as a "part of chromosome clearly distinguishable from adjacent parts by virtue of its lighter or darker staining ability." Each arm consists of a continuous series of dark bands and light bands; by definition, there are no "interbands." Regions and bands are numbered consecutively from the centromere outwards along each chromosome arm (Paris Conference 1971; Gill 1987; Gill *et al.* 1991).

The fraction length (FL) of the diagnostic bands has been calculated based on percent distance from the centromere and is indicated on each chromosome map (Gill *et al.* 1991).

Bands are designated from the centromere outward for each arm. The heterochromatin bisected by the centromere is considered as two bands, each being labeled as band 1, in region 1, of each arm (Paris Conference 1971). If band number exceeded more than nine, then the arm was subdivided into two regions. A divided band of region 1 and 2 is considered as band number 1 of region 2. For designating a particular band, five items are required; the chromosome number, the genome designation, the arm symbol, the region number, and the band number within that region. The region number is separated from the band number by a decimal point (Gill *et al.* 1991).

Nomenclature rules on the subdivision of existing landmark bands declare that "in the event that a band serving as a landmark requires subdivision, all sub-bands derived from it should retain the original region and band number of that landmark." The sub-bands are numbered sequentially from the centromere outward. For example, if the original band 1L1.1 was subdivided into three sub-bands: 1L1.11, 1L1.12, and 1L1.13, with sub-band 1.11 being a sub-band was subdivided, additional digits without punctuation should be used; e.g. sub-band 1L1.11 might be further subdivided into 1L1.111, 1L1.112, etc. As indicated, this proposed nomenclature provides the necessary accuracy and flexibility for describing banded chromosomes and any further additions of new bands and polymorphic bands without changing the designations of original bands and intervening regions (Gill *et al.* 1991).

CHAPTER 3 MATERIALS AND METHODS

1. Plant material

The plants used in this research included three possible diploid progenitors and two allotetraploid species of *Elymus*, *E. canadensis*, and *E. trachycaulus*. Nomenclature and genome designation of species are based on Dewey (1984a) and Löve (1984). Old nomenclature, original sources, and accession information are listed in Table 3.1. The materials used in this study were kindly provided by Dr. D.R. Dewey and Dr. K.B. Jensen, USDA-ARS, Utah State University, Logan, Utah, USA., except *E. trachycaulus* cv. *Revenue* which was obtained from Mr. Hanns Jahn, University of Alberta.

All seeds were germinated on wet filter paper at room temperature for 3-5 days, then transferred to root trainers and kept in the greenhouse. The seedlings were transplanted into pots at the three leaf stage. The day/night temperature was 21-27°C/ 21°C with a 16 h photoperiod and 450 μmol photon m^{-2} s^{-1} light, from high pressure sodium lamps.

2. Standard cytological studies

Pretreatment: The white and plump root tips were selected and cut about 10-20 mm long, then treated with ice water for approximately 24 hours. This treatment gives satisfactory chromosome contraction and a high mitotic index.

Fixing: The root tips were transferred to vials containing Carnoy's solution I (3:1 ethanol-acetic acid) (Carnoy 1886) for 3 days at room temperature or longer in the refrigerator at 0-4 °C. This process causes cell death without disturbing the cell structures.

Table 3.1 Genome symbols, chromosome number, geographic origin, and accession information of the plant material.

Species	Genome	Chromosome number	Origin	Accession information*
Pseudoroegneria spicata (Pursh) A. Löve (=*Agropyron spicatum* (Pursh) Scribner & Smith	S	14	USA.	PI 232127 (MA-87-71-75)
Critesion bogdanii (Wilensky) A. Löve (=*Hordeum bogdanii* Wilensky)	H	14	USSR.	PI 314696 (C-36-6--10)
Critesion californicum (Covas & Stebbins) A. Löve (=*Hordeum californicum* Covas & Stebbins)	H	14	USA.	PI 531778 (C-37-26--30)
Elymus canadensis Linnaeus	SH	28	North America	PI 531564 (= D3231)
Elymus trachycaulus (Link) Gould ex Shinners (=*Agropyron trachycaulum* (Link) Malte ex H.F. Lewis)	SH	28	North America	Cultivar *Revenue*

* Accession number of Dr. D.R. Dewey, USDA-ARS, Utah State University, Logan, Utah, USA.

Staining and Squashing: The fixed root tips were stained in 1% acetocarmine for 1-2 h, and the root cap was then sliced off to remove the meristematic cells in a drop of 45% acetic acid on a glass slide. After placing a cover slip over the drop of 45% acetic acid, the slide was slightly warmed over the flame, placed between the two halves of folded filter paper on a flat surface, and thumb pressure applied directly to the cover glass.

Photomicrography: The slides were examined under a BH2 Olympus microscope. The cells with well spread chromosomes were photographed using Technical Pan 2450 film.

3. Sequential C- and N-banding

The root tips were pretreated, fixed, and squashed in the same manner as that used for standard cytological studies, except that they were not stained in 1% acetocarmine. Instead they were placed in 45% acetic acid for 2-3 min to soften the tissue. After examining for the required cells and recording their locations, the coverslips were removed by using the quick freezing method (Conger and Fairchild 1953).

The C-banding technique employed in this study followed procedures reported by Gill and Kimber (1974c) with the following modifications. After removing the coverslips, the slides were incubated in 0.2 M HCl at 60°C for 2.5 min and rinsed in distilled water. Air-dried slides were incubated in a barium hydroxide solution (5%) at 50 °C for 2.5 min and washed in distilled water. Then slides were subsequently incubated in 2xSSC solution at 60°C for 1 h. After rinsing in water, the slides were stained in 2% Giemsa in 0.15 M phosphate buffer (pH 6.8) for an appropriate time (20-45 min). The slides were checked every 5 min until the optimum staining was obtained. Then slides were briefly rinsed in distilled water,

blotted on f r paper, and air dried. For the preparation of C-banding slides, a coverslip was mounted on a drop of immersion oil, and observations were made under immersion oil.

After the C-banding technique was performed, the slides were destained with xylene, and an ETOH treatment series (96%, 70%, 50%, 30%) for 10 min per each solution, and then washed in distilled water. The N-banding technique used was a modified version of the procedure used by Endo and Gill (1984a). The air dried slides were incubated in 45% acetic acid at 60 °C for 10 min and air dried overnight. Next, the slides were incubated in hot phosphate buffer (1 M NaH_2PO_4) at 92°±2°C for 3 min, rinsed briefly in distilled water, and stained in 2% Giemsa in 0.15 M phosphate buffer (pH 6.8) for 20 min. The observations were also carried out under immersion oil and chromosomes in the same cells were photographed after both C- and N-banding. The N-banded karyotype was established according to the C-banded karyotype.

4. Sequential acetocarmine staining and N-banding

For acetocarmine staining/N-banding analysis, the root tips were first prepared for standard cytological studies. Then morphological observations of cells with well-spread chromosome complements were recorded, and the chromosomes were photographed. After removing the coverslip by freezing, slides were dried overnight in the desiccator. Next, the preparation was treated by the N-banding technique as described above. The incubation time in the hot phosphate buffer ranged from 1.5-3 min, and staining time in Giemsa ranged from 7-20 min. Chromosomes in the same cells were photographed to construct the sequential acetocarmine staining/N-banded karyotype.

5. Karyotyping

Chromosome measurements were made on photomicrographs of well-spread cells (magnification 3,400x). Measurements of each chromosome pair were analyzed with the Chrompac 4.1α computer program developed and supported by Dr. D.M. Green, Redpath Museum, McGill University, Montreal, Quebec and presented for the following parameters.

Length of long arm = Ll

Length of short arm = Ls

Length of chromosome(LT)= Ll + Ls

Relative length $= \frac{\text{Length of chromosome}}{\text{Total length of all chromosomes in genome}}$

$= LT / \Sigma LT$

Arm ratio = Ll / Ls

Centromeric index = Ls / LT

In the morphologic identification of chromosomes, this study followed the nomenclature for centromeric position on chromosomes of Levan *et al.* (1964) as shown in Table 3.2.

For the construction of karyotypes of each diploid species, homologues were paired according to chromosome length, arm ratio, chromosome marker (e.g. satellite), and banding pattern. The sequential acetocarmine staining/N-banding procedures provide detailed observations on chromosome morphology together with banding patterns which allow precise chromosome matching. Only the

Table 3.2 Nomenclature for centromeric position on chromosomes.

Centromeric position	Arm ratio	Chromosome designation	
Median *sensu stricto*	1.0	M	metacentric
Median region		m	
----------	-----1.7-----	----------	----------
Submedian		sm	submetacentric
----------	-----3.0-----	----------	----------
Subterminal		st	subtelocentric
----------	-----7.0-----	----------	----------
Terminal region		t	acrocentric
Terminal *sensu stricto*	∞	T	telocentric

karyotype of *P. spicata* was constructed on the basis of conventional staining and sequential C-/N- banding techniques. For the construction of *Elymus* karyotype ? assignment of chromosomes to the S and H genomes is facilitate ?y ?e application of sequential acetocarmine staining/N-banding techniques. Homologues were paired on the same basis as that used in the diploid species.

Karyotypes were constructed based on lengths, relative lengths, and centromere positions of chromosomes. The chromosomes in each genome were arranged in a standard manner (based on order of decreasing size and centromere position without regard for the satellite position) to facilitate comparisons among all species. The positions of bands were measured from the centromere to the middle position of the band. The fraction length was calculated from the percent distance of a particular band from the centromere.

Chromosome measurement, band position, and band length of each chromosome in the genome was transferred to the Microsoft Excel 4.0 computer

program to draw the idiogram. The nomenclature system for description of chromosome bands was that used by Gill *et al.* (1991) in wheat. The description of a particular band includes the chromosome number, the genome designation, the arm symbol (S or L), the region number, the band number within that region, and the fraction length (FL) of the diagnostic bands. The idiogram of banded chromosome 1D of 'Chinese Spring' wheat from Gill *et al.* (1991) is shown in Figure 3.1. Band numbers are indicated on the left and FL positions on the right of the chromosome. For example, chromosome 1D shows the biggest band, S1.3, at FL.68, and the smallest band, L1.5, at FL.68. The region number is separated from the band number by a decimal point.

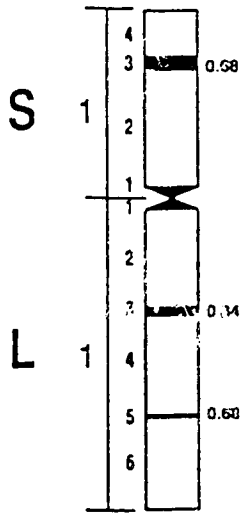

Figure 3.1. Idiogram of banded chromosome 1D of 'Chinese Spring' wheat from Gill *et al.* (1991).

CHAPTER 4 RESULTS

1. Karyotype analysis of diploid species: *Pseudoroegneria spicata* (S genome), *Critesion bogdanii* and *Critesion californicum* (both H genome).

1.1 *Pseudoroegneria spicata*

1.1.1 The cytology of *P. spicata*

P. spicata (2n=2x=14) belongs to the genus *Pseudoroegneria* having a genome designated 'S' by Dewey (1984a). The karyotype of *P. spicata*, based on standard staining and C-banding, is presented in Figure 4.1. The chromosomes, including the satellited chromosome, are arranged by size. The positions and sizes of the satellites and relative arm ratios are important diagnostic markers. The chromosome measurements including lengths of long and short arms, total length, relative length, arm ratio, centromeric index, and total genome length are reported in Table 4.1.

Karyotype analyses show one pair of small satellites on the short arms of chromosome pair 1S and one pair of large satellites on the short arms of chromosome pair 5S. All of the chromosomes are metacentric.

1.1.2. C- and N-banded karyotypes of *P. spicata*

C-banded karyotype

Characteristic C-bands of *P. spicata* are shown in the terminal regions of each chromosome (Figure 4.1). They are located at the terminal ends of the short arms of all chromosomes and at the ends of the long arms of three of the chromosomes in the complement. These characteristic banding patterns along with chromosome lengths and satellited chromosome morphology, allow the identification of each chromosome.

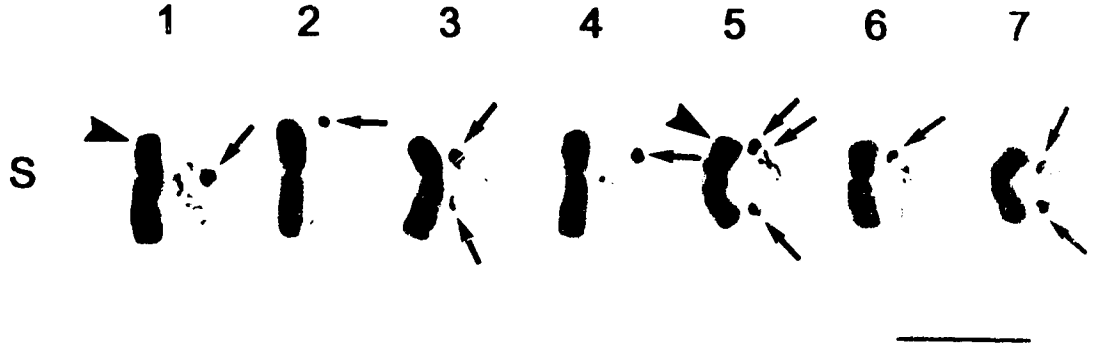

Figure 4.1 Karyotype of the *Pseudoroegneria spicata* genome based on acetocarmine staining (left-hand side) and C-banding (right-hand side) from different cells (arrow heads indicate satellites, arrows indicate C-bands, and scale bar = 10 µm).

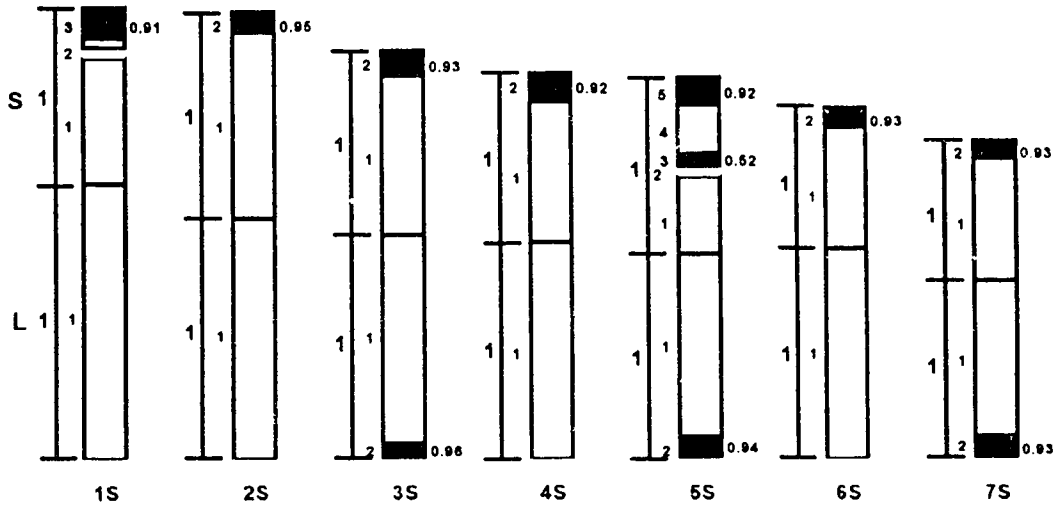

Figure 4.2 Idiogram of C-banded chromosomes of *P. spicata*.

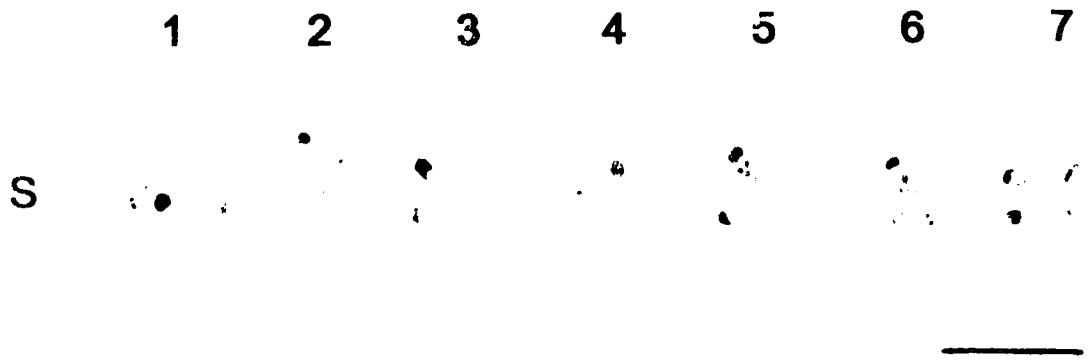

Figure 4.3 Karyotype of the *P. spicata* genome based on sequential C- and N-banding techniques. C-banded chromosomes (left-hand side) and the same chromosomes subsequently stained by the N-banding treatment (right-hand side), arrows indicate C-bands, an arrow head indicates N-band, and scale bar = 10 µm.

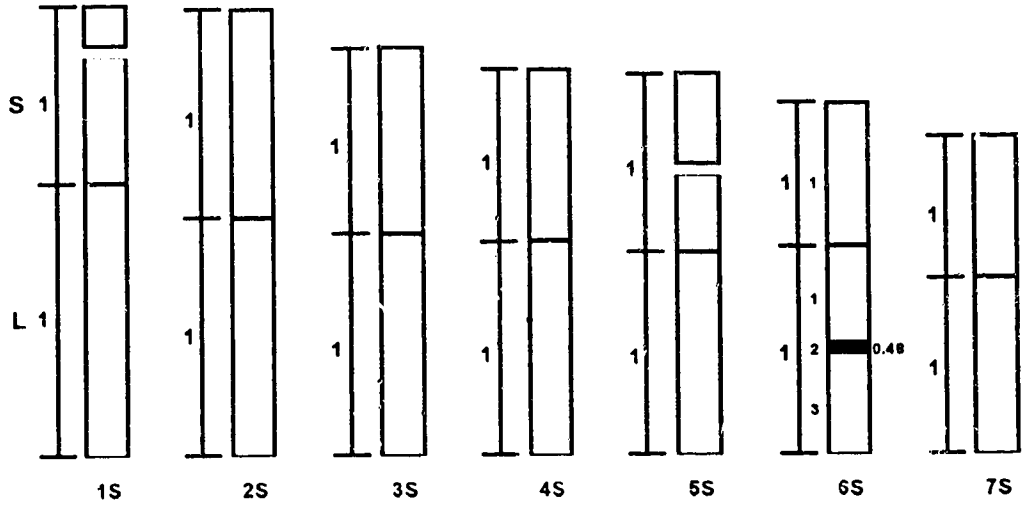

Figure 4.4 Idiogram of N-banded chromosomes of *P. spicata*.

Table 4.1 Measurements of *P. spicata* chromosomes.

Chromosome	Length of long arm (Ll)	Length of short arm (Ls)	Total length	Relative length	Arm ratio (Ll / Ls)	Centromeric index
1S	5.50	3.54 (0.81)*	9.04±0.78**	16.44	1.55	0.39
2S	4.80	4.16	8.96±0.31	16.30	1.15	0.46
3S	4.47	3.72	8.19±0.57	14.89	1.20	0.45
4S	4.32	3.42	7.74±0.38	14.06	1.26	0.44
5S	4.09	3.56 (1.81)*	7.65±0.60	13.92	1.15	0.47
6S	4.20	2.85	7.05±0.51	12.81	1.47	0.40
7S	3.55	2.83	6.38±0.60	11.59	1.26	0.44

Total genome length = 55.01±2.96 μm

* the length of satellites

** standard deviation

Chromosome 1S

This chromosome is the largest and has a small satellite in the short arm. Chromosome 1S also reveals a prominent terminal C-band at the position of the satellite.

Chromosome 2S

Chromosome 2S also shows a large terminal C-band in the short arm, but it has a different arm ratio from chromosome 1S.

Chromosome 3S

Chromosome 3S has terminal C-bands in both arms. The one in the short arm is about twice the size of that on the long arm.

Chromosome 4S

This chromosome has only a large terminal C-band in the short arm which is sirnilar in morphology to the large terminal C-bands in the short arms of chromosomes 1S and 2S. However, it can be distinguished from chromosome 1S by the lack of a satellite and from chromosome 2S on the basis of chromosome length.

Chromosome 5S

Chromosome 5S has a big satellite in the short arm and terminal C-bands in both arms. In addition, the distinct interstitial band (indicated by arrow in the figure 4.3) at the secondary constriction makes it an easily identifiable chromosome.

Chromosome 6S

Chromosome 6S shows a terminal C-band in the short arm which is the same in size as the terminal C-bands in chromosomes 1S, 2S, and 4S. All four of these chromosomes possess different chromosome lengths and arm ratios.

Chromosome 7S

The smallest chromosome with terminal C-bands in both arms.

N-banded karyotype

Figure 4.3 presents the karyotype of *P. spicata* based on sequential C- and N-banding techniques. The difference between C- and N-banding patterns is as follows: only C-bands appeared at the terminal region of all chromosomes

following C-banding. The terminal C-bands disappeared or remained as traces (e.g. in chromosomes 1S, 6S, and 7S) when slides with C-banded chromosomes were subsequently subjected to the N-banding treatment. The N-banding patterns of *P. spicata* rarely show any bands except the small pronounced interstitial band, L1.2, in the long arm of chromosome 6S (indicated by an arrow head).

On the basis of C-banding pattern, 5 of 7 chromosomes of *P. spicata*, 1S, 2S, 3S, 5S, 7S, can be unequivocally identified. The remaining chromosomes (4S and 6S) cannot be distinguished by the banding procedures. These two chromosomes were differentiated on the basis of size and arm ratios.

The C-banding patterns of the chromosomes of *P. spicata* show large terminal bands in both arms of three chromosomes or only the short arm of four chromosomes. The prominent band at the secondary constriction of SAT-chromosome 5S is revealed only with the C-banding technique. Neither of these banding techniques stain this region of SAT-chromosome 1S. C- and N-banding analysis of *P. spicata* demonstrates that most of the heterochromatin is C-banding positive and N-banding negative (C^+N^-). The C-banding negative and N-banding positive (C^-N^+) class of heterochromatin is found only in the proximal half of the long arm of chromosome 6S.

1.1.3 Standard karyotype of *P. spicata*

Idiograms of C-banded chromosomes and N-banded chromosomes of *P. spicata* are presented in Figures 4.2 and 4.4, respectively. Chromosome number with genome designation is presented beneath each idiogram of the banded chromosomes. Vertical lines to the left indicate chromosome length and the short horizontal lines in the middle indicate centromere position. The arm symbols 'S'

for short arm and 'L' for long arm. are on the left. The region number is on the left, while the band number is on the right of the line. Fraction lengths of diagnostic bands are placed on the right of each idiogram of banded chromosomes. Fraction lengths of diagnostic C-bands and N-bands with band lengths of *P. spicata* chromosomes are given in Table 4.2.

Table 4.2 Fraction lengths of diagnostic C-bands and N-bands with band lengths of *P. spicata* chromosomes.

Chromosome	Diagnostic C-bands	Fraction length	Band length (µm)	Diagnostic N-bands	Fraction length	Band length (µm)
1S	1SS1.3	0.91	0.62	-	-	-
2S	2SS1.2	0.95	0.42	-	-	-
3S	3SS1.2	0.93	0.52	-	-	-
	3SL1.2	0.96	0.33			
4S	4SS1.2	0.92	0.57			
5S	5SS1.3	0.52	0.24	-	-	-
	5SS1.5	0.92	0.56			
	5SL1.2	0.94	0.45			
6S	6SS1.2	0.93	0.40	6SL1.2	0.48	0.20
7S	7SS1.2	0.93	0.37	-	-	-
	7SL1.2	0.93	0.47			
	Total length of bands		4.95 µm			0.20 µm

1.2 *Critesion bogdanii*

1.2.1 The cytology of *C. bogdanii*

The genus *Critesion* as defined by Löve (1984) has the genome designation H. The karyotype of the *C. bogdanii* genome based on sequential acetocarmine staining and N-banding is shown in Figure 4.5 and the chromosome measurements are reported in Table 4.3. The karyotype reveals one chromosome with a large satellite that is bigger than the size of the adjacent short arms. Most of the chromosomes are metacentric, and the satellited chromosome (chromosome 1H) is mostly submetacentric.

Table 4.3 Measurements of *C. bogdanii* chromosomes.

Chromosome	Length of long arm (Ll)	Length of short arm (Ls)	Total length	Relative length	Arm ratio (Ll / Ls)	Centromeric index
1H	6.50	3.89 (2.32)*	10.39±0.38**	15.90	1.67	0.37
2H	6.01	4.34	10.35±0.52	15.83	1.38	0.42
3H	5.41	4.57	9.98±0.25	15.26	1.18	0.46
4H	4.89	4.46	9.35±0.20	14.31	1.10	0.48
5H	5.10	4.01	9.11±0.26	13.94	1.27	0.44
6H	4.91	3.54	8.45±0.78	12.94	1.39	0.42
7H	4.28	3.44	7.72±0.61	11.82	1.24	0.45

Total genome length = 65.35±2.46 µm

* the length of satellites

** standard deviation

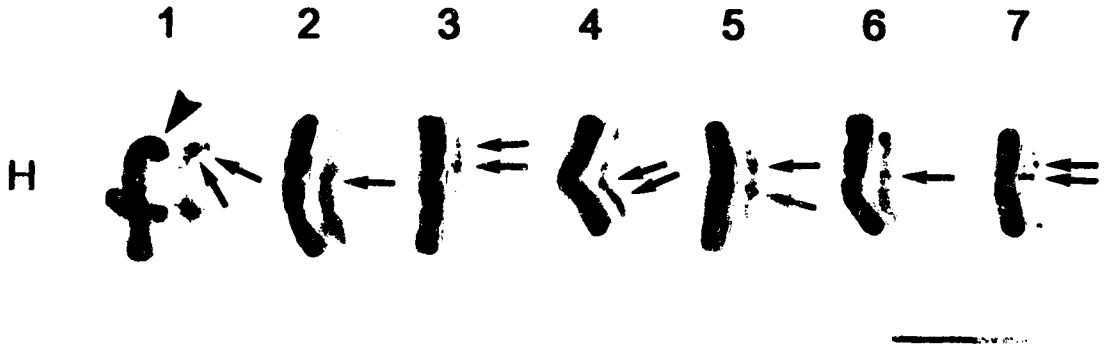

Figure 4.5 Karyotype of the *C. bogdanii* genome based on sequential acetocarmine staining/N-banding techniques. Acetocarmine stained chromosomes (left-hand side) and the same chromosomes subsequently stained by the N-banding treatment (right-hand side), arrows indicate N-bands, an arrow head indicates satellite, and scale bar = 10 µm.

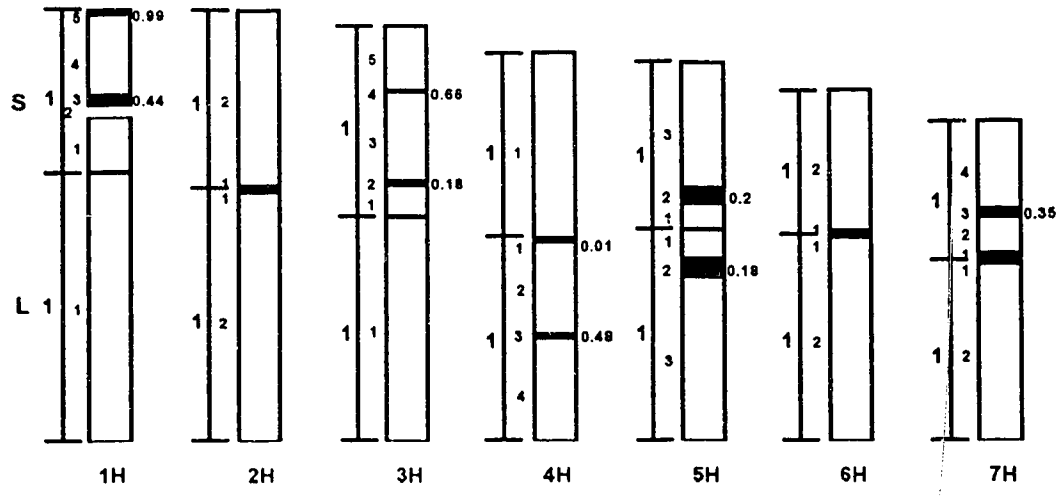

Figure 4.6 Idiogram of N-banded chromosomes of *C. bogdanii*.

1.2.2 N-banded karyotype of *C. bogdanii*

The N-banded karyotype of *C. bogdanii* is presented in Figure 4.5. Characteristic N-bands are found mostly at the centromere and in intercalary regions. Terminal bands are located in the short arm of SAT-chromosome 1H.

Chromosome 1H

This SAT-chromosome has a prominent band at the secondary constriction and a terminal band in the short arm.

Chromosome 2H

This chromosome possesses a centromeric band.

Chromosome 3H

The small distal and proximal N-bands are observed in the short arm.

Chromosome 4H

Chromosome 4H shows a pericentromeric band and a interstitial band in the proximal half of the long arm.

Chromosome 5H

This chromosome has conspicuous interstitial bands in the proximal regions of both arms which provide diagnostic identification.

Chromosome 6H

Chromosome 6H reveals a centromeric band similar to that in chromosome 2H. Chromosome 6H is shorter than chromosome 2H.

Chromosome 7H

A prominent interstitial band is located in the proximal region of the short arm along with a centromeric band.

On the basis of N-banding pattern, chromosomes 2H and 6H which show centromeric bands, cannot be precisely identified. The chromosome sizes assisted in distinguishing these two chromosomes.

1.2.3 Standard karyotype of *C. bogdanii*

The idiogram of N-banded chromosomes is shown in Figure 4.6. Fraction lengths of diagnostic N-bands of *C. bogdanii* are given in Table 4.4. The heterochromatic band bisected by the centromere is considered as two bands, each being labeled as band 1 ,in region 1 of each chromosome arm (e.g. in chromosome 2H).

Table 4.4 Fraction lengths of diagnostic N-bands with band lengths of *C. bogdanii* chromosomes.

Chromosome	Diagnostic N-bands	Fraction length	Band length (μm)
1H	1HS1.3	0.44	0.28
	1HS1.5	0.99	0.11
2H	2HS1.1	c.b.*	0.07
	2HL1.1	c.b.	0.07
3H	3HS1.2	0.18	0.10
	3HS1.4	0.66	0.06
4H	4HL1.1	0.01	0.
	4HL1.3	0.48	0.08
5H	5HS1.2	0.20	0.36
	5HL1.2	0.18	0.42
6H	6HS1.1	c.b.	0.08
	6HL1.1	c.b.	0.08
7H	7HS1.1	c.b.	0.13
	7HS1.3	0.35	0.18
	7HL1.1	c.b.	0.13
		Total length of bands	2.28 μm

* c.b.= centromeric band

1.3 *Critesion californicum*

1.3.1 The cytology of *C. californicum*

The karyotype of the *C. californicum* genome based on sequential acetocarmine staining and N-banding, is presented in Figure 4.7. A large satellite may be seen in the short arm of chromosome 3H. Six of the seven chromosomes are metacentric, and one is submetacentric. The chromosome measurements of *C. californicum* are presented in Table 4.5.

Table 4.5 Measurement . *californicum* chromosomes.

Chromosome	Length of long arm (Ll)	Length of short arm (Ls)	Total length	Relative length	Arm ratio (Ll / Ls)	Centromeric index
1H	5.90	4.43	10.33±0.23**	16.19	1.33	0.43
2H	5.94	3.89	9.83±0.34	15.41	1.53	0.40
3H	5.08	4.54 (2.73)*	9.62±0.35	15.09	1.12	0.47
4H	5.58	3.40	8.98±0.71	14.08	1.64	0.38
5H	4.90	3.94	8.84±0.55	13.87	1.24	0.45
6H	6.12	2.48	8.60±0.65	13.49	2.47	0.29
7H	4.69	2.88	7.57±0.67	11.88	1.63	0.38

Total genome length = 63.77±2.94 µm

* the length of satellites

** standard deviation

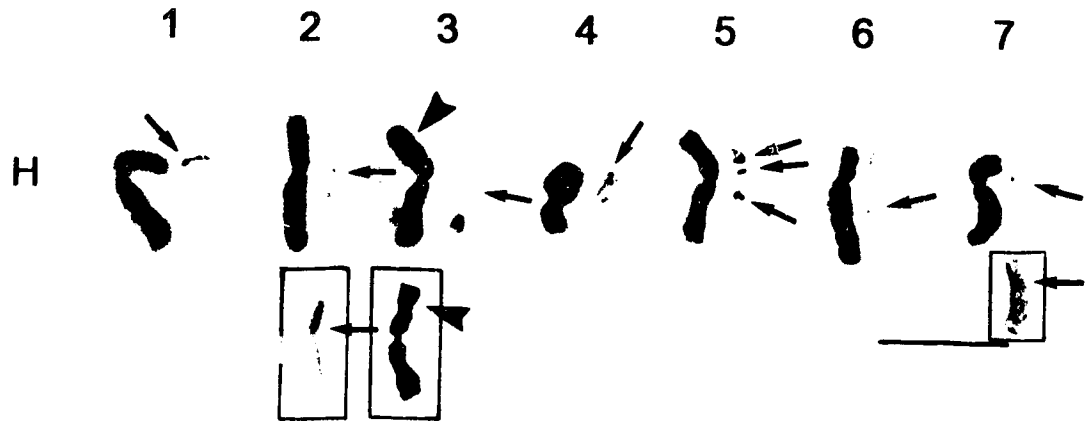

Figure 4.7 Karyotype of the *C. californicum* genome based on sequential acetocarmine staining/N-banding techniques. Acetocarmine stained chromosomes (left hand side) and the same chromosomes subsequently stained by the N-banding treatment (right-hand side), arrows indicate N-bands, arrow heads indicate satellites, insets show N-banded chromosomes 2H and 7H and acetocarmine stained SAT-chromosome 3H from the different cells, and scale bar = 10 μm

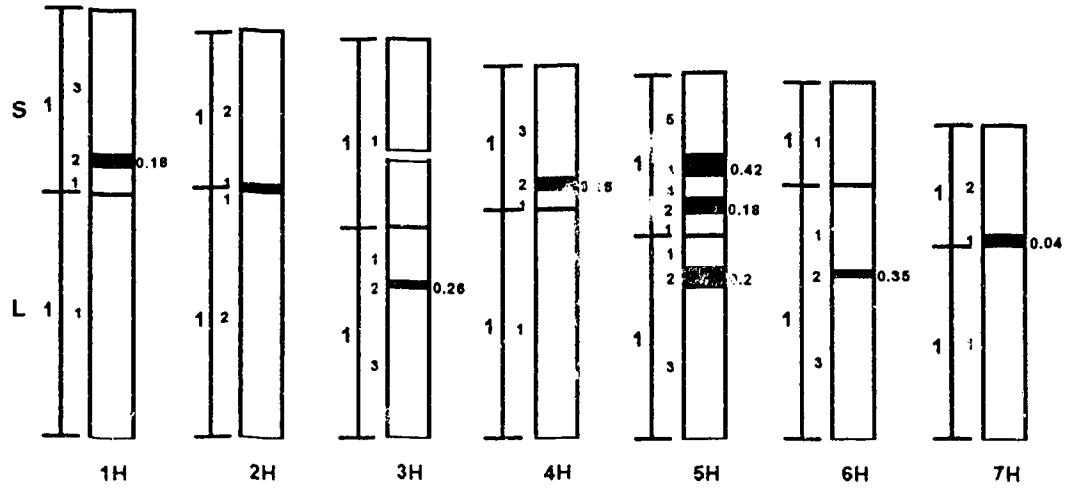

Figure 4.8 Idiogram of N-banded chromosomes of *C. californicum.*

1.3.2 N-banded karyotype of *C. californicum*

The N-banded karyotype of *C. californicum* based on sequential acetocarmine staining and N-banding is shown in Figure 4.7. The N-banding pattern indicates very small to larger bands at centromeric and intercalary regions with the absence of terminal bands.

Chromosome 1H

This chromosome reveals a distinct band in the short arm proximal to the centromere.

Chromosome 2H

A centromeric band is located in this chromosome.

Chromosome 3H

The subdivision of the secondary constriction region in the short arm of this metacentric chromosome cannot be seen using the N-banding technique. It possesses an interstitial band in the proximal region of the long arm.

Chromosome 4H

This chromosome has a prominent proximal band in the short arm.

Chromosome 5H

Two distinct proximal bands are seen in the short arm and a conspicuous proximal band is observed in the long arm of chromosome 5H which aid its differentiation from the other chromosomes. In addition, the larger of the two proximal bands in the short arm is located distally.

Chromosome 6H

Chromosome 6H also exhibits a interstitial band in the proximal region of the long arm like satellited chromosome 3H, but this chromosome is submetacentric.

Chromosome 7H

The distinct pericentromeric band on the short arm is highly diagnostic for its identification.

Chromosomes 1H and 4H cannot be distinguished using the N-banding technique. These two chromosomes were differentiated on the basis of size and arm ratio.

1.3.3 Standard karyotype of *C. californicum.*

The idiogram of N-banded chromosomes and fraction lengths of diagnostic N-bands of *C. bogdanii* are shown in Figure. 4.8 and Table 4.6, respectively.

Table 4.6 Fraction lengths of diagnostic N-bands with band lengths of *C. californicum* chromosomes.

Chromosome	Diagnostic N-bands	Fraction length	Band length (μm)
1H	1HS1.2	0.18	0.26
2H	2HS1.1	c.b.*	0.08
	2HL1.1	c.b.	0.08
3H	3HL1.2	0.26	0.11
4H	4HS1.2	0.16	0.23
5H	5HS1.2	0.18	0.33
	5HS1.4	0.42	0.40
	5HL1.2	0.20	0.44
6H	6HL1.2	0.35	0.12
7H	7HS1.1	0.04	0.22
		Total length of bands	2.27 μm

* c.b.= centromeric band

2. Karyotype analysis of tetraploid species: *Elymus trachycaulus* and *Elymus canadensis*

Eight of the fourteen pairs of *Elymus* chromosomes show N-bands. This study found that all seven chromosome pairs in the two H genomes have N-bands. Only one chromosome in the genome of *P. spicata*, the diploid species with the S genome shows any N-banding and this is a single interstitial band in the long arm of chromosome 6S. Therefore, it can be inferred that the *Elymus* chromosomes with major N-bands probably belong to the H genome.

2.1 *Elymus trachycaulus*

2.1.1. The cytology of *E. trachycaulus*

The karyotype of *E. trachycaulus* based on sequential acetocarmine staining and N-banding is presented in Figure 4.9 and chromosome measurements are reported in Tables 4.7 and 4.8. The karyotype of *E. trachycaulus* shows three pairs of satellited chromosomes, two pairs in the S genome and one pair in the H genome. The SAT-chromosome in the H genome is submetacentric, but the rest of the chromosomes in this genome are metacentrics.

2.1.2. N-banded karyotype of *E. trachycaulus*

In *E. trachycaulus*, of the six chromosome pairs without N-bands, two are metacentric and satellited. The larger of these two chromosome pairs has small satellites on the short arms and the smaller chromosome pair has large satellites on the short arms. These two chromosome pairs are morphologically similar to the two satellited chromosome pairs observed in *P. spicata*. The relative lengths of these two pairs of satellited chromosomes is also similar to the two satellited chromosomes in *P. spicata*. Hence, these six chromosomes were assigned to the S genome.

The other eight chromosome pairs with N-bands contain one pair of submetacentric satellited chromosomes with a proximal band in the long arm, one pair of large chromosomes with distinct centromeric bands, one pair of medium-sized chromosomes with a clear interstitial band at the proximal half of the long arm, and another five chromosome pairs with major N-bands in the centromeric and intercalary regions. The chromosome pair with a distinct centromeric band was found to be the largest of these eight chromosome pairs and has the same banding pattern as N-banded chromosome 3 in the H genome of the *E. trachycaulus* karyotype in Morris and Gill (1987). The N-banded chromosome 3 in their karyotype was also the largest in the H genome. Therefore, this large chromosome pair with a distinct centromeric band was designated as chromosome 1H on the basis of size and N-banding pattern. The medium-sized chromosome pair possesses the same N-banding pattern as chromosome pair 6S of *P. spicata* with an interstitial band at the proximal half of the long arm. This chromosome pair was assigned to the S genome on the basis of its N-banding pattern.

In summary, the S-genome chromosomes consist of six chromosome pairs without N-bands and the medium-sized chromosome pair with an interstitial band in the proximal half of the long arm; the H-genome chromosomes contain one pair of submetacentric satellited chromosomes with a proximal band in the long arm, one pair of the largest chromosomes with a distinct centromeric band, and five chromosome pairs with intense N-bands.

The S genome

The N-banding pattern of the S genome shows a prominent interstitial band in the proximal half in the long arm of chromosome 4S as indicated by an arrow in Figure 4.9.

The H genome

The N-bands of the chromosomes in the H genome are located in the intercalary and centromere regions of the chromosomes (Figure 4.9). The pattern and intensity of the N-bands allows the identification of all seven chromosomes. A more detail description of the N-banding pattern of each chromosome in the H genome is presented below.

Chromosome 1H

This is the largest chromosome in the complement and reveals a distinct centromeric band that facilitates its identification.

Chromosome 2H

A very large interstitial band is located in the proximal half of the short arm with a small band in the proximal region of its long arm.

Chromosome 3H

Chromosome 3H has one prominent interstitial band in the distal region of the short arm.

Chromosome 4H

This satellited chromosome is submetacentric with a faint proximal band in the long arm.

Chromosome 5H

Chromosome 5H exhibits a distinct interstitial band in the proximal half of the short arm with a conspicuous band in the proximal region of the long arm. The specific N-banding pattern makes this chromosome easily identifiable.

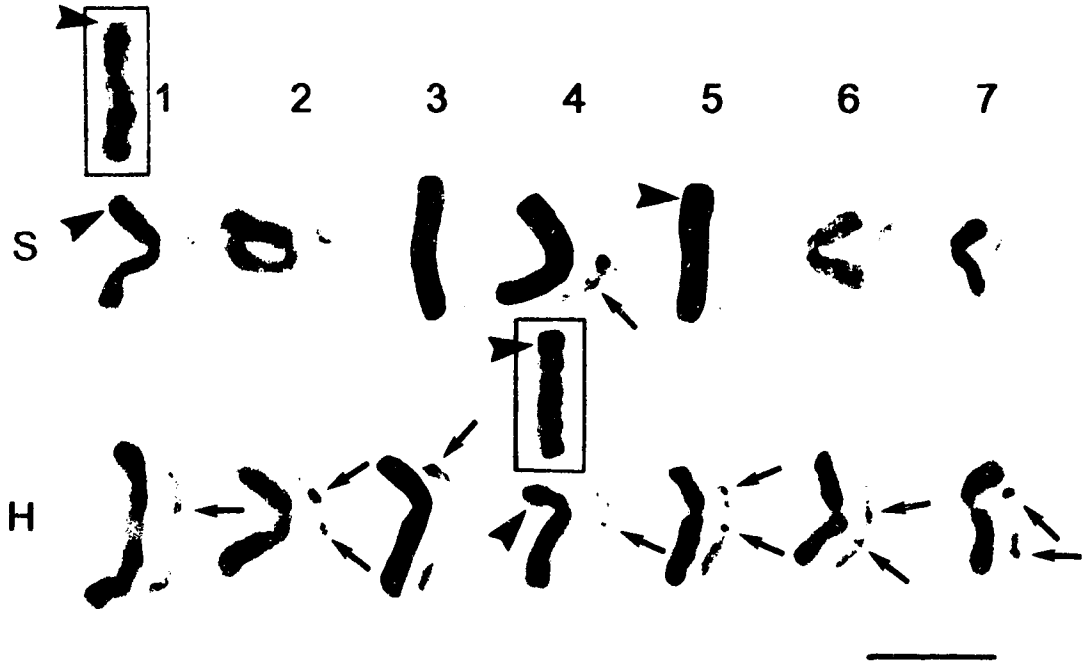

Figure 4.9 Karyotype of the *Elymus trachycaulus* genomes S and H based on sequential acetocarmine staining/N-banding techniques. Acetocarmine stained chromosomes (left-hand side) and the same chromosomes subsequently stained by the N-banding treatment (right-hand side), arrows indicate N-bands, arrow heads indicate satellites, insets over the chromosomes show SAT-chromosomes 1S and 4H from the different cell and scale bar = 10 μm.

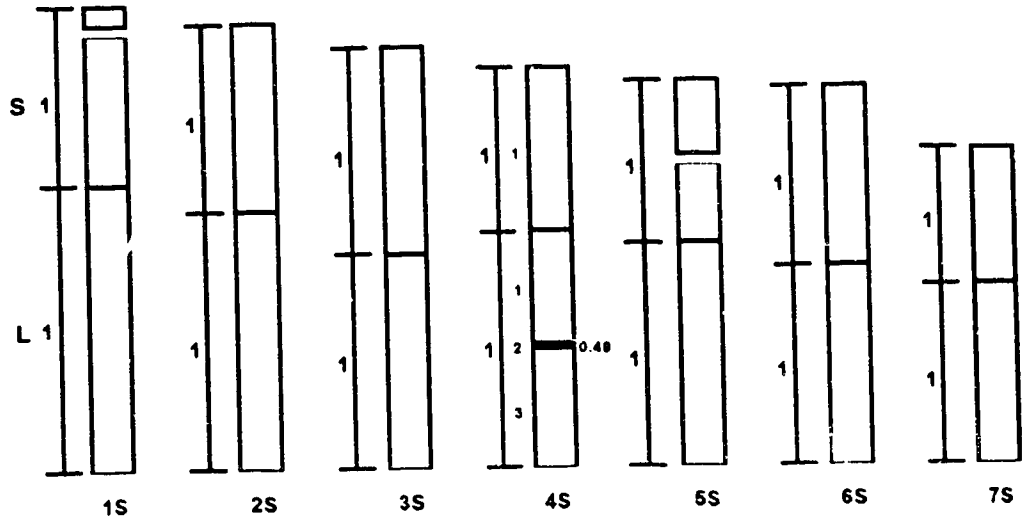

Figure 4.10 Idiogram of N-banded chromosomes of the S genome of *E. trachycaulus*.

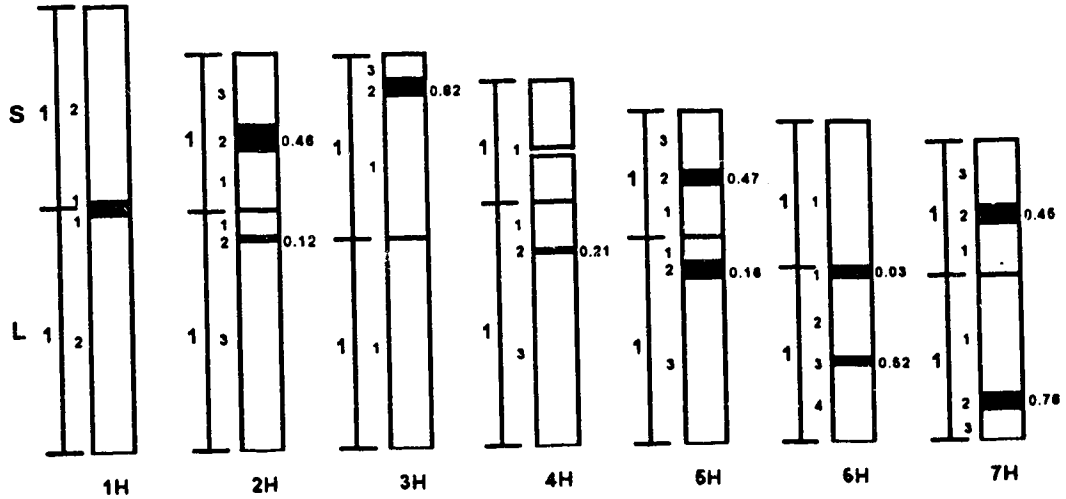

Figure 4.11 Idiogram of N-banded chromosomes of the H genome of *E. trachycaulus*.

Table 4.7 Measurements of the S-genome chromosomes of *E. trachycaulus*.

Chromosome	Length of long arm (Ll)	Length of short arm (Ls)	Total length	Relative length	Arm ratio (Ll / Ls)	Centromeric index
1S	7.00	4.36 (0.50)*	11.36±0.06**	16.53	1.61	0.38
2S	6.33	4.56	10.89±0.37	15.85	1.39	0.42
3S	5.28	5.02	10.30±0.40	14.99	1.05	0.49
4S	5.83	3.94	9.77±0.29	14.21	1.48	0.40
5S	5.50	3.92 (1.80)*	9.42±0.15	13.71	1.40	0.42
6S	4.91	4.34	9.25±0.07	13.46	1.13	0.47
7S	4.45	3.29	7.74±0.66	11.26	1.35	0.43

Total genome length = 68.73±1.47 µm

* the length of satellites
** standard deviation

Table 4.8 Measurements of the H-genome chromosomes of *E. trachycaulus*.

Chromosome	Length of long arm (Ll)	Length of short arm (Ls)	Total length	Relative length	Arm ratio (Ll / Ls)	Centromeric index
1H	6.89	5.65	12.54±0.75**	17.44	1.22	0.45
2H	6.79	4.36	11.15±0.67	15.51	1.56	0.39
3H	5.92	5.16	11.08±0.77	15.41	1.15	0.47
4H	6.89	3.39 (1.88)*	10.28±0.73	14.30	2.03	0.33
5H	5.85	3.54	9.38±0.55	13.05	1.65	0.38
6H	4.80	4.21	9.02±0.60	12.54	1.14	0.47
7H	4.66	3.78	8.44±0.21	11.74	1.23	0.45

Total genome length = 71.89±4.01 µm

* the length of satellites
** standard deviation

Chromosome 6H

Chromosome 6H shows a pericentromeric band with a smaller band in the proximal half of the long arm.

Chromosome 7H

This chromosome is characterized by a big interstitial band in the proximal half of the short arm with a clear band in the distal region of its long arm.

2.1.3 Standard karyotype of *E. trachycaulus*

Figures 4.10 and 4.11 present idiograms of N-banded chromosomes of the S and the H genomes of *E. trachycaulus*. Fraction lengths of N-bands with their band lengths are given in Table 4.9.

Table 4.9 Fraction lengths of diagnostic N-bands with band lengths of *E. trachycaulus* chromosomes.

Chromosome	Diagnostic N-bands	Fraction length	Band length (µm)	Chromosome	Diagnostic N-bands	Fraction length	Band length (µm)
1S	-	-	-	1H	1HS1.1	c.b.*	0.18
					1HL1.1	c.b.	0.18
2S	-	-	-	2H	2HS1.2	0.46	0.68
					2HL1.2	0.12	0.10
3S	-	-	-	3H	3HS1.2	0.82	0.44
4S	4SL1.2	0.49	0.12	4H	4HL1.2	0.21	0.08
5S	-	-	-	5H	5HS1.2	0.47	0.38
					5HL1.2	0.16	0.44
6S	-	-	-	6H	6HL1.1	0.03	0.28
					6HL1.3	0.52	0.18
7S	-	-	-	7H	7HS1.2	0.45	0.48
					7HL1.2	0.76	0.40
Total length of bands			0.12 µm				3.82µm

* c.b. = centromeric band

2.2. *Elymus canadensis*

2.2.1. The cytology of *E. canadensis*

The present study is the first to report karyotype and N-banding analysis of *E. canadensis* chromosomes. Karyotype of *E. canadensis* based on sequential acetocarmine staining and N-banding is shown in Figure 4.12 and chromosome measurements are given in Tables 4.10 and 4.11. The karyotype of *E. canadensis* shows three pairs of satellited chromosomes, two pairs in the S genome and one pair in the H genome, similar to the pattern found in *E. trachycaulus*. Almost all of the chromosomes are metacentric, except chromosome 2S and SAT-chromosome 4H which are submetacentric.

2.2.2. N-banded karyotype of *E. canadensis*

In *E. canadensis*, six chromosome pairs with no N-bands include two pairs of satellited chromosomes. The characteristics of these satellited chromosomes are similar to the satellited chromosomes in *P. spicata*. The larger chromosome pair has small satellites and the smaller chromosome pair has large satellites on the short arms, and both pairs are metacentric. The arm ratios of these satellited chromosomes are similar to those of the satellited chromosomes in *P. spicata*, and all of these chromosomes are devoid of N-bands. Therefore, all six chromosome pairs without N-bands were allocated to the S genome.

Eight chromosome pairs with N-bands consist of one pair of submetacentric satellited chromosomes with distinct interstitial bands in corresponding positions in the long arm, two chromosome pairs (one large and one small) possess prominent centromeric bands, and five chromosome pairs reveal heavy N-bands in the centromeric and intercalary regions. According to the allocation of the H-genome chromosomes in *E. trachycaulus*, the large chromosome pair with a prominent centromeric band, which is the largest of these eight chromosome

pairs, was designated as chromosome 1H. The process of elimination indicated that the small chromosome with a centromeric band should be assigned to the S genome.

In brief, the S-genome possesses six chromosomes without N-bands and one chromosome with a centromeric band; the H-genome consists of one submetacentric satellited chromosome with a distinct interstitial band in the long arm, one large chromosome with a prominent centromeric band, and five chromosomes with heavy N-bands.

The S genome

Most S genome chromosomes seldom exhibit diagnostic N-bands such as the pattern observed in *P. spicata*. Only a clear centromeric band is observed in chromosome 6S as marked by an arrow (Figure 4.12).

The H genome

The N-banding patterns of H genome chromosomes can be characterized by the existence of conspicuous and even large interstitial bands and smaller centromeric bands (Figure 4.12).

Chromosome 1H

A prominent centromeric band provides easy identification.

Chromosome 2H

This chromosome possesses a very large interstitial band in the distal region of its long arm which distinguishes it from all other members of the H genome.

Chromosome 3H

This chromosome has conspicuous interstitial bands in the distal region of both arms as well as a smaller centromeric band.

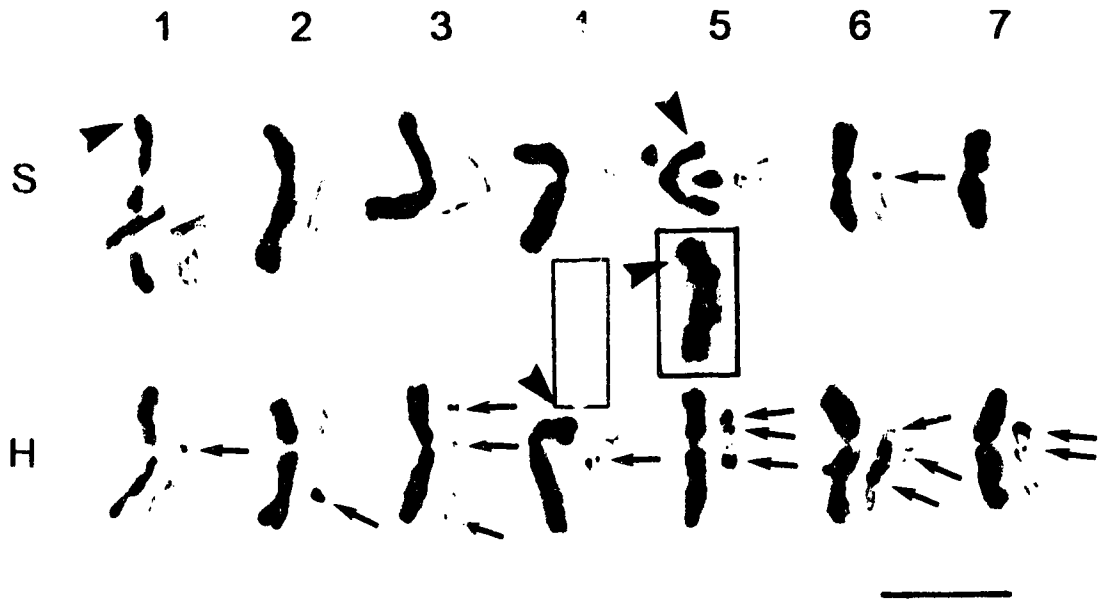

Figure 4.12 Karyotype of the *Elymus canadensis* genomes S and H based on sequential acetocarmine staining/N-banding techniques. Acetocarmine stained chromosomes (left-hand side) and the same chromosomes subsequently stained by the N-banding treatment (right-hand side), arrows indicate N-bands, arrow heads indicate satellites, insets show N-banded chromosome 4S and acetocarmine stained SAT-chromosome 5S from the different cells and scale bar = 10 μm.

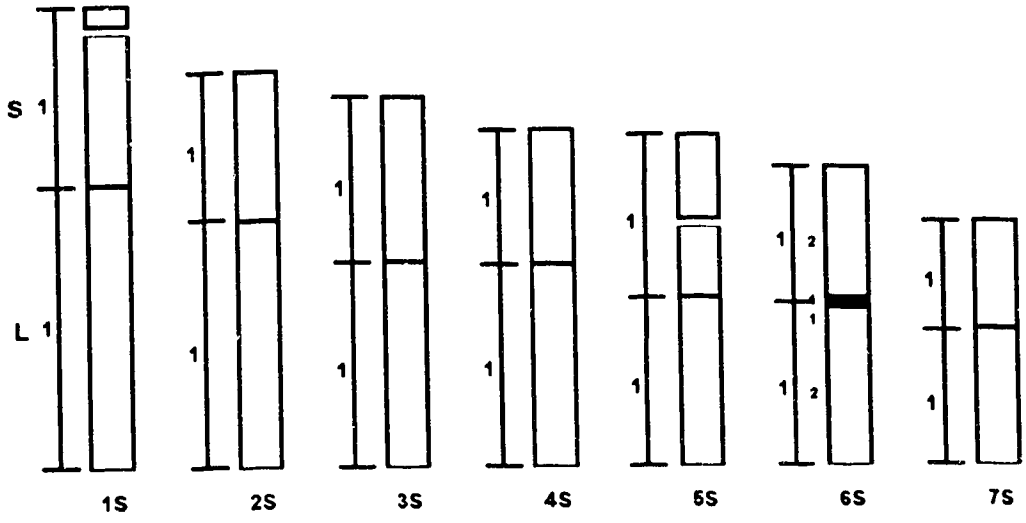

Figure 4.13 Idiogram of N-banded chromosomes of the S genome of *E. canadensis*.

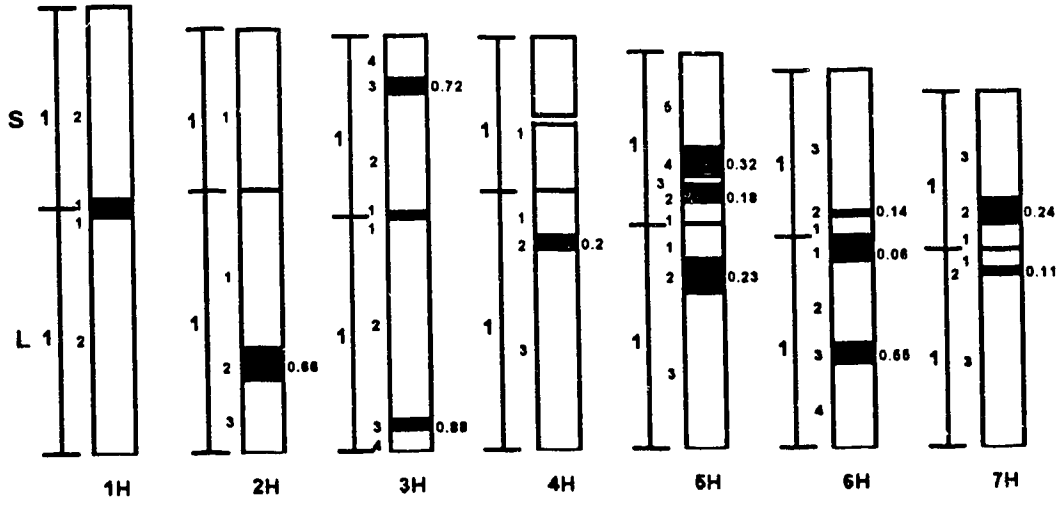

Figure 4.14 Idiogram of N-banded chromosomes of the H genome of *E. canadensis*.

Table 4.10 Measurements of the S-genome chromosomes of *E. canadensis.*

Chromosome	Length of long arm (Ll)	Length of short arm (Ls)	Total length	Relative length	Arm ratio (Ll / Ls)	Centromeric index
1S	8.05	5.12 (0.58)*	13.17±0.69**	18.94	1.57	0.39
2S	7.06	4.21	11.27±0.78	16.21	1.68	0.37
3S	5.87	4.66	10.53±0.85	15.15	1.26	0.44
4S	5.80	3.80	9.60±0.95	13.80	1.53	0.40
5S	4.85	4.60 (2.37)*	9.45±0.62	13.59	1.05	0.49
6S	4.68	3.84	8.52±0.31	12.25	1.22	0.45
7S	3.94	3.04	6.98±0.13	10.05	1.30	0.44

Total genome length = 69.52±3.88 μm

* the length of satellites
** standard deviation

Table 4.11 Measurements of the H-genome chromosomes of *E. canadensis.*

Chromosome	Length of long arm (Ll)	Length of short arm (Ls)	Total length	Relative length	Arm ratio (Ll / Ls)	Centromeric index
1H	5.94	4.82	10.76±0.66**	15.82	1.23	0.45
2H	6.34	3.84	10.18±0.43	14.97	1.65	0.38
3H	5.68	4.31	9.99±0.31	14.69	1.32	0.43
4H	6.26	3.67 (1.88)*	9.93±0.36	14.60	1.70	0.37
5H	5.43	4.07	9.50±0.40	13.96	1.33	0.43
6H	5.11	3.98	9.09±0.70	13.37	1.28	0.44
7H	4.78	3.78	8.56±0.80	12.59	1.26	0.44

Total genome length = 68.01±3.15 μm

* the length of satellites
** standard deviation

Chromosome 4H

This submetacentric SAT-chromosome reveals a prominent interstitial band in the proximal region of the long arm which aids in its identification.

Chromosome 5H

This chromosome is easily differentiated from all others by two easily identifiable interstitial bands in the proximal region of the short arm and a very large proximal band in the long arm. The larger band in the short arm is located distally.

Chromosome 6H

This chromosome possesses a conspicuous pericentromeric band along with a prominent band in the proximal half of the long arm. It also has a very faint proximal band in the short arm.

Chromosome 7H

Chromosome 7H shows a large proximal band in the short arm and a small proximal band in the long arm.

2.2.3. Standard karyotype of *E. canadensis*

Idiograms of N-banded chromosomes of the S genome and the H genome of *E. canadensis* are shown in Figures 4.13 and 4.14, respectively. Table 4.12 describes the fraction lengths of diagnostic N-bands and their band lengths.

Table 4.12 Fraction lengths of diagnostic N-bands with band lengths of *E. canadensis* chromosomes.

Chromosome	Diagnostic N-bands	Fraction length	Band length (μm)	Chromosome	Diagnostic N-bands	Fraction length	Band length (μm)
1S	-	-	-	1H	1HS1.1	c.b.*	0.21
					1HL1.1	c.b.	0.21
2S	-	-	-	2H	2HL1.2	0.66	0.76
3S	-	-	-	3H	3HS1.1	c.b.	0.05
					3HS1.3	0.72	0.35
					3HL1.1	c.b.	0.05
					3HL1.3	0.88	0.22
4S	-	-	-	4H	4HL1.2	0.20	0.32
5S	-	-	-	5H	5HS1.2	0.18	0.41
					5HS1.4	0.32	0.60
					5HL1.2	0.23	0.82
6S	6SS1.1	c.b.	0.15	6H	6HS1.2	0.14	0.11
	6SL1.1	c.b.	0.15		6HL1.1	0.06	0.56
					6HL1.3	0.55	0.48
7S	-	-	-	7H	7HS1.2	0.24	0.58
					7HL1.2	0.11	0.15
Total length of bands			0.30 μm				5.88μm

* c.b. = centromeric band

CHAPTER 5 DISCUSSION AND CONCLUSIONS

1. Chromosome identification of possible diploid progenitors and polyploid species using standard (acetocarmine) staining, C- and/or N-banding.

Giemsa C- and N-banding techniques provide unique banding patterns of plant chromosomes that can aid in their identification. Consequently, C- and N-banded karyotypes have been described in many species of the *Triticeae* tribe, such as *Triticum, Secale, Hordeum, Aegilops, Elymus,* and *Agropyron* (Endo and Gill 1984a, 1984b; Gerlach 1977; Gill 1981; Gill and Kimber 1974a, 1974b; Jewell 1979; Linde-Laursen *et al.* 1980; Morris and Gill 1987; and Seal 1982). Morris and Gill (1987) reported C- and N-banding analyses of the S- and the H-genome chromosomes of *P. spicata, C. bogdanii, C. californicum,* and *E. trachycaulus.* They constructed C- and N-banded karyotypes by pairing C- and N-banded homologous chromosomes within each species according to similarities in their morphologies and (or) banding patterns. The seven chromosomes in each genome were arbitrarily designated by numbers 1-7, and they were not intended to imply homology. Due to the deterioration of chromosomes after the banding treatment, the morphologies of chromosomes, especially the satellites, were difficult to identify. Consequently, it seems that the pairing of C- and N-banded homologous chromosomes within each species by Morris and Gill (1987) was only tentative. The karyotype information of these species required reexamination for more accurate karyotyping.

In this study, the karyotypes of *P. spicata, C. bogdanii, C. californicum, E. canadensis,* and *E. trachycaulus* were constructed by pairing of homologous chromosomes on the basis of morphologies and banding patterns from

sequential acetocarmine staining/N-banding analysis, then arranging the homologous chromosome pairs in each genomes in a standard manner, except for the karyotype of *P. spicata* which is based on conventional staining and sequential C-/N-banding techniques. Their standard banded karyotypes were established to facilitate the study of relationships and evolutionary hierarchy of the S and H genomes among these closely related species.

1.1. Identification of *P. spicata* chromosomes

The conventional karyotype of *P. spicata* has been described previously by Schulz-Schaeffer and Jurasits (1962), Dvórak *et al.* (1984), and Hsiao *et al.* (1986). For the construction of karyotypes by Hsiao *et al.* (1986), the chromosomes were arranged in order of decreasing size. The karyotype pattern of *P. spicata* observed in this study is similar to that reported by Hsiao *et al.* (1986) which indicated two pairs of satellited chromosomes: one pair of small and one pair of large satellites on the short arms of chromosomes designated as 1S and 5S, respectively, in both studies. *P. libanotica, P. stipifolia, P. cognata* and *P. strigosa ssp. aegilopoides* which are widely distributed in the Middle East and central Asia, have a karyotype pattern similar to that of *P. spicata* except for some differences in the order of the satellited chromosomes in the karyotype. Karyotypes of *P. libanotica, P. stipifolia,* and *P. cognata* have one pair of small and one pair of large satellites on the short arms of chromosomes 2S and 5S, respectively (Hsiao *et al.* 1986).

The C-banding patterns of *P. spicata* chromosomes revealed large terminal C-bands in both arms or only the short arms of all seven chromosome pairs, and a distinct interstitial band at the secondary constriction in the short arm of chromosome 5S with the large satellite (Fig. 4.1). The C-banded karyotype of *P.*

spicata shown in Morris and Gill (1987) was taken from a previous study by Endo and Gill (1984b). The plant accessions used by Endo and Gill (1984b) were as follows: *P. spicata* - CS-4-28, PI 232134, and CS-4-14; *P. spicata* var. *inerme* - CS-4-11, while *P. spicata* - PI 232127 was used in the present study. On the basis of chromosome morphologies and C-banding patterns following sequential acetocarmine staining/C-banding techniques, *P. spicata* chromosomes described by Endo and Gill (1984b) can be identified as presented in Table 5.1. Chromosomes A and B in their karyotype can be equated with chromosomes 5S and 1S, respectively, in this study because they have similar chromosome morphologies (e.g. the size of satellites) and C-banding patterns. Chromosomes D and G in their karyotype have different C-banding patterns from chromosomes 2S and 6S, respectively, in this study but they were matched on the basis of size.

Table 5.1 Comparison of nomenclature of *Psathyrostachys* chromosomes by C-banding among Endo and Gill (1984b), Morris and Gill (1987), and the present study.

Endo and Gill (1984b)	B	D?	E	F	A	G?	C
Morris and Gill (1987)	2	4	5	3	1	7	6
The present study	1S	2S	3S	4S	5S	6S	7S

Morris and Gill (1987) observed the total absence of N-banding patterns in all chromosomes of *P. spicata*. This study showed one small interstitial band, L1.2, at FL.48 in chromosome 6S (Figs. 4.3 and 4.4) which demonstrated the presence of the C-banding negative and N-banding positive (C^-N^+) class of heterochromatin. This change of the heterochromatin from N^- (C^-) to N^+ (C^-)

could be due to banding pattern polymorphism and/or technical procedures. *P. spicata* seeds used in both studies were provided from USDA-ARS, Utah State University, Logan, Utah, USA., but they were different plant accessions. Morris and Gill (1987) used plant accession no. 2218 (CS-4-28), while this study used plant accession no. PI 232127 (MA-87-71--75). Banding pattern polymorphism has been reported to exist within other species (Linde-Laursen *et al.* 1980; Linde Laursen and Bothmer 1984; Endo and Gill 1984a, 1984b; Kakeda *et al.* 1991).

Jewell (1981) reported detailed studies on the manipulation of different steps in the N-banding procedure in wheat chromosomes and concluded that temperature and duration of the treatment in 1M NaH_2PO_4 buffer were critical for good quality N-banding. The modified N-banding technique used by Endo and Gill (1984a) with shorter duration (from 3 min to 2 min) of the treatment with 1M NaH_2PO_4 at 94 °C allowed recognition of 16 bread wheat chromosome pairs rather than 9 as previously reported by Gerlach (1977). Kakeda *et al.* (1991) confirmed that the temperature reduction in 1M NaH_2PO_4 (from 94 °C to 90°C) produced an increased numbers of bands and a stable appearance of some bands in barley (*Hordeum vulgare* L.) chromosomes.

In the present N-banding procedure, the temperature of the treatment with 1M NaH_2PO_4 was reduced from 94 °C (Endo and Gill (1984a)'s procedure) to 92 °C for 3 min. Morris and Gill (1987) also followed the N-banding technique of Endo and Gill (1984a) with slight modifications but no details were given. Jewell (1981) found that the heterochromatin at the nucleolar organizers (NORs) on chromosomes 1B and 6B were changed from N^- (C^-) to N^+ (C^-) when the temperature of 1M NaH_2PO_4 treatment was reduced. It indicated that N-banding produced new bands even after the C-banding treatment. In this study, C- and

N-banding analysis of *P. spicata* chromosomes shows mostly C^+N^- heterochromatin; nevertheless, the C^-N^+ heterochromatin was observed in the proximal half in the long arm of chromosome 6S. It suggests that the N-band, L1.2, at FL.48 on chromosome 6S of *P. spicata* in the present study may be due to the temperature reduction in 1M NaH_2PO_4.

1.2. Identification of chromosomes of *C. bogdanii* and *C. californicum*

In the present study, the idiogram of N-banded chromosomes of *C. bogdanii* shows six metacentric chromosomes and one SAT-chromosome as presented in Fig. 5.1, which agrees with the descriptions given by Linde-Laursen *et al.* (1980, 1992), and Hsiao *et al.* (1986). The idiogram of *C. californicum,* presented in Fig. 5.1, shows one SAT-chromosome pair, submetacentric chromosome, and five metacentric chromosomes, as previously reported by Morrison (1959), and Hsiao *et al.* (1986). The presence of one pair of large satellites at the terminal ends of the short arms of one of the seven chromosome pairs of *C. bogdanii* and *C. californicum* was also reported by Rajhathy *et al.* (1964). This study confirms that chromosome 1H in *C. bogdanii* and chromosome 3H in *C. californicum* are satellited as previously described by Hsiao *et al.* (1986).

The N-banding patterns of *C. bogdanii* and *C. californicum* were previously described by Morris and Gill (1987), but chromosomes were not arranged in the standard manner. Therefore, the order of chromosomes in the karyotypes of this study does not correspond with the ones in Morris and Gill (1987). However, some N-banded chromosomes in both studies can be compared on the basis of banding patterns as presented in Tables 5.2 and 5.3.

Table 5.2 Comparison of nomenclature of *C. bogdanii* chromosomes by N-banding between Morris and Gill (1987) and the present study.

Morris and Gill (1987)	3	5	6 or 7	2	4	1
The present study	1H	2H	3H or 4H	5H	6H	7H

From the N-banded karyotype of *C. bogdanii* presented by Morris and Gill (1987), chromosomes 2, 4, and 5 can be identified as chromosomes 5H, 6H, and 2H, respectively, in this study as presented in Table 5.2. Chromosome 5H in this study shows proximal bands on both arms of the chromosome, the same as the pattern found on chromosome 2 in their results. Chromosomes 6H and 2H possess centromeric bands such as those observed in chromosomes 4 and 5 in their results. Chromosome 1 appears to be the smallest chromosome in their karyotype and has a proximal band in the short arm as does chromosome 7H the smallest chromosome in our karyotype. However, additional differences between these chromosomes were observed. Chromosome 7H also has a centromeric band not seen in chromosome 1 in their results, and chromosome 1 has an interstitial band in the long arm. Chromosome 3 in their karyotype appears to be homologous to chromosome 1H in this study because both chromosomes are large and reveal an interstitial band in their short arms. N-bands were not detected on chromosomes 6 and 7 in their results; therefore, they cannot be identified. Although both studies used the same plant accession (PI 314696) of *C. bogdanii*, the N-banding patterns in the two studies also reveal some variations. The present study distinguished all seven chromosomes of *C. bogdanii* by sequential acetocarmine staining/N-banding analysis. N-banding reveals a distinct band at the secondary constriction in the short arm of SAT-

chromosome 1H, while the satellited chromosome cannot be identified in the karyotype of Morris and Gill (1987). The present N-banding pattern of *C. bogdanii* displays the enhancement in the number and intensity of some bands. This is probably due mainly to the modifications of the N-banding technique as described earlier.

Table 5.3 Comparison of nomenclature of *C. californicum* chromosomes by N-banding between Morris and Gill (1987) and the present study.

Morris and Gill (1987)	5	3 or 7	4	2	6	1
The present study	1H	2H or 3H	4H	5H	6H	7H

Chromosomes 1, 2, 4, and 5 in the N-banded karyotype of *C. californicum* in Morris and Gill (1987) can be recognized as chromosomes 7H, 5H, 4H, and 1H, respectively, in the present study as shown in Table 5.3. Chromosomes 7H in this study and chromosome 1 in their study possess a pericentromeric band in the short arm. Each of the chromosomes 1H and 4H in the present study have a proximal band in the short arm similar to chromosomes 5 and 4, respectively, in their study. Chromosome 2 in their study demonstrated the same N-banding pattern (two interstitial bands in the short arm and one interstitial band in the long arm) as chromosome 5H in this study. However, the bands were more intense in our karyotypes than in others. Chromosomes 3, 6, and 7 in Morris and Gill (1987) were not distinguished by N-banding analysis. On the basis of chromosome morphologies, chromosome 6 in the karyotype reported by Morris and Gill (1987) appears to be homologous to chromosome 6H in this study because both chromosomes are submetacentric. The sequential acetocarmine staining/N-banding analysis differentiates all seven chromosomes of *C.*

californicum in the present study. The variations of N-banding patterns could be attributed to the use of different plant accessions [2243(CS-15-26-30) in Morris and Gill (1987) and PI 531778 (C-37-26--30) in the present study] and the modified N-banding techniques.

The similarities and differences in morphologies and N-banding patterns of chromosomes of *C. bogdanii* and *C. californicum* in the present study can be observed in Fig. 5.1. The arm ratios of chromosomes 3H and 5H of *C. bogdanii* and *C. californicum* are almost alike as presented in Table 5.4. Chromosomes 2H in both species have a different arm ratio, but reveal the same N-banding pattern that shows a distinct centromeric band. In both species chromosome 5H reveals proximal bands on both arms. The differences between the two chromosomes are due to the different numbers of interstitial bands in their short arms. There are two bands in *C. californicum*, but one in *C. bogdanii*. The similarities of chromosome morphologies and banding patterns of these chromosomes of *C. bogdanii* and *C. californicum* indicate a close phylogenetic relationship between Asiatic *C. bogdanii* and North American *C. californicum*.

According to Linde-Laursen *et al.* (1992) each SAT-chromosome found in the Asian *C. roshevitzii*, *C. bogdanii*, and in the American diploid species including *C. californicum* resembles each of the three different SAT-chromosome types present in *C. brevisubulatum ssp. brevisubulatum* (2x). They proposed that the self-incompatible Asian *C. brevisubulatum* complex has a close relationship to Asian and American H genome diploid species and a central position in the karyotypic evolution of these species. Their findings were supported by electrophoretic analyses (Jørgensen 1986), SAT-chromosome types (Linde-Laursen *et al.* 1980, 1986, 1989, 1992), and the observations of moderately high to high chromosome pairing in some interspecific hybrids (Bothmer *et al.* 1986).

Evidences indicates that *C. bogdanii* anc *C. californicum* might have a similar evolutionary origin.

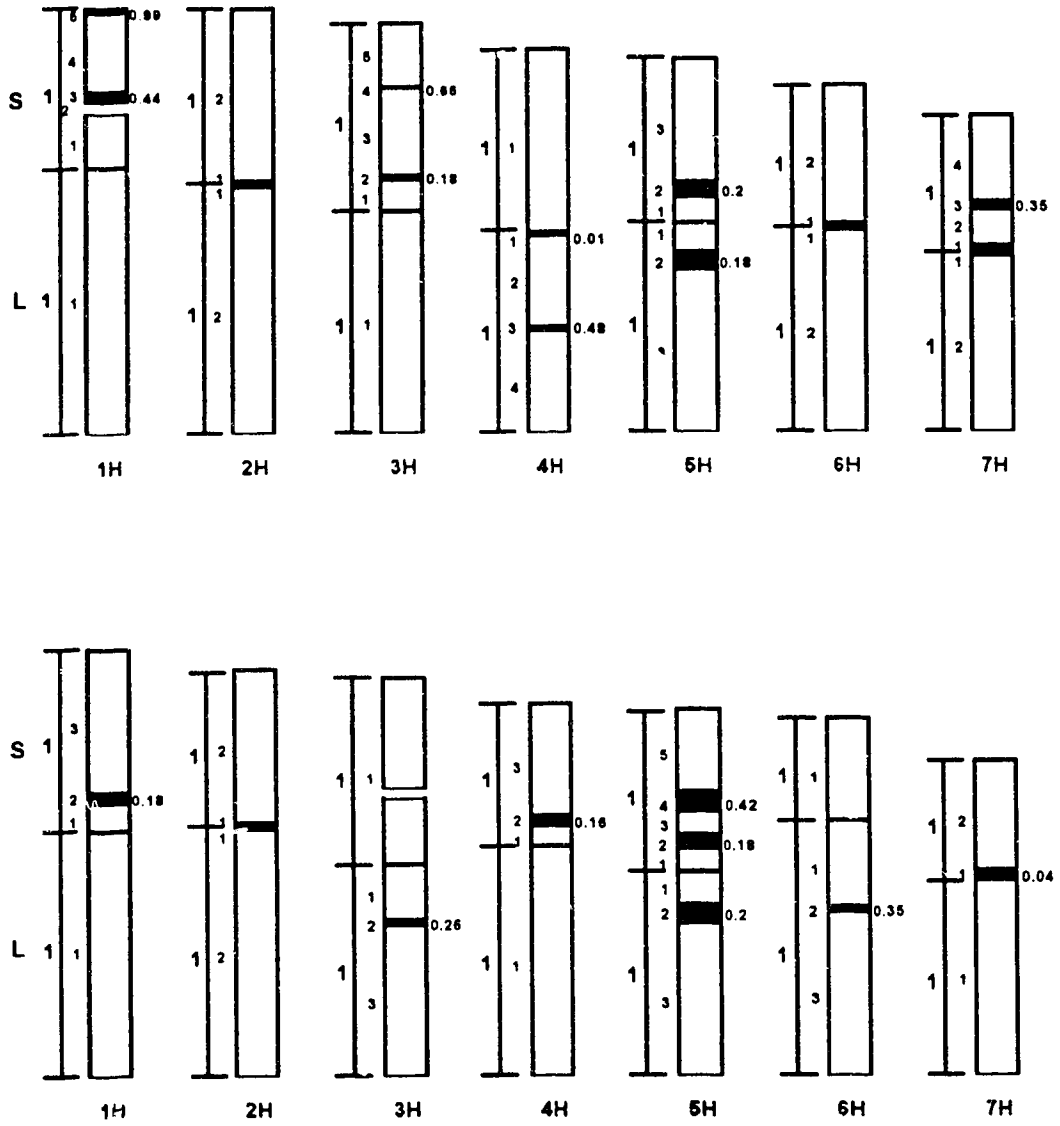

Figure 5.1 Comparison of N-banded idiograms of *C. bogdanii* (top) and *C. californicum* (bottom) chromosomes.

Table 5.4 Relative lengths, arm ratios, and diagnostic N-bands of *C. bogdanii* and *C. californicum* chromosomes.

	C. bogdanii				*C. californicum*			
Chromo-some	Relative length	Arm ratio	Diagnostic N-band	Fraction length	Relative length	Arm ratio	Diagnostic N-band	Fraction length
1H	15.90*	1.67	1HS1.3	0.44	16.19	1.33	1HS1.2	0.18
			1HS1.5	0.99				
2H	15.83	1.38	2HS1.1	c.b.	15.41	1.53	2HS1.1	c.b.**
			1HL1.1	c.b.			2HL1.1	c.b.
3H	15.26	1.18	3HS1.2	0.18	15.09*	1.12	3HL1.2	0.26
			3HS1.4	0.66				
4H	14.31	1.10	4HL1.1	0.01	14.08	1.64	4HS1.1	c.b.
			4HL1.3	0.48			4HS1.2	0.16
							4HL1.1	c.b.
5H	13.94	1.27	5HS1.2	0.20	13.87	1.24	5HS1.2	0.18
			5HL1.2	0.18			5HS1.4	0.42
							5HL1.2	0.20
6H	12.94	1.39	6HS1.1	c.b.	13.49	2.47	6HL1.2	0.35
			6HL1.1	c.b.				
7H	11.82	1.24	7HS1.1	c.b.	11.88	1.63	7HS1.1	0.04
			7HS1.3	0.35				
			7HL1.1	c.b.				

* satellited chromosomes

** c.b. = centromeric band

1.3. Identification of chromosomes of *E. canadensis* and *E. trachycaulus*

The C-banding pattern of *E. canadensis* chromosomes was reported by Park *et al.* (1990); however, the chromosomes were neither assigned to S or H genomes nor arranged in the standard manner. C- and N-banded karyotypes of *E. trachycaulus* were previously studied by Morris and Gill (1987). However, N-banding patterns of *E. canadensis* chromosomes and the standard karyotype and the chromosome measurements of both tetraploid species have not been investigated previously.

The C- and N-banded chromosomes of *E. trachycaulus* in Morris and Gill (1987) were tentatively assigned to the S and H genomes on the basis of similar heterochromatin compositions and positions in these species and its putative progenitor species. Their pairing of homologous chromosomes is questionable without applying the sequential acetocarmine staining/banding techniques. In addition, the assignment of chromosomes 1, 2, and 5 to the S genome on the basis of C-banding patterns is in doubt because they showed terminal, subterminal, and centromeric C-bands which are similar to the C-banding patterns of the H-genome chromosomes and are different from the C-banding patterns of *P. spicata* chromosomes. However, some N-banded chromosomes in their study and the present study can be compared on the basis of size and banding pattern as presented in Table 5.5.

In the karyotype of *E. trachycaulus* presented by Morris and Gill (1987), 11 of the 14 chromosome pairs show N-bands. These eleven pairs, including four pairs in the S genome of *E. trachycaulus* revealed centromeric and proximal N-bands in the long arms. The study of Jiang *et al.* (1994) reported that 8 of the 14 pairs of *E. trachycaulus* chromosomes have major N-bands, but these authors did not

present the karyotype of N-banded chromosomes. The plant accession used by Morris and Gill (1987) and Jiang *et al.* (1994) was Kansas State University accession number 2052. Jiang *et al.* (1994) used the procedure of Gill *et al.* (1991). The differences in N-banding patterns of homologues in these two studies indicate the effects of different N-banding techniques on the same plant accession.

Jiang *et al.* (1994) proposed that the satellited chromosome with one major N-band on the long arm, and other chromosomes without major N-bands, may belong to the S genome. In the present study, N-bands were recorded on 8 of the 14 pairs of *E. trachycaulus* chromosomes. Six chromosome pairs without N-bands include two pairs of satellited chromosomes which have similar relative length to each of the two satellited chromosomes in *P. spicata*. These two pairs of satellited chromosomes were assigned to the S genome. The other pair of satellited chromosom mal band in the long arm was assigned to the H genome. Th r with an interstitial band in the proximal half of the long arm N-banding patte:n as chromosome 6S of *P. spicata*, was

In the H genor ., 2, and 3 in Morris and Gill (1987) can be recognized as chromosomes 7H, 5H, and 1H, respectively, in this study as shown in Table 5.5. Chromosome 7H in this study has an interstitial band in the proximal half of the short arm and a distal band in the long arm which is the same as the band distribution in chromosome 1 in their study. The difference between the two chromosomes is the location of the centromeric band in chromosome 1. Chromosome 5H in this study and chromosome 2 in their study have prominent proximal bands in both arms. Chromosome 1H possesses a

Table 5.5 Comparison of nomenclature of *E. trachycaulus* chromosomes by N-banding between Morris and Gill (1987) and the present study.

	The S genome							The H genome						
Morris and Gill (1987)	2	3?	7?	5?	1	4	6	3	4?	5?	6	2	7?	1
The present study	1S	2S	3S	4S	5S	6S	7S	1H	2H	3H	4H	5H	6H	7H

centromeric band as does chromosome 3 in their study. Chromosome 4H in this study and chromosome 6 in their study have proximal bands in the long arm, but chromosome 6 also has a terminal band in the long arm. Chromosomes 4, 5, and 7 in their karyotype have different N-banding patterns from chromosomes 2H, 3H, and 6H, respectively, in this study; however, they were matched on the basis of size. The variations in N-banding patterns may be due to either the different plant accessions used [Kansas State University accession number 2052 by Morris and Gill (1987), and cultivar 'Revenue' in this present study] or the modification of N-banding techniques.

The comparison of idiograms of N-banded chromosomes of the S genomes of *E. canadensis* and *E. trachycaulus* in the present study are shown in Figure 5.2. The comparison of idiograms of N-banded chromosomes of the H genomes of *E. canadensis* and *E. trachycaulus* are presented in Figure 5.3. There are two SAT-chromosomes (1S and 5S) in the S genome, and one SAT-chromosome (4H) in the H genome of *E. canadensis*. The position of the satellited chromosomes in the S and the H genomes of *E. trachycaulus* are the same as in *E. canadensis*.

The similarities and differences in morphologies and N-banding patterns of S-genome chromosomes of *E. canadensis* and *E. trachycaulus* can be seen in Fig.

5.2. The arm ratios of chromosomes 1S, 4S, and 7S of *E. canadensis* and *E. trachycaulus* are very similar as shown in Table 5.6. Chromosomes 1S and 7S reveal no N-bands, while chromosome 4S of *E. trachycaulus* has a small interstitial band, L1.2, at FL.49. Chromosome 6S of *E. canadensis* possesses a prominent centromeric band. Neither of the SAT-chromosomes (1S and 5S) in *E. canadensis* and *E. trachycaulus* reveal N-bands.

While the arm ratios are similar for H-genome chromosomes 1H, 2H, and 7H of *E. canadensis* and *E. trachycaulus* as presented in Table 5.7, only chromosome 1H in both species have the same N-banding pattern that reveal a distinct centromeric band (Figure 5.3). The differences in arm ratios are observed for chromosomes 3H, 4H, 5H, and 6H in the two species; nevertheless, these chromosomes have very similar N-banding patterns. Chromosomes 3H, 5H, and 6H in *E. canadensis* reveal a larger number of bands than in *E. trachycaulus*. Differences are observed in the locations and intensities of N-bands in all of these four chromosomes.

Hybridization between *E. canadensis* and *E. trachycaulus* is achieved without much difficulty, particularly when *E. canadensis* is used as a pistillate parent (Aung and Walton 1990). In Appendix.1 meiotic pairing data of interspecific hybrids between *E. canadensis* x *E. trachycaulus* show a high mean bivalent frequency (Dewey 1968a; Aung and Walton 1990; Kumar and Walton 1990). Hybrids between these two species are highly sterile in spite of very good chromosome pairing. The sterility of the F_1 hybrids is attributed to gametic sterility due to structural differences between the genomes of the parental species (Kumar and Walton 1990). This conclusion is further substantiated by the studies of Dewey (1968a) and Aung and Walton (1990). They suggested that the genomes of the two species have very close homologies. However, two

interchanges involving three chromosomes, as evidenced by multivalent associations including quadrivalents and hexavalents, were reported in this hybrid by the above workers.

Additional evidence that there are structural differences between the genomes of these two species is apparent since there are some differences in morphologies and N-banding patterns of some chromosomes. Differences are observed in the arm ratios of chromosomes 2S, 3S, 5S, 6S, 3H, 4H, 5H, and 6H of *E. canadensis* and *E. trachycaulus*, the interstitial band, ' 1.2, of chromosome 4S and centromeric band of chromosome 6S, the band location and band intensity of chromosomes 2H, 3H, 5H, 6H, and 7H. Nevertheless, the genomes of *E. canadensis* and *E. trachycaulus* show similarities in morphologies of chromosomes 1S, 4S, 7S, 1H, 2H, and 7H; and N-banding patterns of chromosomes 1H, 4H, 5H, and 6H. This substantiates the close phylogenetic relationship of the two native North American species. However, they possess structural differences which are responsible for the sterility of the F1 hybrids and differences in morphologies and N-banding patterns of some chromosomes between the two species.

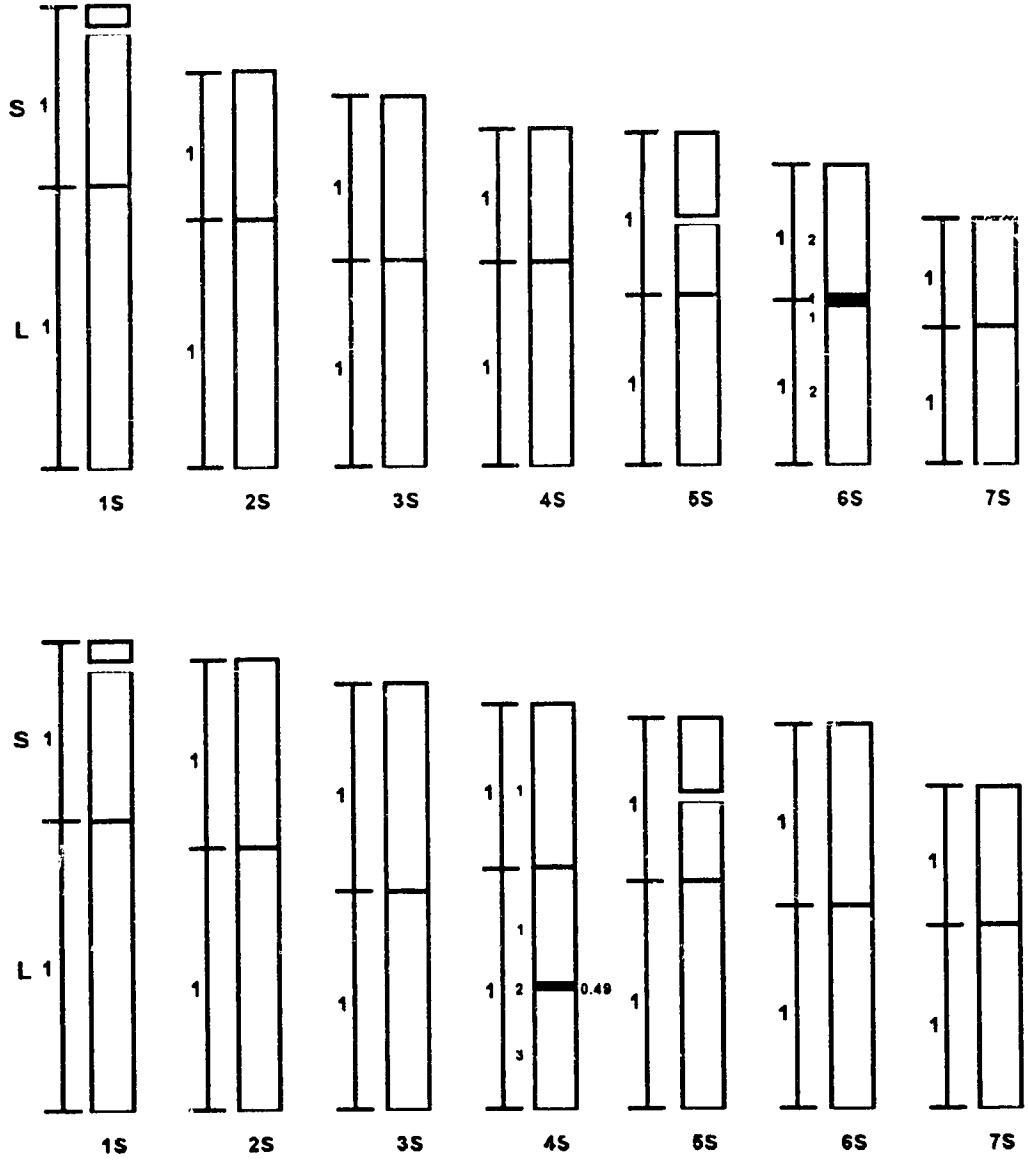

Figure 5.2 Comparison of idiograms of N-banded chromosomes of the S genome of *E. canadensis* (top) and *E. trachycaulus* (bottom).

Table 5.6 Relative lengths, arm ratios, and diagnostic N-bands of the S-genome chromosomes of *E. canadensis* and *E. trachycaulus*.

	E. canadensis				*E. trachycaulus*			
Chromo-some	Relative length	Arm ratio	Diagnostic N-band	Fraction length	Relative length	Arm ratio	Diagnostic N-band	Fraction length
1S	18.94*	1.57	-	-	16.53*	1.61	-	-
2S	16.21	1.68	-	-	15.85	1.39	-	-
3S	15.15	1.26	-	-	14.99	1.05	-	-
4S	13.80	1.53	-	-	14.21	1.48	4SL1.2	0.49
5S	13.59*	1.05	-	-	13.71*	1.40	-	-
6S	12.25	1.22	6SS1.1	c.b.**	13.46	1.13	-	-
			6SL1.1	c.b.				
7S	10.05	1.30	-	-	11.26	1.35	-	-

* satellited chromosomes

**c.b. = centromeric band

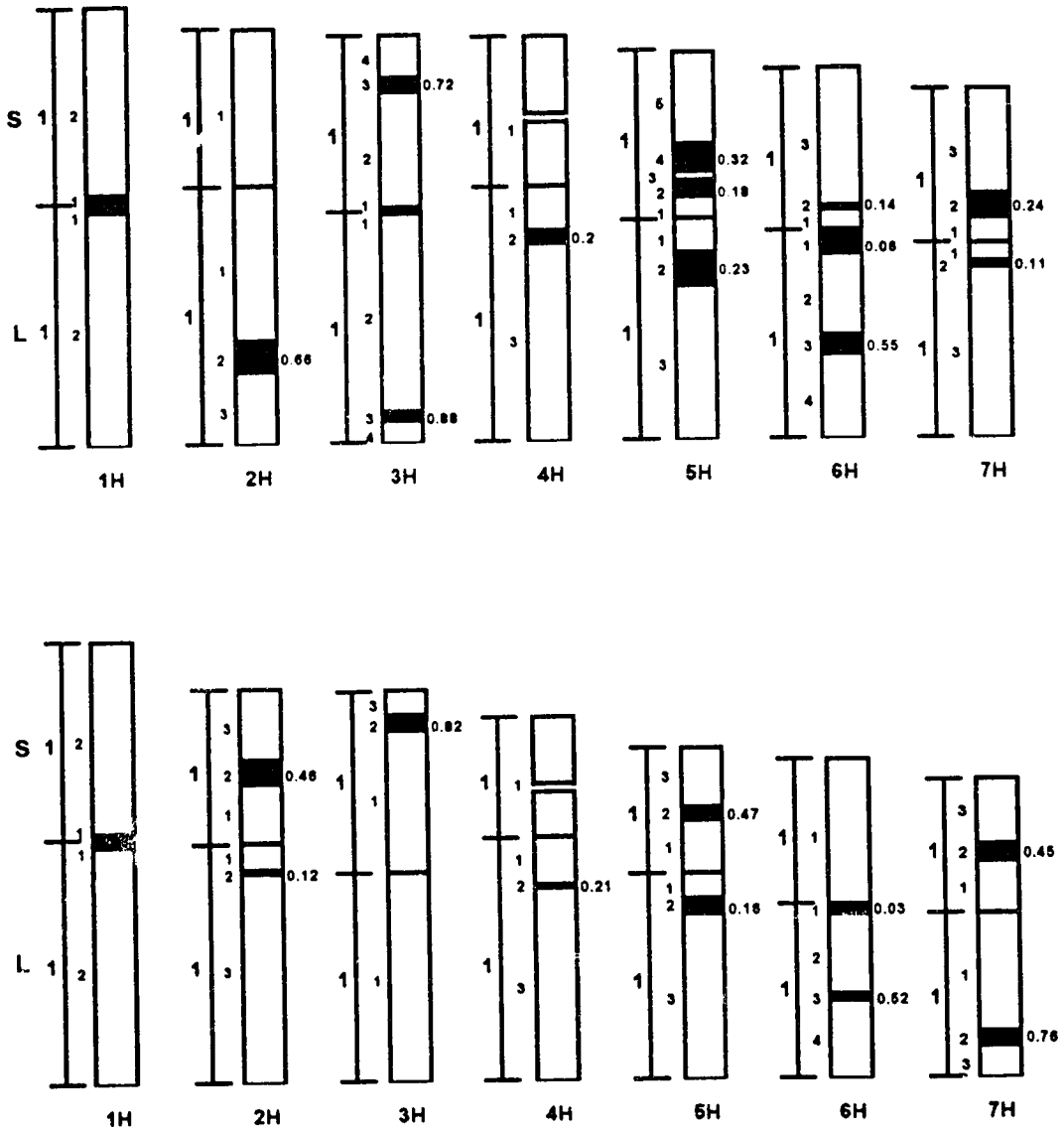

Figure 5.3 Comparison of idiograms of N-banded chromosomes of the H genome of *E. canadensis* (top) and *E. trachycaulus* (bottom).

Table 5.7 Relative lengths, arm ratios, and diagnostic N-bands of the H-genome chromosomes of *E. canadensis* and *E. trachycaulus*.

	E. canadensis				*E. trachycaulus*			
Chromosome	Relative length	Arm ratio	Diagnostic N-band	Fraction length	Relative length	Arm ratio	Diagnostic N-band	Fraction length
1H	15.82	1.23	1HS1.1	c.b.**	17.44	1.22	1HS1.1	c.b.
			1HL1.1	c.b.			1HL1.1	c.b.
2H	14.97	1.65	2HL1.2	0.66	15.51	1.56	2HS1.2	0.46
							2HL1.2	0.12
3H	14.69	1.32	3HS1.1	c.b.	15.41	1.15	3HS1.2	0.82
			3HS1.3	0.72				
			3HL1.1	c.b.				
			3HL1.3	0.88				
4H	14.60*	1.70	4HL1.2	0.20	14.30*	2.03	4HL1.2	0.21
5H	13.96	1.33	5HS1.2	0.18	13.05	1.65	5HS1.2	0.47
			5HS1.4	0.32			5HL1.2	0.16
			5HL1.2	0.23				
6H	13.37	1.28	6HS1.2	0.14	12.54	1.14	6HL1.1	0.03
			6HL1.1	0.06			6HL1.3	0.52
			6HL1.3	0.55				
7H	12.59	1.26	7HS1.2	0.24	11.74	1.23	7HS1.2	0.45
			7HL1.2	0.11			7HL1.2	0.76

* satellited chromosomes
** c.b. = centromeric band

2. Comparisons of chromosomes and genomes of possible diploid progenitors and polyploid species.

2.1. Comparisons of chromosomes of *P. spicata* with S-genome chromosomes of *E. canadensis* and *E. trachycaulus*.

The comparison of idiograms of N-banded chromosomes of *P. spicata* and the S genome of *E. canadensis* is shown in Fig. 5.4. The relative lengths, arm ratios, and diagnostic N-bands of chromosomes of *P. spicata* and the S-genome chromosomes of *E. canadensis* are presented in Table 5.8. The arm ratios of chromosomes 1S, 3S, 5S, and 7S of *P. spicata* are much like those of the 1S, 3S, 5S, and 7S chromosomes in the S genome of *E. canadensis*, and all of these chromosomes are devoid of N-bands. A small interstitial band, L1.2, at FL.48 appears on chromosome 6S of *P. spicata*, while there is a prominent centromeric band on chromosome 6S of *E. canadensis*.

The differences in morphologies and N-banding patterns of some S-genome chromosomes of *P. spicata* and *E. canadensis* indicate structural differences between the two S genomes. It may be that the centromeric band on chromosome 6S of *E. canadensis* is a consequence of a structural rearrangement(s) between chromosomes of the S and H genomes. Additional evidence for this comes from bivalents and multivalents observed in a SH dihaploid of *E. canadensis* (Torabinejad *et al.* 1987). These authors indicated that autosyndetic pairing seldom occurs between chromosomes of the S and H genomes of haploid *E. canadensis*. The rare trivalents and quadrivalents observed in the *E. canadensis* haploid suggest heterozygosity for a reciprocal translocation. This translocation could also be involved in multivalent formation in

hybrids derived from crosses *E. canadensis* x *E. trachycaulus* as described by Dewey (1968a), Aung and Walton (1990), and Kumar and Walton (1990).

Figure 5.5 presents the idiograms of N-banded chromosomes of *P. spicata* and the S-genome chromosomes of *E. trachycaulus*. Table 5.9 gives the relative lengths, arm ratios, and diagnostic N-bands of chromosomes of *P. spicata* and the S genome of *E. trachycaulus*. The sizes and arm ratios of chromosomes 1S, 3S, and 7S of *P. spicata* and the corresponding chromosomes in the S genome of *E. trachycaulus* are much alike. The N-banding patterns are not seen on these chromosomes. Chromosome 4S of *E. trachycaulus* reveals a band, L1.2, at FL.49. This interstitial band in the long arm of chromosome 4S of *E. trachycaulus* could be the result of a reciprocal translocation between the S-genome chromosomes of the diploid progenitor species. However, the differences in the morphologies and N-banding patterns of some chromosomes of *P. spicata* and *E. trachycaulus* suggest that there are also structural differences between the two S genomes.

Karyctype analyses of *P. spicata* and the S genome of *E. canadensis* and *E. trachycaulus* reveal that the two satellited chromosomes (1S and 5S) occupy the same positions in the karyotypes of the two species. There is one pair of small satellites on the short arms of chromosome pair 1S and one pair of large satellites on the short arms of chromosome pair 5S. *P. spicata* was reported to be the S genome donor of polyploid species of *Elymus* on the basis of a high affinity of chromosome pairing in the intergeneric hybrids (Stebbins and Snyder 1956; Dewey 1965, 1972, 1977, 1982, 1984a; Jensen *et al.* 1989; Aung and Walton 1987a). The meiotic pairing data of the triploid hybrids derived from the cross *E. trachycaulus* x *P. spicata* reported by Stebbins and Snyder (1956), Aung and Walton (1987a) showed strong homology between chromosomes of

the two S genomes. The chromosome pairing data of a triploid hybrid from the cross, *E. canadensis* x *P. spicata*, has not been reported. The similarity of karyotypes of *P. spicata* and the S genome of *E. canadensis* and *E. trachycaulus* found in this study confirm that the S genome of these two *Elymus* species is derived from *P. spicata*. However, the differences in morphologies and N-banding patterns of some S-genome chromosomes between *P. spicata* and the two *Elymus* species sho[illegible]at there are structural differences between the S-genome chromosome[illegible] diploid and the tetraploid species. This conclusion is substantiated by the [illegible]ility of the triploid hybrid derived from the cross, *E. trachycaulus* x *P. spicata*, reported by Aung and Walton (1987a) which is attributed to structural differences between the two S genomes.

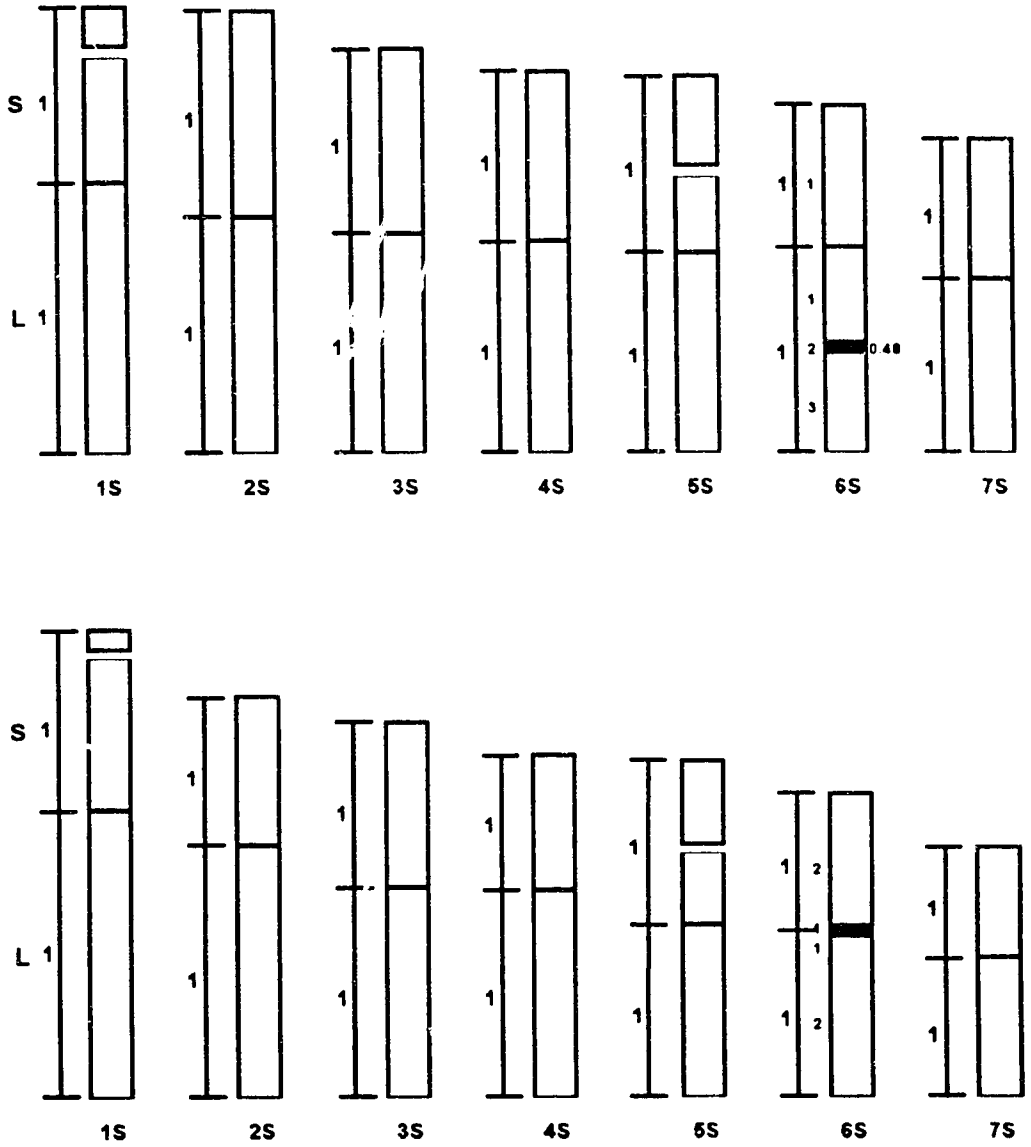

Figure 5.4 Comparison of idiograms of N-banded chromosomes of *P. spicata* (top) and the S genome of *E. canadensis* (bottom).

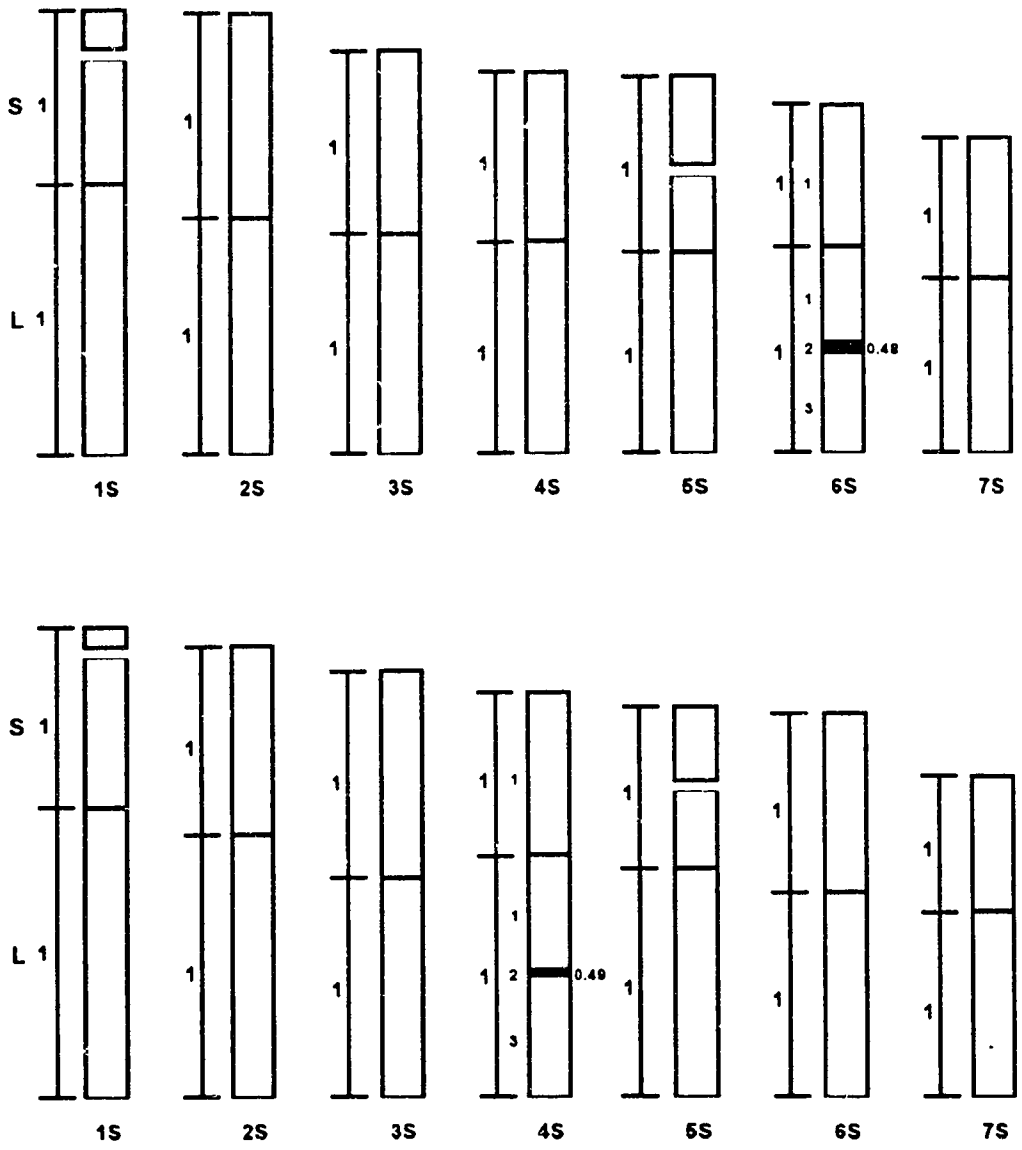

Figure 5.5 Comparison of idiograms of N-banded chromosomes of *P. spicata* (top) and the S genome of *E. trachycaulus* (bottom).

Table 5.8 Relative lengths, arm ratios, and diagnostic N-bands of *P. spicata* chromosomes and the S-genome chromosomes of *E. canadensis*.

	P. spicata				*E. canadensis*			
Chromosome	Relative length	Arm ratio	Diagnostic N-band	Fraction length	Relative length	Arm ratio	Diagnostic N-band	Fraction length
1S	16.44*	1.55	-	-	18.94*	1.57	-	-
2S	16.30	1.15	-	-	16.21	1.68	-	-
3S	14.89	1.20	-	-	15.15	1.26	-	-
4S	14.06	1.26	-	-	13.80	1.53	-	-
5S	13.92*	1.15	-	-	13.59*	1.05	-	-
6S	12.81	1.47	6SL1.2	0.48	12.25	1.22	6SS1.1	c.b.**
							6SL1.1	c.b.
7S	11.59	1.26	-	-	10.05	1.30	-	-

* satellited chromosomes
** c.b. = centromeric band

Table 5.9 Relative lengths, arm ratios, and diagnostic N-bands of *P. spicata* chromosomes and the S-genome chromosomes of *E. trachycaulus*.

	P. spicata				*E. trachycaulus*			
Chromosome	Relative length	Arm ratio	Diagnostic N-band	Fraction length	Relative length	Arm ratio	Diagnostic N-band	Fraction length
1S	16.44*	1.55	-	-	16.53*	1.61	-	-
2S	16.30	1.15	-	-	15.85	1.39	-	-
3S	14.89	1.20	-	-	14.99	1.05	-	-
4S	14.06	1.26	-	-	14.21	1.48	4SL1.2	0.49
5S	13.92*	1.15	-	-	13.71*	1.40	-	-
6S	12.81	1.47	6SL1.2	0.48	13.46	1.13	-	-
7S	11.59	1.26	-	-	11.26	1.35	-	-

* satellited chromosomes

2.2. Comparisons of chromosomes of *C. bogdanii, C. californicum* and H-genome chromosomes of *E. canadensis.*

Comparison of idiograms of N-banded chromosomes of *C. bogdanii* and the H genome of *E. canadensis* is presented in Fig. 5.6. Relative lengths, arm ratios, and diagnostic N-bands of *C. bogdanii* chromosomes and H-genome chromosomes of *E. canadensis* are given in Table 5.10. Similarity in arm ratios is found between chromosomes 3H, 5H, 6H, and 7H in *C. bogdanii* and corresponding H-genome chromosomes in *E. canadensis*; however, their N-banding patterns are not alike. Chromosome 5H in both species possess proximal bands on both arms, however there is only one interstitial band in the short arm of chromosome 5H in *C. bogdanii* and two in the short arm of chromosome 5H of *E. canadensis*. Chromosomes 1H, 2H, and 4H in both species have different arm ratios and N-banding patterns. Chromosome 2H of *C. bogdanii* and chromosome 1H of *E. canadensis* have centromeric bands. SAT-chromosome 1H in *C. bogdanii* and SAT-chromosome 4H in *E. canadensis* reveal differences in N-banding patterns and lengths.

Figure 5.7 presents idiograms of N-banded chromosomes of *C. californicum* and the H genome of *E. canadensis*. The lengths, arm ratios, and diagnostic N-bands of *C. californicum* chromosomes and the H-genome chromosomes of *E. canadensis* are presented in Table 5.11. The arm ratios of chromosomes 1H, 2H, 4H, and 5H of *E. canadensis* are similar to those of the corresponding chromosomes of *C. californicum*. However, there are large differences in the arm ratios of chromosomes 6H and 7H. Chromosome 5H of *E. canadensis* possesses a N-banding pattern corresponding to that of chromosome 5H of *C. californicum*. SAT-chromosome 4H in *E. canadensis* also reveals the same diagnostic band, L1.2, as does the SAT-chromosome 3H in *C. californicum*.

Chromosome 2H in *C. californicum* and chromosome 1H in *E. canadensis* have distinct centromeric bands.

Although the relative ease of hybridizing *E. canadensis* and *C. bogdanii* suggests a close relationship between the two species, the hybrids derived from these crosses are completely sterile (Dewey 1971). Chromosome pairing data shows that the *C. bogdanii* genome appears to be partially homologous with the H genome of *E. canadensis*. Wang and Hsiao (1986) obtained hybrids derived from crosses between *E. canadensis* and *C. californicum* without difficulty when the *Elymus* species was the female parent. Chromosome pairing data showed that the triploid hybrid obtained from the crosses between *E. canadensis* and *C. californicum* (Wang and Hsiao 1986) had a higher mean bivalent frequency (6.48, of which 66% were rings) than in the triploid hybrid derived from the crosses between *E. canadensis* and *C. bogdanii* (5.40, of which approximately 50% were rings) (Dewey 1971). The chromosome pairing data of these two triploid hybrids suggest homologies between chromosomes of *C. bogdanii* and *C. californicum* and the H-genome chromosomes of *E. canadensis*. However, the H genome in *E. canadensis* appears to be more closely related to the H genome of *C. californicum* than to that of *C. bogdanii*.

Additional evidence has also been obtained from finding the same N-banding patterns on SAT-chromosome 3H of *C. californicum* and SAT-chromosome 4H of *E. canadensis,* and on chromosome 5H of the two species. Differences in the arm ratios of chromosomes 6H and 7H, the positions in the karyotypes of the satellited chromosomes, and the location and intensity of some dissimilar N-bands are apparent. The site-specific amplification of repeated DNA sequences, the structural changes such as translocations between S- and H-genome chromosomes may be factors responsible for genome evolution at the polyploid

level. However, evidence now suggests that the H genome of *E. canadensis* is more closely related to the H genome of *C. californicum* than to that of *C. bogdanii*.

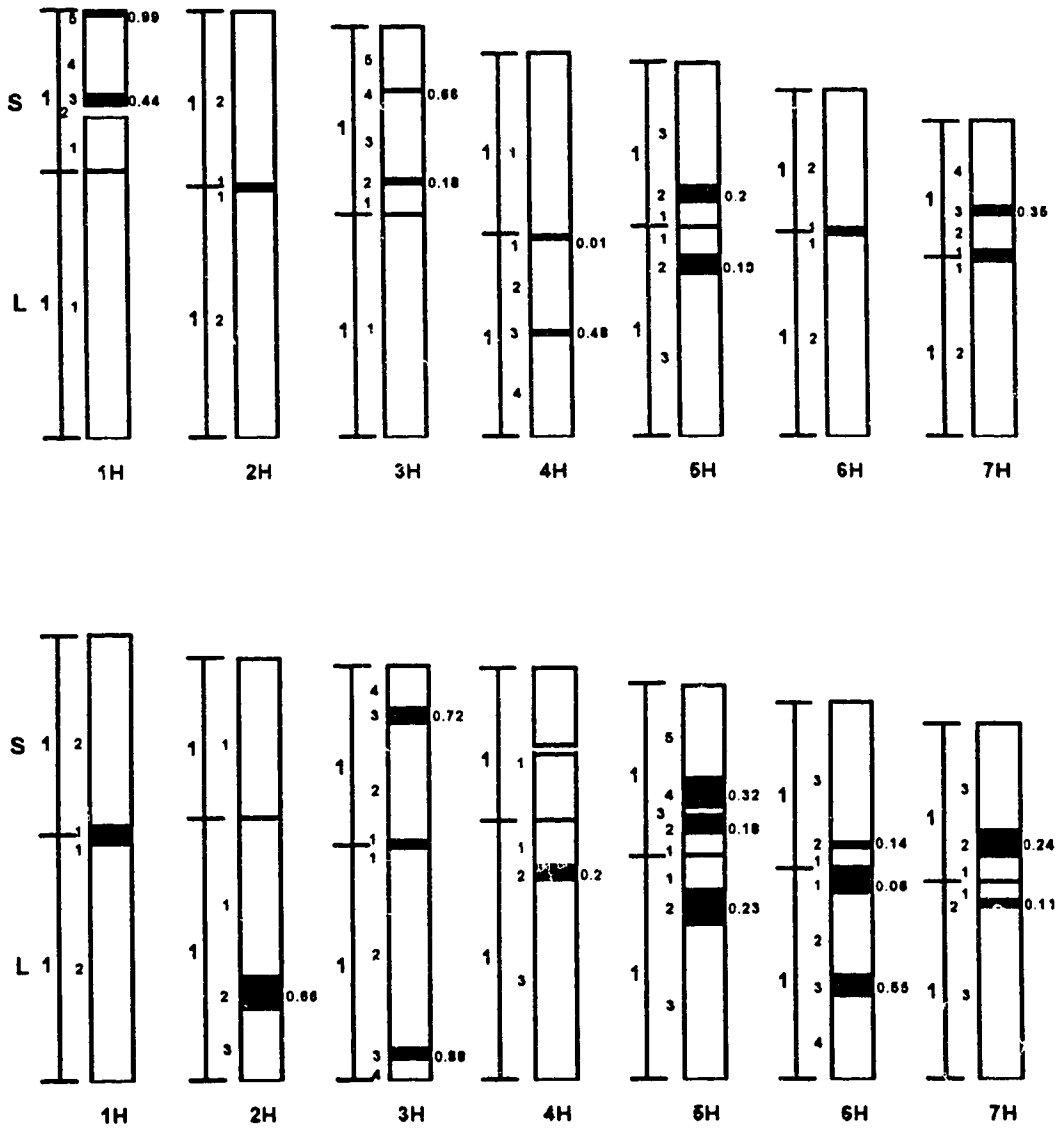

Figure 5.6 Comparison of idiograms of N-banded chromosomes of *C. bogdanii* (top) and the H genome of *E. canadensis* (bottom).

Table 5.10 Relative lengths, arm ratios, and diagnostic N-bands of *C. bogdanii* chromosomes and the H-genome chromosomes of *E. canadensis*.

	C. bogdanii				*E. canadensis*			
Chromosome	Relative length	Arm ratio	Diagnostic N-band	Fraction length	Relative length	Arm ratio	Diagnostic N-band	Fraction length
1H	15.90*	1.67	1HS1.3	0.44	15.82	1.23	1HS1.1	c.b.**
			1HS1.5	0.99			1HL1.1	c.b.
2H	15.83	1.38	2HS1.1	c.b.	14.97	1.65	2HL1.2	0.66
			1HL1.1	c.b.				
3H	15.26	1.18	3HS1.2	0.18	14.69	1.32	3HS1.1	c.b.
			3HS1.4	0.66			3HS1.3	0.72
							3HL1.1	c.b.
							3HL1.3	0.88
4H	14.31	1.10	4HL1.1	0.01	14.60*	1.70	4HL1.2	0.20
			4HL1.3	0.48				
5H	13.94	1.27	5HS1.2	0.20	13.96	1.33	5HS1.2	0.18
			5HL1.2	0.18			5HS1.4	0.32
							5HL1.2	0.23
6H	12.94	1.39	6HS1.1	c.b.	13.37	1.28	6HS1.2	0.14
			6HL1.1	c.b.			6HL1.1	0.06
							6HL1.3	0.55
7H	11.82	1.24	7HS1.1	c.b.	12.59	1.26	7HS1.2	0.24
			7HS1.3	0.35			7HL1.2	0.11
			7HL1.1	c.b.				

* satellited chromosomes

** c.b. = centromeric band

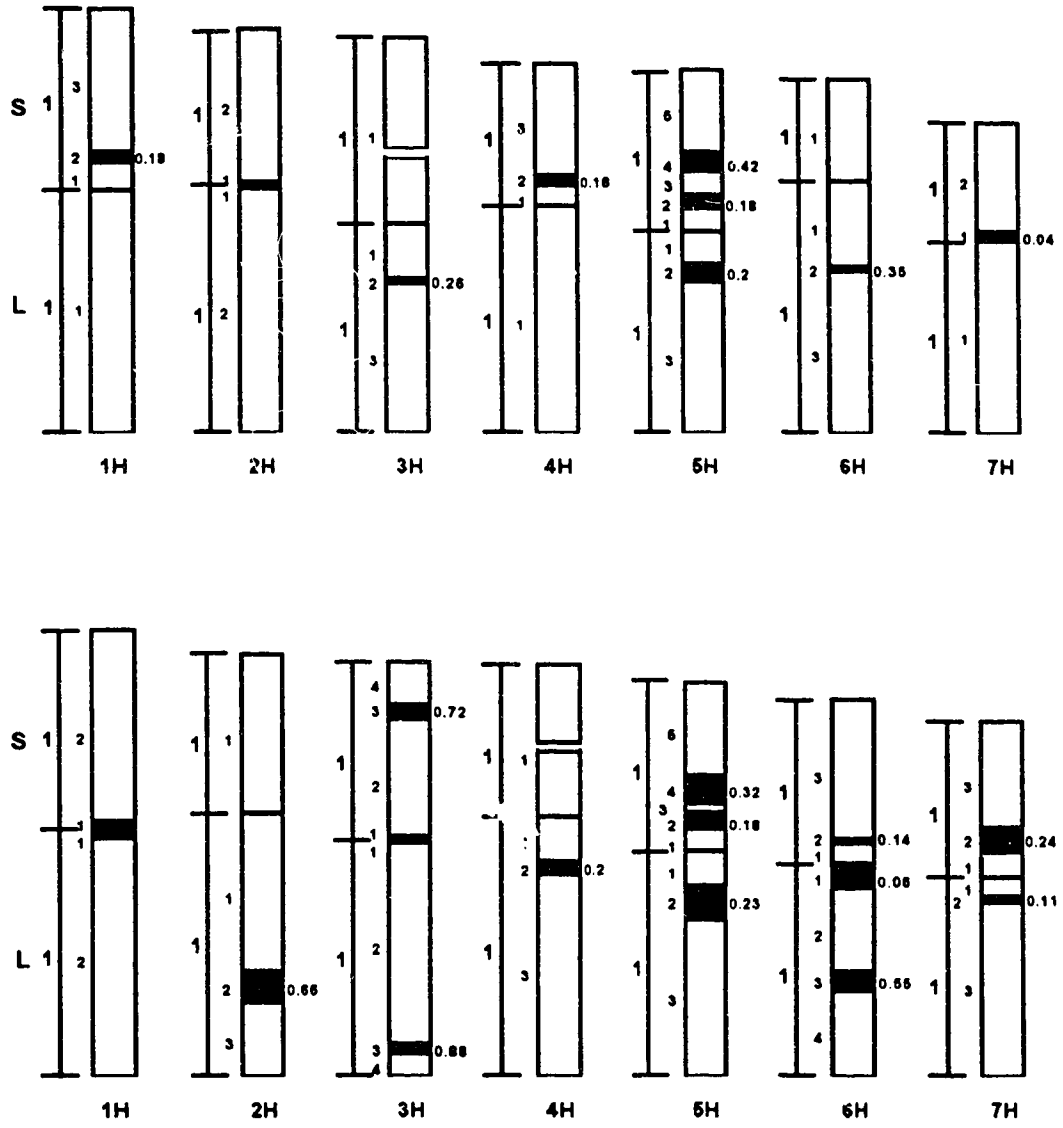

Figure 5.7 Comparison of idiograms of N-banded chromosomes of *C. californicum* (top) and the H genome of *E. canadensis* (bottom).

Table 5.11 Relative lengths, arm ratios, and diagnostic N-bands of *C. californicum* chromosomes and the H-genome chromosomes of *E. canadensis*.

	C. californicum				*E. canadensis*			
Chromosome	Relative length	Arm ratio	Diagnostic N-band	Fraction length	Relative length	Arm ratio	Diagnostic N-band	Fraction length
1H	16.19	1.33	1HS1.2	0.18	15.82	1.23	1HS1.1	c.b.**
							1HL1.1	c.b.
2H	15.41	1.55	2HS1.1	c.b.	14.97	1.65	2HL1.2	0.66
			2HL1.1	c.b.				
3H	15.09*	1.12	3HL1.2	0.26	14.69	1.32	3HS1.1	c.b.
							3HS1.3	0.72
							3HL1.1	c.b.
							3HL1.3	0.88
4H	14.08	1.64	4HS1.1	c.b.	14.60*	1.70	4HL1.2	0.20
			4HS1.2	0.16				
			4HL1.1	c.b.				
5H	13.87	1.24	5HS1.2	0.18	13.96	1.33	5HS1.2	0.18
			5HS1.4	0.42			5HS1.4	0.32
			5HL1.2	0.20			5HL1.2	0.23
6H	13.49	2.47	6HL1.2	0.35	13.37	1.28	6HS1.2	0.14
							6HL1.1	0.06
							6HL1.3	0.55
7H	11.88	1.63	7HS1.1	0.04	12.59	1.26	7HS1.2	0.24
							7HL1.2	0.11

* satellited chromosomes

**c.b. = centromeric band

2.3. Comparisons of chromosomes of *C. bogdanii*, *C. californicum* and H-genome chromosomes of *E. trachycaulus*.

Comparison of idiograms of N-banded chromosomes of *C. bogdanii* and the H genome of *E. trachycaulus* is shown in Fig. 5.8. Comparison of chromosomes of *C. bogdanii* and the H-genome chromosomes of *E. trachycaulus* based on lengths, arm ratios, and diagnostic N-bands are presented in Table 5.12. Chromosomes 3H and 7H in both species are similar in arm ratios. SAT-chromosome 1H in *C. bogdanii* and SAT-chromosome 4H in *E. trachycaulus* have different lengths, arm ratios, and N-banding patterns. The N-banding patterns of chromosomes 5H of the two species are the same except for the position of band, S1.2, in the short arm (at FL.20 in *C. bogdanii*, and FL.47 in *E. trachycaulus*). Likewise, the N-banding pattern and arm ratio of chromosome 4H in *C. bogdanii* is similar to that of chromosome 6H in *E. trachycaulus*. Each of the chromosomes 1H of *E. trachycaulus* and 2H of *C. bogdanii* show a centromeric band.

Comparison of idiograms of N-banded chromosomes of *C. californicum* and the H genome of *E. trachycaulus* is presented in Fig. 5.9. Relative lengths, arm ratios, and diagnostic N-bands of *C. californicum* chromosomes and the H-genome chromosomes of *E. trachycaulus* are shown in Table 5.13. Chromosomes 1H, 2H, and 3H of *C. californicum* and the corresponding chromosomes in *E. trachycaulus* are similar in arm ratios. SAT-chromosome 3H of *C. californicum* and SAT-chromosome 4H of *E. trachycaulus* have different arm ratios, but both chromosomes reveal the similar diagnostic band, L1.2. Chromosomes 5H in *C. californicum* and *E. trachycaulus* have similar N-banding patterns, but differ in the number of bands in their short arms. There are two bands in *C. californicum*, and one in *E. trachycaulus*. Chromosome 2H in *C.*

californicum has a centromeric band which is similar to chromosome 1H in *E. trachycaulus*.

Morris and Gill (1987) reported that the N-bands of H-genome chromosomes of *E. trachycaulus* were characterized by bands in the centromeric and intercalary regions. These authors found that the locations of heterochromatic regions of several *E. trachycaulus* chromosomes were similar to the locations of such chromatin in the chromosomes of both *C. bogdanii* and *C. californicum*. Their results indicated that *E. trachycaulus* appeared closely related to *C. bogdanii* and *C. californicum*, but the H genome donor remained undetermined. They proposed that a possible progenitor of the H genome may be *C. californicum*, since this species and *E. trachycaulus* are indigenous to North America.

There are no reports of meiotic pairing data of intergeneric hybrids between *C. bogdanii*, *C. californicum*, and *E. trachycaulus* to corroborate the findings from N-banding patterns. However, Tsujimoto and Gill (1991) analyzed the chromosomal distribution of the S-genome and H-genome repeated DNA sequences in *E. trachycaulus* and the putative diploid progenitor species by using several new repetitive DNA sequences. They concluded that *E. trachycaulus* most probably originated from diploid S- and H-genome species resembling *P. spicata* and *C. californicum*, respectively. Their findings were supported by DNA analysis, since both *P. spicata* and *C. californicum* share a 350-base pair family and Taq 2.5-family sequences, as do some chromosomes of *E. trachycaulus*. Nevertheless, they also detected differences in the internal locations of some of the telomeric sequences in *E. trachycaulus*, which provided evidence of chromosome structural changes in these polyploid species.

From this study, the N-banding patterns of SAT-chromosome 1H of *C. bogdanii* and SAT-chromosome 4H of *E. trachycaulus* revealed differences in the positions of all of the bands, especially band S1.3 at the secondary constriction of the SAT-chromosome 1H of *C. bogdanii*, which is absent at the secondary constriction of the SAT-chromosome 4H of *E. trachycaulus*. Moreover, SAT-chromosome 4H of *E. trachycaulus* and SAT-chromosome 3H of *C. californicum* possess the same N-banding patterns. Chromosome 5H of *C. californicum* and *E. trachycaulus* shows the same banding patterns based on band intensities and positions, except for the lack of a proximal band in the short arm of chromosome 5H in *E. trachycaulus*. From this additional evidence it appears that the H genome of *E. trachycaulus* has a closer relationship to the H genome of *C. californicum* than to that of *C. bogdanii* which agrees with the conclusions of previous studies by Morris and Gill (1987) and Tsujimoto and Gill (1991).

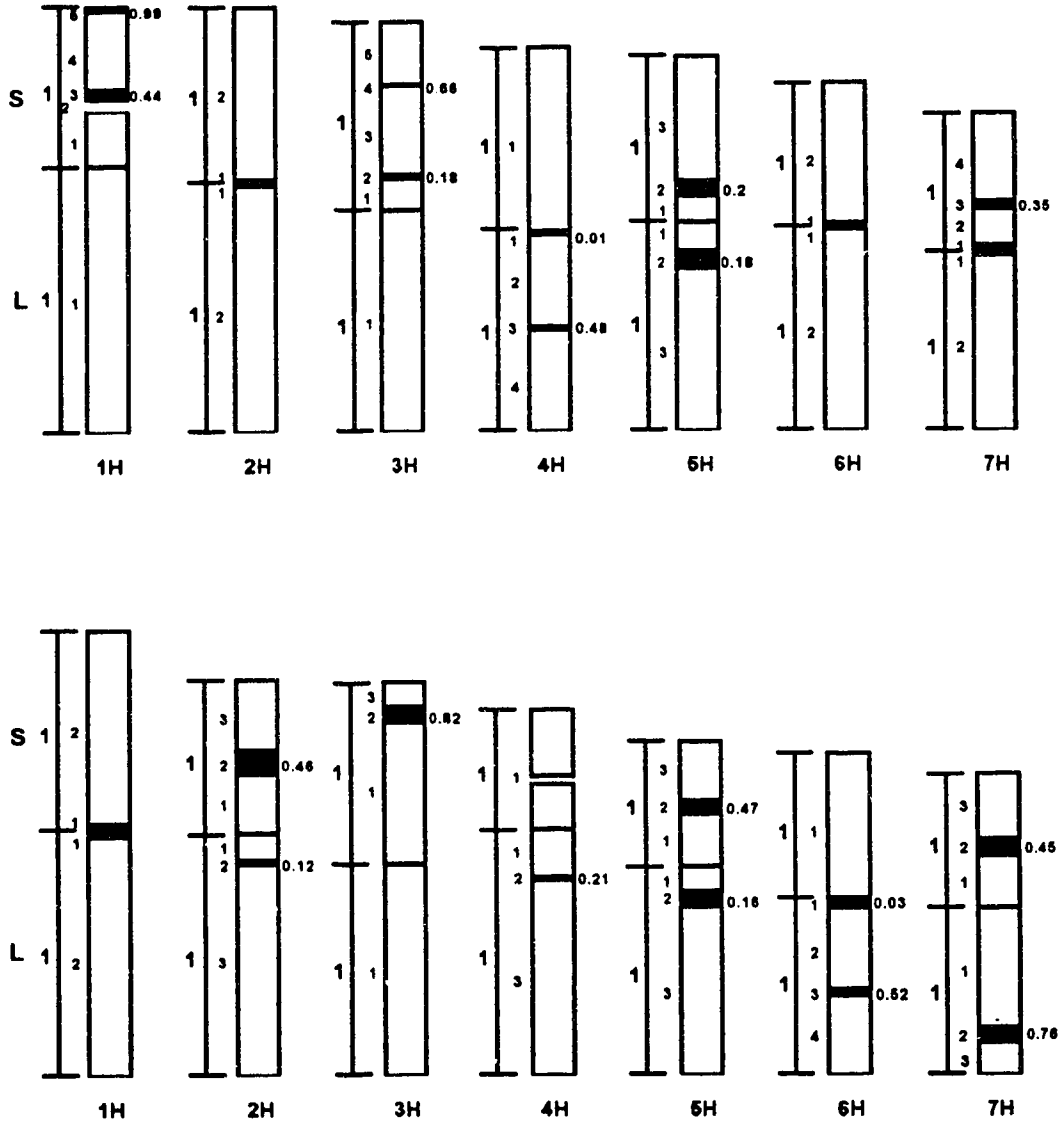

Figure 5.8 Comparison of idiograms of N-banded chromosomes of *C. bogdanii* (top) and the H genome of *E. trachycaulus* (bottom).

Table 5.12 Relative lengths, arm ratios, and diagnostic N-bands of *C. bogdanii* chromosomes and the H-genome chromosomes of *E. trachycaulus*.

	C. bogdanii				*E. trachycaulus*			
Chromosome	Relative length	Arm ratio	Diagnostic N-band	Fraction length	Relative length	Arm ratio	Diagnostic N-band	Fraction length
1H	15.90*	1.67	1HS1.3	0.44	17.44	1.22	1HS1.1	c.b.**
			1HS1.5	0.99			1HL1.1	c.b.
2H	15.83	1.38	2HS1.1	c.b.	15.51	1.56	2HS1.2	0.46
			1HL1.1	c.b.			2HL1.2	0.12
3H	15.26	1.18	3HS1.2	0.18	15.41	1.15	3HS1.2	0.82
			3HS1.4	0.66				
4H	14.31	1.10	4HL1.1	0.01	14.30*	2.03	4HL1.2	0.21
			4HL1.3	0.48				
5H	13.94	1.27	5HS1.2	0.20	13.05	1.65	5HS1.2	0.47
			5HL1.2	0.18			5HL1.2	0.16
6H	12.94	1.39	6HS1.1	c.b.	12.54	1.14	6HL1.1	0.03
			6HL1.1	c.b.			6HL1.3	0.52
7H	11.82	1.24	7HS1.1	c.b.	11.74	1.23	7HS1.2	0.45
			7HS1.3	0.35			7HL1.2	0.76
			7HL1.1	c.b.				

* satellited chromosomes

**c.b. = centromeric band

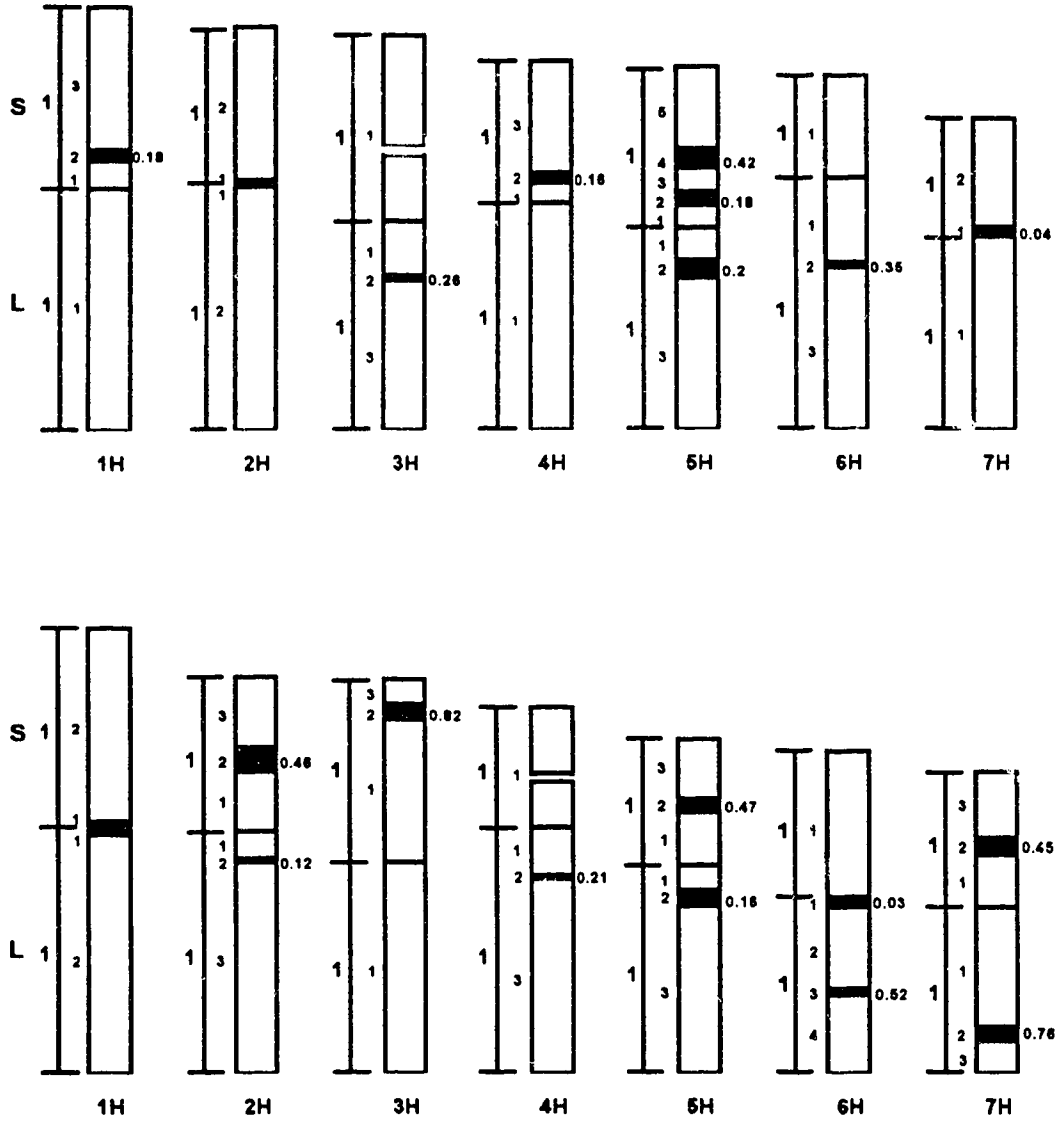

Figure 5.9 Comparison of idiograms of N-banded chromosomes of *C. californicum* (top) and the H genome of *E. trachycaulus* (bottom).

Table 5.13 Relative lengths, arm ratios, and diagnostic N-bands of *C. californicum* chromosomes and the H-genome chromosomes of *E. trachycaulus*.

	C. californicum				*E. trachycaulus*			
Chromosome	Relative length	Arm ratio	Diagnostic N-band	Fraction length	Relative length	Arm ratio	Diagnostic N-band	Fraction length
1H	16.19	1.33	1HS1.2	0.18	17.44	1.22	1HS1.1	c.b.**
							1HL1.1	c.b.
2H	15.41	1.53	2HS1.1	c.b.	15.51	1.56	2HS1.2	0.46
			2HL1.1	c.b.			2HL1.2	0.12
3H	15. *[illegible]	1.12	3HL1.2	0.26	15.41	1.15	3HS1.2	0.82
4H	14.[illegible]	1.64	4HS1.1	c.b.	14.30*	2.03	4HL1.2	0.21
			4HS1.2	0.16				
			4HL1.1	c.b.				
5H	13.87	1.24	5HS1.2	0.18	13.05	1.65	5HS1.2	0.47
			5HS1.4	0.42			5HL1.2	0.16
			5HL1.2	0.20				
6H	13.49	2.47	6HL1.2	0.35	12.54	1.14	6HL1.1	0.03
							6HL1.3	0.52
7H	11.88	1.63	7HS1.1	0.04	11.74	1.23	7HS1.2	0.45
							7HL1.2	0.76

* satellited chromosomes

**c.b. = centromeric band

Summary

The sequential acetocarmine staining/N-banding techniques permit the identification of different chromosome pairs in the standard karyotype, especially the satellited chromosomes which are difficult to identify using banded chromosome preparations. The assignment of *Elymus* chromosomes to the S and H genomes was made using comparisons of detailed observations on chromosome morphology as well as the N-banding patterns. In all karyotypes, the seven chromosomes in each genome are arranged in the standard manner to facilitate comparisons among these closely related species. The standard banded karyotypes allow the study of genome relationships and karyotypic evolution of the two tetraploid *Elymus* species and their possible diploid progenitors.

The karyotype of *P. spicata* consists only of metacentric chromosomes with two pairs of satellited chromosomes, one pair with small and one pair with large satellites on the short arms of chromosomes designated 1S and 5S, respectively, in this study. The observation of an interstitial N-band, L1.2, at FL.48 of chromosome 6S reveals the presence of the C-banding negative and N-banding positive (C^-N^+) class of heterochromatin which was not reported previously by Morris and Gill (1987). The difference in this finding from the study of Morris and Gill (1987) may be due to the modified N-banding technique with the temperature reduction in 1M NaH_2PO_4 treatment.

The karyotype of *C. bogdanii* shows six metacentric chromosome pairs and one pair of satellited chromosomes designated as SAT-chromosome 1H. The karyotype of *C. californicum* shows five metacentric chromosome pairs, one submetacentric chromosome pair, and one pair of satellited chromosomes

designated as SAT-chromosome 3H. SAT-chromosome 1H of *C. bogdanii* reveals a N-banding pattern different from that of SAT-chromosome 3H of *C. californicum*.

According to Linde-Laursen *et al.* (1992), It is possible that *C. bogdanii* and *C. californicum* may have evolved from the Asiatic *C. brevisubulatum ssp. brevisubulatum*. The similarities of N-banding patterns of *C. bogdanii* and *C. californicum* are observed in the present study on chromosomes 2H and 5H with the absence of one interstitial band (S1.4) located distally in the short arm of chromosome 5H in *C. bogdanii*. The structural differences between the chromosomes of the two species are also evident by the differences in the order of chromosomes in the karyotypes, morphologies, and N-banding patterns of satellited chromosomes and the morphologies, positions and sizes of some N-bands of other chromosomes. This indicates that there has been considerable evolution at the karyotypic level that create the distinctiveness of each H genome species.

The close relationship of genomes in *E. canadensis* and *E. trachycaulus* is supported by a high mean bivalent frequency in interspecific hybrids derived from crosses between these two species (Dewey 1968a; Aung and Walton 1990; Kumar and Walton 1990). In this study it was found that the karyotypes of *E. canadensis* and *E. trachycaulus* are similar. In both species, the S-genome chromosomes are all metacentrics including two satellited chromosomes designated as SAT-chromosomes 1S and 5S, respectively. The H genome contains one submetacentric satellited chromosome designated as SAT-chromosome 4H, and six metacentric chromosomes. Although the similarities of chromosome morphologies and N-banding patterns of some S- and H-genome chromosomes, especially the order in the karyotype and N-banding pattern of

satellited chromosomes of *E. canadensis* and *E. trachycaulus* indicate a close phylogenetic relationship between these two species. The differences in morphologies and N-banding patterns of some chromosomes demonstrate that these two species have chromosomal structural differences which are probably due to different kinds of chromosome mutations.

The strong homology between chromosomes of the two S genomes in the triploid hybrids derived from the cross *E. trachycaulus* x *P. spicata* was reported by Stebbins and Snyder (1956) and Aung and Walton (1987a). The karyotypes of *P. spicata* and the S genome of *E. canadensis* and *E. trachycaulus* in the present study also reveal the same positions of the two satellited chromosomes in the karyotypes of these species. The comparison of *P. spicata* chromosomes and the S-genome chromosomes of *E. canadensis* and *E. trachycaulus* shows that the arm ratios of chromosomes 1S, 3S, and 7S in all these species are much alike and all of these chromosomes show no N-bands. Chromosome 6S of *P. spicata* has an interstitial band, L1.2, at FL.48, while chromosome 6S of *E. canadensis* has a prominent centromeric band. This indicates that the structural differences between the two S genomes is likely due to a reciprocal translocation between chromosomes of the S and H genomes in *E. canadensis*. Chromosome 4S in *E. trachycaulus* and chromosome 6S in *P. spicata* show the same N-banding pattern. The structural changes in the S genome of these two species may be the result of a reciprocal translocation between S-genome chromosomes.

The higher chromosome pairing of the triploid hybrid derived from the cross *E. canadensis* x *C. californicum* (Wang and Hsiao 1986) than that of the triploid hybrid produced by crossing *E. canadensis* and *C. bogdanii* (Dewey 1971) suggests that the H genome of *E. canadensis* is more closely related to the H

genome of *C. californicum* than to that of *C. bogdanii* In this study, karyotype analyses of *C. bogdanii* and *C. californicum* and the H genomes of *E. canadensis* and *E. trachycaulus* indicate that the H-genome chromosomes of the two *Elymus* species are more closely related to *C. californicum* than to *C. bogdanii*. SAT-chromosome 3H of *C. californicum* and SAT-chromosome 4H of the two *Elymus* species reveal the same N-banding patterns, but SAT-chromosome 1H of *C. bogdanii* and SAT-chromosome 4H of the two *Elymus* species show different N-banding patterns. Chromosome 2H of *C. bogdanii* and *C. californicum* and chromosome 1H of both *Elymus* species have the same N-banding patterns with centromeric bands. Similarly, chromosome 5H of the two *Critesion* species and both *Elymus* species reveal the same N-banding patterns.

However, the similarities of chromosome morphologies and N-banding patterns of some H-genome chromosomes of these *Elymus* species and those of *C. bogdanii* are apparent. *C. bogdanii* and *C. californicum* have a close relationship and probably have the same origin as discussed earlier. The similarities of the N-banding patterns of chromosomes 2H and 5H of both species are also observed. The differences in the satellited chromosomes in these species indicate significant differences between the two *Critesion* species. The satellited chromosomes present in the two *Elymus* species resemble the one found in *C. californicum*. However, differences in the position in the karyotype and size of the satellited chromosomes, and morphologies and N-banding patterns of some H-genome chromosomes of *C. californicum* and the two *Elymus* species reflect chromosomal structural changes that occurred during and/or subsequent to polyploidization.

The comparison of the standard N-banded karyotypes of both *Elymus* species and their putative diploid progenitors suggests that these allotetraploid *Elymus*

species are derived from an intergeneric hybrid from the cross between *P. spicata* and *C. californicum*. This finding supports the conclusions of Tsujimoto and Gill (1991) who analyzed the chromosomal distribution of the S-genome and H-genome sequences in *E. trachycaulus* and the putative diploid progenitor species by using several new repetitive DNA sequences. The karyotype comparisons in the present study support the conclusion that *E. canadensis* may have evolved from the same species as *E. trachycaulus*. These two *Elymus* species have similar karyotype patterns with the same positions of satellited chromosomes in their karyotypes. The SAT-chromosome types present in the S genome resemble the ones observed in *P. spicata*, and the SAT-chromosome type present in the H genome resemble the one found in *C. californicum*. The structural differences between the two *Elymus* species may be the result of a translocation between the S- and H-genome chromosomes in *E. canadensis*. This finding is supported by the observation that autosyndetic pairing occurs between chromosomes of the S and H genomes in haploid *E. canadensis* (Torabinejad *et al.* 1987) and the N-banding pattern of chromosome 6S of *E. canadensis* in the present study.

The standard banded karyotypes established in *P. spicata*, *C. bogdanii*, *C. californicum*, *E. trachycaulus*, and *E. canadensis* are useful for the construction of genetic and physical maps to locate desirable genes on chromosomes. The cytogenetic maps of chromosomes are useful for effective gene transfers. Five of seven chromosomes of *P. spicata*, *C. bogdanii*, and *C. californicum* can be unequivocally identified using banding procedures. However, the remaining two chromosomes in each diploid species and the N-banded chromosomes in the S genome of the tetraploid species were identified in conjunction with chromosome morphologies. Further studies, such as genomic *in situ* hybridization (GISH),

fluorescence *in situ* hybridization (FISH), and chromosome painting, are recommended to confirm the identification of these chromosomes in each genome, and the origin of the genomes of the two allotetraploid species.

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Appendix1. Cytogenetic data from chromosome pairing at meiosis of interspesific and intergeneric hybrids of species having S or H genomes.

Species and Hybrids combinations	Chro. no.	Proposed Genome	Mean Chromosome Associations per cell									References
			I	II			III	IV	V	VI	VII	
				Rods	Rings	Total						
E. canadensis	28	SSHH	•	•	•	14	•	•	•	•	•	Dewey 1967a
E. lanceolatus	28	SSHH	0.05	•	•	13.96	•	0.007	•	•		"
E. canadensis x E. lanceolatus	28	SSHH	0.42	•	•	13.7	0.02	0.03	•	•	•	"
E. canadensis x C. californicum	21	SHH	7.96	2.2	4.28	6.48	0.03	•	•	•	•	Wang and Hsiao 1986
E. canadensis x C. bulbosum	21	SHH[b]	16.24	0.48	1.81	2.29	0.05	0.01	•	•	•	"
C. bogdanii	14	HH	0.08	•	•	•	6.96	•	•	•	•	Dewey 1971
E. canadensis x C. bogdanii	21	SHH	9.98	•	•	5.4	0.08	•	•	•	•	"
E. canadensis x P. libanotica	21	SSH	9.47	•	•	5.38	0.26	•	•	•	•	Dewey 1974b
E. canadensis x E. siribicus	28	SSHH	4.96	•	•	10.18	0.34	0.31	•	•	•	Dewey 1974a
E. canadensis x E. caninus	28	SSHH	3	•	•	12.4	0.07	•	•	•	•	Dewey 1968a
E. canadensis x E. trachycaulus	28	SSHH	0.1	•	•	11.5	•	0.2	•	0.7	•	"
E. trachycaulus	28	SSHH	•	0.32	13.68	14	•	•	•	•	•	Aung and Walton 1990
E. canadensis	28	SSHH	•	0.56	13.44	14	•	•	•	•	•	"
E. canadensis x E. trachycaulus	28	SSHH	0.46	2.1	9.17	11.27	0.14	0.11	•	0.69	•	"
E. trachycaulus x E. canadensis	28	SSHH	2.05	4.68	7	11.68	0.25	0.19	0.06	0.13	•	"
E. canadensis x E. trachycaulus	28	SSHH	0.3	•	•	12.6	•	0.1	•	0.4	•	Kumar and Walton 1990

Species and Hybrids combinations	Chro. no.	Proposed Genome	Mean Chromosome Associations per cell									References
			I	II			III	IV	V	VI	VII	
				Rods	Rings	Total						
E. canadensis (8x) x	56	SSSSHHHH	1.2	•	•	2	•	2.4	•	•	•	Kumar and Walton 1990
E. trachycaulus (8x)												
E. canadensis	28	SSHH	•	0.85	13.15	14	•	•	•	•	•	Jensen et al. 1989
E. arizonicus	28	SSHH	0.46	3.42	10.35	13.77	•	•	•	•	•	"
E. arizonicus x	28	SSHH	2.67	2.16	10.44	12.6	0.02	•	•	•	•	"
E. canadensis												
E. trachycaulus	28	SSHH	0.06	0.52	13.45	13.97	•	•	•	•	•	"
E. vaillantianus	28	SSHH	•	0.56	13.44	14	•	•	•	•	•	"
E. vaillantianus x	28	SSHH	2.75	3.98	8.58	12.56	0.04	0.005	•	•	•	"
E. trachycaulus												
P. spicata	14	SS	0.03	0.98	6	6.98	•	•	•	•	•	"
E. vaillantianus x	21	SSH	7.53	2.06	4.15	6.21	0.24	0.08	•	•	•	"
P. spicata												
E. canadensis	28	SSHH	•	0.84	13.16	14	•	•	•	•	•	Torabinejad et al, 1987
" *(haploid)*	14	SH	12.97	0.49	0.002	0.49	0.014	0.002	•	•	•	"
E. canadensis (8x)	56	SSSSHHHH	1.11	•	•	20.45	0.95	2.82	•	•	•	Park and Walton 1989
E. canadensis (6x)	42	SSSHHH	3.14	•	•	8.25	8.34	0.41	•	•	•	"
E. trachycaulus (4x)	28	SSHH	•	•	•	14	•	•	•	•	•	Aung and Walton 1987b
E. trachycaulus (5x)	35	SSHH+7	1.18	•	•	8.18	5.82	•	•	•	•	"
E. trachycaulus (6x)	42	SSSHHH	0.84	•	•	0.84	13.16	•	•	•	•	"
E. trachycaulus (8x)	56	SSSSHHHH	0.85	•	•	17.84	0.65	4.38	•	•	•	"
E. trachycaulus (4x) x	21	SHS1	7.37	0.892	5.07	5.962	0.248	0.238	0.002	•	•	Aung and Walton 1987a
P. spicata												
E. trachycaulus (4x) x	21	SHS2	6.02	1.125	4.19	5.315	0.554	0.235	0.25	0.05	0.03	"
P. spicata ssp.inermis												
E. trachycaulus (8x) x	35	SSHHS1	5.22	1.33	10.61	11.94	1.97	•	•	•	•	"
P. spicata												
E. trachycaulus (8x) x	35	SSHHS2	3.5	1.34	8.84	10.18	3.54	0.13	•	•	•	"
P. spicata ssp.inermis												
E. tilcarensis	28	SSHH	0.08	•	•	13.96	•	•	•	•	•	Dewey 1977a

Species and Hybrids combinations	Chro. no.	Proposed Genome	Mean Chromosome Associations per cell									References
			I	II			III	IV	V	VI	VII	
				Rods	Rings	Total						
E. tilcarensis x	28	SSHH	0.58	•	•	12.18	0.36	0.35	•	•	•	Dewey 1977a
E. trachycaulus												"
E. lanceolatus	28	SSHH	0.05	•	•	13.96	•	0.01	•	•	•	Dewey 1972
E. patagonicus	42	SSHHHH	0.14	•	•	19.81	0.01	0.55	•	•	•	"
E. lanceolatus x	35	SSHHH	3.84	•	•	9.97	3.3	0.11	•	•	•	"
E. patagonicus												
E. lanceolatus	28	SSHH	0.01	•	•	13.99	•	•	•	•	•	Dewey 1975
E. trachycaulus	28	SSHH	•	•	•	14	•	•	•	•	•	"
E. lanceolatus x	28	SSHH	0.12	•	•	11.57	0.12	0.17	0.01	0.61	•	"
E. trachycaulus												
E. mutabilis	28	SSHH	0.14	•	•	13.85	•	0.04	•	•	•	Dewey 1979
E. lanceolatus x	28	SSHH	2.55	•	•	12.04	0.36	0.07	•	•	•	"
E. mutabilis												
E. lanceolatus	28	SSHH	•	1.67	12.33	14	•	•	•	•	•	Sadasivaiah and Weijer 1981
E. lanceolatus (5x)	35	SSHH+7	2.27	0.31	9.06	9.37	4.63	•	0.02	•	•	"
E. lanceolatus (6x)	42	SSSHHH	4.21	1.06	5.85	6.91	7.38	0.25	0.03	0.11	•	"
P. spicata x	42	SSSSHH	0.85	1.32	12.8	14.12	0.3	2.92	•	0.06	•	"
E. lanceolatus (double triploid)												
P. spicata (2x) x	21	SSS	2.62	0.05	2.67	2.72	4.29	0.02	•	•	•	"
P. spicata (4x)												
P. spicata x	21	SSH	6.84	•	•	6.95	0.08	•	•	•	•	Dewey 1965a
E. lanceolatus												
P. libanotica x	21	SSH	6.08	•	•	5.96	0.98	0.02	•	•	•	Dewey 1968b
E. lanceolatus												
A. ferganense x	21	SSH	6.74	•	•	6.4	0.49	•	•	•	•	Dewey 1981
E. lanceolatus												
E. tilcarensis x	28	SSHH	0.46	•	•	9.5	0.33	0.71	•	•	•	Dewey 1977a
E. lanceolatus												
E. caninus	28	SSHH	•	•	•	14	•	•	•	•	•	Dewey 1969b

Species and Hybrids combinations	Chro. no.	Proposed Genome	Mean Chromosome Associations per cell									References
			I	II			III	IV	V	VI	VII	
				Rods	Rings	Total						
P. libanotica x E. caninus	21	SSH	12.6	•	•	3.98	0.14	•	•	•	•	Dewey 1969b
E. sibiricus	28	SSHH	•	•	•	14	•	•	•	•	•	Dewey 1974a
E. caninus x E. sibiricus	28	SSHH	1.8	•	•	11.93	0.18	0.45	•	•	•	•
E. mutabilis x E. caninus	28	SSHH	1.41	•	•	13.14	0.04	0.05	•	•	•	Dewey 1979
A. ferganense x E. caninus	21	SSH	8.25	•	•	5.5	0.56	0.01	•	•	•	Dewey 1981
C. californicum x E. caninus	21	HSH^V	10.7	3.27	0.59	3.86	0.86	•	•	•	•	Gupta and Fedak 1985
C. violaceum x E. caninus	21	H^VSH^V	8.34	2.78	2.76	5.53	0.53	•	•	•	•	•
P. spicata	14	SS	0.06	•	•	6.97	•	•	•	•	•	Dewey 1969b
P. libanotica	14	SS	0.24	•	•	6.88	•	•	•	•	•	•
P. libanotica x P. spicata	14	SS	0.53	•	•	6.71	0.02	•	•	•	•	•
E. patagonicus	42	SSHHHH	0.14	•	•	19.81	0.01	0.55	•	•	•	Dewey 1972
P. spicata x E. patagonicus	28	SSHH	5.27	•	•	10.75	0.24	0.07	•	•	•	•
P. libanotica x E. patagonicus	28	SSHH	14.15	•	•	6.68	0.17	•	•	•	•	•
E. tilcarensis x P. libanotica	21	SSH	8.21	•	•	5.28	0.57	0.09	•	•	•	Dewey 1977a
E. tilcarensis x P. spicata	21	SSH	6.7	•	•	4.78	0.64	0.33	•	•	•	•
A. ferganense x P. spicata	14	SS	0.87	•	•	6.21	0.09	0.11	•	•	•	Dewey 1981
A. ferganense x P. libanotica	14	SS	2.72	•	•	5.45	0.09	0.03	•	•	•	•

Species and Hybrids combinations	Chro. no.	Proposed Genome	Mean Chromosome Associations per cell									References
			I	II			III	IV	V	VI	VII	
				Rods	Rings	Total						
P. spicata x C. violaceum	14	SH^V	7.12	2.87	0.32	3.19	0.14	0.02	•	•	•	Wang and Hsiao 1986
P. spicata ssp.inermis	14	SS	•	0.52	6.48	7	•	•	•	•	•	Asay et al. 1987
C. violaceum	14	HH	0.43	1.53	5.25	6.78	•	•	•	•	•	•
P. spicata ssp.inermis x C. violaceum	14	SH	7.28	2.79	0.32	3.11	0.16	0.005	•	•	•	•
C0 amphiploid	28	SSHH	4.01	6.42	4.94	11.37	0.26	0.09	•	•	•	•
C1 amphiploid	28	SSHH	5.31	3.84	7.27	11.13	0.11	0.03	•	•	•	•
E. agropyroides	28	SSHH	0.54	•	•	13.74	•	•	•	•	•	Dewey 1970
P. libanotica	14	SS	0.08	•	•	6.96	•	•	•	•	•	•
P. libanotica x E. agropyroides	21	SSH	11.62	•	•	4.54	0.11	•	•	•	•	•
P. libanotica x E. mutabilis	21	SSH	9.83	•	•	4.96	0.42	0.003	•	•	•	Dewey 1979
C. violaceum x C. bogdanii	14	H^VH	13.9	0.05	•	0.05	•	•	•	•	•	Wang and Hsiao 1986
C. chilense x C. bulbosum	14	H^CH^b	13.25	0.37	•	0.37	•	•	•	•	•	Padilla and Martin 1983
C. pusillum	14	HH	0.16	0.9	6.02	6.92	•	•	•	•	•	Bothmer et al. 1985
C. intercedens	14	HH	•	0.55	6.45	7	•	•	•	•	•	•
C. euclaston	14	HH	0.33	0.68	6.16	6.84	•	•	•	•	•	•
C. flexuosum	14	HH	0.1	0.71	6.24	6.95	•	•	•	•	•	•
C. muticum	14	HH	0.35	1.03	5.8	6.83	•	•	•	•	•	•
C. cordobense	14	HH	0.32	1.24	5.6	6.84	•	•	•	•	•	•
C. stenostachys	14	HH	0.23	0.66	6.23	6.89	•	•	•	•	•	•
C. chilense	14	HH	0.1	0.2	6.75	6.95	•	•	•	•	•	•
C. pusillum - x C. intercedens	14	HH	0.59	1.99	4.72	6.71	0.005	•	•	•	•	•
- x C. euclaston	14	HH	0.77	1.9	4.68	6.53	•	•	•	•	•	•
- x C. flexuosum	14	HH	4.52	3.06	1.59	4.65	0.04	0.01	•	•	•	•

Species and Hybrids combinations	Chro. no.	Proposed Genome	Mean Chromosome Associations per cell									References
			I	II			III	IV	V	VI	VII	
				Rods	Rings	Total						
C. pusillum	14	HH	3.27	2.56	2.78	5.36	•	•	•	•	•	Bothmer et al. 1985
- x C. muticum												
- x C. cordobense	14	HH	2.36	2.98	2.76	5.74	0.01	0.01	•	•	•	•
- x C. stenostachys	14	HH	0.77	2.15	4.46	6.61	•	•	•	•	•	•
- x C. chilense	14	HH	2.13	2.35	3.55	5.9	0.023	•	•	•	•	•
C. intercedens	14	HH	0.51	0.86	5.86	6.75	•	•	•	•	•	•
- x C. euclaston												
- x C. muticum	14	HH	2.84	3.22	2.5	5.72	•	0.03	•	•	•	•
- x C. chilense	14	HH	1.32	2.92	3.36	6.24	0.02	•	•	•	•	•
C. euclaston	14	HH	0.7	1.78	4.82	6.66	•	•	•	•	•	•
- x C. flexuosum												
- x C. muticum	14	HH	0.8	1.47	4.37	5.84	0.08	0.3	•	•	•	•
- x C. stenostachys	14	HH	0.46	1.67	4.58	6.25	0.04	0.23	•	•	•	•
C. flexuosum	14	HH	0.69	1.04	5.6	6.64	0.01	•	•	•	•	•
- x C. muticum												
- x C. cordobense	14	HH	1.7	2.84	3.24	6.06	0.06	•	•	•	•	•
- x C. chilense	14	HH	0.4	0.89	5.96	6.86	•	•	•	•	•	•
C. chilense	14	HH	0.48	0.9	5.86	6.76	•	0.002	•	•	•	•
- x C. cordobense												
- x C. muticum	14	HH	0.71	1.67	4.93	6.6	0.001	0.04	•	•	•	•
- x C. stenostachys	14	HH	0.07	0.32	6.65	6.97	•	•	•	•	•	•
C. cordobense	14	HH	0.89	1.52	5.04	6.56	•	•	•	•	•	•
- x C. muticum												
- x C. stenostachys	14	HH	0.37	0.95	5.76	6.71	•	0.002	•	•	•	•
C. muticum	14	HH	0.89	1.54	5.01	6.55	•	0.002	•	•	•	•
- x C. stenostachys												