THESIS

CHROMOSOME RACES AND POLYPLOID CYTOFORMS IN *DISTICHLIS SPICATA*

Submitted by

Scott D. Reid

Department of Horticulture and Landscape Architecture

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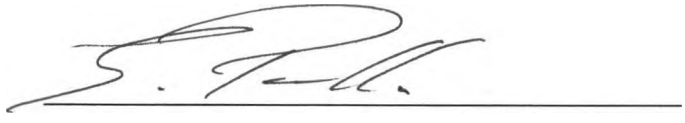
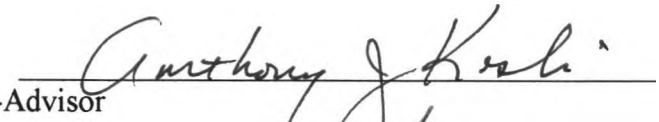
WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY SCOTT D. REID ENTITLED CHROMOSOME RACES AND POLYPLOID CYTOFORMS IN *DISTICHLIS SPICATA* BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work





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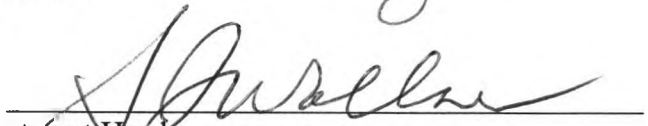

Department Head

ABSTRACT OF THESIS

CHROMOSOME RACES AND POLYPLOID CYTOFORMS IN *DISTICHLIS SPICATA*

Saltgrass (*Distichlis spicata* [L.] Greene) is a salt-tolerant C_4 grass native to the Americas that is currently being evaluated for use as a turfgrass species. Saltgrass has a highly variable morphology in different regional occurrences, and this has led some botanists to consider it as two species or as comprising several botanical varieties over its wide distribution in the U.S. west of the Mississippi River and along coastal regions. Chromosome numbers of 160 saltgrass accessions, primarily from nine western states, were determined. A 38-chromosome saltgrass race with distributions separate from the previously known 40-chromosome type has been identified. Both races are fertile and hybridize readily. The distribution pattern and the cytological behavior of hybrids between the races suggest they are adaptively different. No aneuploidy in the 38-chromosome race has been found, and no deficient aneuploids have been found in the 40-chromosome race. Meiotic irregularities in the 40-chromosome race suggest a pathway for the evolution of the 38-chromosome race, dependant on hypothesized genetic changes that allow fitness in individuals lacking the pair of homologues defining the difference between the races. More research is needed to evaluate broad but varied morphological differences between the races. The consequences of intermating the two cytoraces in breeding efforts are unknown, but karyotypic instability may result in some cases. An octaploid saltgrass cytotype, previously considered rare, has been found to occur more

commonly, distributed among both of the cytoraces. One accession, believed to be a 6x cytotype, represents a ploidy level previously undescribed in saltgrass. Screening to identify individuals of different ploidy levels among collections made for the turfgrass breeding program is advisable. Aneuploidy and multivalent associations in meiosis suggest the higher-order polyploid cytoforms may reproduce with less efficiency sexually, but this has not been adequately studied. Two ca. 74-chromosome accessions showed high estimated pollen viability. B chromosomes are reported for the first time in saltgrass, occurring in some individuals of all three cytotypes.

Scott Douglas Reid
Department of Horticulture and Landscape Architecture
Fort Collins, Colorado 80523
Fall, 2001

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Judy Harrington, my saltgrass research partner, provided invaluable assistance throughout the entire project. Her assistance with techniques, theory, editing, and philosophy is greatly appreciated.

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Introduction

Saltgrass (*Distichlis spicata* [L.] Greene) is a widely distributed, warm-season, dioecious grass (Figure 1) native to the Americas. Saltgrass has vigorous, very sharp rhizomes (Figure 2) which, armored with numerous silica cells (Hansen et al., 1976), can penetrate hard clay, shale, or pavement and, thus, can be quite invasive. Because of this aggressiveness, and other reasons, saltgrass is sometimes considered to be a weed. These same features make it an important pioneer species in certain harsh habitats (Hansen et al., 1976) where resource sharing from established ramets via the long rhizomes can sustain the colonization of areas of extreme conditions in which other plants cannot grow (Shumway, 1995). Saltgrass is generally associated with saline, wetland environments in both coastal and inland regions (USDA-NRCS, 1999), but has moderate to high drought tolerance (Kopec and Marcum, 2001; Uchytil, 1990) conditioned, in part, by adaptations in leaf morphology and anatomical features such as covered stomata (Hansen et al., 1976). Salt glands provide an important salinity tolerance mechanism (Marcum, 1999), and saltgrass is generally considered to be a halophyte, but ecotypic differences in salt tolerance have been found (Wrona and Epstein, 1982).

Figure 1. Female (A, B) and male (C, D) saltgrass panicles from inland collections. Accessions are variable for the number of florets per spikelet, number of spikelets per panicle, and the length of panicle branches. These variations contribute to the often-described differences in inflorescence "congestion" that have been used to differentiate types in various taxonomic treatments.

Figure 2. Saltgrass rhizomes and stolons. Rhizomes can grow aggressively for long distances without branching to colonize new territory (A). Sharp, tough rhizomes can penetrate compacted or rocky soil (B). Some saltgrass accessions are also stoloniferous (C). This trait was used in part by Beetle (1943) to differentiate the variety *Distichlis spicata* var. *stolonifera* based on specimens from California and Oregon coastal habitats, but it has been observed among the material assembled for the present study in accessions from Yuma, Arizona and Delta, Colorado.

A

B

C

Saltgrass is tolerant to selenium (Enberg and Wu, 1995; Wu et al., 1997) and certain heavy metals (Prodgers and Inskeep, 1991), and may be useful for revegetation and stabilization of contaminated sites (Dahlgren et al., 1997; Prodgers and Inskeep, 1991). Seliskar and Gallagher (2000) have suggested that existing diversity among natural saltgrass populations or from somaclonal variants can be exploited to achieve specific goals in salt marsh habitat restoration. On native range, saltgrass is generally considered to have limited forage value (due to low forage quality), but it can provide important late-season forage in arid areas or under drought conditions (Cluff et al., 1983). Research is also being conducted into the use of saltgrass as a domesticated forage for use in desert areas or where brackish water is the only available irrigation source (Pasternack et al., 1993; Gallagher, 1985).

Saltgrass is generally regarded as a poor seed producer (Cluff et al., 1983), a factor potentially limiting its use as a cultivated species where the benefits of seed propagation are often highly valued. Saltgrass is well adapted to persist vegetatively, and this is apparently an important part of its life strategy compared to sexual reproduction (Freas, 1987). Eppley et al. (1998) obtained only 45 flowering plants (about 7.4%) from 601 greenhouse-grown seeds collected from a California site. The factors that contribute to poor seed production and infrequent flowering are not well understood. Natural stands of saltgrass have been reported to have spatial segregation of the sexes (Eppley et al., 1998). Saltgrass pollen dispersal is by wind, but pollen dispersal is also thought to be spatially restricted. Combined with the spatial segregation of the sexes, limited pollen dispersal results in high numbers of unfertilized ovules in some natural populations (Eppley et al., 1998).

Saltgrass is one of several stress-tolerant alternative grasses currently being evaluated for use as turfgrass in stress-limited environments (DePew, 1998; Duncan, 1997; Kopec and Marcum, 2001). Saltgrass can form dense sods that remain low growing in some environmental conditions. Inland occurrences of large patches with a turf-like appearance are common along roadways and other disturbed areas. Saltgrass has good wear tolerance, especially when compared to other native species under drought conditions (Fraser and Anderson, 1980), which is another favorable characteristic for its use as a utility or low-maintenance turf. Cuany (1987) has evaluated saltgrass accessions for turfgrass use that remain less than six inches tall even with irrigation. Kopec and Marcum (2001) have identified several saltgrass selections that might be useful for golf course roughs. Saltgrass is undomesticated, however, and currently available germplasm lacks many qualities required to make it more useful in intensively managed turfgrass situations. Although saltgrass is easily propagated vegetatively, establishment of dense turf stands from sprigs or plugs is slow due to the growth habit of the rhizomes. A major drawback to the development of saltgrass as a cultivated species is that it is an alternate host of the spinach rust fungus, *Puccinia aristidae* Tracy (Schuster and Ray, 1949), a disease that can seriously impact other cultivated crops. Smut caused by *Ustilago trebouxii* has also been reported on inland saltgrass (Watson, 1972). Currently, only one clonally propagated saltgrass cultivar selected for use as a turfgrass is known to be available (NyPa International, 2001). It is expected that continued selection and breeding will improve saltgrass for a number of turfgrass characteristics.

The current cytological study was conducted to provide basic information to support breeding efforts currently underway at Colorado State University to develop

saltgrass cultivars useful as turfgrass. Grass breeding can be challenging for a number of reasons. Polyploidy, aneuploidy, and frequent hybridization are major factors influencing evolution in the grasses. More than 80% of grass taxa are believed to be of polyploid origin (De Wet, 1986), but chromosome numbers for only about one-third of the species in the family are known (Hunziger and Stebbins, 1986). Many grass taxa comprise extensive and sometimes complex polyploid series. This is perhaps most extreme in *Poa*, where species and species complexes are known with chromosome numbers from 2n = 2x = 14 in *Poa trivialis* (Armstong, 1937) to 2n = ca. 38x = 263-265 in *Poa litoralis* (Hunziger and Stebbins, 1986). Polyploidy, especially above the tetraploid level (Appels et al., 1998) often reduces fertility, promoting a strong reliance on vegetative reproduction including apomixis. Meiotic irregularities with variable effects on fertility have been described for numerous grass taxa (Myers, 1947). Monosomics or nullisomics, even at the more buffered tetraploid or hexaploid levels, frequently show reduced zygote viability, poor endosperm development, or other impairments that can reduce fitness (Khush, 1973). Genomic structure in polyploid taxa affects segregation ratios or the complexity of inheritance for many traits (Sleper, 1987).

Although these factors can be troublesome, crosses within a polyploid series or wide crosses among different cytotypes can be exploited for increasing the variability available to breeders in several ways (Sleper, 1987). For example, van Santen et al. (1990) were able to integrate diploid germplasm into tetraploid breeding lines by identifying genotypes in diploid orchard grass with frequent 2n gamete formation, allowing acceptable recovery of the desirable tetraploid form in 2x-4x crosses. Hybridization between different ploidy levels can result in useful cultivars for taxa that

can be vegetatively propagated even when reduced fertility results. Many *Cynodon* and *Zoysia* cultivars used for turf are examples.

Grass breeding is often complicated by the occurrence of multiple ploidy levels that may not be readily distinguishable morphologically but cause reduced fertility when seed production is important (e.g. Johnson et al, 1998). An understanding of the mode of inheritance and knowledge of different ploidy levels or other cytotypes that may occur fundamentally enhances breeding efforts by allowing the design of efficient breeding strategies (Fehr, 1991) and by avoiding complications from inadvertently hybridizing different cytotypes in cases where this may interfere with fertility.

The State of the Saltgrass Taxonomy

Recent taxonomic treatments place the genus *Distichlis* in the grass subfamily Chloridoideae (Watson and Dallwitz, 1992; Soreng et al., 2000), but tribal relationships and those of the genera within this subfamily are not well understood (Kellogg and Campbell, 1986). The basic chromosome number in the Chloridoideae is predominately x = 9, but x = 10 occurs in about 13% of species and x = 7 or x = 8 occurs infrequently (De Wet, 1986). Competing treatments recently have recognized from two to eight tribes in the subfamily (Hilu and Alice, 2001). Among the fluid subfamily and tribal nomenclatures there is a current trend to broadly interpret the tribe Cynodonteae (Jacobs, 1986) to include much of what is sometimes called the "main chloridoid assemblage" (Watson and Dallwitz, 1992) that includes the various suprageneric taxa to which *Distichlis* has been assigned by different workers. In this scheme, Soreng et al. (2000) place *Distichlis* in Cynonteae, subtribe Monanthocloinae, where closely related New

World genera include the dioecious halophytes *Monanthochloë* and *Reederochloa* (Hilu and Alice, 2001). The close relationship of *Distichlis* to *Monanthochloë* is highlighted by the report of a putative hybrid between the two genera from Baja California (Stephensen, 1971). A phylogenetic understanding of the grasses as a whole remains an uncompleted goal (Kellogg and Campbell, 1986), and the subtribe Monanthocloinae is not monophyletic as currently described (Hilu and Alice, 2001).

It is now commonly accepted that there are just two North American species of saltgrass, the widely distributed *Distichlis spicata* (L.) Greene (Soreng et al, 2000; ITIS, 2001; Beetle, 1955; Beetle, 1943) and the quite distinct *D. palmeri* (Vasey) Fassett (Soreng et al., 2000; Fassett, 1925), which is apparently limited in distribution to northern Gulf of California salt marshes (Yensen et al., 1983). *D. texana* (Vasey) Scribn. has been convincingly reassigned to the genus *Allolepis* (Soderstrom and Decker, 1965). Earlier, however, the great variability seen among U.S. saltgrass populations, especially those of the Atlantic and northern Pacific coasts compared to certain regional inland occurrences, had led to the description of *D. stricta* (Torr.) Rydb. (Rydberg, 1905) and *D. dentata* Rydb. (Rydberg, 1909) from the *D. spicata* complex. Although reference was made to these new taxa frequently in the literature of the time, perhaps due to their acceptance by Hitchcock (1935) in the first comprehensive manual of U.S. grasses, they quickly came under attack. Arguing that it was almost impossible to separate *D. stricta* and *D. dentata*, and that male plants were almost always classified as *D. stricta* while female plants were almost always classified as *D. dentata* irrespective of their occurrence using Rydberg's descriptions, Reeder (1943) proposed *D. dentata* did not constitute a valid species, and was synonymous with *D. stricta* (Torr.) Rydb. Beetle (1943) went a step further and

suggested that *D. stricta* was but a regional variant of *D. spicata*, since characters used to separate these species differed only by overlapping degrees. He recognized six botanical varieties within the *D. spicata* complex that are still applied, with some confusion, today. *D. spicata* was limited to types occurring along the Atlantic and Gulf coasts of North America. *D. spicata* var. *stricta* comprised saltgrass of varying character occurring over the vast range from Saskatchewan south to Texas and west to the Pacific coast from the eastern limits of saltgrass distribution in Nebraska, Kansas, and the Dakotas. This variety essentially revived with new authorship *D. spicata* (L.) Greene var. *stricta* (Torr.) Scribn., first described in 1894. *D. spicata* var. *borealis* was of Pacific coast distribution from Vancouver south to Washington. This latter variety, and the varieties *stolonifera*, *nana*, and *divaricata* achieved limited use, probably due to the difficulty in differentiating them in practice as well as disagreement about the legitimacy of the classifications. Interestingly, the second edition of Hitchcock's manual (Hitchcock, 1950), revised by Agnes Chase, retains *D. stricta* as the inland species of saltgrass but includes two of Beetle's varieties, *D. spicata* var. *stolonifera* and *D. spicata* var. *nana*, as infraspecific taxa of seashore saltgrass. Steyermark (1963) also chose to retain the use of *D. spicata* (L.) Greene and *D. stricta* (Torr.) Rydb. two decades after Beetle's revision. Years earlier, Steyermark had discovered what he determined to be *D. spicata* (L.) Greene growing over several acres of saline meadow in central Missouri (Steyermark, 1940). This was reported as the first recorded occurrence of *D. spicata* in the U.S. interior. Steyermark suggested it was a stranded relict species of a formerly much more extensive saline region. Saltgrass is rare in Missouri, and that of the typical inland form is thought to occur as introductions, often along railroad beds (Steyermark, 1940). Many recent

floras (Cronquist et al., 1977; Gould, 1975; Hallsten et al., 1987; Hickman, 1993; Sutherland, 1986) continue to follow Beetle's use of infraspecific taxa, especially regarding *D. spicata* var. *stricta*. The authors often describe the occurrence and range of the varieties quite differently, however, from what was originally proposed by Beetle (1943). Other recent treatments (ITIS, 2001; Soreng et al., 2000) do not recognize infraspecific taxa within *D. spicata*. Reference to *D. spicata* (L.) Greene var. *spicata*, a taxon that apparently has no valid citation, is sometimes seen in the literature to differentiate coastal from inland saltgrass occurrences (e.g. Gould, 1975). There are many synonyms in the literature beyond those discussed here. While it seems clear that there are major geographical variants within *D. spicata*, it is also apparent from examination of the material assembled for the current study that the few, continuously variable characters used to describe them inadequately account for variability that can be observed among, or even within, populations.

Additional *Distichlis* species of other than North American distribution have been proposed. *D. distichophylla* (Labill.) Fassett occurs in coastal and saline interior regions of Australia (Fassett, 1925). While this species designation remains in use (e.g. Conner and Jacobs, 1991), Beetle (1955) considered it synonymous with *D. spicata* var. *stricta*. *D. scoparia* (Kunth) Arech. and *D. humilis* Phil. are species of South American distribution along with *D. spicata* (Beetle, 1955). Beetle (1955) also proposed *D. sudanensis* based on examination of one collection from Sudan, Africa. There has been no recent critical review of these species. *D. humilis* and *D. sudanensis* in particular are not well defined.

The Previous Cytogenetic Understanding of Saltgrass

Stebbins and Love (1941) made the first reports of the chromosome number of saltgrass. They observed 2n = 40 chromosomes in root tip sections of two samples, both collected from Alameda County, California. One of these plants was classified by the authors as *Distichlis spicata* (L.) Greene and the other as *Distichlis stricta* (Torr.) Rydb. They concluded that "both of the California species" were probably tetraploids based on the basic number x = 10. Noting that *Distichlis* chromosomes were among the smallest found in the grass family, they combined their cytological and leaf anatomical observations to suggest that *Distichlis* was part of a transitional group between the Chloridae, members of which it most closely resembled in total, and the Festuceae, to which it was generally assigned at the time based on floral characteristics.

Nielson (1956) observed meiosis from among anther collections of 17 male saltgrass accessions as part of a larger study of variability among *Distichlis stricta* accessions directed toward improving the forage potential of the species. It is unclear if his results included all or just a sample of these 17 plants. Nielson concurred with Stebbins and Love that the chromosome number of *Distichlis stricta* was 2n = 40. None of his observations of diakinesis, the only stage that was interpretable, revealed more than 20 bivalents, but some of the preparations appeared to have fewer than 20 chromosome pairs. No multivalent associations were observed. On the basis of strict bivalent pairing and high pollen fertility (determined by undescribed methods), Nielson suggested saltgrass was probably an allopolyploid. Additionally, he observed no lagging chromosomes or other evidence of meiotic irregularities. The possibility of aneuploidy in some accessions was noted, but the evidence was considered inconclusive. Although the

saltgrass accessions in the study originated from a wide range of western U.S. localities, Nielson did not report from which accessions the cytological data were collected.

Several investigators have published additional chromosome counts for *Distichlis* over the years since these first reports. Bowden (1960) found 2n = 40 chromosomes for one *Distichlis stricta* (Torr.) Rydb. plant collected west of Fort Smith, Northwest Territories, Canada, and Rahn (1961) reported 2n = 40 for one *Distichlis spicata* (L.) Greene accession collected near Laguna Blanca, Mendoza Province, Argentina. John Reeder expanded the number of saltgrass collections sampled and contributed much to our understanding of the potential for variation in chromosome numbers in *Distichlis* in three reports over a ten-year period. Reeder first studied *Distichlis spicata* (L.) Greene collections from four localities in Mexico, and while two of these agreed with previous counts of 2n = 40, plants from two different central Mexico populations had 2n = ca.72 chromosomes (Reeder, 1967). Reeder interpreted the ca. 72-chromosome saltgrass as aneuploids, and said there was little doubt that the basic chromosome number of *Distichlis* was x = 10. The drawing of a pollen mother cell at diakinesis from the ca. 72-chromosome accession from San Luis Potosí, Mexico, appears to depict 36 bivalents but was not specifically interpreted by Reeder. Pointing out that there had been previously published chromosome counts for only four plants of this genus, his observations led him to suggest that "clearly additional cytological data are needed for a better understanding of this wide-ranging taxon" (Reeder, 1967). This assessment did not include the results from Nielson's thesis work, but did specifically exclude the report of 2n = 40 for *Distichlis texana* by Brown (1951), a species that had been reclassified as *Allolepis texana* by Soderstrom and Decker (1965). In light of his statement calling for additional

data, Reeder's next report of chromosome counts for just two additional *Distichlis spicata* collections, one from Mexico and the other from southwest Texas, both with 2n = 40 chromosomes, contained the interesting declaration that the previous counts of 2n = ca.72 were "unusual" (Reeder, 1971). Reeder eventually did, however, find another of these unusual saltgrass plants, as well as plants with additional chromosome variations. In his most recent report on saltgrass, Reeder (1977) made chromosome counts for two accessions from southern Nevada, one each from Clark and Lincoln Counties, four accessions from Albany County in southeastern Wyoming, and one accession each from Merced County, California, Mesa County in western Colorado, Pecos County in southwest Texas, and Salt Lake City, Utah. As in his previous reports, the determinations were made from observations of meiosis in pollen mother cells. Just six of these ten collections had 40 chromosomes, including both of the Nevada and the California and Texas collections. The Colorado collection was reported to have 2n = 72 chromosomes. Two of the four Wyoming collections had 2n = 40 chromosomes, but two had only 2n = 38 chromosomes. The Utah collection had 2n = 42 chromosomes. Reeder commented that the 38- and 42-chromosome plants, apparently aneuploids, should not be considered unexpected discoveries since aneuploidy among tetraploids is not uncommon. It is interesting to note, however, that not only did all three of these cases of aneuploidy seem to involve the loss or gain of one pair of chromosomes, since either 19 or 21 bivalents were observed, but that the extra pair of chromosomes in the 42-chromosome plant did not associate with any other chromosomes. Reeder was more interested in what he called the "rather startling" occurrence of the 72-chromosome plant of "not obvious" origin in Colorado (Reeder, 1977). He reasoned that this plant represented a

chromosome race that had evolved independently from those he had earlier described with 72 chromosomes from central Mexico. Further, he suggested that it might have most plausibly evolved via chromosome loss from a chance octaploid. This was based on the reckoning that since neither hexaploids nor complete octaploids of the species had been found, it was less likely to represent an aneuploid variant of a heptaploid formed by combination of 3x and 4x gametes. Meiosis in the 72-chromosome plant was described as "essentially regular", and "usually no more than one quadrivalent was observed per cell" (Reeder, 1977). There was no mention of whether similar female plants occurred among or near to the 72-chromosome male plants. Reeder's statement that unusual biotypes of saltgrass like these could easily persist for long periods of time given their great capacity for vegetative reproduction was perhaps a suggestion of how it would be possible that eventually breeding populations arose from these rare, chance events.

In summary, chromosome counts for 20 *Distichlis spicata* (*sensu lato*) plants are known from published accounts. Of these, 70% have 2n = 40 chromosomes and 30% are interpreted as aneuploids. Half the cases of aneuploidy, 15% of the total counts, occur among plants also varying in genome number from the presumed tetraploids where x = 10. Additional, but somewhat vague, reports from Nielson (1956) would appear to increase the proportion of 2n = 40 plants observed, but also increase the number of reports of aneuploidy. Only bivalent pairing during meiosis was reported for all 2n = 40 plants (excluding the four plants from which the chromosome number had been determined only from somatic cells) as well as for the aneuploids of the tetraploids. This suggests that the loss or gain of chromosomes involved both of one pair of homologues, but leaves open the question of why, then, multivalent associations were not observed in

the 2n = 40 + 2 individual, a putative tetrasomic based on this reasoning. Multivalent associations are neither certain nor exclusive indicators of the presence of multiple homologues (Sybenga, 1996; Appels et al, 1998; Rees and Jones, 1977), however, and few quadrivalents were observed in what is most likely an autopolyploid variant of the 40-chromosome plants.

Initial cytological examination of saltgrass accessions collected in Larimer County, Colorado, and seven accessions from Dr. Robin Cuany's prior saltgrass breeding efforts at Colorado State University found no plants with 40 chromosomes. All Larimer County collections and five breeding program selections from these preliminary studies had 38 chromosomes. One accession to the breeding program had 74 chromosomes. This plant had been collected from Mesa County in western Colorado, the same region where Reeder had reported finding saltgrass with 72 chromosomes. Another western Colorado accession had 42 chromosomes.

Research Objectives

The identification of different ploidy levels and the exclusive putative aneuploidy existing among saltgrass accessions already selected for synthesis of an initial breeding population defined two major research questions.

First, how common is variation in ploidy level? Are the higher order polyploids indeed rare, as had been previously suggested? Although the inclusion of one of these polyploids among a small number of selections could just be the result of a chance collection from a particular region, it also could be possible they occur more commonly than thought and therefore define an important selection criterion.

Second, what is the nature of the aneuploid variation at the tetraploid level? All cases observed so far vary from the 2n = 40 number by two chromosomes. Very limited evidence exists that this variation may involve both of one pair of homologues. It is unknown if this is representative, or if variations for other chromosomes occur. Is the aneuploidy of practical significance, especially regarding fertility? The chance selection of all aneuploids for the breeding program seems unlikely given previous reports of aneuploid frequency. Could there be explanations other than aneuploidy for the observed variation in chromosome number around 2n = 40? Most of the material from the preliminary investigations originated from a region where no prior studies had been made. Could there be a pattern to the distribution of different cytotypes?

To answer these questions, chromosome counts were made for many saltgrass accessions from diverse western U.S. origins. The distribution of cytotypes was mapped to determine patterns of variation. Additional meiotic data were collected from male plants of different cytotypes. Seed production resulting from hand pollinations among accessions was used to confirm fertility, and additional chromosome counts from among the progeny of these crosses were made to study the transmission of chromosomes in 2n = 38 x 2n = 40, 2n = 38 x 2n= 42, and 2n = 38 x 2n = 38 crosses.

Materials and Methods

Sources of Plant Material

Saltgrass accessions were collected from diverse habitats within several major biogeographical regions. Chromosome counts were made for a total of 160 *Distichlis spicata* accessions. Of these, 127 were vegetatively collected accessions from eight states in the western United States plus one accession from Florida. All of these accessions are thought to be from naturally occurring saltgrass stands at the collection sites, although many were growing in dramatically disturbed and human-influenced environments. Thirty-two accessions were grown from seeds collected from four different western U.S. localities.

The vegetatively collected accessions comprise an unknown number of different genotypes. Although collections were made with the intention of obtaining unique specimens, individual accessions could contain more than one genotype and in some instances ramets of the same genets could have been collected more than once as different accessions. Very little is known about the structure of saltgrass populations or how they may vary from site to site, but Eppley et al. (1998) estimated from a conservative interpretation of RAPD profiles that 1 m^2 quadrats containing either

predominately male or predominantly female *Distichlis spicata* ramets sampled at Tomales Bay, California, contained an average of more than 9 different genotypes and suggested the true number of genotypes per quadrat would be greater. While several of our accessions were collected as single rhizomes or have been re-propagated from single rhizomes separated from the original collection material, it therefore seems possible some accessions could contain more than one genotype when multiple rhizomes were used to establish an accession. However, since the vegetatively collected materials maintained as individual accessions are phenotypically uniform and individual seedling accessions from within populations are usually phenotypically variable, it is assumed that most likely each vegetative accession is a single genotype. Multiple collections of the same genet as different accessions are of more concern when making inferences from this study. Since saltgrass is capable of spreading vegetatively over long distances naturally and by physical transport of rhizomes in disturbed areas, the possibility exists that some collections from a particular area are actually ramets of the same genet. In several cases noted below more than one plant from a particular source was considered as an individual accession. This was done when the chromosome numbers of all samples were the same, no other phenotypic differences were observed, or where prior clonal propagation could have occurred to avoid inflation in the number of plants reported. Undetected duplication could exist among other accessions used in the study.

The following brief descriptions of the number and nature of the saltgrass collections studied and the major ecogeographic associations from which they originated use the descriptors (CE) set forth by the Common Ecological Region National

Interagency Technical Team (CERNITT, 1999). Table 1 lists the saltgrass accessions by state and county of origin. Figures 3 through 5 map the locations of the accessions.

The most extensively sampled region was along the eastern front range of northern Colorado, in a densely human-populated region at the western edge of the Western High Plains (CE30). Seventy-two accessions collected over a distance of approximately 120 kilometers from Denver, Colorado, north to Wellington, Colorado were included in the study. Additional Western High Plains accessions were collected from eastern Wyoming near the town of Lusk, approximately 190 kilometers north of the Colorado front range collection area and near Sterling, Colorado, approximately 145 kilometers east of the northern limit of the front range collection area. Eight additional eastern Colorado accessions were collected about 200 kilometers south of the southern limit of the front range collections near Pueblo, Colorado, in the Southwestern Tablelands (CE31) adjacent to, but south and west of the Western High Plains region. Two accessions from Pueblo, C10 and C11, and the accession from Sterling, C8, are also known as NM-1113, NM-1114, and NM-1100 respectively, and were originally collected by the Los Lunas Plant Materials Center, NRCS, as desert saltgrass, *Distichlis stricta* (Torr.) Rydb. These accessions were the highest-rated native grass accessions in the New Mexico wear tolerance tests under severe wear and moisture-limiting conditions (Fraser and Anderson, 1980). Six Central Great Plains (CE32) accessions were collected near Great Bend, Kansas, and in the nearby Cheyenne Bottoms area. Two collections, considered as one accession, were made at the Arbor Lake Wildlife Management Area near Lincoln, Nebraska, in the transitional region of the Central Great Plains into the Western Cornbelt Plains (CE51) ecological region.

Vegetative collections from the California Central Valley (CE9) region include 10 accessions collected over a 350-kilometer range in the San Joaquin Valley and one accession from the Sacramento Valley in Sutter County about 225 kilometers north of the San Joaquin collections. All but one of these plants were part of a collection contributed by the Lockeford Plant Materials Center, NRCS, from material evaluated for potential use in riparian restoration, and bank and shoreline stabilization. One of the Lockeford accessions, 9032700, was released as selected and tested material under the name LK 517f saltgrass and is available from the Foundation Seed Service, University of California, Davis, as vegetative propagules. The collectors of the Lockeford germplasm classified all accessions as seashore saltgrass, *Distichlis spicata* (L.) Greene. The remaining San Joaquin Valley accession was collected from a golf course green in Fresno, where it was growing as a weed in a salt-affected site.

Saltgrass from broadly defined Great Basin Desert localities include five vegetatively collected accessions and individuals grown from seed collected at three sites. Eleven accessions, representing a population sample from the Goose Lake area in Modoc County, California, were grown from seeds collected in the wild and purchased from Freshwater Farms, Eureka, California. This region is classified as part of the Eastern Cascade Slopes and Foothills (CE7) common ecological region, but it is geographically part of the Great Basin system of terminal lakes and wetlands contained in the vast basin to the southeast. Eight accessions, known as the C66 population, include one vegetatively collected plant and seven plants grown from seeds collected at the same site in the Humbolt Sink area, Nevada, about 220 kilometers south-southeast of the Goose Lake site. This site is in a transitional region in the Central Basin and Range (CE16)

region where shrubland becomes more common than the interspersed shrub and grasslands characteristic of the wetter, lower region just to the north. Twelve accessions, established from seeds purchased from the Granite Seed Company, Lehi, Utah, originated from the eastern part of the Central Basin and Range region in the Great Salt Lake vicinity. The seeds were designated *Distichlis spicata* var. *stricta*, desert saltgrass, by the vendor. Granite Seed Company managers would not disclose the exact location of the natural saltgrass stand that was the source of the Granite population seeds. Three other Central Basin accessions from northwestern Utah and northern Nevada were collected along an approximately 300-kilometer line extending west from the Great Salt Lake toward the C66 population site. One accession was collected from Aberdeen, Idaho, at the transition from the Central Basin into the less mountainous sagebrush steppes of the Northern Basin and Range (CE15) ecological region.

Fourteen accessions were collected from the geologically defined Colorado Plateau. This area contains two common ecological regions, the Colorado Plateaus (CE24) and the Arizona/New Mexico Plateau (CE26) to the south. Both regions are large, variable transition zones. The Colorado Plateaus are generally drier than the region to the south. Two accessions originated from western Colorado at the eastern edge of the Colorado Plateaus and seven accessions were collected from St. George, Utah, and Fredonia, Arizona, two nearby localities at the southwestern limit of this region. The Colorado collections from this region are separated by about 500 kilometers from the St. George and Fredonia collections. Four accessions were examined from collections made near Holbrook, Arizona, in the west-central part of the Arizona/New Mexico Plateau region, located about 275 kilometers southeast of the Fredonia collections.

Six accessions were obtained from two collection sites along the lower Colorado River, one near Yuma, Arizona, and the other about 150 kilometers to the north near Parker, Arizona. Both sites are in the Sonoran Basin and Range (CE18) ecological region. This region is climatically distinguished from the other western regions in which collections were made primarily by its higher temperature regime and the occurrence of both summer and winter rainy seasons. The Sonoran is considered by some to be the most biologically diverse desert region in the world, due in part to these climatic factors. Chromosome counts were made for three additional accessions, referred to as the GZ population. These are plants grown from seeds purchased from Granite Seed Company as coastal saltgrass, *Distichlis spicata* var. *spicata*. The seeds were progeny among an unknown number of parents originally collected from undisclosed locations in "the lower Colorado River drainage" (Yensen, personal communication) that had been selected to develop saltgrass commercially useful as a salt-tolerant forage. Data from these accessions are considered to be only supplementary due to the uncertain origin of the germplasm. Plants from the GZ population are morphologically similar to the plants collected from Parker.

Two accessions of saltgrass originating from two different truly coastal habitats were obtained from commercial sources. Ten plants originating from Florida Gulf Coast collections were purchased from Horticultural Systems, Parrish, Florida, and were considered as one accession. Ten plants originating from collections made along San Francisco Bay, California, purchased from Freshwater Farms, Eureka, California, were also considered as one accession. The precise collection locations of these accessions are not known.

Additional chromosome counts made from progeny of controlled crossing experiments among selected accessions are considered separately.

Cytological Procedures and Data Analysis

Accurate chromosome counts can be difficult to make in plants with numerous, small chromosomes. The method used must result in well spread chromosomes lying flat in one focal plane with a minimum of broken cells. Cell walls, small bits of debris, or even the cytoplasm of several overlapping cells can introduce unacceptable ambiguity. Most of the chromosome counts were made, therefore, from root tip cells prepared by a "splash" technique, similar to the smear methods used for animal cells, that was developed from various protocols to facilitate optimal spreading. Root tips were collected into 1.5 ml microfuge tubes from greenhouse grown plants, rinsed to remove debris in several changes of water, and treated for 3.5 hours in 0.05% aqueous colchicine solution at room temperature. Root tips were then rinsed once with water and fixed in 3:1 95% ethanol and glacial acetic acid for at least 24 hours. The initial fixative was replaced by 1% acetocarmine (1 g carmine refluxed in 45 ml glacial acetic acid, 55 ml deionized water) and samples were stored at 4° C for at least several days to achieve optimal staining. Root tips were prepared for spreading by dissecting the darkly stained meristematic regions, usually about 2 mm in length, and digesting them after rehydration with 2% cellulase, 2% pectinase in 10 mM citrate buffer, pH 4.2, at 37° C. Digestions were done in the wells of ceramic spot plates. From 4 to 20 root tips were digested in approximately 200 µl of enzyme solution, depending on the number of roots. Enzymes of different lots from several suppliers were used, and the time required for digestion

varied. The minimum time required for release of cells for acceptable splashes was 4 hours, but much longer digestions of up to 9 hours were often necessary. The progress of the digestions was monitored by withdrawing the enzyme solution from the wells with a pipette under a dissecting microscope and considered complete when the outer root body remained intact, but inner cells were loosened enough to become free of the collapsing root cylinder when the supporting liquid was removed. At this time, the enzyme solution was replaced with 45% acetic acid, which was changed once before collecting the root tips by Pasteur pipette to 0.5 ml microfuge tubes. The spreading mixture was prepared by adding 80% ethanol, 0.5% Igepal detergent (Sigma) solution to a final ratio of approximately 3:1 after excess acetic acid was removed from the samples (usually about 50 µl roots + acetic acid). Tubes were gently vortexed to facilitate release of cells into the solution and centrifuged briefly, if needed, to move large debris to the bottom of the tubes. Aliquots of approximately 15 µl were dropped onto freezer-cold slides from a height of about 10 cm and slides were allowed to air dry. The total volume of spreading solution or the proportion of ethanol to acetic acid was adjusted as necessary to obtain optimal cell densities, cell spreading, and cell bursting times. Chromosome counts from root tips were also made by a more traditional squash technique similar to that modified for plants with small chromosomes as described by Lapitan (1997) following the colchicine treatment and enzymatic softening as described above. The splash method is more time consuming and subject to failure due to small changes in a number of parameters, but can provide superior results since cells are spread in one layer without overlap and there are few broken cells. The squash technique, limited by the difficulty of not breaking cells that are adequately digested for obtaining flat preparations, was used

primarily when limited material was available or counts from cells of a single root tip were desired.

Observations of meiosis were made for selected male accessions from anthers dissected from inflorescences initially fixed in 3:1 95% ethanol and glacial acetic acid with 0.1% ferric chloride. Some anthers were used directly following this fixation, but most inflorescences were transferred to 70% ethanol and stored at 4° C prior to staining. Extended cold storage in 70% ethanol generally seems to improve results. Individual fixed anthers were stained in acetocarmine for at least several hours. Contents of the anther sacs were extruded from cut anthers and squashed in 45% acetic acid using a technique similar to that described by Goicoechea and Giraldez (1996). Duplicate determinations of the chromosome number of several accessions were also made from mitotic metaphase cells in very young anthers.

Chromosome preparations were examined with an Olympus BX50 microscope using phase contrast. All determinations of chromosome number were made from observations using the 100X oil immersion objective. Photomicrographs to document representative cells or allow further study were made with a 35mm camera attachment using Kodak Technical Pan film. Confirming counts of at least 25 cells judged to be intact with unambiguous chromosome spreads were made to determine the chromosome number of individual accessions. For many accessions, counts of well over 100 cells have been made to validate the methods. Approximate counts for certain accessions where ambiguous preparations have not been resolved beyond doubt are indicated in the discussion. Chromosome measurements, where appropriate, were made from digitized images using MicroMeasure version 3.3 software (Reeves and Tear, 2000). Maps

(Albers Equal-Area Conic Projections) for locating the origin of the accessions were generated using ArcView GIS version 3.2 software (Environmental Systems Research Institute, Inc).

Supporting data from estimates of pollen viability were made primarily using the MTT method (Rodriguez-Riano and Dafni, 2000). The ability of pollen to germinate and grow through the stylar conducting tissue *in vivo* was assessed by hand-pollinating selected accessions and observing pollen tube growth using alkaline aniline blue staining (Kho and Bayer, 1968). Callose fluorescence was observed with an Olympus Vanox epifluorescence microscope equipped with the appropriate filters.

Table 1. Source and 2n chromosome numbers of *Distichlis spicata* accessions.

Locality				Locality				Locality			
State	**County**	**Accession**	**2n**	**State**	**County**	**Accession**	**2n**	**State**	**County**	**Accession**	**2n**
Arizona				Colorado				Colorado			
	Navajo	AZ 203[1]	40		Denver	AZ 37[1]	38		Pueblo	P3[1]	ca. 74
	Navajo	AZ 204[1]	40		Denver	AZ 39[1]	38+1B		Pueblo	P4[1]	ca. 74
	Navajo	AZ 207[1]	ca. 74		Adams	AZ 42[1]	74		Pueblo	P5[1]	38
	Navajo	AZ 209[1]	ca. 74		Denver	AZ 46[1]	38		Logan	C8[1]	38
	Navajo	AZ 212[1]	ca. 74		Denver	AZ 48[1]	38		Pueblo	C10[1]	38
	Coconino	AZ 228[1]	40		Denver	AZ 49[1]	38		Pueblo	C11[1]	38
	Coconino	AZ 229[1]	ca.74		Denver	AZ 50[1]	38		Delta	C12[1]	40+2B
	Coconino	AZ 230[1]	42		Denver	AZ 51[1]	38		Mesa	C92[1]	74
	Yuma	AZ d1[1]	77		Denver	AZ 53[1]	38	Florida			
	Yuma	AZ d5[1]	ca. 76		Denver	AZ 59[1]	38		[4]	Flor1-10[1]	40
	Yuma	AZ d6[1]	76		Denver	AZ 60[1]	38	Idaho			
	Yuma	AZ d7[1]	77		Denver	AZ 61[1]	38		Bingham	C56[1]	38
	La Paz	AZ par6[1]	40		Denver	AZ 65[1]	38	Kansas			
	La Paz	AZ par9[1]	40		Denver	AZ 67[1]	38		Barton	AZ 211[1]	38
California					Denver	AZ 68[1]	ca. 72		Barton	AZ 213[1]	38
	Merced	9032620[1]	40		Denver	AZ 71[1]	38		Barton	AZ 214[1]	38
	Kern	9032698[1]	40		Denver	AZ 73[1]	38		Barton	AZ 215[1]	38
	Sutter	9032717[1]	40		Denver	AZ 75[1]	38		Barton	AZ 216[1]	38
	Kern	9032694[1]	40		Adams	AZ 77[1]	38		Barton	AZ 219[1]	38
	Madera	9032683[1]	40		Adams	AZ 78[1]	38	Nebraska			
	Los Angeles	9032708[1]	40		Adams	AZ 79[1]	38		Lancaster	Neb-1-2[1]	38
	Tulare	9032700[1]	74		Adams	AZ 80[1]	38	Nevada			
	San Luis Obispo	9032610[1]	40		Weld	AZ 89[1]	38		Pershing	C66[1]	38
	Kings	9032696[1]	40		Weld	AZ 94[1]	38		Pershing	sp66-1[2]	38+2B
	Kern	9032699[1]	40		Weld	AZ 95[1]	38		Pershing	sp66-7[2]	38
	Fresno	FresnoGC[1]	40		Weld	AZ 97[1]	38		Pershing	sp66-10[2]	38
	[3]	SF[1]	40		Weld	AZ 103[1]	74		Pershing	sp66-14[2]	38
	Modoc	M1[2]	38		Weld	AZ 104[1]	74		Pershing	sp66-15[2]	38
	Modoc	M2[2]	38+2B		Larimer	AZ 107[1]	38		Pershing	sp66-17[2]	38
	Modoc	M3[2]	38		Larimer	AZ 108[1]	38		Elko	DC29[1]	74
	Modoc	M4[2]	38		Larimer	AZ111[1]	38		Elko	DC31[1]	76

	Modoc	M5[2]	38	Larimer	AZ 114[1]	38	Utah				
	Modoc	M6[2]	38	Larimer	AZ 122[1]	38		Washington	AZ 221[1]	ca. 74	
	Modoc	M7[2]	38	Larimer	AZ 124[1]	38		Washington	AZ 222[1]	ca. 72	
	Modoc	M8[2]	38	Larimer	AZ 126[1]	38		Washington	AZ 225[1]	ca. 74	
	Modoc	M9[2]	38+7B	Larimer	AZ 135[1]	38		Washington	AZ 226[1]	ca. 74	
	Modoc	M10[2]	38	Larimer	AZ 136[1]	38		Tooele	DC33[1]	40	
	Modoc	M11[2]	38+2B	Larimer	AZ 137[1]	38		[5]	G2[2]	41	
Colorado				Larimer	AZ 138[1]	38		[5]	G3[2]	41	
	Arapahoe	AZ 1[1]	38	Larimer	Cat City[1]	38		[5]	G6[2]	40	
	Denver	AZ 2[1]	74+2B	Larimer	Har-7[1]	38		[5]	G7[2]	40+4B	
	Denver	AZ 6[1]	74	Larimer	FC34E[1]	38		[5]	G10[2]	40+2B	
	Denver	AZ 8[1]	74+2B	Larimer	B4[1]	38		[5]	G11[2]	40	
	Jefferson	AZ 10[1]	38	Larimer	B5[1]	38		[5]	G13[2]	42	
	Denver	AZ 11[1]	38+2B	Larimer	D1[1]	38		[5]	G12[2]	40	
	Jefferson	AZ 12[1]	38	Larimer	D4[1]	38		[5]	G28[2]	40	
	Denver	AZ 14[1]	38	Larimer	IBIS3-1[1]	38		[5]	G23[2]	40	
	Denver	AZ 16[1]	38	Larimer	97-7-1[1]	38		[5]	G25[2]	40	
	Denver	AZ 18[1]	38	Larimer	96-3-1[1]	38		[5]	G30[2]	40	
	Denver	AZ 20[1]	74	Larimer	96-4-1[1]	38	Wyoming				
	Denver	AZ 23[1]	38	Larimer	96-5-1[1]	38		Sweetwater	DC43[1]	ca.74	
	Denver	AZ 24[1]	38	Larimer	96-5-2[1]	38		Niobrara	DC86[1]	38	
	Denver	AZ 27[1]	56	Larimer	97-1-1[1]	38					
	Denver	AZ 28[1]	38	Larimer	A[1]	38	[6]		GZ3[2]	40	
	Denver	AZ 29[1]	38	Pueblo	P-RCR[1]	ca. 73	[6]		GZ4[2]	40	
	Denver	AZ 30[1]	38	Pueblo	P1[1]	ca. 72	[6]		GZ6[2]	40	
	Denver	AZ 31[1]	38	Pueblo	P7[1]	38					

[1]Vegetative collection.
[2]Seed collection.
[3]County of origin unknown. Plants collected from the San Francisco Bay area.
[4]County of origin unknown. Plants collected from the Florida Gulf Coast in the Tampa Bay vacinity.
[5]County of origin unknown. Plants grown from seeds harvested from a natural stand in the Great Salt Lake vacinity.
[6]Plants grown from seeds produced by plants originally collected in the Lower Colorado River Drainage.

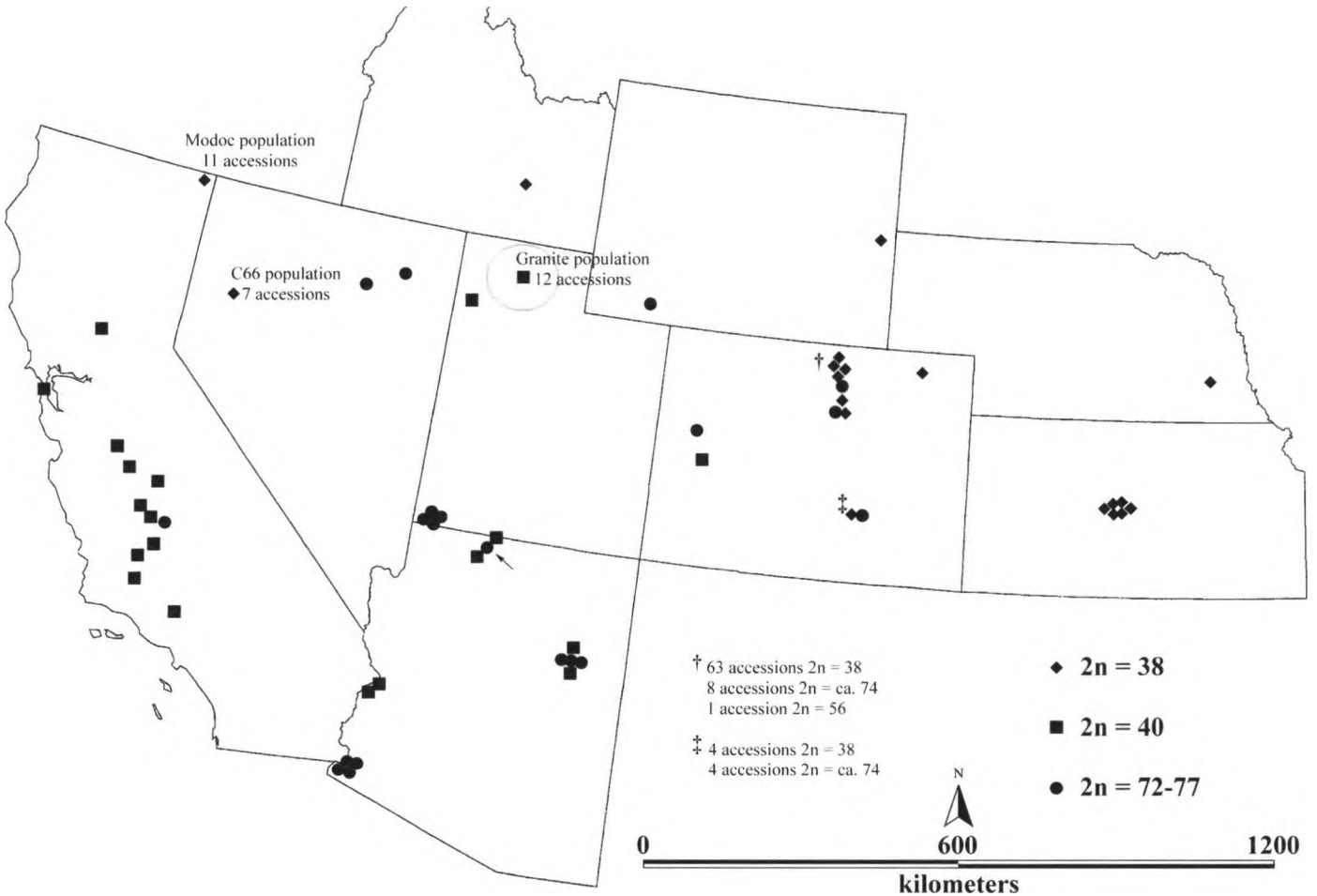


Figure 3. Distribution of cytotypes found among accessions from nine western U.S. states. Saltgrass accessions with 2n = 40 chromosomes were collected primarily from southwestern desert regions while accessions with 2n = 38 were found in Great Plains collections and at the northern edge of the Great Basin. The aneuploid 8x cytotypes were found regionally distributed with both 2n = 40 and 2n = 38 collections. Arrow indicates the lone possible aneuploid (a tri-or tetrasomic) found among vegetative collections of 2n = 40 plants. Shaded areas are detailed in following figures.

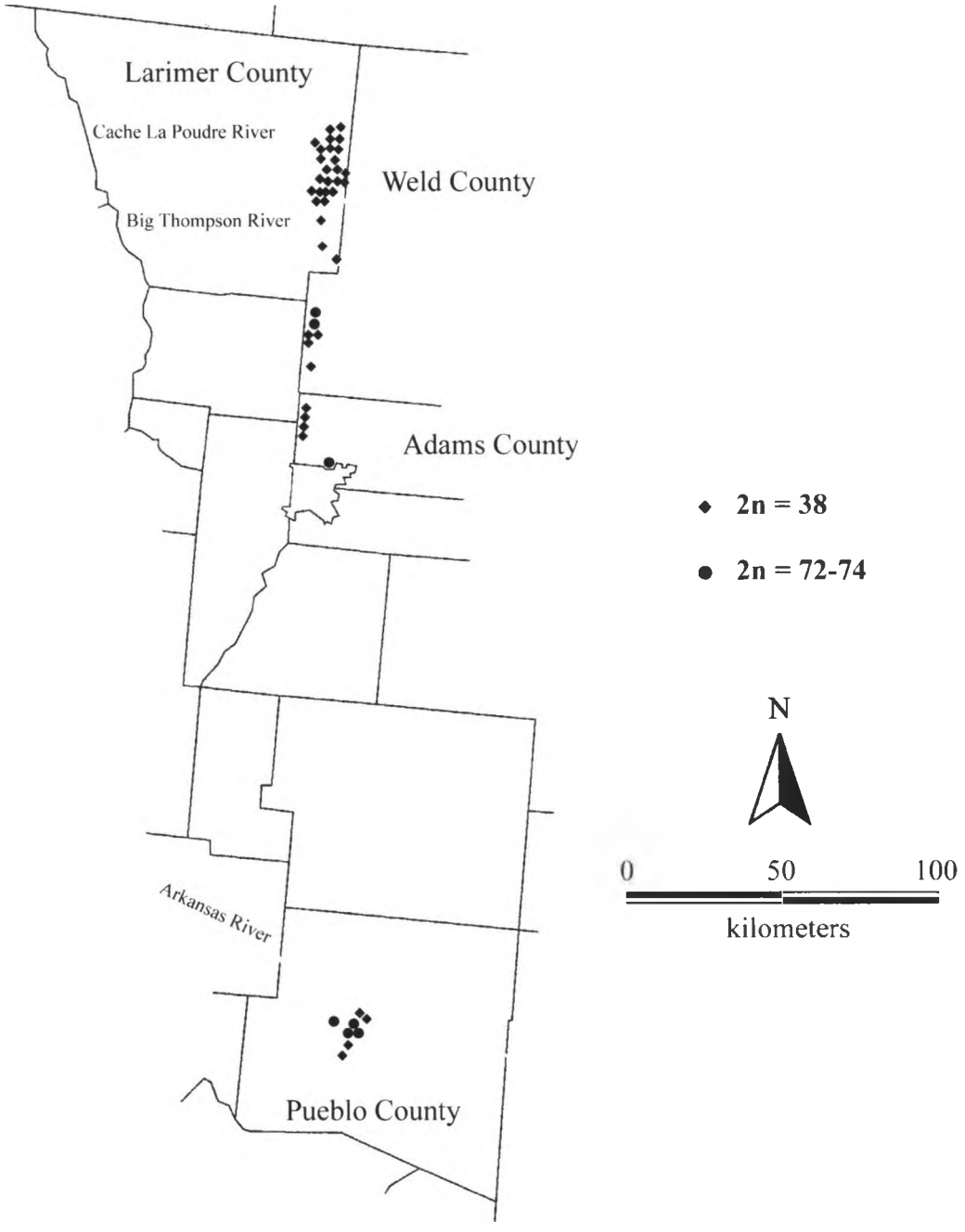


Figure 4. Distribution of saltgrass cytotypes found among accessions collected from the eastern front range of Colorado. Shaded area is detailed in the following figure.

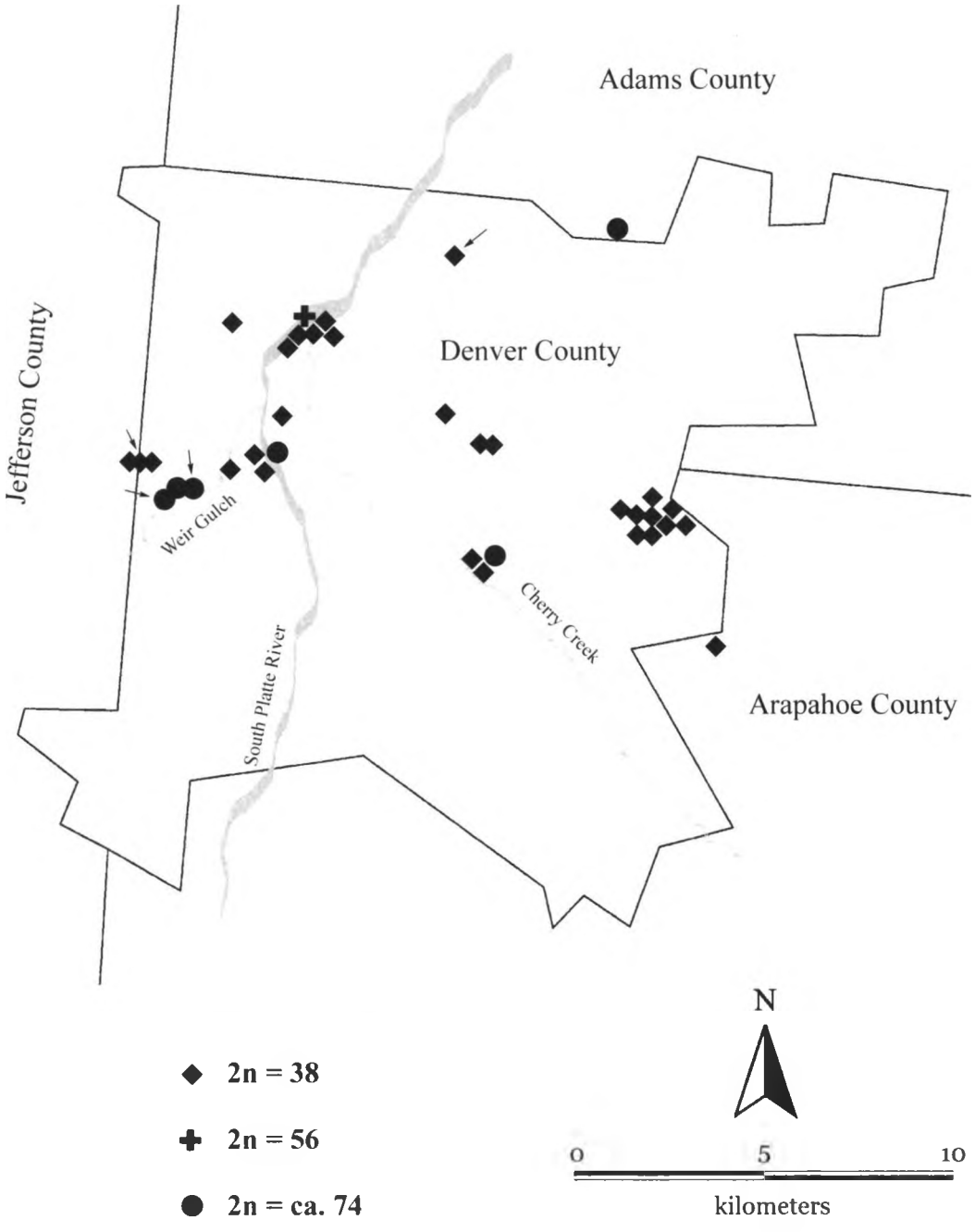


Figure 5. Distribution of saltgrass cytotypes found among accessions collected from Denver County, Colorado, and adjacent counties. Arrows indicate accessions with B chromosomes.

Results and Discussion

Thirteen different somatic chromosome numbers were observed among the 160 accessions studied. This high level of variation in number, however, appears to reflect a very limited and specific set of variations in kind. Nearly 94% of all accessions have chromosome numbers falling into just three number classes. More than 55% of the accessions have 2n = 38 chromosomes, 20% have 2n = 40 chromosomes, and just over 18% have from 2n = 72 to 2n = 77 chromosomes. Table 2 shows the frequency of the total number of somatic chromosomes found in the accessions.

Table 2. Frequency of somatic chromosome numbers observed in 160 *Distichlis spicata* accessions.

	Chromosome number								
	38	39	40	41	42	44	45	56	72, 73, 74, 76, or 77
Number of Accessions	89	1	32	2	4	1	1	1	29

Other than the large number of plants with 38 chromosomes, at least half of the relatively few cases of variation from the previously reported 2n = 40 tetraploid number observed here are thought to be due to the occurrence of B chromosomes in some individuals rather than instances of aneuploid variation. In four cases, imperfect chromosome preparations have as yet prevented the interpretation of one or two additional chromosomes in plants that otherwise appear to have the chromosome complement typical of the 2n = 40 plants. Of these, two 41-chromosome plants could either be trisomics or have one B chromosome. Similarly, two 42-chromosome plants could be trisomic with one B chromosome, be tetrasomic, or have two B chromosomes. Although these 42-chomosome plants could also be trisomic for two different homologues, this is thought not to be the case for reasons discussed below. One accession has 2n = 40 + 4 B chromosomes. The putative B chromosomes also occur among plants with chromosome complements otherwise morphologically indistinguishable from those of typical 2n = 38 plants, and appear to occur among the higher order polyploids in at least two accessions. Variation in number from 2n = 38 due to B chromosomes is thought to explain the chromosome complements of the 39- and 45-chromosome accessions as well as four accessions with 40 total chromosomes. The interpretation of the 38-chromosome plants simply as aneuploid variations of the 40-chromosome plants is disputed by evidence of regional, sometimes extensive, distributions of these fertile populations with karyotypic uniformity in the absence of 40-chromosome plants. If we remove cases of variation due to B chromosomes and recognize the 38-chromosome plants as a previously undescribed chromosome race, the possible cases of aneuploidy at the 4x level (including both 38- and 40-chromosome

races) are only about 3%. Table 1 lists the chromosome numbers for each accession as currently interpreted, along with the locality of origin.

The Chromosome Complements of 2n = 38 and 2n = 40 Plants and B Chromosomes

Although it is difficult to identify all of the small saltgrass chromosomes with certainty from preparations treated to facilitate making chromosome counts, some morphological differences can be recognized. Most of the chromosome pairs can be recognized in favorable prometaphase or less highly-contracted metaphase preparations. An important and consistent difference can be seen, however, in the chromosome complements between the 38- and 40-chromosome plants even in the highly contracted, colchicine-treated metaphase chromosomes. The 38-chromosome plants all lack two small chromosomes found in plants with 40 chromosomes (Figure 6 and 7). The chromosome complements of 38-chromosome plants are similar in gross morphology whether they are from the Modoc or C66 populations of California and Nevada, or from the Idaho or Great Plains accessions. Centromeres of all chromosomes in both cytotypes are median or submedian (using the terminology of Levan et al., 1964), but the arms can be quite variable in appearance due to differential condensation and staining, usually dark in the proximal arm regions and faint in the distal arm regions. Faint, unstable, small condensations (Fukui, 1996) are sometimes clearly visible in several chromosomes as "knobs" or "antennae" and may be useful as identifying features with further study. Using the current techniques, these features are not always visible. The nucleolus organizer region is not obvious, but is thought to occur in one of the smaller pairs of homologues.

Figure 6. Root tip metaphase chromosomes of 2n = 40 and 2n = 38 cytotypes compared to those of both cytotypes with B chromosomes. (A), accession SF, collected from San Francisco Bay area, 2n = 40. Typical of 40-chromosome plants collected from other localities. (B), accession C56, collected near Aberdeen, Idaho, 2n = 38. Typical of 38-chromosome plants collected from other localities. (C), accession AZ 11, collected from Denver, Colorado, 2n = 38 + 2B. This is the only accession with 40 total chromosomes among 91 Great Plains accessions examined to date. (D), accession AZ 39, collected in Denver, Colorado, 2n = 38 + 1B. (E), accession C12, collected from Delta County, Colorado, 2n = 40 + 2B. (F), accession G7, grown from seed collected near the Great Salt Lake, Utah, 2n = 40 + 4B. Arrows indicate B chromosomes. Scale bars represent 10 μm.

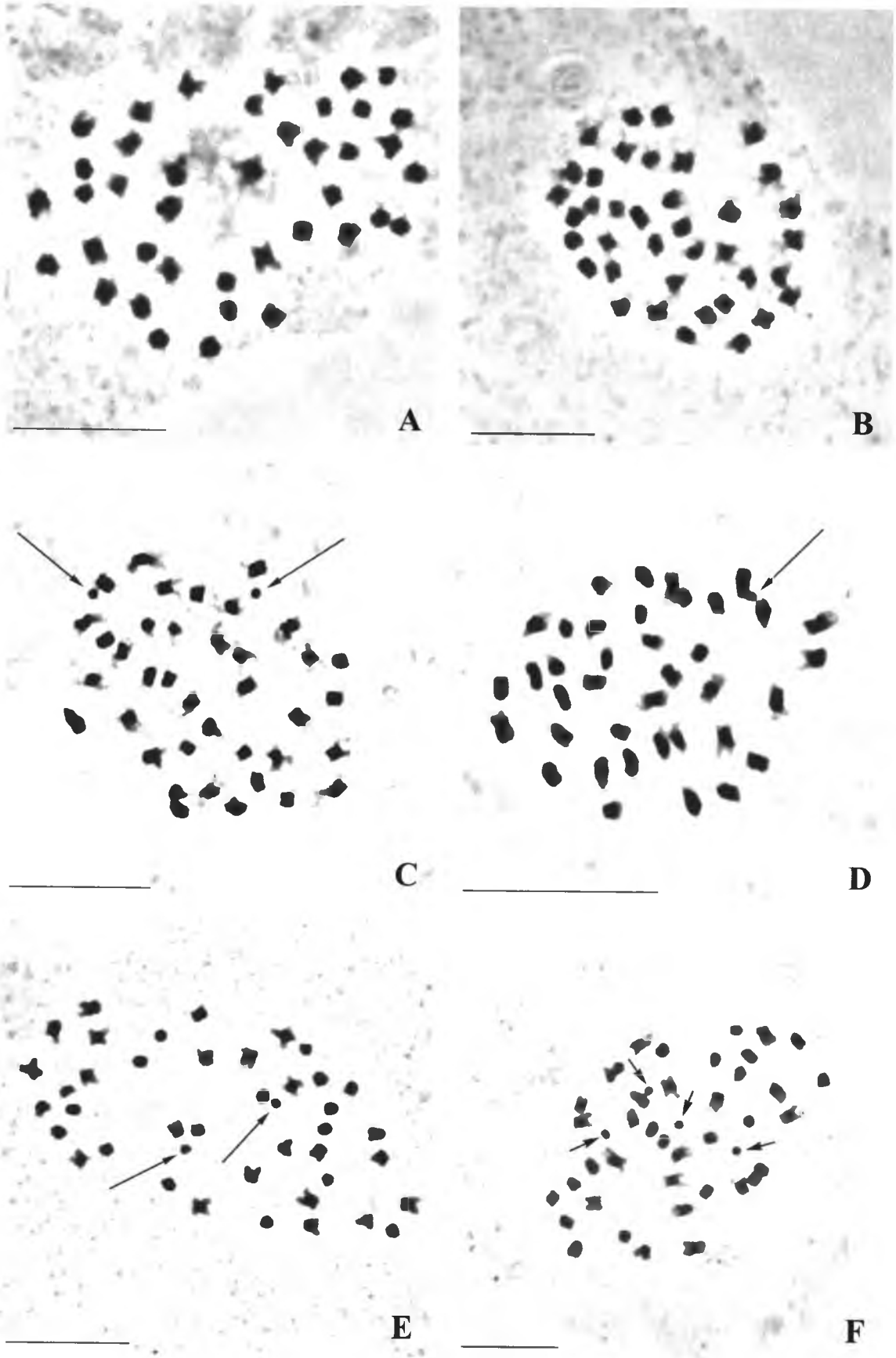
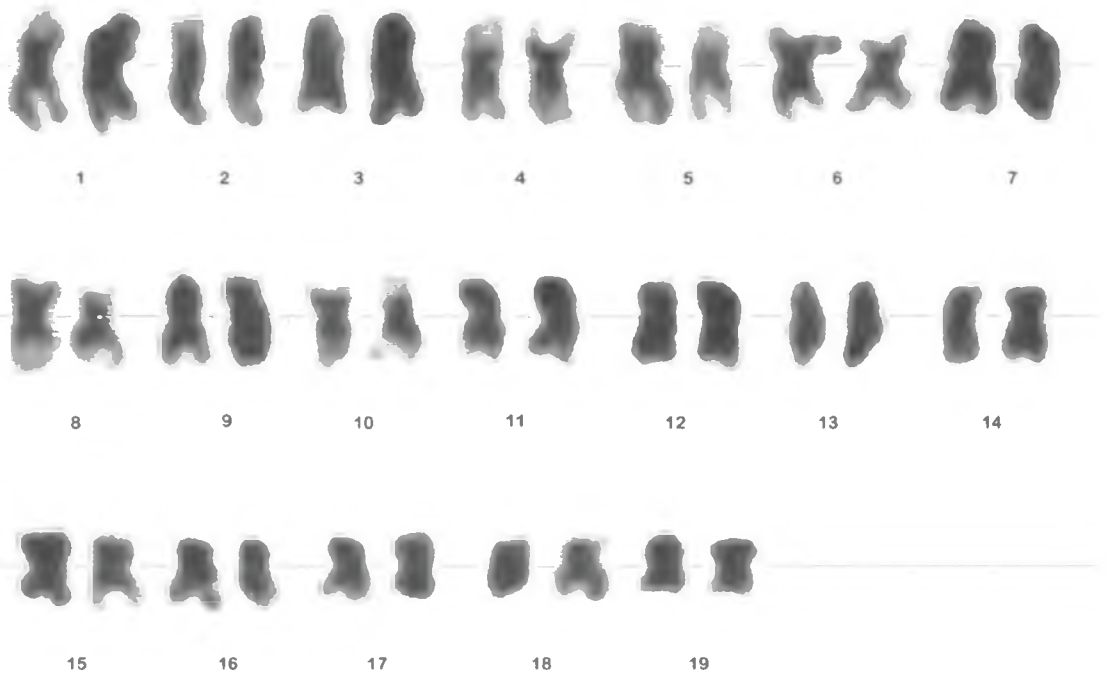
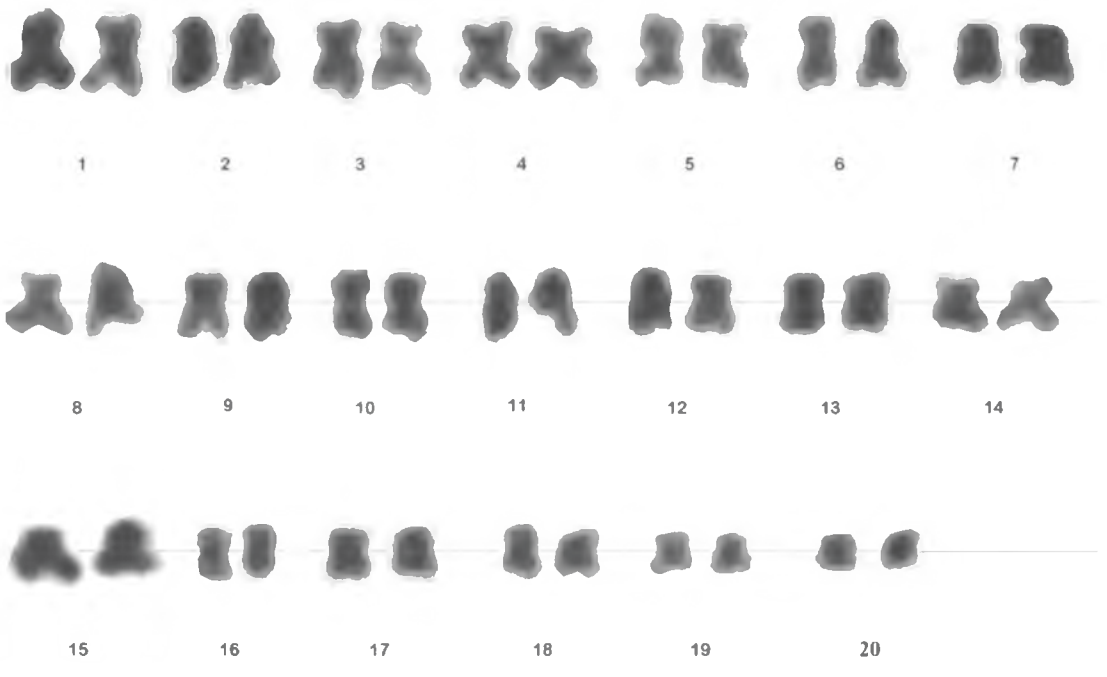

A
B
C
D
E
F

Figure 7. Comparison of the chromosome complements of the 38-chromosome and 40-chromosome cytotypes. Prometaphase chromosomes from accessions AZ 65 (2n = 38) and DC33 (2n = 40) are arranged by length. The chromosomes of DC33 are more highly contracted than the chromosomes of AZ 65. Since some of the longer pairs of prometaphase chromosomes will become differentially more condensed than others, this arrangement does not in all cases allow direct comparison between homologous pairs of the two accessions as numbered here. Based on observations from many cells, as well the lengths and arm ratios of these chromosomes, it is thought the smaller chromosomes, in the third row of each arrangement, are comparable as numbered. The 2n = 38 accessions lack the smallest pair of homologues of the 2n = 40 accessions. Although many chromosomes can be recognized at certain stages in favorable cell preparations, reliable karyotypes will require additional information.



AZ 65

10 µm



DC33

10 µm

Exclusive bivalent formation has been observed at diakinesis in ten 38-chromosome male accessions, most from Great Plains collections, but including accession C56 from Idaho. More limited observations of meiosis have been made from the three available flowering 40-chromosome male accessions, 9032964, 9032708, and C12. Accession C12 also carries two B chromosomes. Meiosis in these accessions is not always regular. At diakinesis, 19 bivalents and 2 univalents are commonly seen in accessions 9032964 and 9032708. In some cells at diakinesis, the univalents are very close to each other, but usually do not appear to be engaged by even one chiasma. Fewer cells appear to have 20 bivalents at diakinesis. Similar figures are observed in accession C12, but interpretations are frequently complicated by the B chromosomes, which are sometimes paired and sometimes not. In a few cells, 20 bivalents thought to be of the A-set, and 2 univalents, presumably the B chromosomes, have been observed. Occasionally in such cells, the unpaired B chromosomes seem to be associated with (or at least lying near) other bivalents. In so far as diplotene or pachytene figures can be interpreted, it appears that all chromosomes form bivalent associations in these three accessions. The univalents, then, appear to arise from precocious separation at diakinesis rather than from asynaptic chromosomes. At metaphase I, one or two univalents frequently are not at the plate with the rest of the chromosomes. Lagging chromosomes are observed as the reduction division proceeds. By late anaphase, laggards are observed that could be variously included in one or another daughter cell, or excluded from either daughter cell (Table 3). Figure 8 illustrates representative pairing at meiosis I in 38- and 40-chromosome cytotypes. Figure 9 shows the irregular univalent behavior in 2n = 40 accessions.

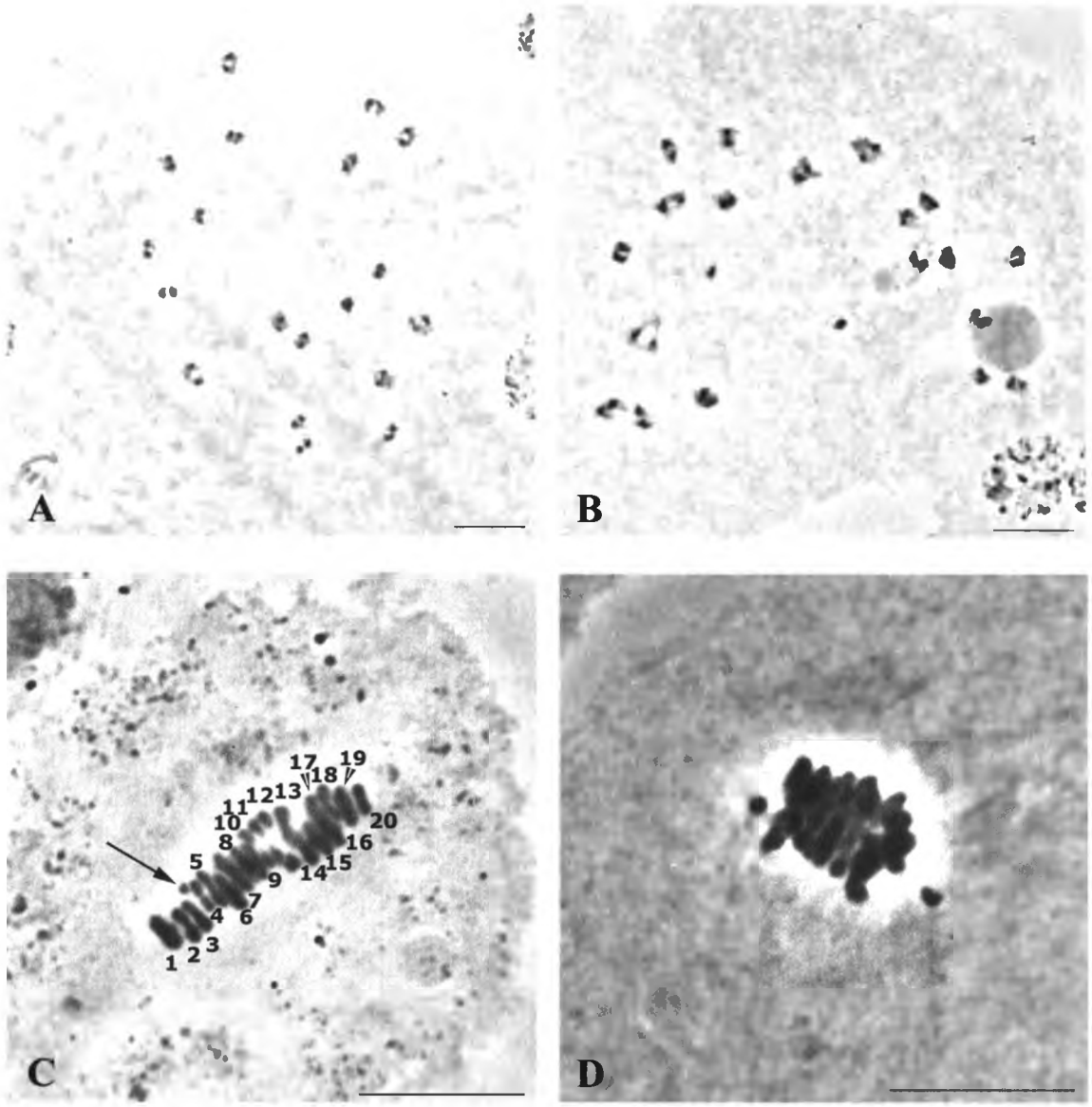


Figure 8. Chromosome pairing in 38- and 40-chromosome cytotypes. All cells observed at diakinesis in 2n = 38 accessions have 19 bivalents (A, accession C56). At diakinesis in 40-chromosome cytotypes, however, 19 bivalents and 2 univalents are observed in many cells (B, accession 9032708) as well as 20 bivalents. At metaphase I in 2n = 40 accessions, chromosomes arrive at the metaphase plate paired (C), or with univalents (D). The univalents frequently do not arrive at the metaphase plate with the bivalents, and one or two may be seen variously distributed in the cytoplasm. Photograph C, of accession C12 with 2n = 40 + 2B chromosomes, is numbered to illustrate the number of bivalents believed to have been present before the smaller pairs separated as the cell enters anaphase. The arrow indicates one unpaired B chromosome. The other B chromosome is not fully visible, but is thought to be located under bivalent 1. The behavior of the B chromosomes during meiosis is not well understood. Scale bars represent 10 μm.

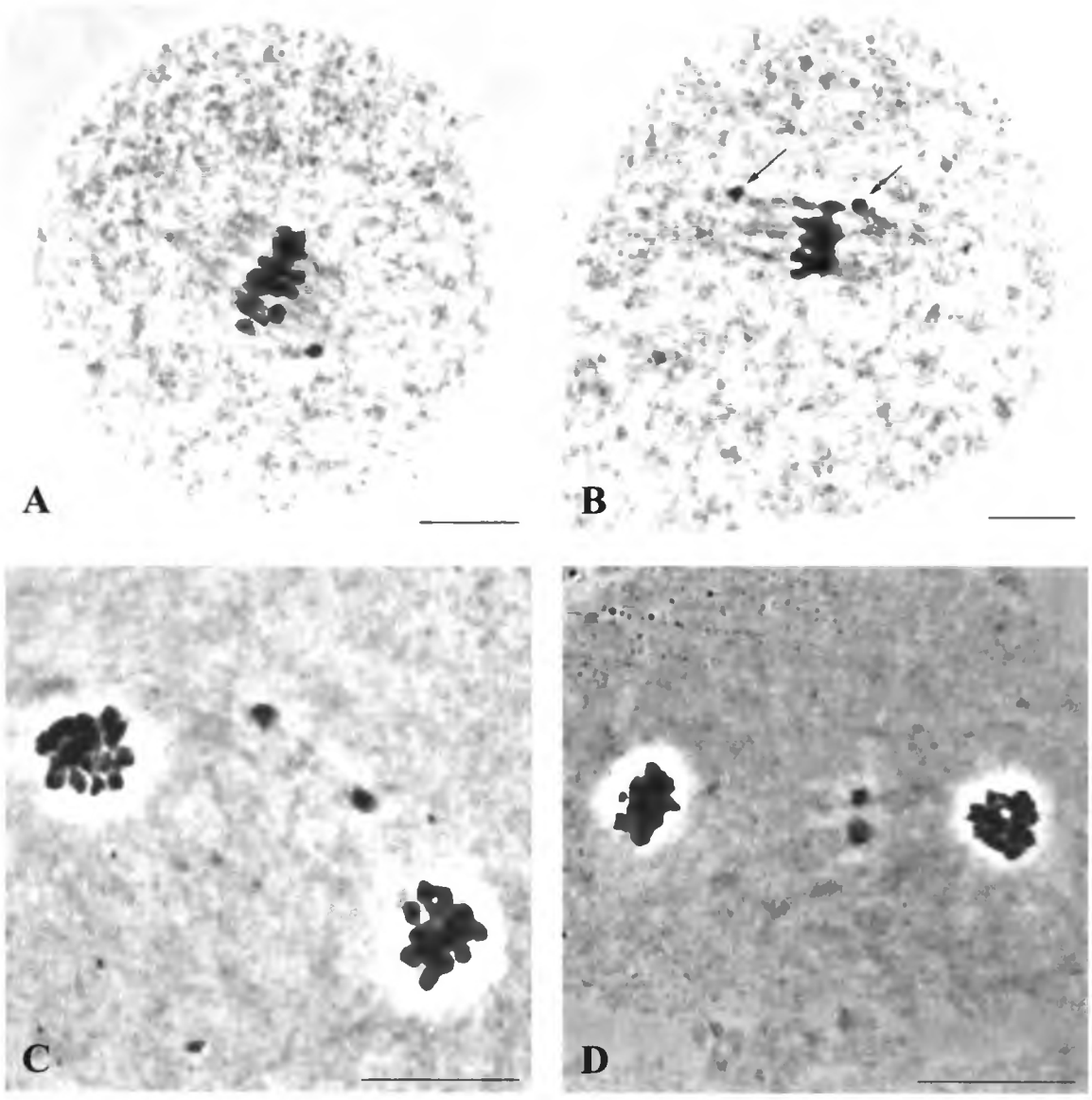


Figure 9. Early and late anaphase I in 2n = 40 accessions. Univalents are often observed either not at the metaphase plate with the bivalents at the start of anaphase I (A, B), or lagging at late anaphase I (C, D), suggesting irregular assortment and ultimately the possibility of irregular gamete formation. Meiosis II has not been observed with adequate clarity to estimate the frequency of irregular assortment or to understand if additional irregularities occur. Scale bars represent 10 μm.

The sum of the meiotic data provides additional evidence that one pair of homologues is missing from the 38-chromosome plants compared to 40-chromosome plants. In each cytotype, 38 chromosomes form bivalents that proceed through the first division normally. The 40-chromosome plants have an extra pair of presumably homologous chromosomes that exhibit irregular behavior that could result in unbalanced gametes. An essentially diploid mode of inheritance is expected in both cytotypes, other than the irregularities resulting from the occasional odd assortment of chromosome 20. Neither the number of chromosomes in cells at the end of meiosis II, nor the regularity of the pollen divisions have been determined. Limited pollen fertility studies suggest higher levels of inviable pollen in the few 40-chromosome plants studied compared to 38-chromosome plants, but the levels do not seem significant enough to define a major limit to fertility.

Table 3. Percent of cells dividing in meiosis I with either 1or 2 univalents not at the metaphase plate (early anaphase) or with lagging chromosomes (late anaphase) that appear likely to be excluded from one or both daughter cells. Total n = 393 observations.

	% of cells	
Accession	**Early anaphase I**	**Late anaphase I**
9032708	36	7
9032694	51	10
C12	40	27

B chromosomes are dispensable, morphologically different, usually smaller chromosomes that are mostly non-homologous to the regular chromosome sets from which they presumably derive (Appels et al, 1998; Beukeboom, 1994; Rees and Jones,

1977). B chromosomes vary in occurrence among populations within species and in number among individuals of populations (Rees and Jones, 1977). They have been reported in about 15% of all cytologically described species (Beukeboom, 1994) and in somewhat over 6% (about 200 species) of characterized grass species (Hunziker and Stebbins, 1986). The effect of B chromosomes apparently varies by species, but it has been proposed that in some cases they may regulate genetic variability in populations by affecting chromosome pairing (Hunziker and Stebbins, 1986; Rees and Jones, 1977). The saltgrass B chromosomes (Figure 6) are on average about 70% the size of the smallest homologues of the 40-chromosome plants, depending on the stage at which the measurements are made. They typically are more condensed earlier in the progression to metaphase than the other chromosomes, making it difficult to identify centromeres. There may be small differences in size among some of the B chromosomes, but their very small size and differences in cell preparations make this difficult to characterize with the methods used here. Because of the size difference between the B chromosomes and the smallest chromosomes of the A set, they are readily detected in chromosome preparations at some stages that are perfectly flat, allowing measurements that are not distorted excessively by chromosomes lying in different focal planes. One, two, four, and seven B chromosomes have been found in different plants. They occur in individuals of all three cytotypes. Among all accessions, they have been observed at a frequency of about 7%. The frequency of B chromosomes among individuals from each of the three population samples grown from seed is higher than that observed in the vegetatively collected plants. The meiotic behavior is still largely unknown, but the B chromosomes sometimes appear to be paired and sometimes exist as univalents at meiosis I. It is not known if there is an

accumulation mechanism operating. A number of crosses with a 2n = 40 + 2B male accession and several different 38-chromosome plants have been made, but detailed studies of B transmission have not yet begun.

Higher Polyploid Variation

Saltgrass plants with from 72 to 77 chromosomes (Figures 10 and 11) have been identified among about 18% of the accessions from many localities. Plants thought to have 74 chromosomes are more common. These higher order polyploids are neither as rare nor limited in distribution as previously described by other researchers. Unlike Reeder's prior description of mostly bivalent pairing in a 72-chromosome plant (Reeder, 1977), nine multivalent associations at diakinesis involving 34 chromosomes are consistently observed in one 74-chromosome accession (Figure 12), and similar multivalent frequencies have been observed in two other 74-chromosome accessions. That these cytotypes are probably derived from autopolyploids of the tetraploids seems likely based on the multivalent pairing observed at diakinesis. The exact chromosome complements of individuals with from 72 to 77 chromosomes have not been determined. The interesting question of how they may vary among individuals both within and among regions remains unanswered at present.

Fertility in the 8x cytotypes has not been studied in detail, but estimates of pollen viability for two ca. 74-chromosome accessions using the MTT method were 86% for accession AZ 104 and 97% for accession P3, similar to that of 38- or 40-chromosome accessions tested. The MTT method may overestimate the number of pollen grains that, although they are metabolically active, can ultimately produce viable zygotes.

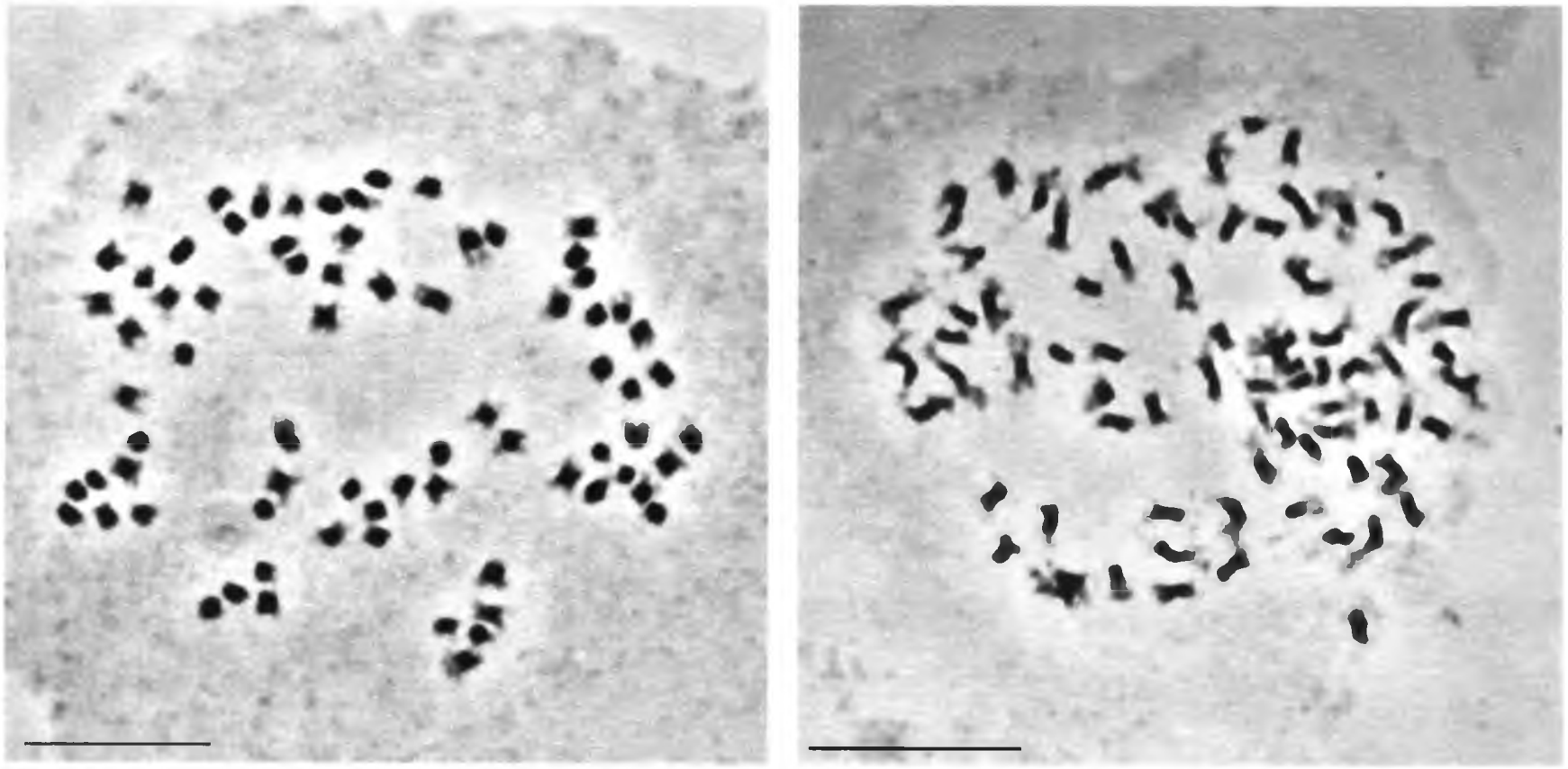


Figure 10. Metaphase root tip chromosomes of octaploid saltgrass aneuploids. Photograph (A) is of the 74-chromosome accession AZ 103 collected near Longmont, Colorado, and (B) is of the 77-chromosome accession AZ d7 collected near Yuma, Arizona. Scale bars represent 10 µm.

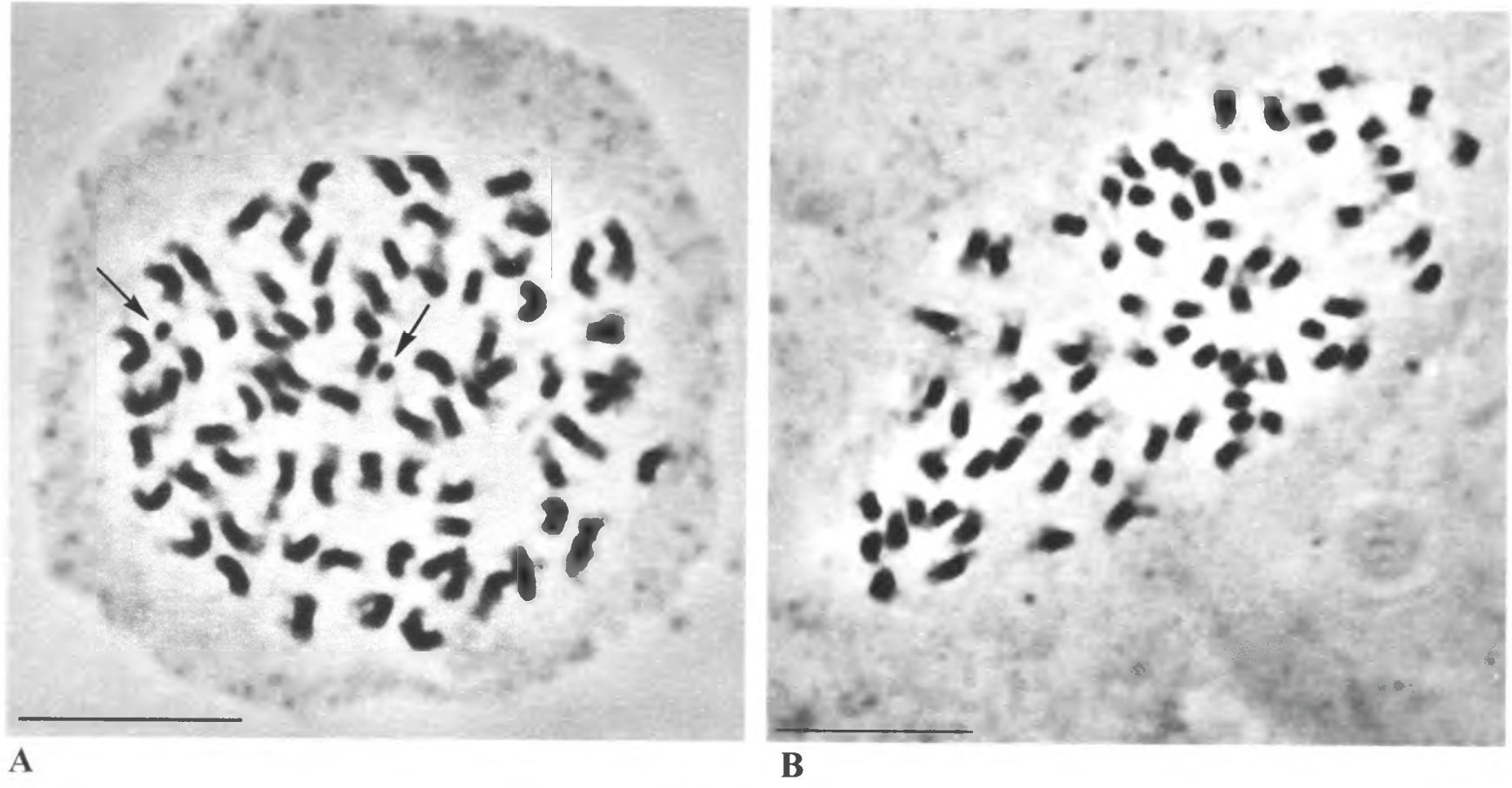


Figure 11. Additional aneuploid variations of octaploid saltgrass. Photograph (A) is of the 74 + 2B-chromosome accession AZ 2 collected in Denver, Colorado, and (B) is of the 73-chromosome accession P-RCR collected near Pueblo, Colorado. Arrows indicate B chromosomes in AZ 2. Scale bars represent 10 µm.

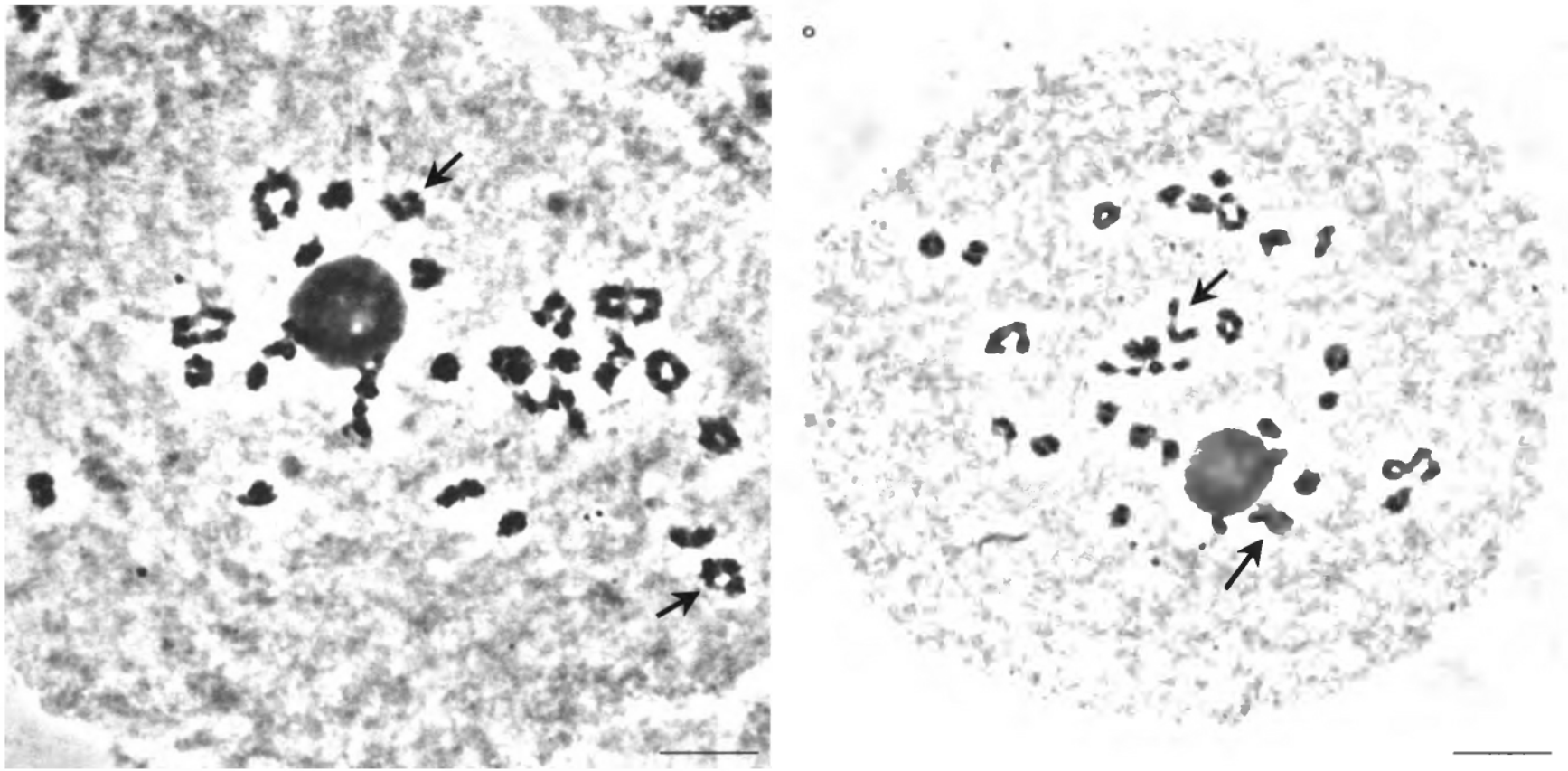


Figure. 12. Diakinesis in the 74-chromosome accession AZ 104. Both cells are interpreted as having 7 IV, 2 III, and 20 II. Arrows indicate trivalents. Scale bars represent 10 μm.

One 56-chromosome plant (Figure 13), collected in Denver County, Colorado, is probably derived from hybridization between a 38-chromosome plant and a ca. 74-chromosome plant or from the combination of a reduced and an unreduced gamete from among plants in the 38-chromosome population. This is the first report of a ploidy level, essentially 6x, in saltgrass other than 4x or 8x. The 6x cytotype replaces the 8x cytotype as the one currently considered as being rare.

It seems reasonable to hypothesize that the 72- to 77-chromosome saltgrass cytotypes are aneuploid derivatives of autopolyploids that may have originated multiple times, perhaps from within both 40-chromosome populations and 38-chromosome populations. Levin (2001) has recently suggested that as an extension of the now commonly accepted theory that polyploid taxa may have multiple, independent origins, there is no reason to reject the idea that even races may arise independently more than once. This could occur in single steps (Ramsey and Schemske, 1998) through either fusion of two unreduced gametes or some form of somatic doubling. No evidence, however, currently exists to support this hypothesis other than that offered by the shared distributions and the understanding that this is a method of autopolyploid formation apparently capable of operating in other species (Ramsey and Schemske, 1998). This mode of origin agrees with Reeder's assessment that the 72-chromosome cytotypes are reduced from 8x forms (Reeder, 1977), which seems especially valid in light of the discovery that many have chromosome numbers closer to 8x than 7x. Plants have been found with 76 chromosomes, the possible "perfect" result of autopolyploidization without chromosome loss or gain from 38-chromosome plants.

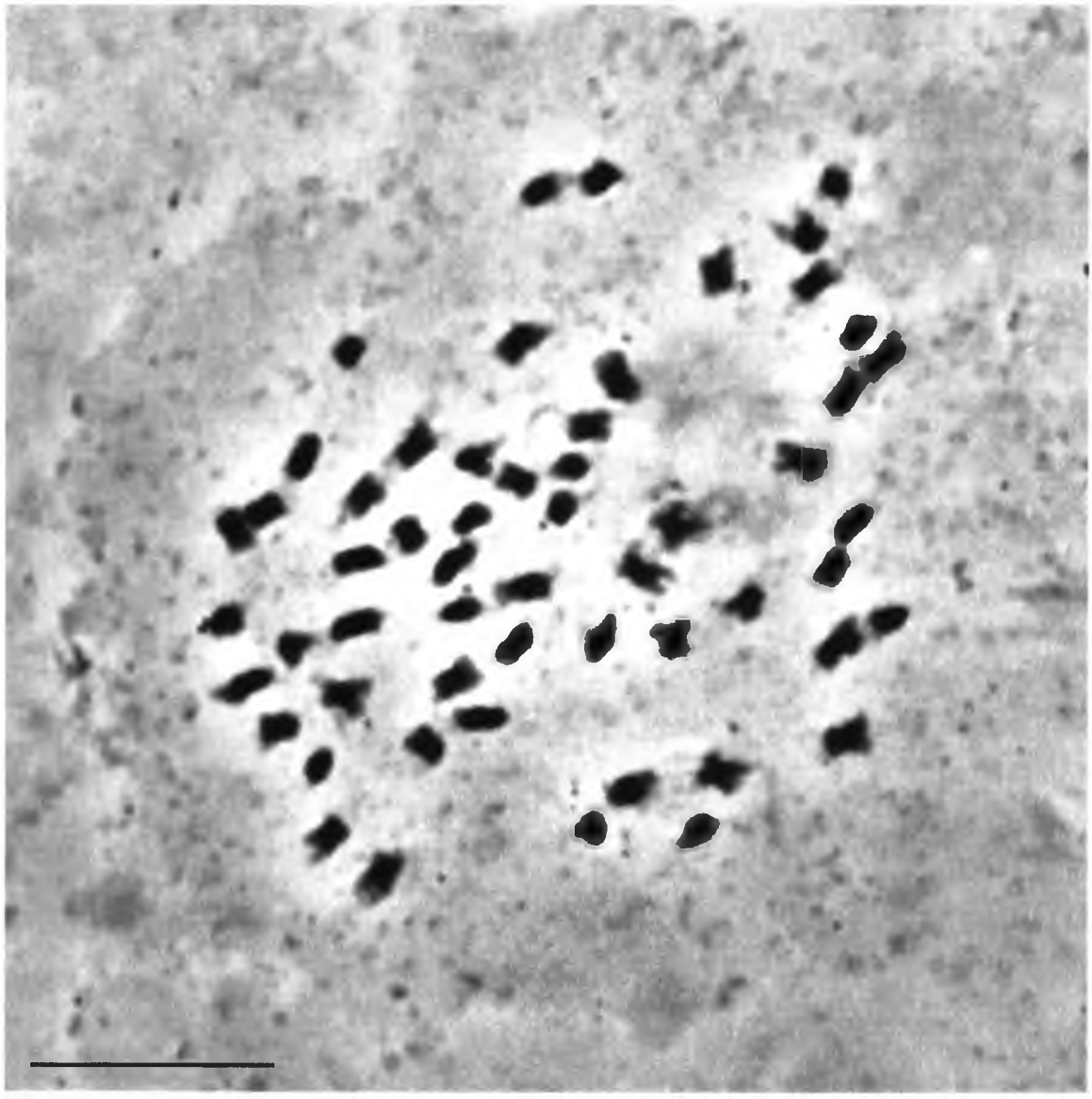


Figure 13. Accession AZ 27, collected from Denver, Colorado has 2n = 56 chromosomes. This accession represents the only known ploidy variation in *Distichlis spicata* other than cytotypes of 4x or 8x levels. Probably derived from either the combination of a normal gamete from a 38-chromosome plant and a gamete from an aneuploid 8x plant, or a normal and an unreduced gamete from within the 38-chromosome saltgrass population with which it occurs, it is essentially 6x. Scale bar represents 10 µm.

These plants have been found, however, either in regions where 2n = 40 plants would be expected or in regions where limited sampling would make it hazardous to predict the likely cytotype of the progenitors.

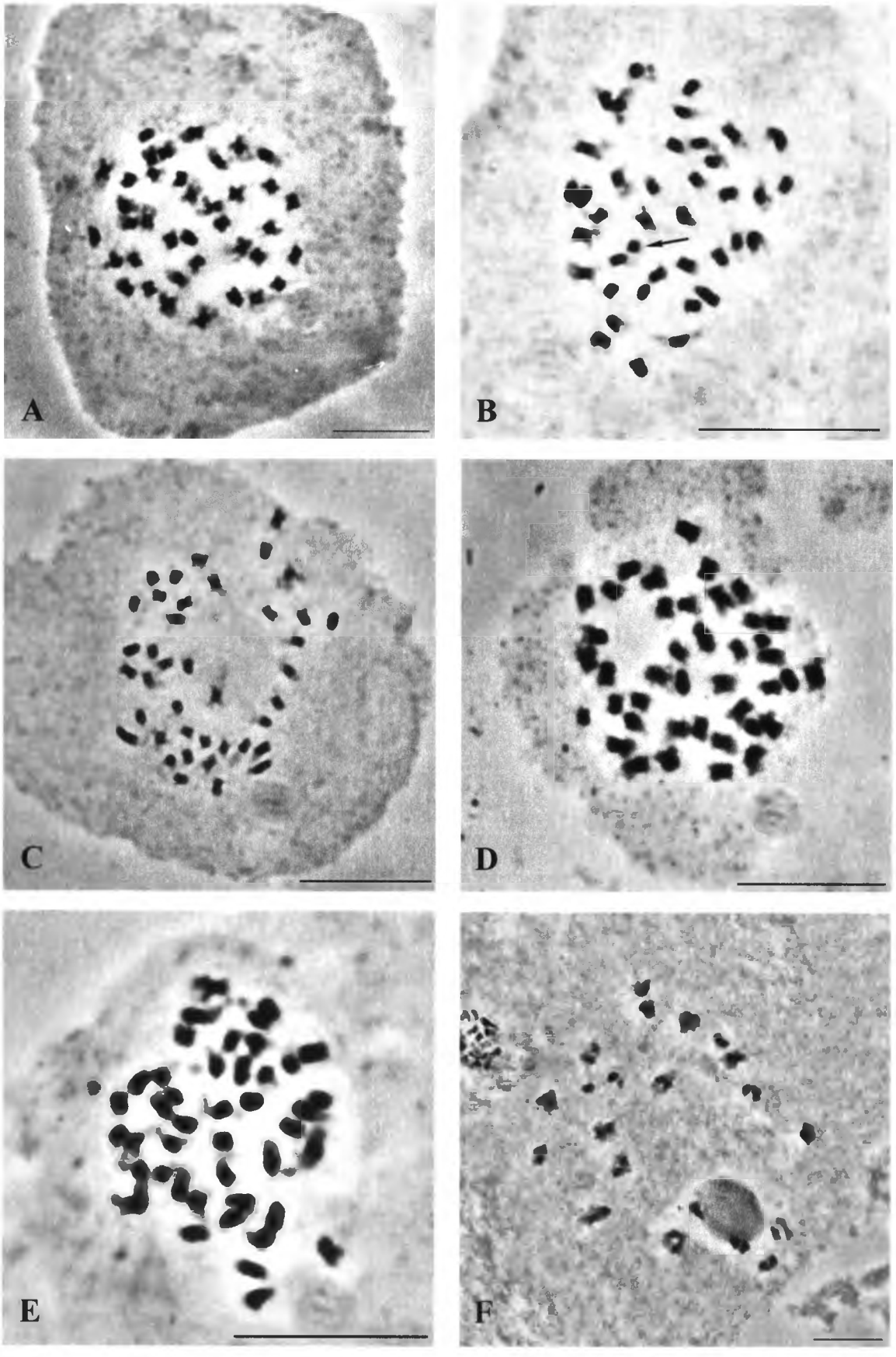
Perhaps an even more interesting question is whether tetraploid saltgrass arose multiple times, both as a 38-chromosome type and a 40-chromosome type under different evolutionary circumstances. The simplest alternative to this, for which at least a possible mechanism could be proposed to exist, is that of aneuploid reduction from 2n = 40 to 2n = 38 made favorable (or possible) by factors such as differential gene silencing, chromosomal rearrangements, chance gene combinations, or mutations.

Chromosome Variation Among 38- x 40-chromosome Progeny

Crosses were made among available 38- and 40-chromosome accessions in an attempt to better understand why 38-chromosome plants are so common, but within collections where 40-chromosome plants occur, or among the 12 accessions studied from a 40-chromosome population, no 2n - 1 or 2n - 2 plants have been found. Based on the observation of exclusive bivalent pairing in meiosis of the 2n = 38 accessions, and the meiotic irregularities sometimes seen in 2n = 40 accessions, it was predicted that plants with 38, 39, or 40 chromosomes could be possible among the progeny of crosses. This assumes the gamete from the 38-chromosome parent is always n = 19, and the possible production of n = 19, 20, or 21 gametes from the 40-chromosome parent, based solely on observed irregularities. If additional irregularities in meiosis occur just among the univalents already sorting irregularly, such as non-disjunction in meiosis II from a cell containing both univalents, it would be possible to add n = 22 gametes to the list that

could be produced from the 2n = 40 plants. Individual progeny from these crosses do have 38, 39, or 40 chromosomes as predicted, along with individuals with 41 chromosomes and some plants that are aneusomatic (Figure 14), often with 42 or 43 chromosomes representing the majority cell type of those from a single root tip (Table 4). Aneusomaty is a phenomenon not seen among any of the collections. Mechanistically, aneusomaty is thought to occur through somatic non-disjunction, precocious chromatid separations, or other mitotic irregularities involving chromosome aggregation or spindle-associated abnormalities (D'Amato, 1995). Further, it may or may not be restricted to different tissues or developmental stages (D'Amato, 1995). Aneusomaty has been reported in many different taxa (D'Amato, 1995), often those with complex backgrounds involving hybridizations such as sugarcane (Tlaskal et al., 1970), or bluegrass (Speckmann and Van Dijk, 1972). The study of extensive aneusomaty in *Clatonia virginica* populations (Lewis, 1962) in part led Lewis to proclaim that "constancy of chromosome number within all individuals is merely a convenient fiction" (Lewis et al., 1971). While it is true that exceptions do exist, in general, constant chromosome numbers are a remarkably stable biological phenomenon (Appels et al, 1998; Swanson et al., 1981). Since aneusomaty is implicated, and the exact karyotype of each of the progeny has not been determined, a more intensive investigation needs to be completed before the nature of transmission of the extra chromosomes from 40-chromosome cytotypes can be known with certainty. Some of the progeny that appear to have stable numbers may have been under the influence of, and then stabilized from, whatever process results in the aneusomaty still observed in others, and thus may not reflect the gametes passed from the 40-chromosome parent.

Figure 14. Different chromosome numbers in progeny of 2n = 38 x 2n = 40 crosses. Progeny have been observed with 38 chromosomes (A), 39 chromosomes (B), 40 chromosomes (C), and 41 chromosomes (D). Additionally, some plants believed to be aneusomatic have as many as 43 (E) or 44 chromosomes in some or the majority of root tip cells. Accessions represented by photographs (A), (B), and (C) appear to have stable chromosome numbers and probably represent the products of irregular univalent segregation observed in the 40-chromosome plants combining with normal gametes from the 38-chromosome plants. The accession shown in (D) may be aneusomatic. Photograph (F) shows diakinesis in a 40-chromosome progeny of a 38 x 40 cross. Like the 40-chromosome male parent, this accession forms 19 bivalents and 2 univalents or 20 bivalents at diakinesis. Scale bars represent 10 μm.


A
B
C
D
E
F

Tlaskal et al. (1970) and Roach and Tlaskal (1970), after describing the difficulties of determining the chromosome number in sugarcane, a difficult subject because of numerous, small chromosomes and aneusomatic clones, have developed an elaborate set of protocols for the purpose of making accurate chromosome assessments in aneusomatic plants (Tlaskal et al., 1971; Tlaskal and Hutchinson, 1974). This detailed approach was not applied here; rather, the same standards for determining chromosome number were used as for the accessions with no apparent variability. Several additional factors can complicate the study of aneusomatic plants. Aneusomaty in some plants can remain fairly stable, even from generation to generation (Zhou et al., 1992), but some aneusomatic plants develop mosaics of tissues with different, but stable, chromosome numbers (D'Amato, 1995). Diplontic selection may occur in diploid or diploidized tetraploid aneusomatic individuals, which results in uniform, normal sporogenous tissue and subsequent normal gamete formation (D'Amato, 1995). This is an area where further study would be appropriate.

Most of the better cell preparations available suggest that the expected karyotypes do exist in some of the 38-, 39-, and 40-chromosome progeny, and that indeed it is the smallest chromosomes of the 2n = 40 plants that exhibit irregular behavior in meiosis and are missing from the 38-chromosome karyotype. Meiosis in one male 40-chromosome progeny of the hybrids has been studied, and in most of the cells at diakinesis there are 19 bivalents and 2 univalents, figures identical to those seen in the 40-chromosome parent.

Speculating with these results, it seems that aneuploid gametes deficient for chromosome 20 from the 40-chromosome plants can be viable, at least when expressed against the genetic background of the 38-chromosome plants. If different genetic

backgrounds can compensate for a lack of aneuploid fitness, for example, via differentially silenced genes from different ancestral genomes, this could explain why monosomics and nullisomics appear in progeny of the hybrids between the cytotypes but none have been found in nature among 40-chromosome collections as yet.

Table 4. Chromosome numbers in progeny of 38- x 38- and 38- x 40-chromosome crosses. The chromosome number of the majority of counted cells is listed for plants believed to be aneusomatic. Chromosome counts in cells of individual aneusomatic plants varied from 38 to 44.

	2n Chromosome Number		
Accession	**Female Parent**	**Male Parent**	**Progeny**
138x136-1	38	38	38
138x136-2	38	38	38
138x136-8	38	38	38
138x136-11	38	38	38
138x111-5	38	38	38
138x111-12	38	38	38
138x111-15	38	38	38
40x127-2	38	38	38
40x127-6	38	38	38
138x708-10	38	40	43, aneusomatic
138x708-11	38	40	41, aneusomatic
126x12-1	38	40 + 2B	41*
126x12-2	38	40 + 2B	40 + 1B
126x12-3	38	40 + 2B	39 + 2B
126x12-4	38	40 + 2B	41*
126x12-5	38	40 + 2B	40 + 1B
11x12-10	38	40 + 2B	39 + 2B
18x12-6	38	40 + 2B	39
66x12-3	38	40 + 2B	41*
138x708-1	38	40	38
138x694-3	38	40	40
138x708-7	38	40	40
138x694-1	38	40	40
138x708-12	38	40	39
138x694-5	38	40	39
138x694-7	38	40	41, aneusomatic
138x708-2	38	40	43, aneusomatic
138x694-2	38	40	39, aneusomatic
66x694-5	38	40	40
66x694-1	38	40	41, aneusomatic
66x694-5	38	40	43, aneusomatic
M3x708-8	38	40	39
M3x708-12	38	40	41, aneusomatic

*Accessions where B chromosomes cannot be differentiated with certainty from the other small chromosomes.

Gametes with two copies of chromosome 20 may effectively be a kind of insurance in the 40-chromosome cytotype, buffering the inviability that might otherwise be greater. Trisomics and tetrasomics may account for nearly 12% of the otherwise 40-chromosome plants in this study, subject to some misidentification due to B chromosomes as discussed earlier.

The occurrence of aneusomaty in some progeny of the 38- and 40-chromosome saltgrass hybrids is a phenomenon similar to that sometimes observed in wide crosses among other species (D'Amato, 1995), and may further reflect the divergence of the 38- and 40-chromosome cytotypes. Progeny sampled from crosses made among 38-chromosome plants all have 38 chromosomes, and while this additional sample is small, together with the data from the collections it suggests meiosis in the 38-chromosome plants is indeed highly regular.

Distribution of Plants Varying for Chromosome Number

Prior to this report, the known occurrences of 38-chromosome plants were so rare compared to 2n = 40 plants they were considered simply as aneuploids (Reeder, 1977). In this study, 2n = 38 accessions are in the majority, and no plants that can be identified as either monosomic or nullisomic have been found regionally distributed among 2n = 40 plants. While the much larger sample size of this study partly explains the increased detection of different cytotypes, and variation due to B chromosomes is recognized for the first time, most of the disparity between this and the prior reports is almost certainly

explained by the extensive inclusion of accessions from previously unsampled regions where 2n = 38 saltgrass predominates.

Among the three commonly occurring cytotypes, two different patterns of geographic distribution exist. The 38- and 40-chromosome plants are regionally separated, at least in the Great Plains distribution of the 2n = 38 cytotype from the broad southwestern U.S. distribution of the 2n = 40 cytotype, and there is evidence of a second regional 2n = 38 distribution. In contrast, the higher order polyploids are represented in all regions sampled, and occur in the same habitats as either the 2n = 38 or 2n = 40 cytotypes in several of the more extensively sampled localities. It has been proposed that in general, different polyploid cytotypes of taxa tend to occupy different regions as a reflection of differential adaptation (De Wet, 1986). Examples of this have been described for *Tripsacum* (De Wet, 1986), *Paspalum* (Norrmann et al., 1989), and *Cynodon* (Silva and Snaydon, 1995). It has been shown, however, that this is not always the case. Different ploidy levels are not always characterized by different regional distributions in *Buchloë dactyloides* (Johnson et al., 1998), *Andropogon gerardii* (Norrmann et al., 1997; Keeler, 1992), or *Panicum virgatum* (Hulquist et al., 1997). In some of these cases, habitat specialization may occur within regions, or no apparent pattern to the distribution is observed.

The region sampled in this study covers just a part of the saltgrass range in the U.S., and there are far too many sampling gaps to confidently propose distribution boundaries. The distribution of cytotypes seen here could be part of a much more complex pattern. However, the Rocky Mountains through Colorado appear to be a barrier on the west for the 2n = 38 saltgrass that may extend eastward for some

considerable distance. Additional Great Plains collections are needed to confirm that the 2n = 38 cytotype may occur continuously (and perhaps exclusively of the 2n =40 cytotype) from the Colorado plains to the eastern-most collections in Kansas and Nebraska. Northern and southern boundaries of this eastern distribution of the 38-chromosome cytotype are unknown. The one report of 2n = 40 from Canada (Bowden, 1960) and the two from southwest Texas (Reeder, 1977; Reeder, 1971) are at present the only other published reports of saltgrass chromosome numbers from east of the Rocky Mountains. Along the northern boundary of the collections west of the Rocky Mountains, the two 2n = 38 populations from northeastern California and western Nevada suggest another regional distribution that may extend eastward, possibly to the Idaho collection, located along the western and northern transitional zones of the upper Great Basin. Reeder's (1977) collections from the Wyoming Basin suggest several possibilities. Both 38- and 40-chromosome plants were reported in Albany County (the precise collection locations are unknown), and could be a point of contact, either a transitional or hybrid zone. Only a more detailed study of the area could resolve the question of whether Reeder's 38-chromosome collections are legitimate aneuploids among other 2n=40 plants in a population, perhaps in a transition zone, or if the saltgrass of the region fits into the current model of distribution. The Wyoming Basin also could be a bridge in the range of the 2n = 38 saltgrass through the Rocky Mountains between the geographically-near Western High Plains region to the east, along the northern frontier of the current collections to the 2n = 38 occurrence in Idaho and onward to the Modoc and C66 populations at the northwestern edge of the Central Basin. Again, more collections are needed in these areas.

It is not possible to reliably predict the frequency at which the higher order polyploids occur among the other cytotypes, in total or within any specific region, from the data gathered in this study. As Norrmann et al. (1997) have pointed out, there are "complex biases" associated with sampling grasses for this kind of determination, and the bulk of the accessions used here were collected for other purposes that did not even attempt to address those concerns. Therefore, the apparently greater proportion of the higher order polyploids, for one example, from central Nevada south through Arizona and east to the Rocky Mountains compared to regions either to the east or west, should be regarded cautiously. Reeder's (1977) report adds two 2n = 40 plants to the list that were collected in southern Nevada from an area where multiple accessions with ca. 74 chromosomes were collected for this study. With the exceptions noted earlier, all previous U.S. chromosome number reports for saltgrass support the distribution patterns observed in this study.

The 38- and 40-chromosome cytotypes in our collections generally are somewhat distinct in appearance. The differences are primarily due to varying canopy architectures resulting from generally more upright leaves in 38-chromosome plants versus leaves declining to as much as 90 degrees from the culm in many 40-chromosome plants (Figure 15). Progeny from crosses of 38-chromosome plants from Colorado and 40-chromosome plants from the Central Valley of California strongly resemble the 40-chromosome parent for this habit. A complete morphometric analysis might better define some consistent differences. These traits are variable and overlapping, however, like so many of the other characters that have been used to define species or varieties within the *Distichlis spicata* complex. It is thought the different appearances may also be rather strongly affected by

environment and phenology. Plants from the 40-chromosome Granite population actually resemble the accessions from near Lincoln, Nebraska, more in gross morphology than any plants from southern California or Arizona. Even within cytotypes, then, highly variable populations occur. The aneuploids of the 8x plants, when grown in the greenhouse, are generally larger, but otherwise are very similar in appearance to other 38- or 40-chromosome plants from regions of shared distribution. They are very difficult to discriminate in nature due to environmental effects, similar to the kind of ecophenic plasticity described by Seliskar (1985) for saltgrass in different environmental extremes of a salt marsh habitat. Because of the current lack of consistent, well-defined morphological differences of even a qualitative nature among the three cytotypes (Stuessy, 1990), it remains difficult to support varieties within the species even considering the new cytological information. Following Stuessy (1990), it may be useful taxonomically to distinguish the 38- and 40-chromosome saltgrass cytotypes as two cytoraces, and the higher order polyploids as cytoforms, based on the major geographic correlation of the former case and the lack of geographic correlation in the latter case.

Summary

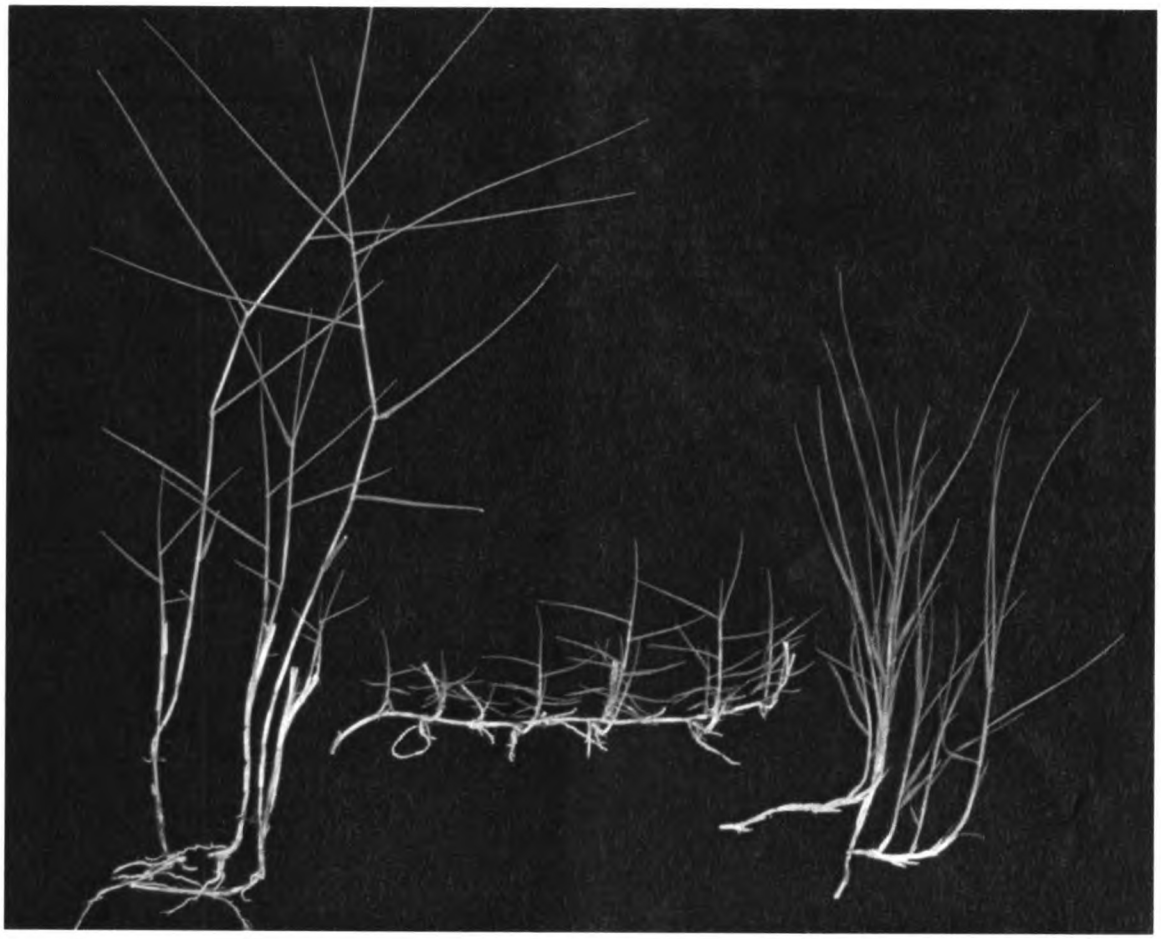
A 38-chromosome saltgrass race with distributions separate from the previously known 40-chromosome type has been identified. Both races are fertile and hybridize readily. The distribution pattern and the cytological behavior of hybrids between the races suggest they are adaptively different. No aneuploidy in the 38-chromosome race has been found, and no deficient aneuploids have been found in the 40-chromosome race. Meiotic irregularities in the 40-chromosome race suggest a pathway for the evolution of

the 38-chromosome race, depending on hypothesized genetic changes that allow fitness in individuals lacking the pair of homologues defining the difference between the races. More research is needed to evaluate broad but variable morphological differences between the races. The consequences of intermating the two cytoraces in breeding efforts are unknown, but karyotypic instability may result in some cases.

The octaploid cytoform, previously considered rare, has been found to occur more commonly, distributed among both of the cytoraces. One accession, believed to be a 6x cytotype, represents a ploidy level previously undescribed in saltgrass. Screening to identify individuals of different ploidy levels among collections made for the breeding program is advisable. Extensive, variable aneuploidy and multivalent associations in meiosis suggest the higher-order polyploid cytoforms may reproduce sexually with less efficiency, but this has not been adequately studied. Two ca. 74-chromosome accessions showed high pollen viability based on color development during MTT reduction reactions.

B chromosomes are reported for the first time in saltgrass, occurring in some individuals of all three cytotypes.

Figure 15. Saltgrass plants with 38 or 40 chromosomes. The plant at right in each photograph has 2n = 38 chromosomes, those on the left have 2n =40 chromosomes. Although saltgrass from different localities is quite variable in appearance and some exceptions have been observed, the different plant habits are remarkably well associated with cytotype among the accessions collected for this study. The large plant at left in the bottom photograph is the 40-chromosome accession from the Gulf coast of Florida, the accession in the center was collected in Fresno, California, and the accession on the right was collected in Denver, Colorado.



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