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The ploidy races of *Atriplex confertifolia* (Chenopodiaceae)

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THE PLOIDY RACES OF *ATRIPLEX CONFERTIFOLIA* (CHENOPODIACEAE)

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ABSTRACT.—Previous accounts of polyploidy in the North American salt desert shrub *Atriplex confertifolia* (shadscale) have dealt with the distribution of polyploidy and the morphological and secondary chemical differences between races. The present study amplifies these studies and reveals additional ploidy-flavonoid races, with ploidy levels known to extend from 2x to 12x, and all except 2x and 12x represented by races with and without 6-methoxylation of flavonol compounds. Results of this study show that diploids across their range have about 113% as much DNA per genome as do polyploids and that parallel variation in monoploid genome size between diploids and accompanying polyploids can be shown in different parts of the species' range. Polyploidy, therefore, appears to have developed independently in several areas of the western United States. Hexaploids are generally not as common as octoploids in shadscale, which could be an indication of diploidization of older tetraploid races.

RESUMEN.—Informes anteriores sobre la poliploidía en el arbusto *Atriplex confertifolia* ("shadscale") del desierto de sal norteamericano, han tratado la distribución de la poliploidía, las diferencias químicas secundarias y las diferencias morfológicas entre razas. El presente estudio amplía los estudios previos y revela razas ploidía-flavonoides adicionales, con niveles de ploidía que se sabe que van de 2x a 12x, y todos, con excepción de 2x y 12x, están representados en razas con y sin 6-metoxilación de compuestos de flavonoles. Durante el estudio descubrí que los diploides a lo largo de su rango de distribución tienen alrededor del 113% más ADN por genoma que los polploides. También descubrí que la variación paralela en el tamaño del genoma monoploide entre los diploides y sus poliploides correspondientes, se puede manifestar en diversas partes del rango de distribución de la especie. La poliploidía, por lo tanto, parece haberse desarrollado de forma independiente en varias regiones del occidente de los EE.UU. Los hexaploides son generalmente menos comunes que lo octoploides en el shadscale, lo cual podría ser indicio de diploidización de razas tetraploides más antiguas.

A remarkable development of polyploid races has been reported in *Atriplex canescens* (Pursh) Nutt. (fourwing saltbush) and *Atriplex confertifolia* (Torr. & Frém.) S. Watson (shadscale), the most important salt desert shrubs of the genus *Atriplex* (Chenopodiaceae) in western North America. Fourwing saltbush, used for reclamation in North America (McArthur and Young 1999) and the Mediterranean and Middle East (Le Houérou 1992), has a race that is 20x, in addition to all even ploidies from 2x to 14x (Stutz et al. 1975, Stutz and Sanderson 1979, Dunford 1984, 1985, Senock et al. 1991, Sanderson and Stutz 1994, 2001, Glenn et al. 1996, 1998, Glenn and Brown 1998, Ruas et al. 2001, Sanderson and McArthur 2004). Shadscale, the subject of this report, is widespread and often dominant in the western United States. (Dayton 1937, Billings 1949, Welsh et al. 2003). However, it is used infrequently in reclamation because of unfavorable seed dormancy characteristics (Meyer et al. 1998, Garvin and Meyer 2003). Shadscale contains races of even ploidies from 2x to 10x (Stutz and Sanderson 1983, Sanderson et al. 1990), and a 12x race is reported herein.

In both species, extensive geographic races characterized by presence or absence of flavonol 6-methoxylation are known for most ploidy levels (Sanderson and Stutz 1983, 2001, Sanderson et al. 1988, 1990). Flavonoid biosynthesis proceeds by lengthening of the carbon chain of the phenylpropanoid compound 4-hydroxy coumarate by the addition of 3 acetyl-CoA units followed by their cyclization (Ebel and Hahlbrock 1982, Winkel-Shirley 2001). Because of this origin, hydroxyl groups normally occur at the 5 and 7 positions of flavone and flavonol molecules. However, in order for oxygenation to occur at the 8 position, or at the 6 position as in this case, the presence of a specific enzyme is necessary (Anzellotti and Ibrahim 2004, Halbwirth et al. 2004).

This report provides additional findings on the distribution of ploidy-flavonoid races in *A. confertifolia* through studies facilitated by the availability of flow cytometry for rapid ploidy measurement. During the study, evidence was

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obtained for a decrease in genome size between diploids and polyploids in this species. Loss of DNA has often been reported in hybridization and allopolyploidization (Leitch and Bennett 2004, Leitch et al. 2008, Tate et al. 2009), but this is an example of its association with apparent autoploidy.

METHODS

Ploidy Determinations

Meiotic chromosome counts were made using acetocarmine squash methods as employed previously for *Atriplex* and other taxa (Sanderson et al. 1990, 1999).

Flow cytometry was carried out using a Partec PAII ploidy analyzer using DAPI (4′, 6-diamidino-2-phenylindole) dye solutions provided by the Partec GmbH company. Their reagent CyStain® UV Ploidy one-step staining solution was used for *Atriplex* because it gave peaks whose position was relatively stable in the presence of interfering secondary chemicals. Leaf samples of 0.5–1 cm^2 were chopped in plastic petri dishes using double-edged razor blades. One sample was chopped with each end of a cutting edge, for a total of 4 samples per blade. Bladder hairs of the leaf are rich in salts (Osmond et al. 1980) and apparently secondary chemicals as well. Improved peaks were usually obtained by wet rubbing each side of the leaf on a fine, abrasive surface to partially remove the indument layer.

DNA content per genome in *A. confertifolia* polyploids is near to, though slightly higher than that in *A. canescens,* and so diploid and tetraploid individuals of *A. canescens* growing on the laboratory grounds were used as reference standards and appropriate corrections were made. Results obtained using the diploid and tetraploid standard plants did not differ significantly from each other.

The 2C nuclear DNA content of a population of diploid *A. canescens,* race Gigantea, has been measured at 1.575 pg, and one, belonging to the tetraploid race Occidentalis, at 3.048 pg (David J. Walker personal communication). Before it was found that there was a sizable difference in per-genome DNA content between diploids and polyploids in the species, flow cytometry standards were mainly run externally for diploid and tetraploid samples but internally for one or more plants per location to provide greater precision for higher ploidies. Later in the study, internal standards were used for diploids and tetraploids as well.

A statistical analysis of relative DNA amounts was carried out using Proc GLM of SAS and the LSD option for comparison of means.

Flavonoid Chromatography

Acid-hydrolyzed flavonoid aglycones were chromatographed on 18.5 × 46-cm half-sheets of thick chromatography paper, as previously described (Sanderson et al. 1999). Compounds that have been reported in shadscale and other North American *Atriplex* species (Sanderson et al. 1988) were identified on chromatograms when examined over longwave ultraviolet (UV) light (i.e., black light), before and after the sheets were dipped in 2% aqueous aluminum chloride and redried (Sanderson et al. 1999). In the very early years of data collection, material from several plants per population was combined in composite samples, but this practice was later changed to examination of 3 separate plants per location. Because of the consistency of flavonoid patterns observed, samples were taken for 30% of the populations examined for ploidy, with a greater emphasis on areas where contrasting flavonoid races were adjacent.

RESULTS

Per-Genome DNA Content of Diploids

Throughout their range, by my measurements, diploids of *A. confertifolia* have a relative per-genome DNA content of about 1.16 times more than that of the *A. canescens* standards (Table 1). Polyploids and diploids showed parallel variation across regions, which agrees with flavonoid evidence below in suggesting local origin of polyploidy in these locations. However, relative per-genome DNA amounts were not significantly different among different ploidy levels in polyploids, nor was the interaction of DNA amount with region. More detailed studies of genome size might be made using propidium iodide as the flow cytometry staining agent (Doležel and Bartoš 2005). This reagent is favorable because the wavelength for analysis falls outside of the general absorbance range of phenolics.

Cold Tolerance in Diploids

Only diploids were found in eastern Montana and adjacent areas of the northern Great Plains (Table 2), which suggests they have a greater

TABLE 1. Apparent relative per-genome DNA content for representative populations of diploids and polyploids of *Atriplex confertifolia*. DNA content is by comparison with my *A. canescens* standards, which have approximately 0.75 pg of DNA per monoploid genome. The Bonneville and Lahontan basins are located in the eastern and western Great Basin, respectively.

	Sites	Mean relative DNA content	SD
By region (diploids)			
Lahontan Basin and Oregon	12	1.1767 a	0.0266
Mojave Desert Borders	5	1.1698 ab	0.0293
Missouri River Basin	12	1.1688 a	0.0230
Bonneville Basin	4	1.1605 ab	0.0181
Colorado Plateau	5	1.1401 bc	0.0199
Mojave Desert	4	1.1256 c	0.0204
By region (polyploids)			
Lahontan Basin and Oregon	77	1.0461 a	0.0209
Mojave Desert Borders	109	1.0361 b	0.0185
Colorado Plateau	33	1.0352 b	0.0198
Bonneville Basin	27	1.0348 bc	0.0166
Mojave Desert	77	1.0256 c	0.0222
All locations			
A. parryi	5	1.2352 a	0.0184
All *A. confertifolia* diploids	42	1.1629 b	0.0279
All *A. confertifolia* polyploids	324	1.0358 c	0.0211

TABLE 2. Number of examined *Atriplex confertifolia* locations of different ploidies in successively southwestward areas of the data set. Numerical proportions for the 4x and 8x major cytotypes are conservative because distributions of the less common 2x, 6x, 10x, and 12x cytotypes were explored in greater detail.

Area	2x	4x	6x	8x	10x	12x
Montana and North Dakota	18	0	0	0	0	0
Wyoming	15	26	0	0	0	0
Colorado Plateau	38	211	41	0	0	0
Great Basin and connected areas	91	354	27	312	43	9
Other: south Arizona, east Colorado, Texas	0	12	0	0	0	0
Total populations (1197)	162	603	68	312	43	9

tolerance for cold. In most areas where both are present, diploids are also found at higher elevations than polyploids (Stutz and Sanderson 1983). *Chamaenerion angustifolium* (Mosquin 1967, Husband and Sabara 2003) and *Castilleja* (Heckard and Chuang 1977, Matthews and Lavin 1998) are examples of other species in which diploids are surmised to be more cold tolerant. The opposite pattern has been observed in some taxa (Soltis and Soltis 1989, Borgen and Hultgård 2003).

Flavonoids of *Atriplex confertifolia*

As reported (Sanderson and Stutz 1983, Sanderson et al. 1988, 1990), the more abundant compounds (Fig. 1) found in *A. confertifolia* were simple flavonols (fluorescent yellow with $AlCl_3$ under black light) and 6-methoxylated flavonols (olive green under the same conditions). Other, less abundant compounds, such as 3-methoxy flavonols and flavones such as the compound tricin, were observed occasionally. Geographic races differing in presence or absence of flavonol 6-methoxylation (Figs. 2–6) are referred to, hereafter, as being of negative and positive (6-methoxy flavonol) chemotype.

Similar to the pattern in several North American perennial *Atriplex* species (Sanderson and Stutz 1983, Sanderson et al. 1990, unpublished data), diploids of shadscale do not show evidence of flavonol 6-methoxylation at the level of my assay conditions (Table 3), although many of the polyploid races evidently derived from them do. This may suggest a change in gene regulation with polyploidy (Galitski et al. 1999, Osborn et al. 2003).

As shown in Table 3, status for production of 6-methoxy flavonols according to ploidy varied across regions of the western United States but was consistent within regions.

Of 379 populations sampled, 251 were represented by 2 or more separate samples. There were few discordant results in flavonoid type among samples from the same population,

TABLE 3. Flavonoid chemotype of races in apparent centers of ploidy development in shadscale. Chemotype refers to presence (Pos) or absence (Neg) of the faculty for 6-methoxylation of flavonol compounds. All diploids showed a 6-methoxyflavonol negative flavonoid pattern.

Geographic area	2x	4x	6x	8x	10x	12x
Colorado Plateau (eastern Utah and the Four Corners region)	Neg	Pos	Pos	—	—	—
Eastern Great Basin (the Bonneville Basin)	Neg	Pos	—	Pos	Pos	Pos
Southeastern Oregon and the northwestern Great Basin (including the main part of the Lahontan Basin)	Neg	Neg	—	Neg	Neg	—
Mojave borders, southwestern Great Basin (including southern parts of the Lahontan Basin)	Neg	Neg	Neg	Pos	Pos	—
Mojave Desert (southernmost Nevada and the High Desert of California)	Neg	Neg	—	Pos	—	—

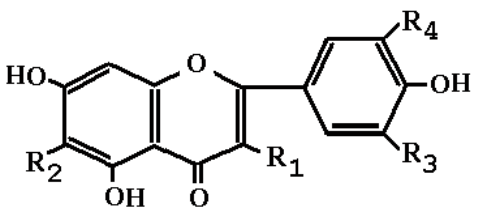

Fig. 1. Aglycone structures of flavonoids found in *Atriplex confertifolia.*

Common compounds:

Yellow Flavonols ($R_1 = OH$, $R_2 = H$): kaempferol (R_3, R_4 = H), quercetin (R_3 = H, R_4 = OH), isorhamnetin (R_3 = H, $R_4 = OCH_3$). Myricetin (R_3, R_4 = OH) usually also appears to be present.

6-Methoxy Flavonols ($R_1 = OH$, $R_2 = OCH_3$): 6-methoxy kaempferol (R_3, R_4 = H), patuletin (R_3 = H, R_4 = OH), spinacetin (R_3 = H, $R_4 = OCH_3$), and probably 6-methoxy myricetin (R_3, R_4 = OH).

Less common compounds:

Flavonol 3-methyl ethers ($R_1 = OCH_3$): 3-methyl ethers of the yellow flavonols, above.

Flavonol 3,6-methyl ethers ($R_1 = OCH_3$, $R_2 = OCH_3$): 3-methyl ethers of the 6-methoxy flavonols.

Flavones ($R_1 = H$, $R_2 = H$): tricin (R_3, $R_4 = OCH_3$) and apparently sometimes its precursors apigenin (R_3, R_4 = H), luteolin (R_3 = H, R_4 = OH), and chrysoeriol (R_3 = H, $R_4 = OCH_3$).

virtually all from locations near the contact zone between 8x-positive and 8x-negative races in western Nevada (Fig. 5). I interpret these as being due to interracial hybridization.

Newly Discovered Races

Reported here for the first time are (1) an additional group of positive chemotype hexaploid populations near the west end of Lake Powell in Utah and Arizona, (2) a negative type hexaploid race in southern Nevada, (3) a positive decaploid race in southern Nevada, and (4) a positive 12-ploid race in southwestern Utah (Figs. 3, 5).

Possible Introgression

The color and morphological aspects of shrubs in the southwestern portion of the *A. confertifolia* species range, compared to that in other regions, may suggest introgression into polyploid shadscale from *A. parryi,* probably its closest relative. I have observed the 1C-value of *A. parryi* to be approximately 19% greater than that of polyploid *A. confertifolia* but have not observed the expected increase of DNA content in suspected introgressants. However, the size of the *A. parryi* genome has some similarity to that of diploids of *A. confertifolia.* Perhaps if introgression does occur, there is also downsizing of the *A. parryi* genome.

DISCUSSION

Parallel geographic variation of genome size between polyploids and diploids, as noted above, suggests that the diploids dispersed and became differentiated in DNA amount before giving rise to existing polyploids. Separate areas of ploidy development may include the Colorado Plateau, the Bonneville Basin (eastern Great Basin), the Lahontan Basin (western Great Basin) plus adjacent southeastern Oregon, western and southern Nevada just north of the Mojave Desert, and the Mojave Desert proper, mostly located in southern Nevada and southeastern California. Flavonoid chemotype of races is mostly consistent within several of these areas (Table 3); for instance, all ploidies in the Lahontan Basin–Oregon area (2x to 10x) are 6-methoxy flavonol negative, whereas all except 2x in the Colorado Plateau (2x to 6x) and the Bonneville Basin (2x to 12x) are of positive type. The Mojave Desert (having 2x, 4x, and 8x) and Mojave Desert border areas (2x to 10x) have a mixture of chemotypes, with 2x to 6x ploidies being negative and 8x to 10x being positive. Even though the

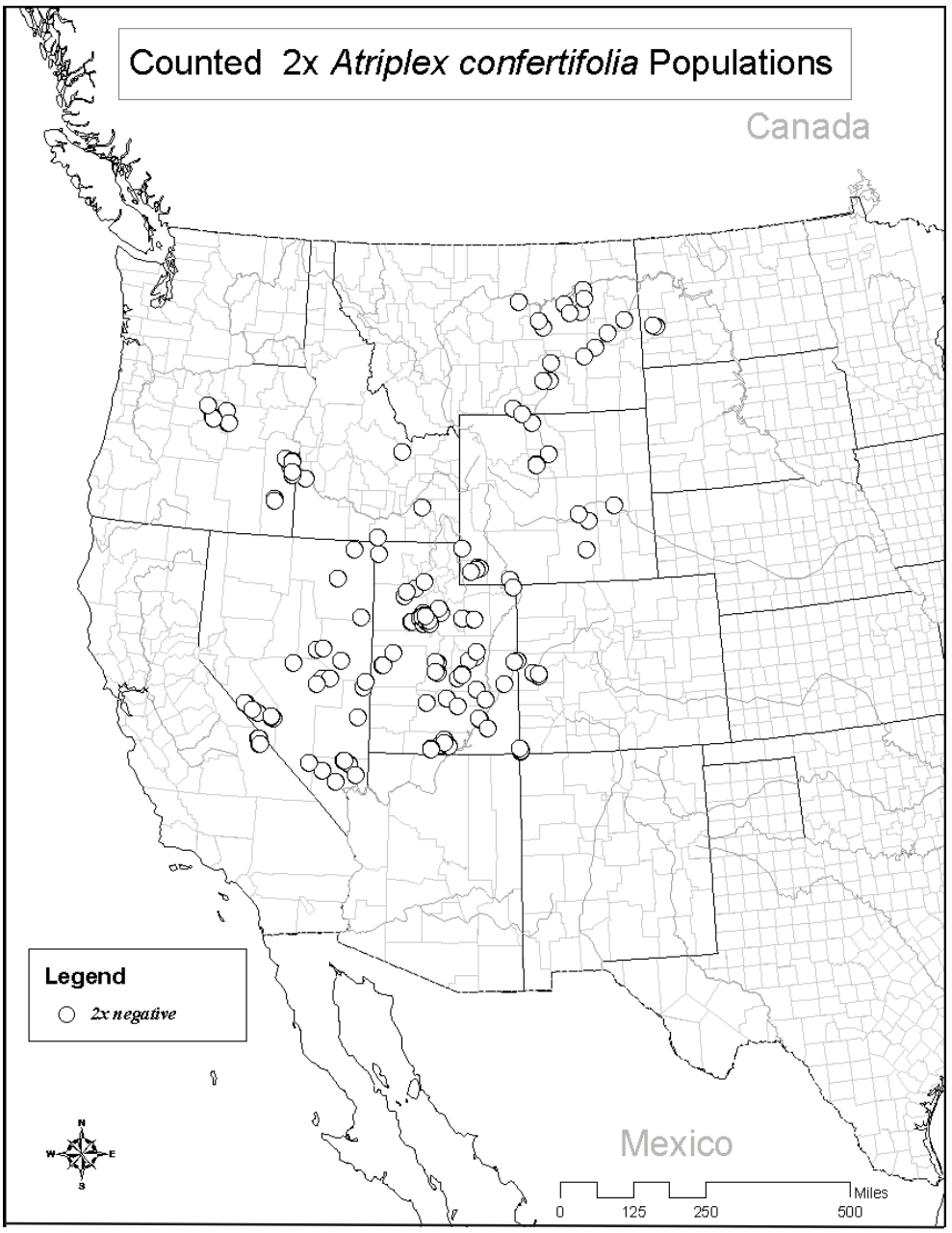

Fig. 2. Distribution of sampled diploid populations of *Atriplex confertifolia.* All populations were 6-methoxy flavonol negative (open circles).

latter 2 areas are adjacent, relative C-values do not suggest that plants of these areas are closely related, since diploids and polyploids of the Mojave have low genome-size values, but plants of the Mojave Desert borders have fairly high values (Table 1).

Races with similar ploidy but opposite flavonoid type are centered in different geographical areas and, for the most part, do not come into contact (Figs. 2–5); but, in cases where they do meet, placement of the contact zones suggests that the differences in flavonoid substitution

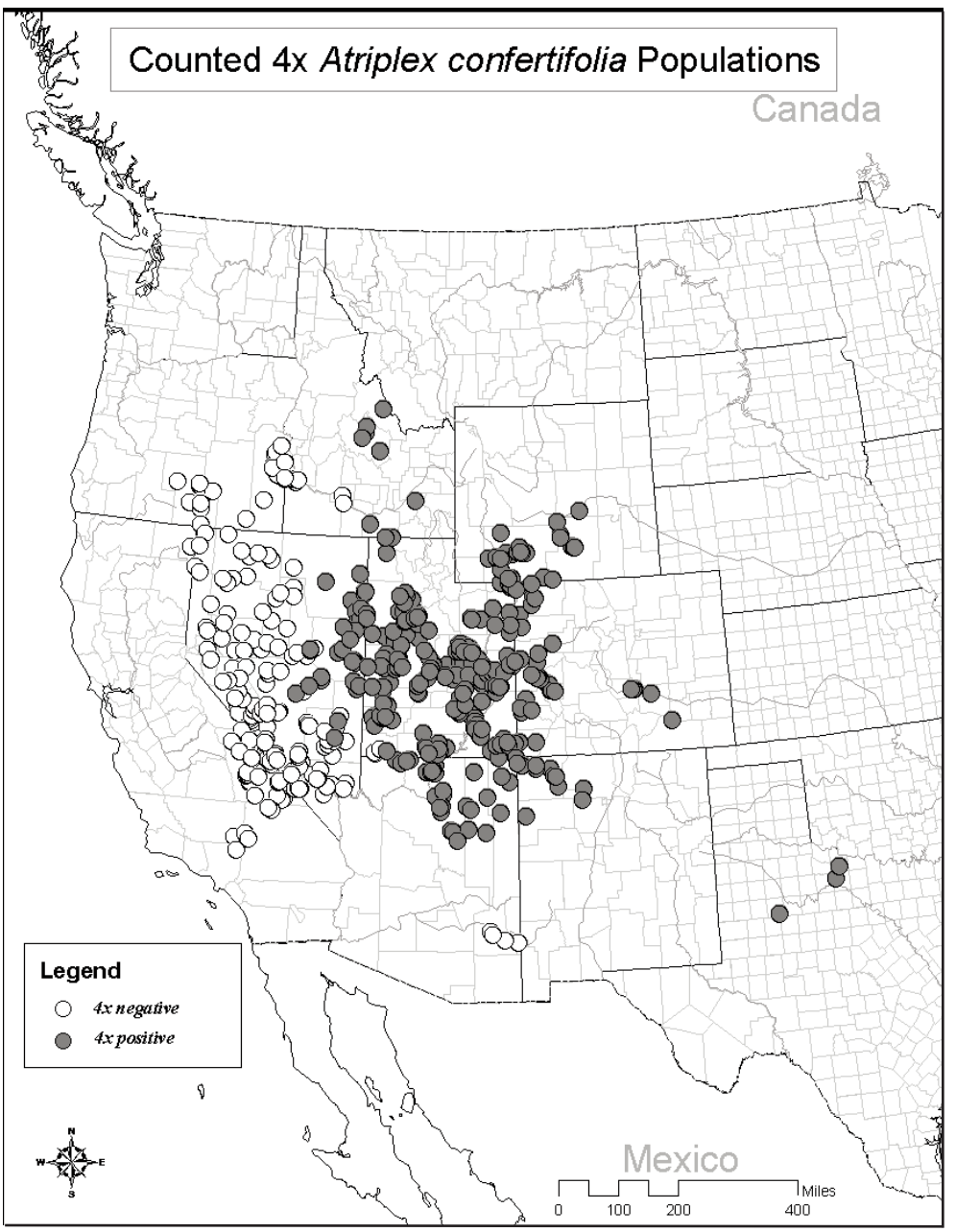

Fig. 3. Distribution of sampled tetraploid populations of *Atriplex confertifolia.* Dark circles represent those that were 6-methoxy flavonol positive; open circles represent those that were 6-methoxy flavonol negative.

are biologically significant. According to hybrid zone theory, boundaries between hybridizing taxa in a tension zone should move to areas of lowest frequency (Barton 1979, Barton and Hewitt 1985). Central Nevada is a higher-altitude region with few shadscale populations, and the boundary in the western Great Basin between the 8x positive race to the south and the 8x negative race to the north runs through it. While it is not obvious that the 6-methoxylation character itself is strongly selected, the location of the hybrid zone in this area is

Fig. 4. Distribution of sampled hexaploid populations of *Atriplex confertifolia.* Dark circles represent those that were 6-methoxy flavonol positive; open circles represent those that were 6-methoxy flavonol negative.

in accordance with the prediction of hybrid zone theory and suggests that either this or other genetic differences between the races do attain selective importance. The east and west boundary between chemotypes in the 4x is likewise associated in part with the central Nevada highlands.

A curious aspect of the distribution of the ploidy races is the relative scarcity of hexaploids (Fig. 4). Some explanations for this deficiency

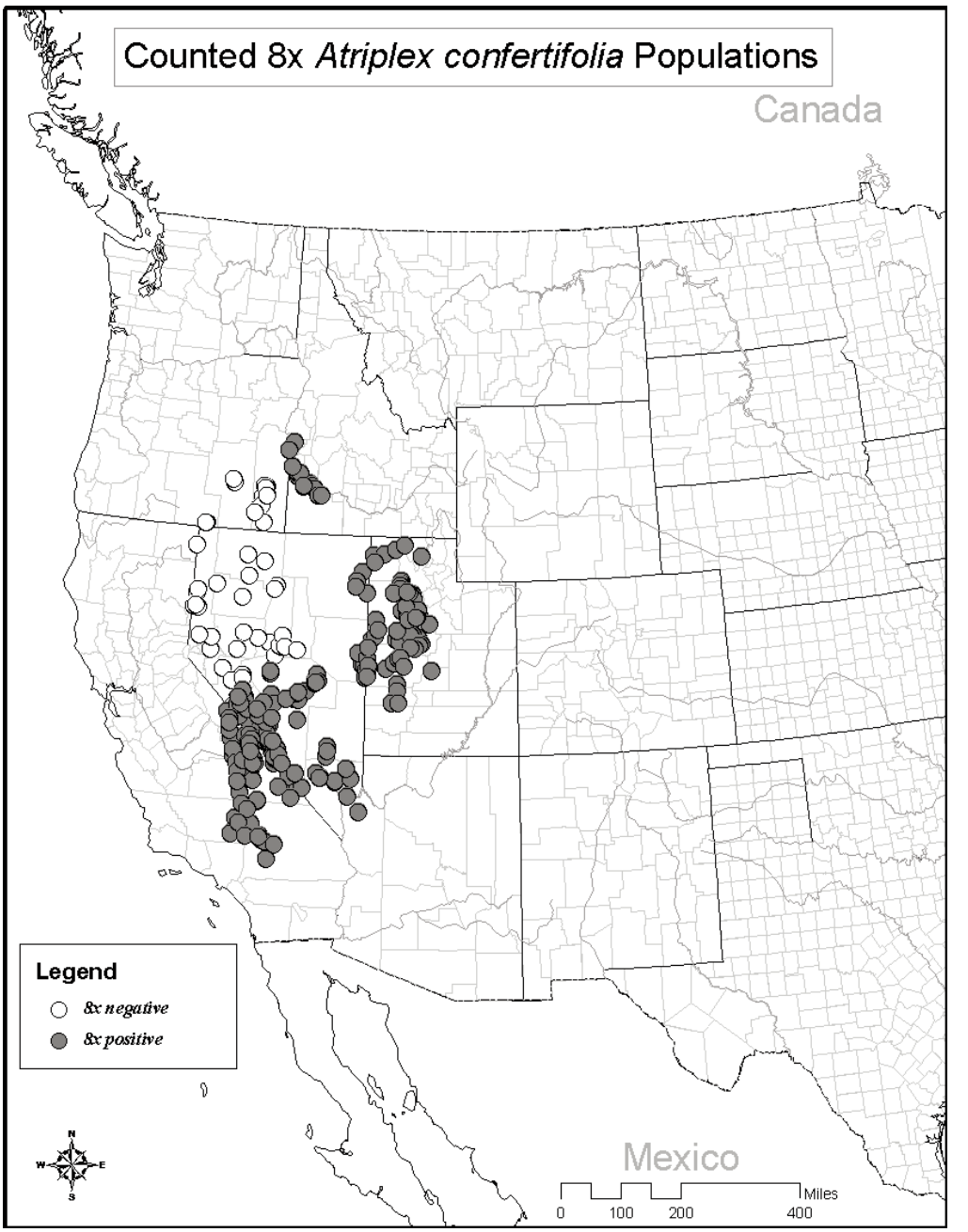

Fig. 5. Distribution of sampled octoploid populations of *Atriplex confertifolia.* Dark circles represent those that were 6-methoxy flavonol positive; open circles represent those that were 6-methoxy flavonol negative.

that might be suggested include polyploidization by means of simultaneous unreduced gametes, polyploidization by somatic doubling, and diploidization of some tetraploid races, causing hexaploid derivatives to suffer from fertility problems.

Since taxa with strong triploid block resulting from the endosperm balance mechanism are limited to polyploidization by simultaneous unreduced gametes (Ramsey and Schemske 1998, Carputo et al. 2003), this might seem a likely mechanism in *A. confertifolia* that would

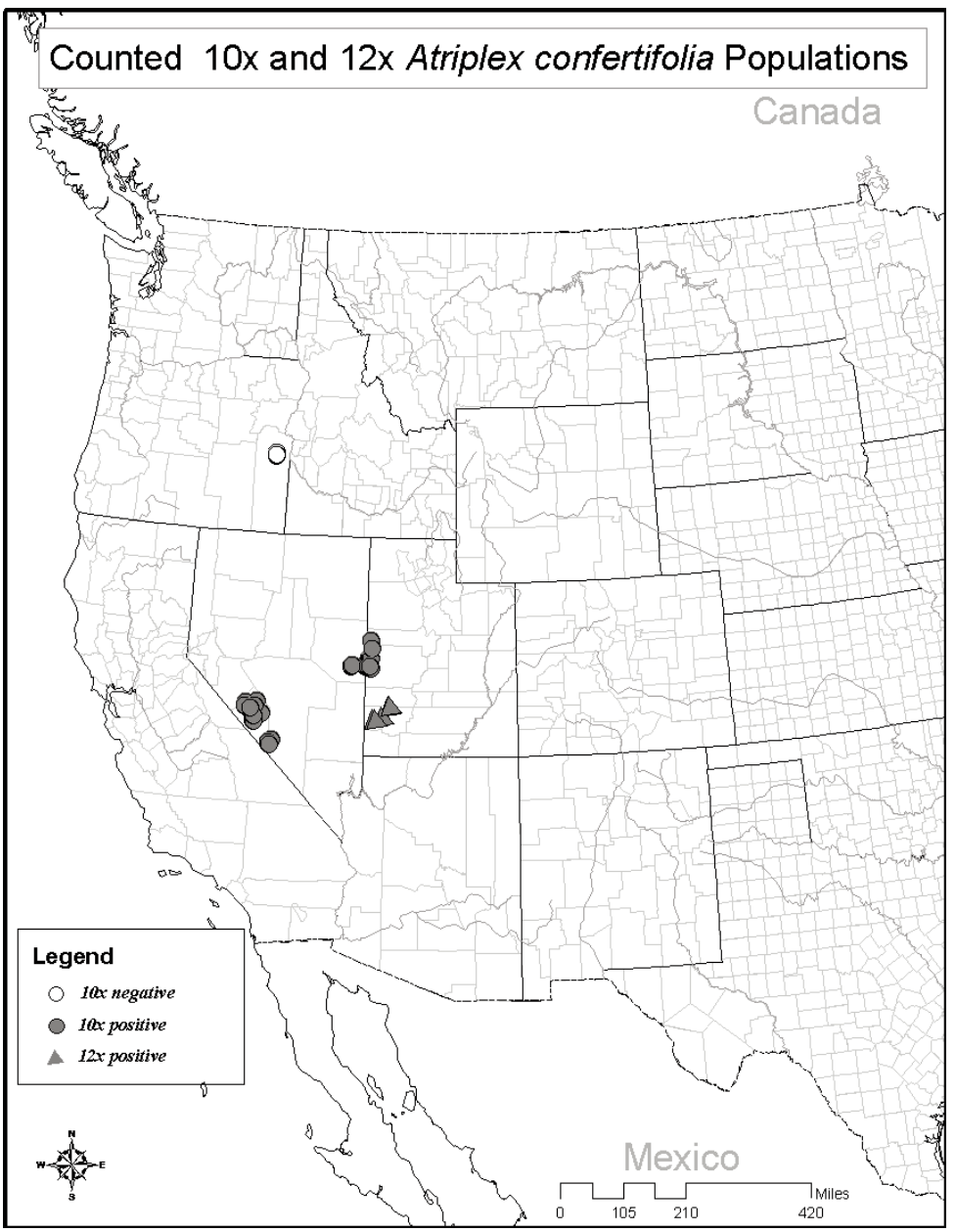

Fig. 6. Distribution of sampled decaploid and 12-ploid populations of *Atriplex confertifolia.* Dark circles represent those that were 6-methoxy flavonol positive; open circles represent those that were 6-methoxy flavonol negative.

lead to a greater frequency of octoploids than hexaploids. However, the Chenopodiaceae and related families have perisperm and very little endosperm (Batygina 2006), which would seem to rule out endosperm balance as an explanation (Ramsey and Schemske 1998). Furthermore, observations on the occurrence of neopolyploidy in nature for this species show, almost exclusively, the products expected from the action of single unreduced gametes (Sanderson in press).

Somatic doubling has been found in *A. tridentata* (Kuntze) H.M. Hall & Clem., but never in *A. confertifolia* or *A. canescens*, despite

observations at hundreds of sites. Therefore, if this process ever occurs in shadscale, it must be much less common than polyploidization by unreduced gametes.

With time, chromosomal changes, or genic changes, or the effects of hybridization can lead to diploidization in autopolyploids, so that functional differences between auto- and allopolyploids decrease (Ramsey and Schemske 2002). In the case of *A. confertifolia,* it seems likely that tetraploid races within the Great Basin may have become diploidized, which might result in triploid-like meiotic behavior in neohexaploid derivatives. As a result, the formation of hexaploid races would be deterred or prevented in this area.

In contrast, the Colorado Plateau has no octoploid shadscale race known, but hexaploid populations are rather frequent. This should indicate that the tetraploid race of that region is young and not diploidized. Ecological and geographic evidence for sensitivity of shadscale polyploids to cold has been mentioned previously. Polyploids in the Colorado Plateau might have been extirpated during colder intervals in the Pleistocene because of the lack of access to Pleistocene refugia of the kind that exist in the Great Basin, because the Colorado Plateau is encircled by highlands (Reveal 1979).

Efforts to test the cold hardiness of polyploids of *A. confertifolia* have produced somewhat equivocal results to the present, because cold-hardened Great Basin or Colorado Plateau plants seem to die uniformly when chilled to –25 to –30 °C in the laboratory, irrespective of ploidy. Nevertheless, foliar freezing damage correlated with ploidy has been observed in plants that had not been cold hardened (Sanderson unpublished data). Injury of such a kind might be important in the case of recent germinants because of their limited amounts of leaf tissue.

Genetic comparison of tetraploids in different regions is needed to verify the hypothesis of diploidization. Also, the fertility of 6x neoploids from the Great Basin and Colorado Plateau could be compared if sufficient numbers of them are discovered.

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