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Sponsored by: Montana Agricultural Experiment Station, Montana State University, Bozeman, MT.

The impact of introgressive hybridization on the weediness of leafy spurge

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Abstract:

Root tip cells of *Euphorbia* accessions, collected from three Canadian provinces, fourteen U.S. states, and five European countries were analyzed according to chromosome number and morphology. Extensive chromosome instability was discovered to be caused by introgressive hybridization between species with different ploidy levels. Introgression between diploid *E. virgata* Waldst. & Kit. ($2n = 20$) and *E. cyparissias* L. ($2n = 20$), tetraploid *E. virgata* ($2n = 40$) and *E. cyparissias* L. ($2n = 40$), and hexaploid *E. esula* ($2n = 60$) has led to the establishment of chromosome races in introgressive *E. X pseudovirgata* (Schur) Soó which range in predominant chromosome numbers from $2n = 46$ to 64. This genetic variability complicates biological and chemical control of leafy spurge and helps explain the variability in vegetative morphology within and among plants of *Euphorbia X pseudovirgata*.

Introduction

Long-term chemical control of leafy spurge has so far proven ineffective (Alley and Messersmith, 1985; Messersmith, 1979) and biological control is only in the developmental stages (Harris, 1979). Alley and Messersmith stated that new herbicides for control of leafy spurge are not available at the present time, and that there is little promise of more in the future. Harris (1979) mentioned that the establishment of a biocontrol agent will not automatically reduce the density of leafy spurge. The difficulty is that insects, for instance, may or may not have much impact on the population dynamics of their host plants. Chromosome instability within and among plants may be one of the causes of the present ineffectiveness of leafy spurge control (Schulz-Schaeffer and Gerhardt, 1987, 1989).

Chromosome instability within plants, termed mosaicism, was reported in plants from Fergus County, MT ($2n = 40$ to 60), Flathead County, MT ($2n = 52$ to 56), Gallatin County, MT ($2n = 58$ to 60), and Teton County, MT ($2n = 62$ to 65) (Schulz-Schaeffer and Gerhardt, 1987). Mosaicism is frequently found in allopolyploids or segmental allopolyploids like *E. X pseudovirgata* (Schulz-Schaeffer and Gerhardt, 1989). Our morphological studies -of leaf characteristics (Schulz-Schaeffer and Gerhardt, 1987) indicated that genetic material of *E. esula*, *E. virgata*, *E. cyparissias*, and *E. uralensis* Fisch. ex Link may be involved in this complex species group. Variability in vegetative morphology, particularly among leaves, has been noted both within-site and within-plant by Bakke (1936), Dunn and Radcliffe-Smith (1980), Groh (1935), Moore (1958), Radcliffe-Smith (1981), and Harvey *et al.*, (1988). Harvey *et al.*, (1988) have been unable to satisfactorily assign North American field grown *E. X pseudovirgata* plants to a specific taxon, using current identification keys. They found evidence of morphological expression of several apparent nominate taxa to be present at most sites.

Chromosome instability among plants of the same population was found in 62 of 107 leafy spurge accessions (Schulz-Schaeffer and Gerhardt, 1987). This kind of instability is believed to be caused by introgressive hybridization between species of different ploidy levels. Introgressive hybridization of two or more species in weedy leafy spurges has been postulated by Croizat (1945) and Radcliffe-Smith (1985). Dunn and Radcliffe-Smith (1980) adopted the name *Euphorbia X pseudovirgata* (Schur) Soó indicating its interspecific hybrid nature. They have shown how widespread this plant type is in the United States in relation to other members of this species aggregate, bearing out Croizat's contention that this is the aggressively invasive entity which has become naturalized and has spread rapidly in Montana, the Dakotas, Nebraska, Kansas, Minnesota, and Iowa. Similar plant material has subsequently been seen from Wyoming, Colorado, Wisconsin, and Michigan. It is also present in some of the northeastern states like New Hampshire, Massachusetts, New York, and New Jersey, but does not appear to be troublesome there. *E. X pseudovirgata* has also been reported in every province in Canada from British Columbia across to Nova Scotia.

Materials and methods

A *Euphorbia* collection was established in the greenhouse at Bozeman, Montana, for cytotaxonomic analysis. The material was collected by weed supervisors and research personnel in Colorado, Idaho, Iowa, Maryland, Michigan, Minnesota, Montana, Nebraska, Nevada, New Jersey, North Dakota, Oregon, Washington, Wyoming, Alberta, British Columbia, and Saskatchewan, and in Austria, Hungary, Italy, Switzerland, and Yugoslavia. The collection consisted of 107 accessions of the weedy *Euphorbia* species *E. X pseudovirgata*, *E. esula*, *E. virgata*, *E. cyparissias*, *E. salicifolia* Host, and *E. seguieriana* Neck. A standard leafy spurge numbering system recommended by the GPC-14 committee at the 1984 Leafy Spurge Symposium at Dickinson, North Dakota, was used for all accessions (Davis, 1985). Root tips grown on a root zone heating pad were harvested from potted plants in the greenhouse. Excised root tips were treated in 0.002 M 8-hydroxy-quinoline for 2 hours at room temperature and for 20 hours at 1°C , for 7 minutes in 0.2 N HCl, fixed in Carnoy's, squashed and stained in aceto-orcein (Gurr) or car-

bol-fuchsin, and observed under phase contrast on the microscope to conduct chromosome counts and morphological chromosome studies.

Results and discussion

1. Introgressive hybridization and its effects in weedy leafy spurges.

As stated, chromosome instability in weedy leafy spurges is believed to be caused by introgressive hybridization. Introgressive hybridization is the incorporation of genes of one species into the gene pool of another species by hybridization and backcrossing (Anderson and Hubricht, 1938). Introgressive hybridization can only occur in that part of a geographic range of a species which overlaps the distribution of closely related species, and then only when the habitat provides an ecological niche for the establishment of introgressive types (Stebbins, 1950). If, therefore, the variation pattern of a species is being altered by introgressive hybridization, this pattern should contain more variability in regions where the ranges of two related species overlap than where either species grows alone. Also, this variability should be greater in newly opened and much-disturbed areas than in old, stable habitats. An example of introgressive hybridization in the U.S. is the study by Abel and Austin (1981) of introgressive types of the wild sweet potato species *Ipomoea trichocarpa* Ell. and *I. lacunosa* L. which was based on corolla width and length.

Radcliffe-Smith (1985) reported some signs of introgressive hybridization of *E. esula* with *E. virgata* in populations of *E. X pseudovirgata*. According to him, the European distribution of *E. X pseudovirgata* includes eastern Austria, southern Czechoslovakia, Hungary, Romania, Bulgaria, Yugoslavia, and Poland. He also noted that the European distribution of *E. esula* is essentially the same as that of *E. cyparissias*. It was noticed that two of the three accessions received by us from Europe designated *E. esula* had narrower leaflets than the majority of *E. X pseudovirgata* and *E. esula* accessions (Schulz-Schaeffer and Gerhardt, 1987). Since narrow leaflets are a distinct characteristic of *E. cyparissias*, these accessions probably were derived from *E. esula X E. cyparissias* hybrids. The Italian *E. esula* accession (1982 I 001) had a chromosome number range of $2n = 48$ to 51 which is between $2n = 40$ for *E. cyparissias* and $2n = 60$ for *E. esula*. Moore (1958) described an artificial hybrid of this nature with $2n = 50$ chromosomes which matched natural European hybrids in all significant morphological characteristics. Moore and Frankton (1969) reported three natural *E. esula X E. cyparissias* hybrids from Ontario, Canada, one of which had $2n = 50$ chromosomes. Another one was analyzed by Parmlee (1962) as $2n = 50$. In Europe the *E. esula X E. cyparissias* hybrid has been reported along waterways of northern and eastern Austria (Dörfler in Moore, 1958), Czechoslovakia, eastern Germany (Reichinger, 1902; Hegi, 1930), Hungary (Dörfler in Moore, 1958), and Romania (Schur, 1866).

Introgressive hybridization in weedy leafy spurges obviously has occurred between species of three different ploidy levels. *E. virgata* and *E. cyparissias* are mainly diploids and tetraploids ($2n = 20, 40$). *E. esula* and *E. X pseudovirgata* should be mainly considered to be hexaploids ($2n = 60$). The hexaploids have taken up genetic material from the diploids and tetraploids in order to colonize new disturbed environments. According to

the literature it was formerly thought that the basic chromosome numbers of the weedy leafy spurge group were $x = 8, 9,$ and 10 (Table 1). We believe that the reports of $2n = 16$ for *E. esula* (Van Loon and DeJong, 1978), $2n = 36$ for *E. cyparissias* (Zhukova, 1967), $2n = 56$ for *E. virgata* (Hurusawa and Shimoyama, 1976), and $2n = 64$ for *E. esula* (Gadella and Kliphuis, 1968) are aneuploid chromosome numbers based on $x = 10$ rather than euploid multiples of $x = 8$ and $x = 9$ (Table 1).

Table 1. Chromosome numbers of the weedy leafy spurge complex as reported in the literature (for references, see Schulz-Schaeffer and Gerhardt, 1987, 1989).

Ploidy level	$x = 8$	$x = 9$	$x = 10$
Diploid	$2n = 16$ <i>E. esula</i>		$2n = 20$ <i>E. virgata</i> <i>E. cyparissias</i>
Tetraploid		$2n = 36$ <i>E. cyparissias</i>	$2n = 40$ <i>E. virgata</i> <i>E. cyparissias</i>
Pentaploid			$2n = 50$ <i>E. cyparissias</i> X
Hexaploid			$2n = 60$ <i>E. esula</i> <i>E. X pseudovirgata</i>
Heptaploid	$2n = 56$ <i>E. virgata</i>		
Octoploid	$2n = 64$ <i>E. esula</i>		

2. Colonization of weedy leafy spurges by means of a pivotal genome.

Zohary (1965) states that the genes of a pivotal basic genome control the preadaptive theme, while the other basic genomes of a polyploid complex provide the wide variation on the theme in the form of modified genomes. We have evidence that a pivotal basic genome of ten chromosomes exists in the weedy leafy spurge species complex. A marker chromosome (satellite chromosome II) may represent this pivotal genome (Schulz-Schaeffer and Gerhardt, 1989). This marker chromosome was most common in *E. X pseudovirgata* but was also present in *E. esula* and tetraploid *E. cyparissias* ($2n = 40$). This common basic genome must have served as a buffer in the process of hybridization.

If hybridization and subsequent introgression of chromosomes of one species into the pool of another species has taken place by backcrossing between tetraploids and hexaploids, and if the hybrids have moved into habitats where the hexaploids predominate, then aneuploid forms may have arisen which were closer in chromosome number to the hexaploids. The predominant hexaploid, aneuploid, and euploid chromosome levels of *E. X pseudovirgata* ($2n = 56$ to 60) in North America may well be the result of such development.

A second marker chromosome (III) was found in the tetraploid *E. cyparissias* ($2n = 40$) and in the hexaploid introgressive *E. X pseudovirgata*, but not in *E. esula*. This marker chromosome may form the basis for the variation in *E. X pseudovirgata*. It may be desirable to study the marker chromosome or chromosomes of diploid *E. cyparissias*, ($2n = 20$) since they may contribute a modified genome. A diploid *E. cyparissias* was reported by Majofsky *et al.*, (1970) in Slovakia, and by Kliphuis and Wieffering (1972) in southern France. Another necessary study is the morphology of the marker chromosomes of diploid and tetraploid *E. virgata* ($2n = 20, 40$). Accessions with such chromosome numbers from Hungary were discovered which may reveal the nature of the basic genomes contributed by *E. virgata* (Schulz-Schaeffer and Gerhardt, 1989).

Zohary (1965) studied the colonization characteristics of wild *Aegilops* and *Triticum* species in the Near East. He found, as a rule, that diploids are more restricted in their distribution than polyploids. Compared with the diploids, the tetraploids and hexaploids are extraordinarily variable. The wide ranges of variation are usually coupled with blurred specific boundaries. In other words, species delimitation on the polyploid level is often difficult and arbitrary, and series of intermediate forms interconnect the major morphological types. Another major difference between diploid and polyploid wild *Triticum* species is the pronounced weediness of the latter.

The leafy spurge chromosome maps of the United States and Canada (Schulz-Schaeffer and Gerhardt, 1987) (Figures 1 and 2) display trends similar to those observed by Zohary in 1965. The highest predominant $2n$ chromosome numbers occur in Montana, specifically in Teton County (58 to 64). Lower chromosome numbers were observed in British Columbia ($2n = 50$), Alberta ($2n = 46$ to 60), Michigan ($2n = 54$), and New Jersey ($2n = 50$ to 56), areas where *E. X pseudovirgata* is less weedy. Areas with plants of intermediate chromosome numbers occur between those regions with both extremes of the variation. Areas most infested with leafy spurge occur in Montana, the Dakotas, and Nebraska where the highest chromosome numbers occur.



Figure 1. Map of predominant 2n chromosome numbers of leafy spurge collected from Idaho, Montana, North Dakota, Oregon, Washington, Wyoming, Alberta, British Columbia, and Saskatchewan (from Schulz-Schaeffer and Gerhardt, 1987).

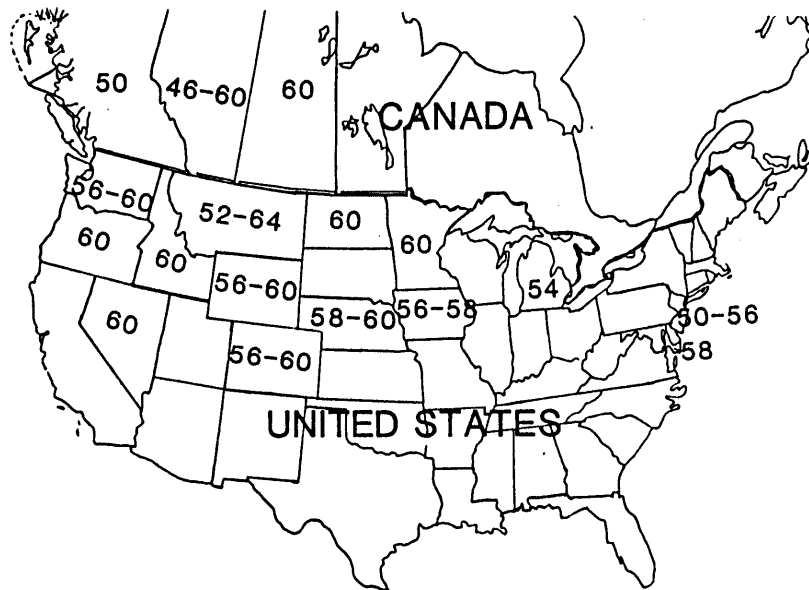


Figure 2. Map of predominant 2n chromosome numbers of leafy spurge arranged according to U.S. States and Canadian provinces (from Schulz-Schaeffer and Gerhardt, 1987).

Conclusions

Chromosome number patterns of weedy leafy spurges in North America confirm earlier morphological observations that this burdensome rangeland weed is extremely variable in its genetic expression. It is evident that plants of diploid, tetraploid, and hexaploid species have intercrossed and backcrossed with plants of the parental species, which has resulted in forms with aneuploid chromosome numbers. Furthermore, different amphiploid forms may have intercrossed which was facilitated by a commonly shared basic pivotal genome. This common genome served as a buffer in the process of hybridization. Such hybridization between closely related amphiploids would be expected to produce intraspecific chromosomal variation as in *E. X pseudovirgata* parallel to morphological variation as expressed for weedy *Aegilops* spp. by Zohary and Feldman (1962). As a result, many chromosome recombinations are possible, mainly between unshared basic genomes, resulting in their differential modifications and the establishment of modified or differential genomes, side by side with the unaltered basic pivotal genome.

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