

POLYPLOIDY AND SEGREGATION ANALYSES IN *DELPHINIUM GYPSOPHILUM* (RANUNCULACEAE)

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ABSTRACT

Delphinium gypsophilum Ewan consists of both diploid and tetraploid individuals, but the type of polyploidy (allo- vs. autopolyploidy) has not been documented. Cytotyping using flow cytometry indicated that some populations were $2n$, others were $4n$, and several had mixed ploidy. Triploid individuals represented approximately 20% of the sampled plants. There appears to be little geographic structuring of cytotypes. Progeny arrays from controlled crosses provided evidence favoring tetrasomic inheritance, and *Delphinium gypsophilum* has allozyme banding patterns that are consistent with autotetraploidy. Genetic data indicate that polyploids may have formed recurrently, but the exact number of origins and specific progenitor-derivative relationships remain uncertain. Conservation efforts should manage the two cytotypes separately, as they represent potentially different evolutionary units.

INTRODUCTION

Polyploidy is an important phenomenon in the evolution of many plant species. Approximately 47–52% of angiosperms and 44–95% of pteridophytes may have polyploid origins (Grant 1981; Vida 1976). Given the pervasiveness of polyploidy, much remains to be learned concerning its evolutionary consequences. Polyploidy has often been considered to be an evolutionary dead end; however, recent research is revealing that polyploidy is a dynamic process and does not necessarily lead the species toward extinction (reviewed in D. Soltis and P. Soltis 1993, 1999; P. Soltis and D. Soltis 2000). Two types of polyploids exist. Allopolyploids combine the genomes of two diploid species via hybridization and subsequent chromosome doubling. Autopolyploids arise intraspecifically from a diploid progenitor. Because of the differences associated with their origin, allopolyploids and autopolyploids have different modes of inheritance and different genetic attributes. Allopolyploids exhibit fixed heterozygosity and disomic-digenic inheritance. Autopolyploids do not exhibit fixed heterozygosity, but have increased levels of heterozygosity due to polysomic inheritance (often detected as unbalanced heterozygotes; reviewed in D. Soltis and P. Soltis 1993). Taxa that exist as both diploid and polyploid individuals offer a natural laboratory in which further studies can be developed to examine the evolutionary consequences of polyploidy (e.g., shifts in mating systems, Cook and Soltis

1999, 2000). This paper presents data on the nature of polyploidy in *Delphinium gypsophilum* Ewan.

Delphinium gypsophilum gypsum-loving larkspur, grows in open grasslands in the southern San Joaquin Valley of central California. Two subspecies of *D. gypsophilum* have been described based on size, and to a lesser degree, flower color and range (Lewis and Epling 1954; Warnock 1997). *Delphinium gypsophilum* subsp. *gypsophilum* typically has white flowers that are larger than those of *D. gypsophilum* subsp. *parviflorum* Lewis & Epling (Warnock 1997). *Delphinium gypsophilum* subsp. *parviflorum* has flowers that are usually white, but are often lavender or pink, and this subspecies is typically located closer to the Pacific coast (Lewis and Epling 1954). *Delphinium gypsophilum* subsp. *gypsophilum* consists of both diploid ($2n = 16$) and tetraploid ($2n = 32$) individuals (Lewis et al. 1951), and the two cytotypes are morphologically indistinguishable (Lewis and Epling 1959; J. Koontz pers. obs.). The morphological similarity between diploid and tetraploid individuals of *D. gypsophilum* suggests autotetraploidy. *Delphinium gypsophilum* subsp. *parviflorum* is apparently only diploid (Warnock 1995). Previous genetic work on *D. gypsophilum* (Koontz 2000) does not support the recognition of subspecies, therefore, we treat both subspecies together as *D. gypsophilum* in this study.

In their study of chromosome numbers of most Californian larkspurs, Lewis et al. (1951) cytotyped 378 individuals from 35 populations of *D. gypsophilum* (the subspecies were not recognized in their study). Sixteen populations were diploid, and 19 were tetraploid. Neither populations of mixed ploidy nor triploid individuals were documented (Lewis et al. 1951). Their survey of *D. gypsophilum* sampled populations from throughout the range of the species as it occurred in the 1950's; however, since that time, many populations have been lost because

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TABLE 1. POPULATIONS OF *D. GYPSOPHILUM* STUDIED, THEIR PLOIDY, METHOD OF DETERMINATION, AND SAMPLE SIZE (*N*).
¹ Numerical population designations are from Koontz (2000). ² Full locality data available from first author. ³ Population grown at WSU.

Population ¹	Locality ²	Ploidy/method/ <i>N</i>
1	Kern Co., Hwy 58	3 <i>n</i> -4 <i>n</i> /flow/6
2	Kern Co., Hwy 58	2 <i>n</i> -3 <i>n</i> /flow/6
3	San Luis Obispo Co., Soda Lake	2 <i>n</i> -3 <i>n</i> -4 <i>n</i> /flow/9
5	Kern Co., Elk Hills	4 <i>n</i> /flow/6
6	Kern Co., Elk Hills	3 <i>n</i> -4 <i>n</i> /flow/7
7	Kern Co., Elk Hills	2 <i>n</i> -4 <i>n</i> /flow/4
9	Kern Co., Elk Hills	2 <i>n</i> -3 <i>n</i> /flow/5
11	San Luis Obispo Co., Hwy 41	3 <i>n</i> -4 <i>n</i> /flow/7
12	San Luis Obispo Co., Hwy 41	2 <i>n</i> -3 <i>n</i> /flow/4
13	San Luis Obispo Co., Cypress Mtn. Rd.	2 <i>n</i> /flow/4
14	San Luis Obispo Co., G14	2 <i>n</i> /flow/3
15	Monterey Co., New Pleyto Rd.	2 <i>n</i> /flow/4
16	Monterey Co., Vineyard Canyon Rd.	3 <i>n</i> /flow/1
17 ³	Merced Co., Los Baños Reservoir	4 <i>n</i> /squash/5
20	Kern Co., A. D. Edmonston Pumping Plant	2 <i>n</i> /flow/11
HR ³	Merced Co., Howard's Ranch, Aqueduct mi. 65	4 <i>n</i> /squash/5
0 ³	Merced Co., O'Neill Forebay	4 <i>n</i> /squash/5

of extensive development in the Central Valley of California. *Delphinium gypsophilum* continues to co-exist with human development, but the California Native Plant Society (CNPS) has placed this species on its List 4, a "watch list" of species that may become threatened or endangered. We reexamined the distribution of ploidy in *D. gypsophilum* because the range of *D. gypsophilum* has changed since 1951 and because additional populations of *D. gypsophilum* are now known. If the range of *D. gypsophilum* continues to change and the species becomes threatened or endangered, these data on ploidy will be useful in management plans to ensure the protection of both cytotypes. Additionally, determining the type of polyploidy in *D. gypsophilum* is important for understanding the evolutionary history of this species. The differences between the genetic attributes of allopolyploids and autopolyploids could affect how the cytotypes are treated taxonomically and ultimately managed in conservation efforts.

This study was designed to determine (1) the number, distribution, and similarity of diploid and tetraploid populations, and (2) the mode of inheritance, and therefore the type of polyploid, in the tetraploid cytotype. Flow cytometry and mitotic root-tip squashes were used to determine ploidy of the samples, and their ploidal distributions were plotted along with data from Lewis et al. (1951). To determine the type of polyploid, controlled crosses were performed to generate progeny arrays to test for inheritance patterns of allozyme markers.

MATERIALS AND METHODS

Number, distribution, and similarity of diploid and tetraploid populations. Fifteen populations of *D. gypsophilum* were sampled (Table 1). Leaf material from these populations was sampled in April and

early May, 1999. Two or three leaves were removed from up to 15 individuals per population. These leaves were wrapped in dry paper towels, placed in labeled plastic sandwich bags and shipped on ice overnight to the University of Arizona (UAZ) for flow cytometry analyses following Galbraith et al. (1997). Briefly, protocols for an arc lamp-based flow cytometer (Partec CCAII, Partec GmbH, Munster, Germany) were used with the Galbraith Homogenization Buffer I and DAPI fluorescent stain. Fluorescent microbeads (Alignflow, Molecular Probes, Inc., Eugene, OR) were used to align the instrument, and then samples of *Nicotiana tabacum* cv. *Xanthi* were run to set up the instrument. Leaf material from known 4*n* *D. gypsophilum* individuals grown at Washington State University (WSU) were sent to UAZ to use as *Delphinium* standards. The samples of known ploidy (either the *Nicotiana* or 4*n* *Delphinium*) were also run at the beginning of each day and rerun at intervals during the day to ensure that the alignment had not drifted. Each sample was run for 10,000–30,000 events. Flow cytometry is an indirect measure of ploidy and is an effective and efficient technique for estimating the ploidy of natural populations (e.g., Burton and Husband 1999; Greilhuber and Obermayer 1999; Husband and Schemske 1998; Keeler 1992; Thompson et al. 1997). Samples from three additional populations of *D. gypsophilum* were maintained in greenhouse culture at WSU. These populations were started in September, 1996, from seed collected in April and May, 1996, from natural populations (Table 1). Mitotic root-tip squashes following Soltis (1980) were performed to determine the ploidy of these populations. Root tips were harvested from five actively growing plants per population. Voucher specimens from each population were collected and are deposited in the Marion

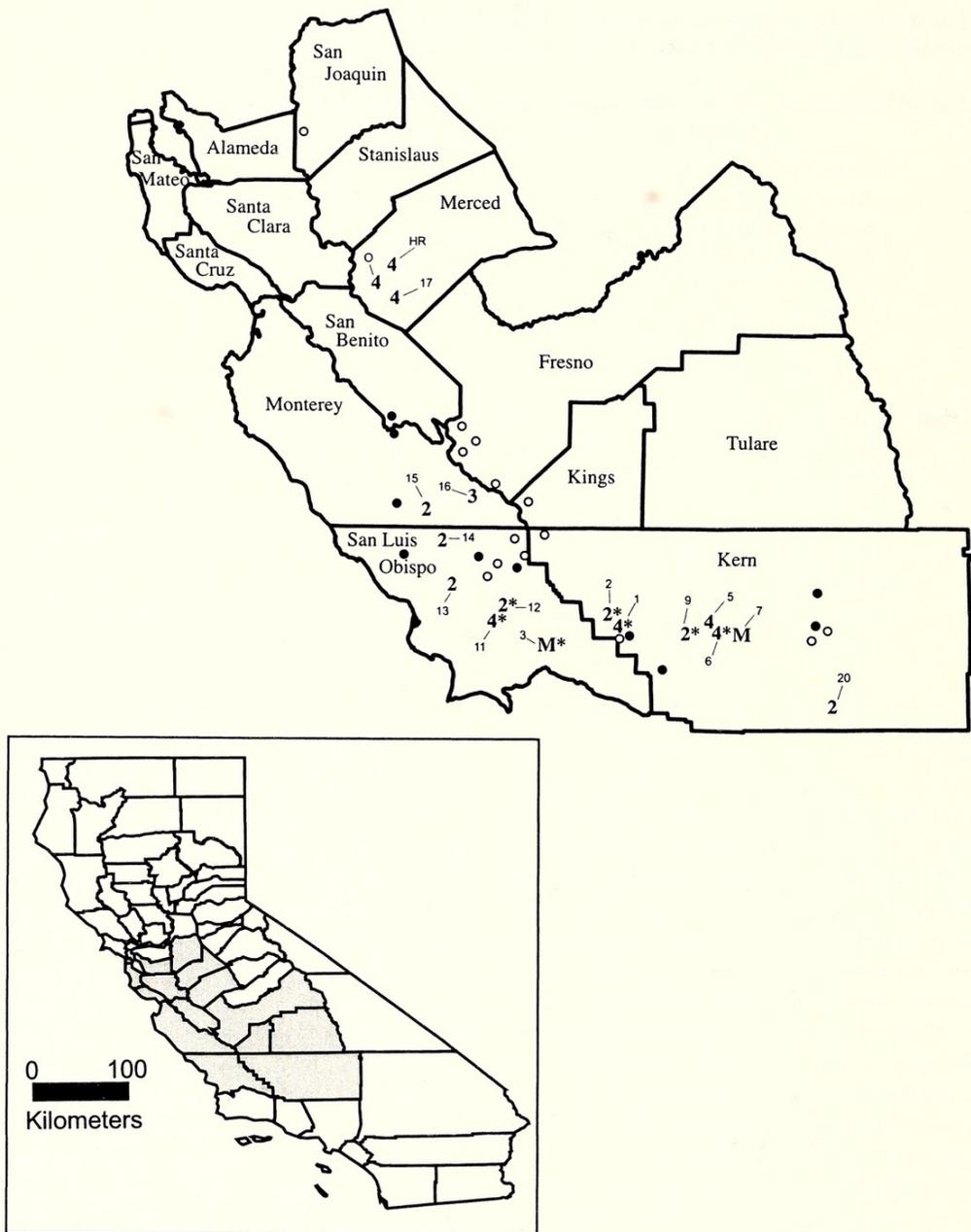


FIG. 1. General location of *D. gypsophilum* populations and their ploidy. Inset: Map of California with counties shaded. Populations from Table 1 are indicated by a connecting line to the ploidy of the samples: 2 = diploid population; 2* = 2n and 3n individuals present; 3 = triploid population (but based on only 1 sample); 4 = tetraploid population; 4* = 4n and 3n individuals present; M = 2n and 4n individuals present; M* = 2n, 3n, and 4n individuals present; open circles = 4n populations surveyed by Lewis et al. (1951); closed circles = 2n populations surveyed by Lewis et al. (1951).

Ownbey Herbarium (WS). Ploidal levels of sampled populations are listed in Table 1. Figure 1 shows the relative locations of the populations studied.

We investigated the similarities among extant populations of *D. gypsophilum* using 12 allozyme loci reported in a previous study (Koontz 2000). These data were originally used to test the hypothesis of hybrid origin of *D. gypsophilum* (Lewis and Epling 1959). The analyses of allozyme data reported here are original. We computed genetic identities (Nei 1972) that were then subjected to clustering analysis (UPGMA) using BIOSYS-1 (Swof-

ford and Selander 1989) to explore which populations were more similar to one another, to determine if the number of origins of tetraploid *D. gypsophilum* could be inferred, and if more than one diploid population was involved.

Mode of inheritance in the tetraploid cytotype. We tested inheritance patterns by crossing cultivated plants of known allozyme genotype to generate progeny arrays. The crossing design, number of progeny scored, and allozyme genotypes are listed in Table 2. Controlled crosses were performed in the Steffen Center Greenhouses, WSU, during Feb-

TABLE 2. CROSSING DESIGN FOR *D. GYPSOPHILUM* TO TEST FOR TETRASOMIC INHERITANCE.

Cross	Source population (individual)	Locus	Cross genotype	No. of progeny scored
1a	O'Neill (30) × O'Neill (42)	<i>Pgm</i>	<i>bbbc</i> × <i>bbbc</i>	71
1b		<i>Aat-1</i>	<i>bbbc</i> × <i>bbbb</i>	84
2	O'Neill (35) × O'Neill (37)	<i>Lap</i>	<i>aacc</i> × <i>aaaa</i>	81
3a	Los Baños (26) × O'Neill (30)	<i>Lap</i>	<i>aacc</i> × <i>aaaa</i>	88
3b		<i>Aat-1</i>	<i>bbbb</i> × <i>bbbc</i>	82
4a	Los Baños (9) × Los Baños (11)	<i>Pgm</i>	<i>bbbd</i> × <i>bbbb</i>	51
4b		<i>Lap</i>	<i>aacc</i> × <i>aaaa</i>	57

ruary–March, 1997. Seven plants were selected based on their previously determined allozyme genotype (Table 2). For each cross, 10 flowers per plant were cross-pollinated in the following manner. Flowers were emasculated just prior to opening. The removal of the anthers triggered the stigmatic surface to become receptive two days later, when pollen was transferred to the stigma. Each cross was performed reciprocally. Fruits were harvested at maturity, just before or at dehiscence (approximately 3–4 weeks after pollination). The fruits and seeds were stored in paper coin envelopes at room temperature. Previous work on this species indicated that the seeds remain viable for many years, but only germinate when planted in the early fall (JAK pers. obs.). The seeds were planted in plastic flats using regular potting soil in September, 1999. As soon as the seedlings had produced their first true leaves, they were harvested for allozyme electrophoresis.

Allozyme procedures followed Soltis et al. (1983), with the exceptions listed below. Up to 96 individuals per cross were harvested. The fresh leaves were ground, and the wicks were frozen as described by Cook and Soltis (1999). All starch gels were 12.5% (w/v). Buffer system 6 was used to resolve aspartate aminotransferase (AAT). System 8, as modified by Hauffer (1985), was used to resolve leucine aminopeptidase (LAP) (stain recipe in McDonald 1985). The morpholine system (Odrzykoski and Gottlieb 1984) at pH 6.1 was used to resolve phosphoglucosmutase (PGM). Isozymes were numbered sequentially starting with the most anodal as 1. Alleles were designated alphabetically, the most anodal as *a*.

A χ^2 test of significance was used to determine if the frequency of progeny genotypes deviated from expected ratios of disomic-digenic and tetrasomic inheritance.

RESULTS AND DISCUSSION

Geographic distribution of cytotypes. Although 15 individuals were sampled from each population for flow cytometry, the leaf material for some individuals was unusable when the flow cytometry was conducted (Table 1). Low sample sizes for some populations may therefore fail to reflect the

proportions of diploid and tetraploid individuals accurately.

Populations were either $2n$, $4n$, or of mixed ploidy (Table 1), and 18 individuals (approximately 20% of the 92 samples cytotyped) were interpreted from the flow cytometric data as $3n$ (in population #16, the only individual was $3n$). In an abstract, Lewis (1946) reported the occurrence of natural triploid hybrid individuals in areas of contact between diploid and tetraploid cytotypes of *D. gypsophilum*. However, Lewis et al. (1951) sampled between 1 and 48 individuals per population (mean = 10, standard deviation = 12) from 35 populations, but they did not detect any $3n$ individuals or populations of mixed ploidy in that study. The high frequency of triploids we detected by flow cytometry is surprising given that Lewis et al. (1951) detected no triploid individuals with broader sampling. Other studies have detected triploids, but at lower frequencies [e.g., 1.4% in *Heuchera grossulariifolia* Rydb. (Thompson et al. 1997), 9% in *Chamerion angustifolium* (L.) Holub (Husband and Schemske 1998), and 11% in *Galax urceolata* (Poin) Brumintt (Burton and Husband 1999)]. The coefficients of variation for the *Delphinium* samples measured on the flow cytometer ranged from 3.3 to 41, the average being 10.5 ± 0.74 (SEM). The CVs were high because some of the field-collected samples had started to degrade and were therefore less than optimal for flow cytometry.

In assigning ploidy to the samples, the values set for each ploidy class were arbitrary, but were calibrated on the values obtained from the known $4n$ samples. To determine the effect of changing the boundaries of the $2n$ and $4n$ ploidy classes on the number of $3n$ samples inferred, we broadened the range of $2n$ and $4n$ classes by 10%, but this change only reduced the triploid frequency to 15%. Given the discrepancy in the number of $3n$ individuals observed here and by Lewis et al. (1951), future work is needed using both flow cytometry and mitotic or meiotic squashes.

Geographic structure among related diploids and polyploids has been commonly reported (e.g., Husband and Schemske 1998; Ness et al. 1989; Soltis 1984). Polyploids often have broader ecological amplitudes, in part due to their increased levels of

genetic variation, that allow them to occupy habitats that are inhospitable to their diploid progenitors. This structuring leads to the successful establishment of the polyploid race by ensuring individuals of the same ploidy do not co-occur. The distributions of the cytotypes reported here show little geographic structure. However, the tetraploid populations are clustered in Merced County and in central San Luis Obispo County to western Kern County (Fig. 1). Diploid populations (containing no $3n$ or $4n$ individuals) appear on both sides of the Monterey-San Luis Obispo County line. Many of the populations analyzed by Lewis et al. (1951) no longer exist, though attempts were made to locate them for use in this study. A tetraploid population from San Joaquin County was Lewis et al.'s (1951) northernmost sample, well separated from the other populations of *D. gypsophilum* they surveyed (Fig. 1). Despite several attempts to locate this population, it appears to have been destroyed; nevertheless, the historical presence of this population makes the tetraploid populations discovered in Merced County less isolated. The data from Lewis et al. (1951) indicate that the tetraploids generally clustered around southwestern Fresno County into Kings County, San Luis Obispo County, and in both western and central Kern County.

Other species that exist as both diploid and polyploid populations often display broad geographic structuring of ploidy, but these species all have a much larger geographic range than *D. gypsophilum*. For example, the tetraploid cytotypes of *Heuchera micrantha* Dougl. ex Lindl. occur in the central part of the range, with diploid populations occurring to the north and south (Ness et al. 1989). A distinct north-south distribution is found in *Tolmiea menziesii* (Pursh) Torn & Groy, in which the tetraploid cytotype occurs from southeastern Alaska to central Oregon and the diploid cytotype occurs from central Oregon into northern California (Soltis 1984). Diploid and tetraploid cytotypes of *Chamerion angustifolium* are also distributed latitudinally, with the diploids occurring at higher latitudes (Husband and Schemske 1998). The geographic structure of cytotypes of *Galax urceolata* is less defined because the diploid and polyploid cytotypes overlap; in general, the frequency of diploids decreases north to south, while tetraploids increase (Burton and Husband 1998). The diploid cytotype of *Heuchera grossularifolia* occurs throughout river systems in Idaho and western Montana, but the tetraploids are more limited in distribution across north-central Idaho into western Montana (Segraves et al. 1999; Wolf et al. 1990).

Multiple origins of polyploid species and cytotypes have been detected in almost all cases that have been investigated (reviewed in Soltis et al. 1992; D. Soltis and P. Soltis 1993, 1999; P. Soltis and D. Soltis 2000). Some of the tetraploid populations in this study cluster geographically with one or more diploid populations (Fig. 1, pops. 1 and 2;

5, 6, and 9; 11 and 12). Additionally, some populations are of mixed ploidy, containing both diploids and tetraploids, as well as some triploids (Fig. 1). These distributions suggest the possibility of multiple origins of the tetraploid cytotype from different diploid progenitor populations.

In previous work, allozyme analyses of multiple populations of *D. gypsophilum* (Koontz 2000; raw data available from first author by request) indicate few differences among tetraploid populations, and DNA sequence divergence in the nuclear ribosomal internal transcribed spacer (ITS) regions between the two diploids and one tetraploid sampled is low ($\sim 0.17\%$) (Koontz 2000). Comparisons of the alleles reported in Koontz (2000) found that neighboring diploid and tetraploid populations e.g., pops. 1 and 2; 5, 6, 7, 8, and 9; and 11 and 12. show similar allele frequencies at most loci. The genetic identities (Nei 1972) among the populations sampled are high ($\sim 88\text{--}99\%$); however, a UPGMA clustering diagram reveals two groups (Fig. 2), one composed mainly of the populations found in eastern San Luis Obispo County, eastern Monterey County, and easternmost Kern County, and the other composed of those populations in western Kern County, northwestern San Luis Obispo County, southwestern Monterey County, and Merced County. Tetraploids and diploids occur in both clusters, and those that occur together geographically [i.e., pops. 1 ($3n/4n$) and 2 ($2n/3n$), 6 ($3n/4n$) and 9 ($2n/3n$), 11 ($3n/4n$) and 12 ($2n/3n$)] generally occur in the same group in the UPGMA phenogram (Fig. 2), consistent with recurrent formation of tetraploid populations from neighboring diploid populations. Other populations from the same geographic area occur in separate groups. Population 7 ($2n/4n$) from the Elk Hills area of Kern County occurs in a cluster separate from other populations from this area [pops. 5 ($4n$), 6 ($3n/4n$), and 9 ($2n/3n$), where pop. 5 is more similar to pops. 1 and 2 than to pops. 6 and 9]. Both populations 3 and 7 contain mixed cytotypes, suggesting that the $4n$ cytotype could have arisen within each of these two populations. These data do not provide conclusive evidence of specific progenitor-derivative relationships; however, they are consistent with more than a single origin of the tetraploid cytotype. To test the hypothesis of multiple origins of polyploidy thoroughly, additional populations will need to be sampled both cytologically and genetically.

Segregation analyses. Although no differences in seed set were observed between reciprocal crosses, the seed produced from parent individuals 9 and 11 (crosses 4a and 4b) had low germination, and only 68 progeny were harvested. The numbers of progeny scored for each cross are lower than the total harvested (Table 2) because some individuals did not express well and could not be scored with confidence.

Allopolyploids are characterized by fixed hetero-

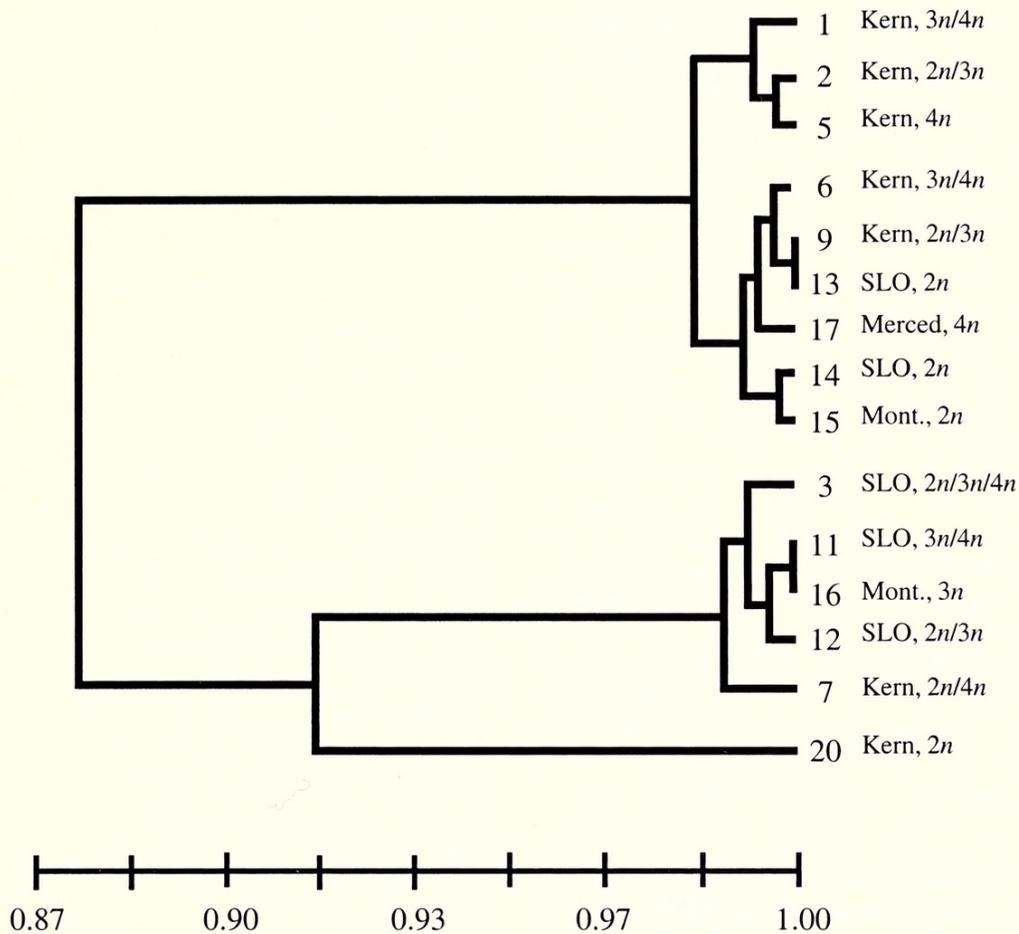


FIG. 2. UPGMA phenogram of genetic identity (Nei 1972) for the populations of *D. gypsophilum*, their county, and ploidy. SLO = San Luis Obispo County; Mont. = Monterey County.

zygosity at allozyme loci (e.g., Roose and Gottlieb 1976). Fixed heterozygosity was not observed at any allozyme loci during this or previous work on *D. gypsophilum* (Koontz 2000). At several loci (e.g., *Pgi*, *Aat*, *Pgm*, *Lap*) the tetraploids exhibited unbalanced heterozygotes, indicating dosage effects that may result from either tetrasomic segregation or disomic-digenic segregation with shared alleles at the two loci. Genotypes from progeny arrays were compared with expectations for both tetrasomic and disomic-digenic segregation.

Crosses 1a-b, 3b, and 4a (Table 2) yielded progeny arrays that were consistent with both disomic-

digenic and tetrasomic inheritance models; therefore, χ^2 scores could not distinguish between the two models. For crosses 2, 3a, and 4b (Table 3), progeny were obtained that could only be expected under the tetrasomic model; however, ratios of the observed progeny did not fit the expected tetrasomic ratios (Table 3). Cross 2 had one progeny with an unexpected genotype of *cccc*. These crosses also had a higher proportion of *aaaa* (2), *aacc* (3a), or both *aacc* and *aaaa* (4b) genotypes than expected, and crosses 2 and 4b also had fewer *aaac* progeny than expected.

The progeny arrays from the crossing experi-

TABLE 3. EXAMPLES OF THE EXPECTED AND OBSERVED PROGENY FREQUENCIES UNDER DISOMIC-DIGENIC AND TETRASOMIC MODELS OF INHERITANCE FOR CROSSES 2, 3A, AND 4B. All are *Lap aacc* × *aaaa*. *aa,ac* = genotype *aa* at one disomic locus and *ac* at the second disomic locus. NA = genotypes present that are not possible under the given model, making a significance test not appropriate. * The *cccc* genotype was not included in the χ^2 computation, but it would actually make the value "NA" for the tetrasomic model.

Progeny genotype	<i>aa,cc</i> × <i>aa,aa</i>	<i>ac,ac</i> × <i>aa,aa</i>	Tetrasomic	2 Observed	3a Observed	4b Observed
<i>aaaa</i>	0.5	0	0.167	0.516	0.159	0.474
<i>aaac</i>	0	1	0.666	0.234	0.5	0.193
<i>aacc</i>	0.5	0	0.167	0.247	0.341	0.333
<i>cccc</i>	0	0	0	0.012	0	0
χ^2	NA	NA	see column under each cross	82.54* P < 0.0005	19.718 P < 0.0005	41.974 P < 0.0005

ments do not offer a clear answer for the mode of inheritance in the tetraploid cytotype. In some cases, the crosses could not distinguish between tetrasomic and disomic-digenic inheritance; these progeny arrays are therefore consistent with both models. In other crosses, the disomic-digenic model could be ruled out because multiple genotypes were recovered that were impossible under the disomic-digenic model without invoking a high frequency of chromatid segregation. However, these same crosses did not statistically fit the tetrasomic model, and one genotype that was not expected under either model appeared in the progeny of one cross. The occurrence of a novel genotype in low frequency in the progeny of a known cross may reasonably be attributed to chromatid segregation (reviewed in Wolf et al. 1989). Additionally, gametic selection has been implicated where progeny arrays derived from the same parents alternately fit perfectly or deviate significantly from expectations when produced and grown in different environments (e.g., Henningsen et al. 1993). Future work should address the possible role of gametic selection.

CONCLUSIONS

Using flow cytometry and root-tip preparations, we mapped the cytotypes of *D. gypsophilum* from throughout its current known range. Unlike the previous study (Lewis et al. 1951), we detected mixed ploidy within some populations, as well as triploid individuals. The evidence from allozyme data and the segregation analyses presented here point to tetraploid *D. gypsophilum* as an autotetraploid. Although diploid and tetraploid populations cluster together both geographically and genetically, suggesting recurrent formation of the tetraploid cytotype, the genetic data do not provide conclusive evidence of specific progenitor-derivative relationships among populations.

Conservation implications. The data presented here and elsewhere (Koontz 2000) do not support the subdivision of *D. gypsophilum* into two subspecies. Populations 13, 14, and 15 (all $2n$) are from localities that Warnock (pers. comm.) has identified as subsp. *parviflorum*. Population 13 is more similar genetically to populations of subsp. *gypsophilum* (Fig. 2, populations 9, 6, and 17;) than to populations 14 and 15. Furthermore, ploidy does not distinguish the two subspecies. Although all of the tetraploids detected occur in *D. gypsophilum* subsp. *gypsophilum* (Lewis and Epling 1954; this study), five $2n$ populations are also recognized as subsp. *gypsophilum*.

The combination of genetic and ploidy data suggest that two subspecies should no longer be recognized, even though the subspecies may be distinguished to some degree by range and flower color (but not size). Both subspecies are currently placed on the CNPS List 4. Because the range of *D. gyp-*

sophilum sensu lato continues to be affected by human development, *D. gypsophilum* should remain a List 4 species. Both cytotypes and all morphological variants should be included in any future conservation efforts for this species; the cytotypes should be managed separately, as they represent potentially different evolutionary units.

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