

Cytogeography and genome size variation in the *Claytonia perfoliata* (Portulacaceae) polyploid complex

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- **Background and Aims** Genome duplication is a central process in plant evolution and contributes to patterns of variation in genome size within and among lineages. Studies that combine cytogeography with genome size measurements contribute to our basic knowledge of cytotype distributions and their associations with variation in genome size.
- **Methods** Ploidy and genome size were assessed with direct chromosome counts and flow cytometry for 78 populations within the *Claytonia perfoliata* complex, comprised of three diploid taxa with numerous polyploids that range to the decaploid level. The relationship between genome size and temperature and precipitation was investigated within and across cytotypes to test for associations between environmental factors and nuclear DNA content.
- **Key Results** A euploid series ($n = 6$) of diploids to octoploids was documented through chromosome counts, and decaploids were suggested by flow cytometry. Increased variation in genome size among populations was found at higher ploidy levels, potentially associated with differential contributions of diploid parental genomes, variation in rates of genomic loss or gain, or undetected hybridization. Several accessions were detected with atypical genome sizes, including a diploid population of *C. parviflora* ssp. *grandiflora* with an 18 % smaller genome than typical, and hexaploids of *C. perfoliata* and *C. parviflora* with genomes 30 % larger than typical. There was a slight but significant association of larger genome sizes with colder winter temperature across the *C. perfoliata* complex as a whole, and a strong association between lower winter temperatures and large genome size for tetraploid *C. parviflora*.
- **Conclusions** The *C. perfoliata* complex is characterized by polyploids ranging from tetraploid to decaploid, with large magnitude variation in genome size at higher ploidy levels, associated in part with environmental variation in temperature.

Key words: *Claytonia perfoliata*, Portulacaceae, flow cytometry, C-value, cytogeography, genome duplication, DNA content.

INTRODUCTION

Whole-genome duplication, or polyploidy, is common and recurrent among flowering plants (Jiao *et al.*, 2011). Nearly all extant angiosperm lineages exhibit genomic signatures consistent with one or more ancient whole-genome duplication event followed by genome reduction (Blanc and Wolfe, 2004; Cui *et al.*, 2006; Soltis *et al.*, 2009). Hypotheses for the ubiquity of polyploidy include advantages such as ecological benefits of increased heterozygosity, ability to invade novel habitats (Ramsey, 2011) and labile expression of divergent parental genomes (Stebbins, 1971; Grant, 1981; Levin, 1983; Comai, 2005; Dubcovsky and Dvorak, 2007), as well as potentially neutral explanations such as a high rate of polyploid formation relative to extinction (e.g. Meyers and Levin, 2006; but see Mayrose *et al.*, 2011). Central to our understanding of the ecological aspects of polyploidy are cytogeographic studies of the distributions of diploids and polyploids across environments, allowing the association of cytotypes with environmental gradients such as altitude and latitude to be studied. Contemporary cytogeographic studies that combine direct chromosome counts

with flow cytometric estimation of genome size are informative not only for geographic patterns of ploidy, but also for understanding patterns of genome size evolution within polyploid complexes. The extensive range of genome sizes observed within polyploid complexes makes them ideal systems for investigating patterns of genome size variation across ploidy levels and across environments.

The ease with which genome size can be estimated through flow cytometry (FCM) has contributed to our understanding of patterns of genome size evolution through the accumulation of genome size estimates across numerous taxa, allowing for comparative examinations. In recent years, advances in FCM methods that control for interference from plant secondary compounds (e.g. Bennett *et al.*, 2008) have enabled rigorous documentation of intraspecific variation among individuals within populations (Šmarda *et al.*, 2008; Marhold *et al.*, 2010). The common trend among both auto- and allopolyploids is a reduction in genome size relative to the sum of the inferred parental diploids (Leitch and Bennett, 2004), although some examples of increased genome size have also been reported (Leitch *et al.*, 2008). However, in autopolyploids,

genome reduction appears to be associated with the restoration of diploid meiotic behaviour, as autopolyploids with polysomic inheritance may more commonly exhibit a pattern of genome size that is additive relative to the inferred parental genome sizes (Eilam *et al.*, 2010; Parisod *et al.*, 2010). Given what appears to be a general trend of selection for reduced genome size among neopolyploids, stochastic variation in the rate of DNA loss among populations may contribute to differences in genome size among polyploid populations.

Variation in the strength of selection across environments may also contribute to variation in genome size. Among the many genetic and ecological hypotheses suggested to explain patterns of variation in genome size, it has been proposed that larger genomes are adaptive in colder environments, where cell expansion rather than division is a more efficient means of growth (e.g. Grime and Mowforth, 1982; Knight and Ackerly, 2002). Thus, polyploids occurring in colder environments may experience reduced selection for genome reduction through the positive association between genome size and cell size. However, numerous other ecological factors have been proposed to influence genome size (Miller, 1978; Miller and Chambers, 2006), including selection for small genomes associated with rapid growth and short life spans, and smaller genomes associated with drought and other stressful environmental conditions (Kalendrar *et al.*, 2000; Jakob *et al.*, 2004; Knight *et al.*, 2005; Veselý *et al.*, 2012). Some of these patterns may be complicated by extensive endopolyploidy of somatic cells, where cells achieve polyploid DNA content through duplication of the genome within the nucleus, without cellular division (Barow, 2006; Lee *et al.*, 2009). With the intense interest in explaining patterns of genome size variation there is a need for further data on genome size variation, especially from groups with elevated variation such as polyploid complexes, to provide raw data for comparative studies across taxa.

In this paper, I report chromosome counts and genome size estimates from populations of the *Claytonia perfoliata* Don ex Wild (Portulacaceae) polyploid complex in western North America, and ask whether variation in genome size within a cytotype is associated with environmental variation in the form of temperature and precipitation. The *C. perfoliata* complex is an ideal system in which to investigate patterns of genome size variation with ploidy and environment, as classical cytogenetic studies document a range of ploidy from diploid to decaploid (Swanson, 1964; Miller, 1978; Miller and Chambers, 2006), and more recent work suggests ecological differentiation across cytotypes (McIntyre, 2012). The group is comprised of three allopatric diploid species, *C. rubra* (Howell) Tidestrom. ssp. *rubra*, *C. perfoliata* ssp. *mexicana* (Rydb.) John M. Miller & K.L. Chambers and *C. parviflora* ssp. *grandiflora* (Rydb.) John M. Miller & K.L. Chambers with a series of auto- and allopolyploid derivatives (Miller and Chambers, 2006; McIntyre, 2012). Apart from diploid *C. parviflora* ssp. *grandiflora*, cytotypes are highly selfing. The origins of many of the polyploid populations in the group are uncertain, but analysis based on chloroplast and internal transcribed spacer (ITS) sequences confirms the presence of *C. rubra* autotetraploids and allotetraploids between *C. parviflora* ssp. *grandiflora* and *C. perfoliata* ssp.

mexicana, while higher ploidy levels contain diverse sequences of the diploids *C. parviflora* ssp. *grandiflora* and *C. perfoliata* ssp. *mexicana*, suggesting multiple formation of polyploid lineages (Rausch, 2008). Polyploids are variously ascribed to a number of sub-specific designations, reflecting complex patterns of morphological variation thought to be associated with hybridization and polyploidy (Miller and Chambers, 2006); however, for the purposes of this study, plants were assigned identity as one of the diploids described above or as a polyploid belonging to *C. perfoliata* ssp. *perfoliata*, *C. parviflora* ssp. *parviflora* or *C. rubra* ssp. *depressa*, based on morphology, as described in Miller and Chambers (2006). Morphological intermediates not easily assigned to a taxon are identified as *Claytonia perfoliata sensu lato*.

MATERIALS AND METHODS

Sampling

A total of 78 populations of *Claytonia perfoliata* were sampled along the west coast of North America, between Washington State in the north and southern California to the south (Fig. 1, Supplementary Data Table S1). Sampling was focused to capture the diversity of reported cytotypes documented for the group from previous cytogenetic studies, spanning the range from diploids to decaploids (Miller, 1978; Miller and Chambers, 2006), but does not represent the entire geographic distribution of the *C. perfoliata* complex. Due to logistical constraints, sampling did not include disjunct diploids and decaploids from Mexico or Guatemala.

Flow cytometry

Genome size was estimated from a total of 121 individuals from the 78 localities using FCM. Many of these localities were previously surveyed for ploidy based on direct chromosome counts in earlier cytogenetic studies of the group (Swanson, 1964; Miller, 1978). Nuclear suspensions for FCM were prepared by grinding 3–6 seeds of *Claytonia* simultaneously with three seeds of an internal standard, *Solanum lycopersicum*, between two sheets of fine (27.9 grit cm⁻²) sandpaper in a 60 mm diameter Petri plate. *Solanum lycopersicum* cultivar Stupicke was used as an internal standard since it yielded clear 2C peaks from seed, and its 2C genome size of 1.96 pg (Dolezel *et al.*, 1992) was relatively close in size to that of the diploid *Claytonia* taxa. Seed rather than leaf tissue was used for analysis due to extensive endopolyploidy found in leaf tissue making determination of the first 2C peak difficult, along with strong interference in estimates of genome size associated with variation in leaf pigmentation and secondary chemistry among accessions. Following co-grinding of the sample and standard, the sandpaper and plate were washed with two 600 µL aliquots of Galbraith's buffer (Dolezel *et al.*, 2007), and the resulting suspension was filtered through two layers of Miracloth (CalBiochem, Pasadena, CA, USA). A 600 µL aliquot of filtered solution was transferred to a 5 mL tube and stained at a propidium iodide concentration of 50 µg mL⁻¹ along with RNase at a concentration of 50 µg mL⁻¹. Samples were kept on ice and protected from light for 30–60 min prior to analysis.



FIG. 1. Map of the western USA depicting locations of diploid, tetraploid, hexaploid and octoploid cytotypes of the *Claytonia perfoliata* complex identified in this study. Precise locations of all populations are provided in the Supplementary Data Table S1.

FCM measurements were performed on a Becton Dickinson FACScan flow cytometer equipped with a 488 nm argon laser maintained at the Optical Biology CORE Facility of the UC Davis Cancer Center. Peak means were identified using Cellquest software (Becton Dickson). Absolute DNA content was calculated for between one and three samples of seed from each of the 121 individual plants (accessions) from 78 localities. Replicate measurements of seeds from the same individual occurred on different days in order to obtain a more precise measure of average genome size of seeds from an individual while controlling for variation due to instrument drift and sample preparation. The average genome size across these replicates is reported for these plants. Readings with $>5\%$ coefficient of variation (CV) or <1000 nuclei were discarded. Genome size was compared among taxa at

the diploid, tetraploid and hexaploid levels using analysis of variance (ANOVA) in R 2.13.1 (R Development Core Team, 2010) followed by the Tukey HSD test for comparisons among groups.

Chromosome counts

Direct chromosome counts were made from meiotic or mitotic material from 65 accessions spanning the range of genome size estimates based on FCM. Mitotic counts were prepared from root tips while meiotic counts were prepared from whole immature flowers. Root tips were collected variously from rapidly growing greenhouse seedlings planted in small pots with the roots allowed to grow into sand, or from germinated seeds placed on filter paper in a refrigerator at 4°C .

Immature flowers were collected from leaf axils of mature plants. Both floral buds and root tips were fixed in 4:3:1 chloroform:ethanol:acetic acid for a minimum of 24 h. Prior to fixation, root tips were treated with 0.002 M 8-hydroxyquinoline for 3 h at 4 °C. Following fixation, plant material was stained in alcoholic hydrochloric acid–carmine for a minimum of 24 h (Snow, 1963), and heated to 60 °C for 1 h prior to preparation of squashes. Plant material was squashed onto a slide with 45 % acetic acid, with or without a drop of Hoyer's solution to make permanent slides.

Environmental variation and genome size

To test whether variation in genome size is associated with environmental variation, I compared genome size within a cytotype with minimum winter temperature and growing season precipitation (October–May, as members of the *C. perfoliata* complex within the study region typically germinate with late autumn or winter rains and flower between March and May). Climate data for accessions were obtained from monthly temperature and precipitation values extracted for each collection locality using the climate downscaling software ClimateWNA v.4.6.1 (Mbogga *et al.*, 2009; Wang *et al.*, 2011) based on the PRISM climate data set for North America (www.prism.oregonstate.edu; Daly *et al.*, 2002). Individual

regressions of genome size vs. temperature and precipitation were performed for all cytotypes with at least five accessions. In order to investigate patterns of genome size variation with environment across the entire complex, 2C genome size was standardized within cytotype (by subtracting the mean 2C genome size for a cytotype and dividing by the standard deviation for the cytotype). Environmental values were also standardized within cytotypes in order to account for relative differences in the range of environmental variation experienced by the different cytotypes. Linear and quadratic regression were used to examine the relationship between variation in standardized genome size and the standardized environmental variables for the entire complex. This approach is similar to that used in phenotypic selection analysis (Lande and Arnold, 1983; Kingsolver *et al.*, 2001). A sequential Bonferroni correction was used to adjust for multiple tests. Regressions were done in R 2.13.1 (R Development Core Team, 2010).

RESULTS

Flow cytometry from seed samples resulted in clear fluorescence peaks (Fig. 2) and revealed 2C DNA content ranging from 1.42 to 7.46 pg for the complex as a whole (Fig. 3, Table 1). The three diploid taxa had distinct 2C genome sizes ($F_{2,21} = 253.3$, $P < 0.001$, Tukey HSD), with *C. rubra*

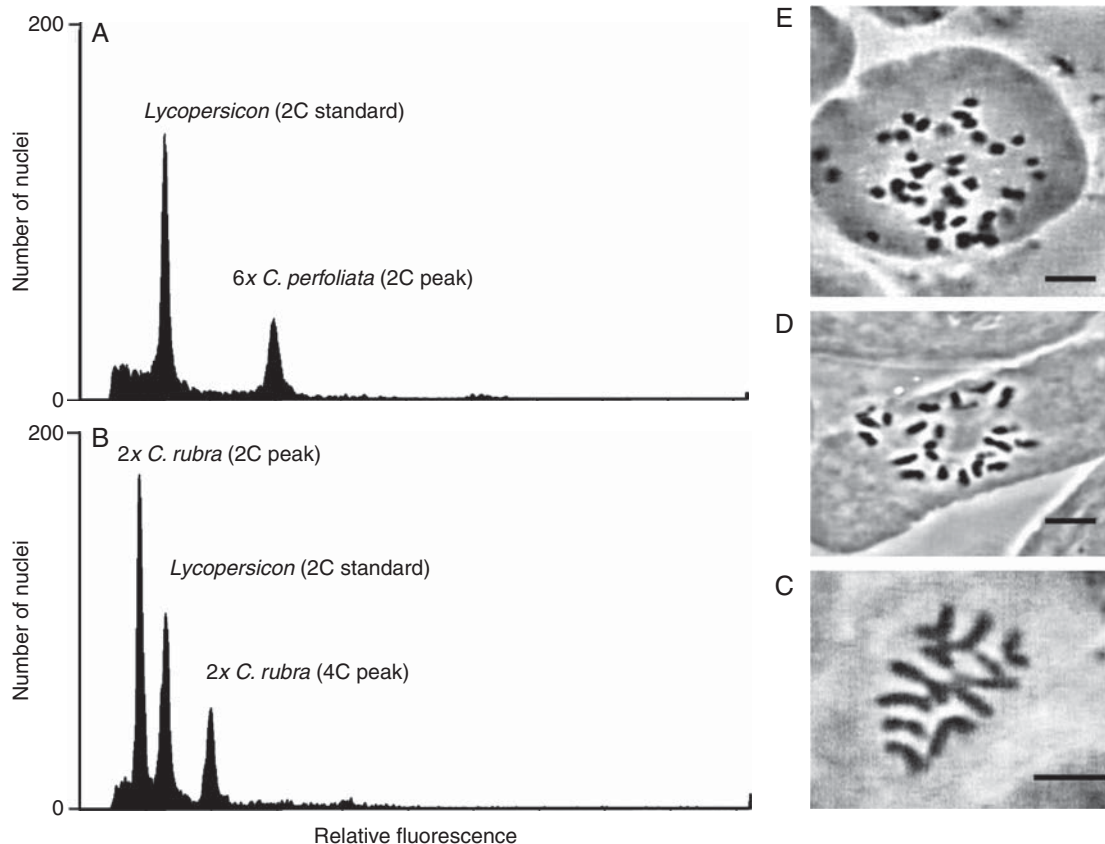


FIG. 2. Representative chromosome counts (mitotic) and flow cytometry results for the *C. perfoliata* complex. (A) Hexaploid *C. perfoliata* ssp. *perfoliata*. (B) Diploid *C. rubra* ssp. *rubra*. (C) Diploid *C. parviflora* ssp. *grandiflora*, $2n = 12$. (D) Tetraploid *C. parviflora* ssp. *parviflora*, $2n = 24$. (E) Octoploid *C. perfoliata* ssp. *perfoliata*, $2n = 48$. Scale bars = 10 μm .

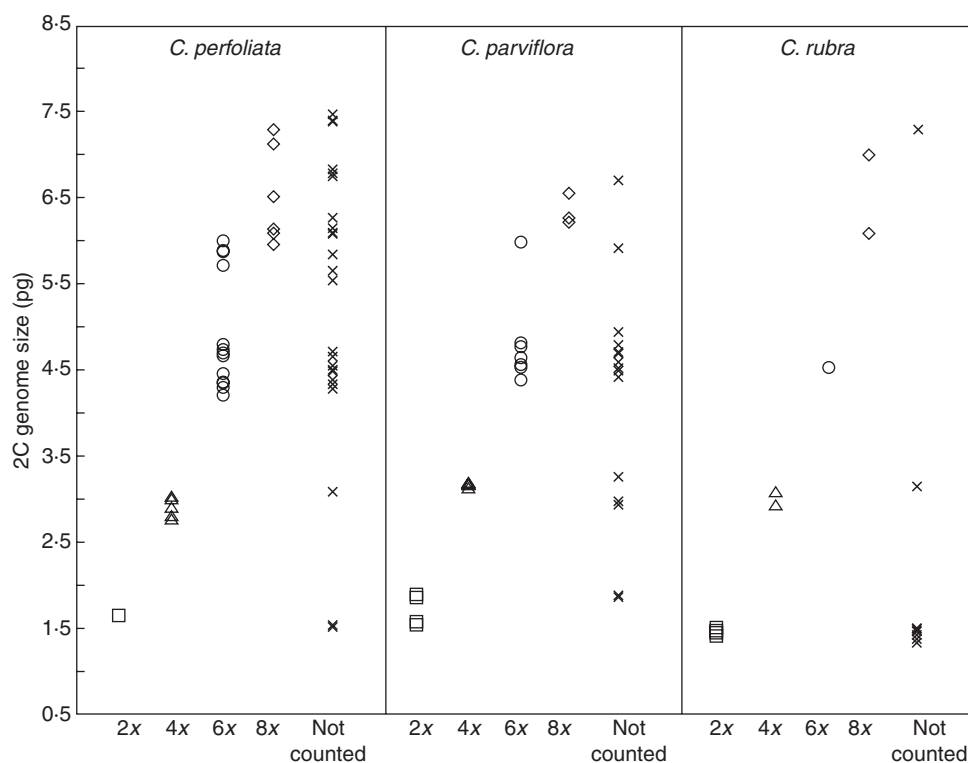


FIG. 3. Genome size (2C) estimates from flow cytometry for all individuals in this study, for *C. perfoliata*, *C. parviflora* and *C. rubra*. Symbols depict ploidy status as determined by direct chromosome counts, or whether values are from individuals without direct chromosome counts. Note the overlap in genome size between hexaploids and octoploids among individuals, preventing clear determination of ploidy from flow cytometry at these ploidy levels.

TABLE 1. Mean 2C genome sizes for cytotypes of the *Claytonia perfoliata* complex, with standard errors for genome size estimates, and inferred monoploid genome size (for a haploid set of six chromosomes). Taxa may be listed multiple times due to different ploidy levels or genome sizes within the same taxon

Taxon	Populations (individuals)	Inferred ploidy	2C genome size (pg)	s.e.	Inferred monoploid (1Cx) genome size (pg)
<i>C. perfoliata</i> subsp. <i>mexicana</i>	3 (7)	2x	1.53	0.01	0.77
<i>C. parviflora</i> subsp. <i>grandiflora</i>	5 (11)	2x	1.87	0.01	0.94
<i>C. parviflora</i> subsp. <i>grandiflora</i> – atypical	1 (3)	2x	1.55	0.01	0.78
<i>C. rubra</i> subsp. <i>rubra</i>	9 (12)	2x	1.44	0.01	0.72
<i>C. perfoliata</i> subsp. <i>perfoliata</i>	4 (5)	4x	2.94	0.05	0.73
<i>C. rubra</i> subsp. <i>depressa</i>	4 (5)	4x	3.06	0.06	0.76
<i>C. parviflora</i> subsp. <i>parviflora</i>	6 (7)	4x	3.12	0.05	0.78
<i>C. perfoliata</i> subsp. <i>perfoliata</i>	17 (23)	6x	4.52	0.07	0.75
<i>C. perfoliata</i> subsp. <i>perfoliata</i> – atypical	3 (3)	6x	5.81	0.04	0.97
<i>C. parviflora</i> subsp. <i>parviflora</i>	15 (18)	6x	4.62	0.04	0.77
<i>C. parviflora</i> subsp. <i>parviflora</i> – atypical	2 (2)	6x	5.99	0.06	1.00
<i>C. rubra</i> subsp. <i>depressa</i>	1 (2)	6x	4.58	0.06	0.76
<i>C. perfoliata</i> subsp. <i>perfoliata</i>	5 (6)	8x	6.44	0.20	0.81
<i>C. parviflora</i> subsp. <i>parviflora</i>	2 (2)	8x	6.42	0.11	0.80
<i>C. rubra</i> subsp. <i>depressa</i>	2 (2)	8x	6.53	0.45	0.82
<i>C. perfoliata sensu lato</i> -higher polyploids	11 (13)	8x or 10x	6.97	0.14	0.70–0.87

Sample sizes are given as the number of populations sampled, with the total number of individuals measured in parentheses.

ssp. *rubra* having the smallest genome and *C. parviflora* ssp. *grandiflora* the largest. One population of diploid *C. parviflora* ssp. *grandiflora* from the southern Sierra Nevada mountains (population ‘Trim-7’, Supplementary Data Table S1) had a genome size 18% smaller than that of other populations. In contrast to a typical 2C genome size of 1.87 pg for diploid

C. parviflora, this anomalous population had a 2C genome size of 1.55 pg based on FCM of seed from two individuals (FCM results on leaf tissue from three additional members of this population were consistent with this smaller genome size, but were not included in genome size estimates due to high CVs). Genome sizes were not significantly different

between *C. parviflora* and *C. perfoliata* for tetraploids ($F_{1,10} = 4.206$, $P < 0.067$) or hexaploids ($F_{1,23} = 0.006$, $P < 0.937$). *Claytonia rubra* was not included in comparisons of polyploid genome sizes due to small sample sizes for polyploids.

The majority of hexaploid plants had a 2C genome size between 4.2 and 4.7 pg; however, a subset of hexaploids in *C. perfoliata* and *C. parviflora* had a 2C genome size of approx. 5.9 pg, about 30% larger than typical of most hexaploids, and within the range of genome sizes for octoploids (Fig. 3, Table 1). These hexaploids were confirmed through multiple chromosome counts, and the elevated 2C content confirmed through multiple bouts of flow cytometry. These hexaploids contributed to a pattern of increased variation in genome content with ploidy level. Variation in 2C genome size was significantly greater for hexaploid cytotypes than diploids (Fig. 3), Levene's test ($F_{1,78} = 13.1$, $P = 0.005$). There was no general pattern of reduced monoploid (1C \times) genome content with higher ploidy, as mean monoploid genome content ranged from 0.72 to 0.94 pg among diploids and from 0.73 to 1.00 pg among polyploids. Several accessions had a genome content consistent with decaploidy, and decaploids are well documented from previous studies of this complex (Miller, 1978). However, chromosome squashes in the present study from accessions with putative decaploid levels of DNA suffered from such extensive overlap among chromosomes that a precise count was not possible (estimates ranged between 52 and 64). Chromosome counts documented a range of ploidy levels from diploid ($2n = 12$) to octoploid ($2n = 48$) based on a haploid set of six chromosomes as previously documented for the *C. perfoliata* complex (Fig. 2, Supplementary Data Table S1) (Swanson, 1964; Miller, 1978). No decaploid counts were confirmed in this study, although several accessions were consistent with a decaploid genome size in FCM, but with such poor spread and extensive overlap among chromosome preparations that an accurate count was not possible. The distribution of ploidy across the landscape was similar to that documented by previous researchers. Hexaploids of *C. parviflora* and *C. perfoliata* were widespread, while diploids of *C. rubra* were restricted to high latitudes or elevations, diploids of *C. perfoliata* were restricted to southern and coastal regions, and diploids of *C. parviflora* were restricted to the Sierra Nevada foothills (Fig. 1). Other cytotypes were sporadic in occurrence. In contrast to previous work, a mixed population of diploid and tetraploid *C. perfoliata* ssp. *mexicana* was documented in Cleveland National Forest in southern California. Although meiotic behaviour of populations was not generally recorded in this study (as the focus was ploidy determination), the meiotic behaviour of tetraploids from this population was distinctly aberrant, with the majority of observed meioses (seven of 12) exhibiting behaviours such as asynchronous meiosis, lagging chromosomes and multiple spindle orientations (e.g. Vasek, 1962) leading to unbalanced gametes. Nearly all meiotic events from other populations were normal, apart from occasional lagging chromosomes, as reported from previous observations of *C. perfoliata* polyploids (Swanson, 1964; Miller, 1978).

Comparisons of genome size with environmental variables revealed a significant but weakly negative linear relationship between variation in genome size and minimum winter

TABLE 2. Regression and partial regression coefficients (values from simple and multiple regression, respectively) for the linear and quadratic relationships between variation in genome size and variation in environmental factors across the *Claytonia perfoliata* complex

	Regression coefficient		Partial regression coefficient	
	Linear	Quadratic	Linear	Quadratic
Winter temperature	-0.26**	-0.12	-0.23*	-0.088
Growing season precipitation	0.19	-0.127	0.129	-0.11

Genome size and environmental variables were standardized within cytotypes prior to regression analysis.

* $P < 0.05$; ** $P < 0.01$.

TABLE 3. Results of regressions of genome size on winter temperature and growing season precipitation

Cytotype	<i>n</i>	Minimum winter temperature		Growing season precipitation	
		<i>B</i>	r^2	<i>B</i>	r^2
<i>C. parviflora</i> 2x	13	-0.470	0.225	0.164	0.027
<i>C. parviflora</i> 4x	7	-0.974**	0.939	0.152	0.023
<i>C. parviflora</i> 6x	16	-0.060	0.004	0.403	0.163
<i>C. perfoliata</i> 2x	6	-0.686	0.470	0.633	0.401
<i>C. perfoliata</i> 4x	8	-0.562	0.317	0.240	0.058
<i>C. perfoliata</i> 6x	30	-0.019	0.001	-0.168	0.028
<i>C. rubra</i> 2x	14	-0.056	0.003	0.196	0.039
8x (combined)	25	-0.105	0.011	-0.247	0.060

Slope values (*B*) are standardized coefficients, in order to compare values of slope across the two environmental factors. Significance tests were adjusted using the sequential Bonferroni procedure.

** $P < 0.01$.

temperature for the complex as a whole ($R^2 = 0.049$, $B = -0.26$, $P = 0.003$; Table 2). However, when individual cytotypes were examined, this relationship was significant only for tetraploid *C. parviflora*, for which minimum winter temperature was strongly negatively correlated with genome size ($R^2 = 0.939$, $B = 0.974$, $P = 0.006$; Table 3). The relationship for the complex as a whole was not driven solely by tetraploid *C. parviflora*, as the results changed only slightly when these were excluded from the analysis ($R^2 = 0.023$, $B = -0.21$, $P = 0.033$). No significant correlations were found between genome size and precipitation for the complex as a whole (Table 2) or for individual cytotypes (Table 3).

DISCUSSION

Chromosome counts revealed a similar geographic distribution of cytotypes to that previously reported (Swanson, 1964; Miller, 1978), with geographically widespread and co-occurring hexaploids of *C. parviflora* and *C. perfoliata*, scattered octoploids, and allopatric diploids of *C. rubra*, *C. perfoliata* and *C. parviflora*, restricted, respectively, to northern and montane, southern and coastal, and Sierran

foothill environments (Fig. 2D). FCM, however, suggested previously undocumented, large-magnitude variation in genome size within several cytotypes. In addition, the presence of tetraploids within a diploid population of *C. perfoliata* ssp. *mexicana* suggests the possibility of ongoing formation of polyploids, and highlights the potential for contemporary gene flow from diploid to polyploid populations via newly formed polyploids. Previous investigations of *C. perfoliata* ssp. *mexicana* have not documented mixed diploid–tetraploid populations for this taxa, nor meiotic irregularities among tetraploids (Swanson, 1964; Miller, 1978; Miller and Chambers, 2006). The irregular meiosis observed from these tetraploids suggests the possibility that they are recently formed autotetraploids. Although irregular gamete formation is often a poor indicator of autopolyploidy vs. allopolyploidy and time since ploidy formation (Ramsey and Schemske, 2002), normal meiotic behaviour is typical of *C. perfoliata* polyploids, suggesting that recent autopolyploidy should be investigated as an explanation.

Extensive variation in genome size within cytotypes was found for hexaploids of *C. parviflora* and *C. perfoliata*, and for diploids of *C. parviflora* ssp. *grandiflora*. For diploid *C. parviflora* ssp. *grandiflora*, members of one population were found to have a genome size 18% smaller than typical (1.55 pg. vs. 1.87 pg), while exhibiting typical diploid chromosome counts ($n = 6$). This population was located in the southern Sierra Nevada foothills and was morphologically distinct from other populations of *C. parviflora* ssp. *grandiflora* in that the plants possessed strongly pigmented red foliage and paler than typical pink floral colour. There are several possibilities that may explain the markedly reduced genome size of this population. Individuals at this site may be a cryptic species, may represent individuals from an otherwise typical *C. parviflora* ssp. *grandiflora* genomic background which has experienced genome reduction, or may be hybrids between *C. parviflora* ssp. *grandiflora* and a congener with a smaller genome. Alternatively, hybridization among other populations of this taxa might have led to an increase in genome size (Baack *et al.*, 2005; Ungerer *et al.*, 2009). Phylogenetically informative DNA sequence information from this population and other members of the *C. perfoliata* complex and closely related lineages would allow discrimination among these hypotheses. Although secondary compounds associated with red pigmentation such as anthocyanins or betalains may reduce fluorescence readings and lead to spuriously small genome size estimates (Dolezel *et al.*, 2007; Bennett *et al.*, 2008), readings were consistently lower for this population from both leaf and seed tissue and for an alternative buffer solution containing β -mercaptoethanol to reduce interference from cytosolic compounds (Ramsey, 2007).

The typical range of genome size for hexaploid *C. perfoliata* (approx. 4.4–4.7 pg) agrees with the previously reported genome size of 4.35 pg for a plant with a hexaploid chromosome count (Bennett and Smith, 1976). However, a portion of hexaploid plants confirmed by chromosome counts were documented with a genome size 30% larger than typical (approx. 5.9 pg), placing them within the range of octoploid populations. These plants were widespread, occurring between the Southern Coast Ranges of California and the northern Sierra Nevada foothills. These plants were extreme

examples of a general pattern of increased genome size variation at higher ploidy levels (Fig. 3). A potential explanation for varying genome size among higher polyploids includes differential genomic contributions of diploid *C. parviflora* ssp. *grandiflora*, *C. perfoliata* ssp. *mexicana* and *C. rubra* ssp. *Rubra*, which have monoploid genomes of 0.94, 0.77 and 0.72 pg, respectively, to distinct hexaploid lineages. However, the monoploid genome content of atypical hexaploids averaged 0.97 pg, slightly larger than the monoploid content of *C. parviflora* ssp. *grandiflora* (0.94), while an intermediate monoploid genome content would be expected if differential contributions of the diploid genomes of other species were accounting for hexaploids with high 2C values. One possibility is that other species of *Claytonia* with larger genomes may hybridize with members of the *C. perfoliata* complex. *Claytonia sibirica* represents a potential source of introgression, as it is hypothesized to have hybridized with *C. perfoliata* to form the allotetraploid *C. washingtoniana* (Fellows, 1976), and is sympatric in portions of California, Washington and Oregon with members of the *C. perfoliata* complex (Miller *et al.*, 1984). In addition, genomic contributions from putatively extinct diploid lineages have been documented in polyploid complexes (e.g. Brokaw and Hufford, 2010; Jakob and Blattner, 2010), representing an additional source of historic introgression. An additional possibility would be within-lineage factors associated with genome size, such as the spread of retrotransposons within some populations leading to increased genome size (e.g. Bennetzen *et al.*, 2005; Hawkins *et al.*, 2006; Ungerer *et al.*, 2009). The use of seeds rather than adults in this study might result in an increase in measured variation within lines, and might result in differences between genome size estimates from adult tissue, as individual seeds may vary in genome size and also differ in their likelihood of survival. However, variation within lines would be unlikely to affect the comparisons in this study which are based on differences among maternal lines and across ploidy levels, unless there were very strong biases in viability of seeds or fitness of offspring among maternal lines with different genome size, which was not observed in field experiments involving these same maternal lines (McIntyre, 2011).

Environment and genome size

Environmental variation in the form of mean winter temperature was weakly negatively correlated with genome size across the complex, and strongly so for *C. parviflora* tetraploids, but not for any other individual cytotype. It is unclear why such a strong relationship would be found for only one cytotype; however, the sampling distribution of cytotypes across the landscape may influence the potential to detect such a correlation. Relative to other cytotypes, sampling for *C. parviflora* covered a wide latitudinal gradient, with little variation in whether populations were inland or coastal. It is possible that variation in whether a population is inland or coastal may reflect other environmental conditions altering selection on genome size. Alternatively, *C. parviflora* might be subject to stronger selection for genome size in relation to winter temperature than other members of the complex. Previous analysis of the distributions of cytotypes on the landscape suggests niche differentiation across ploidy levels

(McIntyre, 2012), and it would be interesting to investigate whether variation in genome size is also associated with ecological differentiation, or if similar ecological factors are associated with variation in both ploidy and genome size. More extensive measurements of genome size from across the distribution of the *C. perfoliata* complex would be informative in determining whether genome size is associated with other environmental factors for individual cytotypes, as the number of individuals sampled in the study (121) did not allow for fitting multivariate relationships within cytotypes.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: detailed information for all individual plants utilized in the study, including sampling location, ploidy level, n , $2n$ and $2C$ data.

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