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ARTICLE

# Geographical parthenogenesis in Pacific Northwest hawthorns (*Crataegus*; Rosaceae)

E.Y.Y. Lo, S. Stefanović, and T.A. Dickinson



**Abstract:** We have demonstrated geographical parthenogenesis in *Crataegus* series *Douglasianae*, an agamic complex comprising exclusively tetraploid *Crataegus douglasii* sensu lato and the morphologically distinct *Crataegus suksdorfii* complex that comprises diploids and polyploids. Here we characterize ploidy level and breeding system by detailed flow cytometric measurements of the 2C nuclear DNA content of leaf, embryo, and endosperm tissues from 282 black-fruited hawthorns (*Crataegus* series *Douglasianae*) representing 33 localities in the Pacific Northwest, one in the Cypress Hills, and three more in the upper Great Lakes basin. We use existing climate and molecular data to place our flow cytometry results in an environmental and evolutionary context. *Crataegus douglasii* occupies more widely distributed sites that experience more extreme temperature and moisture regimes than do the sites occupied by diploid *C. suksdorfii*.

*Key words:* geographical parthenogenesis, gametophytic apomixis, nuclear DNA content, flow cytometry, climate, cytotype, hawthorn.

**Résumé :** Les auteurs ont démontré la présence de parthénogenèse géographique chez des *Crataegus* de la série *Douglasianae*, un complexe agame comportant le *Crataegus douglasii* exclusivement tétraploïde sensu lato et le complexe morphologiquement distinct *Crataegus suksdorfii* comportant des diploïdes et des polyploïdes. Les auteurs caractérisent ici le degré de ploïdie et le système de croisement suite à des mesures détaillées par cytométrie en flux de l'ADN nucléique 2C de la feuille, de l'embryon et des tissus de l'endosperme, à partir de 282 aubépines noires (*Crataegus* série *Douglasianae*) provenant de 33 localités du Nord-Ouest Pacifique, une des Cypress Hills, et trois autres de la partie supérieure du bassin des Grands Lacs. Ils utilisent le climat actuel et les données moléculaires pour situer leurs résultats de cytométrie dans un contexte environnemental et évolutif. Le *C. douglasii* occupe des sites plus largement distribués, expérimentant des températures et des régimes hydriques plus extrêmes que ne le connaissent les sites occupés par le *C. suksdorfii*. [Traduit par la Rédaction]

*Mots-clés :* parthénogenèse géographique, apomixie gamétophytique, teneur en ADN nucléique, cytométrie en flux, climat, cytotype, aubépine.

## Introduction

Geographical parthenogenesis is a phenomenon in which asexual individuals generally tend to be more geographically widespread than their sexual relatives (Vandel 1928, 1940), where the latter are usually self-sterile (Schön et al. 2000; Law and Crespi 2002). Asexual reproduction has been hypothesized to be particularly important to organisms that inhabit environments subjected to disturbance, whether natural (e.g., flood plains) or man-made (e.g., abandoned or poorly managed agricultural land), or in marginal habitats where populations are overly fragmented (Stebbins 1985; McLellan et al. 1997; Morris et al. 2004). When disturbance is frequent and local populations go extinct, or when the potential for severe inbreeding depression exists, asexuals might be favored because of their ability to reproduce autonomously, without loss of heterozygosity (Haag and Ebert 2004).

In plants, apomicts are almost exclusively polyploids, and the geographical pattern occurs mostly in apomictic allopolyploid complexes (see reviews by Bierzychudek (1985), Hörandl (2006), Hörandl et al. (2008), and Whitton et al. (2008)). With few exceptions (e.g., Thomas 1997), geographical parthenogenesis has been reported mostly in herbaceous species such as Asteraceae (Houliston and Chapman 2004; Verduijn et al. 2004; Thompson and Whitton 2006) and Ranunculaceae (Paun et al. 2006; Cosendai and Hörandl 2010). Although there exists an ontogenetic link between apomixis and polyploidy (Ramsey and Schemske 1998; Whitton et al. 2008; Talent 2009), there are few answers to the questions of how these two processes, together with hybridization, influence distribution within a group and whether variation in the environment has played a role contributing to contrasting geographical patterns. Here we document the association of apomixis, polyploidy, and differences in the areal and environmental extent of cytotype distributions, using data from woody perennials in *Crataegus* series *Douglasianae* (Loud.) Poletiko (section *Douglasianae* Loud.), the black-fruited hawthorns of the Pacific Northwest.

As in most other Rosaceae in which agamospermy occurs, the underlying developmental process in hawthorns is generally apospory. This is the form of gametophytic apomixis in which cells of the sporophyte (with the unreduced number of chromosomes) develop into gametophytes without the occurrence of meiosis (Talent 2009; Supplementary Fig. 1[1]) The unreduced female gametes produced in this way develop directly into embryos with-

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out the occurrence of fertilization (parthenogenesis) and give rise to seed, but usually only if endosperm fertilization occurs (pseudogamy). Therefore, successful asexual seed production still depends on pollination taking place. However, in Rosaceae, self-incompatibility is of the gametophytic type, and thus in polyploids, self-pollination can be as effective as cross pollination in eliciting seed set (Dickinson and Phipps 1986; Dickinson et al. 1996). Because female gametes continue to be produced in apospory, albeit by mitotic divisions, it is also possible for fertilization to occur, resulting in not only endosperm, but also an embryo, with increased ploidy levels (Talent and Dickinson 2007*a*; see Supplementary Fig. 1, but note that fertilization of an unreduced female gamete is not shown).

In this study, the DNA content of nuclei extracted from vegetative tissues is used to indicate ploidy level of an individual (Talent and Dickinson 2005). The nuclear DNA content of seed tissues, and hence the relative ploidy levels of embryo and endosperm, are used to infer the breeding system (Matzk et al. 2000; Talent and Dickinson 2007*a*,2007*b*). Together with information about the maternal cytotype, comparison of embryo and endosperm ploidy levels tells us whether the megagametophyte from which a seed developed was reduced or unreduced, as well as whether and what kinds of fertilizations were involved in the development of the embryo and of the endosperm (Supplementary Fig. 1). By documenting ploidy level and breeding system, we seek to compare diploids and polyploids in *Crataegus* series *Douglasianae* with respect to environmental factors operating at the sites where they occur and to the areal extent of their geographic distributions. Furthermore, we use existing molecular data (Lo et al. 2009*a*) to examine genetic relationships among the populations that we studied to place differences in ploidy level and reproductive behavior in a historical context and to compare differentiation between these populations to features of the environment.

## Materials and methods

### Study system and plant sampling

In the Pacific Northwest (Fig. 1), *Crataegus* series *Douglasianae* comprises two morphologically distinct entities that have been recognized since at least the early 20th century: *C. douglasii* Lindl. (with approximately 10 stamens per flower) and *C. suksdorfii* (Sarg.) Kruschke. (with approximately 20 stamens per flower). *Crataegus douglasii* has been shown so far to be uniformly tetraploid (Talent and Dickinson 2005). Recently, some segregates of *C. douglasii* have been described as morphological species (Phipps and O'Kennon 1998, 2002), but the differences between them do not include differences in ploidy level (Talent and Dickinson 2005; N. Talent, unpublished data, 2011). Also, genetic data have not distinguished these segregate taxa (Lo et al. 2009*a*). Hence, we consider them collectively as representatives of *C. douglasii* sensu lato in this study (Table 1). *Crataegus suksdorfii*, on the other hand, does vary in ploidy level (Table 1; Dickinson et al. 1996; Talent and Dickinson 2005) but has not yet been dismembered into segregate taxa. Both auto- and allo-triploids are known, as are allotetraploids (and putative autotetraploids), all of which resemble diploid *C. suksdorfii* to the extent that they have around 20 stamens per flower. The allopolyploids appear to have arisen as a result of introgression from *C. douglasii* (Lo et al. 2009*a*, 2010). Geographically, *C suksdorfii* occurs from the Rocky Mountains west to the Pacific Ocean and north to British Columbia and the southern tip of the Alaska panhandle (fig. 2, second panel, in Dickinson et al. 2008). *Crataegus douglasii* sensu lato has a distribution similar to that of *C. suksdorfii* but is the only taxon found further east in the Cypress Hills of southern Alberta and Saskatchewan and in the upper Great Lakes basin (Marquis and Voss 1981; fig. 1 in Brunsfeld and Johnson 1990; fig. 2, top panel, in Dickinson et al. 2008; Coughlan 2012). Zarrei et al. (2012) have shown that *C. douglasii* arose by hybridizations between ancestral *C. suksdorfii* and red-fruited members of *Crataegus* section *Coccineae* Loud. emend. J.B. Phipps (Phipps et al. 1990), as proposed by Brunsfeld (Brunsfeld and Johnson 1990).

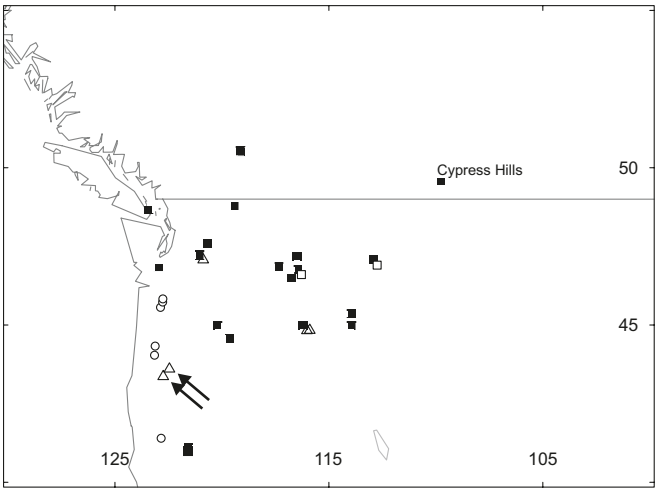



**Fig. 1.** Geographic distribution of *Crataegus* series *Douglasianae*; North American sites studied here (Table 1; not shown, three sites in Ontario, Canada). Solid squares denote sites at which tetraploid *C. douglasii* sensu lato was sampled. Open circles, open triangles, and open squares denote sites where *C. suksdorfii* diploids, triploids, and tetraploids, respectively, were sampled. Arrows: sites at which *C. suksdorfii* was determined to be autopolyploid (Dickinson et al. 1996; Lo et al. 2009*a*).

We sampled a total of 282 individuals of *C. suksdorfii* and *C. douglasii* collected from 33 localities in the Pacific Northwest, one in the Cypress Hills of Saskatchewan, Canada, and three in Ontario, Canada (Fig. 1; Table 1). Where local populations were sufficiently large, sampling of individuals was randomized (Lo et al. 2009*a*). Great-circle distances between collecting sites (Table 1) were calculated according to the haversine formula (Veness 2010). Distances were averaged for each cytotype to compare spatial ranges among cytotypes in a manner analogous to Levene's test for equality of dispersion (Van Valen 1978; Schultz 1985). Mature leaves and fruits were collected in the fall of 2004–2007, and the number of individuals collected corresponded approximately to the number of trees present per site. Because fruit production failed or because we were unable to return to some sites, fruits were collected in 25 of the 37 localities (Table 1). With one to nine seeds per tree and one to 10 trees in each locality, nuclei from a total of 417 seeds were examined by flow cytometry. Our sample also included five population samples representing closely related *Crataegus* series *Cerrones* (section *Douglasianae*; Table 1). These were used as outgroups for our sample of *Crataegus* series *Douglasianae* (see below). Except as noted in Table 1, vouchers of all studied trees are deposited in the Green Plant Herbarium of the Royal Ontario Museum (TRT).

### DNA quantification from leaf and seed tissues

Leaves and fruits were kept at 4 °C after collection. Leaves were processed within a week after collection, and fruits were processed within 2–3 months. All of the pyrenes in each fruit were opened to extract any seed present. Seeds of *C. suksdorfii* and *C. douglasii* have a white embryo covered with a thin translucent layer of endosperm usually adhering to the brown seed coat when fully developed. For each seed, the endosperm and embryo were extracted and analyzed. The preparation of nuclear suspensions from leaf and seed materials and the determination of DNA content followed the protocol by Talent and Dickinson (2005), using a FACSCalibur flow cytometer (Becton Dickinson) equipped with an argon laser and detector (585 nm wavelength) for fluorescence of propidium iodide stained samples. Nuclear DNA content was es-

**Table 1.** Geographic coordinates and elevations of collecting sites (Fig. 1) and total number of leaf and seed samples of *C. douglasii* sensu lato and *C. suksdorfii* sensu lato in the present study.

| Species | Site label | Latitude (°N) | Longitude (°W) | Elevation (m) | State or province; county; locality | No. of individuals | No. of seeds |
|---|---|---|---|---|---|---|---|
| *C. douglasii* | BC3 | 50.55 | 119.13 | 360 | BC; Enderby | 1 | 6 |
| | BC14 | 50.51 | 119.10 | 396 | BC; 8.1 km SSE of Enderby | 3 | 0 |
| | BC67 | 48.67 | 123.42 | 13 | BC; Saanich Peninsula | 1 | 0 |
| | CAR2 | 41.11 | 121.57 | 1015 | CA; Shasta; Dana | 1 | 5 |
| | CAR3 | 40.98 | 121.56 | 841 | CA; Shasta; Hat Creek | 3 | 0 |
| | CA3 | 41.01 | 121.60 | 835 | CA; Shasta, Dusty Campground | 1 | 5 |
| | ID2 | 46.77 | 116.45 | 811 | ID; Latah; Little Boulder Creek | 7 (8) | 17 (4)+15 (3) |
| | ID3 | 47.18 | 116.49 | 786 | ID; Benewah; St. Maries River, Santa Creek | 2 (2) | 6 (2)+3 (1) |
| | ID6 | 44.99 | 116.19 | 1420 | ID; Adams; Last Chance Campground, near Meadows | 17 (3) | 35 (10) |
| | ID15 | 44.97 | 113.94 | 1292 | ID; Lemhi; US 93 S of Gibbonville | 2 (3) | + 5 (1) |
| | ID16 | 45.37 | 113.95 | 1122 | ID; Lemhi; US 93 N of Salmon | 4 (1) | 9 (3) |
| | ID20 | 46.52 | 116.73 | 280 | ID; Nez Perce; Little Potlatch Creek | 7 (3) | 7 (3)+5 (2) |
| | MT2 | 47.07 | 112.91 | 1356 | MT; Powell; Kleinschmidt Flat | 14 (1) | 26 (6) |
| | ON18 | 48.45 | 89.19 | 200 | ON; Thunder Bay | 1 (2) | + 10 (2) |
| | ON20 | 44.75 | 80.95 | 225 | ON; Grey; Big Bay, Colpoy's range | 6 (16) | 8 (3)+11 (3) |
| | ON21 | 44.90 | 81.20 | 250 | ON; Bruce; Barrow Bay | 4 | 3 (1) |
| | OR | 44.58 | 119.64 | 655 | OR; Grant; John Day River, South Fork | 1 | 0 |
| | OR31 | 44.99 | 120.20 | 845 | OR; Wheeler; Fossil | 2 | 0 |
| | SK | 49.58 | 109.77 | 1219 | SK; Cypress Hills | 2 | 0 |
| | WA | 47.58 | 120.66 | 100 | WA; Chelan | 3 | 0 |
| | WA20 | 47.24 | 121.04 | 747 | WA; Kittitas; Cle Elum | 5 | 24 |
| | WA21 | 46.84 | 122.98 | 64 | WA; Thurston; Mound Prairie | 10 (13) | 8 (3)+9 (2) |
| | WA22 | 46.85 | 117.34 | 666 | WA; Whitman; South of Colfax | 4 (3) | 4 (2)+2 (1) |
| | WA24 | 48.79 | 119.40 | 259 | WA; Okanogan; Ellisforde | 1 | 7 |
| *C. suksdorfii* | CAR5 | 41.40 | 122.84 | 871 | CA; Siskiyou; Fay Lane | 8 | 35 (8) |
| | ID2 | 46.77 | 116.45 | 811 | ID; Latah; Little Boulder Creek | 5 | 14 (3) |
| | ID5 | 45.00 | 116.06 | 1524 | ID; Valley; North Beach, Payette Lake | 5 (1) | 15 (4) |
| | ID6 | 44.99 | 116.19 | 1420 | ID; Adams; Last Chance Campground, near Meadows | 15 (5) | 14 (3) |
| | MT2 | 47.07 | 112.91 | 1356 | MT; Powell; Kleinschmidt Flat | 13 (9) | 33 (7)+7(2) |
| | OR1 | 44.33 | 123.12 | 88 | OR; Linn; Cogswell Foster Reserve | 7 (3) | 18 (5) |
| | OR2 | 44.04 | 123.15 | 119 | OR; Lane; Bertelson Rd., West Eugene | 2 | 0 |
| | OR4 | 43.53 | 122.91 | 1250 | OR; Douglas; Elk Meadows RNA | 5 | 0 |
| | OR6 | 43.77 | 122.62 | 1295 | OR; Lane; Patterson Mountain Prairie | 20 | 0 |
| | OR7 | 45.56 | 122.87 | 55 | OR; Washington; Hillsboro. | 1 | 0 |
| | OR11 | 45.73 | 122.77 | 10 | OR; Columbia; Sauvie Island | 11 (8) | 35 (6) |
| | WA20 | 47.24 | 121.04 | 747 | WA; Kittitas; Cle Elum | 2 | 7 (2) |
| | WA7 | 45.83 | 122.76 | 15 | WA; Clark | 1 (10) | 0 |
| Outgroups | | | | | | | |
| *C. rivularis* | ID13 | 42.32 | 111.21 | 1951 | ID; Bear Lake; Montpelier Canyon | — | — |
| | ID13a | 42.32 | 111.25 | 1859 | ID; Bear Lake; E of Montpelier | — | — |
| *C. saligna* | CO1 | 40.03 | 107.86 | 1926 | CO; Rio Blanco; E of Meeker | — | — |
| | CO6 | 40.03 | 108.13 | 1798 | CO; Rio Blanco; W of Meeker | — | — |
| | UT5 | 40.21 | 110.41 | 1722 | UT; Duchesne; Duchesne River valley | — | — |
| **Total counts** | | | | | | **254** | **398** |

**Note:** Sample sizes for flow cytometric ploidy level determinations from leaf tissue are indicated as follows: vouchered individuals and, in parentheses, unvouchered individuals. The numbers of seeds studied by flow cytometry is given together with the number of vouchered individuals that provided those seeds (in parentheses). Plus signs designate seeds from unvouchered individuals (numbers of individuals in parentheses). Site labels comprise the standard two-letter acronyms for the USA states and the Canadian provinces and serial numbers that are consistent with earlier studies.

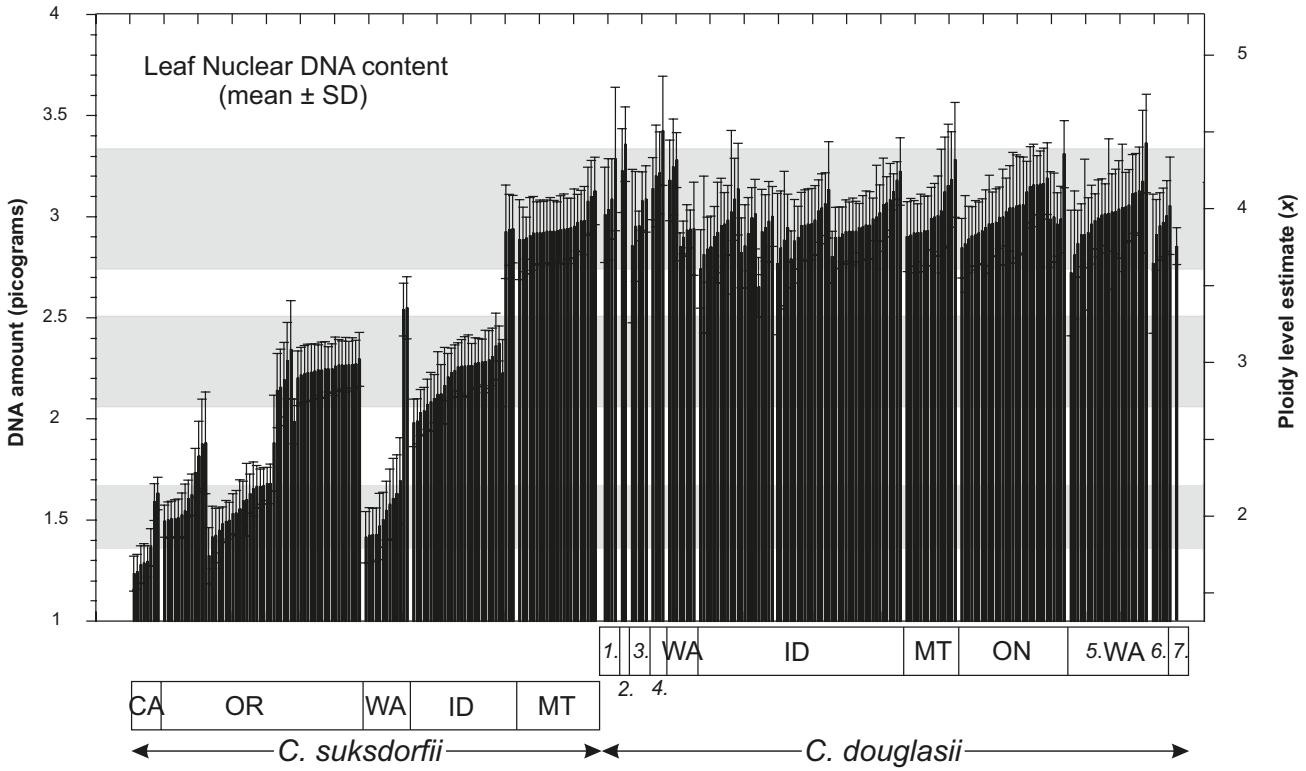


timated as a ratio between the fluorescence of the *Crataegus* samples and the standard *Pisum sativum*, with a 2C value of 9.56 pg DNA per *Pisum* nucleus (Johnston et al. 1999). Because the peak of *Pisum* may overlap with that of the endosperm of some *Crataegus* polyploids (Talent and Dickinson 2007*a*) when both are mixed, we used the embryo as an internal standard for the endosperm. Separately, we checked that the embryo ploidy level was well within an euploid range using *Pisum* as an external standard (Talent and Dickinson 2007*a*). This approach is considered suitable for routine estimation of ploidy level, if not necessarily adequate for the detection of aneuploidy (Doležel et al. 2007). Histograms showing fluorescence height of the selected particles were used for statistical analyses. In addition to the mean 2C values ($\mu$), the standard deviation ($\sigma^2$) in each measurement was calculated following the methods of Dickson et al. (1992). Measurements with a ratio of standard deviation to mean ($\sigma^2/\mu$) greater than 15% were discarded because they might represent inaccurate estimation.

### Statistical analyses

All data analyses and graphics were produced using R (R Development Core Team 2008), except as noted. The Mann–Whitney test was used to compare the distances between the *C. douglasii* sites with those between the *C. suksdorfii* ones. DNA content of the endosperm ($\mu_{end}$) was divided by that of the embryo ($\mu_{emb}$) in each seed sample as an index of whether a seed was sexually or asexually produced (Supplementary Fig. 1). To examine relationships between nuclear DNA content (as a continuous variable indicative of ploidy level) and environmental attributes, monthly values of six climate variables (75% probability precipitation, mm/month; mean daily air temperature, degrees Fahrenheit (°F); percent rel-

**Fig. 2.** Leaf nuclear DNA content for individuals of *Crataegus douglasii* sensu lato and *C. suksdorfii* sensu lato (Table 1). Provenances of the individuals sampled are indicated in the horizontal bars: CA, California; OR, Oregon; WA, Washington; ID, Idaho; MT, Montana; ON, Ontario, Canada; (1) British Columbia (BC), Canada; (2) Saskatchewan, Canada; (3) California; (4) Oregon; (5) individuals segregatable as *C. castlegarensis* (WA21); (6) individuals segregatable as *C. okennonii* (WA22); and (7) one individual segregatable as *C. shuswapensis* (BC3). Shaded areas show the range for each ploidy level derived from an average estimate of 0.76 pg/genome ± 10%. We define values of 1.37–1.67 pg as diploids, 2.05–2.51 pg as triploids, and 2.74–3.34 pg as tetraploids (cf. Talent and Dickinson 2005).






ative humidity; percent maximum sunlight hours, mean wind speed, m/s; and days with ground frost) were obtained from the International Water Management Institute's Online Climate Summary Service Portal (http://www.iwmi.cgiar.org/WAtlas/) for each of the localities (Table 1). These data were then averaged to give single, annual values that were used in all subsequent analyses. In view of the inherent multicollinearity exhibited among climate variables, we also used principal component analysis (PCA; R function prcomp, data made commensurate by standardizing to unit variance) to reduce data dimensionality and display the relationship between the climate variables and site–cytotype combinations in the form of a biplot. The proportions of the total variance accounted for by the PC axes were compared with critical values of the broken-stick criterion, calculated as described by Legendre and Legendre (1998).

### Genetic relationships

With data from 13 microsatellite loci (Lo et al. 2009*a*), we computed the $\Delta\mu^2$ distance values between each pair of populations using SPAGEDI version 1.2 (Hardy and Vekemans 2002). Because microsatellites are likely evolving via a stepwise mutation model (SMM; Slatkin 1995), we used this model, which assumes that the allelic size difference is proportional to the number of mutational events that have occurred since their most recent common ancestor. A neighbor-joining (NJ) tree based on the $\Delta\mu^2$ distances was constructed in PHYLIP version 3.66 (Felsenstein 2006) to visualize genetic relationships. Samples of *C. rivularis* and *C. saligna* were used to root this tree because these two species, in series *Cerrones*, have been shown to be sister to *C. suksdorfii* and *C. douglasii* (Lo et al. 2009*b*; Table 1).

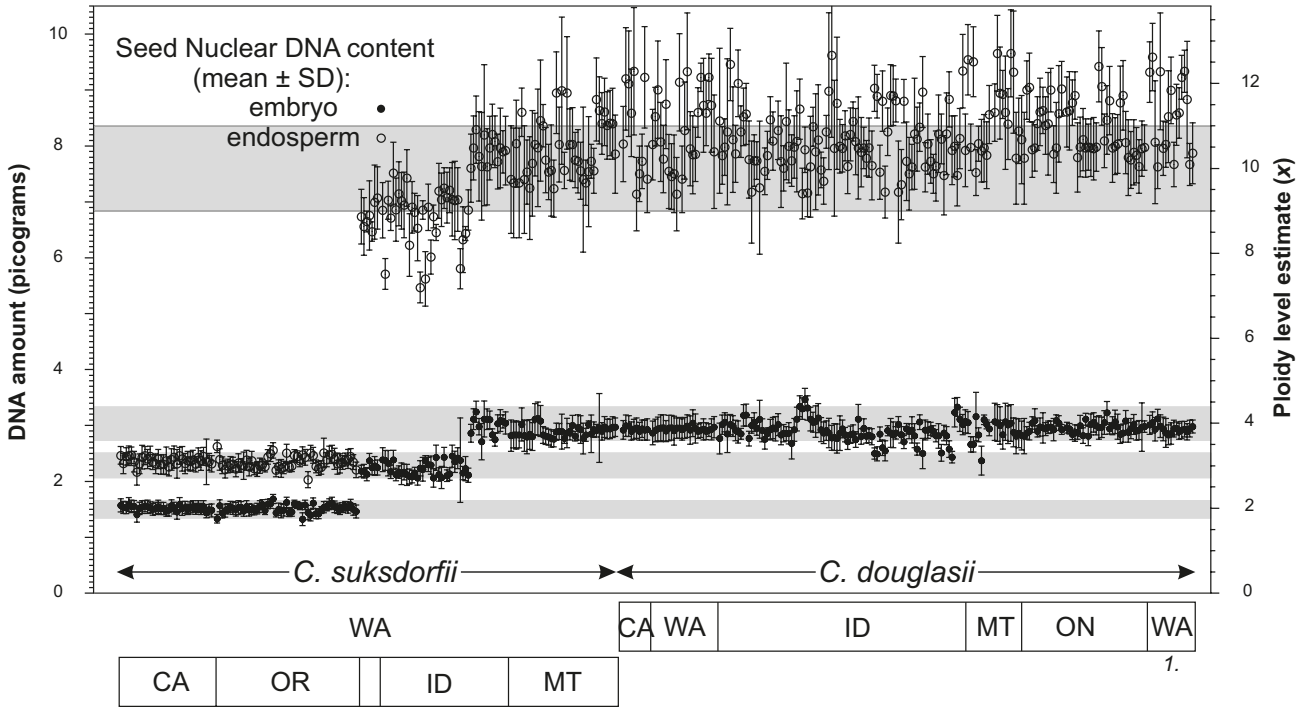
## Results

### Variation in nuclear DNA content

*Crataegus* series *Douglasianae* comprises diploid, triploid, and tetraploid individuals (Fig. 2; Supplementary Table 1). All three ploidy levels are present in *C. suksdorfii* sensu lato, the 20-stamen, black-fruited taxon of the Pacific Northwest, whereas its more widely distributed sister taxon, *C. douglasii* and its segregates, are exclusively tetraploid (Figs. 1, 2; Supplementary Table 1). The highest leaf DNA content (average 2C value = 3.01 ± 0.12 pg) was detected among samples of *C. douglasii*, and these samples were all confidently identified as tetraploids using the ranges observed by Talent and Dickinson (2005). By contrast, leaf DNA content of *C. suksdorfii* varied considerably between sites. Most samples were found within ranges previously determined to correspond to diploids (2*n* = 34; average 2C value = 1.54 ± 0.14 pg), triploids (2*n* = 51; average 2C value = 2.23 ± 0.12 pg), or tetraploids (2*n* = 68; average 2C value = 2.95 ± 0.06 pg) (Talent and Dickinson 2005). Flow cytometry of seeds enabled us to confirm ploidy determinations, e.g., as follows: five *C. suksdorfii* individuals from site CAR5 show 2C values of 1.24–1.30 pg, below the lower limit of 1.37 pg for diploids (Talent and Dickinson 2005). However, embryos of these individuals all show 2C values that clearly fall into the diploid range (Fig. 3). This discrepancy between the leaf and embryo 2C values could be due to experimental error or to physiological differences between metabolically active leaf tissues and dormant seeds.

Comparisons of seed embryo and endosperm nuclear DNA content (Fig. 3) demonstrate that seeds of diploid individuals of *C. suksdorfii* contain diploid embryos (mean = 1.52 ± 0.06 pg/2C) with

**Fig. 3.** Embryo and endosperm nuclear DNA content (mean and standard deviation (SD)) for seeds from diploid, triploid, and tetraploid individuals of *Crataegus douglasii* sensu lato and *C. suksdorfii* sensu lato (Table 1). Provenances of the individuals sampled are indicated in the horizontal bars: CA, California; OR, Oregon; WA, Washington; ID, Idaho; MT, Montana; ON, Ontario, Canada; and (1) seeds from individuals segregatable as *C. castlegarensis* (WA21). Shaded areas show the ranges for the 2*x*, 3*x*, and 4*x* levels as in Fig. 2; the uppermost band represents 6.84–8.36 pg (10*x*).




triploid endosperm (mean = 2.35 ± 0.15 pg/2C; Fig. 3). Seeds of triploid *C. suksdorfii* individuals (Fig. 1; Supplementary Table 1) contain triploid embryos (mean = 2.20 ± 0.23 pg/2C) and endosperm varying from approximately octaploid to decaploid (5.71 ± 0.27 pg/2C to 7.51 ± 0.55 pg/2C; Fig. 3). The average 2C value of embryos in seeds from tetraploid individuals of both *C. suksdorfii* and *C. douglasii* (Fig. 1; Supplementary Table 1) is 2.93 ± 0.14 pg/2C, clearly in the tetraploid range (Fig. 3). Endosperm from these seeds has an average nuclear DNA content of 8.07 ± 0.82 pg/2C (decaploid to dodecaploid).

Together with these data on DNA amounts in embryo and endosperm nuclei, the $\mu_{end}$:$\mu_{emb}$ ratios for seeds from *C. suksdorfii* diploids (1.5–1.7; Supplementary Fig. 2) suggest that seeds are produced sexually by double fertilizations involving reduced male and female gametes (Supplementary Fig. 1). Overall, no sexually produced seeds were detected in our triploid and tetraploid samples. For triploid *C. suksdorfii* individuals, the $\mu_{end}$:$\mu_{emb}$ ratio was calculated to be 2.8–3.5 (Supplementary Fig. 2), suggesting that the embryos in the seeds of these triploids developed parthenogenetically from unreduced female gametes. The corresponding endosperm likely was fertilized by reduced or unreduced male gametes from tetraploids. We infer the role of pollen from tetraploids because of (*i*) the co-occurrence of the triploids that we studied with *C. douglasii* (Table 1), (*ii*) the poor pollen stainability of triploids compared with that of tetraploids (Dickinson et al. 1996; Talent 2009; T.A. Dickinson, unpublished data, 2010), and (*iii*) the evidence that crossing between *C. suksdorfii* and *C. douglasii* has occurred in the past (Lo et al. 2009*a*, 2010). Finally, the $\mu_{end}$:$\mu_{emb}$ ratio of seeds from tetraploids of both species is centered on the range 2.5–3 (Supplementary Fig. 2). This also suggests that the embryos in the seeds of these tetraploids likely developed from unreduced female gametes, whereas the endosperm of their seeds likely resulted from fertilization by reduced or unreduced male gametes from the same (tetraploid *C. douglasii* has been shown to be self-compatible, unlike diploid *C. suksdorfii*; Dickinson et al. 1996; Love and Feigen 1978) or another tetraploid individual. None of the seeds from triploid and tetraploid individuals shows signs of having been produced sexually (Fig. 3; Supplementary Fig. 2).

## Spatial range of cytotypes

The six diploid sexual *C. suksdorfii* collection sites (Fig. 1; Table 1; Supplementary Table 1) span what is presently known of the range of this cytotype and have an average distance apart of 212 km (Table 2). Autotriploids are known from only two sites (OR4 and OR6, indicated by arrows in Fig. 1; Table 1; Lo et al. 2009*a*) that are adjacent to the range of the diploids and 35 km apart; the average distance between the combined diploid and autotriploid sites is 192 km (Table 2). The five sites at which allotriploid and allotetraploid apomictic *C. suksdorfii* were collected (Fig. 1; Supplementary Table 1; Lo et al. 2009*a*) span most of the United States range of these cytotypes and are separated by 323 km on average (the two tetraploid *C. suksdorfii* sites are 271 km apart). In contrast, the 24 collection sites for *C. douglasii* tetraploid apomicts span most of the North American range of this species complex and are, on average, 1007 km apart (Fig. 1; Tables 1, 2; Supplementary Table 1). Overall, the between-site distances for *C. douglasii* are significantly different from those for all of the *C. suksdorfii* sites sampled (Table 2).

## Environment and cytotypes

Elevation, relative humidity, and temperature (mean daily air temperature, days with ground frost) show the greatest differentiation between diploid and polyploid cytotypes in plots of nuclear DNA content against single environmental variables (Fig. 4). The northern California diploids (CAR5; Fig. 1) are outliers in that this population occurs at a higher elevation and experiences lower temperatures and humidities than do the other diploids

**Table 2.** Average great circle distances (km) between sites (Fig. 1; Table 1) at which different cytotypes in *Crataegus* series *Douglasianae* are found.

| Cytotype | Average great circle distances (km) |
|---|---|
| *C. douglasii* sensu lato (tetraploid; $N = 24$) | 1007 |
| *C. suksdorfii* | |
| Diploid ($N = 6$) | 212 |
| Diploid+autotriploid ($N = 8$) | 192 |
| Triploid ($N = 5$) | 399 |
| Tetraploid+allotriploid ($N = 5$) | 323 |
| All ploidy levels ($N = 13$) | 409 |

**Note:** $H_0$: the distances between all *C. douglasii* sites are not different in location from the distances between the *C. suksdorfii* ones is rejected ($p \ll 0.001$) using the Mann–Whitney test.

(Fig. 4). With all of the climatic variables, polyploids occupy the widest range of conditions (Fig. 4).

The principal components analysis (PCA) of the standardized annual averages of the climate variables demonstrates that the Oregon and Washington diploid sites, together with two Washington *C. douglasii* sites, are warmer and more mesic than any of the remaining polyploid sites (Fig. 5a). Again, compared with the other diploid localities (Table 1), the high-elevation California diploid site (CAR5) is an exception. Even with this exceptional site, however, the sites with diploid and autotriploid *C. suksdorfii* (OR4, OR6) and the allotriploid *C. suksdorfii* (ID5, ID6) occupy much smaller areas in the plane of the first two climate PC axes (74% of the total sample variation) than do those with tetraploid *C. douglasii* (Fig. 5*b*). All six climate variables appear to contribute more or less equally to the scatter of sites in this plane, and except for relative humidity (*H*) and 75% probability precipitation (*P*), they do so in different directions. Mean daily air temperature (*T*) and days with ground frost (*F*) also have parallel effects, but these differ in sign. Although sampling sites of polyploid *C. suksdorfii* are limited, they span the known range of these cytotypes (Fig. 1; Coughlan 2012). It is interesting to note that the sites with allopolyploids that combine genomes from *C. suksdorfii* and *C. douglasii* (Lo et al. 2009*a*, 2010) occupy a largely different area of the PC1–PC2 plane than do the sites with diploid and autotriploid *C. suksdorfii* (Fig. 5*a*).

In the $\Delta\mu^2$-based NJ tree (Fig. 5*c*), diploid (OR1, OR2, OR7, OR11, OR11, WA7, and CAR5) and autotriploid (OR4, OR6) samples of *C. suksdorfii* are clustered together (branch A, Fig. 5*c*). The allotriploid (ID5, ID6) and allotetraploid (ID2, MT2) samples of *C. suksdorfii* from east of the Cascades are distinct (branch B, Fig. 5*c*), but are intermingled with the tetraploid samples of *C. douglasii* (e.g., WA21, ID2, ID6, ID15, and MT2) that span the Rocky Mountains and extend to the Great Lakes Basin (ON18, ON20, ON21; branch B, Fig. 5*c*). Differences in length observed between branches A and B are open to a range of possible explanations and are not discussed further.

## Discussion

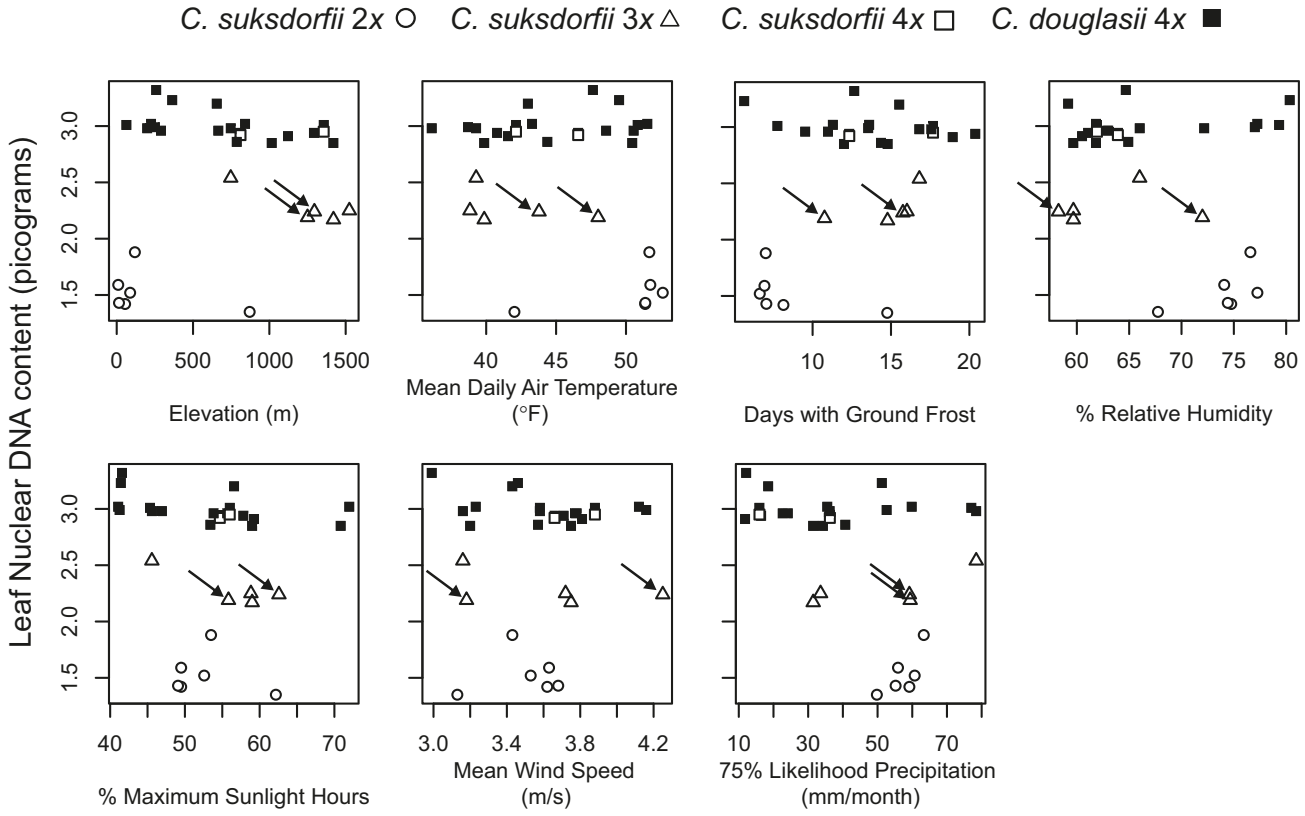
The role of abiotic environmental factors in mediating spatial distribution in asexual lineages and their sexual relatives lacks empirical evidence (Peck et al. 1998; Haag and Ebert 2004), especially in woody perennials. Our survey of ploidy level variation in *Crataegus* series *Douglasianae* is the most comprehensive survey to date of an ostensibly monophyletic group within *Crataegus*, a woody genus of shrubs and small trees. Our comparison of the geographic distribution of the tetraploid apomict *C. douglasii* sensu lato relative to that of its sister taxon, the sexual diploid *C. suksdorfii*, clearly demonstrates the phenomenon of geographical parthenogenesis: polyploid asexual individuals are more geographically widespread than their diploid sexual relatives (Fig. 1; Table 2). The same is true of triploid *C. suksdorfii* compared with the diploids (Fig. 1; Table 2). We have also documented environmental factors operating at our sampling sites, and here too polyploid apomicts occupy a wider range of environmental conditions than diploids (Figs. 4*a*, 4*b*), as would be expected from their occurrence mainly in the rain shadow east of the Cascades and in the forested Rocky Mountains and upper Great Lakes basin (Fig. 1; Table 1). Although the sample size on which these conclusions are based is limited, we know from recent fieldwork that apart from the lack of data from British Columbia (where both species are represented only by polyploids), our sample here is representative of the ranges of each cytotype (Coughlan 2012). The diploid count from the Queen Charlotte Islands (Taylor and Mulligan 1968; second panel of fig. 2 in Dickinson et al. 2008) has been shown to be in error (T.A. Dickinson, unpublished data, 2010).

The lack of evidence for sexual reproduction seen in our data (Fig. 3; Supplementary Fig. 2) is consistent with molecular data (Lo et al. 2009*a*) showing the greatest genetic diversity in diploid populations but the lowest in triploid ones. Although tetraploids, in general, show moderate genetic diversity within populations, it is unclear whether this is due to occasional sexual events or frequent dispersal among populations.

Gametophytic apomixis has the potential to contribute to the phenomenon of geographic parthenogenesis in at least two ways. First, like self-compatibility, apomixis provides reproductive assurance by enhancing the ability of individuals to set seed whether or not other conspecific (or even congeneric) individuals are nearby. Our data indicate that apomictic reproduction is the rule among the polyploids in our sample (Fig. 3; Supplementary Fig. 2), and so pseudogamy is no obstacle to seed set, given the breakdown of gametophytic self-incompatibility with polyploidy (Talent and Dickinson 2007*a*, 2007*b*). Second, pseudogamous gametophytic apomixis means that endosperm fertilization occurs and, it would appear, often may involve both sperm nuclei (Supplementary Fig. 1). Polyploidy and pseudogamy combine to produce endosperm with proportionally higher nuclear DNA content than is found in sexual diploids (Fig. 3; Supplementary Fig. 2). Besides enabling us to detect the asexual or sexual origin of a given seed, the proportionally higher nuclear DNA content of the endosperm compared with the embryo of seeds of polyploids may condition rapid nutrient uptake and transfer (Shuter et al. 1983; MacGillivray and Grime 1995). Increased DNA content in the cells of both the sporophyte and the endosperm of apomictic polyploid individuals may thus confer selective advantages by enhancing growth rate and nutrient capacity, both of which may, in turn, contribute to the increased ecological amplitude of *C. douglasii* seen in our data (Figs. 4, 5*b*).

In a review of geographical parthenogenesis, Hörandl (2006) proposes four theoretical scenarios for the linkage between dispersal, ecology, and genetic variation and their effects on cytotype distribution. Three of these scenarios appear particularly relevant in light of what we know about the evolution of *Crataegus* series *Douglasianae*. The first of these (fig. 3*a* in Hörandl 2006) is long-distance dispersal of single individuals. Compared with equivalently dispersed sexual individuals, apomictic polyploid hawthorns will be able to produce clonal lineages in new areas thanks to asexual, uniparental reproduction. Dependence on pollination for successful seed set will have little effect on the establishment of these apomictic individuals because of their self-fertility. Tetraploid plants of *C. douglasii* are found more widely dispersed and in environments that are overall more heterogeneous than those occupied by either diploid and autotriploid *C. suksdorfii* or the *C. suksdorfii* allopolyploids (Figs. 4, 5). Given wide dispersal, the other effects of polyploidy such as high rates of growth and metabolic activity may come into play and enable survival even under conditions inhospitable to diploids. Similar spatial patterns have been reported in herbaceous plants (e.g., Verduijn et al. 2004; Cosendai and Hörandl 2010). Hence, we suggest that regardless of differences in metabolic rates, life histories, and generation times between woody and herbaceous plants,

**Fig. 4.** Population average leaf nuclear DNA content for *Crataegus douglasii* sensu lato and *C. suksdorfii* sensu lato plotted against elevation and six climate variables. Open symbols, cytotypes of *C. suksdorfii*, as shown; solid symbols, *C. douglasii* sensu lato. Arrows: sites at which *C. suksdorfii* was determined to be autopolyploid (Lo et al. 2009*a*).



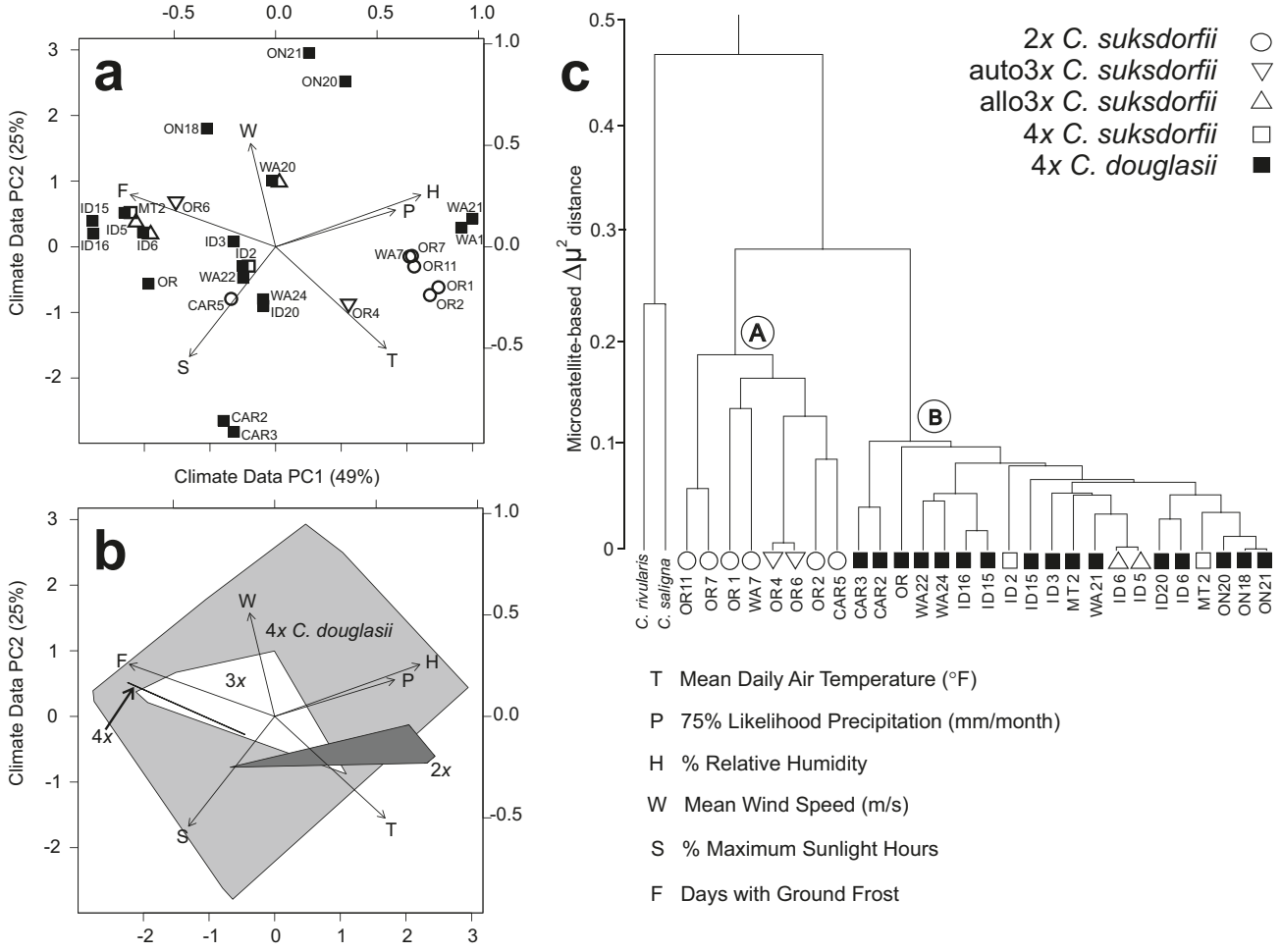

reproductive behavior appears to play a determining role in cytotype distribution. This conclusion also suggests the need for additional information about the dispersal process in hawthorns.

The second scenario (fig. 3*b* in Hörandl 2006) refers to comigration of apomicts and sexuals, with gene flow between the two groups. Sexual *C. suksdorfii* is not presently known to occur in sympatry with apomicts, yet gene flow resulting in the incorporation of apomixis and wide ecological amplitude into the sexuals is documented (e.g., allotriploid *C. suksdorfii* derived predominantly from fertilization of reduced *C. douglasii* female gametes by reduced male ones from diploid *C. suksdorfii* or allotetraploid formation via a triploid bridge; Lo et al. 2010). Introgression from *C. douglasii* evidently contributes some differences in ecological amplitude, enabling allopolyploids to occupy colder and less mesic habitats than those in which the diploids are found (Figs. 4, 5*a*). Moreover, the NJ tree (Fig. 5*c*) suggests that this introgression may have occurred more than once, in different places, implicitly involving different genotypes of one parent, if not both. A greater proportion of heterozygous loci resulting from hybridization may contribute to heterosis, conferring greater biomass, speed of development, and fertility than is seen in either parent (Rhode and Cruzan 2006). Several studies have also demonstrated that hybrids exhibit higher fitness than their inbred and outbred parents along an environmental gradient (e.g., Campbell and Waser 2001; Johnston et al. 2001; Mercer et al. 2006). Thus, hybridization and related ploidy level changes could enhance colonization and survivorship of allopolyploids, leading to their wider distribution.

Only these first two scenarios have any likely bearing on geographical parthenogenesis in *Crataegus*. Because the third scenario (fig. 3*c* in Hörandl 2006) lacks gene flow between apomicts and sexuals, its relevance to *Crataegus* series *Douglasianae* is limited. The fourth scenario (fig. 3*d* in Hörandl 2006), however, includes both dispersal and niche differentiation as explanations of geographical parthenogenesis. As suggested above, dispersal of hawthorns warrants further investigation as an explanation for differences in geographic distribution between apomicts and sexuals. However, because diploid and polyploid *C. suksdorfii* have yet to be found in sympatry, this scenario appears not to apply to *Crataegus* series *Douglasianae*. In addition, this scenario focuses on contrasts between selfers and apomicts in its exploration of niche differentiation; as yet, we have no evidence that selfing plays a role in *Crataegus* series *Douglasianae*.

Finally, we note the way in which our documentation of geographical parthenogenesis in *Crataegus* series *Douglasianae* differs from that of most other studies on flowering plants. Studies on Ranunculaceae (e.g., Cosendai and Hörandl 2010) and Asteraceae (e.g., Thompson and Whitton 2006) have focused on contrasts between previously glaciated and unglaciated portions of a taxon's range. Occupation of the former by polyploid apomicts has been interpreted in terms of rapid colonization of newly available and environmentally harsh habitat by cytotypes endowed with multiple genomes and capable of reproducing themselves uniparentally. Those studies have also dealt predominantly with herbaceous taxa occurring as chamaephytes, hemicryptophytes, or cryptophytes, plants of relatively small stature, with their apical meristems often at ground level or below, protected from environmental insult. In contrast, the hawthorns that we have studied are woody phanerophytes, frequently capable of heights in excess of 2 m. Apical meristems in these plants, notably those producing reproductive structures, are borne well above ground level, protected only by enclosing bud scales during the most adverse climatic conditions. In addition, while the currently known range of diploid *C. suksdorfii* was not ice-covered during the last glacial period, the range of *C. douglasii* and allopolyploid *C. suksdorfii* in-

**Fig. 5.** Principal components biplots based on yearly averages of six climate variables (Fig. 4) standardized to unit variance, showing (*a*) individual sites (Table 1) and (*b*) convex hulls enclosing sites with 2*x*, 3*x*, and 4*x* individuals. Critical values of the broken-stick criterion for these two axes are 41% and 24%, respectively. (*c*) Neighbor-joining (NJ) tree for microsatellite-based $\Delta\mu^2$ distances between the population samples of *C. douglasii* sensu lato and *C. suksdorfii* sensu lato (Lo et al. 2009*a*; sites lacking microsatellite data are omitted). *Crataegus rivularis* and *C. saligna* (both series *Cerrones*; data from (Lo 2008) are employed as outgroups to root the tree.




cludes both glaciated and extensive unglaciated areas (compare maps in Dickinson et al. 2008 with fig. 4 in Kahle et al. 2005). This suggests that geographical parthenogenesis in woody plants such as the larger genera in Rosaceae subtribe Pyrinae (Dickinson et al. 2007) is less likely to involve only postglacial history than may be the case in predominantly herbaceous groups such as the Asteraceae and Ranunculaceae. Rather, differences in breeding system and nuclear DNA content are likely historically to have interacted with the environment in very complex ways.

In summary, our study indicates that polyploid apomicts in *Crataegus* series *Douglasianae*, and *C. douglasii* in particular, have a wider range (Fig. 1; Table 2) and exhibit a broader ecological amplitude than those of closely related diploid sexuals belonging to *C. suksdorfii* (Fig. 4). Polyploid apomictic lineages in *C. suksdorfii* apparently arose independently and occupy habitats that are different and more varied environmentally than are those occupied by diploids (Figs. 5*b*, 5*c*). This suggests that expansion into new environmental niches not exploited by the diploids is enabled by the *C. douglasii* genomes such as those found in allopolyploid *C. suksdorfii* (Lo et al. 2009*a*, 2010). In *Crataegus*, the phenomenon of geographic parthenogenesis is now demonstrated for series *Douglasianae*, and our results suggest how all aspects of this agamic complex, polyploidy, hybridization, and apomixis are implicated in the establishment of the distributional patterns making up this phenomenon. Reproductive behavior in *Crataegus* thus plays a prominent role in the formation and distribution of cytotypes, much as it does in herbaceous groups, despite differences in life form and generation times.

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