Black-fruited hawthorns of western North America — one or more agamic complexes?¹,²

Timothy A. Dickinson, Eugenia Y.Y. Lo, Nadia Talent, and Rhoda M. Love

Abstract: Black-fruited hawthorns in North America comprise two taxonomic groups within the genus *Crataegus*, section *Brevispinæ* and section *Douglasianæ*. The first of these has recently been shown to be monospecific, consisting of the blueberry haw, *Crataegus brachyacantha* Sarg. & Engelm., of Louisiana and Texas. *Crataegus* section *Douglasianæ*, however, comprises several taxa in a single clade that is not closely related to section *Brevispinæ*, and that is now one of the best-studied groups of hawthorns at least in North America. Most taxa in the group are found in, or west of, the Rocky Mountains. They include diploids, triploids, and tetraploids that can be ascribed to four or more species that differ in distribution and ecology, thorn morphology, leaf shape, and floral architecture. Diploids are self-incompatible, whereas polyploidy is associated with pseudogamous, gametophytic apomixis and self compatibility. Molecular data suggest that polyploids have arisen repeatedly, both within and from crosses between ploidy levels. We suggest that *Crataegus* section *Douglasianæ* represents at least two agamic complexes that may serve as models for understanding the biology of, the relationships within, and the appropriate taxonomic treatment of other such groups that may make up much of the rest of the genus.

Key words: *Brevispinæ*, *Douglasianæ*, *Crataegus*, Rosaceae, apomixis, leaf shape.

Résumé : Les aubépines à fruits noirs de l’Amérique du Nord comportent deux groupes taxonomiques dans le genre *Crataegus*, la section *Brevispinæ* et la section *Douglasianæ*. On a récemment montré la nature monospecifique de la première section constituée de la cenelle à fruits bleus, *Crataegus brachyacantha* Sarg. & Engelm., de la Louisiane et du Texas. Cependant, les *Crataegus* de la section *Douglasianæ* comprennent plusieurs taxons appartenant à un même clade qui est faiblement relié à la section *Brachyacantha*, et qui constitue maintenant un des groupes d’aubépines les mieux étudiés, du moins en Amérique du Nord. On retrouve la majorité des taxons du groupe dans ou à l’ouest des montagnes Rocheuses. Ils comportent des entités diploïdes, triploïdes et tétraploïdes attribuables à au moins quatre espèces différentes par leur distribution et leur écologie, la morphologie des épines, la forme des feuilles et l’architecture florale. Les entités diploïdes sont autoincompatibles alors que les polyploïdes, montrant de la pseudogamie et de l’apomixie gamétophytique, sont autocompatibles. Les données moléculaires suggèrent que les entités polyploïdes sont apparues de façons répétées à la fois par croisements et degrés de ploidies. Les auteurs proposent que la section *Douglasianæ* des *Crataegus* représente au moins deux complexes agames qui peuvent servir de modèles pour comprendre la biologie des relations internes et le traitement taxonomique approprié d’autres groupes similaires qui pourraient fort bien constituer la majorité des genres résiduels du reste du genre.

Mots-clés : *Brevispinæ*, *Douglasianæ*, *Crataegus*, Rosaceae, apomixie, forme foliaire.

Introduction

An association between taxonomic complexity, polyploidy, and gametophytic apomixis has been documented repeatedly over the past century, especially in Asteraceae, Poaceae, and Rosaceae (Phipps and Muniyamma 1980; Campbell et al. 1991; Asker and Jerling 1992) and in some well-studied genera from other families (e.g., *Ranunculus* L.; Hörandl and Paun 2007). Frequently, these cases involve interspecific hybrids in which asexual reproduction compensates for reduced sexual fertility, often in such a way that changes in ploidy level and further hybridization ensue. In many of these cases, biosystematic studies have resolved taxonomically complex groups into a small number of more or less readily distinguishable ancestral sexually reproducing diploids and a larger number of poly-
ploids that reproduce asexually for the most part, and in which diagnostic features of the diploids are found in novel combinations.

During the first half of the twentieth century it became apparent that many of the hundreds of species of North American hawthorns (Crataegus L.; Rosaceae, Spiraeoideae, tribe Pyreae) had been described on the basis of differences in relatively small numbers of seemingly minor details of margination, pubescence, and anther colour together with subtle (and poorly characterized) differences in leaf shape and fruit colour (Schneider 1906; Palmer 1925; Rickett 1936, 1937). Since it had also been shown that many of these hawthorns were pollen infertile (Standish 1916) and polyplloid (Longley 1924), it was subsequently all too easy to ascribe the taxonomic complexity of Crataegus to hybridization, polyplloid, and asexual reproduction without any of these processes actually having been documented in greater detail; indeed, embryological studies on hawthorns did not begin until the 1970s.

Hybridization in Crataegus was studied experimentally by Love and Feigen (1978) and subsequently by Wells and Phipps (1989), and by Talent and Dickinson (2007a), although it appears that unpublished studies were carried out much earlier (e.g., Brown 1910). The embryology of Crataegus apomixis and sexual reproduction was documented by Muniyamma and Phipps (1979b, 1984, 1985), Ptk (1986, 1989), and others (Dickinson and Phipps 1986; Smith and Phipps 1988; Dickinson et al. 1996). Pollination experiments demonstrated that the success of apomictic reproduction is dependent on pollination (pseudogamy; Dickinson and Phipps 1986; Talent and Dickinson 2007c). These studies also demonstrated that tetraploid hawthorns are self-compatible. Phipps and co-workers used chromosome counts to further explore the taxonomic distribution of polyplloid in North American hawthorns (Muniyamma and Phipps 1979a; Dickinson and Phipps 1986; Smith and Phipps 1988). Flow cytometric studies of variation in nuclear DNA content have built upon this work and greatly increased the coverage of species for which ploidy level can be inferred (Talent and Dickinson 2005).

Hawthorn species have been accommodated in about 15 morphologically well-differentiated sections and 40 series (Loudon 1838; Sargent 1892; Beadle 1902; Schneider 1906; Palmer 1925, 1963; Phipps et al. 1990; Phipps et al. 2003). These groups have been the subject of recent taxonomic revisions (e.g., Christensen 1992; Phipps 1998a), and at least some of them are well supported by DNA sequence data (Verbylaite et al. 2006; Albarouki and Peterson 2007; Lo et al. 2007).

As in other fleshy-fruited Rosaceae, fruit colour varies considerably in Crataegus. Red fruits predominate, but yellow, orange, purple, and black fruits are also found, variously as colour morphs of typically red-fruited species (e.g., Crataegus punctata Jacq., and its occasional yellow-fruited var. aurea Aiton; Phipps and Muniyamma 1980). Variation in fruit colour also characterizes several species in the otherwise red-fruited western Eurasian section Crataegus (Christensen 1992), and in relatively isolated, monophyletic groups such as the North American section Douglasianae (Lo et al. 2007).

In North America there are two groups with “black” (i.e., black, blue, purple, or reddish purple) fruits that are easily recognizable when their fruit is ripe. Monospecific section Brevispiniae comprises the blueberry haw of east Texas and Louisiana, USA, Crataegus brachycantha Sarg. & Engl. (Figs. 1a–1d; Lo et al. 2007). In the Pacific northwest, Rocky Mountains, and upper Great Lakes basin of the USA and Canada, section Douglasianae Loudon, a group of at least four distinctive hawthorns, includes Crataegus douglasii Lindl., Crataegus rivularis Nutt., Crataegus saligna Greene (Figs. 1c–1f), and Crataegus suksdorfii (Sarg.) Kruschke (Fig. 1g).

Two series have been described within section Douglasianae (Table 1) to recognize contrasts between the species pairs C. rivularis and C. saligna (series Cerrones J.B. Phipps; three species) and C. douglasii and C. suksdorfii (series Douglasianae; five species), and to accommodate a group of six species that is for the most part newly described and known only from southern interior British Columbia and adjacent Montana and Washington (series Purpureofructi J.B. Phipps & O’Kennon). The former two series also include additional species, some described much earlier and others described in the past decade (Table 1). Each of the species pairs referred to above also exhibits a contrast common in North American (but not Eurasian) Crataegus species, namely in the number of stamens.
per flower (approximately, either 20 or 10; Dickinson et al. 1996). Species in series Purpureofructi uniformly have 10-stamen flowers (Phipps and O’Kennon 2002). All of the species thus included in section Douglasianae have fruits that ripen to a black or purple colour, although they may pass through either a brownish or reddish phase before becoming fully ripe. In addition, hybrids are found in Oregon between C. suksdorfii and naturalized Crataegus monogyna Jacq. introduced from Europe (Love and Feigen 1978). These hybrids set fruit at a rate comparable to open pollinated fruit (Love and Feigen 1978). These hybrids were georeferenced using gazetteers, atlases, and a variety of methods to obtain an approximately unbiased sample from each site.

A total of 324 of these herbarium specimens (see supplementary data, Appendix A) were included vouchers from these studies in our sample. The 106 vouchers from our studies include subsets of vouchers for topodeme samples of Crataegus species Douglasianae individuals from three sites in Oregon, Idaho, and Montana (OR6, ID6, and MT2, respectively; Dickinson et al. 1996). These samples were drawn using “ignorant man” random sampling (Ward 1974) to obtain an approximately unbiased sample from each site.

Materials and methods

Plant materials

Several hundred herbarium specimens of North American hawthorns known to have black fruit (Table 1), including types, were borrowed from mainly North American herbaria (see supplementary data, Appendix A). They were then augmented with collections made by R.E. Dotterer in the late summer of 1995. To link the results of our phenetic studies here of the loan material and earlier collections to those from studies of breeding system, ploidy level variation, and DNA sequence variation in section Douglasianae (Talent and Dickinson 2005, 2007b, 2007c; Lo et al. 2007; E. Lo, N. Talent, S. Stefanović, and T.A. Dickinson, unpublished data, 2008; Lo 2008; Lo et al. 2008a; Lo et al. 2008c), and to earlier ones (Taylor and Mulligan 1968; Brunsfeld and Johnson 1990) we included vouchers from these studies in our sample. The 106 vouchers from our studies include subsets of vouchers for toponeme samples of Crataegus species Douglasianae individuals from three sites in Oregon, Idaho, and Montana (OR6, ID6, and MT2, respectively; Dickinson et al. 1996). These samples were drawn using “ignorant man” random sampling (Ward 1974) to obtain an approximately unbiased sample from each site.

A total of 324 of these herbarium specimens (see supplementary data, Appendix A) are the operational taxonomic units (OTUs) in this study. They represent all of the species recognized in series Cerrones and Douglasianae by Phipps et al. (2003), as well as one from series Purpureofructi (Table 1; supplementary data, Appendix A). Of these, 216 were georeferenced using gazetteers, atlasses, and a variety

Table 1. Taxa of black-fruited North American Crataegus in sections Brevispinæ and Douglasianæ to which the North American herbarium specimens studied here were ascribed (only one of the species in series Purpureofructi J.B. Phipps & O’Kennon is included in the sample studied here).

<table>
<thead>
<tr>
<th>Group</th>
<th>Taxon</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Section Brevispinæ Beadle ex C.K.Schneid.</strong></td>
<td></td>
</tr>
<tr>
<td>Series</td>
<td>Brevispinæ (Beadle ex C.K. Schneid.) Rehder</td>
</tr>
<tr>
<td>C. brachycantha Sarg. &amp; Engelm., Figs. 7 and 8 in Phipps 1998a; Phipps 1999; Lo et al. 2007</td>
<td></td>
</tr>
<tr>
<td><strong>Section Douglasianæ Loud.</strong></td>
<td></td>
</tr>
<tr>
<td>Series</td>
<td>Cerrones J.B. Phipps</td>
</tr>
<tr>
<td>C. erythropoda Ashe, Figs 6 and 7 in Phipps 1998b; Phipps 1999</td>
<td></td>
</tr>
<tr>
<td>C. rivularis Nutt., Fig 3 in Dickinson and Love 1997; Fig 2 in Phipps 1999; plate 56 in Phipps et al. 2003</td>
<td></td>
</tr>
<tr>
<td>C. saligna Greene (syn. C. douglasii var. duchesnensis Welsh), Fig. 3 in Phipps 1999; Beatty et al. 2004</td>
<td></td>
</tr>
<tr>
<td>Series</td>
<td>Douglasianæ (Loud.) Poletiko</td>
</tr>
<tr>
<td>C. castlegarenseis J.B. Phipps &amp; O’Kennon, Figs. 3 and 4 in Phipps and O’Kennon 2002</td>
<td></td>
</tr>
<tr>
<td>C. douglasii Lindl., Figs 1d, 1e, and 2 in Dickinson and Love 1997; Phipps and O’Kennon 2002</td>
<td></td>
</tr>
<tr>
<td>C. okennonii J.B. Phipps, Figs. 2 and 3 in Phipps and O’Kennon 1998</td>
<td></td>
</tr>
<tr>
<td>C. shuswapensis J.B. Phipps &amp; O’Kennon, Fig. 5 in Phipps and O’Kennon 2002</td>
<td></td>
</tr>
<tr>
<td>C. suksdorffii (Sarg.) Kruschke (syn. C. douglasii var. suksdorffii Sarg., C. punctata var. brevispina Douglas ex Hook.), Figs. 1a–1c, 5, and 6 in Dickinson and Love 1997; Phipps and O’Kennon 2002</td>
<td></td>
</tr>
<tr>
<td>Series</td>
<td>Purpureofructi J.B. Phipps &amp; O’Kennon</td>
</tr>
<tr>
<td>C. phippsii O’Kennon, Fig. 7 in Phipps and O’Kennon 1998</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** References are given for illustrations, distribution maps, and other pertinent information.

---

4 Supplementary data for this article are available on the journal Web site (http://botany.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3777. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.
of online tools (Fig. 2). Subsamples of 170 and, for leaf descriptors only, 190 and 210 fruiting specimens were scored for a limited suite of seven morphological descriptors described below (Table 2). The additional subsample of 106 vouchers from our recent fieldwork was scored for these and 13 additional descriptors (Table 2) to be able to incorporate results from studies of ploidy level and microsatellite allelic variation, and to capture variation underlying recognition of some newly described species. All four subsamples cover most of the ranges of the taxa studied here (Fig. 2) and together represent all of the major geopolitical units (states, provinces) represented in the larger sample (see supplementary data, Appendix A).

**Data collection and analysis**

Outlines of one to several short-shoot leaves were digitized using the program MorphoSys (Meacham and Duncan 1991) and an image capture system based on the PCvision-plus framegrabber from Imaging Technology Inc., Woburn, Mass. It was not possible to control for short-shoot heteroblasty (Dickinson and Phipps 1984) by measuring leaves at only a single position on the shoot when sampling herbarium specimens. Leaf outlines were summarized as elliptic Fourier coefficients for 20 harmonics, and as the corresponding Fourier amplitudes (Rohlf and Archie 1984; Meacham and Duncan 1991; McLellan and Endler 1998). Outlines were also edited by adding landmarks so as to make linear measurements of leaf blade length above and below the widest point (each recorded as a proportion of the width of the leaf in question), petiole length, and to obtain leaf perimeters and areas. This allowed us to calculate the inverse leaf dissection index (invDI; Table 2) as the leaf area divided by the leaf perimeter standardized to a circle of unit area (Table 2; Kincaid and Schneider 1978; Dickinson 2003; Dorken and Barrett 2004). To make comparisons with other studies of variation in hawthorn leaf shape we also obtained a series of nine widths for each leaf, taken at equal intervals along the length of the leaf blade (Phipps 1997; Macklin and Phipps 2006), and recorded them as proportions of the leaf blade length. Secondary venation of short-shoot leaves was visualized on X-ray negatives prepared using a Hewlett-Packard Faxitron X-ray system and Kodak Industrex film. The brightness and contrast of scans of these negatives were adjusted using PhotoShop (Adobe Corp.) to make the venation more discernible.

Additional descriptors of thorns and flowers (Table 2) were scored by hand, using a flexible ruler where necessary. Fruits and flowers were softened by microwaving for 1–2 min in a small dish of water; after scoring the descriptors they were allowed to dry and were placed in packets at-
Table 2. Non-Fourier descriptors of short-shoot leaves, thorns, flowers, and fruits of North American black-fruited hawthorns that were used to discover the group structure present in the sample studied here.

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>invDI</td>
<td>Inverse of the dissection index of Kincaid and Schneider (1978), calculated as $2(A\pi)^{1/2}/P$, where $A$ is the leaf area and $P$ is the leaf perimeter; this ratio of the leaf area to its perimeter reduces to 1.0 for a perfect circle regardless of size, and approaches zero as the leaf perimeter increases with increased lobing</td>
</tr>
<tr>
<td>relX</td>
<td>Leaf blade length above the widest point divided by leaf width at the widest point</td>
</tr>
<tr>
<td>relZ</td>
<td>Leaf blade length below the widest point divided by leaf width at the widest point</td>
</tr>
<tr>
<td>W1</td>
<td>Leaf blade width at 10% of distance from apex to base</td>
</tr>
<tr>
<td>W2</td>
<td>Leaf blade width at 20% of distance from apex to base</td>
</tr>
<tr>
<td>W3</td>
<td>Leaf blade width at 30% of distance from apex to base</td>
</tr>
<tr>
<td>W4</td>
<td>Leaf blade width at 40% of distance from apex to base</td>
</tr>
<tr>
<td>W5</td>
<td>Leaf blade width at 50% of distance from apex to base</td>
</tr>
<tr>
<td>W6</td>
<td>Leaf blade width at 60% of distance from apex to base</td>
</tr>
<tr>
<td>W7</td>
<td>Leaf blade width at 70% of distance from apex to base</td>
</tr>
<tr>
<td>W8</td>
<td>Leaf blade width at 80% of distance from apex to base</td>
</tr>
<tr>
<td>W9</td>
<td>Leaf blade width at 90% of distance from apex to base</td>
</tr>
<tr>
<td>THNL</td>
<td>Thorn length (mm)</td>
</tr>
<tr>
<td>THND</td>
<td>Thorn diameter at base (mm)</td>
</tr>
<tr>
<td>THNS</td>
<td>Thorn curvature: 0, straight; 1, moderately curved; 2, very curved</td>
</tr>
<tr>
<td>INFP</td>
<td>Inflorescence axis pubescence along the secondary veins: 0, completely glabrous; 1, scattered hairs, or hairs dense only locally; 2, dense enough to overlap; 3, hairs very dense, over the entire region</td>
</tr>
<tr>
<td>HOVP</td>
<td>Leaf adaxial surface pubescence along the secondary veins: 0, completely glabrous; 1, scattered hairs, or hairs dense only locally; 2, dense enough to overlap; 3, hairs very dense, over the entire region</td>
</tr>
<tr>
<td>KLOP</td>
<td>Calyx lobe pubescence: 0, completely glabrous; 1, scattered hairs, or hairs dense only locally; 2, dense enough to overlap; 3, hairs very dense, over the entire region</td>
</tr>
<tr>
<td>KLOT</td>
<td>Calyx lobe toothing: 0, no toothing; 1, only 1–2 teeth on a single lobe; 2, several teeth on most lobes; densely toothed on each lobe</td>
</tr>
<tr>
<td>KLOS</td>
<td>Calyx lobe shape: 0, short, lobes generally less than 2.5 mm long, tip obtuse; 1, elongate, lobes generally more than 2.5 mm long, tip acute</td>
</tr>
<tr>
<td>STAM</td>
<td>Number of stamens in the flower from which the fruit developed</td>
</tr>
<tr>
<td>STYL</td>
<td>Number of pyrenes per fruit</td>
</tr>
<tr>
<td>FRKR</td>
<td>Fruiting calyx retained: 0, no; 1, yes</td>
</tr>
</tbody>
</table>

Note: Italics denote leaf shape descriptors compared with Fourier descriptors; boldface denotes descriptors analyzed for the 275 OTU sample. The remaining descriptors were analyzed for the 106 and 72 OTU subsamples.

In some cases where flowering specimens from the same tree were available, descriptors that could not be scored on the fruiting specimen were scored on flowering material (e.g., thorn measurements, stamen and style numbers). Several of these descriptors are binary or multistate, rather than ratio-scale (Table 2). Most of the former, however, were scored on more than one thorn, leaf, flower, or fruit per specimen, and so the integer scores (0, 1, or 0, 1, 2, 3) for each replicate were averaged to describe the specimen as a whole. Only leaf lobing (LFLO), inflorescence pubescence (INFP), and hypanthial ovary pubescence (HOVP) were given a single, integer score for each specimen as a whole.

DNA was extracted according to the modified protocol of Tsumura et al. (1995) from young leaves collected onto silica gel in the field. Twenty-three microsatellite loci located on 10 of the 17 linkage groups of *Malus domestica* Borkh. (Liebhard et al. 2002) were selected for preliminary primer testing because these primers were shown by Liebhard et al. (2002) to be transferable to *Crataegus*. Nine of these 23 loci (on linkage groups 12 and 14; CH01F02, 28 alleles; CH03C02, 23 alleles; CH04P06, 13 alleles;
CH04G04, 19 alleles; CH05D03, 24 alleles; CH05D04, 19 alleles; CH05D11, 20 alleles; CH05G07, 20 alleles; CH05G11, 36 alleles) proved to be variable in a larger sample encompassing additional species from other sections of the genus (Lo et al. 2008b), and these data are analyzed here for a subsample of 72 OTUs that intersects with the set for which we have complete morphological data (Table 2). Prior to fragment analyses, we sequenced the PCR products of these primer pairs from two to three individuals to confirm and identify the types of tandem repeats (Lo et al. 2008b). PCR amplifications were performed in a 15 μL volumes containing ~20 ng of genomic DNA, 1.5 μL 10× PCR buffer (Fermentas, Burlington, Ont.), 0.2 mmol/L of each dNTP, 1.5 mmol/L MgCl₂, 1 U of Taq polymerase (Fermentas), and 0.5 μmol/L each of the forward and reverse primers. All forward primers were end-labelled with either FAM or HEX fluorescent dyes. We followed the PCR conditions of Gianfranceschi et al. (1998) and, because the same annealing temperature was used for all SSR primers, two primer pairs were combined in PCR amplifications (multiplexing). PCR products were analysed on an ABI3700 automatic sequencer (Applied Biosystems). Peaks were scored using the program GeneMapper version 3.5 (Applied Biosystems) and the resulting allelic data were analyzed either directly (Lo et al. 2008b) or, as here, as binary allelic presence/absence data.

Morphological data were organized using spreadsheet programs, and imported into R (The R Development Core Team 2006) and S-Plus (Insightful Corporation 2005) for analysis. Distribution maps were produced using the R package cluster (average linkage) and polythetic divisive (function ape) clustering based on Euclidean distance data. For PCAs involving additional ratio-scale descriptors (THNL, THND, STAM, STYL, InvDI; Table 2) the raw data were made commensurate by ranging (subtracting the descriptor minimum, and dividing the difference by the descriptor range) so as to transform each descriptor’s values to a [0, 1] interval. The same result was achieved by using Gower’s resemblance coefficient for mixed data (function daisy, in the R package cluster) to carry out agglomerative (average linkage) and polythetic divisive (function diana, in the R package ape) clustering based on Euclidean distances calculated from these same descriptors. Gower’s coefficient was also used to summarize data for the complete suite of 20 morphological descriptors that includes ratio-scale, binary, and multistate descriptors (Table 2). In this case principal coordinates analysis (PCoA) was used for ordination instead of PCA. Canonical variates analysis (CVA) was used to compare the success with which the three leaf shape data sets alone could recover the group structure found in the complete data set (i.e., which included data from thorns and fruits). CVA was also used with the largest data set (270 OTUs, following removal of 5 OTUs comprising the two smallest species samples) to evaluate the extent to which species in series Cerrones and Douglasianae could be discriminated from each other using the smaller suite of seven ratio-scale leaf, thorn, and fruit characters (Table 2). CVA was carried out using the Syn-Tax 2000 package (Podani 2001). Dimensionality of the PCAs and CVAs was evaluated using the broken-stick criterion calculated with R according to the formula given by Legendre and Legendre (1998). All of the ordination results (PCA, PCoA, CVA) were interpreted using biplots (Gabriel 1971), in which vectors representing the contributions of individual descriptors to the scatter of OTUs along the ordination axes are superimposed on the scatterplot.

Microsatellite allele presence/absence data were summarized as a 72 × 72 OTU–OTU resemblance matrix using Jaccard’s coefficient (also produced using daisy). This resemblance matrix was summarized as an average-linkage dendrogram and compared with one calculated for the same 72 OTU subsample from the complete suite of morphological descriptors (Table 2) by means of the TreeDyn package (Chevenet et al. 2006). To do this, dendrograms were output in the Newick format using the functions as.phyl and write.tree in the R package ape.

Classification trees (Clark and Pregibon 1992) as implemented in both R and S-Plus were used as an alternative means by which to evaluate species discrimination. Whereas CVA assumes ratio-scale measurements with some semblance of multivariate normality, classification trees can be built from binary and multistate data as well as from measurement data. For this reason, and because construction of classification trees represents monothetic divisive (rather than agglomerative) clustering their results were also used in developing an identification key. Key construction was also assisted by reference to histograms and boxplots constructed using R.

Results

Identification

Given the complete locality information and morphological data, few specimens were hard to classify to species. Monospecific section Brevispinae has a pattern of short-shoot leaf secondary venation (Figs. 1a–1d) quite distinct from that in section Douglasianae (Figs. 1e–1g), so much so that it appears to be unique in the genus, commensurate with its position as sister to all or almost all other species of Crataegus in the phylogeny of Lo et al. (2007). Accordingly, what follows will deal exclusively with section Douglasianae.

Except for the small samples of Crataegus erythropoda Ashe (N = 10, but representing only two locations), Crataegus shuswapensis J.B. Phipps & O’Kennon (N = 4), and Crataegus phippsii O’Kennon (N = 1), the OTUs in the nine species samples were representative of those species’ ranges (Fig. 2; see supplementary data; Appendix A). In the cases of Crataegus castlegarenis J.B. Phipps & O’Kennon, C. erythropoda, Crataegus okononii J.B. Phipps, Crataegus rivularis Nutt., and C. saligna, this can be confirmed by reference to published distribution maps (Phipps 1998a; Phipps and O’Kennon 1998; Phipps 1999). Comparable maps are not available for the entire ranges of C. douglasii and C. suksdorfi, but comparison can be made with information from floras (e.g., Hitchcock and Cronquist 1961; Soper et al. 1989; Hickman 1993) and biogeographic or taxonomic studies (Marquis and Voss 1981; Brunsfeld and Johnson 1990). These suggest that the samples of the two species studied here (Fig. 2) effectively cover the ranges of these two species.
Table 3. Comparison of non-Fourier (Table 2) and Fourier descriptors of short-shoot leaf shape with respect to their ability to discriminate species of North American black-fruited hawthorns in a sample of 192 specimens representing four species (77 C. douglasii, 29 C. okennonii, 37 C. rivularis, and 49 C. suksdorfii). The $X^2$ statistic reported is for the test of the equality of the species mean vectors ($\mu_k$), i.e., $H_0: \mu_1 = \mu_2 = \cdots = \mu_4$, for $k = 1 \ldots 4$, where $\mu$ is the grand mean vector over all species; Wilks’ $\Lambda$ is the ratio of dispersion matrix determinants, $W/|W + A|$, where $W$ is the pooled within-species dispersion matrix, and $A$ is the among-species dispersion matrix (Gittins 1985; Legendre and Legendre 1998).

<table>
<thead>
<tr>
<th>Data set</th>
<th>$X^2$</th>
<th>df</th>
<th>Wilks’ $\Lambda$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data set 1 (relX, relZ)</td>
<td>264.02</td>
<td>6</td>
<td>0.2455</td>
</tr>
<tr>
<td>Data set 2 (W1–W9)</td>
<td>336.42</td>
<td>27</td>
<td>0.1615</td>
</tr>
<tr>
<td>Data set 3a (all 39 Fourier amplitudes calculated for 20 harmonics)</td>
<td>494.67</td>
<td>117</td>
<td>0.0540</td>
</tr>
<tr>
<td>Data set 3b (the first nine of 39 Fourier amplitudes calculated for 20 harmonics)</td>
<td>341.61</td>
<td>27</td>
<td>0.1570</td>
</tr>
</tbody>
</table>

Variation in leaf shape

Data on leaf shape were obtained from samples of 1–10 (occasionally more; modal range 3–9) leaves per specimen, and summarized as specimen means. Size-independent shape data were compiled using two landmark-based approaches (Tables 2 and 3; two leaf blade lengths relative to width, and nine leaf blade widths relative to length), and one outline-based method (elliptic Fourier amplitudes). These data were summarized as follows: (i) by PCA of the shape descriptors available for the subsample of 212 OTUs, excluding the invDI, so as to plot invDI against PC1 (not shown) as well as PC2 against PC1 (Fig. 3), and (ii) by CVA of the 192 OTU subsample (not shown; see Table 3), so as to compare the ability of the shape descriptors, excluding the invDI, to discriminate a priori groups for which we have larger sample sizes (C. douglasii, C. okennonii, C. rivularis, C. saligna, and C. suksdorfii; $N = 99, 23, 42, 8$, and 71, respectively).

In the PCAs of leaf blade lengths and widths (Figs. 3a and 3b), and in the PCA of the raw Fourier amplitudes (Fig. 3c) only the eigenvalue corresponding to the first principal component accounted for a significant proportion of the sample total variance according to the broken-stick criterion. In all three analyses this axis corresponds to a contrast between the narrow, elliptic short-shoot leaves of C. rivularis and C. saligna and the almost isodiametric ones of C. okennonii (Fig. 3). Short-shoot leaves of C. douglasii and C. suksdorfii are intermediate between these two extremes, and overlap in shape (Fig. 3). Plotting the inverse of the dissection index (invDI) against PC1 (as in Dickinson 2003; not shown) for each data set did not provide any greater contrasts between a priori groups than did the corresponding (nonsignificant) PC2 axis.

The extent to which the CVA axes for these data sets permit discrimination of the a priori groups (Table 3; as indicated by the magnitude of and significance of the $X^2$ values for the degrees of freedom shown, and by the smallness of the values of Wilks’ $\Lambda$) varies in a manner similar to the variation between the data sets in the magnitude of the percent total variance accounted for by the first PC axes (Fig. 3). The two leaf blade lengths provide the crudest differentiation (Data set 1, Table 3), and separated only the short-shoot leaves of C. rivularis from those of the other three species in the comparison (Table 3). This discrimination along the first CV axis improved with the nine leaf blade widths (Data set 2, Table 3), and was greatest with the 39 Fourier amplitudes (Data set 3a, Table 3). Since it could be argued that with the fixed sample size in these CVAs ($N = 192$) the much larger number of Fourier amplitudes would overfit the model in this analysis (Gittins 1985), the analysis was repeated using only the first nine Fourier amplitudes (Data set 3b, Table 3; cf. Figure 3c). When this was done the discrimination afforded by the Fourier amplitudes still showed a modest increase over that obtained with the same number of leaf widths. In these latter three analyses there was a trend also to discriminate C. douglasii and C. suksdorfii from C. okennonii along the second CV axis.

In the CVA of the 39 Fourier amplitudes so much variance was accounted for by the second axis at the expense of the first that, of the first three CV axes, only the second was significant according to the broken-stick criterion (eigenvalues as percent total variance = 59, 30, and 10, compared to critical percentages of 61, 28, and 11, respectively, under the broken-stick model).

Variation in seven morphological descriptors

Principal components analysis of seven ranged descriptors of leaf, thorn, and fruit morphology (Table 2) suggested three or more clusters in the 275 OTU sample, as a result of variation in all seven descriptors (Fig. 4). In this analysis, the eigenvalues corresponding to the first two principal components accounted for a significant proportion of the total variance in the sample according to the broken-stick criterion. Average linkage clustering based on Euclidean distances calculated from the ranged data (not shown) provided the basis for recognizing four major clusters (Fig. 4) corresponding to PC2 because of the broken-stick criterion. Stamen and style numbers are correlated with thorn dimensions and with PC1, and are clearly bimodal in both series (Figs. 4 and 5). Differentiation of the two series Cerrones clusters is correlated with PC2 because of their narrower leaves (Figs. 4 and 6).

Polythetic divisive clustering was more successful in resolving many individuals of C. castlegarensis and C. okennonii into separate clusters (not shown). Crataegus douglasii and C. suksdorfii OTUs were split into two or more clusters, so that the topology of the dendrogram reflected not only stamen number bimodality but also differences in leaf shape and thorn dimensions.
correspond substantially to species samples in this data set: mixed data produced seven clusters (Fig. 7). These clusters summarized using Gower’s resemblance coefficient for complete suite of 20 morphological descriptors (Table 2) and (c) 39 Fourier amplitudes calculated from coefficients for 20 elliptic Fourier harmonics. In a–c, only the first PC axis accounts for more than the proportion expected under the broken-stick model (compare the percentages shown with critical values of 75% and 25%, 31% and 20%, and 11% and 8% in a–c, respectively). In each case, plotted points correspond to mean vectors calculated over all of the leaves sampled for each OTU. Convex hulls enclose clusters formed by taxa in the sample; the hull for two unlabeled C. douglasii resembling C. okennonii is a line. A single OTU (de91; supplementary data, Appendix A) resembling both C. douglasii and C. erythropoda is not shown, but is located within the C. douglasii–C. okennonii overlap.

Variation in 20 morphological descriptors

Average linkage clustering and PCoA based on the complete suite of 20 morphological descriptors (Table 2) summarized using Gower’s resemblance coefficient for mixed data produced seven clusters (Fig. 7). These clusters correspond substantially to species samples in this data set: C. saligna, C. suksdorfii (two clusters), C. rivularis, C. erythropoda, C. douglasii, and C. castlegarenensis. In this subsample there are only six OTUs of C. okennonii, and three of these are found as singletons (including an isotype, o324; Fig. 7), as is the sole C. phippsii OTU, while the remaining three C. okennonii form a subcluster nested among the C. douglasii (Fig. 7). Similarly, three of the four C. shuswapensis OTUs form a subcluster nested among the C. erythropoda (Fig. 7). OTUs of none of the nine species occupy exclusively a single cluster. Rather, five clusters are heterogeneous to some degree, with only OTUs of C. saligna and C. rivularis forming single-species clusters. In addition to stamen number bimodality in this sample, the other pronounced trends are in leaf abaxial surface pubescence (LFBPalong, LFBPbetween), and in calyx lobe shape and margination (KLOS, KLOT; Table 2, Fig. 8). The former two descriptors appear to account to a considerable extent for the way in which there is a cluster of C. castlegarenensis OTUs here that failed to form in the 275 OTU analysis in which pubescence descriptors were not included. Polythetic divisive clustering of this subsample (not shown) yielded a dendrogram topology that reflected primarily stamen number bimodality, and thus was simpler overall than that in the divisive clustering of the 275 OTU sample. Most of the OTUs of C. castlegarenensis and of C. erythropoda formed distinct clusters. Clustering of the C. suksdorfii OTUs reflected differences in thorn and leaf dimensions, as well as in leaf pubescence, and calyx lobe margination and shape.

Microsatellite data

Visual comparison of dendrograms derived from the 20 morphological descriptors and from the microsatellite allele presence/absence data for the 72 OTU subsample reveals substantial differences in dendrogram topology and cluster composition between the two data sets (Fig. 9). The microsatellite data support differentiation of series Cerrones (A; Fig. 9a) and Douglassianae (B, C; Fig. 9a), whereas the morphological data largely reflect the contrast in stamen number between C. saligna and C. suksdorfii on one hand (B; Fig. 9b), and the 10-stamen taxa on the other (A, C; Fig. 9b). Clusters found in the microsatellite dendrogram are variously broken up, or else preserved in the morphological one. Microsatellite clusters of C. erythropoda, Ontario C. douglasii, Idaho C. douglasii, and triploid Idaho C. suksdorfii are all dispersed in the morphology dendrogram (Fig. 9). On the other hand, clusters comprising all four C. saligna OTUs, 7 of 10 C. rivularis ones, three Montana C. douglasii, five Montana tetraploid C. suksdorfii, and four triploid Oregon C. suksdorfii are found in both dendrograms. These last three clusters correspond to topodome random samples at sites MT2 and OR6, respectively (Dickinson et al. 1996; supplementary data, Appendix A).

Discussion

Geographic distribution

The ranges of the species studied here can be associated with vegetation types that are indicative of the ecological amplitude of the different species. The range of C. suksdorfii extends into the boreal forest in the north,
while its southern extent corresponds broadly to that of the mesic conifer forest (terminology of Detling 1968; Brunsfeld and Johnson 1990). Typically, the dominant species in this forest type is *Pseudotsuga menziesii* (Mirb.) Franco, and the distribution of this species at least in Washington, Idaho, and Montana (Thompson et al. 1999) approximates that of *C. suksdorfii* (Fig. 2). The distribution of *C. douglasii* (Fig. 2) extends into considerably more xeric vegetation types, e.g., in the Columbia basin and in southeastern Idaho where it overlaps with that of *C. rivularis*. *Crataegus douglasii* also extends farther east than any of the other species considered here (Fig. 2), occurring in the Cypress Hills of southern Alberta and Saskatchewan, and in the upper Great Lakes basin (Phipps and Muniyamma 1980; Marquis and Voss 1981). The ranges of *C. castlegarensis* and *C. okennonii* (Fig. 2; Phipps and O’Kennon 1998) appear to be subsets of that of *C. douglasii*. *Crataegus rivularis* (Fig. 2) occupies habitats apparently more xeric than *C. douglasii* can tolerate. The range of *C. saligna* (Fig. 2), however, is a small subset of that of *C. rivularis*. This species appears to be restricted to more mesic, riparian habitats (Beatty et al. 2004), but this difference in distribution could also be a consequence of differences in breeding system and colonizing ability.

**Morphological variation**

This is the first study to survey variation in leaf shape, thorns, and floral architecture across the ranges of the North American black-fruited hawthorns that make up series *Cerrones* and *Douglasianae* (Fig. 2). Phenetic analyses of just the leaf shape data from these specimens illustrate only a simple contrast in overall leaf shape, between narrowly elliptic leaves (*C. rivularis, C. saligna*) and the nearly isodiametric ones of *C. okennonii* (Fig. 3). In part, this is likely due to sampling short-shoot leaves without regard to their position along the shoot (Dickinson and Phipps 1984; Dickinson 1986). Nevertheless, increasing the resolution with which shape variation was captured from four to 20 to hundreds (in the case of the outline-based Fourier analyses) of landmarks produced correspondingly increased discriminat-
The inverse of the dissection index of Kincaid and Schneider (1978) has proven useful in reflecting marked differences in leaf lobing (Dickinson 2003) but here (Fig. 10), where variation in leaf lobing is much less pronounced, the second principal component axis for each data set provided better contrasts between the groups than did the descriptor invDI (Table 2).

Phenetic analyses of the simplest leaf shape descriptors (relX, relZ, invDI; Table 2) combined with thorn dimensions and the floral architecture descriptors (stamen number and style number) were much more effective than the leaf data alone in resolving the group structure of the data (Figs. 4 and 7). However, neither agglomerative nor divisive clustering could consistently resolve the OTUs into exclusively single-species clusters. Partly this appears to be due to the problems inherent with both these approaches to clustering: local similarities join disparate OTUs with agglomerative clustering, while even polythetic divisive clustering may separate similar OTUs because of the global criterion that is active early on in clustering. Agglomerative clustering was especially bad in this respect (Fig. 4). It also appears that the species studied here overlap more than keys and descriptions.
suggest is the case. For example, some individuals identified as *C. castlegarensis* (in part because they have more than one thorn at a node) may actually be referable to *C. douglasii*.

The dominant feature of these results was the marked distinction between the 10- and 20-stamen taxa along the PC1 axis (Sargent 1907; Brunsfeld and Johnson 1990) and, within the 10-stamen OTUs, the more pronounced separation between *C. rivularis* and the *C. douglasii-C. okennonii* clusters (Fig. 4). These results also corroborate Brunsfeld and Johnson’s (1990) characterization of the contrast in thorn length between *C. douglasii* and *C. suksdorfii* (Figs. 4 and 5d).

Variation within what might be termed *C. douglasii* sensu lato (black fruit, 10 stamens per flower) has been given taxonomic recognition by Phipps and O’Kennon (1998, 2002). Their earlier paper recognized a widespread (Fig. 2), more round-leaved (Fig. 3) form of *C. douglasii* as *C. okennonii*, while in the second paper they described two additional segregate species, *C. castlegarensis* and *C. shuswapensis*, both of which they placed in series *Douglasianae* together with *C. douglasii*, *C. suksdorfii*, and *C. okennonii* (Phipps et al. 2003). *Crataegus castlegarensis* is distinguished by its pubescent pedicels, and by occasionally producing more than a single thorn at a node. Like *C. okennonii*, *C. castlegarensis* is quite widespread in the northwestern United States (Phipps and O’Kennon 2002; Zika 2004). In contrast, *C. shuswapensis* appears to be restricted to the Shuswap drainage in southern British Columbia (Phipps and O’Kennon 2002). All three of these species have black fruit that pass through a stage that is conspicuously reddish or chestnut-coloured (Phipps and O’Kennon 1998, 2002).

The other five species and one variety also with about 10 stamens per flower that are described by Phipps and O’Kennon in these two papers make up their newly described series *Purpureofructi* (Phipps and O’Kennon 2002), a group that is characterized in part by fruit that go through a conspicuous reddish colour phase before maturing to dark red, purple or nearly black. Most of the members of this new series have a relatively limited distribution in the southern interior of British Columbia, or in the Flathead and Clark Fork drainages in northwestern Montana (Crataegus...
The sample studied here does not include members of this group, other than a single individual of *C. phippsii*.

With the sole exception of *C. shuswapensis*, all of these species studied so far which segregated from *C. douglasii* (*C. castlegarenensis*, *C. enderbyensis*, *C. okennonii*, *C. phippsii*) are, like *C. douglasii* itself, tetraploid (Talent and Dickinson 2005). A single accession of *C. shuswapensis* available for flow cytometric study, however, proved to be diploid (Talent and Dickinson 2005).

**Agamic complexes?**

Because much of the sample studied here was drawn from loan material so as to represent the geographic extent of the distribution of black-fruited hawthorns, only limited data on local patterns of phenetic variation could be recovered (i.e., for OTUs from sites ID6, MT2, OR6 in Dickinson et al. 1996; supplementary data, Appendix A). Nevertheless, these limited data can be taken together with the overlapping patterns of variation seen in *C. douglasii* and its segregate species (Figs. 4–8, 10, and 11), and with results (Fig. 9) that reflect those of recent studies of breeding system and ploidy level variation (Talent and Dickinson 2005, 2007a, 2007b, 2007c; E. Lo, N. Talent, S. Stefanović, T.A. Dickinson, unpublished data, 2008).

In series *Cerrones*, *C. saligna* is less well known, but it has 20 stamens per flower and appears to be exclusively a sexual diploid (Talent and Dickinson 2007c). *Crataegus rivularis* is tetraploid (Talent and Dickinson 2005) and is consistent in reproducing apomictically (Lo 2008). *Crataegus rivularis* differs from *C. douglasii* in that it appears to be much more morphologically uniform across its range. The only species with which it might be confused is *C. erythropoda*, which is also tetraploid but has leaves that are more lobed, and fruit that ripen to at most a dark red (Beatty et al. 2004) or purple colour (Phipps 1999; see also Key below). This small series appears to be monophyletic, on the basis of chloroplast and nuclear DNA sequence variation (cf. Fig. 9; Lo et al. 2008c), corroborating results from an earlier phylogenetic analysis of exclusively morphological data (Phipps 1999). We speculate that *C. rivularis* could have arisen as an autoployploid derivative of *C. saligna*. The wider geographic range of *C. rivularis* (Fig. 2) could thus be due to the combination of apomixis and polyploidy (the latter possibly conferring both self–compatibility, hence better colonizing ability, and greater tolerance of xeric conditions). *Crataegus erythropoda* could have arisen through hybridization between *C. rivularis* and one of the sympatric, red-fruited species in section *Coccineae* (*Crataegus chrysocarpa* Ashe, *Crataegus macracantha* Lodd. var. *occidentalis* (Britton) Egglestone, *Crataegus phippsii* Eggl.; Phipps 1998b) that are frequently tetraploid (Talent and Dickinson 2005). In this way, series *Cerrones* serves as a simple model for what may have happened with much more complexity in series *Douglasianae*. 

---

**Fig. 8.** Variation in leaf pubescence (*a* and *b*) and calyx lobe morphology (*c* and *d*) in North American black-fruited hawthorns; box plots of integer scores for four descriptors (Table 2), averaged over replicates within OTUs, for taxa (Table 1) in the 106 OTU sample (Fig. 7).
Key to the black-fruited hawthorns of North America where terminology of leaf shape and venation follows Leaf Architectue Working Group (1999), and see Table 1 for taxon authorities and references consulted, Figs. 5, 6, 8, 10, and 11 for univariate descriptor data.

1a Stamens averaging (15–)20(–22) per flower on an individual; secondary venation of short-shoot leaves weakly brochidodromous, semicraspedodromous, or craspedodromous; calyx lobes variable ........................................... 2

1b Stamens averaging (5–)10(–12) per flower on an individual; secondary venation of short-shoot leaves craspedodromous; calyx lobes longer than 2.5 mm, usually with marginal teeth on each lobe. ............ Section Douglasianae in part, 5

2a Thorns very short (typically less than 10 mm long), strongly recurved, short-shoot leaf blades narrowly elliptic to narrowly ovate, with weak brochidodromous secondary venation (Figs. 1a–1d); petals turning light orange upon drying; radial surfaces of pyrenes smooth. Louisiana, eastern Texas ......................... Crataegus brachycantha (section Brevispinae)

2b Thorns typically 10 mm or longer, straight or only slightly recurved, short-shoot leaf blades with semicraspedodromous to craspedodromous secondary venation (Figs. 1e–1g); petals not turning light orange upon drying; radial surfaces of pyrenes variously smooth or excavated except as noted below .................................. Section Douglasianae in part, 3

3a Short-shoot leaf blades consistently (narrowly) elliptic to oblong in overall outline, with semicraspedodromous secondary venation (Figs. 1e and 1f); thorns usually 18 mm or longer; calyx lobes shorter than 2.5 mm, usually untoothed; radial surfaces of pyrenes excavated. Colorado, eastern Utah. .................. Crataegus saligna (Series Cerrones in part).

3b Short-shoot leaf blades variable in shape but typically elliptic to obovate in overall outline, with semicraspedodromous or craspedodromous secondary venation (Figs. 1e–1g); calyx lobes variable; southern Alaska, British Columbia, California, central and northern Idaho, Montana, Oregon, Washington .................................. Series Douglasianae in part, 4

4a Short-shoot leaf blades broadly elliptic to broadly obovate in overall outline and markedly lobed, secondary venation craspedodromous; thorns usually 18 mm or longer; calyx lobes longer than 2.5 mm, densely toothed on the margins of each lobe; stamen number variable, (10–)20; radial surfaces of pyrenes smooth. Southern interior British Columbia. .................................................. Crataegus shuswapensis

4b Short-shoot leaf blades variable in shape but typically elliptic to obovate in overall outline and sometimes lobed, secondary venation semicraspedodromous or craspedodromous; thorns usually 10–18 mm; calyx lobes shorter than 2.5 mm, usually untoothed; stamens (15–)18–22; radial surfaces of pyrenes excavated. Southern Alaska, British Columbia, California, central and northern Idaho. Washington. .................................. Crataegus suksdorffii

5a Short-shoot leaf blades consistently narrowly elliptic in overall outline and unlobed; calyx lobes sometimes toothed; radial surfaces of pyrenes excavated. Arizona, Colorado, southern Idaho, Nevada, northern New Mexico, Utah, Wyoming. .................. Crataegus rivularis (Series Cerrones in part)

5b Short-shoot leaf blades variable in shape, frequently obovate to isodiametric in overall outline, and usually lobed in the upper half; calyx lobes variously toothed; southern Alberta, British Columbia, California, central and northern Idaho, Montana, Oregon, southern Saskatchewan, upper Great Lakes basin, Washington, Wyoming. .................. 6

6a Inflorescence axes pubescent; short-shoot leaf blades often approaching rhombic in overall outline; sometimes with more than one thorn developing at a node; calyx lobes occasionally toothed; radial surfaces of pyrenes “usually pitted” (Phipps and O’Kennon 2002). Absent from the upper Great Lakes basin .......... Crataegus castlegarenensis (Series Douglasianae)

6b Inflorescence axes glabrous; short-shoot leaf blades variable in shape, frequently obovate in overall outline; multiple thorns at a node infrequent ........................................... 7

7a Fruit ripening to black, not passing through a reddish phase first; calyx lobes sometimes toothed. ........................................... Series Douglasianae in part, 8

7b Fruit ripening to dark red, purple, or black, passing through a (dark) red phase first ........................................... 9

8a Short-shoot leaf blades obovate, glabrous abaxially except sparsely pubescent along veins; thorns often (9–18–23(–30) mm long; radial surfaces of pyrenes excavated; fruit not passing through a chestnut-coloured phase ........ C. douglasii

8b Short-shoot leaf blades broadly obovate to almost isodiametric in overall outline, pubescent abaxially along and between veins; thorns often (11–14–19(–24) mm long; radial surfaces of pyrenes “variably eroded or sometimes plane” (Phipps and O’Kennon 1998); fruit passing through a chestnut-coloured phase. Absent from the upper Great Lakes basin .......... C. okennonii

9a Calyx lobes with margins densely toothed; radial surfaces of pyrenes excavated. Colorado, New Mexico, Wyoming ........................................... C. erythropoda (Series Cerrones)

9b Calyx lobes toothed; radial surfaces of pyrenes smooth, excavated, or variable. Southern interior British Columbia and adjacent Montana, Washington. ........................................... Series Purpureoefructi
In common with some other 20-stamen *Crataegus* taxa, *C. suksdorfii* comprises diploid, triploid, and tetraploid individuals (Dickinson et al. 1996; Talent and Dickinson 2005;5) that vary in breeding system as do most other Rosaceae that exhibit polyploidy (Dickinson et al. 2007); diploids are self-incompatible and reproduce sexually, whereas triploids and tetraploids exhibit gametophytic apomixis (Love and Feigen 1978; Dickinson and Phipps 1986; Dickinson et al. 1996; Talent and Dickinson 2007b, 2007c). As noted above, however, 10-stamen *C. douglasii* s.l. comprises (with the apparent exception of *C. shuswapensis*) exclusively tetraploid entities that, to the extent of our knowledge, produce seeds apomictically or by selfing (tetraploids are self-compatible and reduced gametes may be produced; Talent and Dickinson 2007b, 2007c). Of course, these individuals may also participate in outcrossing (hence possibly also hybridization) as either ovule or pollen parents (pollen stainability is frequently quite high; Dickinson et al. 1996).

In connection with the use of fruit colour in distinguishing segregates of *C. douglasii*, one of us (R.M.L.) has observed fruits of hybrids between *C. suksdorfii* and red-fruited *C. monogyna* in the Willamette valley passing through a crimson phase prior to ripening fully black. The red, brown, and purple colours seen during fruit ripening in *C. castlegarensis*, *C. okemoni*, *C. shuswapensis*, and series *Purpureofructi*, as well as the variation in pyrene radial surface that occurs in these taxa (see also, Key) all suggest the possibility of their hybrid origin.

Morphological variation in series *Douglasianae* (Figs. 3–8, 10, and 11) involves not only the variability seen in individual descriptors (Figs. 5, 6, 8, 10, and 11) but also the way in which portions of the descriptor ranges are recombined in different ways. For example, the within-species variation in *C. suksdorfii* (Figs. 4 and 7) results in part from specimens in which, say, 20-stamen flowers are found together with lobed leaves and (or) longer thorns. In Fig. 9 the clusters representing tetraploid OTUs of *C. douglasii* and *C. suksdorfii* from site MT2 illustrate how similar these 10- and 20-stamen individuals are, such that in the morphology dendrogram the *C. suksdorfii* specimens are actually part of the cluster of 10-stamen OTUs (C; Fig. 9b). If stamen number is not checked at this site, these two species would be almost indistinguishable. Such recombination of character states typical of different species suggests the possibility of reticulate relationships within the sample, and the existence of such relationships is in fact supported by molecular studies (Lo 2008; Lo et al. 2008a, 2008b). The implications of variation in vegetative morphology and ploidy level within the *C. suksdorfii* complex will be treated in greater detail elsewhere (T.A. Dickinson, R.M. Love, E. Lo, and N. Talent, unpublished data, 2008).

Taken together, these lines of evidence support the common supposition that some *Crataegus* species represent (parts of) agamic complexes. Because of their mixed mating system (pseudogamous gametophytic apomixis, selfing, and outcrossing in varying proportions that depend at least in part on ploidy level) some of the lineages (“species”) making up these complexes apparently are able to remain distinct. The same mixed mating system also likely contributes to the formation and perpetuation of hybrid genotypes. Such genotypes, with intermediate morphologies, or with novel combinations of descriptor states, appear to be responsible for much of the taxonomic difficulty seen in North American *Crataegus*. Implications of this conclusion for hawthorn taxonomy are discussed in the following section.

### Species concepts

Previous monographic work on *Crataegus* (Phipps and Muniyamma 1980; Christensen 1992) has employed a morphological species concept (SC; Du Rietz 1930; Heslop-Harrison 1967). Dickinson (1999) reviewed alternative SCs and their applicability to agamic complexes in plants. No explicit recommendation was made at that time other than to argue that the results of applying a particular SC should be comparable regardless of whether reproduction was predominantly or only infrequently sexual. This would restrict formal taxonomic recognition of species (using the biological SC sensu Coyne and Orr 2004) to entities in which multiple genotypes conform broadly to a distinctive common phenotype. Within such species there may be genotypes (cytotypes, microspecies) that predominantly reproduce by apomixis, and if these have distinctive phenotypes they can be named at an infraspecific level, or else informally (Burt 1970; Campbell and Wright 1996; Dibble et al. 1998). At the same time it seemed that the level in the existing taxonomic hierarchy at which biological species could be circumscribed might be that of the section or series (Dickinson 1999).

Strict application of the biological SC (Coyne and Orr 2004) may seem impractical in Rosaceae tribe Pyreae because of the interfertility of morphologically well-differentiated clades (genera) that are supported to a considerable extent by DNA sequence data (Campbell et al. 2007). Likewise, within many of the larger genera of Pyreae, morphologically and ecologically well-differentiated species are capable of hybridizing (Dickinson et al. 2007). In the case of *Crataegus*, phylogenetic analyses of DNA sequence data demonstrate the presence of at least six well-supported, morphologically well-differentiated clades corresponding to sections *Brevispiniae*, *Mespilus* T.A. Dickinson & E.Y.Y. Lo, *Crataegus*, *Sanguineae* Zabel ex C.K. Schneid., *Douglasianae*, and a group of poorly resolved, mainly eastern North American species (Lo et al. 2007; Lo et al. 2008c). Introduction and naturalization of diploid *C. monogyna* (Sect. *Crataegus*) in North America has led to hybridization between it and North American diploids *C. punctata* (the poorly resolved eastern North American clade; Wells and Phipps 1989) and *C. suksdorfii* (Sect. *Douglasianae*; Love and Feigen 1978). The biological SC also seems impractical to apply because the common-sense criterion suggested by Coyne and Orr (2004; do supposedly distinct species maintain their distinctness in sympathy?) is difficult to evaluate. Hawthorns are ruderal, relatively long-lived woody perennials that colonize open, often early successional, high light intensity habitats subject to frequent disturbance (e.g., erosion surfaces, flood plains, streambanks, forest margins, abandoned or poorly-managed agricultural land, etc.). It is unlikely that a single human

---

observer can detect whether sympatric hawthorn “species” will maintain their distinctness at a given site.

Alternatively, however, this criterion could be applied so as to recognize many hawthorns as biological species on the grounds of their regional sympatry (e.g., in the Pacific Northwest, the Rocky Mountains, or eastern North America) and the fact that over more than a century of human observation they have maintained their distinctness. With this approach what would be recognized most restrictively as conforming to the biological SC would be the one or more diploid entities associated with morphologically defined taxonomic series, e.g., Cerrones (C. saligna), Douglasianae (diploid C. suksdorfii), etc. Limited relaxation of this criterion could admit polyploid entities such as C. douglasii and C. rivularis on the basis of their historical persistence as distinct entities. Further study is required to determine whether the genotypic diversity of C. rivularis is comparable with that of C. douglasii (Lo 2008; Lo et al. 2008a, 2008b). Other entities, that appear to share (or to be likely to share) genomes from two or more species (e.g., allotriploid and tetraploid C. suksdorfii; Lo 2008; Lo et al. 2008a), would be recognized at an infraspecific level (or equivalently as microspecies sensu Gustafsson 1947), as part of an agamic species complex. We suggest informally (see also, Key) that this is also an appropriate treatment for C. castlegarensis and C. okennonii, since their distinctiveness appears (Figs. 4 and 7) to rest on only a few descriptors, and they appear to occur sporadically across the range of C. douglasii (Fig. 2). More data are needed before taking a similar step with C. shuswapensis and C. erythropoda.

A critical component of this approach is recognition of the likelihood that hybridization does occur in Crataegus. In a recent review of this question, Phipps (2005) has argued that in Missouri fewer than 15% (20% for the northeastern United States) of all possible cases of interserial hybridization have occurred. Moreover, “… the numbers of hybrid individuals on the ground appear to be low at best and perhaps, more commonly at this time, non-existent” (Phipps 2005). Phipps goes on to suggest that frequent, implicitly interserial, hybridization is thus not the root cause of taxonomic confusion in North American hawthorns. In doing so, however, he accepts the possibility that hybridization between similar entities may not have been detected (Phipps 2005). Elsewhere we have documented not only evidence for wide hybridization (Lo et al. 2007; Talent and Dickinson 2007a; Lo et al. 2008c), but also for just the kind of reticulation referred to by Phipps, in series Douglasianae here (Fig. 9) and elsewhere (Lo et al. 2007; Lo et al. 2008a). Given the frequency of polyploidy in North American Crataegus (Talent and Dickinson 2005; Lo 2008), the widespread occurrence of gametophytic apomixis and self-compatibility (Muniyamma and Phipps 1979b, 1984; Dickinson and Phipps 1986; Smith and Phipps 1988; Dickinson et al. 1996; Talent and Dickinson 2007c), and the evidence for gene flow between ploidy levels (Talent and Dickinson 2007a; Lo et al. 2008a), it seems likely that many North American species complexes will prove similar to the situations we infer here in series Cerrones and Douglasianae in which morphological and molecular data implicate “wide” and not so wide hybridization in the origin of some degree of taxonomic complexity. We suggest that it will be possible to accommodate these situations at an infraspecific level using the adaptation of the biological SC suggested above.

**Conclusion**

Land clearing and abandonment in eastern North America over the past 300 years is thought to have episodically increased the availability of hawthorn habitat, thus increasing the frequency with which different species are found in sympatry. Hawthorns have unspecialized flowers that are visited by generalist pollinators, and the principal isolating mechanisms identified to date are phenological ones (Phipps and Muniyamma 1980; and see Fig. 1 in Campbell et al. 1991). Other mechanisms may also operate (Talent and Dickinson 2007c), but can only be detected by controlled crosses. As a result, anthropogenic habitats are thought to have been responsible for increasing the opportunities for hawthorns to hybridize in eastern North America. Many of the entities with narrow distributions recently segregated from C. douglasii have been described (Phipps and O’Kennon 1998, 2002) from areas in which there have been substantial changes in land use over the past 50 or more years. For example, hydroelectric development on the Columbia River in British Columbia led to the abandonment of agriculture in many areas (J. Lee, personal communication, 2007). This could have had an effect on the availability of hawthorn habitat and hence on the frequency with which different species occurred in sympathy and hybridized. Also in British Columbia, agricultural activity and other forms of human disturbance over the past century in the Slocan Valley appear to have been followed by extensive hawthorn colonization (T. Steen, A. Anderson, personal communication, 2008). Further evaluation of the possibility that western North American hawthorn groups (species complexes, series, sections) are in fact agamic complexes will need to take into account not only patterns of morphological and genetic variation, but also the history of land use, especially in areas of supposedly high species diversity or endemism.

**Acknowledgements**

The authors are indebted to the curators of the herbaria whose specimens are listed in Appendix A (supplementary data4) for their loans of the specimens without which the study reported here would have been impossible. This study reports on a research program that is in a dialectical relationship with that of James B. Phipps, of the University of
Western Ontario; Phipps’ voluminous studies of North American hawthorns have provided a framework within which much of our work has been done, in an attempt to integrate morphological taxonomy with data from reproductive biology and molecular systematics. Rebecca Dotterer compiled much of the sample studied here, and digitized

© 2008 NRC Canada
Fig. 10. Variation in leaf morphology (a and b) in North American black-fruited hawthorns; box plots of two ratio-scale descriptors (Table 2) for taxa (Table 1) in the 106 OTU sample (Fig. 7).

their leaves. These specimens also include ones from her fieldwork with Bill Dotterer. Jenny Bull, Keiko Kato, Dale Leadbeater, Cheying Ng, and Annabel Por painstakingly compiled the additional morphological data, databased specimen label data, and georeferenced specimens. The authors also thank Christopher S. Reid and Peter Zika for plant collection and identification, and Mildred Arnot, Steve Brunsfeld, Adam Dickinson, John Dickinson, Fannie Gervais, Jack Maze, Sophie Nguyen, and Toby Spribille for help with fieldwork. April Anderson, Jeanette Lee, and Tammy Steen kindly provided T.A.D. with information about land use history in southern interior British Columbia. Cary Gilmour demonstrated to T.A.D. the efficacy of X-ray imaging for documenting leaf venation pattern. Patricia Ross prepared the leaf X-rays studied here, and Brian Boyle and co-workers in the ROM’s Ivey Imaging Center digitized them. We are also indebted to James Macklin and an anonymous reviewer for their comments on an earlier draft of this paper; their comments have helped greatly to refine and strengthen the arguments presented here. Financial support from an Natural Sciences and Engineering Research Council of Canada Discovery Grant A3430 to T.A.D., the Botany Department of the University of Toronto, and the Royal Ontario Museum (ROM) is gratefully acknowledged; this project was also made possible, in part, by an award from the ROM Foundation to T.A.D. for the image capture equipment used to digitize leaf outlines, by their grant to Mark Engstrom and T.A.D. for the purchase of the thermocycler used in part of this work, and by the generous support of the ROM Reproductions Acquisitions and Research Fund.

Fig. 11. Variation in three binary or multistate descriptors (Table 2) for taxa (Table 1) in the 106 OTU subsample (Fig. 7). Note that hypanthial ovary pubescence was scored on spring collections only, where these were available. Numbers indicate the number of specimens exhibiting the states present.
References


