Talent, Nadia and Dickinson, Timothy A. 2005. Polyploidy in *Crataegus* and *Mespilus* (Rosaceae, Maloideae): evolutionary inferences from flow cytometry of nuclear DNA amounts. Canadian Journal of Botany 83(10): 1268–1304.

Polyploidy in *Crataegus* and *Mespilus* (Rosaceae, Maloideae): evolutionary inferences from flow cytometry of nuclear DNA amounts

Nadia Talent and Timothy A. Dickinson

Abstract: Hawthorns and medlars are closely related genera in Rosaceae subfamily Maloideae, whose taxonomy remains poorly understood. Gametophytic apomixis occurs in polyploids, and diploids are sexual out-crossers, so ploidy level is of great interest, but suitable material for chromosome counts is of limited availability each year. The promise of flow cytometry is that it permits rapid measurement of nuclear DNA amounts from most tissues, and ploidy level can be inferred if climatic and taxonomic differences do not interfere. Our DNA measurements cover most of the taxonomic series in *Crataegus*, adding cultivated and naturalized Eurasian plants to the many wild plants collected mainly from south-central Canada and the southeastern and northwestern United States. We found that some variation in DNA amount per genome copy distinguishes certain taxa, but ploidy-level estimates are at least as clear as the published chromosome counts, especially in the most common diploid–triploid–tetraploid range, and to the single published higher (hexaploid) chromosome count, we add evidence of pentaploids. By comparing ploidy evaluations to morphology, we hypothesize that both autopolyploidy and allopolyploidy contribute to the taxonomic complexity. We compared DNA amounts in Maloideae with those in *Gillenia*, a likely sister genus to the subfamily, which has a smaller chromosome number.

Key words: apomixis, *Crataegus*, flow cytometry, *Gillenia*, *Mespilus*, polyploidy.

Résumé : Les aubépines et les nèfles sont des genres voisins de Rosaceae, de la sous famille des Maloideae, dont la taxonomie demeure mal comprise. L'apomixie gamétophytique survient chez les polyploïdes, et les diploïdes sont à croisements externes, de sorte que le degré de ploïdie est d'un grand intérêt, mais le matériel adéquat pour le décompte des chromosomes est d'accessibilité limitée chaque année. L'intérêt de la cytométrie en flux est qu'elle permet de mesurer rapidement les quantités d'ADN, à partir de la plupart des tissus, et qu'on peut en déduire le degré de ploïdie, si les différences climatiques et taxonomiques n'interfèrent pas. Les mesures d'ADN effectuées par les auteurs couvrent la majeure partie des séries taxonomiques chez les *Crataegus*, y incluent des plantes cultivées et naturalisées d'origine eurasienne, ainsi que plusieurs plantes sauvages, récoltées surtout dans le centre sud du Canada, et le sud est et le nord ouest des États-Unis. On constate qu'une certaine variation dans la quantité d'ADN par copie du génome distingue certains taxons, mais que les estimations du degré de ploïdie sont au moins aussi claires que les décomptes de chromosomes publiés, surtout dans la gamme la plus commune diploïde–triploïde–tétraploïde; les auteurs ajoutent à un unique compte de nombre chromosomique supérieur (hexaploïde), la preuve de pentaploïdie. En comparant les estimés de ploïdie avec la morphologie, les auteurs formulent l'hypothèse que l'autopolyploïdie et l'alloploïdie contribuent à la complexité taxonomique. Ils comparent les quantités d'ADN chez les Maloideae avec les *Gillenia*, un genre vraisemblablement voisin de la sous-famille, lequel possède un plus petit nombre de chromosomes.

Mots clés : apomixie, *Crataegus*, cytométrie en flux, *Gillenia*, *Mepilus*, polyploïdie.

[Traduit par la Rédaction]

Introduction

The hawthorns and medlars form a distinct group within Rosaceae subfamily Maloideae, but their taxonomic history has been complex. They are now divided between two closely related genera, with just two species in *Mespilus* L. and the remainder in *Crataegus* L. The distribution of *Mespilus* is strangely disjoint since the Eurasian medlar *M. germanica* L., though widely naturalized in Europe, was apparently native to Azerbaijan, Armenia, Georgia, and northern Turkey (Baird and Thieret 1989) whereas *M. canescens* J.B. Phipps is known from a single site in Arkansas, USA. The species now placed in *Crataegus* are found in Eurasia, North and Central America, and northern South America (Phipps et al. 1990, 2003*b*). It is currently unclear how much genetic diversity exists in these plants, which makes conservation priorities difficult to set, although their ecological importance is considerable and some are valued for fruit, medicinal extracts, or horticulture. The total number of species has been estimated as between 140 and 200 (Phipps et al. 2003*b*), but the work of resolving the vast list

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of published names is far from complete (approximately 1700 species names may have been validly published; IPNI 2004). Approximately 40 taxonomic series have been identified that are grouped with less certainty into about 14 sections, but morphological analysis has so far yielded little insight into the relationships between these groups, and phylogenetic analyses have not yet incorporated molecular data.

Historically it was suggested that hybridization is primarily responsible for the systematic difficulty (Eggleston 1908; Brown 1910; Standish 1916), but although hybridization is well documented, its extent is now debated (Phipps 2005). Thus, it is unclear at this stage whether a phylogenetic analysis using molecular data would be hampered by reticulate relationships. What has long been inferred (Palmer 1932; Rickett 1936) and has since been demonstrated cytologically (Muniyamma and Phipps 1979*b*; Dickinson and Phipps 1986; Smith and Phipps 1988; Dickinson et al. 1996) is that gametophytic apomixis complicates the taxonomy. Apospory occurs in triploids and tetraploids, while diploids and some tetraploids are sexual (Muniyamma and Phipps 1985; Ptak 1986). In most of the many plant groups where apomixis occurs, it is associated with polyploidy and with heterozygosity, which suggests a hybrid origin (Nogler 1984; Carman 2001). The distributions of polyploidy and apomixis within the hawthorns, the details of the mechanisms, and the success rate of apomictic reproduction, as well as the extent of hybridization, all require further study.

One of the major lineages within subfamily Rosoideae of the Rosaceae includes some genera, notably *Rubus* and *Potentilla* sensu lato (Morgan et al. 1994), where apomixis coexists with high polyploid series from diploid to 12-ploid or higher. As far as we are aware, *Crataegus* and other members of subfamily Maloideae are a contrast, having yielded only diploid, triploid, and tetraploid chromosome counts until the recent addition of a hexaploid count for *Crataegus pinnatifida* (Gu and Spongberg 2003). It is plausible that the Maloideae are a group of recent origin where high polyploids have yet to evolve, but it is equally plausible that different mechanisms of genome duplication exist in the two groups (Nogler 1984). An investigation of this question might require a large number of ploidy-level determinations, which could also serve as a foundation for another important goal: a molecular phylogeny of *Crataegus* and *Mespilus* needs a good survey of polyploidy as a first step to determining the numbers of copies of nuclear genes.

Existing data

There already exists a considerable collection of chromosome counts from *Crataegus* (details below), enough to indicate that ploidy level probably varies within a morphological group, and that a full survey would require a large number of counts. Published chromosome counts are sometimes considered unreliable because of uncertain identifications or inadequate taxonomic coverage (Merxmüller 1970; Nelson-Jones et al. 2002; Funk et al. 2005) and in Maloideae, the difficulty of obtaining appropriate tissue for mitotic figures has caused some authors to resort to using data from root tips of seedlings rather than data from the tree itself, which is clearly problematic where hybridization between ploidy levels is known to occur. The most common practice is to obtain countable metaphase figures from pollen meiosis and

from mitosis in meristematic tissues (e.g., petals), both of which are available only during a very narrow window of time. The precision of chromosome counts in *Crataegus* and *Mespilus* is also hampered by the small size of the chromosomes (0.6–2.5 µm at mitotic metaphase; Byatt and Murray 1977; Ptak 1986; Dickinson et al. 1996), but although many of the chromosome counts are approximate, we found that a re-examination of the older data is valuable.

The single largest collection of counts for New World taxa was made by Longley (1924) using sections of pollen mother cells, a particularly difficult technique that often yielded only approximate counts. The aim was to refine a previous conclusion that problems in pollen meiosis indicated hybrid origin (Standish 1916) by checking whether the supposed hybrids were polyploid. Longley followed earlier work by Meyer (1915) in accepting the basic euploid chromosome number as 16 rather than 17, a number that is currently accepted as a feature shared with the genera of Maloideae sensu stricto (Evans and Campbell 2002), but his assessments of polyploidy are in line with other work. For the small number of dedicated workers who continue to wrestle with the taxonomic complexity of *Crataegus*, Longley's chromosome counts might provide a clue to identifying taxa that could be extinct and, as we shall see below, there may be clues to evolutionary behaviour in these data even if some of the plants are no longer readily available for study.

Longley obtained counts from 79 "species" at a time when over 1100 species names were in use for North American *Crataegus*. The counts were made from the living collection at the Arnold Arboretum and voucher specimens were not kept. But the labeling of the trees is described in the paper as "accurate," and at that time much care was taken with that arboretum collection as it was central to taxonomic work on North American *Crataegus*. We have grouped the taxa studied by Longley at the species level according to the most recent taxonomic revisions available (Table 1) to compare them to other publications and to our own results.

Flow cytometry might help

Flow cytometry is an established technique for plant as well as animal cells and is particularly appropriate for establishing the DNA content of free plant nuclei extracted from chopped tissue (Arumuganathan and Earle 1991). We hoped that DNA measurements per genome might be sufficiently uniform across *Crataegus* and *Mespilus*, or at least across many of the taxa, that they could translate to ploidy-level determinations, although this is not guaranteed since the DNA amount can vary greatly within the diploids of a single genus (e.g., *Gossypium*, Wendel et al. 2002). However, it is necessary to check that DNA measurements from a particular plant group are consistent under different growth conditions (Michaelson et al. 1991; Price et al. 2000). *Crataegus* has also been one of the more difficult subjects for flow cytometry (E.E. Dickson, personal communication, 1992). For practical use with a large number of plants, it may be necessary to use a high rate of flow through the cytometer, which reduces the precision of the DNA measurement. Thus we found it necessary to develop some adjustments to the protocol for tissue preparation and to verify the utility of DNA measurements in *Crataegus* and *Mespilus*. We have the pub-

lished chromosome counts from taxa that we can identify with certainty with which to calibrate cytometric measurements, and a few of the counts are from individual trees that are still accessible to us.

Materials and methods

Materials

Leaves were collected in the field and in botanical gardens April–September 2001–2004 (Table 1). We tagged all source trees growing outside botanical gardens with aluminum tags, and voucher specimens from all plants are filed in the Green Plant Herbarium of the Royal Ontario Museum (TRT). At sites distant from Toronto, leaf samples were sealed in plastic bags together with labels identifying the source tree, and (following the suggestion of E.E. Dickson) with no added moisture. Leaves packaged in this way yielded useful data in the cytometer up to 2 weeks later if refrigerated for most of that time.

Determinations of wild trees are by the authors unless other names are listed, and these are the names of colleagues who have lent their special expertise to particular groups. Determinations of cultivated trees were made by the arboretum staff of each institution, or their suppliers, and are accepted as plausible by the authors. Since there is a risk with living collections of mislabeling or misidentification, we have been cautious to use only such plants as seem to be correctly labeled according to our understanding of the taxa. For some Ontario taxa, we have attempted to sample morphological variation and for this we have used the discussions of variation in the recent revisions by Phipps and Muniyamma (1980) and by Voss (1985). At the present time it is not always possible to identify some plants below the level of taxonomic series, and we found it necessary to omit some plants from the sample because even that level of identification proved impossible.

Nomenclature

For the sake of simplicity, we have grouped varieties and cultivars to the species level. The bi-level classification of *Crataegus* into sections and series is based on the world checklist by Phipps et al. (1990) and on Christensen's (1992) monograph. Some adjustments to that classification come from later publications (Kasumova 1991; Phipps 1995, 1997; Gladkova 1996; Phipps and O'Kennon 2002; Phipps et al. 2003a, 2003b), and J.B. Phipps (personal communication, 2005) has suggested the placement of *C. shuswapensis* within series *Douglasianae*, *C. enderbyensis* within series *Purpureofructi*, and *C. munda* within series *Lacrimatae*. For known hybrids, we have used nothosections and nothoseries rather than follow those authors who group hybrids with one of the parents. The placement of series *Flavae* within section *Lacrimatae* is our best guess, and we follow Palmer's recommendation (Rehder et al. 1938) to use the name *C. calpodendron* (Ehrh.) Medik. rather than the ambiguous *C. tomentosa* L.

If a chromosome count exists for an American microspecies name that has not been included in Phipps et al.'s checklist (1990), we have used Kruschke's assessment of its affinity if he offered an opinion (Kruschke 1965). For Eurasian species and varieties that were not included in the

checklist or in Christensen's (1992) monograph, we have followed Cinovskis (1971). If one of these microspecies or varieties would be synonymized to a different species by Kruschke or Cinovskis, the names appear in braces with the original name followed by the revision, for example, {*C. treleasei* Sarg. / *C. dispessa* Ashe}. Other microspecies that have not received a recent revision, but were placed into series by Palmer (1925), are shown in braces, for example, {*C. apposita* Sarg.}.

Flow cytometry protocol

Preparation of the plant tissue involved mixing a buffer solution containing propidium iodide stain (buffer B) with free nuclei extracted (using buffer A) from tissue chopped with a razor blade, as detailed below. The DNA amount was calculated by comparison with that of *Pisum sativum*, which is a well-established standard with a signal close enough to the *Crataegus* values for accurate comparison but still readily distinguished from them (Greilhuber and Ebert 1994; Price and Johnston 1996; Temsch and Greilhuber 2001). Calculations used a value of 8.84 pg DNA for *Pisum sativum* (Greilhuber and Ebert 1994).

For leaf, petal, and bud tissue, the *Pisum* standard was internal: aliquots of prepared, unstained, *Pisum* suspension were mixed with the *Crataegus* preparation as the staining buffer was added.

Buffer solutions

Following Burton and Husband (1999), we used a chopping buffer (buffer A) from Bino et al. (1992) and a staining buffer (buffer B) from Arumuganathan and Earle (1991). The chopping buffer contained 15 mmol/L HEPES, 1 mmol/L EDTA, 80 mmol/L potassium chloride, 20 mmol/L sodium chloride, 300 mmol/L sucrose, 0.2% (*w/v*) triton X-100, 0.1% β-mercaptoethanol, and 0.5 mmol/L spermine. The staining buffer contained 10 mmol/L magnesium sulphate heptahydrate, 1 mg/mL dithiothreitol, 10% (*w/v*) triton X-100, 2% propidium iodide stock solution (5 mg/mL) and 0.24% DNAase-free RNAase. However, because of solubility problems in the propidium iodide stock solution, we routinely added twice as much to the final buffer.

Preparation of material

Preparation of the *Pisum sativum* standard follows Burton and Husband (1999). Approximately 0.5 cm^2 of leaf tissue was chopped in 0.5 mL of buffer over crushed ice. Then 1.0 mL of buffer was added before filtering first through coarse fabric of about 200-μm mesh, then through 37-μm mesh, and finally through 15-μm mesh. The filtrate was centrifuged at 13 000 rpm for 20 s. The pellet was resuspended in 120 μL of resuspension buffer (buffer B without the propidium iodide stain) and then divided into aliquots of 10 μL, one for each of 12 *Crataegus* samples.

For *Crataegus* leaf tissue, the volume of chopping buffer was increased to reduce the viscosity that can destroy the nuclei during filtering, centrifuging times were increased, and more care was necessary to remove a sticky precipitate that formed in some samples and caused problems with the cytometer. No more than 0.5 cm^2 of leaf tissue was chopped in 0.5 mL of chopping buffer, then 2.5 mL of chopping buffer was added before filtering through 37-μm mesh. The fil-

trate was centrifuged at 13 000 rpm for 4 min, and the pellet with an aliquot of the *Pisum* standard was resuspended in 170 μm of staining buffer. After standing on ice for 20–30 min, any precipitate was discarded.

Crataegus petals were prepared in the same way as leaf tissue, although less dilution was required. We found that winter buds are more problematic than leaves and benefitted from even more dilution.

Cytometry

The flow cytometer, located in the University of Toronto Faculty of Medicine, is a Becton Dickinson machine from BD Biosciences, Franklin Lakes, New Jersey, USA, model FacsCalibur. Since our emphasis was on extensive surveys of polyploidy rather than on detecting small variation in DNA amounts, we ran the samples through the machine at high speed.

The side-scatter measurement plotted against fluorescence intensity has proved to be most useful for recognizing a signal from the nuclei in order to reject the signals from particles other than intact nuclei (Fig. 1A; Matzk et al. 2000)). Doublet signals (nuclei that are stuck together) were selected out using a plot of the height of the fluorescence signal against width, as is normal practice (Fig. 1B). The final histogram shows a peak for each plant with the mean and standard deviation for each interval selected (Fig. 1C). It is usual with *Pisum*, and uncommon with *Crataegus* leaf, to obtain a second peak at twice the fluorescence of the first, which would correspond to cells in the G2 phase of the cell cycle or to endopolyploid nuclei.

Calculations

The variance of the fluorescence intensity from particles observed by the cytometer is sensitive to how well the preparation is flowing through the machine and will increase if the machine is partly clogged. The mean of the measurements may also be affected. There is no independent measure of obstruction in the machine, so the variance is used as a guide (Ormerod 1994). We have followed the method contributed by Warren Lamboy (Dickson et al. 1992) to calculate the standard deviation of the fluorescence measurement from the distributions of both the *Crataegus* signal and the *Pisum* standard. The peaks corresponding to nuclei from the two plants did not overlap, so they were measured in the same preparation (Fig. 1C). The adjusted variance (σ^2) and the adjusted mean (μ) were calculated as follows:

$$\sigma^2 = \sigma_1^2/\mu_2^2 + \sigma_2^2 \times \mu_1^2/\mu_2^4$$

$$\mu = 8.84 \times \mu_1/\mu_2$$

where subscripts 1 and 2 refer to the sample and the standard, respectively.

To map fluorescence measurements to ploidy levels, we chose a core sample of trees growing in Ontario (Figs. 2 and 3). These are taxa that we are confident about assigning by morphology to groups whose ploidy level has been at least moderately well established in the publications listed in Table 1. This group includes three individuals for which we have chromosome counts (4x: D950 and D652 (listed incorrectly as D752), and 3x: D668 = JBP4454; Muniyamma

and Phipps 1979*a*; Dickinson and Phipps 1986). Trees growing outside Ontario were excluded, even if morphologically well-defined, on the grounds that climatic differences might affect the DNA measurement. We also included a segregate of *C. succulenta* found at two separate localities that is distinctive in appearance, and because it is male-sterile, we suspected that it might be triploid. The last addition is a possible *C. macracantha – C. punctata* intermediate of unknown ploidy but very distinctive in appearance and very fertile.

In Figs. 2 and 3, it is clear that there are three groups of measurements that mirror the known ploidy levels, except that a few near-diploid aneuploids might be present. There is some variation within taxa, but with error bars to represent the standard deviation calculated from both the sample and the DNA standard, there is no overlap between these groups (Fig. 3). We initially chose by eye some conservative ranges as initial estimates of ploidy level, omitting the apparently anomalous individuals at the high end of the diploid range and at both ends of the tetraploid range, reasoning that these might include the aneuploids that have sometimes been reported with 34+1 or with 68+4 chromosomes.

These initial ranges were 1.4–1.65 pg with a midpoint of 1.525 pg, and a range of 0.25 pg for diploids; 2.2–2.4 pg with a midpoint of 2.3 pg, and a range of 0.2 pg for triploids; 2.8–3.2 pg with a midpoint of 3 pg and a range of 0.4 pg for tetraploids. Using the midpoint of these ranges to estimate the DNA amount per genome gives three independent estimates: 0.76, 0.77, and 0.75 pg, and an average of 0.76 pg/genome.

The standard deviations of the measurements depend on how well the cytometer is running. The adjusted standard deviations from the core taxa measurements mostly fall within 10% of the mean. We have chosen ±10% as the deviation from the midpoints of the ranges as an estimate of which plants fall into each ploidy group. This allowance seems generous for this group of tetraploids, but it omits some plants close to the diploid range (Fig. 2 and Table 2).

Extending these estimates to pentaploid (3.42–4.18 pg) and hexaploid (4.10–5.02 pg) produces some confidence in our ability to distinguish pentaploids and hexaploids from tetraploids, but for single measurements from each plant, these ranges should not be used to distinguish a hexaploid from a pentaploid.

Results

DNA measurements

DNA measurements are listed in Table 1, along with ploidy-level estimates from the ranges derived from our core group of taxa (Figs. 2 and 3, and Table 2). We have used ±12% as a guide to which plants fall just outside one of the euploid ranges rather than between two ranges. These ploidy interpretations are compared with the published chromosome counts and placed on the best current estimate of the classification of the two genera as extracted from recent taxonomic revisions (Table 1).

Comparison with previous measurements

Previous measurements of nuclear DNA exist for two of the species studied here, one from *Mespilus germanica*

Fig. 1. Cleaning the signal in the flow cytometer; the sample shown is from NT306, a still unidentified *Crataegus* seedling, with *Pisum* nuclei as the internal standard. (A) Recognizing the nuclei by the narrow band of their side-scatter values (a measurement that relates to the light-reflecting properties of the particles) and partly cleaning the signal by selecting region R5 for exclusion. (B) Excluding signals with a greater width (duration) than usual. These probably represent particles stuck together. (C) The final histogram of the cleaned fluorescence signal is used to select intervals for statistical analysis, from which the arithmetic means of the fluorescence peaks are combined and adjusted as described in the text. Interval M1 is from the *Crataegus* sample and M2 is from the *Pisum* standard. The third peak has twice the fluorescence of the main *Pisum* peak and represents endotetraploid nuclei or nuclei resting in G2 phase of the cell cycle. A G2-phase peak from *Crataegus* leaf tissue appears only rarely but is more common with other tissues such as petals.
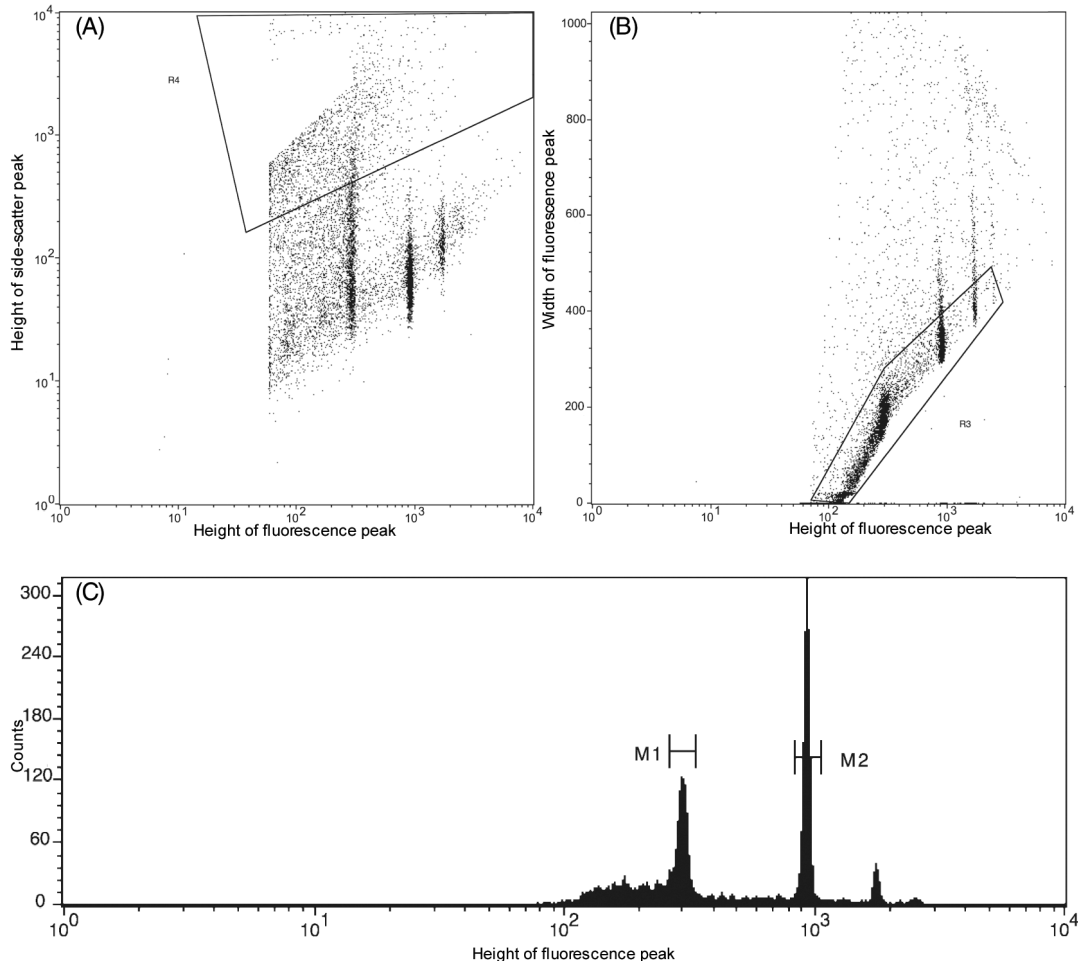
(1.48 pg) and one from *Crataegus crus-galli* (2.71 pg) (Dickson et al. 1992). Dickson et al. (1992) used chicken red blood cells (2.33 pg DNA) rather than a plant DNA standard, which should not produce a discrepancy (Temsch and Greilhuber 2001), but for comparison we also measured some other members of the family (Table 3). Our measurements correspond well to those of Dickson et al. (1992) except for some instances where it is likely that the plants are different species or cultivars.

Dickson et al.'s (1992) measurement of 2.71 pg DNA (SD = 0.24) for a sample of *C. crus-galli* (ploidy level unknown but expected to be triploid or tetraploid) falls a little below our estimate of the usual tetraploid range, but two of our own measurements from this group are similar, and it is notable that the *C. crus-galli* s. l. measurements are low compared with members of other series, the diploids even more so than the tetraploids. For *M. germanica*, our measurements also agree very well, though there is some variation within them: two of the cultivated plants gave measurements higher than our diploid range and higher than the plants grown at the Morton Arboretum from wild-collected seed. These are cultivated selections with large fruit (NT210, probably 'Nottingham', is also extremely parthenocarpic), which may be unusual genotypes. Another possibly exceptional genotype was one of the plants from wild-collected seed, which gave a lower measurement than the other plants.

Uniformity of the measurements

To supplement the measurements from different trees (Table 2), we repeated measurements from two trees of *C. monogyna* at different times of year, in different years, and in petal and bud tissue as well as leaves (Table 4). It is more difficult to get a clean preparation of nuclei from winter buds, but it is possible to use this tissue if necessary.

Crataegus punctata is one of the better-studied taxa, and certain plants have been shown to be sexual diploids (Longley 1924; Muniyamma and Phipps 1985; Dickinson

Fig. 2. Using a group of core *Crataegus* taxa from Ontario, the DNA measurements (calculated as described in the text from data as in Fig. 1C) suggest diploid, triploid, and tetraploid groups, from which we derived an average estimate of 0.76 pg/genome. The ranges shown for each ploidy level derived from this average ±10% are 1.37–1.67 pg (1.52 pg) for diploids, 2.05–2.51 pg (2.28 pg) for triploids, and 2.74–3.34 pg (3.04 pg) for tetraploids. These taxa are known diploids: *C. punctata* (34 trees), *C. monogyna* – *C. punctata* hybrids (10 trees), *C. monogyna* (9 trees), *C. ×lavallei* (1 tree), *C. ×media* (1 tree), *Mespilus germanica* (1 tree); these taxa are known triploids: *C. ?grandis* (1 tree), to which is added four trees of *C. succulenta* that are male-sterile and suspected to be triploid (Table 1); these taxa are known tetraploids: *C. crus-galli* var. *pyracanthifolia* (34 trees), *C. douglasii* (17 trees), *C. ×persimilis* (19 trees), *C. macracantha* var. *macracantha* (9 trees) to which is added six trees of a very fertile, apparently tetraploid, possible *C. macracantha* – *C. punctata* intermediate.

Ploidy estimates from the core taxa
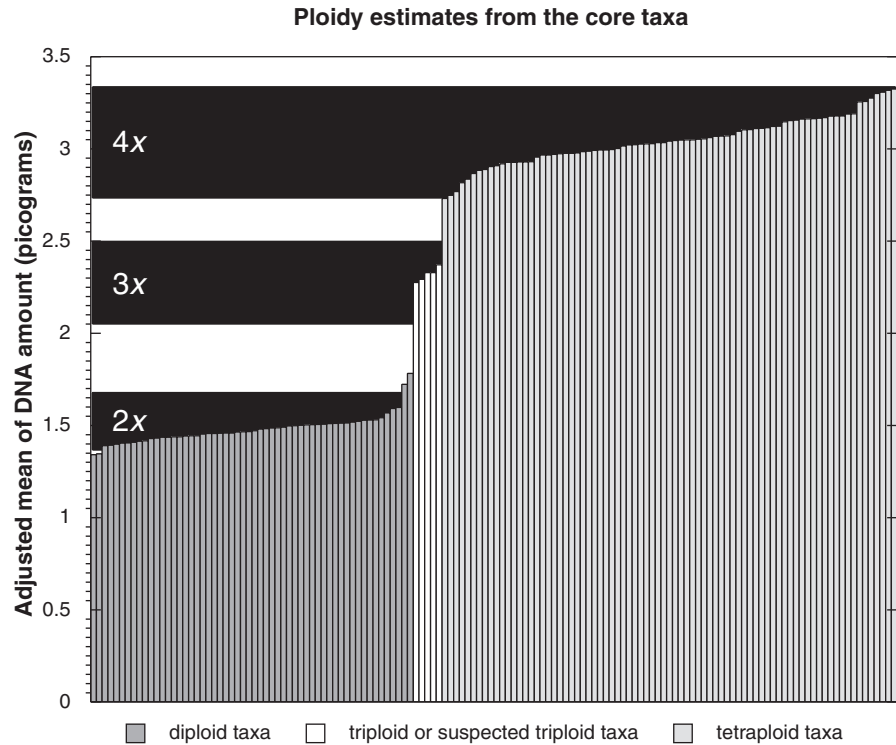
and Phipps 1986; Wells and Phipps 1989). We have sampled this species extensively in Ontario and attempted to cover the spectrum of morphological variation, which is far greater than in most hawthorn taxa. Most of our plants have rather narrow leaves with pointed lobes and double teeth, but some had relatively broad lobed leaves (e.g., NT52, NT226), and one plant had unlobed leaves with single teeth (NT57). Anther colors include white, pale pink, medium pink, dark pink, and deep red. Most of the fruit were various shades of red, some elongated, some not, about 1–1.5 cm in diameter, with varying textures when ripe. Some have exceptionally large fruit (e.g., NT186, at 2 cm diameter). Some had yellow fruit about 1.5 cm in diameter (*C. punctata* var. *punctata* f. *aurea* (Aiton) Rehder, e.g., NT47, NT54, NT55), and some had yellow fruit with a red blotch (*C. punctata* var. *punctata* f. *intermedia* Kruschke, e.g., NT68). Not only are all the plants diploid, but their DNA measurements vary less than the spectrum of diploid values as a whole (Table 2, Fig. 2).

The DNA measurements from tetraploids occupy a broader range of values than those from diploids and triploids, and some of this variation is traceable to the identity of the plant (Figs. 2, 4). Chromosome counts of $2n = 72$ rather than $2n = 68$ have been noted (Table 1), including in *C. phaenopyrum* from which we sampled both cultivated and wild forms. Thus, we speculate that the range of DNA measurements from tetraploids could reflect real differences

in chromosome number, although the accuracy of the measurements is not sufficient to be certain without repeating measurements from the same tree.

Comparison to chromosome counts

The ploidy levels derived from the DNA measurements accord very well with the published chromosome counts (Table 1). For the two species of *Mespilus*, there are previous chromosome counts that have *M. germanica* as diploid (Moffett 1931) and *M. canescens* as triploid (M. Muniyamma, personal communication, 1990) and these also accord well with our estimates calculated from *Crataegus*.

Some taxa do not fall neatly into the ploidy categories estimated from our core group of plants (Fig. 4, Table 1), but for some of these groups we have enough samples with sufficient consistency to suggest that the DNA amount is different, and that could be taxonomically interesting. Such groups are *C. uniflora*, *C. brachyacantha*, the two cultivated specimens of *C. hupehensis*, and the southern plants that we currently consider to be diploids from *C. crus-galli* sensu lato.

Discussion

Chromosome number

A comparison with published chromosome counts shows that cytometric determination of DNA amount in leaf tissue

Fig. 3. The same group of core *Crataegus* taxa from Ontario as in Fig. 2 with errors bars representing the standard deviation of the mean adjusted to take account of the variance of the *Pisum* standard, as described in the text. With these taxa, discrimination between the ploidy levels is good, although proportionality of the error would make higher ploidy levels less distinct from one another.

Core taxa, DNA amount with standard deviation
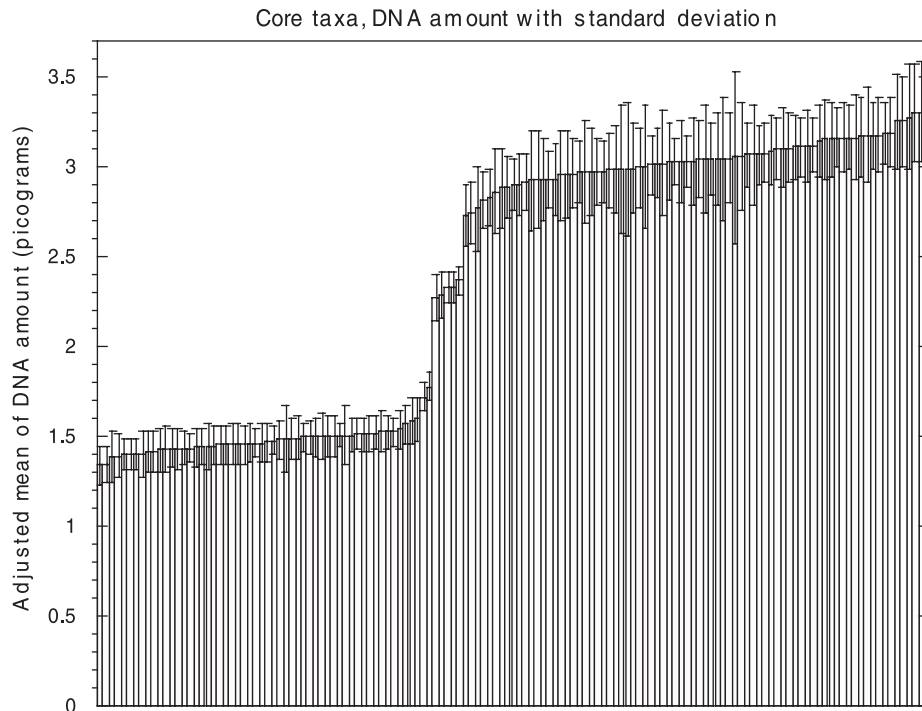
can be a convenient and effective substitute for direct chromosome counts to determine ploidy level (although not necessarily exact chromosome number) for both Eurasian and North American *Crataegus* and *Mespilus* species (Table 1). Chromosome counts require tissue that is difficult to obtain, but flow cytometry has no such restriction. As with other Rosaceae, *Crataegus* tissues can be a frustrating subject for flow cytometry (E.E. Dickson, personal communication, 1992), but we have found that consistent results can be obtained by diluting to reduce the viscosity of the chopped-tissue suspension before filtering.

Our measurements from *Crataegus* and other selected Rosaceae are similar to previous measurements (Dickson et al. 1992). The cytometry involves a certain amount of error, as can be seen by repeated measurements. However, the uncertainty of the cytometry is not great enough to affect the assignment of most of the measurements into ploidy groups, and unassigned values may represent aneuploid individuals. We have verified that the cytometric results are repeatable at different times of year and in different years. By surveying representatives from the majority of taxonomic series in both *Crataegus* (30 series, 7 nothoseries, 72 or more species and nothospecies) and *Mespilus* (2 species), it is apparent that the DNA amount for each 1*x* block of 17 chromosomes varies little. Our range of 1.37–1.67 pg of DNA for the diploids encompasses the measurements for several other diploid Maloideae: *Amelanchier*, *Cydonia*, and *Pyracantha coccinea* (Table 3), as well as measurements by Dickson et al. (1992) for *Sorbus alnifolia*, *Sorbus americana*, and *Eriobotrya japonica*. Some other Maloideae have less DNA than this (Dickson et al. 1992), but with the exception of the cul-

tivar *Pyracantha* 'Royal' (only 0.99 pg), the measurements are close to one another, averaging 1.17 pg: *Pyrus communis* 'Bartlett' (1.11 pg), *Pyrus calleryana* (1.26 pg), *Cotoneaster melanocarpa* (2.24 pg, 4*x*), and *Chaenomeles speciosa* (1.20 pg). Goldblatt (1976) and, more recently, Evans and Campbell (2002) consider the most likely origin of the *x* = 17 base chromosome number of the Maloideae to be from members of subfamily Spiraeoideae with *x* = 9, by doubling followed by the loss of one chromosome. We therefore checked the DNA amount in *Gillenia*, which Evans and Campbell (2002) placed as a sister to Maloideae sensu lato (the clade that contains both the pome-fruited Maloideae sensu stricto and the non-pome-fruited taxa with *x* = 17). The measurements from the two *Gillenia* species are significantly different from one another and they do not appear to represent a polyploid series, but rather suggest that the genome size differs in the two species (Table 3). If we accept our measurement as typical of diploid *G. trifoliata*, further assume that all chromosome sizes are equal, and calculate an equivalent genome size for a diploid *Crataegus* with *x* = 17, the resulting figure of 1.57 pg of DNA is surprisingly close to the 1.53 pg that we estimated as the midpoint of the range for diploid *Crataegus*. (An equivalent calculation for *G. stipulata* gives 1.91 pg, between the 2*x* and 3*x* ranges for *Crataegus*.) On the basis of this very slight evidence, we hypothesize that the genome size of *Crataegus* and of several other Maloid genera might have changed little from an ancestral state.

Of the measurements reported here, 38 species match published chromosome counts for the same species, and for 12 of the 30 series and nothoseries, our assessment of poly-

Table 1. Synopsis of ploidy-level variation in *Crataegus* and *Mespilus*, based primarily on flow cytometric nuclear DNA measurements.

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
Mespilus L.				
M. germanica L.	**Morton Arboretum**, Chicago, Illinois; cultivated trees	M518-37/95-40	1.62±0.12	34 (Moffett 1931; Sax 1931) 2x
		M645-80/21-34	1.62± 0.11	2x
		M645-80/32-28	1.62±0.09	2x
		M665-80/89-47	1.63±0.14	2x
		M682-80/50-62	1.57±0.10	2x
		M682-80/52-72	1.39±0.07	2x
	Ontario, Toronto (*M. germanica* probably 'Nottingham'); cultivated tree	NT210	1.72±0.08	2–3x
	Jardin Botanique de Montréal, Montréal, Québec; cultivated trees	2000-42	1.73±0.18	2–3x
M. canescens **J.B.Phipps**				Triploid (M. Muniyamma, personal communication, 1990)
	Arkansas, Prairie; det. J.B.Phipps	2003-35-03	2.30±0.16	3x
		2003-36-11	2.28±0.17	3x
		2003-37-13	2.25±0.15	3x
		2003-38-17	2.21±0.15	3x
		2003-39-18	2.27±0.16	3x
		2003-40-19	2.26±0.17	3x
		2003-41-20	2.19±0.16	3x
		2003-42-22	2.28±0.14	3x
		2003-43-24	2.28±0.17	3x
Crataegus L.				
sect. *Mexicanae* Loudon				
ser. *Mexicanae* (Loudon) Rehder				
C. mexicana DC.				Diploid (Wells 1985)
C. stipulosa (Kunth) Steud.				34 (Moffett 1931)
ser. *Henryanae* (Sarg.) J.B. Phipps				
C. scabrifolia (Franch.) Rehder				34 (Gu and Spongberg 2003; Guo and Jiao 1995)
sect *Parvifoliae* **Loudon**				Triploid or tetraploid (Longley 1924)
{*C. smithii* Sarg.}				51 (Gladkova 1968)
C. uniflora **Muench.**	**Alabama**, Autauga; det. Ron Lance	2003-26	2.49±0.22	3x
	Virginia, Franklin	2003-49	2.67±0.21	3–4x
		2003-51	2.66±0.29	3–4x
		2003-52	2.57±0.22	3–4x
	Arnold Arboretum, Boston, Massachusetts; cultivated trees	AA52-88	2.74±0.22	4x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
sect. *Crataegus*				
ser. *Crataegus*				
C. ambigua ?Becker				51 (Christensen 1992)
C. ×armena Pojark.				51 (Christensen 1992)
C. caucasica K.Koch				51 (Christensen 1992)
C. ×kyrtostyla Fingerh.				34, 51, 68 (Christensen 1992)
C. ?×kyrtostyla				51 (Christensen 1992)
***C. laevigata* (Poir.) D.C.**				34 (Christensen 1992)
	Washington, San Juan; det. Peter Zika	Zika18470	1.40±0.13	2x
		Zika18472	1.40±0.11	2x
		Zika18473	3.12±0.27	4x
C. ×macrocarpa Hegetschw.				34, 51, 52, 68 (Christensen 1992)
C. ?×macrocarpa Hegetschw.				48 (Christensen 1992)
***C. ×media* Bechst.**				34 (Christensen 1992)
	Ontario, Toronto	NT214	1.57±0.11	2x
C. meyeri Pojark.				51, 68 (Christensen 1992)
C. microphylla K.Koch				34 (Christensen 1992)
***C. monogyna* Jacq.**				34 (Al-Bermani et al. 1993; Christensen 1992; Moffett 1931; Muniyamma and Phipps 1979a, 1985; Wells 1985); 68 (Gladkova 1968)
	Ontario, Durham	2002-15	1.54±0.10	2x
		NT113	1.51±0.16	2x
	Ontario, Toronto	D1697	1.51±0.09	2x
		NT114	1.52±0.10	2x
		NT116	1.78±0.08	2–3x
		NT117	1.46±0.11	2x
	Ontario, Middlesex	EH59	1.51±0.09	2x
	Oregon, Lane; det. Rhoda Love	LoveC-2003-25	2.27±0.13	3x
	Oregon, Linn	EL74	1.47±0.10	2x
		EL78	1.35±0.09	2x–
		EL80	1.41±0.09	2x
		EL83	1.41±0.09	2x
{*C. oxyacantha* L. nom. rej.}				34 (Moffett 1931)
C. pseudoheterophylla Pojark.				51, 68 (Christensen 1992)
C. rhipidophylla Gand.				34, 51, 68 (Christensen 1992)
***C. songarica* K.Koch**				68 (Christensen 1992; Gu and Spongberg 2003)
	Arnold Arboretum, Boston, Massachusetts; cultivated trees	AA198-65A	2.95±0.25	4x
C. sphaenophylla Pojark.				51 (Christensen 1992; Guo and Jiao 1995)
{*C. monogyna* var. *cabulica* Hort. / *C. turkestanica* Pojark}				51 (Moffett 1931)

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
C. ulotricha Pojark. ex V.N.Gladkova				68 (Gladkova 1968)
ser. *Apiifoliae* (Loudon) Rehder				
***C. marshallii* Eggl.**				
	Alabama, Montgomery	2003-08	1.69±0.11	2x+
	Alabama, Dekalb	2003-5	1.71±0.12	2–3x
	Mississippi, Scott	2003-30	1.75±0.19	2–3x
ser. *Orientales* (C.K.Schneid.) Pojark.				
C. azarolus L.				34, 68 (Christensen 1992)
***C. heldreichii* Boiss.**				34 (Christensen 1992)
	Arnold Arboretum, Boston, Massachusetts; cultivated trees	AA238-71A	1.59±0.10	2x
C. orientalis M.Bieb.				51, 68 (Christensen 1992)
ser. *Pentagynae* (C.K.Schneid.) Russanov				
C. atrofusca (K.Koch) T.A.Kasumova				51 (Gladkova 1968)
C. pentagyna Waldst. & Kit.				34 (Christensen 1992)
nothoser. *Crataegynae* K.I.Chr.				
C. ×zangezura Pojark.				
nothoser. *Orientaegus* K.I.Chr.				68 (Christensen 1992)
C. caucasica K.Koch				51 (Christensen 1992)
nothoser. *Orientagynae* K.I.Chr.				
C. ×pseudazarolus Popov				68 (Christensen 1992)
sect. *Sanguineae* Zabel ex C.K.Schneid.				
{*C. atrocarpa* / *C. chlorosarca* Maxim.}				34 (Gladkova 1968)
***C. chlorosarca* Maxim.**				34 (Gladkova 1968; Moffett 1931)
	Arnold Arboretum, Boston, Massachusetts; cultivated trees	AA281-71A	1.63±0.14	2x
***C. dahurica* Koehne**				34 (Gladkova 1968; Gu and Spongberg 2003; Guo and Jiao 1995)
	Arnold Arboretum, Boston, Massachusetts; cultivated trees	AA71-73A	1.65±0.11	2x
C. hissarica Pojark.				34 (Gladkova 1968)
C. kansuensis E.H.Wilson				34 (Gu and Spongberg 2003; Guo and Jiao 1995)
C. maximowiczii C.K.Schneid.				32 (Gu and Spongberg 2003); 34 (Gladkova 1968; Gu and Spongberg 2003; Guo and Jiao 1995); 51 (Gu and Spongberg 2003); 68 (Gladkova 1968; Guo and Jiao 1995)
***C. nigra* Waldst. & Kit.**	**Jardin Botanique de Montréal**, Montréal, Quebec; cultivated trees	JBM2318-50	1.68±0.16	34 (Byatt and Murray 1977); 2x+

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
C. pinnatifida Bunge				34 (Gladkova 1968; Gu and Spongberg 2003; Guo and Jiao 1995); 51, 68 (Guo and Jiao 1995); 102 (Gu and Spongberg 2003)
	Royal Botanical Gardens, Burlington, Ontario; cultivated trees	RBG54719	1.56±0.14	2x
	Jardin Botanique de Montréal, Montréal, Quebec; cultivated trees	JBM1691-49 tree 1	3.18±0.34	4x
		JBM1691-49 tree 2	3.20±0.29	4x; 34 (Gladkova 1968)
C. remotilobata Raikova ex Popov				
C. sanguinea Pall.				Diploid (Longley 1924); 51, 68 (Gladkova 1968; Gu and Spongberg 2003; Guo and Jiao 1995)
C. wattiana Hemsl. & Lace (incl. *C. altaica* Lange)				68 (Byatt and Murray 1977; Gladkova 1968; Gu and Spongberg 2003; Guo and Jiao 1995)
	Jardin Botanique de Montréal, Montréal, Quebec; cultivated trees	JBM1401-52	2.35±0.24	3x
		JBM1280-50	3.26±0.24	4x; 68 (Gladkova 1968)
C. ?wattiana ({*C. altaica* var. *incisa* (Regel) C.K. Schneid. / *C. korolkowii* L.Henry})				
C. wilsonii Sarg.				34 (Gu and Spongberg 2003; Guo and Jiao 1995)
	Arnold Arboretum, Boston, Massachusetts; cultivated trees	AA749-74A	1.61±0.13	2x
		AA271-84A	1.55±0.12	2x
	Jardin Botanique de Montréal, Montréal, Québec; cultivated trees	JBM22322-50	1.51±0.14	2x
sect. Hupehenses J.B.Phipps				
C. hupehensis Sarg.				34 (Gu and Spongberg 2003; Guo and Jiao 1995)
	Arnold Arboretum, Boston, Massachusetts; cultivated trees	AA356-81B	1.80±0.13	2–3x
		AA361-81	1.94±0.16	2–3x
		AA356-81	2.30±0.30	3x
C. shensiensis Pojark.				34 (Gu and Spongberg 2003; Guo and Jiao 1995)
sect. Cuneatae Rehder ex C.K.Schneid.				
C. cuneata Siebold & Zucc.				34 (Gu and Spongberg 2003; Guo and Jiao 1995)
sect. Cordatae Beadle ex Eggl.				

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
C. phaenopyrum (L.f.) Medik.				72 (Moffett 1931)
	North Carolina, Buncombe; det. Ron Lance	NT294	2.38±0.14	3x
		NT296	2.37±0.12	3x
		NT297	2.36±0.12	3x
		NT298	2.42±0.16	3x
		NT301	2.92±0.21	4x
	Ontario, Toronto; cultivated trees	NT311	2.87±0.20	4x
		NT312	2.89±0.25	4x
		2002-01	3.41±0.20	High
		2002-10	3.14±0.29	4x
	Royal Botanical Gardens, Burlington, Ontario; cultivated trees	RBG73481	3.68±0.48	High
sect. *Virides* (Beadle ex Sarg.) **C.K.Schneid.**				
C. nitida (Engelm.) Sarg.	**North Carolina Arboretum**, Asheville, North Carolina; cultivated trees	NC1994-263	2.03±0.11	Diploid (Longley 1924) 3x–
C. viridis L.	**Arkansas**, Prairie	2003-44	1.69±0.19	2x+
		2003-45	1.74±0.16	2–3x
	Arnold Arboretum, Boston, Massachusetts; cultivated tree	AA6316A	2.52±0.23	3x+
sect. *Microcarpae* **Loudon**				
C. spathulata Michx.	**Alabama**, Lowndes	NT277	1.39±0.06	2x
	Alabama, Montgomery	2003-11	1.82±0.15	2–3x
	Georgia, Floyd	2003-6	1.85±0.17	2–3x
	Georgia, Houston	NT280	1.36±0.10	2x-
	Louisiana, Bossier	2003-34	1.81±0.15	2–3x
	Louisiana, Sabine	NT215	1.86±0.13	2–3x
sect. *Lacrimatae* (J.B.Phipps) **J.B.Phipps**				
ser. *Lacrimatae* **J.B.Phipps**				
C. munda **Beadle**	**Georgia**, Rabun; det. Ron Lance	Lance 2313 seedling 2	2.30±0.20	3x
		Lance 2313 seedling 1	2.36±0.14	3x
		Lance 2313 seedling 2	2.49±0.20	3x
C. ?	**Alabama**, Dallas; det. Ron Lance	2002-02	1.71±0.12	2–3x
ser. *Flavae* (Loudon) **Rehder** {*C. agrestina* **Beadle**}		2002-03	2.48±0.23	3x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
C. aprica Beadle	**Alabama**, Lowndes; det. Ron Lance	2003-20	3.26±0.25	4x
	Sherwood Fox Arboretum, University of Western Ontario; cultivated trees	SFA5-12	2.01±0.10	3x–
{*C. ?lassa* Beadle}	**Alabama**, Dallas; det. Ron Lance	2003-18	3.87±0.27	High
{*C. lepida* Beadle}	**Woodlander's Nursery**, Aiken, South Carolina; cultivated tree.	Lance 2003-21	2.60±0.20	3–4x
C. ?	**Georgia**, Houston	NT282	2.43±0.26	3x
	Virginia, Franklin	2003-50	2.50±0.22	3x
		2003-46	3.30±0.24	4x
		2003-47	3.37±0.26	4x+
sect. *Aestivales* (Sarg.) C.K.Schneid.				
C. aestivalis (Walter) Torr. & A. Gray	**North Carolina Arboretum**, Asheville, North Carolina; cultivated trees	NC1992-250	1.25±0.08	Low
	North Carolina, Buncombe, cultivated tree; det. Ron Lance	NT321	1.26±0.09	Low
C. opaca Hook. & Arn. ex Hook.	**Louisiana**, Sabine; cultivated tree (*C. opaca* 'Super Spur')	2003-33	1.59±0.13	2x
	North Carolina Arboretum, Asheville, North Carolina; cultivated trees	NC1992-264A	1.25±0.09	Low
C. rufula Sarg.	**North Carolina Arboretum**, Asheville, North Carolina; cultivated trees	NC1992-425	1.95±0.08	2–3x
	North Carolina, Buncombe, cultivated tree; det. Ron Lance	NT323	2.03±0.15	3x–
sect. *Brevispinae* Beadle ex C.K.Schneid.				
C. brachyacantha Sarg. & Engelm.	**Louisiana**, Morehouse; det. Christopher S. Reid	MORE1-1	1.71±0.11	2–3x
	Louisiana, Ouachita; det. Christopher S. Reid	OUAC1-1	1.74±0.09	2–3x
		OUAC1-2	1.78±0.10	2–3x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
		OUAC2-1	1.73±0.11	2–3x
		OUAC2-2	1.73±0.10	2–3x
		OUAC2-3	1.72±0.10	2–3x
	Louisiana, Sabine; det. Tray Lewis	2003-32	1.88±0.14	2–3x
C. saligna **Greene**				
	Colorado, Rio Blanco	2004-6	1.56±0.07	2x
		2004-7	1.53±0.08	2x
		2004-8	1.71±0.12	2–3x
	Utah, Duchesne	2004-5	1.67±0.10	2x
sect. *Douglasianae* Loudon				
C. castlegarensis **J.B.Phipps**				
	Idaho, Latah	EL140	2.90±0.15	4x
		EL141	3.14±0.23	4x
	Idaho, Lemhi	EL189	2.93±0.21	4x
		EL192	3.00±0.15	4x
	Washington, Okanagan; det. Peter Zika	Zika19233	3.11±0.33	4x
		Zika19243	3.22±0.36	4x
	Washington, Thurston; det. Peter Zika and T.A. Dickinson	Zika18488 Tree A	3.18±0.35	4x
		Zika18488 Tree B	3.05±0.32	4x
		Zika18488 Tree C	2.81±0.32	4x
		Zika18488 Tree D	2.72±0.31	4x–
		EL86	3.12±0.20	4x
		EL86a	2.99±0.19	4x
		EL87	3.02±0.19	4x
	Washington, Whitman; det. Peter Zika	Zika18390	3.65±0.37	High
C. ?castlegarensis				
	Oregon, Grant; det. Rhoda Love	Love C-2003-23	3.30±0.36	4x
	Washington, Thurston	EL88	3.01±0.15	4x
		EL89	3.01±0.18	4x
		EL90	3.03±0.21	4x
		EL91	3.04±0.24	4x
		EL92	2.98±0.14	4x
		EL97	3.06±0.18	4x
		EL100	3.05±0.17	4x
C. douglasii Lindl. s.l.				Triploid (Longley 1924); 68 (Gladkova 1968)
C. douglasii **Lindl.** *s. str.*	**British Columbia**, Capital; det. Peter Zika	Zika18456	3.01±0.24	68 (Brunsfeld and Johnson 1990; Dickinson et al. 1996) 4x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
	British Columbia, North Okanagan; det. Peter Zika	Zika19246	3.00±0.28	4x
		Zika18450	3.09±0.20	4x
		Zika18452	3.29±0.35	4x
		Zika18453	3.04±0.25	4x
	Idaho, Benewah	EL147	2.94±0.17	4x
		EL148	2.77±0.35	4x
		EL149	2.89±0.34	4x
		EL150	2.85±0.28	4x
	Idaho, Lemhi	EL190	2.94±0.17	4x
		EL191	2.98±0.20	4x
		EL193	2.65±0.15	3—4x
		EL194	3.01±0.17	4x
		EL195	2.92±0.18	4x
		EL196	2.82±0.17	4x
		EL197	2.85±0.21	4x
		EL198	2.99±0.15	4x
	Idaho, Nez Perce	EL121	2.96±0.18	4x
		EL123	2.98±0.17	4x
		EL124	2.88±0.21	4x
		EL125	3.04±0.17	4x
		EL126	2.97±0.18	4x
		EL127	2.96±0.18	4x
	Ontario, Bruce	D1416	3.05±0.26	4x
		D1418	3.12±0.15	4x
		RCE009	3.04±0.21	4x (Evans and Dickinson 1996)
		2003-76	3.00±0.22	4x
		2003-78	2.99±0.24	4x
		NT191	3.15±0.20	4x
		NT189	3.16±0.20	4x
		NT192	3.16±0.16	4x
		NT194	3.16±0.18	4x
		NT190	3.19±0.18	4x
		EL4	2.97±0.17	4x
		EL5	2.87±0.24	4x
		EL7	2.93±0.16	4x
		EL8	2.91±0.17	4x
		EL9	2.90±0.15	4x
		EL10	2.89±0.17	4x
		EL11	2.92±0.15	4x
	Oregon, Grant; det. Rhoda Love	Love C-2003-20	3.22±0.20	4x
	Oregon, Wheeler; det. Rhoda Love	Love C-2003-21	3.03±0.31	4x
		Love C-2003-16	3.20±0.25	4x
C. rivularis Nutt.		Love C-2003-17	3.43±0.27	High

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
	Idaho, Bear Lake	2001-10	2.80±0.15	4x
		2001-11	2.86±0.15	4x
		EL199	2.81±0.15	4x
		EL203	3.16±0.21	4x
		EL204	2.82±0.15	4x
		EL205	3.06±0.17	4x
		EL206	3.11±0.21	4x
		EL207	2.74±0.15	4x
		EL210	2.82±0.14	4x
	Utah, Duchesne	2001-5	3.04±0.17	4x
		2001-6	2.78±0.19	4x
	Wyoming, Glenrock	2001-42	3.27±0.22	4x
		2001-43	2.91±0.16	4x
	North Carolina Arboretum, Asheville, North Carolina; cultivated trees	NC96-220B	2.71±0.13	4x–
***C. shuswapensis* J.B.Phipps & O'Kennon**	**British Columbia,** North Okanagan; det. Peter Zika	Zika18449	1.45±0.14	2x
***C. suksdorfii* (Sarg.) Kruschke s. l.**				34 (Brunsfeld and Johnson 1990; Dickinson et al. 1996); 51, 68 (Dickinson et al. 1996)
	British Columbia, Capital; det. Peter Zika	Zika18455	2.18±0.19	3x
	Montana, Powell	EL29	2.92±0.15	4x
		EL30	2.92±0.16	4x
		EL33	2.92±0.15	4x
		EL36	2.91±0.19	4x
		EL37	2.94±0.15	4x
		EL41	2.93±0.13	4x
		EL43	2.94±0.17	4x
		EL44	2.93±0.15	4x
		EL45	2.95±0.18	4x
	Oregon, Columbia	EL104	1.45±0.11	2x
		EL105	1.53±0.10	2x
		EL109	1.49±0.10	2x
	Oregon, Douglas; det. Rhoda Love	Love C-2003-35	2.29±0.19	3x
		Love C-2003-37	2.14±0.18	3x
		Love C-2003-38	2.16±0.19	3x
		Love C-2003-39	2.19±0.18	3x
	Oregon, Lane; det. Rhoda Love and T. A. Dickinson	Love C-2003-10	1.88±0.24	2–3x
		Love C-2003-11	1.82±0.17	2–3x
		Love C-2003-14	1.88±0.22	2–3x
		Love C-2003-15	1.88±0.25	2–3x
		Love C-2003-24	1.65±0.13	2x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
		EL48	2.27±0.12	3x
		EL49	2.25±0.12	3x
		EL50	2.25±0.11	3x
		EL51	1.99±0.11	2–3x
		EL52	2.20±0.13	3x
		EL53	2.23±0.14	3x
		EL54	2.27±0.12	3x
		EL55	2.30±0.13	3x
		EL56	2.23±0.14	3x
		EL57	2.25±0.14	3x
		EL58	2.27±0.12	3x
		EL59	2.24±0.14	3x
		EL60	2.27±0.13	3x
		EL61	2.24±0.14	3x
		EL62	2.22±0.14	3x
		EL63	2.26±0.14	3x
		EL64	2.27±0.13	3x
		EL65	2.22±0.14	3x
		EL66	2.24±0.13	3x
		EL67	2.27±0.14	3x
	Oregon, Linn	EL68	1.50±0.09	2x
		EL69	1.51±0.09	2x
		EL70	1.49±0.08	2x
		EL71	1.54±0.13	2x
		EL72	1.51±0.09	2x
		EL75	1.52±0.11	2x
	Oregon, Washington; det. Peter Zika	Zika18483	1.42±0.13	2x
	Washington, King; det. Peter Zika	Zika19227	2.54±0.28	3x+
sect. *Crus-galli* Loudon				
ser. *Crus-galli* (Loudon) Rehder				Triploid? (Longley 1924)
{*C. rotunda* Sarg. / *C. arborea* Beadle}				Diploid (Longley 1924); triploid? (Longley 1924), 51 (Dickinson and Phipps 1986; Muniyamma and Phipps 1979*a*; Wells 1985); 51?, 64 (Muniyamma and Phipps 1979*a*), 68 (Dickinson and Phipps 1986; Muniyamma and Phipps 1979*a*)
C. crus-galli L. s. l. (**including *C. tenax* Ashe**)				
	Alabama, Lowndes	2003-15	3.20±0.20	4x
		NT274	2.61±0.19	3–4x
		NT276	2.66±0.20	3–4x
	Alabama, Montgomery	NT213a	1.61±0.13	2x
		NT214a	1.33±0.10	Low
		NT272	1.34±0.11	Low
		NT273	1.31±0.09	Low

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
	Georgia, Houston	NT278	1.25±0.09	Low
		NT279	1.24±0.06	Low
		NT281	1.21±0.09	Low
		NT283	1.20±0.07	Low
		NT284	1.23±0.10	Low
		NT285	1.24±0.09	Low
		NT286	1.20±0.09	Low
	Mississippi, Scott	2002-06	3.44±0.31	High
		2003-28	3.18±0.23	4x
	Ontario, Lambton	D652	3.11±0.18	4x (Dickinson and Phipps 1986, as D752)
	Ontario, Middlesex	?D668	3.03±0.14	4x (Dickinson and Phipps 1986)
		D950	2.98±0.17	4x (=JBP4454, Muniyamma and Phipps 1979a)
	Ontario, Niagara	NT165	2.93±0.20	4x
		D1021	2.97±0.18	4x
		NT28	3.15±0.20	4x
		NT35	2.99±0.21	4x
		NT72	3.03±0.21	4x
		NT81	3.07±0.28	4x
		NT82	3.03±0.13	4x
		NT83	2.98±0.19	4x
		NT85	3.02±0.19	4x
		NT168	3.30±0.27	4x
		NT170	2.93±0.23	4x
		NT171	2.97±0.24	4x
		NT184	3.10±0.19	4x
		NT250	2.97±0.20	4x
		NT254	3.05±0.20	4x
		NT255	2.99±0.37	4x
		NT257	2.73±0.17	4x–
		NT262	2.93±0.28	4x
		NT265	3.05±0.30	4x
		NT266A	3.06±0.48	4x
		NT267A	3.06±0.30	4x
		NT269A	3.05±0.34	4x
		NT268A	3.00±0.34	4x
	Ontario, Toronto, planted? *C. crus-galli* var. *pyracanthifolia*	SC06	3.08±0.17	4x
	North Carolina Arboretum, Asheville, North Carolina; cultivated trees	NT32	2.88±0.22	4x
		NT62	2.77±0.23	4x
		NT66	3.07±0.16	4x
		NC1992-255	2.79±0.18	4x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
	Royal Botanical Gardens, Burlington, Ontario, cultivated trees	RBG62354	3.09±0.32	4x
{C. lawrencensis Sarg.}		RBG770636	3.02±0.29	4x
{C. phlebodia Sarg.}				34 (Sax 1931)
{C. rivalis Sarg.}				Triploid? (Longley 1924)
ser. **Punctatae** (**Loudon**) **Rehder**				Triploid? (Longley 1924)
C. collina Chapm.				Diploid (Longley 1924)
	North Carolina, Swain	NT293	2.00±0.12	2–3x
	North Carolina, Buncombe; det. Ron Lance	NT300	2.48±0.16	3x
		NT303	2.56±0.11	3–4x
		NT304	2.57±0.17	3–4x
		NT305	2.51±0.16	3x+
C. disperma Ashe				Triploid or tetraploid (Longley 1924)
{C. pausiaca Ashe / C. disperma Ashe}				Triploid (Longley 1924)
C. ?grandis Ashe				51 (Dickinson and Phipps 1986)
C. punctata Jacq.	**Ontario**, Niagara	NT29	2.29±0.13	3x
				Diploid (Longley 1924), 34 (Dickinson and Phipps 1986; Muniyamma and Phipps 1979a; Wells 1985); 34?, 34+1? (Muniyamma and Phipps 1979a)
	Ontario, Bruce	BB4	1.43±0.12	2x
		EL6	1.35±0.11	2x–
	Ontario, Durham	2002-14	1.47±0.08	2x
	Ontario, Middlesex	NT135	1.44±0.09	2x
		NT136	1.40±0.09	2x
	Ontario, Haldimand-Norfolk	NT267	1.49±0.18	2x
	Ontario, Toronto	NT270	1.34±0.11	2x–
		D1696	1.39±0.12	2x
		NT42A	1.44±0.11	2x
		NT42B	1.50±0.11	2x
		NT42C	1.45±0.10	2x
		NT42E	1.47±0.11	2x
		NT42F	1.50±0.11	2x
		NT42G	1.47±0.11	2x
		NT42H	1.48±0.11	2x
		NT42I	1.46±0.11	2x
		NT42J	1.40±0.08	2x
		NT50	1.41±0.13	2x
		NT54	1.50±0.13	2x
		NT55	1.39±0.14	2x
		NT69	1.46±0.11	2x
		NT158	1.43±0.12	2x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
	Ontario, Wellington	NT226	1.42±0.12	2x
		NT227	1.44±0.11	2x
		NT92	1.49±0.12	2x
		NT93	1.48±0.08	2x
		NT120	1.49±0.11	2x
		NT121	1.44±0.08	2x
		NT122	1.51±0.09	2x
		NT186	1.53±0.09	2x
		NT187	1.52±0.09	2x
		NT231	1.46±0.12	2x
	Ontario, York	NT138	1.46±0.11	2x
		NT141	1.46±0.10	2x
{*C. vicina* Sarg.}				Triploid (Longley 1924)
sect. Coccineae				
ser. *Bracteatae* (E.J.Palmer) Rehder				
C. ashei **Beadle**	**Alabama**, Autauga, det Ron Lance	2003-25	3.13±0.23	4x
	Alabama, Lowndes, det Ron Lance	2003-14	3.49±0.28	High
		NT275	2.89±0.17	4x
		Lance2309 seedling 1	3.18±0.24	4x
		Lance2309 seedling 3	3.19±0.19	4x
		Lance2309 seedling 2	3.39±0.24	4x+
	Mississippi, Scott	2002-07	3.36±0.26	4x+
		2003-29	3.28±0.21	4x
C. harbisonii **Beadle**	**North Carolina Arboretum**, Asheville, North Carolina; cultivated trees	NC98-73A	2.96±0.24	4x
		NC98-75A	2.97±0.25	4x
		NC99-117A	2.98±0.25	4x
		NC2001-93A	3.03±0.26	4x
		NC98-74A	3.18±0.26	4x
ser. *Brainerdianae* Eggl. ex E.J.Palmer				
C. scabrida Sarg.				Triploid (Longley 1924)
C. sylvestris				68 (Muniyamma and Phipps 1979*a*)
ser. *Coccineae* (Loudon) Rehder				
C. coccinea L.				Tetraploid (Macklin 2001)
C. corusca Sarg.				68, 72? (Muniyamma and Phipps 1979*a*), Triploid? (Longley 1924), tetraploid (Macklin 2001)
{*C. eamesii* Sarg.}				Triploid? (Longley 1924)
C. ellwangeriana Sarg.				Tetraploid (Macklin 2001)
C. holmesiana Ashe				34 (Muniyamma and Phipps 1979*a*); triploid?

Table 1. (continued).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
				(Longley 1924), tetraploid (Macklin 2001)
{C. tardipes Sarg. / C. holmesiana Ashe}				Triploid? (Longley 1924)
C. pedicellata Sarg.				Triploid? (Longley 1924), 68 (Moffett 1931)
{C. assurgens Sarg., C. sertata Sarg. / C. pedicellata Sarg.}				Triploid? (Longley 1924)
C. pringlei Sarg.				Triploid? (Longley 1924), 68 (Muniyamma and Phipps 1979a), tetraploid (Macklin 2001)
{C. exclusa Sarg. / C. pringlei Sarg.}				Triploid? (Longley 1924)
{C. lobulata Sarg. / C. pringlei Sarg.}				Triploid? (Longley 1924)
C. ?				34, 68 (Muniyamma and Phipps 1979a)
ser. *Dilatatae* (Sarg.) E.J.Palmer				
C. coccinioides Ashe				Triploid (Longley 1924)
ser. Greggianae J.B.Phipps				
C. greggiana Eggl.	**Sherwood Fox Arboretum**, University of Western Ontario; cultivated trees	SFAC03-03	2.74±0.14	4x
ser. Intricatae (Sarg.) Rehder				
{C. apposita Sarg.}				Tetraploid (Longley 1924); 68 (Moffett 1931)
{C. bartoniana Sarg.}				Tetraploid (Longley 1924)
C. biltmoreana Beadle				Triploid (Longley 1924)
{C. bisselii Sarg.}				Triploid (Longley 1924)
C. boyntonii Beadle				Triploid (Longley 1924)
{C. cuprea Sarg.}				Diploid (Longley 1924)
{C. delosii Sarg.}				Triploid (Longley 1924)
{C. flavida Sarg.}	**Arnold Arboretum**, Boston, Massachusetts; cultivated trees	AA220-89A	3.53±0.42	High
{C. infera Sarg.}				Triploid (Longley 1924)
C. intricata Lange or C. harveyana Sarg.				Triploid (Longley 1924)
{C. bealii Sarg. / C. intricata Lange or C. harveyana Sarg.}				Triploid (Longley 1924)
{C. diversifolia Sarg. / C. intricata Lange or C. harveyana Sarg.}				Triploid (Longley 1924)
{C. inducta Ashe / C. intricata Lange or C. harveyana Sarg.}				Triploid (Longley 1924)
{C. meticulosa Sarg. / C. intricata Lange or C. harveyana Sarg.}				Triploid (Longley 1924)
{C. painteriana Sarg. / C. intricata Lange or C. harveyana Sarg.}				Triploid (Longley 1924)
{C. modesta Sarg.}				Triploid (Longley 1924)

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
C. padifolia Sarg.				Triploid (Longley 1924)
{*C. pallens* Beadle}				Tetraploid (Longley 1924)
{*C. peckii* Sarg.}				Tetraploid (Longley 1924)
{*C. pusilla* Sarg.}				Triploid (Longley 1924)
{*C. pygmaea* Sarg.}				Triploid (Longley 1924)
{*C. verecunda* Sarg.}				Triploid (Longley 1924)
C. sargentii Beadle				Triploid (Longley 1924)
	Georgia, Floyd; det. Ron Lance	NT288	1.98±0.13	2-3x
C. ?				
	Alabama, Dekalb	2003-4	3.49±0.34	High
	Virginia, Franklin	2003-48	3.52±0.28	High
ser. Macracanthae (Loudon) Rehder				
C. calpodendron (Ehrh.) Medik.				Diploid (Longley 1924), 34 (Muniyamma and Phipps 1979*a*; Muniyamma and Phipps 1985); 34+1, 51?, 68 (Muniyamma and Phipps 1979*a*)
	Ontario, Middlesex	NT166	1.61±0.08	2x
		MP32	1.65±0.18	2x
	Ontario, Niagara	NT172	1.58±0.08	2x
	Arnold Arboretum, Boston, Massachusetts; cultivated trees	AA277-68A	1.82±0.19	2–3x
	Jardin Botanique de Montréal, Montréal, Quebec; cultivated trees	JBM1234-49	1.62±0.19	2x
{*C. chapmanii* (Beadle) Ashe}				Triploid (Longley 1924)
{*C. deweyana* Sarg.}				34 (Sax 1931)
{**C. finitima Sarg.**}				
	Royal Botanical Gardens, Burlington, Ontario, cultivated trees	RBG46596	2.30±0.21	3x
C. macracantha Lodd. ex Loudon				68 (Muniyamma and Phipps 1979*a*)
	Ontario, Durham	NT101	3.18±0.21	4x
		NT102	3.10±0.17	4x
		NT104	3.02±0.16	4x
		NT105	2.98±0.24	4x
		NT107	3.05±0.25	4x
		NT130	3.28±0.29	4x
	Ontario, Haliburton	NT198	3.01±0.19	4x
	Ontario, Kawartha Lakes	NT197	3.32±0.16	4x
	Ontario, Niagara	NT180	3.34±0.30	4x
		NT181	3.31±0.17	4x
	Ontario, Peterborough	NT199	3.35±0.22	4x+
	Ontario, Toronto	NT5	3.16±0.18	4x
		NT6	3.21±0.21	4x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
		NT30	3.04±0.22	4x
		NT163	3.19±0.25	4x
		NT203	3.31±0.25	4x
		NT205	3.31±0.27	4x
		NT224	3.04±0.25	4x
		NT225	3.03±0.23	4x
	Ontario, York	NT137	3.14±0.20	4x
		NT139	3.19±0.22	4x
		NT142	3.21±0.22	4x
	Washington, Okanagan; det. Peter Zika	Zika19236	3.10±0.35	4x
C. ?macracantha		Zika19237	3.14±0.43	4x
C. succulenta **Ashe**		Zika19240	3.25±0.40	4x
				72? (Muniyamma and Phipps 1979*a*)
				34+1?, 51 (Muniyamma and Phipps 1979*a*)
	Ontario, Toronto	NT221	2.38±0.16	3x
		NT241	2.40±0.17	3x
		NT242	2.42±0.17	3x
	Ontario, Haldimand-Norfolk	NT268	2.28±0.13	3x
ser. *Molles* (Beadle ex Sarg.) Rehder				
C. arnoldiana **Sarg.**				Triploid? (Longley 1924)
	Royal Botanical Gardens, Burlington, Ontario, cultivated trees	RBG54703	2.87±0.25	4x
{*C. treleasei* Sarg. / *C. dispessa* Ashe}				Diploid (Longley 1924)
C. lanuginosa Sarg.				Triploid (Longley 1924)
{*C. sera* Sarg. / *C. mollis* Scheele}				Diploid (Longley 1924)
C. mollis **Scheele**				Diploid (Longley 1924), 34 (Muniyamma and Phipps 1979*a*; Muniyamma and Phipps 1985)
	Alabama, Lowndes; det. Ron Lance	2003-13	2.42±0.14	3x
	Ontario, Middlesex	2003-16	2.41±0.18	3x
		D1655	1.72±0.17	2–3x
	Wisconsin, Madison	NT208	1.46±0.10	2x
C. submollis **Sarg.**				68 (Gladkova 1968)
	Ontario, Toronto	NT13	3.33±0.32	4x
		NT19	3.06±0.22	4x
		NT212	3.07±0.21	4x
	Jardin Botanique de Montréal, Montréal, Québec; cultivated trees	JBM2457-61	3.06±0.37	4x
C. viburnifolia **Sarg.**				
	North Carolina, Buncombe; det. Ron Lance	NT322	2.50±0.10	3x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
ser. *Pruinosae* (Beadle ex Sarg.) Rehder				
{*C. delawarensis* / *C. pruinosa* Sarg.}				Triploid (Longley 1924)
{*C. fusca* Sarg.}				Triploid (Longley 1924)
{*C. placiva* **Sarg.**}				
	North Carolina Arboretum, Asheville, North Carolina; cultivated trees	NC1994-589	1.94±0.13	2–3*x*
	Royal Botanical Gardens, Burlington, Ontario, cultivated trees	RBG46597	2.96±0.31	4*x*
C. pruinosa (**H.L.Wendl.**) **K.Koch** *s. l.*				Triploid (Longley 1924); 68 (Moffett 1931), 51, 51?, 68, 68, or 72? (Muniyamma and Phipps 1979*a*)
	Ontario, Middlesex	NT167	2.38±0.10	3*x*
	Ontario, Niagara	NT169	3.26±0.17	4*x*
		NT173	3.11±0.18	4*x*
		NT174	3.34±0.26	4*x*
	Ontario, Wellington	NT188	3.11±0.15	4*x*
	Royal Botanical Gardens, Burlington, Ontario, cultivated trees	RBG77073	3.06±0.28	4*x*
	Sherwood Fox Arboretum, University of Western Ontario; cultivated trees	SFAC04-52	2.63±0.13	3–4*x*
{*C. cognata* Sarg. / *C. pruinosa* (**H.L.Wendl.**) **K.Koch**}				
ser. *Pulcherrimae* (Beadle ex E.J.Palmer) K.R.Robertson				
C. ?				68 (Moffett 1931)
ser. *Rotundifoliae* (Eggl. ex Eggl.) Rehder				
C. chrysocarpa **Ashe**	**Alabama**, Montgomery; det. Ron Lance	2003-10	2.40±0.20	3*x*
				Triploid?, tetraploid (Longley 1924); 68 (Moffett 1931); 68, 68? (Smith and Phipps 1988)
	Ontario, Grey	EL1	2.89±0.18	4*x*
		EL3	2.89±0.15	4*x*
C. chrysocarpa var. *piperi* (**Britton**) **Kruschke**	**Washington**, Klickitat	EL117	2.87±0.15	4*x*
		EL118	3.02±0.23	4*x*
		EL119	2.87±0.15	4*x*
		EL120	2.88±0.24	4*x*
		EL122	2.96±0.15	4*x*
C. chrysocarpa var. *aboriginum* (**Sarg.**) **Kruschke**				

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
C. chrysocarpa var. phoenicea Palmer	**Sherwood Fox Arboretum,** University of Western Ontario; cultivated trees	SFAC01-59	2.54±0.14	3x+
{C. dacrioides Sarg.}				Triploid (Longley 1924)
C. dodgei Ashe	**Sherwood Fox Arboretum,** University of Western Ontario; cultivated trees	SFAC01-61	2.61±0.12	3–4x; Triploid, triploid? (Longley 1924); 34, 34?, 51?, 68? (Muniyamma and Phipps 1979a); 51, 51?, 68, 68? (Sax 1931); 51, 51?, 68, 68? (Smith and Phipps 1988)
C. ?dodgei	**Ontario,** Grey	EL2	2.93±0.15	4x
C. irrasa Sarg.	**Ontario,** Toronto	NT12	3.18±0.17	4x
C. lumaria Ashe	**Ontario,** Grey	NT193	3.06±0.22	4x; 34 (Muniyamma and Phipps 1979a)
C. margaretta Ashe		NT307	2.92±0.16	4x; Triploid? (Longley 1924); 51?, 68, 68? (Smith and Phipps 1988)
ser. *Silvicolae* (Beadle ex Sarg.) Rehder				
C. iracunda				Diploid (Longley 1924); 34, 34? (Muniyamma and Phipps 1979a)
ser. *Suborbiculatae* (Kruschke) **J.B. Phipps**				
C. compacta Sarg.				Triploid? (Longley 1924); 68, 72 (Muniyamma and Phipps 1979a); 34 to 42?, 34+1?, 68 (Muniyamma and Phipps 1979a)
C. suborbiculata Sarg.	**Ontario,** Lambton	D654	2.36±0.10	3x; 68 (Muniyamma and Phipps 1979a); 68? (Muniyamma and Phipps 1985)
ser. *Tenuifoliae* (Beadle ex Sarg.) **Rehder**				
{C. paucispina Sarg. / C. apiomorpha Sarg.}				
C. flabellata (Bosc.) K.Koch (as C. flabellata Sarg.)				Triploid (Longley 1924)
C. fluviatilis Sarg.				Triploid? (Longley 1924)
{C. forbesae Sarg.}				Triploid? (Longley 1924)
C. macrosperma Ashe	**Ontario,** Toronto	NT15	3.11±0.19	Tetraploid? (Longley 1924); 68 (Muniyamma and Phipps 1979a; Muniyamma and Phipps 1985; tetraploid (Longley 1924); 4x
{C. pentandra Sarg. / C. macrosperma Ashe}				Triploid (Longley 1924)
C. schuettei Ashe				51, 51? (Muniyamma and Phipps 1979a)
{C. tarda Sarg. / C. schuettei Ashe}				
C. ?				Triploid (Longley 1924)

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
ser. *Triflorae* (Beadle) Rehder				
***C. triflora* Chapm.**	**Ontario**, Toronto	NT162	3.10±0.24	4x
	Alabama, Autauga; det. Ron Lance	2003-09	2.68±0.21	Triploid or tetraploid (Longley 1924); 4x–
		2003-22	1.72±0.16	2–3x
		2003-23	1.73±0.13	2–3x
	Georgia, Floyd; det. Ron Lance	NT287	2.07±0.08	3x
		NT290	1.35±0.06	2x–
	Georgia, Walker; det. Ron Lance	NT291	1.46±0.11	2x
	Mississippi, Newton; det. Ron Lance	NT292	1.42±0.09	2x
		Lance2314 seedling 3	2.35±0.16	3x
		Lance2314 seedling 4	2.38±0.20	3x
		Lance2314 seedling 5	2.38±0.18	3x
		Lance2314 seedling 2	2.38±0.18	3x
		Lance2314 seedling 1	2.44±0.21	3x
	Mississippi, Scott	2002-08	2.47±0.22	3x
No section assignment				
ser. *Purpureofructi* J.B.Phipps & O'Kennon				
***C. enderbyensis* J.B.Phipps & O'Kennon**	**British Columbia**, North Okanagan; det. Peter Zika	Zika18445	3.04±0.27	4x
		Zika18446	3.12±0.32	4x
		Zika18451	2.86±0.25	4x
		Zika18454	3.07±0.22	4x
***C. okennonii* J.B.Phipps**	**Washington**, Okanagan; det. Peter Zika	Zika19239	3.34±0.42	4x
C. ?okennonii	**Washington**, Okanagan; det. Peter Zika	Zika19235	3.28±0.46	4x
***C. phippsii* O'Kennon**	**Washington**, Okanagan; det. Peter Zika	Zika19238	3.04±0.33	4x
		Zika19291	3.17±0.31	4x
nothosect. *Crataeguineae* K.I.Chr.				
***C.* ×*dsungarica* Zabel ex Lange**	**Arnold Arboretum**, Boston, Massachusetts; cultivated trees	AA1196-65A	3.15±0.25	68 (Gladkova 1968); 4x
nothosect. unnamed (*Crataegus* × *Crus-galli*)				
nothoser. unnamed (*Crataegus* × *Punctatae*)				
C. monogyna* × *C. punctata		AA1196-65B	3.19±0.21	34 (Wells and Phipps 1989); 4x

Table 1. (*continued*).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
	Ontario, Durham	2002-12	1.51±0.12	2x
		2002-13	1.53±0.08	2x
	Ontario, Peel	NT3	1.60±0.12	2x
		NT124	1.59±0.13	2x
		NT125	1.53±0.11	2x
	Ontario, Middlesex	NT128	1.41±0.09	2x
		NT129	1.50±0.07	2x
		NT134	1.44±0.10	2x
		EH52	1.50±0.09	2x
		EH55	1.51±0.06	2x
nothosect. unnamed (*Crataegus* × *Douglasianae*)				
C. *monogyna* × C. *suksdorfii*				
	Oregon, Columbia; det. Peter Zika	Zika18482	1.46±0.14	2x
	Oregon, Lane; det. Rhoda Love	LoveC-2003-12	1.60±0.12	2x
	Oregon, Linn	LoveC-2003-13	1.66±0.16	2x
		EL73	1.43±0.09	2x
		EL76	1.48±0.09	2x
		EL77	1.43±0.09	2x
		EL79	1.40±0.10	2x
		EL81	1.49±0.09	2x
		EL82	1.48±0.09	2x
		EL84	1.39±0.10	2x
		EL85	1.44±0.11	2x
nothosect. unnamed (*Crataegus* × *Coccineae*)				
nothoser. unnamed (*Mexicanae* × *Macracanthae*)				
C. ×*lavallei* **Lavallée**	**Ontario**, Toronto; cultivated tree	NT246	1.44±0.13	32 (Sax 1931; diploid (Wells 1985) 2x
nothoser. unnamed (*Crataegus* × *Macracanthae*)				
C. ×*mordenensis* **Boom 'Toba'**	**Ontario**, Toronto; cultivated tree	NT211	1.66±0.08	2x
	Royal Botanical Gardens, Burlington, Ontario; cultivated trees	RBG810471	1.69±0.17	2x+
C. ×*mordenensis* **Boom 'Snowbird'**	**Royal Botanical Gardens**, Burlington, Ontario; cultivated trees	RBG810614?	2.53±0.35	3x+
nothosect. unnamed (*Crus-galli* × *Coccineae*)				
nothoser. unnamed (*Crus-galli* × *Macracanthae*)				
C. ×*persimilis* **Sarg.**	**Ontario**, Niagara	NT34	2.93±0.27	68 (Dickinson and Phipps 1986), tetraploid (Wells 1985) 4x
		NT36	3.07±0.18	4x

Table 1. (concluded).

Taxon	Locality	Sample No.	DNA amount (pg)	Ploidy level or chromosome count
nothoser. unnamed (*Punctatae* × *Bracteatae*)				
C. harbisonii × *C. collina*		NT38	3.31±0.28	4x
		NT175	3.11±0.22	4x
		NT176	3.26±0.25	4x
		NT177	3.11±0.17	4x
		NT178	3.26±0.27	4x
	Tennessee, Davidson; det. Ron Lance	Lance 2306	2.24±0.16	3x
	North Carolina Arboretum, Asheville, North Carolina; cultivated trees	NC98-76A	2.16±0.20	3x
Incertae sedis				
C. aemula **Beadle** (?*Silvicolae*)				
	Georgia, Floyd; det. Ron Lance	NT289	2.55±0.15	3x+
	Georgia, Rabun; det. Ron Lance	Lance 2311 seedling 5	3.01±0.27	4x
		Lance 2311 seedling 2	3.06±0.22	4x
		Lance 2311 seedling 3	3.09±0.22	4x
		Lance 2311 seedling 4	3.11±0.24	4x
		Lance 2311 seedling 6	3.16±0.22	4x
C. ×*peregrina* **Sarg.**				
	Royal Botanical Gardens, Burlington, Ontario; cultivated trees	RBG54716	1.52±0.15	2x
C. pinetorum Beadle (?*Pulcherrimae*)				Triploid (Longley 1924)

Note: Taxa and localities in Canada and the United States that are in boldface are ones for which we report new nuclear DNA amounts, together with the inferred ploidy level (x = 17). DNA amounts are the 2C values, that is, the unreplicated amount of DNA in a somatic nucleus irrespective of the ploidy level. The ploidy level is estimated using the Ontario core taxa, as described in the text. Ploidy estimates followed by a plus sign or minus sign (e.g., 2x–) are within 12% rather than 10% of the midpoint of the estimated range. Since the ranges for 5x and 6x cannot be unambiguously distinguished, all plants that measured more than 4x have the same annotation "high". Where compilations of chromosome counts have been published, the compilation is cited rather than the original references, and if more than one paper by the same authors gives the same chromosome counts for the same taxon, only one paper is cited.

Table 2. DNA amounts (ranges) that we chose to accept as representing each ploidy level in *Crataegus*.

Ploidy level	DNA amount (pg)
2x	1.368–1.672
3x	2.052–2.508
4x	2.736–3.344
5x	3.420–4.180
6x	4.104–5.016

ploidy is the first that we are aware of. Rather than matching Gu and Spongberg's (2003) report of a hexaploid, our data suggest the presence of pentaploids.

Although the small chromosome size and the variability in the measurement makes the detection of aneuploids uncertain, they appear to be rare. Previous chromosome counts have included some individuals with 72 rather than 68 chromosomes (Table 1), and this appears to be mirrored in the DNA measurements, but we found only individuals rather than groups with this higher measurement. It is interesting that by flow cytometry our *C.* ×*lavallei* plant has a similar DNA reading to other diploid *Crataegus*; this plant is considered diploid (e.g., Wells 1985) but Sax (1931) saw only 16 pairs of chromosomes and cited it along with irregular meiosis in some other plants to suggest that counts of 32 rather than 34 from earlier workers were not necessarily mistaken. It should be possible to survey for aneuploidy using flow cytometry, even close to the diploid number of chromosomes, by mixing unknown samples with a reference sample. For example, we have mixed samples from tetraploid *C. phaenopyrum* (tree 2002-10, separately measured at 3.14 pg DNA with SD = 0.29) and *C. crus-galli* (tree NT32, 2.96 pg DNA, SD = 0.22) to give a double peak. However, since the fluorescence distributions from the two trees overlap, and since the numbers of nuclei extracted from the two samples need to correspond, false negatives are likely.

Tetraploids form the majority of our sample of native North American plants, and diploids are the next most frequent. This is a contrast to Longley's results (1924) where triploids predominated amongst the taxa that had been collected and propagated at the Arnold Arboretum. We have concentrated on the forms whose morphological identification and taxonomic position are less problematic with the aim of detecting cryptic polyploidy, and we believe that the early emphasis by taxonomists on building a collection of unusual forms was responsible for the perception that most hawthorns are triploid.

Some interesting "species"

The data on polyploidy that we have so far are a rich source of research questions about particular taxa, some of which we will outline here.

Until the discovery of *Mespilus canescens* in North America (Phipps 1990), the genus was considered monotypic, consisting only of Eurasian *M. germanica*. The triploid chromosome count from *M. canescens* (M. Muniyamma, personal communication, 1990), which is now confirmed by DNA measurements from nine individuals, raises the question of whether the range extension of the genus is due to diploid–tetraploid hybridization of *M. germanica* with a native *Crataegus* species following very early settlement of Europeans in Arkansas. We hope that it will prove feasible to test this hypothesis, using DNA sequences, against the rather more exciting possibility that the two genera dispersed independently in prehistoric times between continents.

Crataegus collina is included in *C. punctata* by some authors, but in North Carolina the two taxa appear to be distinct, with *C. collina* blooming early and *C. punctata* blooming late, though *C. punctata* occurs at higher elevations (R.W. Lance, personal communication, 2004). *Crataegus collina* has been previously recorded as diploid, but we found only polyploids in the parts of North Carolina that we sampled, whereas in a much larger but very distant area of Ontario, the *C. punctata* appear to be diploid only. Our survey has also extended the records of diploids in series *Crus-galli* from two cultivated plants to wild populations in two US states; tetraploids that are classified in that series are present in the same general area as well as much further away. So we see that in section *Crus-galli*, the two series *Punctatae* and *Crus-galli* each contain at least one diploid–polyploid sequence, and the putative hybrids between diploid *C. punctata* and tetraploid *C. crus-galli* in Ontario (Dickinson 1983), if they in fact are hybrids, may have formed between rather distantly related parents rather than between a diploid and its autotetraploid. Section *Crus-galli* would therefore be a worthy candidate for quite extensive phylogenetic and phylogeographic investigation.

Crataegus uniflora has a quite uniform DNA amount across a considerable geographic range and appears to be triploid, or a tetraploid with an exceptionally small genome; the one chromosome count for it is triploid. If it is triploid, then it would be likely to reproduce by apomixis and may be a highly successful clonal plant. There is morphological variation by locality (Phipps et al. 2003*b*) that suggests multiple origins, but we are unaware of any candidates for the diploid or tetraploid ancestors of this plant. Some triploid apomictic plants are known to achieve genetic diversity through pollen transfer to diploids or via a tetraploid generation to re-create apomictic triploids (e.g., *Taraxacum officinale*, Asteraceae; Tas and Van Dijk 1999; Verduijn et al. 2004), but once again we know of no indication from morphology of likely partners for *C. uniflora* in such a reproductive strategy. This plant invites thorough investigation.

Diploid–diploid hybridization is known to occur between Eurasian species (Byatt 1975) and between Eurasian *C. monogyna* and two diploid North American species (Love and Feigen 1978; Wells and Phipps 1989). It has long been suggested that sympatric diploid American species are hybridizing (Camp 1942), but actual evidence is slight. A survey of polyploidy by flow cytometry would be a foundation for a study to find possible cases of diploid–diploid hybridization and eventually to quantify its occurrence. For example, *C. spathulata* is the only species currently placed in series *Microcarpae* and it is diploid in our sample. It has been suggested that in a number of attributes it resembles *C. phaenopyrum*, the sole species in series *Cordatae* (Beadle 1913; Phipps 1998), but that species has so far proved polyploid. If we suppose that the degree of dissection of the

Fig. 4. DNA amounts from leaves of all *Crataegus* and *Mespilus* individuals. When all sampled trees (695 individuals) are included, the 2*x*, 3*x*, and 4*x* groups are less clearly distinguished and some very high and very low values appear. As discussed in the text, although some of the intermediate readings are likely to correspond to aneuploid individuals, it is clear that some taxa consistently fall in these ranges. (A) The low measurements (more than 12% below the midpoint of the diploid range, below 1.337 pg DNA) are 10 of the 11 southeastern diploid *C crus-galli* (series *Crus-galli*), and half of our measurements from series *Aestivales*, namely both *C. aestivalis* and one of the *C. opaca*. (B) The interval between 2*x* and 3*x* (between 1.702 and 2.006 pg DNA) contains all eight individuals of *C. brachyacantha* (*Brevispinae*), both *C. hupehensis* (sect. *Hupehenses*), and the single *C. sargentii* (*Intricatae*). It also contains some individuals of *Mespilus germanica, C. monogyna* (*Crataegus*), *C. marshallii* (*Apiifoliae*), *C. viridis* (sect. *Virides*), *C. spathulata* (sect. *Microcarpae*), series *Lacrimatae, C. rufula* (*Aestivales*), *C. saligna* (*Brevispinae*), *C. suksdorfii* (*Douglasianae*), *C. collina* (*Punctatae*), *C. triflora* (*Triflorae*), *C. mollis* and *C. viburnifolia* (*Molles*), and *C. calpodendron* (*Macracanthae*). (C) The interval between 3*x* and 4*x* (between 2.554 and 2.675 pg DNA) contains three of the four *C. uniflora* (*Parvifoliae*), two *C. crus-galli* (*Crus-galli*) that are the southeastern United States form that is sometimes referred to as *C. berberifolia* Torr. & A. Gray, as well as individuals of *C. collina* (*Punctatae*), *C. pruinosa* (*Pruinosae*), and *C. chrysocarpa* (*Rotundifoliae*). (D) The high values (above 3.405 pg DNA) include the possible *C. lassa* of series *Flavae*, our highest DNA measurement, as well as individuals from other series: three of the four measurements from series *Intricatae* of which one is labeled *C. flavida* at the Arnold Arboretum, two individuals each of *C. ashei* (*Bracteatae*) and *C. phaenopyrum* (*Cordatae*), and in series *Douglasianae* one each of *C. douglasii* and *C. castlegarensis*. The figure appears on the following two pages.

Table 3. DNA measurements from other Rosaceae.

Species	Voucher No.	2C value (pg)	SD	2C value from Dickson et al. (1992) (pg)	SD from Dickson et al. (1992)	Ploidy level[a]
Amelanchier alnifolia Nutt. 'Smoky'	NT216	2.68	0.27	—	—	4*x*
Amelanchier arboria (Michx. f.) Fernald	2003-01	1.39	0.12	1.31	0.25	2*x*
Aronia pyrifolia Lam.	—	—	—	2.57	0.20	2*x*, 4*x*
Aronia sp.	2003-02	3.03	0.22	—	—	—
Cydonia oblonga Mill.	NT324	1.54	0.08	1.45	0.14	2*x*
Gillenia stipulata (Willd.) Nutt.	2000-51	1.01	0.19	—	—	—
Gillenia trifoliata (L.) Moench	2000-52	0.83	0.09	—	—	2*x* (*x*=9)
Photinia parvifolia (E. Pritz) C.K.Schneid.	—	—	—	2.29	0.26	—
Potentilla indica (Andr.) Focke	NT136a	3.58	0.26	3.00	0.14	6*x*, 12*x* (*x*=7)
Pyracantha coccinea M.Roem.	2003-12	1.49	0.12	1.41	0.31	2*x*

Note: Ploidy levels are those compiled from the literature by Dickson et al. (1992) except for *Gillenia trifoliata* (Robertson 1974 as *Porteranthus trifoliatus*) and *Amelanchier alnifolia* 'Smoky', which has been reported to occur in nature as diploids or tetraploids (Pruski et al. 1991). We infer from the DNA measurements that our specimen of *A. alnifolia* 'Smoky' is tetraploid and that *A. arborea* is diploid. 2C values, the unreplicated amount of DNA in a somatic nucleus irrespective of the ploidy level.

[a]*x* = 17 except as noted.

Table 4. Uniformity of DNA amount in repeated measurements from *Crataegus monogyna*.

Voucher No.	Collection date	Tissue	DNA (pg)	SD
NT114	22 August 2002	Leaf	1.52	0.09
NT114	21 August 2003	Leaf	1.59	0.15
NT114	11 September 2003	Leaf	1.57	0.10
NT114	1 October 2003	Leaf	1.53	0.14
NT114	9 January 2004	Buds	1.48	0.09
NT117	7 July 2003	Leaf	1.46	0.11
NT117	7 July 2003	Petals	1.47	0.11

leaves is a better clue to relationships than other characters such as overall leaf shape, then a hypothesis of diploid–diploid hybridization could be considered, with *C. spathulata* a diploid hybrid between one of the diploid species with narrow entire-margined leaves and diploid *C. marshallii* (of the monotypic series *Apiifoliae*), which has highly dissected leaves and is sympatric with *C. spathulata*.

Mixed ploidy levels

The data about polyploidy that we have accumulated to

(A)
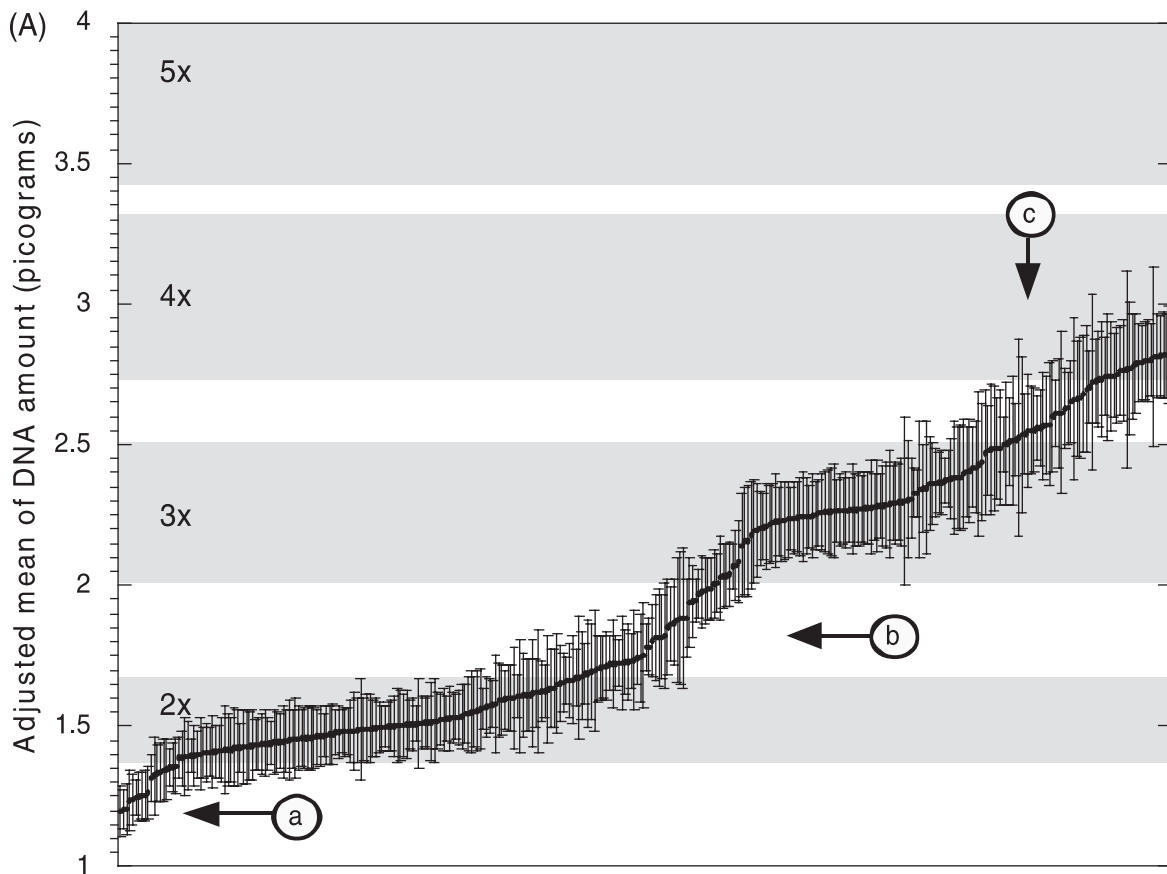
this point leave us with a major question about these plants: why is it that certain groups in certain geographical areas consist of a variety of ploidy levels, but this is not the case throughout the distribution of each "species"? It seems that the answer may have several parts and it would be advisable to study different groups separately.

Some *Crataegus* "species" are geographically widespread but seem to consist of only one ploidy level; examples are *C. punctata* sensu stricto (diploid), *C. uniflora* (triploid), and *C. rivularis* and *C. douglasii* sensu stricto (tetraploid). Many other groups that are currently classified as species contain a range of ploidy levels, for example North American *C. suksdorfii*, *C. calpodendron*, and *C. triflora*, and Eurasian *C. pinnatifida*. In some cases we have found that ploidy-level differences are better predicted by locality than by morphology, since nearby sites may hold plants of different ploidy levels that are not readily separable by morphology; *C. collina* and *C. triflora* are examples. Thus we have a situation that has been seen in other plants, truly cryptic polyploidy where there is no apparent effect on the phenotype from the extra copy or copies of the genome. This could conceivably be autopolyploidy, but investigation of the repetitive DNA in some other plants whose morphology suggests autopolyploidy has instead suggested cryptic allopolyploidy (e.g., *Hordeum mirinum* Taketa et al. 2000).

It seems likely that morphology could equally well lead us to falsely infer allopolyploidy if the ancestral diploid plants had varied morphology and extinctions have obscured the relationships. Autopolyploids with multiple origins could vary in morphology if they derived from variable diploids, and *C. calpodendron* and *C. suksdorfii* are possible exam-

ples that could repay further investigation. We hope to gain more insight on this question with work in progress on section *Douglasianae*.

Allopolyploidy in *Crataegus* is indicated by some triploids (*C. ?grandis*) that are considered to be hybrids between diploid *C. punctata* and tetraploid *C. crus-galli* sensu stricto (Dickinson 1983; Dickinson and Phipps 1986), two species that we decided above are rightly considered members of different series. More investigation will be needed to discover whether allopolyploidy is a common origin for triploids. It has been suggested that some tetraploids are inter-series hybrids (e.g., *C. ×persimilis* as a hybrid of *C. crus-galli* sensu lato and *C. succulenta* sensu lato; Eggleston 1908; Palmer 1950; Phipps 1988*b*; Phipps et al. 2003*b*), but it is not known whether this is a tetraploid–tetraploid sexually derived hybrid or an allopolyploid derived from the diploid or triploid members of the ancestral "species". Beyond the mechanisms of diploid-diploid hybridization and diploid–tetraploid hybridization to form allotriploids, we know little about interbreeding.

At this stage it is likely that the lack of diploids in some groups is a sampling artifact since diploids are relatively rare in some other groups (Table 1): only one diploid from series *Coccineae* has been recorded (without a species identification, Table 1), a diploid count for *C. cuprea* is exceptional in series *Intricatae*, and series *Crus-galli*, for which Longley's diploid chromosome count had seemed anomalous (Longley 1924), still has a vast geographic range in which only polyploids have been recorded. However, with the information that we have so far, some taxonomic series appear to consist only of triploids and tetraploids; two exam-

(B)
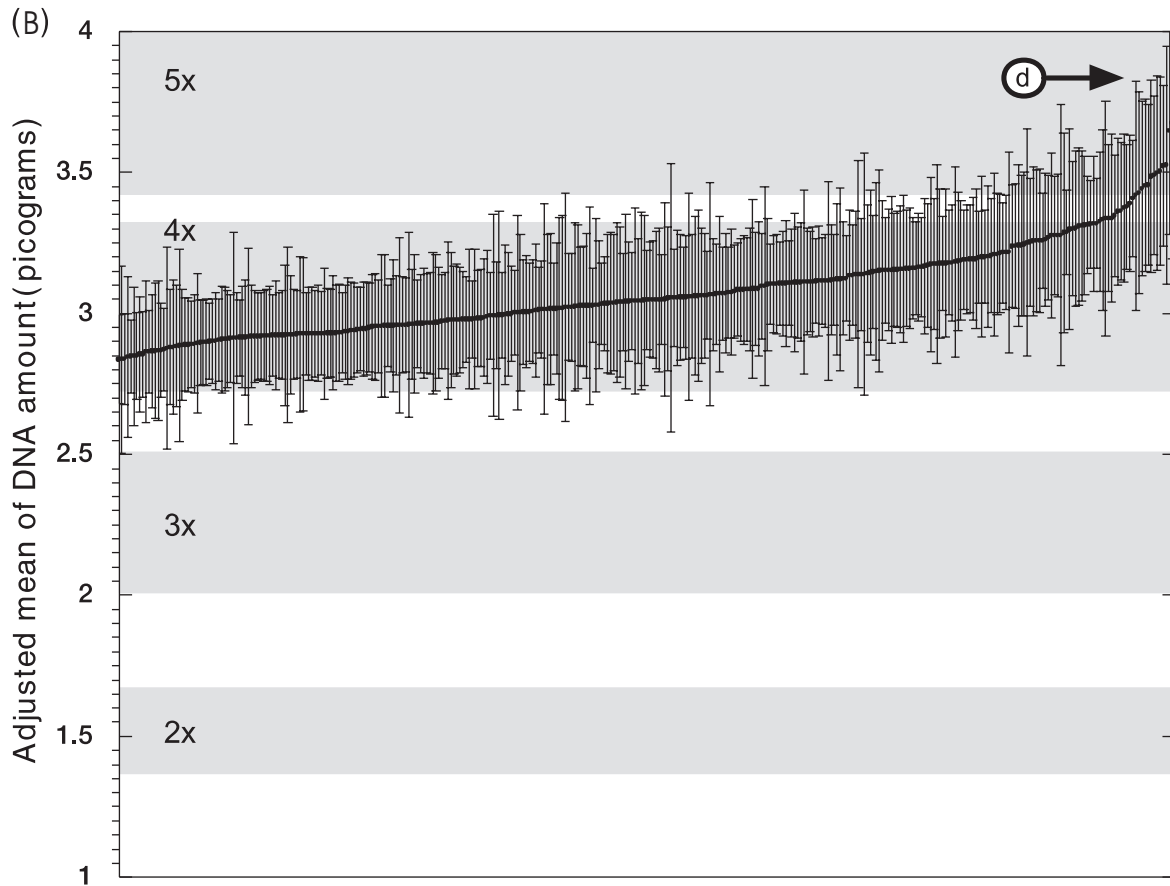
ples are *Pruinosae*, and *Tenuifoliae*, groups that have proved particularly difficult to partition by morphology and that have received different treatments from different authors. In contrast to these, series *Cordatae* and *Parvifoliae* are much less variable. It is tempting to look for differing biology between the variable and the non-variable polyploid groups: perhaps some of them readily cross among themselves and with diploids and tetraploids from other series, while others act as solid biological species. It will be very interesting to discover the true evolutionary role of apomixis in this genus, which on the one hand might be to reduce the potential for hybridization while creating a multitude of true-breeding variant forms, but on the other hand might enhance the potential for hybridization and for some rather dramatic changes of chromosome numbers as has been noted in some Poaceae (e.g., Clausen 1961; de Wet and Harlan 1970).

Geographic distributions

The relative distributions of diploids and polyploids in *Crataegus* are intriguing, but more data are needed and larger geographical areas should be surveyed for each taxon. There is much evidence from other plants that the distributions of polyploids and of apomicts can spread well beyond the distributions of their ancestral diploids (much of the evidence was reviewed, for example, by Lewis (1980) and Grant (1981), and in North America we suspect that Pleistocene refugia might produce a very strong pattern of this type. Probably the strongest evidence so far to suggest this pattern is series *Crus-galli*, where the known diploids are restricted to the southeastern United States, but polyploids spread up into Canada (Table 1). In Ontario series *Molles*

and series *Macracanthae,* the species that appear to be largely or entirely diploid (*C. mollis* and *C. calpodendron*) are distributed in the more southerly Carolinian zone, while the putative tetraploids (*C. submollis* and *C. macracantha*) spread North of the 45th parallel (Table 1, and Phipps and Muniyamma 1980). Not all of the most northerly species are polyploid, however, as *C. punctata* appears to be uniformly diploid even as far North as our most northerly samples from southern Bruce Co. Ontario (44.54°N).

Hybrid derivatives also might spread beyond their ancestors into equally warm areas, and our data on ploidy levels add an intriguing twist to a particularly neat example of hybridization in the southeastern United States that has been studied morphologically by Phipps (1988*a*). "Occurring as it does at the exact interface of *C. aestivalis* (more easterly) and *C. opaca* (more westerly), as well as being intermediate in almost all respects between those two species, *C. rufula* represents a presumed hybrid swarm or its descendants, probably with some elements fixed by apomixis." Our samples from the parental species in this complex were diploid, and the *C. rufula* samples appeared to be triploid, which further suggests that polyploid apomixis has indeed played role in the development of this geographically separated hybrid.

Models of evolution in *Crataegus*

Camp (1942) proposed a hybridization-based model of the development of taxonomic complexity in *Crataegus*. Building on the insight of Sax and Rickett that apomixis was prevalent in the genus (Sax 1931; Rickett 1936), and on Longley's finding more triploids than either diploids or tetraploids (Longley 1924), he drew parallels to the then re-

cently published model of *Crepis* (Babcock and Stebbins 1938) and began with primary hybrids between diploid species, noting that: "in other groups such hybrids are notorious for the production of at least some unreduced gametes. It would therefore seem likely that, in the North American population of *Crataegus*, there is a series of basic sexual diploids on which is super-posed a large and morphologically complex group of derived, asexual, apomictic triploids, to which may be appended a scattered series of tetraploids, some of which may be sexual and others apomictic."

Although we have seen above that cryptic polyploidy might be common, and that this might include autopolyploidy, we find ourselves in agreement with Camp (1942) in thinking it very likely that polyploid offshoots of diploid species can be triggered by some kind of hybridization event and that apomictic triploids would have a strong selective advantage over sexually sterile triploids. But are hybridization and polyploidy likely to be simultaneous or consecutive events, and how do apomictic tetraploids fit into the picture?

Various authors have studied the patterns evident in other taxa, most often the Asteraceae, and have tended to favor different sequences of events. Since apomixis requires multiple mutations (at least two and perhaps three) to achieve a fully functional system, Stebbins (1980) saw even-multiple polyploidy, not triploidy, as a precursor for apomixis. He noted that polyploidy can correlate with a perennial habit (though the strength of this correlation has been debated; Fagerlind 1944) and this would provide greater opportunity for successful reproduction (Stebbins 1942). Stebbins also saw the duplicate genes of polyploids as providing greater opportunity for the mutations necessary for apomixis to arise, while providing a buffering effect against deleterious mutation. The buffering effect extended to environmental adaptation and meant that polyploids could invade marginal habitats where reproductive isolation could produce selection for apomixis. He concludes that "There is good reason for believing that among angiosperms sexual polyploidy precedes apomixis" and "hybridization not only increases the number of different kinds of alleles that are present and might interact favorably with each other, but it also reduces the number of alleles present as duplicates that would minimize the effects of many mutations" (Stebbins 1980). So his view is that polyploids are derived from chromosome doubling, and that apomixis is subsequently more likely to evolve, particularly in polyploids of hybrid origin.

Other authors have seen the question quite differently. It has been argued that a diploid apomict is likely to produce polyploid offspring through fertilization of the meiotically unreduced egg cell (Fagerlind 1944), and so apomixis, whether as a result of hybridization or not, would predate polyploidy. Since Fagerlind's time, however, the scarcity of diploid apomicts has become more firmly established as biological fact rather than as a sampling artifact and there is also more evidence that polyploids originate from unreduced gametes in predominantly sexual diploids, whether those parents were hybrids or not (Harlan and Wet 1975; Ramsey and Schemske 1998). We do not know, of course, that fertilization of the egg cells in diploid apomictic *Crataegus* could result in higher-ploidy offspring, and since diploid apomicts of any flowering plant are so rare, there is no added support

for this phenomenon from other diploid plants with pseudogamous apomixis (Nogler 1984). We do, however, have some unpublished data (Talent and Dickinson, in preparation) to show that fertilization of the meiotically unreduced egg cell is possible in triploid and tetraploid apomictic *Crataegus*.

It has long been argued that apomixis offers an "escape from sterility" for otherwise sterile hybrids (Darlington 1939, 1958; Stebbins 1941). It has even been argued that production of unreduced female gametophytes will confer a selective advantage because they are an evolutionary step towards apomixis (Marshall and Brown 1981), even though other mutations would be needed to produce a fully functional system of apomixis. *Crataegus* is equipped with a multicellular archesporium, a prerequisite for apospory, one of the mechanisms for producing chromosomally unreduced female gametophytes (Nogler 1984). Marshall and Brown's model is a modification of Fagerlind's (1944) in which hybridization is followed by multistep selection for apomixis, which is followed by polyploidy. As well as the difficulty presented by multi-mutation selection where the individual mutations are deleterious (bypassing meiosis or avoidance of fertilization of the egg cell, if they are separate mutations, would change the ploidy of the offspring, whereas autonomous endosperm development might have less severe consequences), this model presents the same difficulty as Fagerlind's (1944) , that we do not (yet) have evidence that successful diploid hybrid apomicts occur in *Crataegus*.

Importance of triploids and pollination

Camp's (1942) model is unusual in specifying that the first polyploids would be triploid, whereas Fagerlind (1944) and Marshall and Brown (1981) do not specify whether they would be triploid or tetraploid or a mixture of these. A triploid stage of evolution has the very appealing property that selection for genetic combinations that confer apomixis could be very strong among triploids that would otherwise be largely sterile. It has frequently been suggested that since apomixis is often associated with a hybrid origin, a tendency to apomixis might result from unusual gene combinations or from developmental instability in hybrids (Nogler 1984; Carman 2001), so it is plausible that some allotriploids could perpetuate themselves through apomictic reproduction without necessarily needing to accumulate all the mutations necessary for efficient seed production.

Evidence from other plant groups (where apomixis is not necessarily present), suggests a mechanism for producing triploid, rather than diploid, hybrids directly from particular combinations of distantly related diploid parent species when mismatched genetic components fail to act together to abort triploid embryos (Håkansson and Ellerström 1950; Lin 1984; Carputo et al. 1999). Paradoxically for this discussion, the mechanism involved is known as the "triploid-block" effect since it prevents the formation of intraspecific triploid embryos through failure in the endosperm. This argument suggests that the putative partly apomictic allotriploids might have been the immediate result of the diploid–diploid hybridization.

The first tetraploids might have arisen from triploids either through sexual hybridization or through fertilization of an unreduced egg cell by pollen from a diploid. There is no

need to suppose that these first tetraploid derivatives were strongly apomictic, but they might have had some such ability. It has often been noted that apomixis has the advantage of rapid multiplication of a well-adapted genotype (the Ford Model-T analogy; Clausen 1954) and under simple genetic models will spread to fixation except for a residual capacity for some sexual reproduction (e.g., Stebbins 1980; Marshall and Brown 1981; Mogie 1992; Noirot 1993) and apomixis in tetraploid *Crataegus* appears to offer still other advantages that might have further enhanced its selective advantage. Seed development is pseudogamous, that is, fertilization is still required for development of the endosperm, and pollen meiosis is generally normal. It seems that it might be possible for the apomictic tetraploids to pollinate diploids and thus confer apomixis genes on a new generation of triploid offspring with eventual gene transfer to tetraploids.

Crataegus species are ruderal shrubs, and the first polyploids might be isolated genetically, even if they could interbreed with diploids. The pollen self-compatibility in some tetraploid apomictic *Crataegus* is in strong contrast to the self-incompatibility of diploids (Dickinson and Phipps 1986; Dickinson et al. 1996; Macklin 2001), but it would seem to be disadvantageous for sexual tetraploids to risk inbreeding depression by self compatibility. Pollen self compatibility is likely to be an immediate result of polyploidy, as has been seen to occur in other dicot taxa with a 1-locus gametophytic system of self-incompatibility (Grant 1981; Ramsey and Schemske 2002), including *Pyrus communis*, another member of Rosaceae subfamily Maloideae (Crane and Lewis 1942), and in this connection it is interesting that the pollen-to-ovule ratios of the few self-compatible polyploid *Crataegus* that have been examined (Dickinson and Phipps 1986; Dickinson et al. 1996) do not show the dramatic reduction that Cruden suggests would be expected with such a change in the breeding system (Cruden 1977), and pollen production is well above the level needed to produce some pseudogamous apomictic seed. This may be selected for by the need to continue to attract pollinating insects, and by the reproductive success that paternity confers. Pollinators are required for selfing these flowers, as well as for transporting pollen to and from unrelated plants for what we hypothesize is a significant rate of out-crossing whenever a megagametophyte is fertilized.

Since we have not yet encountered any groups where we can demonstrate Camp's (1942) model in operation with diploid hybrids subsequently giving rise to triploid apomicts, we would tentatively amend his model by suggesting that triploid first-generation hybrids from diploid parents may prove to be at least as important as diploid hybrids. In subsequent generations the triploids that derive from diploid–tetraploid crosses may also figure in gene transfer. Selection for apomixis in both triploids and tetraploids might be particularly strong forces in this genus.

Further research on polyploidy

Because it provides such a wealth of groups in which it seems that parallel events may have occurred relatively recently, *Crataegus* (possibly including *Mespilus*) may be one of the more promising genera in which to investigate the origins of polyploidy and of apomixis. A logical next step in research would be to derive a phylogeny of the known diploids in the group, which will likely not prove as difficult a task as Camp's (1942) original model with rampant diploid hybridization would have implied. Although we are inclined to think that recent authors are refining the groupings within *Crataegus* in such a way that most series will eventually prove to contain diploid members, this might not be true of some "hybrid polyploid series" that may be complicating the overall picture of the genus (possible examples are series *Pruinosae* and *Tenuifoliae*).

To investigate allopolyploidy, it seems wise not just to rely on phylogenetic reconstructions but also to study some species groups in depth. Unfortunately for the task of finding likely study groups, taxonomic authors rarely include hints about the degree of morphological variation within a group. Some hints that variation exists might be found where later authors have grouped species names as taxonomic synonyms, but there are many other reasons for synonymy, some of which result from the sheer volume of names within these genera. Variety descriptions may be a more fruitful source of clues about morphological variation than synonymy would be.

It is to be hoped that we will soon have a complete picture of the relationship between *Mespilus* and *Crataegus* and the other Maloideae, and of the genetic relationships between the major groups of hawthorn taxa, so that characters such as apomixis can be placed in an evolutionary context. Is it true that only polyploids are apomictic? Are there "culprit" species that have hybridized with many others and spread apomixis and polyploidy through the hawthorn taxa of two continents? Does the reduced stamen number of many American tetraploids correlate with a shared ancestry or will we see it as the result of a slight relaxation in selection for pollen production in apomictic plants with self-compatible pollen (Dickinson et al. 1996)? The research questions in these plants are of great theoretical interest and flow cytometry will be a fundamental tool in what promises to be a much more satisfying and efficient attack on "the *Crataegus* problem" than has ever before been possible.

Acknowledgements

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