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Collection, Description, Taxonomic Relationships, Fruit Biochemistry, and Utilization of Aronia melanocarpa, A. arbutifolia, A. prunifolia, and A. mitschurinii

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Collection, Description, Taxonomic Relationships, Fruit Biochemistry, and Utilization of *Aronia melanocarpa*, *A. arbutifolia*, *A. prunifolia*, and *A. mitschurinii*.

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Abstract. *Aronia* (Rosaceae) or chokeberry is a genus of deciduous shrubs originally native to eastern North America. The taxonomy of this genus is complex and species boundaries are not clear. The genus is thought to be composed of the wild *A. melanocarpa*, *A. prunifolia*, and *A. arbutifolia*, with an additional cultivated taxon, *A. mitschurinii*, originating from the former Soviet Union. *Aronia* is grown as an ornamental and for fruit. Over 100 accessions of *Aronia* were obtained from cultivated sources, germplasm repositories, and wild collection. Three studies were conducted using this plant material.

The first study revealed *A. melanocarpa*, *A. prunifolia*, and *A. arbutifolia*, are distinct from each other and from *A. mitschurinii*. Significant differences in morphological values were found. Accessions of the same species clustered together using unweighted pair group method with arithmetic mean. Geographic origin, ploidy, and morphology used in principal coordinate analysis also supported these *Aronia* species as distinct.

A second study examined biochemically active beneficial compounds found in wild *Aronia* in comparison to *A. mitschurinii*. This first major analysis of wild type

Aronia showed that *A. mitschurinii* generally has the lowest antioxidant (ORAC) value and has about the same or slightly lower total anthocyanin amounts compared to *A. melanocarpa*. Harvest date affected fruit biochemical composition. Later harvested fruit showed higher anthocyanin levels. *Aronia prunifolia* and *A. arbutifolia*, though low in anthocyanin, were found to have the highest phenolic compound levels. *Aronia melanocarpa*, both tetraploid and diploid, in some cases showed higher ORAC, anthocyanin, or phenolic compounds, and had different amounts of individual anthocyanins than *A. mitschurinii* types. These findings reveal that wild *Aronia* species all have potentially useful biologically active compounds.

The last study of this dissertation explored the potential gene pool available to *Aronia* breeders. Seven hundred pollinations were conducted between *Aronia*, *Sorbus*, *Pyrus*, and ×*Sorbaronia*. Compatibility was found between diploid *Aronia*, *Sorbus* and ×*Sorbaronia* resulting in three and four species intergeneric combinations. Backcrosses of ×*Sorbaronia* to both *Aronia* and *Sorbus* were easily accomplished. Some compatibility was found between *Pyrus* and *Aronia*, this intergeneric combination has not been reported previously.

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Collection, Description, Taxonomic Relationships, Fruit Biochemistry, and Utilization of *Aronia melanocarpa*, *A. arbutifolia*, *A. prunifolia*, and *A. mitschurinii*.

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TABLE OF CONTENTS

LIST OF TABLES

Chapter 1. Morphological, Geographical, and Cytological Differentiation of *Aronia* taxa with special focus on the origin of *A. prunifolia*

Chapter 2. Beneficial Biologically Active Compounds in *Aronia*

Chapter 3. Interspecific and Intergeneric Hybridization as Pathways to Improved *Aronia* Cultivars

LIST OF FIGURES

Chapter 1. Morphological, Geographical, and Cytological Differentiation of *Aronia* taxa with special focus on the origin of *A. prunifolia*

Chapter 3. Interspecific and Intergeneric Hybridization as Pathways to Improved *Aronia* Cultivars

General Introduction

Black, red, and purple chokeberries -- *Aronia melanocarpa* (Michx.) Elliot , *A. arbutifolia* (L.) Pers., and *A. prunifolia* (Marshall) Rehder respectively, are all native eastern North American deciduous shrubs of the Rose family (Rosaceae) (Fernald, 1950; Gleason and Cronquist, 1991; and Hardin, 1973). The genus *Aronia* belongs to the subfamily Maloideae, (subtribe Pyrinae) that is rife with taxonomic difficulties (Campbell et al., 2007; Potter et al., 2007; Robertson et al., 1991). This group contains approximately 950 species in 30 genera including *Sorbus* (mountain ash), *Malus* (apple), *Pyrus* (pear), *Amelanchier* (shad bush), *Crataegus* (hawthorn) and several other woody plants with pomes or apple like fruits (Campbell et al., 2007; Gleason, 1991; Robertson et al.,1991; Evans and Campbell, 2002). The phylogeny of the Pyrinae is not well resolved; it appears to have evolved from the same common ancestor as the genus *Gillenia* an herbaceous Spiraeoid. This divergence was followed by cladogenesis early in the history of the group. The radiation was perhaps complicated by a reticulating relationship through intergeneric hybridization (Campbell et al., 2007).

Hybridization between species within genera of Maloideae is not uncommon, along with polyploidy, and apogamy (Campbell et al., 2007; Persson at al., 2004). This complex of phenomena results in complicated reproductive systems that mix sexual and apogamous reproduction, allowing for hybrids and triploids to disperse via non-sexual seed. These apomictic entities behave, in an evolutionary context that is similar to species. The base number of chromosomes for the tribe is n=17 (Sax, 1929; Postman, 2011), diploids being 2n=34 and tetraploids 4n=68 (Darlington and Janaki, 1945; Persson et al., 2004; Brand, 2010; Leonard, 2011). Tetraploids in *Aronia* as a rule reproduce

apogamously and diploids seem to reproduce sexually. Taxa in the Pyrinae tend not to have more than four sets of chromosomes and aneuploidy is rare (Campbell and Berquist, 1987). Sixteen genera are reported to be involved with intergeneric crosses in the Pyrinae (Postman, 2011). Some are known from the wild such as ×*Sorbaronia* (*Sorbus* × *Aronia*) and ×*Amelasorbus* (*Amelanchier* × *Sorbus*), (Connolly, 2009; Haines and Vining, 1998; Haines, 2011; Postman, 2011). Many others are only known from cultivation, e.g. ×*Sorbopyrus* (*Sorbus* × *Pyrus*), ×*Sorbocotoneaster* (*Sorbus*× *Cotoneaster*), and ×*Sorbocrataegus* (*Sorbus*× *Crataegus*) (Facciola, 1990; Postman, 2011; Sax, 1929). Abundant seed set has been observed in ×*Sorbaronia* along with normal chromosome pairing (Sax, 1929), indicating that a high degree of true sexually produced seed also is possible in crosses between genera.

Aronia flowers are borne in a corymb with pentamerous organs, with five sepals and petals, anthers usually 20 in multiple whorls of five, a single pistil of five fused carpels and five free styles (Krussman, 1986; Gleason and Cronquist, 1991; Haines, 2011; Leonard, 2011). Flowers are protogynous, they are self-compatible, and in the case of apomictic polyploids, they are pseudogamous with perhaps some weak parthenocarpy (Hardin, 1973; Campbell and Berquist, 1987; Leonard, 2011). Apogamy is aposporous producing an unreduced megagametophyte (Campbell et al., 1991).

The taxonomic history of the genus is complex; the species currently in *Aronia* have been placed in six other genera at various times. *Aronia* has been included in *Mespilus* (Linnaeus, 1753), *Pyrus* (Linnaeus, 1782), *Crataegus* (Lamarck, 1783), *Sorbus* (Heinhold, 1841) and *Adenorachis* (Nieuwland, 1915). Recently Robertson et al. (1991) placed *Aronia* within the genus *Photinia* due to similarity in floral morphology, but

Campbell et al. (2007) showed there was little molecular genetic affinity between the two genera. Additionally, it has been pointed out that the name *Aronia* is older than *Photinia* and therefore if the two genera were to be merged it should be under the name *Aronia* (Haines, 2011). Therefore, *Aronia* will be used in all the text following. These name changes though do make one wonder if some of the intergeneric hybridization is really just a case of semantics. One taxonomic issue within the genus is the status of purple chokeberry. It has been considered a hybrid *Aronia* ×*prunifolia* (Marshall) Rehder, and described as a species under three different names: *Aronia atropurpurea* Britton, *A. floribunda* (Lindl.) Spach, and *A. prunifolia* (Marshall) Rehder (USDA, 2009; International Plant Names Index, 2012). Additionally, this taxon has also been called a variety of red chokeberry *Aronia arbutifolia* (L.) Pers. var. *atropurpurea* (Britton) Seymour (USDA, 2009)

Aronia mitschurinii (A.K. Skvortsov and Maitul.), a cultivated species derived from hybridization of *A. melanocarpa* and *Sorbus aucuparia* L., has been selected for larger fruit and is used extensively in Scandinavia and Eastern Europe as a wine, jam, and juice crop (Jeppsson, 2000; Gasiorowski et al., 1997). *Aronia* propagation for commercial production can be from seed, cuttings (Dirr, 2009), or tissue culture (Brand and Cullina, 1992). It is considered to be essentially pest free (Robert and Skirvin, 2007), though it is likely susceptible to several minor diseases of the Rosaceae, and ring spot virus has been an issue in some regions (Bremer, 1984). Antioxidants are credited with cancer prevention, diminishing age related memory loss, and better coronary health (Kokotkiewicz et al. 2010). *Aronia mitschurinii* contains very high levels of antioxidants, in the form of anthocyanin and proanthocyanidin (Benvenuti et al., 2004; Gasiorowski et

al., 1997; Wu et al., 2004), and products processed from this fruit are increasingly sold as 'nutraceuticals'. *Aronia* has the highest known Oxygen Radical Absorbance Capacity, a method of measuring antioxidant capacity of any fruit (Zheng and Wang, 2003). Previous work has been mostly on the improved black chokeberry cultivars of *A. mitschurinii*; only a limited amount of work has been done assessing the antioxidant capacity of wild black and purple *Aronia*.

This dissertation is comprised of three studies 1) understanding the morphological, geographical, and cytological differentiation of *Aronia* taxa, with special focus on the origin of tetraploid *A. prunifolia* and *A. mitschurinii*, 2) exploring wild *Aronia* germplasm for beneficial biologically active compounds, 3) finding and using germplasm for future *Aronia* improvement.

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Chapter 1

Morphological, Geographical, and Cytological Differentiation of *Aronia* taxa with special focus on the origin of *A. prunifolia*

INTRODUCTION

The genus *Aronia* belongs to the Rosaceae subfamily Maloideae, also known as subtribe Pyrinae. The Pyrinae is rife with taxonomic difficulties (Robertson et al., 1991; Campbell et al., 2007; Potter et al., 2007). Hybridization in this subtribe between species within genera is common, along with polyploidy, and apogamy (Persson et al., 2004; Campbell, 2007). This results in complex reproductive strategies that mix sexual and apogamous reproduction, allowing for hybrids and triploids to establish and disperse via non-sexual seed in ways that are analogous to species. Taxa in the Pyrinae tend to have four sets of chromosomes or fewer, and aneuploidy is rare (Dickson, 1986; Campbell, 1987). The chokeberries or *Aronia* species, like other genera of the Pyrinae often do not conform to the common concept of a biological species. The biological species concept is based on reproductive isolation between taxonomic units (Judd et al., 2002). In the case of apogamous or agamic complexes, morphologically divergent taxa can cross giving rise to unique forms. These forms can subsequently propagate through asexual seed. Though these forms are generally sexually sterile, they may occasionally backcross to their parent taxa or produce a rare sexual seedling giving rise to additional unique forms that can also reproduce apogamously.

The focus of this study is to investigate the relationships of wild North American *Aronia* to each other, and to large-fruited cultivated forms from Europe. Special focus will be given to the origin of purple chokeberry, *A. prunifolia*.

Black, red, and purple chokeberries, *Aronia melanocarpa* (Michx.) Elliot , *A. arbutifolia* (L.) Pers., and *A. prunifolia* (Marshall) Rehder respectively, are all native eastern North American deciduous rhizomatous shrubs of the Rose family (Rosaceae) (Fernald, 1950; Hardin, 1973; Gleason and Cronquist, 1991). Collectively the three *Aronia* entities have been documented from 33 United States (AL, AR, CT, DC, DE, FL, GA, IA, IL, IN, KY, LA, MA, MD, ME, MI, MN, MO, MS, NC, NH, NJ, NY, OH, OK, PA, RI, SC, TN, TX, VA, VT, WI, WV), the genus also ranges into Canada and can be found in the provinces of LB, NB, NF, NS, ON, PE, and QC (USDA, 2009).

There is considerable overlap in the geographic range and morphology of these taxa making positive identification difficult, but there are generalities that allow some distinctions. *Aronia melanocarpa* is more northern, ranging from Southern Canada to the northern mountains of Georgia west to Missouri and Iowa. This species is ecologically adaptable and can be found growing in bogs, swamps, woodlands, beach dunes and rock outcrops. *Aronia melanocarpa* can be separated morphologically from *A. arbutifolia* by its ovate to obovate glabrous leaves, shorter stature (<2 meters with dwarf forms known), and sepals that lack glands (Gleason and Cronquist, 1991). The juicy black fruits of *A. melanocarpa* are rounder and larger then its congener's, ranging 6-9 mm in diameter, and ripen from late July to mid-August. The fruits of *A. melanocarpa* are not persistent through late fall and winter.

In contrast *A. arbutifolia* tends to be a southern U.S. and coastal plain species absent from the Midwest, inhabiting a range from coastal southeastern Canada to Florida and Texas (USDA, 2009). *Aronia arbutifolia* almost exclusively grows in bogs and swamps. *Aronia arbutifolia* is pubescent on the stems, leaves, and pedicels (Hardin,

1973; Krussmann, 1986). *Aronia arbutifolia* is taller than black chokeberry reaching five meters, with narrow elliptical/lanceolate to narrowly ovate or obovate leaves. The fruits of *A. arbutifolia* are smaller than those of *A. melanocarpa*, pulpy rather then juicy, and ripen in October and November with the bright red pomes persisting into the winter (Gleason and Cronquist, 1991; Rosell and Kesgen, 2003; Dirr, 2009). Both *A. melanocarpa* and *A. arbutifolia* are cited as diploid, at 2n=34 (Gleason and Cronquist, 1991). Persson et al. (2004) recently documented triploid and tetraploid accessions, but did not morphologically identify plants, so ploidy could not be associated with species.

Hardin (1973) demonstrated that *A. melanocarpa* and *A. arbutifolia* can be cross pollinated and result in fruit set, though resulting seeds were not tested for viability, germination, or subsequent plant vigor. Persson et al. (2004) also used Random Amplified Polymorphic DNA (RAPDs) to document lack of genetic variation in the offspring of tetraploid *Aronia*, but found diploid plants had genetically variable offspring that were both triploid and diploid. These results suggest tetraploid apogamy or asexual reproduction. Apogamy is a syndrome where a sporophyte embryo is produced from a gametophyte or maternal tissue without fertilization resulting in clonal plants produced from seeds. Apogamy can result in preservation and propagation of hybrid entities through asexual means even if plants are sexually sterile or possess unbalanced ploidy levels. Through apogamy, hybrid entities can exist indefinitely and expand beyond the geographic range of the parental species.

Aronia prunifolia or the purple chokeberry is an enigmatic third taxon. It is generally intermediate in characteristics between *A. arbutifolia* and *A. melanocarpa*. The nomenclature of purple chokeberry is unclear. It has been considered a hybrid *Aronia*

×*prunifolia* (Marshall) Rehder (pro sp.) (Hardin,1973; Gleason and Cronquist, 1991), but has also been described as a species under three different names: *Aronia atropurpurea* Britton, *A. floribunda* (Lindl.) Spach, and *A. prunifolia* (Marshall) Rehder. Additionally, purple chokeberry has also been considered a variety of red chokeberry *A. arbutifolia* var. *atropurpurea* (Britton) Seymour (USDA, 2009). In my studies (Connolly unpubl.), *A. prunifolia*, this possible hybrid, tends to be found in wetlands similar to *A. arbutifolia*. The reported range of *A. prunifolia* essentially mirrors that of *A. melanocarpa*, though it is absent from the far western edge of that distribution (USDA, 2009). The existence of *A. prunifolia* has caused considerable taxonomic confusion for some field botanists, leading them to believe that *Aronia* taxa are difficult to identify, and to the hypothesis that the *Aronia* complex constitutes an example of introgressive hybridization (Jenkins et al., 2008). For simplicity, purple chokeberry will be referred to as *A. prunifolia* for this study. As early as 1991, Gleason and Cronquist's manual suggest that *A. prunifolia* is at least a partially stabilized hybrid entity and it is likely to be apogamous and polyploid. Apogamous fruit set in *A. prunifolia* has only been demonstrated once with very limited data (Hardin, 1973), and seeds were not planted to test their viability or to examine the variation among seedlings or the relationships between the seedlings and the maternal plant.

Cultivated, dark-fruited *Aronia* plants from Eastern Europe further confound the taxonomic picture. Plants are sold as *A. melanocarpa* in the trade, but are morphologically distinct with larger, rounder leaves, larger diameter stems, a non-rhizomatous habit, and very large fruits (Kask, 1987; Brand, 2010; Leonard, 2011). The cultivated entities were developed by the Soviet plant breeder Ivan Michurin by

hybridizing *A. melanocarpa* with Eurasian *Sorbus aucuparia* (Kask, 1987; Leonard, 2011). The intergeneric cross of these species has been named ×*Sorbaronia fallax* (C. K. Schneid.) C. K. Schneid (USDA, 2009). The first generation hybrid of *A. melanocarpa* and *S. aucuparia* has deeply lobed to irregularly compound leaves and ripe fruits that are maroon in color (Connolly unpubl.). The large-fruited forms of *Aronia* are likely F_2 progeny of ×*Sorbaronia fallax* or backcrosses of the F_1 to *A. melanocarpa* (Leonard, 2011). The leaves of the large-fruited types are bigger and more orbicular than typical *A. melanocarpa* and can show an occasional lobe, but are generally simple. These forms developed by Michurin have been described as a fourth species, *A. mitschurinii* (Skvortsov and Maitulina, 1982). *Aronia mitschurinii* will be the name used for these large-fruited types for this study.

As a result of the complexity in the genus *Aronia*, numerical taxonomy in the form of univariate and multivariate statistical analysis will be applied to sorting out the variation in the wild and cultivated *Aronia*. These methods have been useful in separating taxa in other Pyrinae genera, specifically *Amelanchier*, *Sorbus*, and *Crataegus* that have diploid sexual species as well as apogamous complexes (Dickson, 1986; Campbell and Wright, 1996; and Aldasoro et al., 1998). In this work I use geographic distribution, ploidy determination, and a common garden study of phenology and morphology to determine if the three wild *Aronia* entities form a continuous interbreeding gene pool that collectively contains diploids, triploids, and tetraploids, with continuous overlapping morphological features, or if they constitute separable distinct entities. The cultivated entities originating from Eastern Europe will also be analyzed morphologically to determine if they are a distinct taxon, different from the native species. Because the

hierarchical rank and relationship of these entities is not known I will call them operational taxonomic units OTUs for analysis of the experimental results.

An additional hypothesis that will be investigated in-depth is that *Aronia prunifolia* is of hybrid origin, resulting from crosses between *Aronia arbutifolia* and *A. melanocarpa*. It is believed that this taxon is clearly identifiable and at least partially reproductively isolated from both parents. Scenarios will be developed about the formation of this species/entity using evidence from biogeographical, cross pollination, flow cytometry, reproductive output levels, flowering time, and morphological analysis.

METHODS

Sampling

Sampling sites were located using herbarium specimens, literature-based data, and field surveys. Dunes, pitch pine forest, rock outcrops, heath barrens, swamps, and sphagnum bogs were studied intensively as they are known *Aronia* habitat. As a result, these habitats were targeted for field study. Accessions in addition to the ones Connolly or Brand collected were obtained from United States Department of Agriculture Germplasm Resources Information Network (http://www.ars-grin.gov/). Accessions were also obtained from colleagues in distant parts of the *Aronia* range. Initial assignment of names was carried out using the keys from Fernald (1950), Gleason and Cronquist (1991), and Haines (2011).

Ploidy Determination

Flow cytometry is an automated way to evaluate plant ploidy. A modified version of the protocol in Arumuganathan and Earle (1991) summarized in Lehrer et al. (2008) was followed. Briefly, the analysis depends on using a standard plant with a known cytotype (e.g., a diploid is compared with an unknown of the same or a closely related species). Two to three newly emerged leaves were macerated using a fresh razor blade in nuclei suspending solution in a 55mm petri dish on a freeze pack. The methods were then modified in accordance with Meng and Finn (1999) by adding 2 g of PVP-10 per 50 ml of extraction buffer and fluorescently staining released nuclei after filtering with propidium iodine, instead of during maceration. Relative fluorescence of total DNA was measured using a Becton-Dickson FACS Calibur Dual Laser Flow Cytometer (Becton, Dickson and Co., Franklin Lakes, NJ) at the Flow Cytometry and Confocal Imaging Facility at the University of Connecticut in Storrs, CT. The cytometer was equipped with an Argon ion laser emitting radiation at 488 nm. For each sample, 10,000-20,000 particles were measured. Data were logged and displayed in histograms by BD Cellquest ^{TM} software (Becton, Dickson and Co., Franklin Lakes, NJ). Standard tetraploid and diploid sample histogram peaks were compared to samples of unknown ploidy. Peaks of the unknowns could be compared to the standards and categorized as diploid or tetraploid. If fluorescent peaks were intermediate in value, the sample was determined to be triploid. Any plants with ambiguous peaks were run multiple times and compared to several knowns until a clear ploidy level could be determined.

Mapping

Accession collection location was recorded using a Garmin™ 62 hand-held GPS unit (Garmin Ltd. Olathe, KS), or latitude and longitude later estimated using aerial imagery from Google™ Earth (Google Inc. Mountain View, CA). Associated species, soil moisture, and ecological setting were recorded. Latitude and longitude were converted to decimal degrees and locations were mapped using the ARCGIS® 10 program (ESRI, Inc. Redlands, CA).

Growth and Field Establishment

Populations were sampled by collecting fruit, soft wood cuttings, or by dividing out small vegetative shoots from wild parental plants. I preferred collecting fruits because they were more likely to give a higher degree of genetic diversity. The *Aronia* fruits were harvested, fermented in water until soft, crushed, and the seeds washed and dried. Germination methods were according to Leonard (2011) as follows: in 2008, 2009, 2010, and 2011 seeds were cold stratified in moist sand for 90 days in 50ml conical centrifuge tubes or polyethylene bags at 5ºC. After 90 days of stratification, seeds were germinated in potting medium with a ratio of 5:3:1 composted bark mulch, sphagnum peat moss, and sand that had been sifted using .5 cm hardware cloth screen. The woody plant growth media and seeds were placed in 32 oz. clear plastic salad trays with dome lids. The environmental conditions were approximately 24ºC with cool white fluorescent light (40 µmol·m-2·s-1). Seedlings were transferred to standard 50 cells per flat plug trays with the same 5:3:1 mix, and placed in a heated (18ºC-27ºC) glass greenhouse.

Small vegetative shoots, which were composed of an above ground portion of stem with leaves, rhizome, and roots, were separated from large shrubs in the field, placed in plastic bags with moist paper towels and transported to the UConn Floriculture greenhouses. The shoots were planted in the bark mulch, sphagnum peat moss, and sand media and kept under intermittent mist for 1-2 weeks, prior to being placed in a greenhouse.

Soft wood cuttings were taken if seeds and small vegetative offshoots were not available. Portions of shoot 15 cm long were cut from plants in the field and placed in plastic bags with moist paper towels and transported to the UConn Floriculture greenhouses. Soft wood cuttings were wounded at the bottom and for 1 cm along one side then dipped in Hormex #2 powder (Brooker Chemical Corporation Chatsworth, CA) or Hormodin #2 (3000 pm indole-3-butyric acid IBA, OHP, Inc.Mainland, PA) and stuck in the 5:3:1 media then placed under intermittent mist for approximately a month or until firmly rooted.

After plants reached a height of approximately 15-20 cm, they were transplanted into Jumbo Junior 4.5" pots with the same potting medium. When seedlings, vegetative shoots, and soft wood cuttings reached 45 cm they were moved into 2 gallon pots. Pots were placed outside in May, put on trickle irrigation and fertilized with slow release Osmocote® (formulation N-P_20_5- K_2O 17-6-10, 8-9 month formulation).

In late October, all the plants were moved to an unheated milky plastic covered hoop house for the winter. The following spring plants were established in the field at the University of Connecticut Plant Science Research and Education Facility located in Storrs, CT, USA 41º47'40.26"N 72º13'39.61"W, USDA plant hardiness zone 6a (USDA

Plant Hardiness Zone Map, 2012). Soil series for the planting plot is either Woodbridge fine sandy loam or Paxton and Montauk fine sandy loams (Soil Survey Staff NRCS, 2012). Three replicates of each accession were laid out in a completely randomized design. Plants were placed in rows with 2m within row spacing and 3m between row spacing.

Morphological Data Collection

In 2010 and 2011, morphological and phenological characteristics were recorded from late April until November. Phenological characters, e.g. peak flowering date and fruit ripe date, were observed weekly or bi-weekly. Height, width, and base stem diameter was measured for each replicated plant of each accession. The following leaf characteristics were recorded: leaf pubescence, new stem growth pubescence, phyllotaxy. Leaf pubescence and new growth pubescence was rated on a scale of 0-6; 0 having no pubescence, 1 slight pubescence, 2 slight-moderate, 3 moderate, 4 moderate-dense, 5 dense, and 6 being very densely pubescent. Phyllotaxy was scored as distichous for two-ranked foliar arrangement or polystichous for spirally displayed leaves. Leaf perimeter, leaf width, leaf factor (ratio of leaf area to the perimeter), leaf ratio (ratio of leaf length to width), and leaf length were measured for 5 leaves, at least 3 nodes from the branch tip, per replicated plant with a CI-203 Laser Area Meter (manufactured by CID, Inc. Vancouver, Washington State, USA).The following fruit and flowering characteristics were measured or recorded: inflorescence number per 30 cm of branch; flower diameter; peak flowering date; ripe fruit color; fruit ripe date; ripe fruit length and width; the weight of 25 ripe fruits.

Due to ongoing collection of accessions, plants were in various stages of maturity and the number of accessions per species or OTU varied depending on the size and stage of the plant. For example, plants that were too young to be reproductive were left out of flowering and fruiting analyses, but were included in mapping and ploidy analysis. Table 2 shows accessions used for each analysis.

Univariate Character Analysis

Plant morphological and phenological characteristics were analyzed using SAS for Windows 9.3 (SAS Institute Inc., Cary, N.C.). An analysis of variance using the PROC GLM function was used to compare the mean difference between *Aronia arbutifolia*, *A. melanocarpa*, *A. prunifolia*, and *A. mitschurinii*. Least square (LS) means were used to separate mean values ($p < 0.05$).

Phenogram Construction and Principal Coordinates Analysis

Data collected on leaf pubescence, stem pubescence, fruit weight per 25 g, fruit color, phyllotaxy, plant height, inflorescences per 30 cm of stem, leaf area and fruit ripe date were converted into qualitative discrete traits and placed in a matrix. Traits were categorized as follows: leaf pubescence, 0-2 low, 2.1-3.7 moderate, 3.8-6.0 heavy; stem pubescence, 0-3.4 low, 3.5-4.0 moderate, 4.1-6.0 heavy; fruit weight per 25 in g, 4.5-9.9 small, 10.0-17.1 medium, 17.2-26.6 large; fruit color, black, purple, light purple, and red; phyllotaxy, distichous or polystichous; plant height in cm, 24-99 short, 100-114 medium, 115-204 tall; inflorescences per 30 cm of stem, 1.5-3.3 few, 3.4-12.0 medium, 12.1-19.4 many; leaf area cm^2, 6.9-9.7 small, 9.8-16.8 medium, 16.9-33 large; and fruit ripe in days

from July 1st , 25-43 very early, 44-50 early, 51-73 mid, 74-103 late, 104-120 very late. Similarity matrices were constructed using the SIMQUAL function in NTSYSpc 2.21 software (Exeter Software, Setauket, NY, USA)(Rohlf, 2005). Phenograms were constructed in NTSYSpc using an unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis. Bootstrap values were computed using Winboot (Yap and Nelson, 1996), n=100 replicates.

Principal coordinates analysis was performed using NTSYSpc 2.21. Data were transformed using the DCENT function, eigen vectors were then extracted using the Eigen ordination function, and the scatter plots projected using the graphic function MXPLOT for a 2 dimensional display or MOD3D for 3 dimensional display.

Cross Pollination to Test for Apogamy

In spring of 2007, mature flowering plants conforming clearly to *A. arbutifolia* and *A. melanocarpa* morphologically were reciprocally cross-pollinated. *Aronia arbutifolia* was represented by the commercial cultivars 'Brilliantissima'(UC001) and 'Erecta'(UC021). *Aronia melanocarpa* types that were used in these experiments were the cultivars 'Morton' Iroquois Beauty ™ (UC004), 'Autumn Magic' (UC005), 'Viking' *A. mitschurinii* (UC003), and a wild accession from Maine (UC012). *Aronia* is protogynous (Hardin, 1973) and anthers are pink prior to pollen dehiscence, yellow post dehiscence, and turn from yellow to brown as they wither and pollen is no longer available. Pollinations were done in a pollinator-free greenhouse. Flowers on the maternal plants were emasculated while flowers still had pink anthers, fresh yellow pollen coated-stamens were removed from the paternal plants and pollen was deposited

on the emasculated maternal flower stigmas. Pedicels of pollinated flowers were marked with paper tags. Fruits were allowed to mature and were harvested when they reached ripeness. Seed extraction and seedling establishment followed the methods in the "Growth and Field Establishment" section. All seeds produced from these crosses were sown. When plants were approximately 50 cm tall, their leaf morphology, growth form, and pubescence levels were observed to determine if they were hybrids produced from true sexual reproduction or identical clones of the maternal plant produced by apomixis. Seedlings that appeared to be apogamous clones of the maternal plants were discarded.

Determination of Hybrid Origin for *A. prunifolia*

Seed of *A. melanocarpa* (UC012) × *A. arbutifolia* 'Brilliantissima'(UC001) were planted and established according to the "Growth and Field Establishment" methods. These plants were grown for three years and allowed to be open-pollinated. Second generation seeds from these plants were germinated and examined for variation to determine reproductive mode.

Reproductive output comparison for Aronia species

Fruit number per plant was counted for five *A. arbutifolia* accessions, five natural *A. prunifolia* accessions, and eight plants from the hand- made crosses described above (*A. melanocarpa* UC012 × *A. arbutifolia* 'Brilliantissima' that set fruit in 2011), fruit number per plant was not determined for diploid or tetraploid *A. melanocarpa* because fruits quickly fell off the plant after ripening and an accurate assessment for overall plant fruit set could not be made. Enough fruit could be gathered from *A. melanocarpa*

accession for germination experiments. Seeds per 100 fruits of five accessions of *A. melanocarpa* diploid, *A. melanocarpa* tetraploid, *A. arbutifolia*, and natural *A. prunifolia* were compared to the number of seeds per fruit for ten plants of *A. melanocarpa* UC012 × *A. arbutifolia* 'Brilliantissima'. Germination rate of 100 seeds were compared from: five accessions each of *A. melanocarpa* diploid, *A. melanocarpa* tetraploid, *A. arbutifolia*, natural *A. prunifolia,* and *A. melanocarpa* UC012 × *A. arbutifolia* 'Brilliantissima'. Seeds were cleaned, stratified, and germinated using the same method as in the "Growth and Field Establishment".

PROC GLM function was used to compare the mean difference, LS means was used to separate mean values, only p values of 0.05 or less were considered significant.

RESULTS

Sampling

Wild samples were collected from 105 locations (Table 1). In the northeastern U.S. in xeric habitats, it was observed that *Aronia* commonly occurs with low bush blueberry (*Vaccinium angustifolium*), bearberry (*Arctostaphylos uva-ursi*), bear or scrub oak (*Quercus ilicifolia*), and pitch pine (*Pinus rigida*). In wetter areas I have found that *Aronia* occurs with black gum (*Nyssa sylvatica*), leather leaf (*Chamaedaphne calyculata*), pitcher plants (*Sarracenia purpurea*), high bush blueberry (*Vaccinium corymbosum*), cranberries (*Vaccinium macrocarpon*), red maple (*Acer rubrum*), and Atlantic white cedar (*Chamaecyparis thyoides*). An additional 11 accession of wild origin were obtained from USDA GRIN (http://www.ars-grin.gov/npgs/index.html). These

samples totaled 19 *A. arbutifolia* accessions, 57 *A. melanocarpa* accessions, and 41 *A. prunifolia* accessions. Seven *A. mitschurinii* accessions were obtained from commercial sources and the USDA GRIN.

Ploidy

All 19 wild and three cultivar accessions of *A. arbutifolia* were shown to be tetraploid (see Table 1 for ploidy), contrary to the literature that reports this species as diploid. *Aronia melanocarpa* had the most diversity in cytotypes, 39 wild accessions and 1 cultivar ('Professor Ed' UC023) were found to be diploid. One wild triploid *A. melanocarpa* (UC 019) from Albany, NY was identified, and 13 wild and four cultivar accessions were found to be tetraploid. Some 40 *A. prunifolia* accessions were tetraploid, and one accession was found to be triploid (UC 011) from Damariscotta, ME**.** This triploid *A. prunifolia* from coastal Maine was found growing mixed with diploid *A. melanocarpa*. All seven accessions of *A. mitschurinii* were tetraploid. After observing the differences in ploidy levels in *A. melanocarpa,* it was split into two distinct OTUs for geographical and morphological analysis (Table 1).

Aronia melanocarpa was the only species that had significant variability in its ploidy level being diploid, triploid, and tetraploid. The cytotypes showed a very distinct pattern of distribution. Diploid black *A. melanocarpa* were only found in the northeastern United States north of the glacial maximum. Tetraploid *A. melanocarpa* were found throughout the Midwestern United States and into the southern Appalachian mountains. Only one tetraploid *A. melanocarpa* was found in New England and that was in Windsor, Connecticut (UC031). The one triploid black accession from Albany, New York (UC091)

was collected from the suture zone between the tetraploid and diploid ranges for *A. melanocarpa* (Figures 1-6). All 19 progeny produced from pollinating UC012 *A. melanocarpa* × UC 001 *A. arbutifolia* 'Brilliantissima' that had intermediate morphology were determined to all be triploid.

Mapping

One hundred fifteen accessions from eastern North America were mapped. There were 17 locations for *Aronia arbutifolia*, 57 for *A. melanocarpa*, and 41 for *A. prunifolia*. In the northeastern United States, diploid *A. melanocarpa* plants can commonly be found in proximity to *A. prunifolia.* A single location in Windsor, Connecticut had tetraploid *A. melanocarpa* and *A. prunifolia* growing together. In eastern Tennessee, tetraploid *A. melanocarpa* and *A. arbutifolia* overlap. Only one site in southeastern Connecticut, Pachaug State Forest, had all three species represented: *A. arbutifolia*, diploid *A. melanocarpa*, and *A. prunifolia*. *Aronia arbutifolia* showed a distinct pattern of distribution growing throughout the southeastern United States then along the Atlantic Coastal Plain in the northern part of its range. Plants that conformed to *A. arbutifolia* morphology were collected from southeastern Connecticut to Florida and east Texas (Figure 1). North of Connecticut, *A. arbutifolia* was not found (Figure 2.) Red Chokeberry stays east of the Appalachians except for the southern portions of its range. Black Chokeberry *A. melanocarpa,* has nearly the inverse range. That is, it only occurs west of the Appalachians from Connecticut southward, and is common on the Atlantic Costal Plain only in Connecticut and northward. In our sampling, *A. prunifolia* was only common in southern New England and Long Island. A USDA collection of *A. prunifolia*

from the southern Appalachian Mtns. of Virginia was the only sample disjunct from the other members of this species (Figures 1-6).

Morphological Data Collection

Twenty one *Aronia arbutifolia* , 63 *A. melanocarpa*, and 41 *A. prunifolia*, and 7 *A. mitschurinii* cultivars were used to study morphology. Individual accession mean values for characteristics with standard error were calculated (Table 3a-f). A summary of the morphological attributes of each species follows.

Aronia mitschurinii stood out in the univariate characters: plants had the highest mean values for plant height, plant width, all leaf size characteristics, and all fruit characteristics (Table 4, 5, and 6). *Aronia mitschurinii* was distinct in reproductive characters: it had the fewest number of inflorescences per 30 cm, averaging 2.4 corymbs. Compared with other *Aronia* species, fruit color, fruit ripe date, and pubescence were not useful in separating this entity. All accessions of *A. mitschurinii* had black fruit. The new stem growth and leaves of *A. mitschurinii* had low to moderate pubescence and a moderately early fruit ripening date.

Aronia arbutifolia had the highest pubescence levels for leaves and new growth, with means of 4.9 and 5.1 respectively. Fruits of *A. arbutifolia* were bright red (with the exception of *A. arbutifolia* var. *purpurea* with maroon fruits) that remained red into winter. The species had very late fruit maturation beginning late in September with one accession not ripening until December. This fruit ripening date character is one of the major features distinguishing *A. arbutifolia* from the other species in the genus (Figure 7). *Aronia arbutifolia* has the second largest leaves (the largest found in *A. mitschurinii).*

The leaves of this species are larger on average in leaf area, leaf length, and leaf width than *A. prunifolia* and both *A. melanocarpa* ploidy levels. For example, leaf area of *A. arbutifolia* is 17.8 cm^2, while 4n *A. melanocarpa* is 16.4 cm^2, *A. prunifolia* 15.9 cm^2, and *A. melanocarpa* 2n 12.8 cm^2. *Aronia arbutifolia* and *A. mitschurinii* had the largest stem diameters: 1.91 cm and 2.2 cm respectively.

Aronia prunifolia is similar to *A. arbutifolia* in morphology, but was significantly separated from this species by earlier fruit ripening in *A. prunifolia* (September as opposed to October to December). Additionally, *A. prunifolia* fruit all eventually ripen to purple or black (as opposed to bright red fruit in *A. arbutifolia*), and it bears significantly shorter leaves, and has moderate stem pubescence.

Aronia melanocarpa is easily distinguished from *A. mitschurinii* by the much smaller leaves in *A. melanocarpa* (12.8 cm^2 leaf area compared to 28.5 cm^2 respectively). Generally, *A. melanocarpa* is smaller in stature than *A. mitschurinii*, (mean height of 92.5 cm in contrast to 142.8 cm respectively). The early (July to August) black ripening fruit of *A. melanocarpa* clearly separate *A. melanocarpa* from *A. arbutifolia* with its late September to December maturing bright red pomes. Additionally, all *A. melanocarpa* had a low pubescence score with a mean of 1.1 for both cytotypes, while *A. arbutifolia* had dense pubescence with an average score of 4.9. Discriminating *A. melanocarpa* from *A. prunifolia* can be difficult, but the higher pubescences levels, especially on new stem growth, are diagnostic: *A. prunifolia* has a mean score of 4.1, as opposed to 1.4 for diploid *A. melanocarpa* and 1.0 for tetraploid *A. melanocarpa*. *Aronia prunifolia* fruits ripened much later; August/September versus the end of July/early August for *A. melanocarpa*.

The two cytotypes of *A. melanocarpa* showed consistent distinct features unique to each of them. The tetraploid plants tended to be wider, a mean of 136.1 cm for tetraploids versus 92.5 cm for diploids. Additionally, tetraploid *A. melanocarpa* had distichous or two ranked foliar arrangement. The diploids were generally smaller in stature, width, and leaf size. For example, the average leaf area of diploid *A. melanocarpa* is 12.8 cm^2, leaf length 5.5 cm, leaf width 3.1 cm, and leaf perimeter 15.2 cm compared to tetraploid *A. melanocarpa* with: leaf 16.4 cm^2, leaf length 6.0 cm, leaf width 3.7 cm, and leaf perimeter 18.9 cm. Diploid *Aronia melanocarpa* has a polystichous leaf arrangement. Diploids had fewer inflorescences per 30 cm of branch; 9.5 on average in contrast to 13.5 for tetraploids. Diploid *A. melanocarpa* has a wide range of variation in habit; several dwarf plants and prostrate accessions were collected such as UC012, UC020, UC034, and UC054, that were less than 0 .5 m in height. The origin of these accessions is from dry rocky balds or xeric heath lands. In contrast, collections of diploid *Aronia melanocarpa* from wetlands (e.g., UC015 and UC016) are much taller, reaching well over 1 m. These wetland types were not only taller, but also had the earliest fruit ripe dates of any *Aronia* (Tables 4,5,6). No significant difference was seen between peak flowering dates, all taxa overlapped (figure 8).

Phenogram construction and principal coordinates analysis

The phenogram constructed using UPGMA supported the distinctiveness of all the hypothesized OTUs (Figure 9). Using European mountain ash *Sorbus aucuparia* as an out group, the first major split in the tree was between the *A. prunifolia*/*A. arbutifolia* groups and the *A. mitschurinii*/ *A. melanocarpa* diploid/ *A. melanocarpa* tetraploid group.

This division was well supported with a bootstap value of 79. The split between *A. prunifolia* and *A. arbutifolia* also had strong support with a bootstrap value of 74. All black fruited types clustered together, but *A. mitschurinii* was placed on a different branch than the combined cytotypes of *A. melanocarpa* with a bootstrap value of 65. Diploid and tetraploid *A. melanocarpa* were placed sister to each other in the phenogram, but were distinguished from each other with a bootstrap value of 58. Within diploid *A . melanocarpa*, dry habitat accessions and wetland collections did not separate clearly. But a few genotypes clustered closer to other plants of the same habitat type rather than with accessions of the other ecotype. For example accessions UC020 and UC012 from dry rocky balds grouped together; and also wetland types UC029, UC015, and UC016 are on the same short branch of the phenogram.

Principal coordinates analysis revealed a relationship similar to the phenogram (Figure 10). The outgroup taxon, *Sorbus aucuparia*, was removed from two projections (Figures 10 and 11) because its presence in the scatter plots reduced the resolution between the *Aronia* OTUs. The first two principal coordinates analyses place *A. prunifolia* and *A. arbutifolia* closest, but as distinct entities. The one exception is the Virginia accession (PI 603107) of *A. prunifolia* that clusters with *A. arbutifolia*. This plant was also geographically disjunct from all other *A. prunifolia* accessions. The diploid and tetraploid *A. melanocarpa* OTUs also clustered together again with *A. mitschurinii*. In the two dimensional plot, it appears that *A. mitschurinii* is intermediate between the diploid and tetraploid *A. melanocarpa* and is closer to them than they are to each other. When viewed in 3D (Figure 11), a large spatial gap between *A. mitschurinii* and the two cytotypes of *A. melanocarpa* is evident. The 3D projection is more congruent with the

phenogram in regard to the relationship between the *A. melanocarpa* cytotypes and *A. mitschurinii*. In one projection (Figure 12), the outgroup species *S. aucuparia* was added back to the principal coordinates analysis. This scatter plot projection reduces the general resolution but shows there is a closer affinity between *A. mitschurinii* and *S. aucuparia*, than between *S. aucuparia* and any other *Aronia* OTU.

Cross Pollination

Only diploid maternal plants produced variable sexual offspring (Table 7). The F1plants as a result of hand pollination from the cross of *A. melanocarpa* UC012 × *A. arbutifolia* 'Brilliantissima' had a similar level of pubescence, plant size, leaf area, fruit color as the wild *A. prunifolia*. The F1 fruits were slightly earlier ripening, and some individuals had smaller fruit size. When these F1 hybrids were included in phenogram and principal coordinates analyses, they clustered in all cases with *A. prunifolia* (figures 13, 14).

Reproductive Output Comparison

Wild tetraploid *A. prunifolia* had a larger number of fruits per plant, seeds per fruit, and germination rate than the pooled natural triploid UC011 *A. prunifolia* / artificial triploid *A. melanocarpa* UC012 × *A. arbutifolia* 'Brilliantissima', and *A. arbutifolia* (Table 8). A fertility index was calculated to estimate an average number of seedlings each plant of an OTU could produce. This fertility index was approximated by multiplying the mean number of (fruits per plant × seeds per fruit) × germination rate. Wild tetraploid *A. prunifolia* had a fertility index approximately seven times that of the

combined triploid wild *A. prunifolia* and triploid *A. melanocarpa* UC012 × *A. arbutifolia* 'Brilliantissima'. Additionally wild tetraploid *A. prunifolia* had a fertility rate over twice that of *A. arbutifolia*.

DISCUSSION

Hybridization and apogamy make plants of the Pyrinae difficult to assign to species or taxonomic rank. Of all *Aronia* taxa, only diploid *A. melanocarpa* fits the conventional definition of a biological species with fertility gaps between it and the other *Aronia* species i.e., a genetically isolated entity (Mayr and Ashlock, 1991; Futuyma, 1998; Judd et al., 2002). Tetraploid *A. melanocarpa* and *A. arbutifolia* are each clearly diagnosable from other *Aronia* taxa, do not appear to show morphological influence from other OTUs, and have distinct geographic ranges. Because these OTUs are tetraploid and there are minor morphological variations among accessions it is likely each of these polyploid forms of both species is a composite of several similar apogamous lineages or microspecies. Variation in highly apogamous lineages is generated by rare sexual reproduction or somatic mutations (Loveless and Hamrick 1984). Though there are minor differences among plants, there are more similarities that unite accessions. It could be argued that tetraploid *A. melanocarpa* and tetraploid *A. arbutifolia* are each distinct morphological species via the phenetic species concept that defines a species as a group of individuals that are morphologically distinct and have a gap in variation separating it from other groups of similar organisms (Sokal and Crovello 1970). That is, there are not necessarily any breeding barriers. The phenetic species concept has been commonly

used, appropriately given the difficulties in recognizing breeding, in apogamous lineages and agamic complexes (Bayer and Stebbins 1982; Dickinson and Phipps 1985; Dickinson 1986; Aldasoro et al. 1998; Dibble et al. 1998; McAllister 2005, Nybom and Bartish 2007). Because differences are very subtle between tetraploid and diploid *A. melanocarpa*, and there is no morphological evidence of influence from another *Aronia* or *Sorbus* entity, it is likely that the tetraploid is an autopolyploid. Thus, the *A. melanocarpa* tetraploids would be best treated as a morphologically distinct subspecific taxon rather than an entirely new species. *Aronia arbutifolia* is dramatically distinct from all other *Aronia* types; it can clearly stand as a morphological species due to its heavy stem and leaf pubescence in combination with late ripening red fruit. There is no evidence of *A. arbutifolia* being of hybrid origin; no candidate parental taxa exist. It is possible that *A. arbutifolia* could be an ancient hybrid with one or both parents now extinct. Currently it is clearly different from all extant *Aronia* taxa and deserves to be recognized as a species. Both *A. mitschurinii* and *A. prunifolia* type chokeberries are morphologically distinct and likely merit some sort of recognition, and, based on our present level of understanding, they are best treated as distinct species. It is always best to treat variants as distinct taxa, species in this case, until they are shown not to be distinct. The recognition calls attention to them and helps promote further work.

The key below is proposed as a way to diagnose the *Aronia* taxonomic entities.

Key to *Aronia* taxa

1a. Fruits ripening to bright red or rarely burgundy or maroon in late September through December

Aronia arbutifolia

1b. Fruits purple to purple black maturing late July through September

2a. Plants with large orbicular leaves greater than 7 cm long and 5 cm wide; non-suckering habit; and fruits larger than 10 mm in diameter.

Aronia mitschurinii

2b. Plants with leaves smaller than 7 cm long and 5 cm wide; leaf shape ovate, obovate, or elliptical; plant suckering; and fruits smaller than 10 mm in diameter

3a. Leaves at maturity covered with moderate to dense pubescence on underside

Aronia prunifolia

3b. Leaves at maturity glabrous or sub-glabrate on underside

4a. Leaves on new growth with distichous phyllotaxy; plants primarily of the Midwestern and Southeastern United States

Aronia melanocarpa **tetraploid**

4b. Leaves on new growth with polystichous phyllotaxy; plants of the Northeastern United States

Aronia melanocarpa **diploid**

4a. Dwarf plants often less than 0 .5 m in height at maturity of xeric habitats, fruits ripening early August

Aronia melanocarpa **diploid, xeric ecotype**

4b. Tall plants of wetlands 1-2+ m tall at maturity, fruits ripening late July

Aronia melanocarpa **diploid, wetland ecotype**

Taxon descriptions

Aronia mitschurinii

Cultivated robust plants with shoots arising from a confined crown, not highly rhizomatous and spreading; leaves large orbicular to sub-orbicular often slightly pubescent, large stipules sometimes present, seedling leaves and leaves on fast growing shoots occasionally show a single shallow lobe and pronounced irregular serrations. New growth also often somewhat pubescent, hairs tending to be partially upright and curled as opposed to appressed in other taxa, twigs stout; fruits large averaging 12 mm in diameter and 10 mm long, dark purple, ripening in August tending to remain on plant without withering for several weeks, with a highly indented distal end. This species is derived from hybridization of *A. melanocarpa* and the Eurasian *S. aucuparia*, plants tetraploid.

Aronia prunifolia

Plants tending to be more northern in distribution than *A. arbutifolia*, found primarily in New England, New York, and New Jersey. Plants generally inhabit wetlands; leaves moderately to occasionally highly pubescent on undersides with appressed straight hairs; fruits averaging 8-8.5mm in diameter, ripening in late August through September, dark purple or black when completely ripe often transitioning from green to maroon to black. This species is likely derived from hybridization of *Aronia melanocarpa* and *A. arbutifolia*, plants mostly tetraploid, rarely triploid.

Aronia arbutifolia

Plants of the Atlantic Coastal Plain north to Connecticut (and possibly Massachusetts) south to Florida and throughout the southeastern U.S., west to Texas. Plants frequently found in wetlands. Leaves are highly pubescent on undersides with appressed straight hairs. Fruits average 8-8.5mm in diameter, ripening to bright red (or rarely maroon) September to December. The maroon fruits may be derived from backcrossing with *A. prunifolia*. Tetraploid taxon with no known diploids.

***Aronia melanocarpa* tetraploid**

Plants are often more vigorous and robust than *A. melanocarpa* diploids. This cytotype found primarily west of the Appalachian Mountains in the Midwest and South, mostly absent from the Atlantic Coastal Plain. Plants can be found in xeric or wetland habitats. Leaves are glabrous on underside and tend to have distichous phyllotaxy. Fruits average 8mm in diameter, ripening directly from green to black in August. This cytotype likely an autopolyploid and apogamous form derived from diploid *A. melanocarpa*. This cytotype shows consistent differences from diploid plants, the two ploidy levels could arguably be different species. Splitting theses two entities needs further investigation, the type specimen of *A. melanocarpa* needs to be examined to determine if it is a diploid or tetraploid. Additionally, AFLP data (Samuel Obae unpublished) is showing that there may be two separate tetraploid entities, for a total of three potential wild black chokeberries species in North America.

Aronia melanocarpa diploid

Plants of the northeastern U.S. centered east of the Appalachian Mountains, not known west of the Appalachian Mountains or south of Pennsylvania. Plants of xeric habitats tending to be dwarf below 0.5 m or even prostrate with small leaves. Commonly associated with *Pinus rigida* and *Vaccinium angustifolium*; plants of shrub swamps and fen edges tending to be taller 1-2+ m with larger leaves, and have very early ripening fruits. Leaves of both types tend toward polystichous phyllotaxy, glabrous or occasionally slightly pubescent on undersides. Fruits average 7-7.5mm; wetland types ripening late July, plants of xeric habitats ripening slightly later in early August. Diploid types are known only from the Northeast, the two ecotypes have retained their morphology in common garden, though plants from mesic habitats form a morphological continuum between the two extreme xeric and hydric ecotypes.

Taxonomic status of hybrid derived lineages

Aronia mitschurinii and *A. prunifolia* are apogamously reproducing polyploid hybrids and do not fit cleanly into traditional Linnaean hierarchical taxonomic ranks. Following, I present arguments for and against assigning different ranks to these entities.

A. mitschurinii

All *A. mitschurinii* type accessions appear to be a single clone or a series of very closely related clones based on morphological appearance and also AFLP, ISSR, and RAPD data (Hovmalm et al., 2004; Celka and Szkudlarz, 2011; Leonard, 2011). This

species is morphologically distinct with larger fruit and leaves. This entity deserves recognition, at some level, as distinct from other black fruited *Aronia* types. *Aronia mitschurinii* in the phenogram forms its own distinct branch with a high bootstrap value separating it from the wild type *A. melanocarpa* plants. There are four possible levels it could be recognized at: 1) cultivar, 2) variety or subspecies, 3) hybrid, or 4) species. Currently in the United States it is generally sold as *A. melanocarpa* cv. 'Viking' or cv. 'Nero'; these two cultivars appear to be identical. Because this entity has been shown by genetic and historical sources to be derived from an interspecific hybrid of *A. melanocarpa* and *Sorbus aucuparia*, the use of cultivar seems to be an inadequate level of distinction. Variety, e.g. *A. melanocarpa* var. *mitschurinii* may be an appropriate descriptor for this entity because they show obvious differences in morphology from *A. melanocarpa*, form a distinct branch on the phenogram and cluster more closely with *A. melanocarpa* than either *Sorbus*, or the *A. prunifolia* or *A. arbutifolia* clades. Subspecies is generally a rank that has a geographic or ecological provenance associated with it, and therefore seems inappropriate for a single clone, especially one likely derived from a human-made intergeneric cross. Variety on the other hand has been used with other Rosaceae to describe a unique clone within the species e.g. *Prunus maritima* var. *gravesii* (Anderson, 1980). But there is a distinction in that *P. maritima* var. *gravesii* is not derived from an artificial intergeneric hybridization event. 'Hybrid' is another possible designation that may fit *A. mitschurinii*. This plant is a likely backcross of ×*Sorbaronia fallax* to *A. melanocarpa*. According to International Code of Botanical Nomenclature (Vienna Code) (McNeill et al., 2006) it would be a hybrid:

"H.4.1. When all the parent taxa can be postulated or are known, a nothotaxon is circumscribed so as **to include all individuals (as far as they can be recognized) derived from the crossing of representatives of the stated parent taxa (i.e. not only the F_1 but subsequent filial generations and also back-crosses and combinations of these).** There can thus be only one correct name corresponding to a particular hybrid formula; this is the earliest legitimate name (see Art. 6.3) in the appropriate rank (Art. H.5), and other names to which the same hybrid formula applies are synonyms of it."

Based on this portion of the ICBN, ×*Sorbaronia fallax* cv 'Viking' may be the best designation for this plant. However, from our cross pollination experiments (chapter 3), *A. mitschurinii* is essentially reproductively isolated from diploid *A. melanocarpa*. This argues that a species level recognition may be appropriate. *Aronia mitschurinii* was proposed in 1982 (Skvortsov and Maitulina, 1982). Synthetic crosses only known in cultivation, where fertility has been restored have been given species epithets e.g. *Solanum indianense* (Heiser et al., 2005). This *S. indianense* example appears to set the most similar precedent in treating "Viking" type *Aronia*; that is, an artificial hybridization event followed by an increase in ploidy that stabilizes the new forms. *Aronia mitschurinii* is best treated as a human derived polyploid species. The hybrid was likely a tetraploid *A. melanocarpa* and diploid Eurasian *S. aucuparia*. This would yield a triploid ×*Sorbaronia fallax*. Following that I would hypothesize that an unreduced triploid egg cell united with a 1n sperm cell to result in the 4n *A. mitschurinii*. The morphological stability of *A. mitschurinii* produced from seed (even though apogamous), its two stage origin

(hybridization followed by increased chromosome number), and its apparent reproductive isolation from *A. melanocarpa,* all support treatment as a species.

A. prunifolia taxonomic status

To summarize, *A. prunifolia* has a complex nomenclatural history. This entity has been considered a hybrid under two different names *A.* ×*prunifolia* and *A.* × *floribunda*. Additionally this entity has been described as a species under three different names: *Aronia atropurpurea*, *A. floribunda*, and *A. prunifolia*. And to further complicate matters, it has also been called a variety of red chokeberry *Aronia arbutifolia* (L.) Pers. var. *atropurpurea* (Britton) Seymour (USDA, 2009).

Our crossing research, morphological analysis, and RFLP information indicate that this entity is likely polyphyletic, originating several times. Persson et al. (2004) showed that diploid *A. melanocarpa* plants produced diploid and triploid progeny, very likely resulting from diploid *A. melanocarpa* plants commonly crossing with tetraploids. Additionally, our research here showed that hybrids between *A. arbutifolia* and diploid *A. melanocarpa* are very easy to synthesize. Furthermore, the flowering dates of all *Aronia* in the common garden research field shows that all entities overlap in flowering time. *Aronia*, like most other members of the Rosaceae, are subject to generalist pollination: flower morphology does not present barriers to pollinators that could isolate species. Geographically, our sampling showed that *A. prunifolia* is most numerous near where diploid *A. melanocarpa* and *A. arbutifolia* ranges overlap each other in southern New England. The obvious conclusion is that *A. prunifolia* is the product of hybridization. The confounding problem is that almost all wild *A. prunifolia* are tetraploid. The naturally

occurring tetraploid *A. prunifolia* may be a polyploid derivative of triploid hybrids and could thus be considered a distinct, polyploidy - stabilized natural hybrid species. The triploid accessions could be considered the results of an F1 hybrid. The F1 triploid hybrid could bear one of the hybrid names given to this taxon. Having a named sterile hybrid and a named fertile allopolyploid has precedence. The fern genus *Asplenium* hybridizes freely and allopolyploid species formation is common. *Asplenium* does contain one such pair: *A. platyneron* × *A. rhizophyllum* as a sterile F1 had been called *A.* ×*ebenoides* and the rare fertile allopolyploid from Hale county Alabama has recently been named *A. tutwilerae* (Keener and Davenport 2007; Haines 2011). The difficulty with applying this example to *Aronia* is that differences between the triploid hybrids and the tetraploid derivates are few if any, and cannot be distinguished morphologically.

In conclusion from our morphological, cytological, and geographic studies, it is clear that the OTUs *A. melanocarpa*, *A. prunifolia*, *A. arbutifolia*, and *A. mitschurinii* are all distinct from each other and should stand as species.

Origin of *A. prunifolia*

The vast majority of *A. prunifolia* samples obtained for this study were from southern New England and Long Island, New York. Though this species occurs in Virginia and probably elsewhere, *A. prunifolia* seems to be associated frequently with its probably maternal parent: the diploid form of *A. melanocarpa*. A likely biogeographical scenario is that diploid *A. melanocarpa* existed north of or approximately equal to the glacial maximum on offshore sands. These sands were exposed due to low ocean levels caused by large amounts of the world's water being locked up in the glaciers. The

hypothesis that the coast was further east on the North American continental shelf has been documented by fossil mammoth and mastodon teeth being pulled up by shell fishing boats. Coastal salt marsh peats are also known well off the coast (Whitemore et al., 1967). During glaciation, *A. arbutifolia,* the probably paternal parent of *A. prunifolia,* likely was pushed south and occurred on the southern coastal plain of the eastern United states. Approximately 11,000 ybp (Deevy and Flint, 1957) as the glacier receded; simultaneously areas on the current mainland of the Northeast become habitable while the offshore sands were flooded as ocean levels rose. At this time, diploid *A. melanocarpa* dispersed from the now- submerged areas to its approximate current range. *Sagittaria teres*, a globally rare aquatic plant that grows in sandy bottom ponds (often glacial kettle holes) and *Scirpus longii* (an eastern Atlantic coastal plain endemic) are also likely to have inhabited these offshore sands during the Pleistocene (see range maps Flora of North America Editorial Committee, eds.,1993+). The diploid was likely the lone *Aronia* in the Northeast post glaciation until the hypsithermal or Holocene climatic optimum approximately 9,000 ybp to 2,600 ybp (Deevy and Flint, 1957). Temperatures at this time were warmer than they are currently. Sometime during the hypsithermal, it is likely *A. arbutifolia* spread northward along the Atlantic coastal plain and came in contact with diploid *A. melanocarpa (*figure 15). *Aronia arbutifolia* in the past may have grown further to north than its present distribution and then retreated to its modern range as temperatures cooled to current levels. As shown by our data, *Aronia arbutifolia* and *A. melanocarpa* diploids flower at approximately the same time. Red and black chokeberry flowers are essentially identical to each other and do not have any sort of physical difference that would restrict pollinators, additionally to human senses there are no major

morphological or scent features that would suggest a different pollination syndrome. This implies that pollen could move freely between the species.

The cross pollination data from *A. melanocarpa* UC012 × *A. arbutifolia* 'Brilliantissima' show that using diploid *A. melanocarpa* as a maternal plant and *A. arbutifolia* as the pollen parent produces progeny. F1 hybrids are easy to obtain and do not require any special techniques (e.g. embryo rescue) to grow. This reveals that there are no barriers to natural hybridization and growth of F1 triploid hybrids. Persson et al. (2004) also demonstrated that progeny of diploid *A. melanocarpa* plants when grown with tetraploids presumably of the same and other species (Persson et al. did not assign species names to their accessions) yield triploid offspring. These authors did not distinguish if the hybrids were the product of *A. melanocarpa* 2n × A. *melanocarpa* 4n, *A. melanocarpa* 2n × *A. prunifolia* 4n or *A. melanocarpa* 2n × *A. arbutifolia* 4n or all three. Some of the artificial triploid *A. melanocarpa* UC012 × *A. arbutifolia* 'Brilliantissima' F1s that were created were stunted and had deformed leaves, other were quite robust but had low reproductive output, but four out of the 10 triploids produced over 400 fruits per plant. These fruits had fewer seeds per fruits than any other *Aronia* groups. The F1 seeds also had lower germination rates than tetraploid *A. prunifolia*. The triploids had better germination than *A. arbutifolia*, but overall reproductive output was lower than *A. arbutifolia*. The reproductive index shows tetraploid *A. prunifolia* could produce nearly seven times the number of viable seedlings than triploid F1 crosses, and over twice the potential number of seedlings than *A. arbutifolia* under our experimental conditions. This means that there would be a huge reproductive advantage for a plant to

increase its ploidy level from 3n to 4n. This is likely why tetraploids were commonly found in this study and only two wild triploids located; of which one was *A. prunifolia*.

There are three alternate paths to tetraploidy in *A. prunifolia* -- and this species is likely to have multiple origins. It is possible that diploid *A. melanocarpa* produced an unreduced 2n egg that united with a 2n sperm produced from *A. arbutifolia*. The production of a tetraploid hybrid via this method is possible but uncommon. For example, in *Vitis* only two tetraploid seedlings were derived from 8,057 pollinations (Park et al., 2002). In our study, 10 triploid *Aronia* hybrids were obtained from approximately 50 pollinations. I hope that the second generation seedlings produced from open pollinated F1 triploid plants will reveal if the triploids can easily produce tetraploids. In *Crataegus*, a genus closely related to *Aronia*, 143 pollinations of triploid *C. succulenta* × diploid *C. monogyna* yielded 8 tetraploid seeds (Talent and Dickson 2007). In apple (*Malus)*, another member of the Pyrinae, triploid cultivars also been shown to yield tetraploid offspring; Bergstrom (1938) demonstrated 4 of 70 seedlings of a triploid were tetraploid. The two step hybridization of diploid *A. melanocarpa* × tetraploid *A. arbutifolia* to produce a 3n hybrid that is then pollinated again by diploid *A. melanocarpa* to produce a tetraploid offspring seems more likely than a single step event with an unreduced *A. melanocarpa* egg. Production of tetraploids from unreduced triploid egg cell is known in other angiosperm groups. For example, triploid populations have been known to produce tetraploid offspring when pollinated by diploid paternal plants in families other than the Rosaceae, e.g. *Chamerion angustifolium* (Onagraceae) where triploid plants pollinated by diploid pollen set 1-10% seed, and progeny were diploid 18%, triploid 56%, and tetraploid 27% (Burton and Husband, 2001). A related hypothesis is that triploid F1 *A.*

melanocarpa × *A. arbutifolia* produced an unreduced triploid egg that was self fertilized with 1n sperm to produce a 4n embryo. Triploids in the Pyrinae generally have some pollen production, likely due to selective pressure to stimulate apogamy through pseudogamous self pollination.

A third route to tetraploidy may be represented by the Virginia *A. prunifolia* accession from the USDA. This Virginia *A. prunifolia* accession probably has a separate origin; this plant is disjunct from the range of other *A. prunifolia* plants that were found in New York and New England. *Aronia melanocarpa* is tetraploid in the southern Appalachian Mountains. Therefore, this Virginia *A. prunifolia* is likely a rare sexual product of a tetraploid, where tetraploid *A. melanocarpa* hybridized with tetraploid *A. arbutifolia*. Tetraploids in the Pyrinae are mostly apogamous, but tetraploid *Amelanchier* is known to occasionally produce sexual offspring (Campbell et al., 1987).

Morphological Principal Coordinates Analysis clustering and the UPGMA phenogram both show that the triploid artificial crosses are most similar to *A. prunifolia*, again supporting the hybridization hypothesis. There were some differences between the artificial 3n and natural 4n populations. The 3n had an earlier fruit ripening date but it is possible that this was because the 3n plants had been recently transplanted from a greenhouse out into the field. Additionally, the large 3n plants that were not stunted and malformed had a more spreading wider growth form than typically wild *A. prunifolia*. This spreading wider form could have been caused by using UC012, a dry land *A. melanocarpa* ecotype plant, which has a semi-prostrate habit. It is much more likely that the more upright wetland ecotype of diploid *A. melanocarpa* would have been growing in

proximity to *A. arbutifolia* a plant that is also most often found in wetlands habitats, and these two plants would hybridize eventually giving rise to 4n *A. prunifolia*.

Chromatography (Hardin 1973) showed that the *A. prunifolia* had more compounds than *A. melanocarpa* and *A. arbutifolia*. More compounds often imply that a taxon is a hybrid (Alston et al. 1965). This also gives support to *A. prunifolia* being a product of an *A. melanocarpa* × *A. arbutifolia* chokeberry hybridization event.

The 4n *A. prunifolia* plants are very successful in the northeastern United States being far more common than either *A. arbutifolia* or 3n plants. *Aronia prunifolia* found north of the current *A. arbutifolia* range may have been formed during the hypsithermal and remained behind while *A. arbutifolia* retreated south. Alternatively, *A. prunifolia* could have occurred near the current overlap of diploid *A. melanocarpa* and *A. arbutifolia*, and after becoming a successful tetraploid, spread north and west from the point of origin. *Aronia prunifolia* is abundant in southern New England and Long Island where it may have displaced diploid *A. melanocarpa*. No samples of diploid *A. melanocarpa* were found on Cape Cod, Long Island, Rhode Island, Martha Vineyard, or Nantucket, though diploid forms could be located relatively close to the coast in Connecticut.

The three likely pathways to the formation of *A. prunifolia* have been described above. They are follows:

1) diploid *A. melanocarpa* × tetraploid *A. arbutifolia* , easily obtained in our cross pollination experiments followed by F1 triploid plant producing an unreduced 3n egg fertilized by a 1n sperm to produce a 4n progeny. This route is commonly

seen in Rosaceae and other angiosperms (Ramsey and Schemske, 1998; Talent and Dickson, 2007). Likely the process that lead to the majority of *A. prunifolia*.

2) diploid *A. melanocarpa* producing an unreduced 2n egg that unites with a diploid sperm cell from a tetraploid *A. arbutifolia*: this is likely to be a rare event. There is no evidence to support this hypothesis for the formation of *A. prunifolia*.
3) tetraploid *A. melanocarpa* producing a 2n egg cell that is joined with a 2n sperm cell from *A. arbutifolia* (or vice versa, the gametes for the taxa could be reversed in this scenario), this may be the origin of the Virginia *A. prunifolia* accession from the USDA. In Virginia only tetraploid *A. melanocarpa* is known.

Aronia prunifolia is likely to have arisen several times from the hybridization of diploid *A. melanocarpa* and tetraploid *A. arbutifolia*. The process is likely to have occurred in two stages, the formation of a triploid F1 that then produces an unreduced 3n egg cell that is fertilized by a 1n sperm cell. The two step process restores fertility, with the 4n *A. prunifolia* plants having seven times the reproductive capacity of the triploid F1 hybrids. *Aronia prunifolia* also clusters together morphologically as a unit. The range of *A. prunifolia* also differs from at least its paternal parent (*A. arbutifolia)* and therefore is self sustaining. *Aronia prunifolia* should be considered a species because it is a polyploid derivative of a F1, has morphological diagnosability, and is self sustaining in the absence of at least one parent.

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Table 1. Location of wild *Aronia* collections.

UConn #	Secondary ID	Species	Town	State	~Elevation	latitude	longitude	Ploidy
UC 007a	Halls Pond	melanocarpa	Chaplin	CT	518'	41.8417	72.1078	2n
UC 008	Priester Pond	prunifolia	North Tisbury	MA	42'	41.4022	70.6783	4n
UC 009	Damariscotta #1	melanocarpa	Nobleboro	ME	53'	44.1344	69.4703	2n
UC 010	Damariscotta #2	melanocarpa	Nobleboro	ME	53'	44.1346	69.4705	2n
UC 011	Damariscotta #3	prunifolia	Nobleboro	ME	59'	44.1478	69.4797	3n
UC 012	Birch Pt.	melanocarpa	South Thomaston	ME	4'	44.0383	69.0953	2n
UC 014	Warren TX	arbutifolia	Warren	TX	380'	32.5333	94.9000	4n
UC 015	Willington bog 320	melanocarpa	Willington	CT	741'	41.8822	72.2675	2n
UC 016	Mansfield Hollow	melanocarpa	Mansfield	CT	256'	41.7561	72.1739	2n
UC 017	Hockamock Pt.	melanocarpa	Bremen	ME	4'	43.9814	69.4189	2n
UC 018	Creamer Property	melanocarpa	Nobleboro	ME	55'	44.1489	69.4483	2n
UC 019	Mt. Battie #1 (tall)	prunifolia	Camden	ME	755'	44.2217	69.0697	4n
UC 020	Mt. Battie dwf	melanocarpa	Camden	ME	702'	44.2203	69.0703	2n
UC 022	Mt. Misery	melanocarpa	Voluntown	CT	411'	41.5889	71.8728	2n
UC 025	Mashapaug Lake #1	prunifolia	Union	CT	709'	42.0075	72.1358	4n
UC 026a	Mashapaug Lake #2	melanocarpa	Union	CT	705'	42.0103	72.1336	2n
UC 027	Montville #1	prunifolia	Montville	CT	190'	41.4203	72.1811	4n
UC 028	Montville #2	prunifolia	Montville	CT	190'	41.4203	72.1811	4n
UC 029	Montville #3	melanocarpa	Montville	CT	190'	41.4206	72.1825	2n
UC 030	Salem	melanocarpa	Salem	CT	392'	41.4606	72.3267	2n
UC 031	NW Park	prunifolia	Windsor	CT	180'	41.8992	72.7122	4n
UC 031b	NW Park	melanocarpa	Windsor	CT	180'	41.8992	72.7122	4n
UC 032	X-Lot	prunifolia	Mansfield	CT	583'	41.8081	72.2592	4n
UC 033	Bluff Pt.@ Pt	prunifolia	Groton	CT	41'	41.3150	72.0364	4n
UC 034	"Steuben, ME"	melanocarpa	Steuben	ME	142'	44.4569	67.9292	2n
UC 035	Willington Bog railroad	melanocarpa	Willington	CT	407'	41.8919	72.2944	2n
UC 036	Pachaug Forest	prunifolia	Pachaug	CT	282'	41.5939	71.8686	4n
UC 038	Ragged Mt.	melanocarpa	Southington	CT	412'	41.6125	72.8236	2n
UC 039	Lantern Hill	melanocarpa	Ledyard	CT	320'	41.4589	71.9447	2n
UC 040	Johnson/Scituate	prunifolia	Johnson/Scituate	RI	359'	41.8350	71.5697	4n
UC 041	Devil's Triangle Island	melanocarpa	Damariscotta	ME	53'	44.1372	69.4867	2n
UC 042	Holden/Princeton	melanocarpa	Holden/Princeton	MA	779'	42.4364	71.8381	2n
UC 043	"Mashpee, Cape Cod"	prunifolia	Mashpee	MA	5'	41.5531	70.5058	4n
UC 044	Montague	melanocarpa	Montague	MA	349'	42.5806	72.5156	2n
UC 045	"Westerly, Dunn's Corner"	prunifolia	Westerly	RI	49'	41.3728	71.7742	4n
UC 046	Plainfield #1	melanocarpa	Plainfield	MA	1666'	42.5139	72.9456	2n
UC 047	Plainfield #2	prunifolia	Plainfield	MA	1878'	42.5069	72.9564	4n
UC 048	Mohawk Mt.	prunifolia	Cornwall	CT	1486'	41.8328	73.3022	4n
UC 049	Essex	melanocarpa	Essex	CT	30'	41.3503	72.4044	2n
UC 050	Voluntown rhody	arbutifolia	Voluntown	CT	287'	41.5964	71.8672	4n
UC 051	Sudbury	melanocarpa	Sudbury	MA	117'	42.4072	71.3894	2n
UC 052	Cattus Island	arbutifolia	Toms River	NJ	4'	39.9691	74.1144	4n

Table 1. Location of wild *Aronia* collections.

UConn #	Secondary ID	Species	Town	State	~Elevation	latitude	longitude	Ploidy
UC 053	Pease Wildlife Area	arbutifolia	Millville	NJ	58'	39.3987	74.9057	4n
UC 054	High Pt State Park playground	melanocarpa	Montague	NJ	1571'	41.3175	74.6706	2n
UC 055	High Pt State Park Lake Marcia	prunifolia	Montague	NJ	1581'	41.3181	74.6678	4n
UC 056	Calvert Cliff State Park	arbutifolia	Bertha	MD	57'	38.4014	76.4258	4n
UC 057	Manahawkin WMA	arbutifolia	Manahawkin	NJ	8'	39.6835	74.2262	4n
UC 058	Milmay	arbutifolia	Milmay	NJ	57'	39.4406	74.8078	4n
UC 059	Lebanon power line wetland	melanocarpa	Lebanon	CT	384'	41.6496	72.2345	2n
UC 060	Shelter Harbor Beach	prunifolia	Shelter Harbor	RI	28'	41.3288	71.7462	4n
UC 061	Waquoit Cape Cod	prunifolia	Waquoit	MA	10'	41.5603	70.5355	4n
UC 062	Crane WMA Cape Cod	prunifolia	Falmouth	MA	101'	41.6403	70.5590	4n
UC 063	Southern Worcester Co. Black	melanocarpa	Brookfield	MA	898'	42.1738	72.1194	2n
UC 064	Plattsburg	melanocarpa	Plattsburg	NY	383'	44.6979	73.5309	4n
UC 065	Northboro	melanocarpa	Northboro	MA	339'	42.3061	71.6440	2n
UC 066	Jct rte 6 and 102 RI	prunifolia	Chopmist	RI	646'	41.8271	71.6708	4n
UC 067	Swansea	prunifolia	Swansea	MA	49'	41.7647	71.2386	4n
UC 068	Wareham	prunifolia	Wareham	MA	3'	41.7660	70.7473	4n
UC 069	Riverhead Long Island #1	arbutifolia	Riverhead	NY	19'	40.8977	72.6776	4n
UC 070	Riverhead Long Island #2	prunifolia	Riverhead	NY	19'	40.8977	72.6776	4n
UC 071	Long Pond Dunbarton	prunifolia	Dunbarton	NH	649'	43.0789	71.5981	4n
UC 072	Echo Rd.	prunifolia	Mansfield Center	CT	262'	41.7663	72.1900	2n
UC 073	Stearns Rd.	prunifolia	Mansfield	CT	397'	41.7459	72.2447	4n
UC 074	Westborough WMA	melanocarpa	Westborough	MA	309'	42.2987	71.6224	2n
UC 075	Shrewsbury	melanocarpa	Shrewsbury	MA	379'	42.2466	71.7282	2n
UC 076a	Newbury	prunifolia	Newbury	MA	3'	42.7902	70.9163	4n
UC 077	Fire Island Lighthouse	prunifolia	Fire Island	NY	3'	40.6183	73.2189	4n
UC 080	Flanders Long Island	prunifolia	Flanders	NY	2'	40.9025	72.6019	4n
UC 081	Southhampton LI Barrier Island	arbutifolia	Southhampton	NY	4'	40.8539	72.4417	4n
UC 082	Manorville comm. cranberry bog	prunifolia	Manorville	NY	47'	40.8653	72.7931	4n
UC 083	Sayville Grassland USFWS	prunifolia	Sayville	NY	30'	40.7429	73.1031	4n
UC 084	Perry Hill Rd. Ashford	prunifolia	Ashford	CT	517'	41.8778	72.1631	4n
UC 085	Bluff Pt. woods	prunifolia	Groton	CT	41'	41.3341	72.0314	4n
UC 086	Manchester	prunifolia	Manchester	NH	380'	43.0449	71.4961	4n
UC 087	Lynnfield	melanocarpa	Lynnfield	MA	79'	42.5473	71.0559	2n
UC 088	Florida red	arbutifolia	Gainesville	FL	72'	29.5892	82.3339	4n
UC 091	Albany Pine Bush	melanocarpa	Albany	NY	340'	42.7181	73.8623	3n
UC 092	No Man's Island	prunifolia	Chilmark	MA	40'	41.2569	70.8064	4n
UC 093	Sheffield MA	melanocarpa	Sheffield	MA	850'	42.1290	73.3384	2n
UC 094	SUNY Binghamton	melanocarpa	Vestal	NY	1032'	42.0806	75.9659	4n
UC 095	Eastern PA	melanocarpa	Long Pond	PA	1840'	41.0353	75.4634	2n
UC 096	Delaware	arbutifolia	Lewes	DE	10'	38.7742	75.0886	4n
UC 097	Stoney Man	melanocarpa	Luray	VA	4000'	38.5985	78.3736	4n
UC 098	Cranberry Glades	melanocarpa	Hillsboro	WV	3370'	38.1985	80.2737	4n

Table 1. Location of wild *Aronia* collections.

UConn #	Secondary ID	Species	Town	State	~Elevation	latitude	longitude	Ploidy
UC099	Brown's lake bog	melanocarpa	Shreve	Ohio	950'	40.6757	82.0590	4n
UC100	Black Moshannon Bog	melanocarpa	Phillipsburg	PA	1865'	40.9011	78.0586	4n
UC 102	Pinhook Bog	melanocarpa	Indiana Dunes	IN	822'	41.6144	86.8513	4n
UC 104	Madaket Bike Trail	prunifolia	Nantucket Island	MA	30'	41.2837	70.1420	4n
UC 105	"St #1, Power line, Sandhill"	arbutifolia	West End	NC	489'	35.2526	79.4910	4n
UC 106	"St #2, Longleaf Pine ecosystem"	arbutifolia	Jackson Springs	NC	461'	35.2105	79.5712	4n
UC 107	"St #1, power line area Red"	arbutifolia	Caryville	TN	1744'	36.3578	84.2185	4n
UC 108	"St #1, power line area Black"	melanocarpa	Caryville	TN	1744'	36.3578	84.2185	4n
UC 109	"St #2, rock ridge Red"	arbutifolia	Newcomb	TN	1775'	36.5375	84.1558	4n
UC 110a	"St #2, rock ridge Black"	melanocarpa	Newcomb	TN	1775'	36.5375	84.1558	4n
UC 110b	"St #2, rock ridge Black"	melanocarpa	Newcomb	TN	1775'	36.5375	84.1558	4n
UC 111	Shelby Co AL	arbutifolia	Shelby County	AL	500'	33.3139	86.5286	4n
UC 112	"E.Central AL, near Auburn"	arbutifolia	Lee County	AL	524'	32.5053	85.4273	4n
UC 113	Windham Cedar Bog	prunifolia	Windham	CT	267'	41.7434	72.1624	4n
UC 114	Westerly RI Purple	prunifolia	Westerly	RI	34'	41.3804	71.7771	4n
UC 115	Black Mtn	melanocarpa	Guilford	VT	1191'	42.9228	72.6054	2n
UC 116	St. Dennis Cemetery	melanocarpa	Ashburnham	MA	1300'	42.6455	71.8994	2n
UC 117	Munson	prunifolia	Munson	MA	612'	42.0402	72.3212	4n
UC 118	Sharon Moose Hill	prunifolia	Sharon	MA	390'	42.1288	71.2189	4n
UC 160	"Colchester, bog natural area"	melanocarpa	Colchester	VT	99'	44.5471	73.2858	2n
UC 161	Southern Worcester Co. “Red”	prunifolia	Brookfield	MA	898'	42.1738	72.1194	4n
Ames 27010	"USDA, Ames, IA"	melanocarpa		MI	770'	44.2169	85.9581	4n
Ames 27615	“USDA, Ames, IA"	melanocarpa		MN	900'	45.1403	93.1814	4n
Ames 27649	“USDA, Ames, IA"	arbutifolia		SC	1250'	34.9286	82.8676	4n
PI 545682	"USDA, Ames, IA"	melanocarpa		ONT	580'	42.6167	82.4833	4n
PI 545686	"USDA, Ames, IA"	melanocarpa		ONT	600'	42.4667	82.1225	4n
PI 545687	"USDA, Ames, IA"	melanocarpa		MI	800'	43.0100	85.5047	4n
PI 578096	"USDA, Ames, IA"	arbutifolia		VA	1840'	38.2444	79.3367	4n
PI 603106	"USDA, Ames, IA"	melanocarpa		TN	1900'	35.0833	85.6761	4n
PI 603107	"USDA, Ames, IA"	prunifolia		VA	1840'	38.2444	79.3367	4n
PI 613016	"USDA, Ames, IA"	melanocarpa		MA	1040'	42.4875	72.1875	2n
PI 618684	"USDA, Ames, IA"	melanocarpa		WI	1750'	46.0667	89.4667	4n

Table 2. *Aronia* accessions included in each analysis.

	Flow cytometry	mapping	Height	Stem and leaf pubescence	inflorescences per branch	fruit width and Length	weight per 25 fruits g	Flower diameter	stem diameter	ecology	leaf length, width, area, perimeter, factor	phenogram and PCoA
UConn Accession #												
Ames 26194	x		x	x	x	x	x	x	x		x	x
Ames 26195	x		x	x	x	x	x	x	x		x	x
Ames 27010	x	x	x	x	x	x	x	x	x		x	x
Ames 27615	x	x	x	x	x	x	x	x	x		x	x
Ames 27649	x	x	x	x	x	x	x	x	x		x	x
PI 323957	x		x	x	x	x	x	x	x		x	x
PI 545682	x	x	x	x	x			x	x		x	x
PI 545686	x	x	x	x	x	x	x	x	x		x	x
PI 545687	x	x	x	x	x	x	x		x		x	x
PI 578096	x	x	x	x	x	x	x	x	x		x	x
PI 586591	x		x	x	x	x	x	x	x		x	x
PI 596375	x		x	x	x	x	x	x	x		x	x
PI 603106	x	x	x	x	x	x	x	x	x		x	x
PI 603107	x	x	x	x	x	x	x	x	x		x	x
PI 613016	x	x	x	x	x	x	x	x	x		x	x
PI 618684	x	x	x	x	x	x	x	x	x		x	x
PI 631247	x		x	x	x	x	x	x	x		x	x
PI 636375	x		x	x	x	x	x	x	x		x	x
UC 001	x		x	x	x	x	x	x	x		x	x
UC 002	x		x	x	x	x	x	x	x		x	x
UC 003	x		x	x	x	x	x	x	x		x	x
UC 004	x		x	x	x	x	x	x	x		x	x
UC 005	x		x	x	x	x	x	x	x		x	x
UC 006	x		x	x	x	x	x	x	x		x	x
UC 007	x	x	x	x	x	x	x	x	x	x	x	x

UC 008	x	x	x	x	x	x	x	x	x	x	x	x
UC 009	x	x	x	x	x	x	x	x	x	x	x	x
UC 010	x	x	x	x	x	x	x	x	x	x	x	x
UC 011	x	x	x	x	x			x				
UC 012	x	x	x	x	x	x	x	x	x	x	x	x
UC 013	x	x	x	x	x	x	x	x	x		x	x
UC 014	x	x	x	x	x			x	x		x	x
UC 015	x	x	x	x	x			x	x	x	x	x
UC 016	x	x	x	x	x	x	x	x	x	x	x	x
UC 017	x	x	x	x					x	x	x	
UC 018	x	x	x	x					x	x	x	
UC 019	x	x	x	x	x	x	x	x	x	x	x	x
UC 020	x	x	x	x	x	x	x	x	x	x	x	x
UC 021	x	x	x	x	x	x	x	x	x		x	x
UC 022	x	x	x	x					x	x	x	
UC 023	x	x	x	x	x	x	x	x	x		x	x
UC 024	x	x	x	x	x	x	x	x	x		x	x
UC 025	x	x	x	x					x	x	x	
UC 026	x	x	x	x					x	x	x	
UC 027	x	x	x	x					x	x	x	
UC 028	x	x	x	x	x	x	x	x	x	x	x	x
UC 029	x	x	x	x	x			x	x	x	x	x
UC 030	x	x	x	x					x	x	x	
UC 031	x	x	x	x		x	x		x			
UC 032	x	x	x	x	x	x	x	x	x	x	x	x
UC 033	x	x	x	x	x	x	x	x	x	x	x	x
UC 034	x	x	x	x					x	x	x	
UC 035	x	x	x	x					x	x	x	
UC 036	x	x								x		
UC 037	x	x								x		
UC 038	x	x								x		
UC 039	x	x								x		
UC 040	x	x								x		
UC 041	x	x								x		
UC 042	x	x								x		

UC 043	x	x								x		
UC 044	x	x								x		
UC 045	x	x								x		
UC 046	x	x								x		
UC 047	x	x	x	x					x	x	x	
UC 048	x	x	x	x					x	x	x	
UC 049	x	x	x	x					x	x	x	
UC 050	x	x	x	x					x	x	x	
UC 051	x	x								x		
UC 052	x	x								x		
UC 053	x	x								x		
UC 054	x	x								x		
UC 055	x	x								x		
UC 056	x	x								x	x	
UC 057	x	x	x	x					x	x	x	
UC 058	x	x	x	x					x	x	x	
UC 059	x	x	x	x						x		
UC 060	x	x								x		
UC 061	x	x								x		
UC 062	x	x								x		
UC 063	x	x								x		
UC 064	x	x								x		
UC 065	x	x								x		
UC 066	x	x								x		
UC 067	x	x								x		
UC 068	x	x								x		
UC 069	x	x								x		
UC 070	x	x								x		
UC 071	x	x								x		
UC 072	x	x	x	x					x	x	x	
UC 073	x	x	x	x					x	x	x	
UC 074	x	x	x	x					x	x	x	
UC 075	x	x								x		
UC 076	x	x	x	x					x	x	x	
UC 077	x	x								x		

UC 078	x	x										
UC 079	x	x										
UC 080	x	x										
UC 081	x	x										
UC 082	x	x								x		
UC 083	x	x								x		
UC 084	x	x	x	x					x	x	x	
UC 085	x	x	x	x					x	x	x	
UC 086	x	x	x	x					x	x	x	
UC 087	x	x	x	x						x	x	
UC 088	x	x	x	x					x	x	x	
UC 089	x	x										
UC 090	x	x								x		
UC 091	x	x										
UC 092	x	x								x		
UC 093	x	x								x		
UC 094	x	x								x		
UC 095	x	x								x		
UC 096	x	x								x		
UC 097	x	x								x		
UC 098	x	x								x		
UC 099	x	x										
UC 100	x	x								x		
UC 101	x	x										
UC 102	x	x								x		
UC 103	x	x										
UC 104	x	x								x		
UC 105	x	x								x		
UC 106	x	x								x		
UC 107	x	x								x		
UC 108	x	x								x		
UC 109	x	x								x		
UC 110a	x	x								x		
UC 110b	x	x								x		
UC 111	x	x								x		

UC 112	x	x								x		
UC 113	x	x								x		
UC 114	x	x								x		
UC 115	x	x								x		
UC 116	x	x								x		
UC 117	x	x								x		
UC 141	x											
UC 142	x											
UC 143	x											
UC 144	x											
UC 145	x											
UC 146	x											
UC 147	x											
UC 148	x											
UC 149	x											
UC 150	x											
UC 151	x											
UC 152	x											
UC 153	x											
UC 154	x											
UC 155	x											
UC 156	x											
UC 157	x											
UC 158	x											
UC 159	x											
UC 160	x	x								x		
UC 161	x	x								x		

Table 3a. Individual accession means for stem and leaf pubescence.

Accession	Leaf Pubescence mean st error	Stem Pubescence mean st error
AMES26194	2.3 ± 0.3	2.7 ± 0.3
AMES26195	3.0 ± 0.0	3.00 ± 0.0
Ames27010	2.0 ± 0.0	3.3 ± 0.3
AMES27615	0.0 ± 0.0	0.0 ± 0.0
AMES27649	4.7 ± 0.3	4.7 ± 0.3
PI323957	2.7 ± 0.3	3.0 ± 0.0
PI545682	1.7 ± 0.3	1.7 ± 0.3
PI545686	1.3 ± 0.7	0.0 ± 0.0
PI545687	1.3 ± 0.7	1.7 ± 0.9
PI578096	4.0 ± 0.0	4.0 ± 0.0
PI586591	0.3 ± 0.3	0.0 ± 0.0
PI596375	2.7 ± 0.3	2.7 ± 0.3
PI603106	1.3 ± 1.3	1.3 ± 1.3
PI603107	3.3 ± 0.3	4.0 ± 0.0
PI613016	0.0 ± 0.0	0.0 ± 0.0
PI618684	0.7 ± 0.7	0.7 ± 0.7
PI631247	2.0 ± 0.0	2.3 ± 0.3
PI636375	1.3 ± 0.7	1.3 ± 0.7
UC001	4.7 ± 0.3	4.7 ± 0.3
UC002	2.7 ± 0.3	3.0 ± 0.0
UC003	2.7 ± 0.3	2.7 ± 0.3
UC004	0.7 ± 0.7	0.0 ± 0.0
UC005	2.3 ± 0.3	1.0 ± 1.0
UC006	1.0 ± 0.6	0.7 ± 0.7
UC007	1.7 ± 0.3	0.0 ± 0.0
UC008	3.7 ± 0.3	5.0 ± 0.6
UC009	1.3 ± 0.7	3.3 ± 0.3
UC010	0.0 ± 0.0	2.33 ± 0.3
UC011	3.0 ± 0.6	4.0 ± 0.6
UC012	2.0 ± 0.6	1.0 ± 0.6
UC013	5.0 ± 0.0	5.0 ± 0.0
UC014	6.0 ± 0.0	6.0 ± 0.0
UC015	0.7 ± 0.7	0.0 ± 0.0
UC016	1.3 ± 0.7	3.3 ± 0.3
UC017	1.3 ± 0.7	3.3 ± 0.3
UC018	0.7 ± 0.7	0.0 ± 0.0
UC019	2.7 ± 0.7	4.0 ± 0.6
UC020	0.7 ± 0.7	1.0 ± 1.0
UC021	5.0 ± 0.0	5.0 ± 0.0
UC022	1.7 ± 0.3	3.3 ± 0.3
UC023	0.0 ± 0.0	0.0 ± 0.0
UC024	1.0 ± 1.0	1.0 ± 1.0
UC025	3.7 ± 0.3	4.7 ± 0.3
UC26a	0.0 ± 0.0	0.0 ± 0.0
UC027	3.5 ± 0.5	4.0 ± 0.0
UC028	2.0 ± 0.0	3.7 ± 0.3
UC029	1.3 ± 0.7	2.7 ± 0.3
UC030	2.0 ± 1.0	3.3 ± 0.3
UC031	0.7 ± 0.7	1.3 ± 1.3
UC032	0.0 ± 0.0	4.0 ± 0.0
UC033	3.3 ± 0.7	4.0 ± 0.0
UC034	0.7 ± 0.3	1.0 ± 0.6
UC035	0.7 ± 0.7	1.3 ± 0.7
UC047	3.0 ± 0.0	3.7 ± 0.3

Table 3a continued. Individual accession means for stem and leaf pubescence.

Accession	Leaf Pubescence mean st error	Stem Pubescence mean st error
UC048	2.3 ± 0.3	3.7 ± 0.3
UC049	2.3 ± 0.3	2.0 ± 1.0
UC050	4.7 ± 0.3	4.7 ± 0.3
UC057	5.0 ± 0.0	4.7 ± 0.3
UC058	5.0 ± 0.0	5.0 ± 0.0
UC059	5.0 ± 0.0	5.0 ± 0.0
UC072	1.0 ± 0.6	0.0 ± 0.0
UC073	3.0 ± 0.0	3.7 ± 0.3
UC074	0.7 ± 0.7	2.0 ± 1.0
UC076	2.0 ± 0.0	3.5 ± 0.5
UC084	4.0 ± 0.0	4.0 ± 0.0
UC085	3.3 ± 0.3	4.0 ± 0.0
UC086	0.0 ± 0.0	0.7 ± 0.7
UC087	0.0 ± 0.0	1.0 ± 1.0
UC088	5.0 ± 0.0	5.0 ± 0.0

Table 3b. Individual accession means for plant height, stem diameter, and plant width.

Accession	Height in cm mean st error	Stem dia cm mean st error	Width cm mean st error
AMES26194	129 ± 4	1.7 ± 0.2	166 ± 9
AMES26195	130 ± 7	1.9 ± 0.1	199 ± 8
Ames27010	102 ± 0	2.0 ± 0.1	147 ± 10
AMES27615	80 ± 1	1.3 ± 0.2	169 ± 9
AMES27649	150 ± 6	2.8 ± 0.3	180 ± 5
PI323957	116 ± 7	1.7 ± 0.1	168 ± 8
PI545682	104 ± 6	1.4 ± 0.1	151 ± 2
PI545686	99 ± 4	1.7 ± 0.3	163 ± 17
PI545687	102 ± 4	0.5 ± 0.3	160 ± 7
PI578096	141 ± 5	1.7 ± 0.2	130 ± 8
PI586591	96 ± 9	1.9 ± 0.1	134 ± 7
PI596375	116 ± 4	1.9 ± 0.3	166 ± 4
PI603106	81 ± 7	1.1 ± 0.2	134 ± 4
PI603107	132 ± 5	1.8 ± 0.2	200 ± 3
PI613016	102 ± 5	1.2 ± 0.2	157 ± 2
PI618684	94 ± 5	1.8 ± 0.2	201 ± 4
PI631247	128 ± 8	1.7 ± 0.1	170 ± 5
PI636375	124 ± 5	2.0 ± 0.2	165 ± 8
UC001	182 ± 12	3.2 ± 0.2	196 ± 16
UC002	177 ± 7	3.1 ± 0.6	201 ± 5
UC003	204 ± 5	3.1 ± 0.2	229 ± 13
UC004	137 ± 6	2.6 ± 0.1	198 ± 9
UC005	161 ± 6	2.6 ± 0.4	179 ± 20
UC006	137 ± 8	2.5 ± 0.2	185 ± 7
UC007	85 ± 14	2.1 ± 0.5	149 ± 13
UC008	138 ± 8	2.9 ± 0.1	197 ± 16
UC009	144 ± 4	3.0 ± 0.1	196 ± 14
UC010	116 ± 4	2.2 ± 0.1	207 ± 8
UC011	141 ± 11	1.7 ± 0.5	119 ± 17
UC012	25 ± 1	1.2 ± 0.2	100 ± 15
UC013	147 ± 6	2.1 ± 0.2	175 ± 13
UC014	156 ± 7	2.6 ± 0.1	230 ± 14
UC015	141 ± 2	1.9 ± 0.1	144 ± 12
UC016	112 ± 6	1.9 ± 0.1	168 ± 1
UC017	58 ± 10	1.5 ± 0.3	151 ± 4
UC018	90 ± 9	1.1 ± 0.1	89 ± 0
UC019	97 ± 5	1.7 ± 0.2	143 ± 7
UC020	58 ± 4	1.3 ± 0.4	97 ± 7
UC021	153 ± 17	1.9 ± 0.5	129 ± 35
UC022	94 ± 8	1.1 ± 0.1	107 ± 3
UC023	113 ± 8	2.3 ± 0.4	135 ± 3
UC024	118 ± 4	1.4 ± 0.0	142 ± 5
UC025	137 ± 30	1.8 ± 0.2	130 ± 8
UC026	113 ± 1	1.7 ± 0.2	105 ± 14
UC027	54 ± n/a	1.0 ± n/a	91 ± 8
UC028	114 ± 4	1.8 ± 0.2	157 ± 2
UC029	98 ± 14	1.3 ± 0.2	134 ± 5
UC030	76 ± 10	1.0 ± 0.1	117 ± 0
UC031	89 ± n/a	1.0 ± n/a	116 ± 1
UC032	106 ± 7	1.4 ± 0.0	175 ± 8
UC033	138 ± 2	2.0 ± 0.3	168 ± 8
UC034	38 ± n/a	0.5 ± n/a	71 ± n/a
UC035	95 ± 1	1.0 ± 0.0	105 ± 1
UC047	89 ± 3	1.1 ± 0.1	121 ± 9
UC048	90 ± 1	1.1 ± 0.1	110 ± 6

Table 3b. Individual accession means for plant height, stem diameter, and plant width.

Accession	Height in cm mean st error	Stem dia cm mean st error	Width cm mean st error
UC049	69 ± n/a	1.0 ± n/a	97 ± n/a
UC050	85 ± 9	1.2 ± 0.2	127 ± 10
UC057	141 ± 1	2.0 ± 0.2	152 ± 5
UC058	133 ± 6	1.5 ± 0.1	117 ± 5
UC059	109 ± 8	0.9 ± 0.1	119 ± 18
UC072	126 ± 1	1.3 ± 0.2	124 ± 3
UC073	146 ± 6	1.5 ± 0.1	141 ± 14
UC074	89 ± 5	1.2 ± 0.1	109 ± 8
UC076	102 ± 20	1.4 ± 0.3	113 ± 17
UC084	105 ± 4	1.6 ± 0.1	123 ± 1
UC085	144 ± 4	1.7 ± 0.2	135 ± 3
UC086	65 ± 4	1.1 ± 0.2	123 ± 9
UC087	38 ± 3	1.1 ± 0.1	90 ± 1
UC088	99 ± n/a	1.2 ± n/a	124 ± n/a

Table 3c. Individual accession means for leaf length, width, and area.

	length	**width**	**area**
Accession	**mean st error**	**mean st error**	**mean st error**
AMES26194	6.88 ± 0.23	5.36 ± 0.10	26.66 ± 1.11
AMES26195	7.69 ± 0.12	5.72 ± 0.16	32.34 ± 1.66
Ames27010	5.76 ± 0.23	3.71 ± 0.17	15.98 ± 1.15
AMES27615	6.04 ± 0.28	3.64 ± 0.10	15.51 ± 1.24
AMES27649	7.32 ± 0.06	3.89 ± 0.05	19.86 ± 0.07
PI323957	7.47 ± 0.24	5.67 ± 0.05	31.10 ± 1.40
PI545682	6.22 ± 0.17	3.56 ± 0.03	16.15 ± 0.62
PI545686	5.98 ± 0.29	3.84 ± 0.53	16.80 ± 2.97
PI545687	6.14 ± 0.07	3.72 ± 0.02	16.44 ± 0.39
PI578096	5.36 ± 0.18	3.15 ± 0.08	12.03 ± 0.75
PI586591	6.23 ± 0.47	3.08 ± 0.28	14.51 ± 2.49
PI596375	7.41 ± 0.31	5.42 ± 0.05	30.13 ± 1.31
PI603106	6.67 ± 0.26	3.69 ± 0.14	17.36 ± 1.24
PI603107	5.77 ± 0.29	3.44 ± 0.03	13.77 ± 0.84
PI613016	6.23 ± 0.98	3.05 ± 0.12	14.22 ± 2.41
PI618684	6.46 ± 0.35	3.93 ± 0.06	18.66 ± 1.18
PI631247	7.86 ± 0.22	5.68 ± 0.08	33.71 ± 1.28
PI636375	6.24 ± 0.24	4.79 ± 0.11	21.90 ± 0.68
UC001	6.06 ± 0.26	3.54 ± 0.49	14.64 ± 1.66
UC002	6.65 ± 0.23	4.39 ± 0.31	21.54 ± 1.89
UC003	6.54 ± 0.34	4.96 ± 0.19	24.15 ± 1.50
UC004	5.57 ± 0.31	2.96 ± 0.18	12.30 ± 1.32
UC005	4.51 ± 0.09	3.25 ± 0.12	11.18 ± 0.73
UC006	5.30 ± n/a	3.14 ± n/a	12.51 ± n/a
UC007	5.00 ± 0.25	2.68 ± 0.27	9.72 ± 1.34
UC008	5.23 ± 0.40	3.21 ± 0.18	12.42 ± 1.46
UC009	4.45 ± 0.08	2.67 ± 0.12	9.07 ± 0.78
UC010	4.77 ± 0.05	2.63 ± 0.03	9.34 ± 0.18
UC012	4.21 ± 0.19	2.29 ± 0.08	6.98 ± 0.36
UC013	5.66 ± 0.22	3.32 ± 0.27	13.92 ± 1.58
UC014	7.91 ± 0.23	4.32 ± 0.14	23.78 ± 0.31
UC015	6.13 ± 0.09	3.17 ± 0.03	14.39 ± 0.02
UC016	5.69 ± 0.17	3.48 ± 0.21	14.73 ± 0.71
UC017	4.87 ± 0.07	2.87 ± 0.16	10.46 ± 0.95
UC018	5.53 ± 0.84	3.63 ± 0.24	15.88 ± 2.61
UC019	5.04 ± 0.24	4.06 ± 0.13	15.05 ± 1.50
UC020	4.34 ± 0.11	2.59 ± 0.10	8.74 ± 0.38
UC021	6.68 ± 0.32	3.21 ± 0.05	15.27 ± 1.06
UC022	5.33 ± 0.28	3.28 ± 0.11	13.19 ± 0.08
UC023	5.23 ± 0.12	3.12 ± 0.07	12.48 ± 0.41
UC024	6.74 ± 0.39	4.96 ± 0.37	23.84 ± 2.92
UC025	6.56 ± 0.16	3.79 ± 0.08	17.94 ± 0.28
UC026	7.31 ± 1.04	3.63 ± 0.87	19.86 ± 6.99
UC027	4.58 ± n/a	3.53 ± n/a	12.76 ± n/a
UC028	7.88 ± 0.14	3.91 ± 0.07	21.58 ± 1.05
UC029	6.28 ± 0.13	3.43 ± 0.04	15.82 ± 0.28
UC030	4.93 ± 0.25	2.78 ± 0.13	9.85 ± 0.76
UC032	6.22 ± 0.20	3.55 ± 0.14	16.44 ± 1.18
UC033	6.09 ± 0.15	3.57 ± 0.08	15.93 ± 0.73
UC034	4.18 ± n/a	2.42 ± n/a	7.82 ± n/a
UC035	6.49 ± 0.41	3.61 ± 0.21	17.53 ± 1.98

Table 3c. Individual accession means for leaf length, width, and area.

	length	**width**	**area**
Accession	**mean st error**	**mean st error**	**mean st error**
UC047	6.14 ± 0.09	3.46 ± 0.07	15.79 ± 0.01
UC048	5.65 ± 0.19	3.59 ± 0.13	15.66 ± 1.15
UC049	4.67 ± n/a	3.03 ± n/a	10.75 ± n/a
UC050	6.00 ± 0.07	3.87 ± 0.02	17.55 ± 0.25
UC056	5.94 ± 0.26	3.22 ± 0.13	13.64 ± 1.09
UC057	5.96 ± 0.35	3.90 ± 0.17	17.02 ± 1.25
UC058	6.12 ± 0.10	3.64 ± 0.01	16.76 ± 0.50
UC072	6.52 ± 0.32	3.20 ± 0.01	14.84 ± 0.96
UC073	6.38 ± 0.16	4.20 ± 0.22	19.26 ± 0.85
UC074	5.98 ± 0.10	3.78 ± 0.11	16.69 ± 0.65
UC076	5.43 ± 0.42	3.38 ± 0.15	13.50 ± 1.83
UC084	5.59 ± 0.44	3.44 ± 0.23	14.82 ± 2.16
UC085	5.57 ± 0.08	3.44 ± 0.00	13.83 ± 0.08
UC086	5.87 ± 0.02	4.53 ± 0.02	20.11 ± 0.34
UC087	6.23 ± 0.09	3.45 ± 0.14	16.46 ± 0.68
UC088	9.31 ± 0.46	4.93 ± 0.14	32.31 ± 1.99

Table 3d. Individual accession means for perimeter, ratio, and factor.

	perimeter	ratio	factor
Accession	**mean st error**	**mean st error**	**mean st error**
AMES26194	34.76 ± 1.57	1.28 ± 0.02	0.28 ± 0.02
AMES26195	33.92 ± 3.64	1.35 ± 0.02	0.52 ± 0.16
Ames27010	20.21 ± 3.27	1.62 ± 0.03	0.61 ± 0.14
AMES27615	19.61 ± 2.09	1.67 ± 0.06	0.59 ± 0.14
AMES27649	18.13 ± 0.34	1.90 ± 0.01	0.79 ± 0.01
PI323957	36.54 ± 0.71	1.32 ± 0.03	0.35 ± 0.06
PI545682	20.98 ± 2.17	1.75 ± 0.04	0.58 ± 0.10
PI545686	19.65 ± 3.16	1.69 ± 0.08	0.65 ± 0.05
PI545687	18.43 ± 2.28	1.66 ± 0.01	0.70 ± 0.12
PI578096	14.49 ± 0.37	1.71 ± 0.05	0.79 ± 0.03
PI586591	15.54 ± 0.95	2.06 ± 0.07	0.73 ± 0.02
PI596375	26.81 ± 0.73	1.37 ± 0.05	0.67 ± 0.08
PI603106	21.27 ± 1.66	1.82 ± 0.04	0.62 ± 0.07
PI603107	20.10 ± 0.91	1.69 ± 0.08	0.52 ± 0.05
PI613016	15.77 ± 1.25	2.07 ± 0.25	0.73 ± 0.02
PI618684	19.35 ± 0.19	1.65 ± 0.07	0.72 ± 0.01
PI631247	26.73 ± 3.51	1.38 ± 0.02	0.80 ± 0.18
PI636375	23.19 ± 1.85	1.32 ± 0.07	0.63 ± 0.04
UC001	20.16 ± 5.16	1.77 ± 0.16	0.60 ± 0.11
UC002	21.00 ± 1.68	1.55 ± 0.14	0.72 ± 0.15
UC003	23.03 ± 3.16	1.32 ± 0.02	0.84 ± 0.19
UC004	13.91 ± 0.60	1.90 ± 0.07	0.79 ± 0.03
UC005	14.07 ± 0.52	1.41 ± 0.05	0.92 ± 0.19
UC006	15.15 ± n/a	1.69 ± n/a	0.76 ± n/a
UC007	14.94 ± 2.40	1.91 ± 0.11	0.62 ± 0.07
UC008	15.27 ± 2.36	1.63 ± 0.04	0.75 ± 0.07
UC009	11.98 ± 0.29	1.67 ± 0.05	0.79 ± 0.03
UC010	12.78 ± 0.37	1.82 ± 0.03	0.73 ± 0.04
UC012	11.59 ± 0.79	1.87 ± 0.11	0.69 ± 0.07
UC013	16.17 ± 1.13	1.73± 0.08	0.71± 0.03
UC014	23.91 ± 0.39	1.83± 0.11	0.64 ± 0.01
UC015	15.70 ± 0.70	1.94± 0.06	0.75 ± 0.05
UC016	18.17 ± 2.49	1.65± 0.15	0.68 ± 0.09
UC017	13.43 ± 0.13	1.70± 0.07	0.75 ± 0.04
UC018	15.93 ± 2.38	1.52± 0.13	0.81 ± 0.04
UC019	18.54 ± 1.71	1.24± 0.02	0.64 ± 0.15
UC020	11.51 ± 0.19	1.68± 0.07	0.82 ± 0.01
UC021	16.90 ± 0.38	2.10± 0.06	0.69 ± 0.02
UC022	15.23 ± 0.04	1.63± 0.14	0.73 ± 0.00
UC023	15.71 ± 0.90	1.68± 0.04	0.69 ± 0.03
UC024	24.43 ± 4.84	1.37± 0.04	0.66 ± 0.13
UC025	18.32 ± 1.83	1.74± 0.08	0.73 ± 0.11
UC026	21.93 ± 6.77	2.06± 0.20	0.62 ± 0.08
UC027	13.47 ± n/a	1.30± n/a	0.88 ± n/a
UC028	23.50 ± 2.34	2.05± 0.04	0.61 ± 0.09
UC029	19.00 ± 1.21	1.83± 0.04	0.61 ± 0.05
UC030	13.71 ± 1.71	1.78± 0.01	0.73 ± 0.06
UC032	19.51 ± 1.63	1.76± 0.04	0.67 ± 0.04
UC033	18.69 ± 1.77	1.71± 0.01	0.64 ± 0.07
UC034	11.03 ± n/a	1.74± n/a	0.80 ± n/a

Table 3d. Individual accession means for perimeter, ratio, and factor.

Accession	perimeter mean st error	ratio mean st error	factor mean st error
UC035	16.50 ± 0.88	1.80± 0.01	0.80 ± 0.00
UC047	16.00 ± 0.27	1.77± 0.06	0.77 ± 0.02
UC048	15.50 ± 0.65	1.57± 0.01	0.82 ± 0.00
UC049	12.80 ± n/a	1.55± n/a	0.82 ± n/a
UC050	17.44 ± 0.49	1.55± 0.03	0.75 ± 0.04
UC056	15.18 ± 0.42	1.85± 0.01	0.75 ± 0.01
UC057	17.90 ± 2.51	1.54± 0.02	0.75 ± 0.08
UC058	17.57 ± 0.88	1.69± 0.02	0.70 ± 0.07
UC072	18.08 ± 1.51	2.04± 0.09	0.65 ± 0.07
UC073	20.80 ± 4.25	1.52± 0.04	0.66 ± 0.18
UC074	16.29 ± 0.26	1.59± 0.07	0.78 ± 0.04
UC076	16.10 ± 1.93	1.62± 0.05	0.73 ± 0.03
UC084	18.04 ± 4.22	1.63± 0.01	0.69 ± 0.13
UC085	15.05 ± 0.62	1.63± 0.03	0.79 ± 0.05
UC086	24.73 ± 5.61	1.30± 0.01	0.56± 0.22
UC087	16.61 ± 0.87	1.82± 0.04	0.78± 0.02
UC088	29.58 ± 4.52	1.89± 0.04	0.61± 0.09

Table 3e. Individual accession means for fruit width, length, and weight per 25 fruits.

Accession	Fruit width mm mean st error	Fruit length mm mean st error	Fruit weight per 25 g mean st error
AMES26194	12.5 ± 0.3	10.7 ± 0.2	26.2 ± 1.1
AMES26195	12.2 ± 0.3	10.9 ± 0.3	23.8 ± 1.8
Ames27010	9.5 ± 0.1	8.7 ± 0.1	12.5 ± 0.4
AMES27615	7.5 ± 0.3	6.5 ± 0.2	6.9 ± 0.6
AMES27649	7.2 ± n/a	7.8 ± n/a	7.1 ± n/a
PI323957	11.8 ± 0.4	10.7 ± 0.1	23.5 ± 0.0
PI545682	-- --	-- --	-- --
PI545686	8.8 ± 0.1	8.2 ± 0.1	10.4 ± 0.2
PI545687	8.8 ± 0.1	7.7 ± 0.2	10.5 ± 0.2
PI578096	7.3 ± 0.1	6.8 ± 0.1	6.9 ± 0.2
PI586591	8.7 ± 0.1	7.4 ± 0.0	9.9 ± 1.0
PI596375	12.2 ± 0.1	10.7 ± 0.2	23.8 ± 0.5
PI603106	9.4 ± 0.1	8.8 ± 0.2	11.8 ± 0.6
PI603107	8.5 ± 0.1	8.6 ± 0.5	10.5 ± 0.4
PI613016	9.2 ± 0.3	8.9 ± 0.4	4.5 ± 0.2
PI618684	8.1 ± 0.3	7.0 ± 0.3	9.2 ± 0.9
PI631247	11.9 ± 0.3	10.4 ± 0.3	24.2 ± 1.6
PI636375	10.1 ± 0.3	8.4 ± 0.4	14.8 ± 0.1
UC001	8.2 ± 0.1	7.8 ± 0.0	8.6 ± 0.2
UC002	12.1 ± 0.3	10.6 ± 0.1	25.2 ± 1.0
UC003	12.3 ± 0.0	10.8 ± 0.1	26.6 ± 0.8
UC004	9.0 ± 0.1	7.3 ± 0.1	11.1 ± 0.6
UC005	9.7 ± 0.2	8.1 ± 0.0	12.5 ± 0.6
UC006	9.2 ± 0.1	8.5 ± 0.2	12.1 ± 0.8
UC007	9.6 ± 0.3	8.2 ± 0.2	11.0 ± 1.1
UC008	7.5 ± 0.1	7.3 ± 0.2	8.6 ± 0.4
UC009	9.0 ± 0.1	7.9 ± 0.1	9.7 ± 0.4
UC010	8.8 ± 0.2	7.6 ± 0.2	10.1 ± 0.7
UC012	7.7 ± 0.2	9.4 ± 0.2	8.4 ± 0.4
UC013	7.8 ± 0.2	6.8 ± 0.2	7.7 ± 0.2
UC014	-- --	-- --	-- --
UC015	-- --	-- --	-- --
UC016	8.3 ± 0.1	7.7 ± 0.1	9.7 ± 0.6
UC017	-- --	-- --	-- --
UC018	-- --	-- --	-- --
UC019	8.6 ± 0.0	7.5 ± 0.2	9.2 ± 0.3
UC020	8.2 ± 0.4	7.2 ± 4.1	8.0 ± 1.5
UC021	8.5 ± 0.3	8.8 ± 0.1	11.1 ± 0.8
UC022	-- --	-- --	-- --
UC023	9.2 ± 0.1	8.0 ± 0.2	9.4 ± 0.4
UC024	10.3 ± 0.2	8.8 ± 0.1	17.1 ± 0.2
UC025	-- --	-- --	-- --
UC027	-- --	-- --	-- --
UC028	8.7 ± 0.2	7.9 ± 0.1	9.8 ± 0.4
UC029	-- --	-- --	-- --
UC030	-- --	-- --	-- --
UC031	12.8 ± 0.3	10.7 ± 0.0	27.7 ± 1.5
UC032	8.7 ± 0.2	7.0 ± 0.1	9.1 ± 0.2
UC033	8.3 ± n/a	7.9 ± 0.1	9.8 ± 0.1

Table 3f. Individual accession means for number of inflorescences per 30 cm of branch and individual flower diameters mm.

Accession	Inflorescences per branch mean st error	Flower diameter mm mean st error
AMES26194	2.3 ± 0.0	12.2 ± 0.1
AMES26195	1.9 ± 0.1	12.9 ± 0.3
Ames27010	8.3 ± 1.4	14.7 ± 0.4
AMES27615	12.8 ± 4.2	9.0 ± n/a
AMES27649	7.6 ± 2.3	14.6 ± 0.4
PI323957	2.2 ± 1.2	12.3 ± 0.0
PI545682	15.3 ± 3.5	11.8 ± 0.5
PI545686	11.9 ± 0.6	12.3 ± 1.1
PI545687	13.8 ± 1.5	-- --
PI578096	12.4 ± 0.4	12.2 ± 0.2
PI586591	16.9 ± 2.7	12.7 ± 0.0
PI596375	1.6 ± 0.3	12.6 ± 0.2
PI603106	12.9 ± 0.3	10.0 ± 0.3
PI603107	4.4 ± 0.6	13.6 ± 0.3
PI613016	8.8 ± 1.3	13.7 ± 0.0
PI618684	19.3 ± 1.6	11.0 ± n/a
PI631247	3.0 ± 0.2	12.7 ± 0.3
PI636375	14.8 ± 3.4	13.0 ± 0.4
UC001	6.8 ± 0.4	13.4 ± 0.2
UC002	3.2 ± 0.7	12.7 ± 0.0
UC003	2.7 ± 0.5	13.1 ± 0.5
UC004	12.1 ± 1.7	13.4 ± 0.2
UC005	10.2 ± 0.6	14.6 ± 0.6
UC006	18.9 ± 1.3	12.6 ± 0.2
UC007	10.3 ± 1.1	12.6 ± 0.1
UC008	8.7 ± 0.6	13.5 ± 0.2
UC009	4.6 ± 0.3	13.2 ± 0.8
UC010	7.0 ± 1.3	12.2 ± 0.2
UC011	6.4 ± 1.3	12.2 ± 0.1
UC012	12.00 ± 1.1	11.0 ± 0.9
UC013	9.6 ± 0.7	14.4 ± 0.2
UC014	3.1 ± 0.1	13.8 ± 0.3
UC015	7.89 ± 1.5	12.0 ± 0.5
UC016	11.1 ± 3.2	14.9 ± 0.1
UC017	-- --	-- --
UC018	-- --	-- --
UC019	8.1 ± 2.1	14.7 ± 0.2
UC020	10.9 ± 1.5	9.8 ± 0.5
UC021	8.7 ± 0.7	13.2 ± 0.2
UC022	-- --	-- --
UC023	12.9 ± 0.2	11.2 ± 0.1
UC024	7.8 ± 0.8	13.3 ± 0.7
UC025	-- --	-- --
UC027	-- --	-- --
UC028	6.3 ± 1.7	13.6 ± 0.7
UC029	9.0 ± 1.7	12.3 ± 1.1
UC030	-- --	-- --
UC031	-- --	-- --
UC032	9.7 ± 1.7	13.7 ± 0.3
UC033	8.7 ± 0.9	13.2 ± 0.4

Table 4. Whole plant univariate character mean separations.

Largest Stem Diameter

OTU group	No.	mean cm
mitschurinii	7	2.2 a
arbutifolia	11	1.9 a
melanocarpa 4n	14	1.8 a
prunifolia	17	1.6 ab
melanocarpa 2n	20	1.5 b

Plant Width

OTU group	No.	mean cm
mitschurinii	7	185 a
melanocarpa 4n	14	158 a
arbutifolia	11	153 ab
prunifolia	17	136 bc
melanocarpa 2n	20	129 c

Plant Height

OTU group	No.	mean cm
mitschurinii	7	143 a
arbutifolia	14	136 a
melanocarpa 4n	11	108 b
prunifolia	17	106 b
melanocarpa 2n	20	92 b

Table 5. Leaf univariate character mean separations.

Stem Pubescence scale	**No.**	**0-6 scale**
OTU group		**mean**
arbutifolia	6	5.0 a
prunifolia	6	4.1 a
mitschurinii	7	2.8 b
melanocarpa 2n	10	1.4 c
melanocarpa 4n	13	1.0 c

Leaf Pubescence		**0-6**
OTU group	**No.**	**mean**
arbutifolia	6	4.9 a
prunifolia	6	3.0 b
mitschurinii	7	2.6 b
melanocarpa 4n	5	1.1 c
melanocarpa 2n	13	1.1 c

Leaf area		
OTU group	**No.**	**mean**
mitschurinii	7	28.5 a
arbutifolia	11	17.9 b
melanocarpa 4n	13	16.4 b
prunifolia	15	15.9 b
melanocarpa 2n	21	12.8 c

Leaf length		
OTU group		**mean**
mitschurinii	7	7.2a
arbutifolia	11	6.6 a
melanocarpa 4n	13	6.0 ab
prunifolia	15	5.9 bc
melanocarpa 2n	21	5.5 c

Leaf Width		
OTU group	**No.**	**mean**
mitschurinii	7	5.3 a
arbutifolia	11	3.7 b
melanocarpa 4n	13	3.7 b
prunifolia	15	3.7 b
melanocarpa 2n	21	3.1 c

Leaf perimeter		
OTU group	**No.**	**mean**
mitschurinii	7	29.0 a
melanocarpa 4n	13	19.0 b
arbutifolia	11	18.9 b
prunifolia	16	18.2 b
melanocarpa 2n	21	15.2 c

Leaf ratio		
OTU group		**mean**
melanocarpa 2n	21	1.8 a
arbutifolia	11	1.8 a
melanocarpa 4n	13	1.7 ab
prunifolia	15	1.6 b
mitschurinii	7	1.4 c

Table 6. Fruit and flower univariate character mean separation.

Fruit wt/25 2010			**Fruit wt/25 2011**		
OTU	**No.**	**Mean g**	**OTU**	**No.**	**Mean g**
mitschurinii	7	24.7 a	*mitschurinii*	7	21.8 a
melanocarpa 4n	12	11.6 b	*prunifolia*	17	9.5 b
prunifolia	6	9.5 c	*arbutifolia*	18	8.6 b
melanocarpa 2n	8	8.8 c	*melanocarpa* 4n	13	7.7 bc
arbutifolia	5	8.3 c	*melanocarpa* 2n	16	6.2 c

Fruit Width	**2010**		**Fruit length 2010**		
OTU	**No.**	**Mean mm**	**OTU**	**No.**	**Mean mm**
mitschurinii	7	12.2 a	*mitschurinii*	7	10.7 a
melanocarpa 4n	12	9.1 b	*melanocarpa* 2n	8	8.1 b
melanocarpa 2n	8	8.7 b	*melanocarpa* 4n	12	7.9 b
prunifolia	6	8.4 bc	*prunifolia*	6	7.7 b
arbutifolia	5	7.8 c	*arbutifolia*	5	7.6 b

Fruit Width 2011			**Fruit length 2011**		
OTU	**No.**	**Mean mm**	**OTU**	**No.**	**Mean mm**
mitschurinii	16	11.8 a	*mitschurinii*	7	10.1 a
prunifolia	26	8.5 b	*prunifolia*	26	7.9 b
arbutifolia	18	8.2 b	Triploids	8	7.7 b
melanocarpa 4n	17	8.2 b	*arbutifolia*	18	7.6 b
Triploids	8	7.7 c	*melanocarpa* 4n	17	7.5 b
melanocarpa 2n	16	7.3 c	*melanocarpa* 2n	16	6.8 c

Table 6 continued. Fruit and flower univariate character mean separation.

Inflorescences per 30 cm 2010			**Flower Diameter**		
OTU	**No.**	**Mean**	**OTU**	**No.**	**Mean**
melanocarpa 4n	13	13.5 a	*prunifolia*	6	13.7 a
melanocarpa 2n	10	9.5 b	*arbutifolia*	6	13.6 ab
prunifolia	6	7.7 b	*mitschurinii*	7	12.6 ab
arbutifolia	6	8.0 b	*melanocarpa* 4n	12	12.4 b
mitschurinii	7	2.4 c	*melanocarpa* 2n	10	12.3 b

Fruit Ripe Date Day from July 1		
OTU	**No.**	**Mean days**
arbutifolia	18	102 a
prunifolia	21	80 b
melanocarpa 4n	13	58 c
mitschurinii	7	46 cd
melanocarpa 2n	17	31 d

Table 7. Crosses of *Aronia melanocarpa* and *A. arbutifolia* to test for apogamy or sexual reproduction.

Maternal cultivar/accession	**Ploidy/SP**	**Paternal**	**Ploidy/Sp cultivar/accession**	**Seedlings**	**Reproduction**
'Brilliantissima'	*arbutifolia*	UC012	*melanocarpa* 2n	48 identical	apogamy
'Brilliantissima'	*arbutifolia*	'Viking'	*mitschurinii*	21 identical	apogamy
'Erecta'	*arbutifolia*	'Autumn Magic'	*melanocarpa* 4n	17 identical	apogamy
'Viking'	*mitschurinii*	'Brilliantissima'	*arbutifolia*	20 identical	apogamy
'Viking'	*mitschurinii*	UC012	*melanocarpa* 2n	9 identical	apogamy
'Viking'	*mitschurinii*	open	unknown	15 identical	apogamy
'erecta'	*arbutifolia*	'Iroquois Beauty'	*melanocarpa* 4n	5 identical	apogamy
UC012	*melanocarpa* 2n	'Brilliantissima'	*arbutifolia*	20 variable	sexual
UC012	*melanocarpa* 2n	'Viking'	*mitschurinii*	1 variable	sexual

Table 8. Reproductive out put of *Aronia* taxa.

Fruits Per Plant			**Seeds Per Fruit**		
OTU	**No.**	**Mean**	**OTU**	**No.**	**Mean**
prunifolia	10	410.4 ns	*melanocarpa* 4n	5	3.4 a
arbutifolia	10	406.1 ns	*melanocarpa* 2n	5	3.3 a
Triploid	10	235.7 ns	*prunifolia*	5	3.0 a
			arbutifolia	5	2.6 a
			Triploid	10	1.4 b

Germination Rate			**Fertility Index**		
OTU	**No.**	**Mean**	**OTU**	**No.**	**Mean**
melanocarpa 4n	5	48.0 ns	*prunifolia*	n/a	403.3
prunifolia	5	33.2 ns	*arbutifolia*	n/a	178
melanocarpa 2n	5	31.0 ns	Triploid	n/a	57.8
Triploid	5	17.4 ns			
arbutifolia	5	16.6 ns			

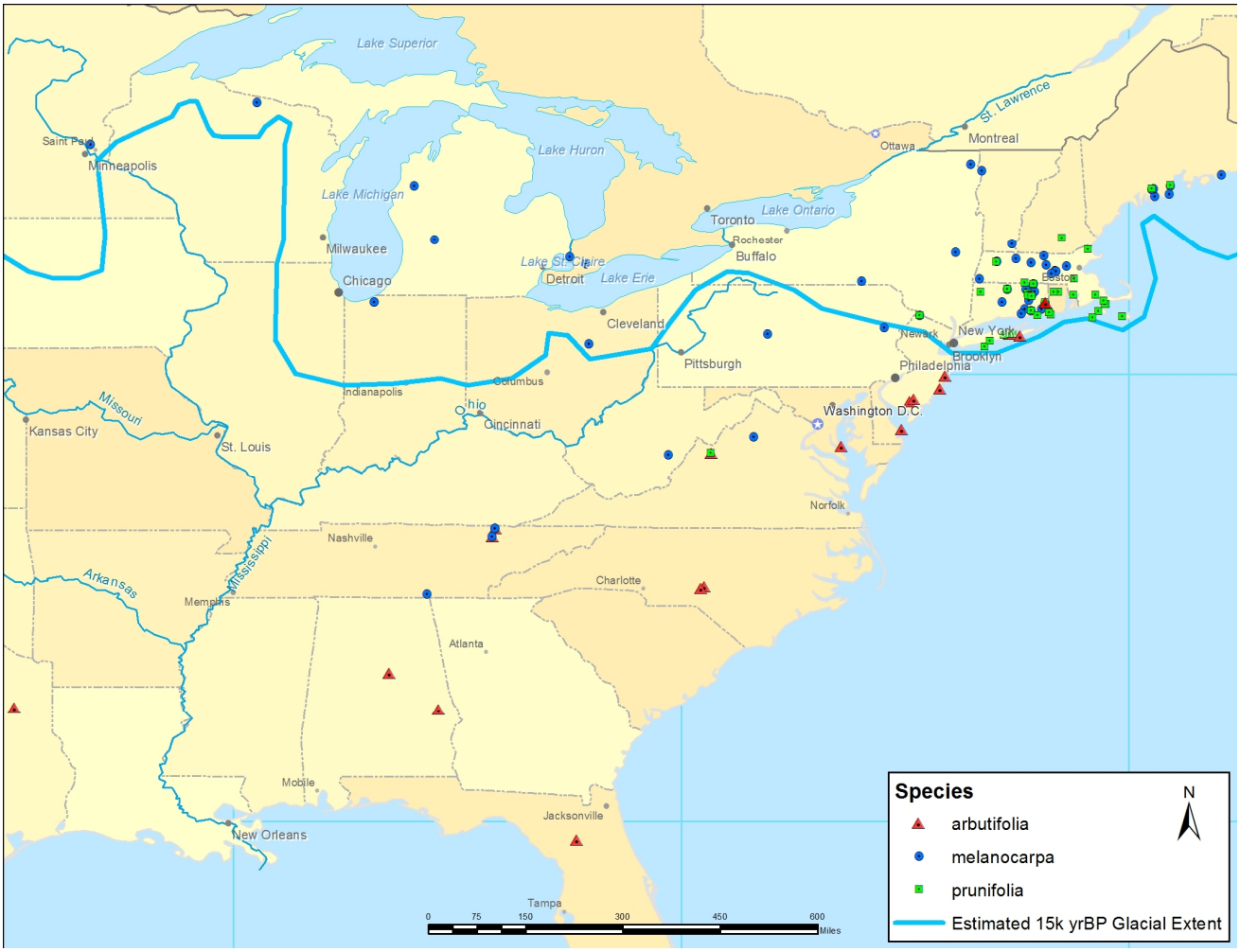

Figure 1. Distribution of *Aronia arbutifolia*, *A. melanocarpa*, and *A. prunifolia* in eastern North America.

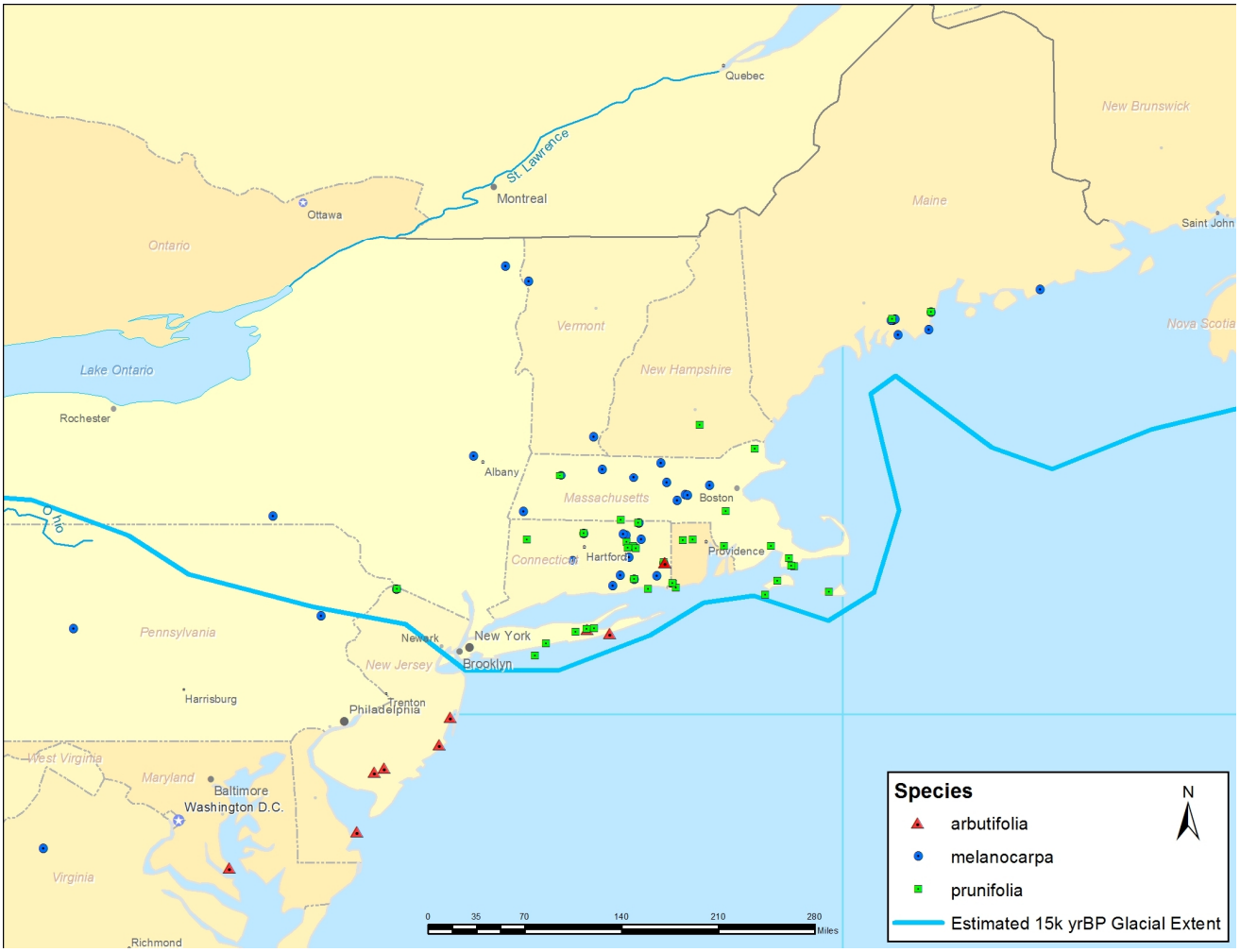

Figure 2. Distribution of *Aronia arbutifolia*, *A. melanocarpa*, and *A. prunifolia* in the northeastern United States.

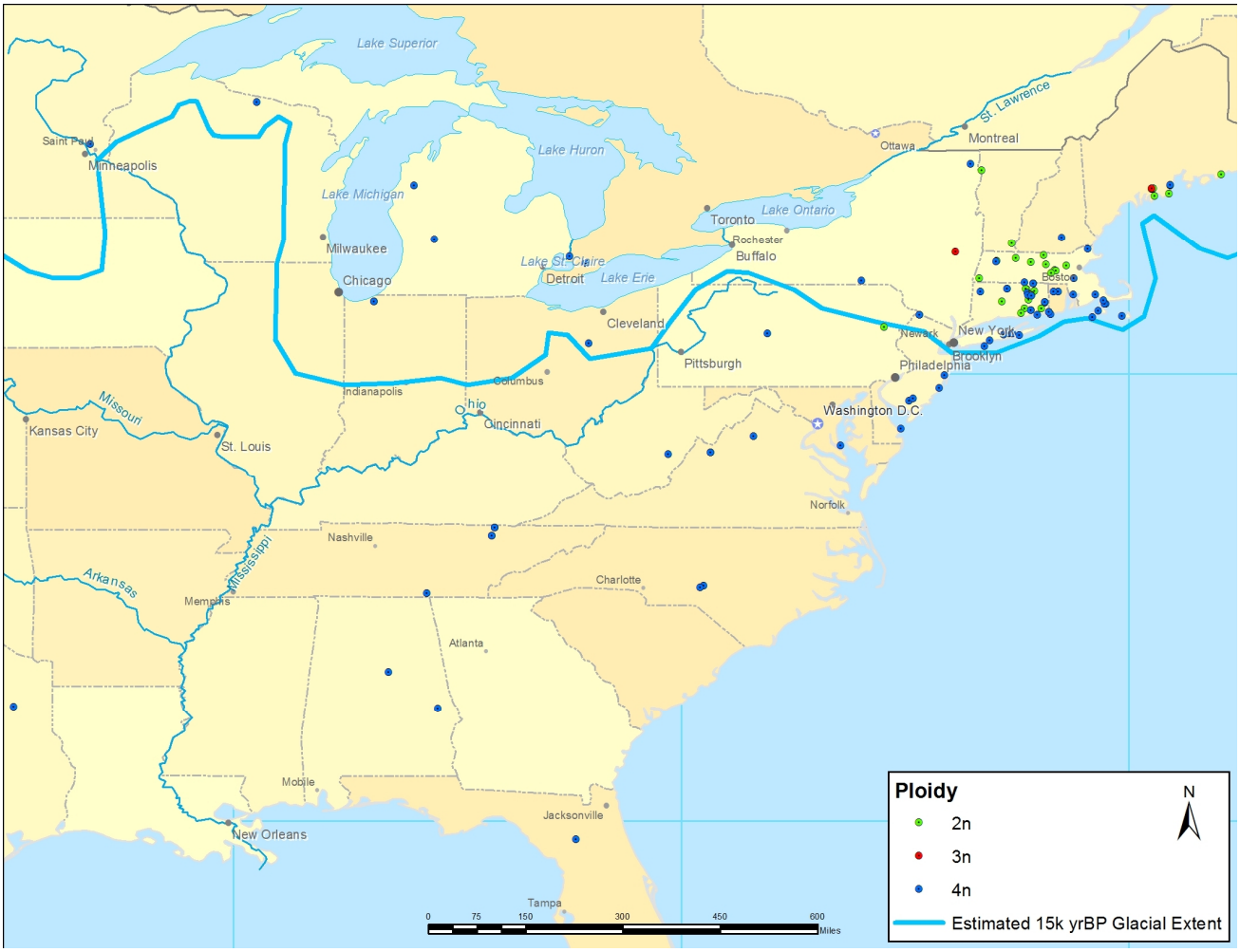

Figure 3. Ploidy of combined wild OTUs for eastern North America.

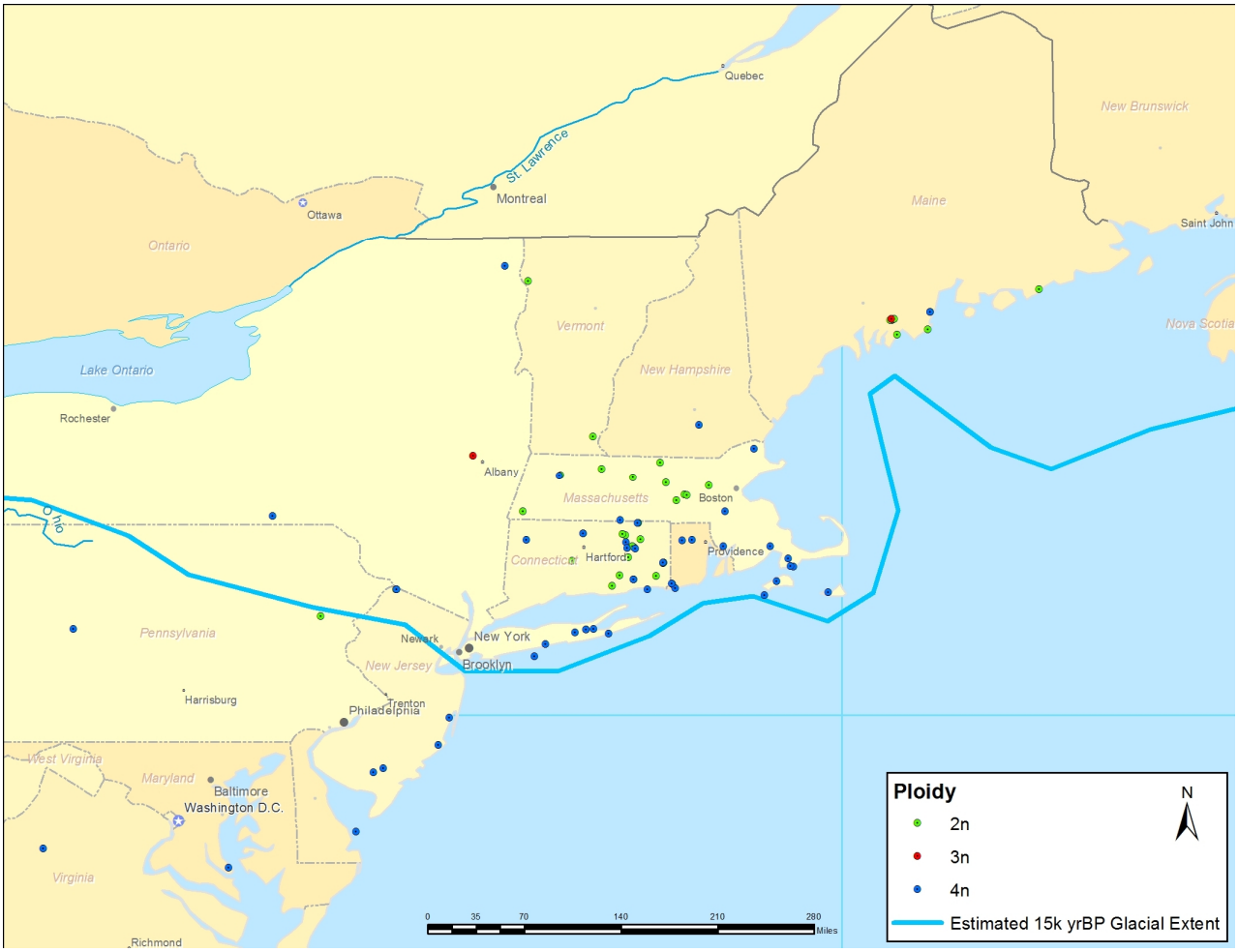

Figure 4. Ploidy of combined wild OTUs for northeastern United States.

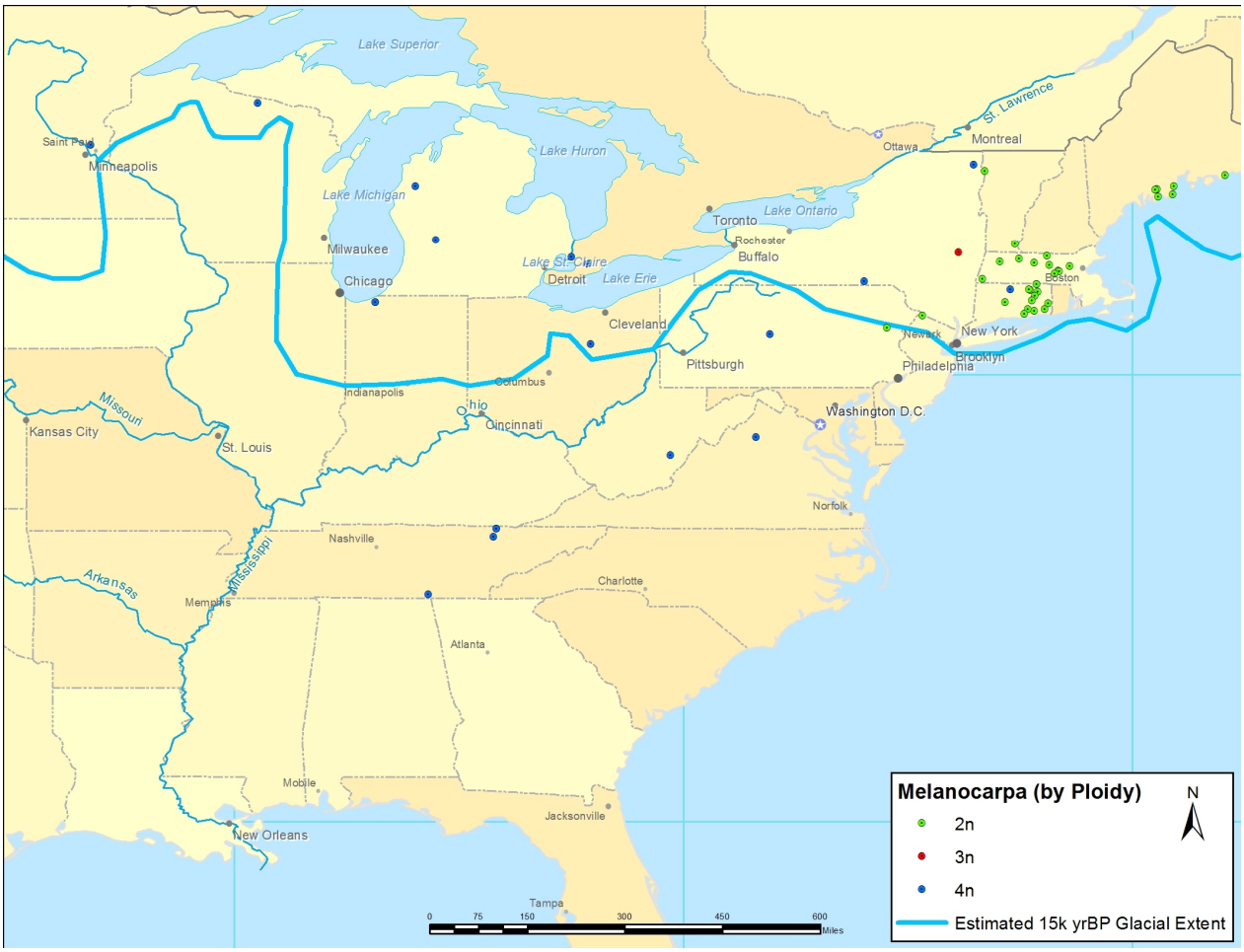

Figure 5. Ploidy distribution of *A. melanocarpa* only, eastern North America.

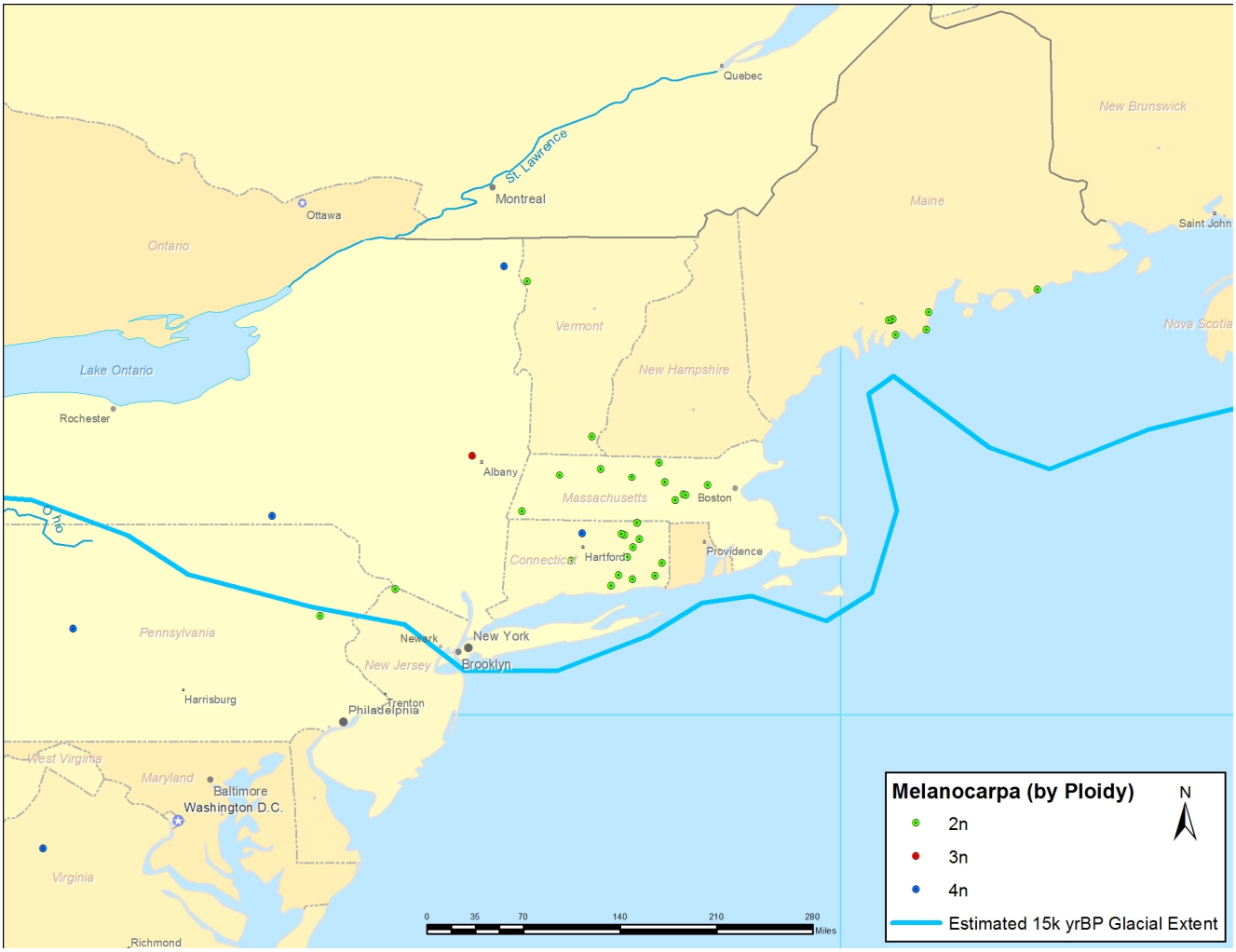

Figure 6. Ploidy distribution of *A. melanocarpa* only, northeastern United States.

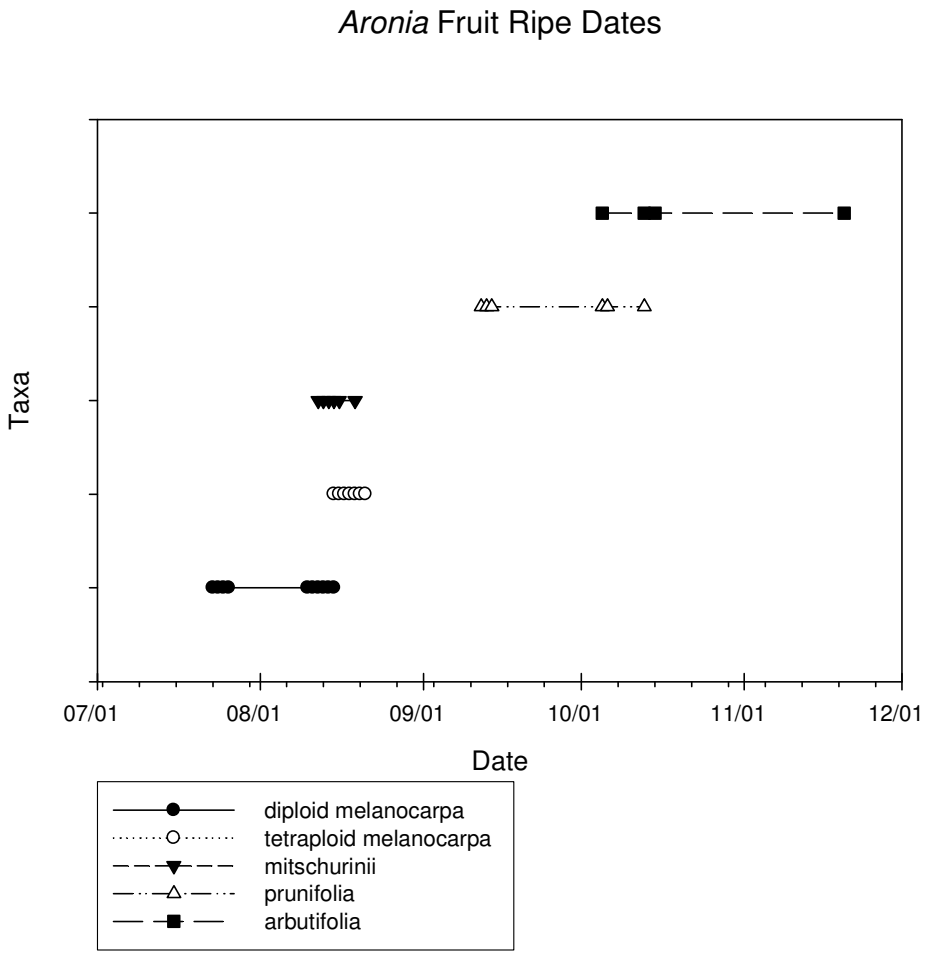

Figure 7. Fruit maturation dates of representative accessions of each OTU. 2n= *A. melanocarpa* diploid, 4n=*A. melanocarpa* tetraploid, Purple=*A. prunifolia*, Red= *A. arbutifolia*.

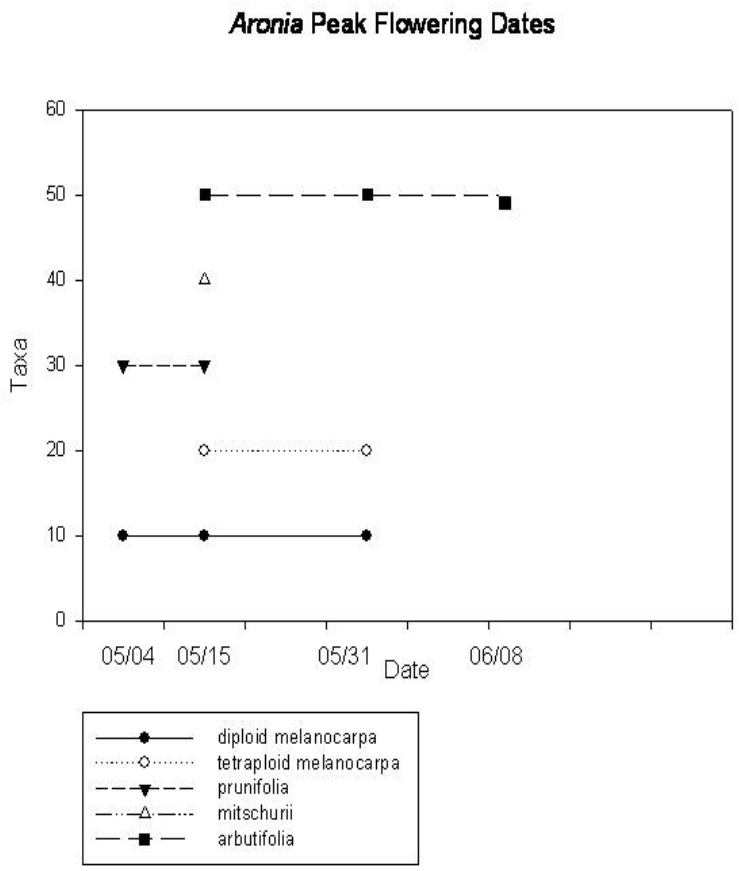

Figure 8. Peak flowering dates of representative accessions of each OTU. 2n= *A. melanocarpa* diploid, 4n=*A. melanocarpa* tetraploid, Purple=*A. prunifolia*, Red= *A. arbutifolia*.

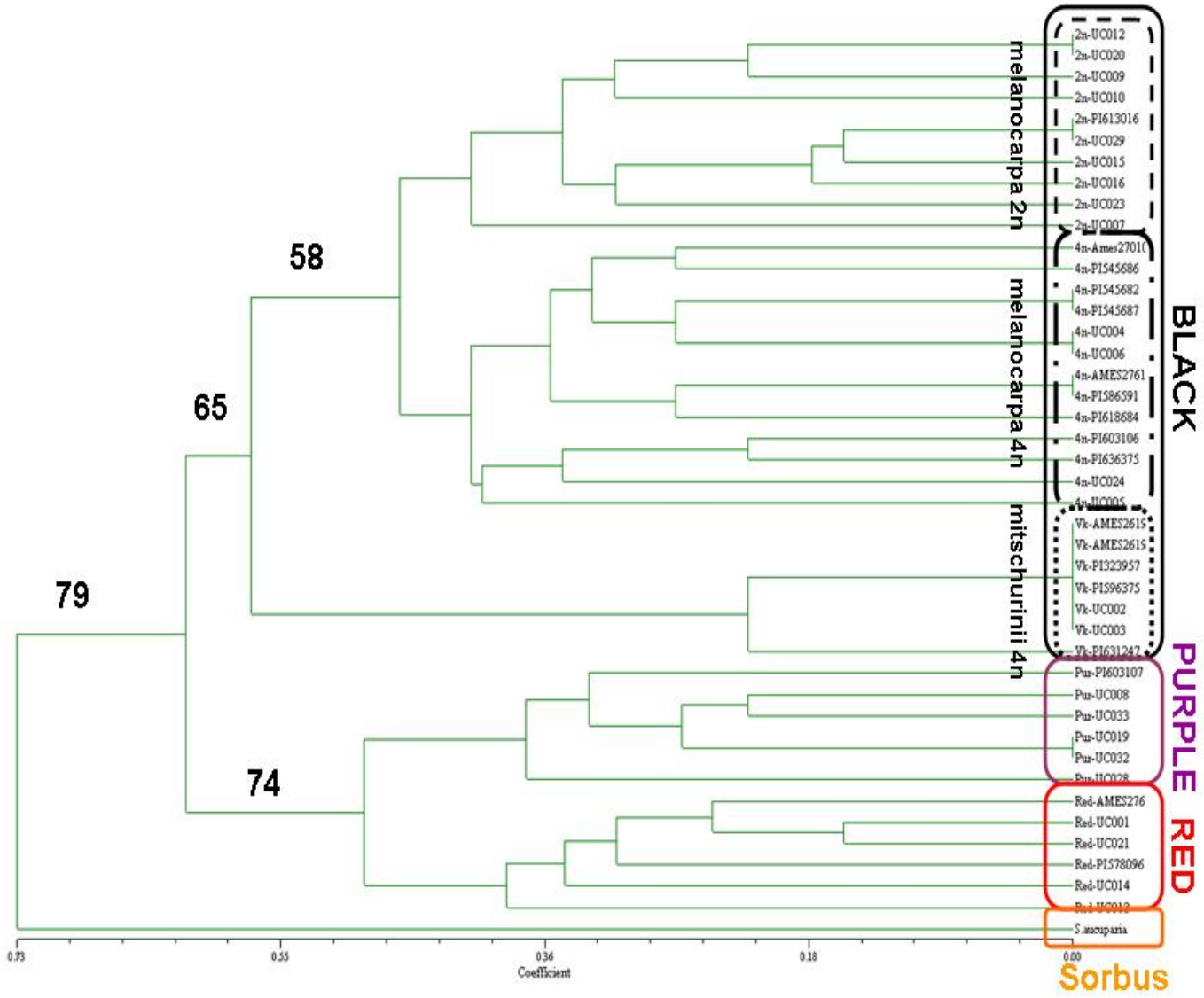

Figure 9. Phenogram of *Aronia* accession with bootstrap values shown for major OTU nodes.

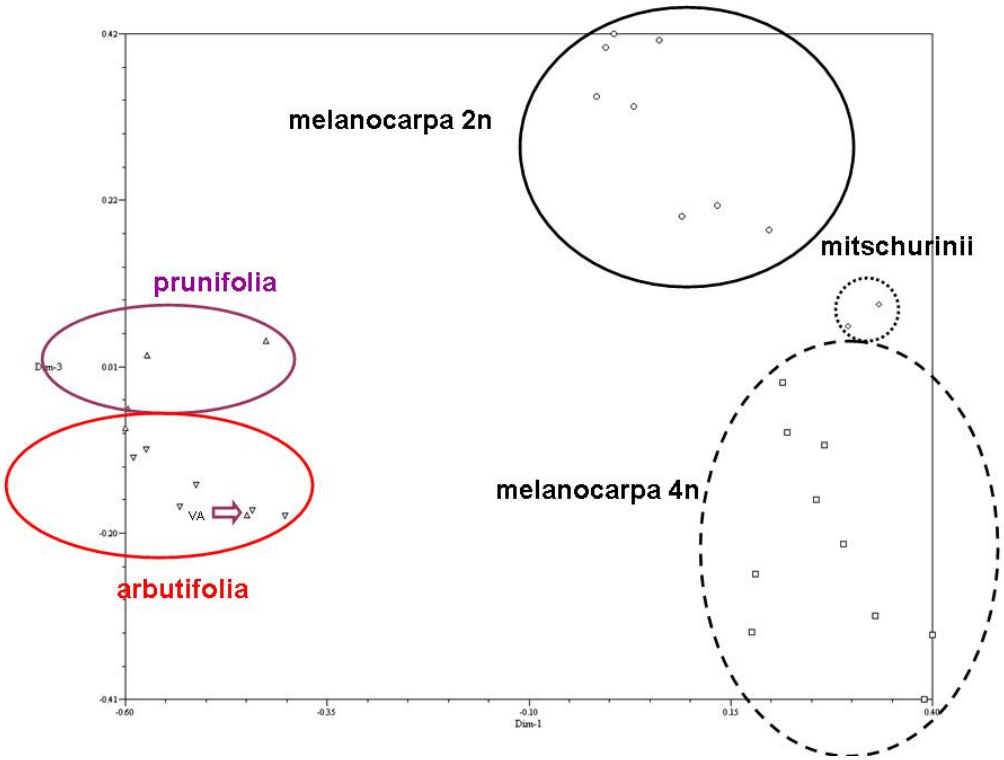

Figure 10. Principal Coordinates Analysis 2D scatter plot of select *Aronia* accessions. Purple arrow indicates a single *A. prunifolia* accession that clustered with *A. arbutifolia*.

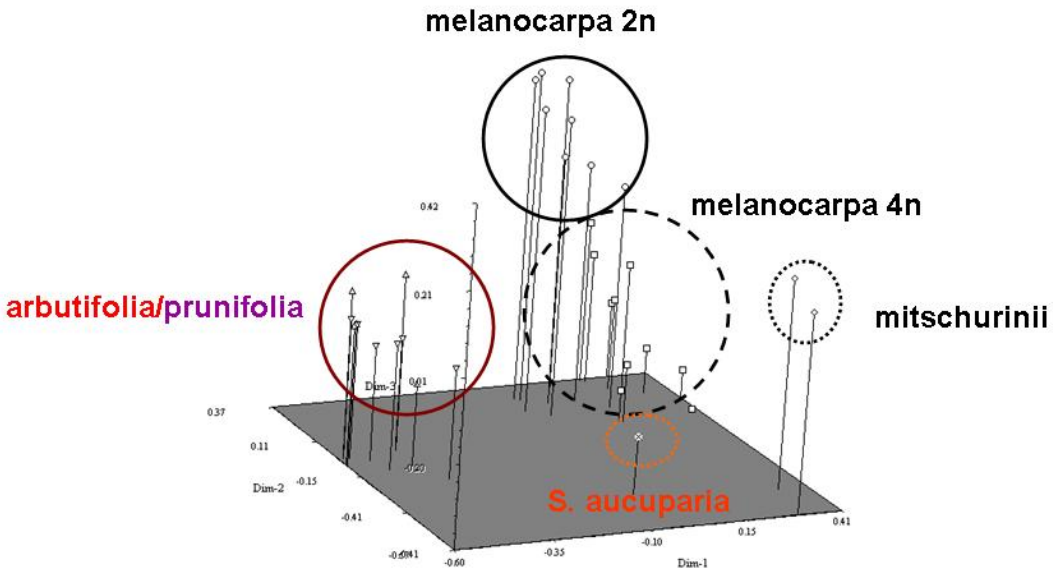

Figure 11. Principal Coordinates Analysis 3D scatter plot of select *Aronia* accessions

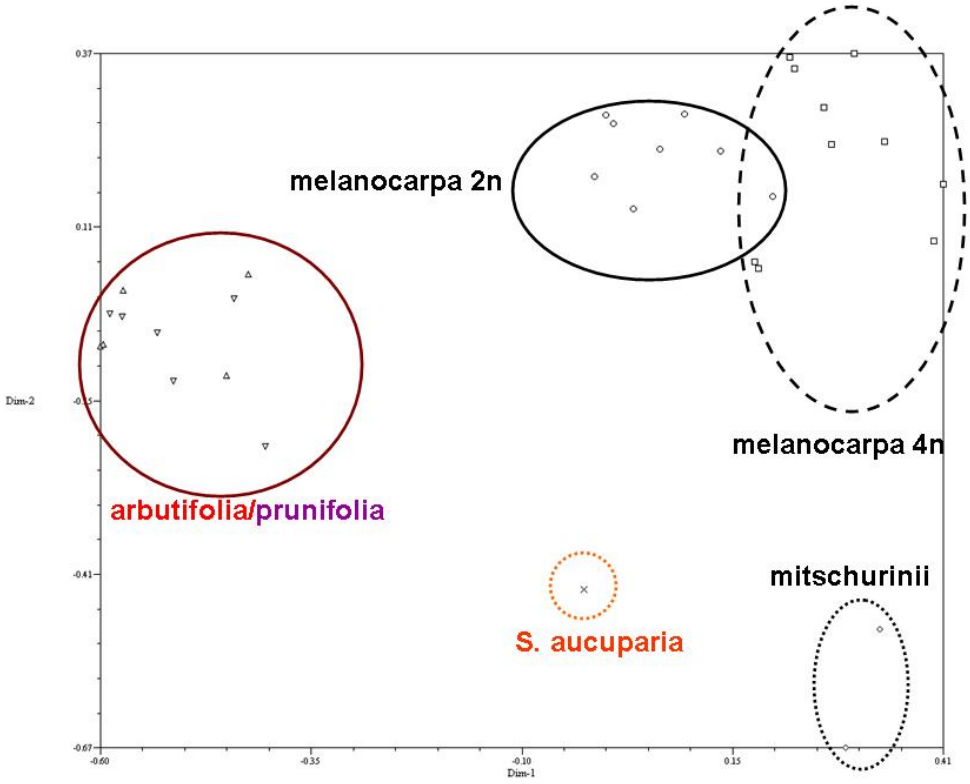

Figure 12. Principal Coordinates Analysis 2D scatter plot of select *Aronia* accessions with *Sorbus aucuparia*

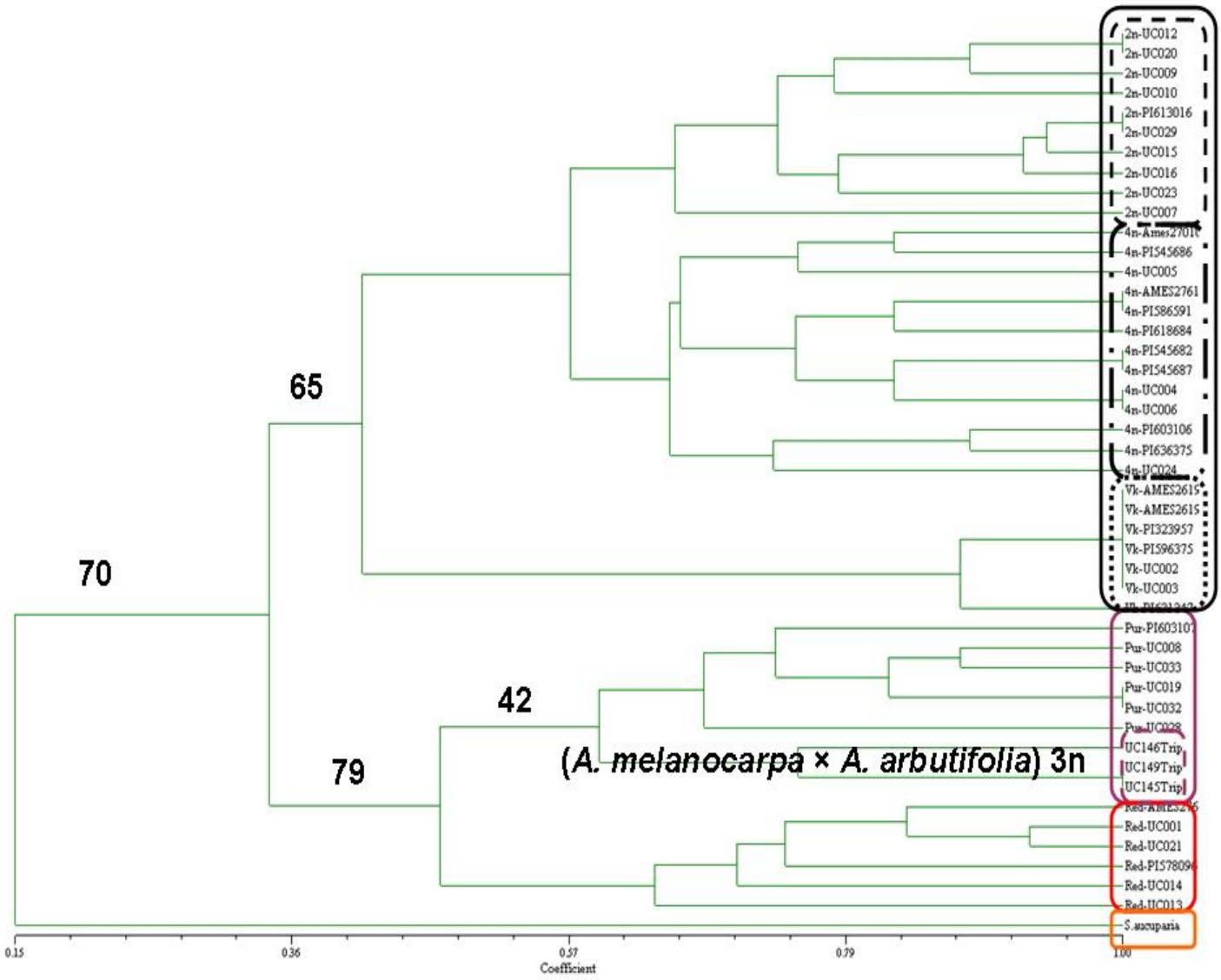

Figure 13. *Aronia* phenogram with triploids (2n *A. melanocarpa* × 4n *A. arbutifolia*) included that were created by intentionally hand pollination.

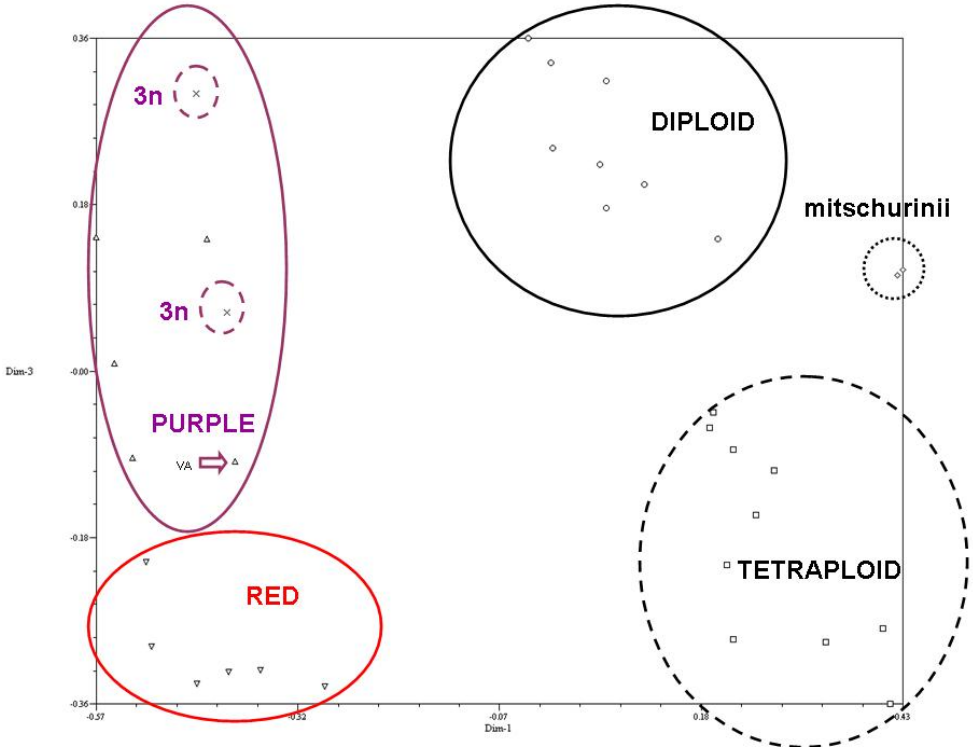

Figure 14. Principal Coordinates Analysis 2D scatter plot of select *Aronia* accessions with artificial triploid (2n *A. melanocarpa* × *A. arbutifolia*) included.

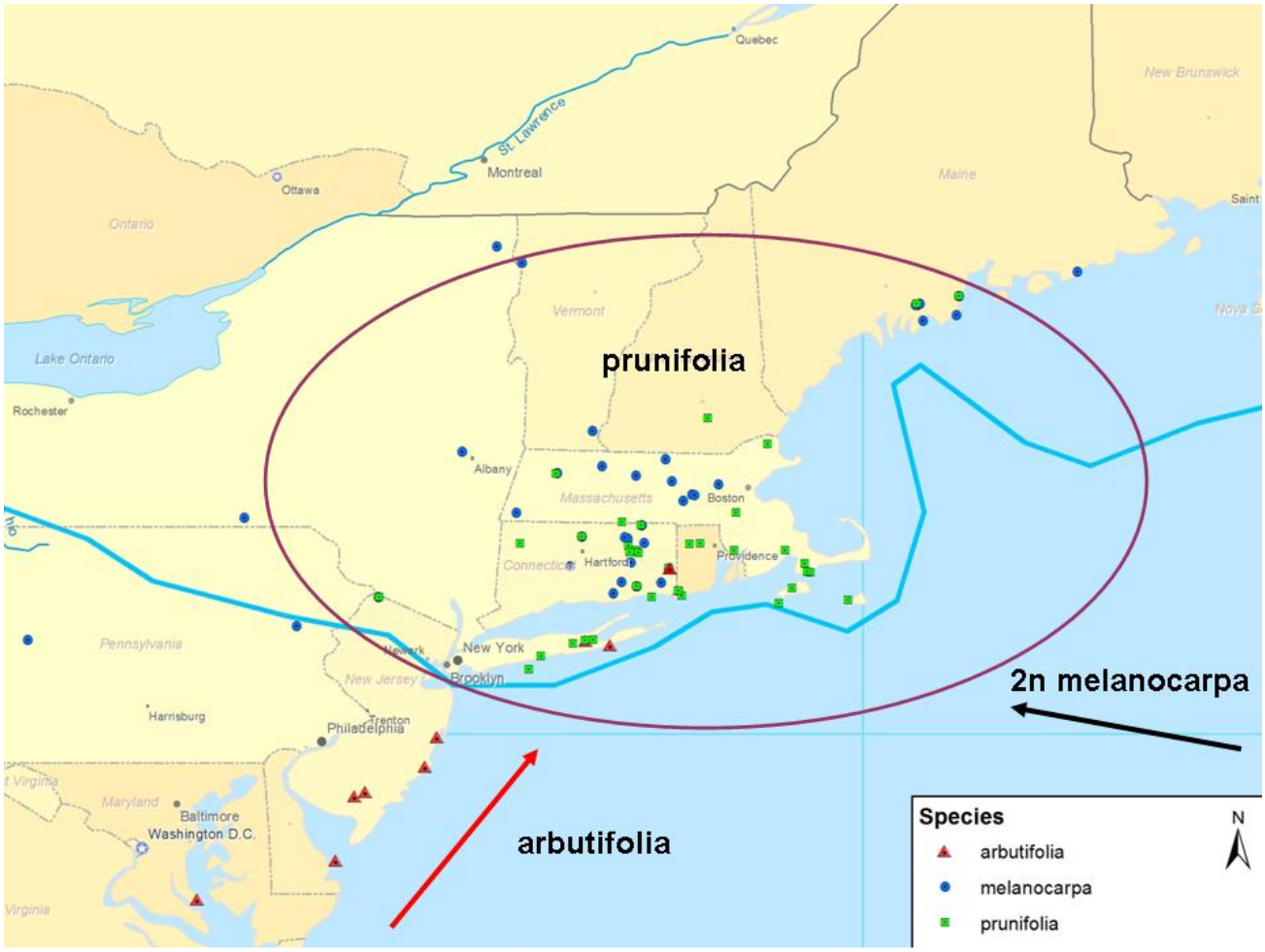

Figure 15. Hypothesized migration routes of *A. arbutifolia* and diploid *A. melanocarpa* into the overlap zone where hybridization and formation of *A. prunifolia* is likely to have occurred.

Chapter 2

Beneficial Biologically Active Compounds in *Aronia*

INTRODUCTION

Black chokeberry, *Aronia melanocarpa*, has a long history of use by humans as food and for medicine. Native Americans were familiar with the plant and the Potawatomi Indians were known to use the berries in pemmican, and to treat colds (Kokotkiewicz et al., 2010). Though native to eastern North America (Fernald, 1950; Hardin, 1973; Gleason and Cronquist, 1991; Haines, 2011), *Aronia* is seldom cultivated in its homeland, but the plant found fertile ground in Europe and became a major berry crop. Records exist of the genus being cultivated in Britain in 1700 (Loudon, 1838) and in the Botanical Garden of St. Petersburg, Russia by 1835 (Kask, 1987). *Aronia melanocarpa* was mainly cultivated as a curiosity until Russian plant breeder Ivan Michurin obtained seeds of it from Germany. Michurin hybridized *Aronia melanocarpa* with European Mountain Ash (*Sorbus aucuparia*) and created the cultivar 'Likernaya' in 1905. A seedling from 'Likernaya' was likely produced by backcrossing to *A. melanocarpa* and this offspring became the large-fruited, improved form we know in cultivation today (Kask, 1987; Leonard, 2011; Leonard et al., 2013). This improved *Aronia* cultivar did not come into wide cultivation until after Michurin's death when his colleague Mikhail Lisavenko began distributing cuttings, and promoting the plant (Skvortsov et al., 1983; Leonard, 2011). These larger fruited cultivars have been given their own species name of *Aronia mitschurinii* (Skvortsov and Maitulina, 1982).

Fresh fruits of *Aronia* are edible (Brand, 2010), but are sour and astringent (Scott and Skirvin, 2007), *A. melanocarpa* and A. *mitschurinii* are the species typically consumed. The processed fruit are utilized for juice, jam, pickles, wine, pies and baked goods (Kask, 1987; Facciola, 1990; Scott and Skirvin, 2007; Brand, 2010). The fruits are high in pectin making them suitable for mixed jams with other low pectin fruits (Facciola, 1990; Scott and Skirvin, 2007). By 1984, 17,800 hectares of *Aronia* were in cultivation in the Soviet Union, from the Baltic region to Sakhalin. In addition, it was the most common garden shrub in European Russia (Kask, 1987). In the 1970's, Finland began a breeding program with seed stock from Estonian germplasm (Kask, 1987). The cultivation of *Aronia* continued to expand globally. The Swedish began cultivating it in 1986 to find an effective food colorant (Kask, 1987; Jeppsson, 1999). *Aronia* was introduced to Japan, and has become popular in Poland, the Czech Republic, and Slovakia.

The darkly colored fruit of *Aronia melanocarpa* and *A. mitschurinii* are high in antioxidants in the form of anthocyanin and proanthocyanidin (Gasiorowski et al., 1997; Benvenuti et al., 2004; Wu, 2004a), and products processed from this fruit are increasingly sold as 'nutraceuticals'. *Aronia melanocarpa* and *A. mitschurinii* are commonly confused in the literature, with fruits of *A. mitschurinii* being the most commonly analyzed, but they have been reported as *A. melanocarpa.* Regardless, the antioxidants in *Aronia* are credited with cancer prevention, diminishing age-related memory loss, and better coronary health. *Aronia melanocarpa* has the highest known ORAC (Oxygen Radical Absorbance Capacity—a measure of antioxidant capacity), of

any fruit (Zheng, 2003). No toxic side effects of *Aronia* have been observed (Kokotkiewicz et al., 2010).

A review article by Kokotkiewicz et al. (2010) summarized the studies related to human health using *Aronia* fruit dry extract, concentrate, commercial extract, commercial dry extract, acetone extracts, leaf extract, nectar, juice, and sugar free juice. The authors report antimutagenic affects in the form of activity against benzo[a]pyrene and 2-aminofluorene, decreased genotoxity of benzo[a]pyrene and mitiomycin C, and reduced peroxide DNA strand breakage (Gasiorowski et al., 1997; Pool-Zobel et al., 1999). Anticancer properties of *Aronia* products are widely reported, with studies showing inhibition of colon cancer, carcinoma cells, and leukemia cells (Atanasova-Goranova et al., 1997; Sueiro et al., 2006; Bermudez-Soto et al., 2007a; Bermudez-Soto et al., 2007b; Saruwatari et al., 2008; Skupien et al., 2008). There are also a myriad of cardiovascular related studies on *Aronia*. Black chokeberry products have been shown to lower: total cholesterol, low density lipoproteins, high density lipoproteins, and triglycerides (Broncel et al., 2007; Naruszewicz et al., 2007; Skoczynska et al., 2007; Jurgonski et al., 2008; Valcheva-Kuzmanova et al., 2007a&b; Wroblewska et al., 2008). Additionally, *Aronia* has anti-hypertensive qualities and has been documented to lower arterial pressure (Broncel et al., 2007; Skoczynska et al., 2007). Extracts of this fruits are anti-aggregatory or blood clot preventing (Ryszawa et al., 2006). *Aronia* juice and nectar have hepatoprotective qualities. Rats given the toxic chemicals carbon tetrachloride, aminopyrine, and sodium nitrite had reduced histopathological changes in their livers (Atanasova-Goranova et al., 1997). Chemically induced gastric lesions in rats were also reduced in number, area, and severity with black chokeberry juice (Neidworok et al.,

1997; Hara et al., 2004; Matsumoto et al., 2004). Products from these fruits have also proven to be anti-diabetic, reducing glucose and fasting glucose blood levels in healthy and diabetic lab animals (Maslov et al., 2004; Simeonov et al., 2002; Jurgonski et al., 2008; Valcheva-Kuzmanowa et al., 2007a&b). *Aronia* is anti-inflammatory with 100 g of extract having comparable effects to 10 g of prednisolone in one study (Ohgami et al., 2005). Black chokeberry products have also been shown to be antibacterial, antiviral, radioprotective, and immunomodulatory (Andryskowski et al., 1998; Andryskowski et al., 1998b; Neidworok et al., 1999; Yaneva et al., 2002).

Previous work has been mostly on the improved black chokeberry *A. mitschurinii* or 'Viking' type cultivars. Only a very limited amount of work has been done assessing the antioxidant capacity of wild *Aronia*. One study in Illinois investigated the medicinal qualities of non-cultivated *Aronia* using local plants from two populations (Sueiro et al., 2006). This publication reported higher inhibitory effects of wild *Aronia* extract on leukemia cells than found for cultivated forms.

The Brand lab's collection of nearly 100 wild genotypes of *Aronia* provided a unique opportunity to carry out a study investigating the beneficial biologically active compounds in many *Aronia* genotypes including: ORAC; anthocyanin; and total phenol content. It is hypothesized that diploid and tetraploid black wild accessions of *Aronia* will have higher anthocyanin content and ORAC, because of their very dark purple-black ripe fruits, than *A. mitschurinii*. *Aronia prunifolia* and *A. arbutifolia* are thought to have much lower amounts of anthocyanins, likely resulting in lower ORAC and lower general phenol content. Our hope is that information from these assessments will lead to identifying *Aronia* genotypes with higher beneficial compound content that can be used

directly in products, or find accessions that could be candidates for a breeding program to enhance these compounds in future cultivars.

METHODS

Sampling

Sampling sites were located using herbarium specimens, literature-based data, and field surveys. Dunes, pitch pine forest, rock outcrops, heath barrens, swamps, and sphagnum bogs were studied intensively as they are known *Aronia* habitats. Accessions in addition to the ones Connolly or Brand collected were obtained from United States Department of Agriculture Germplasm Resources Information Network (http://www.ars-grin.gov/). Accessions were also obtained from colleagues in distant parts of the *Aronia* range. Initial assignment of species names was carried out using the keys from Fernald (1950), Gleason and Cronquist (1991), and Haines (2011).

Ploidy Determination

Flow cytometry is an automated way to evaluate plant ploidy. A modified version of the protocol in Arumuganathan and Earle (1991) summarized in Lehrer et al. (2008) was followed. Briefly, the analysis depends on using a standard plant with a known cytotype (e.g., a diploid is compared with an unknown of the same or a closely related species). Two to three newly emerged leaves were macerated using a fresh razor blade in nuclei suspending solution in a 55mm petri dish on a freeze pack. The methods were then modified in accordance with Meng and Finn (1999) by adding 2 g of PVP-10 per 50 ml

of extraction buffer and fluorescently staining released nuclei after filtering with propidium iodine, instead of during maceration. Relative fluorescence of total DNA was measured using a Becton-Dickson FACS Calibur Dual Laser Flow Cytometer (Becton, Dickson and Co., Franklin Lakes, NJ) at the Flow Cytometry and Confocal Imaging Facility at the University of Connecticut in Storrs, CT. The cytometer was equipped with an Argon ion laser emitting radiation at 488 nm. For each sample, 10,000-20,000 particles were measured. Data were logged and displayed in histograms by BD Cellquest ™ software (Becton, Dickson and Co., Franklin Lakes, NJ). Standard tetraploid and diploid sample histogram peaks were compared to samples of unknown ploidy. Peaks of the unknowns could be compared to the standards and categorized as diploid or tetraploid.

Growth and Field Establishment

Populations were sampled by collecting fruit, softwood cuttings, or by dividing out small vegetative shoots from wild parental plants. Collecting fruits was preferred because they were more likely to give a higher degree of genetic diversity. The *Aronia* fruits were harvested, fermented in water until soft, crushed, and the seeds washed and dried. Germination methods were according to Leonard (2011) as follows: in 2008, 2009, 2010, and 2011 seeds were cold stratified in moist sand for 90 days in 50ml conical cetrifuge tubes or polyethylene bags at 5ºC. After 90 days of stratification, seeds were germinated in potting medium with a ratio of 5:3:1 composted bark mulch, sphagnum peat moss, and sand that had been sifted using 0.5 cm hardware cloth screen. The woody plant growth media and seeds were placed in 32 oz. clear plastic salad trays with dome

lids. The environmental conditions were approximately 24ºC with cool white fluorescent light (40 µmol·m-2·s-1). Seedlings were transferred to standard 50 cells per flat plug trays with the same 5:3:1 mix, and placed in a heated (18ºC-27ºC) glass greenhouse.

Small vegetative shoots, which were composed of an above-ground portion of stem with leaves, rhizome, and roots, were separated from large shrubs in the field, placed in plastic bags with moist paper towels and transported to the UConn Floriculture greenhouses. The shoots were planted in the bark mulch, sphagnum peat moss, and sand media and kept under intermittent mist for 1-2 weeks, prior to being placed in a greenhouse.

Softwood cuttings were taken if seeds and small vegetative offshoots were not available. Portions of shoot 15 cm long were cut from plants in the field and placed in plastic bags with moist paper towels and transported to the greenhouses. Softwood cuttings were wounded at the basal end and for 1 cm along one side then dipped in Hormex #2 powder (Brooker Chemical Corporation Chatsworth, CA) or Hormodin #2 (3000 pm indole-3-butyric acid IBA, OHP, Inc.Mainland, PA) and stuck in the 5:3:1 media then placed under intermittent mist for approximately a month or until firmly rooted.

After plants reached a height of approximately 15-20 cm, they were transplanted into Jumbo Junior 4.5 inch pots with the same potting medium. When seedlings, vegetative shoots, and soft wood cuttings reached 45 cm they were moved into 2 gallon pots. Pots were placed outside in May, put on trickle irrigation and fertilized with slow release Osmocote® (formulation N-P_20_5- K_2O 17-6-10, 8-9 month formulation).

In late October, all the plants were moved to an unheated milky plastic-covered hoop house for the winter. The following spring, plants were established in the field at the UConn Plant Science Research and Education Facility located in Storrs, CT, USA 41º47'40.26"N 72º13'39.61"W, USDA plant hardiness zone 6a (USDA Plant Hardiness Zone Map, 2012). Soil series for the planting plot is either Woodbridge fine sandy loam or Paxton and Montauk fine sandy loams (Soil Survey Staff NRCS, 2012). Three replicates of each accession were laid out in a completely randomized design. Plants were placed in rows with 2m within row spacing and 3m between row spacing.

Harvest

Fruits were harvested in July, August, and September of 2010 and 2011 when pomes were assessed to be visually ripe. Berries were hand picked, placed in plastic freezer bags, and transported in a cooler with ice. In the lab fruits were placed in a -80 ºC freezer within 24 hours. Additionally, five accessions were sampled when visually ripe, than again two weeks later, *A. mitschurinii* types UC002 and UC003, *A. melanocarpa* diploids UC009 and UC023, and *A. melanocarpa* tetraploid PI545682 were selected for this subsampling. These accessions were tested for all anthocyanin types and phenolics twice to ascertain if antioxidant levels change with a later harvest date. Samples were shipped overnight on dry ice to the Analytical and Biological Chemistry Lab at the John F. Kennedy Space Center, Orlando, FL where analyses were performed. Upon arrival, samples were unpacked from the dry ice and immediately stored at -80 °C until analysis. Before analysis, pedicels and other debris were removed from the *Aronia* fruit. Fruits were then placed in a 50ml tube and weighed to determine fresh weight (FW). The samples were held on dry ice until they were placed in the lyophilizer. Fruits were

lyophilized to complete dryness followed by determination of dry weight (DW). Freeze dried berries were ground with a Wiley Mill under a stream of dry gaseous nitrogen to prevent the adsorption of moisture from the air. Powdered fruits of each sample were divided into two aliquots and placed in two sample vials, one for anthocyanin determination and one for ORAC/phenolic analysis. Sample vials were tightly closed and stored in a -80 C freezer until analysis.

Total anthocyanins and anthocyanin profile were determined by spectrophotometry and by HPLC (High-performance liquid chromatography), respectively. Approximately 100 mg (the sample was accurately weighed and documented for subsequent calculation) of each dry *Aronia* sample powder was placed in an ASE350 cell of the Dionex Automated Solvent Extraction System (Dionex Corporation Sunnyvale, California) and extracted with a solvent mixture of methanol:water:acetic acid 85:14.5:0.5(V/V/V). Extraction was carried out under 160 PSI of nitrogen at 100°C oven temperature for 10 minutes with a total volume of 14 ml. Extracts were filtered through 0.45 µm nylon membrane filters and subjected to HPLC analysis as described in Wu et al. (2004a) with minor modification to optimize the separation of individual anthocyanin. The sample extract (10 µl) was injected onto the HPLC and its constituents were separated on an analytical column (Zorbax SB-C18, 250 mm x 4.6 mm with an Altima C18 5µm guard column) using a mobile phase mixture consisting of 10% acetic acid (Mobile Phase A) and 100% methanol (Mobile Phase B) with varying proportions over time at a flow rate of 0.8 ml/min and column oven temperature of 30 ^{o}C. Anthocyanins were detected by a diode array UV-Vis detector at 520 nm with a bandwidth of 10 nm. Anthocyanin standards purchased from Sigma

Aldrich were analyzed in the same way as the sample extract, and used to identify the anthocyanin constituents in the sample extract based on the match of retention time, UV spectrum and mass spectrum. A precise calibration curve (a relationship between the HPLC peak area and anthocyanin quantity) was established using the standard Kuromanin Chloride (cyanidin-3-O-glucoside), subsequently all other anthocyanin constituents were calculated as the equivalent amount of cyanidin-3-O-glucoside. The same sample extracts were subjected to spectrophotometric analysis at 530 nm for the quantification of total anthocyanin content as cyanidin-3-O-glucoside equivalence (Kleinhenz et al., 2003).

A separate extract was prepared for the determination of Oxygen Radical Adsorption Capacity (ORAC) and total phenolics in *Aronia* berries. Twenty five milligrams (25 mg) of the freeze dried *Aronia* powder was placed in an ASE350 cell and extracted with acetone:water:acetic acid at the ratio of 70%:29.5%:0.5%. The sample extract of 0.2 ml was subsequently reacted with Folin-Ciocalteu phenol reagent for the determination of total phenolics by the modified Folin-Ciocalteu assay (Prior et al., 2005). A series of known concentrations of gallic acid was prepared and reacted with the same reagent to create a calibration curve that was in turn used to determine the concentration in the sample extracts. Consequently, the total phenolics were expressed as gallic acid equivalent. Another sample of 0.2 ml of 1/50 diluted extract was subjected to ORAC assay on a 96 well plate format as described in (Wu et al., 2004b; Prior et al., 2005).

Univariate Character Analysis and Correlations

ORAC, anthocyanin, and phenolic values were analyzed using SAS for Windows 9.3. An analysis of variance using the PROC GLM function was used to compare the mean difference between diploid *A. melanocarpa*, tetraploid *A. melanocarpa*, *A. prunifolia*, *A. mitschurinii*, and *A. arbutifolia*. Least squares (LS) means were used to separate mean values, where p values of 0.05 or less were considered significant.

RESULTS

All individual accession means and standard errors for all beneficial biochemical compounds analyzed are reported in Tables 1 & 2. Significant differences were found in 2010 and 2011 fruit samples between diploid *A. melanocarpa*, tetraploid *A. melanocarpa*, *A. mitschurinii*/Viking types, and *A. prunifolia* (Table 3). Average accession ORAC reported as µM Trolox Eq/g dry weight in 2010 ranged from PI60310 tetraploid *A. melanocarpa* (1091.5) to PI631247 *A. mitschurinii* (519.6). ORAC by species was highest for diploid *A. melanocarpa* (881.4) that was significantly different than all other *Aronia* taxa, tetraploid *A. melanocarpa* (763.5) had the second highest mean that was significantly different than *A. mitschurinii* (621.8), but the tetraploid *A. melanocarpa* was not different than *A. prunifolia* (710.3), and *A. prunifolia* and *A. mitschurinii* also were not statistically different. Mean anthocyanin levels, in µmol/g DW, were highest in accession UC023 diploid *A. melanocarpa* (103.5) and lowest in UC019 *A. prunifolia* (7.8). By species anthocyanin levels were greatest on average in diploid *A. melanocarpa* (69.6) that again were statistically higher than all other *Aronia* types, *A. mitschurinii* (51.5), and tetraploid *A. melanocarpa* (49.0) shared a mean, while the lowest levels were

found in purple chokeberry *A. prunifolia* 25.4 (Table 3).Total phenolics were highest in accessions UC011 *A. prunifolia* (982.1) and lowest in UC003 *A. mitschurinii* (468.3) . Ranked by taxa *A. prunifolia* had relatively high levels of total phenolics (813. 2 mol/g DW), but only statically higher than *A. mitschurinii* (626.7 mol/g DW). Large fruited *A. mitschurinii* types contained significantly more water than the other *Aronia* species in 2010.

For the four separate anthocyanin types in 2010 there were also differences between the taxa. Anthocyanin types have been abbreviated as follows Cy3Gal = cyanidin-3-galactoside, Cy3Glu = cyanidin-3-glucoside, Cy3A = cyanidin-3-arabinoside, CyX = cyanidin-3-xyloside. All means in units of (µmol/g DW) are also reported in Table 3. Diploid *A. melanocarpa* (47.9) had the highest average for Cy3Gal , followed by *A. mitschurinii* (34.2) and tetraploid *A. melanocarpa* (32.5) that were not significantly different from each other but were separate from *A. prunifolia* (16.2). *Aronia mitschurinii* (1.77) fruits contained the most Cy3Glu followed by diploid *A. melanocarpa* (1.2) and tetraploid *A. melanocarpa* (1.0) that were not significantly different from each other, but all were statistically above *A. prunifolia* (0.6). Cy3A showed no difference between diploid *A. melanocarpa* (18.9), tetraploid *A. melanocarpa* (14.3), and *A. mitschurinii* (13.9), though all were higher than *A. prunifolia* (7.8). *Aronia mitschurinii* (1.6) and diploid *A. melanocarpa* (1.6) had higher levels of CyX, than *A. prunifolia* (0.7), but tetraploid *A. melanocarpa* (1.2) overlapped with both the higher and lower means. Total phenols also reported as (µmol/g DW) showed significant variation: *A. prunifolia* (813.2), was higher than *A. mitschurinii* (626.7), though tetraploid *A. melanocarpa* (732.1) and diploid *A. melanocarpa* (726.2) shared both the higher and lower averages.

The five *Aronia* accessions that were sampled when visual ripe and again two weeks later in 2010, though not statistically tested, showed a few interesting trends. Sample sizes were small and a more comprehensive study is needed to show statistical significance. The apparent trends are increasing levels of all anthocyanin types between the first and second sampling dates, while at the same time total phenolic compounds generally decreased. These trends can be seen in four out of five of the *Aronia* accessions tested (Table 4).

In 2011, *A. arbutifolia* accessions were added to the sampling, but was not included in some of the analyses due to small sample size. The sum of anthocyanin was highest in accession PI578096 *A. arbutifolia* (82.1) and lowest in UC022 diploid *A. melanocarpa* (7.2). The species rank for sum anthocyanins was as follows: *A. mitschurinii* (43.0) and diploid *A. melanocarpa* (42.2), with tetraploid *A. melanocarpa* (38.2) over lapping with the higher mean and *A. arbutifolia* (21.25), while *A. prunifolia* (13.0) had the lowest value (Table 3). The ORAC values in 2011 were from UC074 diploid *A. melanocarpa* (937.9) to UC012 diploid *A. melanocarpa* (449.3). The taxa were in the same order for ORAC in 2011 and 2010, in 2011 only two taxa were significantly different, diploid *A. melanocarpa* (713.8) and *A. mitschurinii* (574.6). Individual anthocyanins in units of μmol/g DW for 2011 were as follows: Cy3Gal diploid *A. melanocarpa* (28.0) and *A. mitschurinii* (27.9) being significantly higher than *A. prunifolia* 14.65, with tetraploid *A. melanocarpa* (24.6) sharing both means. For Cy3Glu *A. mitschurinii* (1.5) was higher than all other *Aronia* types. Cy3A diploid *A. melanocarpa* (12.6) and *A. mitschurinii* (12.3) had the highest value and *A. prunifolia* (5.4) the lowest. CyX content was greatest in *A. mitschurinii* (1.4), and least in *A.*

arbutifolia (0.6), and *A. prunifolia* (0.4). In 2011 total phenols µmol/g DW were highest in accession UC081 *A. arbutifolia* (891.0) and lowest in Ames26195 tetraploid *A. melanocarpa* (424.6), and by species phenolics were highest in *A. arbutifolia* (661.1), and lowest diploid *A. melanocarpa* (521.3).

DISCUSSION

Viking or *A. mitschurinii* types are the only *Aronia* varieties grown on an industrial scale. These types have made up nearly all the material used in studies of *Aronia* health benefits. Here we have shown that Viking types generally have the lowest ORAC values, and have about the same or slightly lower total anthocyanin amounts than *A. melanocarpa* wild accessions. Noteworthy, wild accessions with high anthocyanin or ORAC values content are UC023, UC007, and UC009 all diploid *A. melanocarpa* types. These three accessions will likely be valuable for future breeding efforts for both their biologically active compounds and the fact that they can produce true sexual seed. PI603106 a tetraploid *A. melanocarpa* accession with high anthocyanin content may also have some limited use in a breeding program.

Harvest date did affect fruit composition. The same accessions harvested at visual ripeness and two weeks later generally showed higher anthocyanin levels in the later harvest sample. In contrast total phenolics were lower from later harvested fruits. These differences in biochemistry may have practical applications if there are different therapeutic uses for anthocyanins versus phenolics.

Surprisingly, *A. prunifolia* and *A. arbutifolia* were found to have some of the highest phenolic compound levels; before our investigations these species had not been previously tested for beneficial biochemically active compounds. The results we report for *A. prunifolia* and *A. arbutifolia* open up new avenues of research for perhaps entirely new medicinal benefits of this genus. *Aronia prunifolia* and *A. arbutifolia* are generally lower in anthocyanin relative to *A. mitschurinii* and *A. melanocarpa*, but appear to be rich in other antioxidants. *Aronia prunifolia* and *A. arbutifolia* have recently been shown to have higher levels of proanthocyanins and phenolic acids (Taheri et al., 2013) and these are compounds that may be responsible for the high ORAC values. Even though genetic studies have shown all varieties of *A. mitschurinii* to be closely related clones (Persson et al., 2004), some accessions were significantly different from each other in ORAC and anthocyanin levels. In this case it is difficult to separate out genetic differences and environemental factors. Soil fertility and different fertilizers have been shown to induce different pigment levels in *Aronia* fruit, many of which are antioxidants (Jeppsson, 1999; Jeppsson, 2000a&b). Further comparisons of the *A. mitschurinii* types needs to be done to clarify if there are real genetic differences between the named varieties. Wild *A. melanocarpa*, both tetraploid and diploid, in some cases showed higher ORAC, anthocyanin, or phenolics compounds, and had different amounts of individual anthocyanins levels than *A. mitschurinii* types. This means that wild types of *Aronia melanocarpa* may be more effective treatment for human health issues than the *A. mitschurinii* types. These wild types though present agronomic challenges. Viking/*A. mitschurinii* types have much larger, longer lasting, and disease resistant fruits. The wild type berries are small, do not stay turgid on the shrubs for nearly as long, and often get an

unidentified rot in wet weather. Wild types have a very narrow window when the fruits are fully ripe before the fruit wither and fall off. As a general rule diploids had higher ORAC scores and more anthocyanin. Thus, diploid *A. melanocarpa* types are key to improving the ORAC values, though a few wild tetraploid *A. melanocarpa* scored relatively well. The diploid *A. melanocarpa* are also the only plants that can produce true sexual offspring; crosses between wild tetraploid *A. melanocarpa* and Viking/ *A. mitschurinii* types would be nearly impossible. Though diploid types yield sexual seed, it is not easy to successfully produce them with Viking types. Chapter 3 deals with pathways to improvement for *Aronia*, as part of that research, 135 crosses between *A. mitschurinii* types and diploid *A. melanocarpa* produced five seedlings, only one of these seedlings has been confirmed as *bona fide* hybrid, the rest may be products of accidental self pollination. It appears that *A. mitschurinii* has low pollen viability, pollen germination studies need to be performed in the future. *Aronia mitschurinii* are superior in fruit production and it is probably the combination of *Aronia* and *Sorbus* genetics that gives it the turgid fruits that stay for weeks on the plants.

The data reported here is the first major analysis of wild *Aronia* genotypes for biochemically active beneficial compounds. Now that these promising accessions have been identified, the sexual reproduction of the genus is fairly well understood (chapter 1). We understand what taxa can be crossed with each other (chapter 3), and the origin of the improved *A. mitschurinii* types has been elucidated (Leonard, 2011; Leonard et al., 2012). The ground work has been laid, and the general way forward for a breeding program is now clear. We have now begun the route to improvement using diploid ×*Sorbaronia fallax* (*Sorbus aucuparia* × *A. melanocarpa*) and back crossing it to high

ORAC, high anthocyanin diploid *A. melanocarpa*. The first round of cross pollination of UC023, UC007, and PI603106 to ×*Sorbaronia fallax* was started in the spring of 2013. We hope that offspring of these crosses will yield vigorous plants, with large fruits that persist on the shrubs without rotting, and also contain high levels of beneficial compounds.

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Table 1. Summary of anthocyanin content in units of (μmol/g DW) means ± standard error for *Aronia* accessions in 2010 and 2011.

		Cy3Gal†		Cy3Glu		Cy3A		CyX		Sum	
		2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Accession	sp. *	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean
Ames26194	mit	36.4±3.8	n/a	2.1±0.2	n/a	14.2±1.3	n/a	1.8±0.2	n/a	54.5±5.4	n/a
Ames26195	mit	34.8±4.1	29.7±n/a	1.6±0.3	1.6±n/a	14.6±1.6	13.5±n/a	1.5±0.2	n/a	52.6±5.8	46.4±n/a
Ames27010	me4n	23.1 ±1.0	20.9±1.5	0.9±0.0	0.7±0.1	11.4±0.6	11.8±1.0	1.2±0.1	1.2±0.3	36.5±1.8	33.6±2.6
Ames27615	me4n	36.4 ±3.7	n/a	1.0±0.1	n/a	17.6±1.8	n/a	1.2±0.1	n/a	56.1±5.7	n/a
PI323957	mit	33.2±n/a	25.4±1.8	1.9±n/a	1.3±0.1	13.3±n/a	11.1±0.7	1.7±n/a	1.3±0.1	50.2±n/a	39.2±2.6
PI545682	me4n	n/a	n/a	0.5±n/a	n/a	8.4±n/a	n/a	0.5±n/a	n/a	30.7±n/a	n/a
PI545687	me4n	23.9±1.1	n/a	1.0±0.0	n/a	9.6±0.4	n/a	0.6±0.0	n/a	35.1±1.6	n/a
PI578096	arbu	n/a	8.2±0.9	n/a	0.3±0.1	n/a	2.4±0.3	n/a	0.1±0.1	n/a	7.3±3.7
PI586591	me4n	22.6±1.8	14.7±n/a	0.8±0.0	0.5±n/a	13.3±0.3	9.6±n/a	1.1±0.1	0.8±n/a	37.8±2.2	25.6±n/a
PI596375	mit	32.1±2.7	25.1±1.0		1.3±0.1	12.3±0.6	11.7±0.4	1.3±0.2	1.2±0.1	47.0±2.6	39.4±1.8
PI603106	me4n	17.9±0.9	n/a	1.3±0.1	n/a	7.1±0.4	n/a	0.8±0.0	n/a	27.1±1.4	n/a
PI603107	prun	8.2 ± 0.4	8.8±0.5	0.4±0.0	0.4±0.0	4.6±0.2	4.5±0.2	0.4±0.0	0.4±0.0	13.6±0.6	14.0±0.8
PI613016	me2n	n/a	22.1±5.1	n/a	0.7±0.1	n/a	12.5±2.7	n/a	1.0±0.3	n/a	36.2±8.0
PI618684	me4n	38.6±0.9	n/a	1.1±0.0	n/a	18.1±0.6	n/a	1.2±0.1	n/a	58.9±1.6	n/a
PI631247	mit	29.0±0.8	25.4±1.0	1.7±0.1	1.3±0.0	11.5±0.2	11.1±0.5	1.4±0.1	1.3±0.1	43.7±1.2	39.0±1.6
PI636375	me4n	43.8±12.2	24.0±1.8	1.6±0.8	0.6±0.0	17.2±4.7	11.8±0.7	1.9±0.9	0.8±0.0	64.5±18.4	37.2±2.6
UC002	mit	35.2±3.6	27.7±1.4	1.6±0.2	1.5±0.1	14.4±2.2	12.4±0.7	1.6±0.3	1.4±0.1	52.9±6.2	42.9±2.2
UC003	mit	32.8±4.4	35.7±3.4	2.1±0.3	1.8±0.2	14.9±1.8	15.3±1.5	2.1±0.3	1.7±0.2	51.9±6.7	54.6±5.2
UC004	me4n	30.7±2.0	6.6±1.1	1.1±0.1	0.6±0.1	13.2±0.6	9.0±0.5	1.1±0.1	0.6±0.0	46.1±2.7	26.8±1.7
UC005	me4n	42.1±4.5	26.6±0.9	1.0±0.1	0.6±0.0	16.7±1.4	13.1±0.4	1.5±0.1	0.9±0.0	61.3±6.2	41.1±1.3
UC006	me4n	41.5±2.7	n/a	0.8±0.1	n/a	14.0±0.9	n/a	0.8±0.1	n/a	57.2±3.8	n/a
UC007	me2n	59.2±4.0	27.8±2.0	1.7±0.1	0.9±0.1	32.6±2.8	17.4±1.1	3.6±0.4	1.5±0.1	97.0±7.3	47.6±3.3
UC008	prun	30.1±4.0	n/a	1.1±0.4	n/a	12.5±1.8	n/a	1.2±0.4	n/a	44.9±6.2	n/a
UC009	me2n	58.9±1.3	69.1±4.9	0.8±0.0	1.0±0.0	1.1±0.0	1.3±0.0	0.0	0.0	60.7±1.3	71.3±4.9
UC010	me2n	48.1±1.6	n/a	1.0±0.2	n/a	16.0±0.6	n/a	1.1±0.2	n/a	66.2±2.1	n/a
UC011	prun	20.8±1.2	9.3±0.7	0.7±0.1	0.3±0.0	11.1±0.9	4.8±0.6	1.2±0.1	0.5±0.0	33.8±2.3	14.9±1.3
UC012	me2n	23.4±0.2	11.1±1.9	0.7±0.0	0.3±0.0	11.7±1.0	5.6±0.7	0.8±0.1	0.4±0.0	36.6±1.1	17.4±2.7
UC015	me2n	n/a	29.8±1.1	n/a	0.8±0.0	n/a	16.0±0.6	n/a	1.5±0.0	n/a	48.0±1.7
UC016	me2n	39.5±2.6	n/a	1.1±0.1	n/a	22.0±1.4	n/a	1.5±0.1	n/a	64.1±2.4	n/a
UC019	prun	4.9±0.4	n/a	0.3±0.0	n/a	2.3±0.2	n/a	0.4±0.0	n/a	7.8±0.6	n/a
UC020	me2n	42.4±10.2	n/a	1.2±0.4	n/a	19.5±5.5	n/a	2.0±1.1	n/a	65.2±16.7	n/a
UC022	me2n	n/a	41.0±n/a	n/a	1.0±n/a	n/a	23.7±n/a	n/a	1.5±n/a	n/a	67.1±n/a
UC023	me2n	69.0±3.7	n/a	1.6±0.1	n/a	30.4±1.2	n/a	2.5±0.1	n/a	103.5±5.0	n/a
UC024	me4n	51.3±0.7	n/a	1.2±0.0	n/a	20.3±0.0	n/a	1.9±0.0	n/a	74.7±0.7	n/a
UC026	me2n	30.0±3.2	n/a	n/a	0.9±0.1	n/a	13.4±1.4	n/a	0.7±0.1	n/a	45.1±4.8
UC028	prun	11.9±0.7	n/a	0.4±0.0	n/a	6.2±0.6	n/a	0.5±0.1	n/a	19.1±1.4	n/a
UC029	me2n	n/a	37.9±4.9	n/a	1.0±0.3	n/a	16.2±6.6	n/a	1.4±0.8	n/a	56.6±11.0

Table 1 continued. Summary of anthocyanin content in units of (μmol/g DW) means ± standard error for *Aronia* accessions in 2010 and 2011.

		Cy3Gal†		Cy3Glu			Cy3A		CyX		Sum	
		2010	2011	2010	2011	2010	2011		2010	2011	2010	2011
Accession	**sp.***	**mean**	**mean**	**mean**	**mean**		**mean**	**mean**	**mean**	**mean**	**mean**	**mean**
UC030	me2n	n/a	11.1±0.5	n/a	0.5±0.0		n/a	8.0±0.4	n/a	0.5±0.0	n/a	20.0±0.9
UC031	me4n	41.2±0.8	18.6±0.5	1.3±0.0	0.6±0.0		21.3±0.2	11.5±0.6	2.3±0.0	0.8±0.0	66.2±0.9	31.5±1.2
UC032	prun	20.4±0.9	n/a	0.5±0.0	n/a		6.7±0.3	n/a	0.5±0.0	n/a	28.0±1.3	n/a
UC033	prun	17.1±0.8	6.4±0.6	0.8±0.0	0.3±0.0		11.6±0.2	3.6±0.3	0.8±0.0	0	30.3±1.0	10.3±1.0
UC035	me2n	n/a	35.8±5.4	n/a	1.1±0.1		n/a	22.8±3.4	n/a	1.5±0.2	n/a	61.1±9.0
UC047	prun	n/a	11.9±1.3	n/a	0.3±0.0		n/a	0.5±0.0	n/a	0.0±0.0	n/a	12.6±1.3
UC049	me2n	n/a	22.2±6.1	n/a	0.6±0.1		n/a	12.9±3.4	n/a	0.8±0.2	n/a	36.6±9.7
UC067	prun	n/a	38.8±1.3	n/a	1.1±0.1		n/a	20.0±0.6	n/a	1.6±0.1	n/a	61.4±2.1
UC072	me2n	n/a	19.7±1.1	n/a	0.6±0.0		n/a	10.5±1.7	n/a	1.0±0.1	n/a	31.8±2.7
UC074	me2n	n/a	20.4±3.0	n/a	0.5±0.0		n/a	7.9±0.6	n/a	0.5±0.0	n/a	29.3±3.4
UC080	prun	n/a	11.1±7.5	n/a	0.4±0.1		n/a	3.8±3.4	n/a	0.2±0.2	n/a	15.3±11.2
UC081	arbu	n/a	7.4±0.4	n/a	0.3±0.0		n/a	3.9±0.1	n/a	0.3±0.0	n/a	11.9±0.5
UC082	prun	n/a	16.5±3.7	n/a	0.4±0.1		n/a	0.7±0.1	n/a	0.0±0.0	n/a	17.5±3.8
UC087	me2n	n/a	14.5±3.9	n/a	0.4±0.1		n/a	7.5±2.1	n/a	0.4±0.2	n/a	22.7±6.2
UC099	me4n	n/a	28.8±n/a	n/a	0.5±n/a		n/a	5.8±n/a	n/a	0.0±n/a	n/a	35.2±n/a
UC105	arbu	n/a	27.8 ± n/a	n/a	0.7±n/a		n/a	14.9±n/a	n/a	1.3±n/a	n/a	44.7±n/a
UC108	me4n	n/a	53.8 ± n/a	n/a	1.5±n/a		n/a	23.8±n/a	n/a	3.0±n/a	n/a	82.1±n/a
UC110B	me4n	n/a	12.1 ± 0.6	n/a	0.5±0.0		n/a	7.7±0.7	n/a	0.6±0.0	n/a	22.2±1.2

* Sp. = species, mit = *A. mitschurinii*, me4 = *A. melanocarpa* tetraploid, arbu = *A. arbutifolia*, prun = *A. prunifolia*, me2n = *A. melanocarpa* diploid.

†Cy3Gal = cyanidin-3-galactoside, Cy3Glu = cyanidin-3-glucoside, Cy3A = cyanidin-3-arabinoside, CyX = cyanidin-3-xyloside, Sum = sum of anthocyanins.

Table 2. Summary of means ± standard error for *Aronia* accessions in 2010 and 2011.

Accession	sp.*	H_2O(%)†		Total Anthocyanins		Total Phenol		ORAC	
		2010 mean	2011 mean	2010 mean	2011 mean	2010 mean	2011 mean	2010 mean	2011 mean
Ames26194	mit	81±0.4	n/a	9.7±1.0	n/a	564.7±13.0	n/a	609.8±25.8	n/a
Ames26195	mit	81.1±0.7	76.4±n/a	8.9±0.9	9.1±n/a	565.6±12.4	424.6±n/a	551.5±6.6	n/a
Ames27010	me4n	72.5±0.1	71.6±2.2	6.7±0.1	7.3±0.0	642.9±5.9	510.4±86.8	617.4±6.5	48.3±n/a
Ames27615	me4n	69.2±1.7	n/a	8.8±0.6	n/a	747.0±30.7	n/a	827.8±51.7	n/a
PI323957	mit	80.2±n/a	76.8±0.3	8.9±n/a	7.1±1.3	666.8±n/a	545.1±22.4	588.9±n/a	557.2±49.5
PI545682	me4n	68.3±n/a	n/a	5.6±n/a	n/a	811.7±n/a	n/a	893.7±n/a	n/a
PI545687	me4n	72.8±0.8	n/a	6.3±0.0	n/a	630.8±90.7	n/a	735.3±34.0	n/a
PI578096	arbu	n/a	74.7±0.2	n/a	3.8±0.7	n/a	607.2±27.6	n/a	503.0±33.9
PI586591	me4n	69.1±2.0	68.0±n/a	6.1±0.1	4.9±n/a	730.0±44.3	659.0±n/a	622.7±30.3	n/a
PI596375	mit	79.8±0.8	77.0±0.3	7.9±1.7	7.7±0.8	583.3±39.9	565.4±4.0	725.0±31.1	565.7±26.1
PI603106	me4n	74.1±0.4	n/a	5.2±0.1	n/a	875.2±75.9	n/a	1091.5±81.0	n/a
PI603107	prun	73.2±0.7	71.4±1.2	4.7±0.3	4.6±0.4	720.4±73.9	644.4±9.0	619.2±14.9	601.7±11.0
PI613016	me2n	n/a	73.2±0.3	n/a	6.6±1.0	n/a	460.8±80.8	n/a	727.3±105.3
PI618684	me4n	72.5±0.4	n/a	9.2±0.6	n/a	769.0±43.0	n/a	697.1±15.8	n/a
PI631247	mit	81.2±0.3	76.6±0.3	7.9±0.4	8.4±1.0	588.9±3.1	534.9±14.1	519.6±11.1	611.1±51.7
PI636375	me4n	75.3±0.8	69.9±0.6	9.5±0.7	7.4±0.7	914.7±31.6	766.7±20.4	820.0±91.6	788.9±30.5
UC002	mit	79.1±1.0	76.8±0.8	10.6±0.9	8.7±1.2	657.0±21.5	580.9±38.2	718.0±34.7	626.9±53.5
UC003	mit	70.3±1.8	78.1±0.6	9.1±1.1	10.4±0.1	468.3±32.8	565.8±58.5	587.3±21.0	512.1±9.0
UC004	me4n	76.6±0.6	71.4±0.6	7.6±0.4	5.2±0.4	677.4±7.6	596.2±37.9	653.3±43.9	619.6±42.1
UC005	me4n	73.0±1.1	72.9±0.6	10.2±0.7	7.0±1.0	933.6±30.7	764.1±32.6	748.9±12.8	701.8±46.9
UC006	me4n	74.0±0.3	n/a	9.5±0.2	n/a	714.2±25.6	n/a	767.6±16.6	n/a
UC007	me2n	76.8±0.8	72.8±0.8	13.7±0.3	8.2±0.2	719.2±11.8	595.8±22.5	893.8±31.1	789.1±37.8
UC008	prun	71.2±1.0	n/a	5.6±0.3	n/a	831.3±80.0	n/a	631.1±39.6	n/a
UC009	me2n	77.7±0.7	68.3±1.0	10.9±0.3	12.7±0.3	657.0±15.8	516.4±61.8	823.1±59.0	692.0±47.5
UC010	me2n	75.2±0.8	n/a	11.3±0.8	n/a	674.1±5.9	n/a	794.3±45.7	n/a
UC011	prun	69.7±0.1	64.6±0.7	5.8±0.4	3.3±0.1	982.1±81.1	545.9±21.9	725.0±70.6	549.3±12.2
UC012	me2n	72.1±1.0	69.2±2.4	7.6±0.4	4.7±0.4	770.9±47.7	485.9±77.8	822.5±8.0	449.3±106.4
UC015	me2n	n/a	73.0±0.5	n/a	7.6±0.2	n/a	490.6±5.9	n/a	633.6±48.0
UC016	me2n	68.3±0.5	n/a	9.6±0.7	n/a	790.3±38.1	n/a	846.9±46.3	n/a

Table 2 continued. Summary of means ± standard error for *Aronia* accessions in 2010 and 2011.

Accession	sp.*	H_2O(%)†		Total Anthocyanins		Total Phenol		ORAC	
		2010 mean	2011 mean	2010 mean	2011 mean	2010 mean	2011 mean	2010 mean	2011 mean
UC019	prun	65.3±1.7	n/a	3.3±0.3	n/a	687.9±58.5	n/a	804.6±17.9	n/a
UC020	me2n	72.4±0.5	n/a	10.7±2.6	n/a	779.8±55.9	n/a	800.9±101.9	n/a
UC022	me2n	n/a	68.7±n/a	n/a	10.5±n/a	n/a	546.6±n/a	n/a	n/a
UC023	me2n	72.7±0.1	n/a	16.9±1.5	n/a	660.4±23.2	n/a	1067.6±38.9	n/a
UC024	me4n	75.4±0.2	n/a	11.1±0.2	n/a	769.6±8.5	n/a	753.6±46.7	n/a
UC026	me2n	n/a	71.5±0.8	n/a	8.1±0.7	n/a	485.2±96.9	n/a	719.3±62.9
UC028	prun	71.7±2.2	n/a	5.4±0.6	n/a	827.0±6.9	n/a	664.3±21.6	n/a
UC029	me2n	n/a	70.3±0.8	n/a	10.4±0.4	n/a	531.2±32.5	n/a	793.7±82.4
UC030	me2n	n/a	63.3±0.4	n/a	5.3±0.2	n/a	600.7±32.5	n/a	695.1±30.1
UC031	me4n	80.2±0.1	73.8±0.2	10.2±0.1	6.2±0.5	483.6±7.7	518.8±29.2	758.5±3.0	520.8±3.8
UC032	prun	70.2±1.3	n/a	5.8±0.2	n/a	770.5±50.4	n/a	636.7±34.9	n/a
UC033	prun	66.5±1.5	69.4±0.3	6.7±0.5	3.9±0.2	873.1±26.3	667.1±75.2	891.1±19.7	679.9±49.9
UC035	me2n	n/a	66.2±2.0	n/a	10.4±1.4	n/a	454±69.9	n/a	690.7±33.8
UC047	prun	n/a	64.4±0.5	n/a	3.9±0.2	n/a	502.6±30.9	n/a	626.8±62.7
UC049	me2n	n/a	65.1±3.0	n/a	7.0±1.3	n/a	429.5±49.2	n/a	721.4±92.6
UC067	prun	n/a	75.7±0.4	n/a	11.0±1.2	n/a	746.8±72.1	n/a	820.4±77.3
UC072	me2n	n/a	69.9±1.4	n/a	6.2±0.4	n/a	451.9±26.7	n/a	615.3±53.9
UC074	me2n	n/a	68.5±0.8	n/a	6.2±0.6	n/a	682.4±38.5	n/a	937.9±94.3
UC080	prun	n/a	75.4±1.4	n/a	4.7±0.9	n/a	791.0±0.7	n/a	782.9±46.0
UC081	arbu	n/a	73.8±0.9	n/a	4.3±0.6	n/a	891.0±15.0	n/a	749.5±17.1
UC082	prun	n/a	74.4±1.1	n/a	5.1±0.2	n/a	591.8±77.9	n/a	509.3±24.5
UC087	me2n	n/a	72.0±0.7	n/a	5.9±0.8	n/a	567.2±141.9	n/a	673.2±23.8
UC099	me4n	n/a	70.9±n/a	n/a	7.7±n/a	n/a	810.8±n/a	n/a	n/a
UC105	arbu	n/a	71.8±n/a	n/a	7.9±n/a	n/a	485.2±n/a	n/a	n/a
UC108	me4n	n/a	70.3±n/a	n/a	18.4±n/a	n/a	859.4±n/a	n/a	n/a
UC110B	me4n	n/a	73.4±0.5	n/a	4.2±0.1	n/a	577.8±60.8	n/a	658.9±76.4

*Sp. = species, mit = *A. mitschurinii*, me4 = *A. melanocarpa* tetraploid, arbu = *A. arbutifolia*, prun = *A. prunifolia*, me2n = *A. melanocarpa* diploid.

†Units H2O(%) = water in percent, Total Anthocyanins = (µmol/g DW) as Cyanidin 3-O-glucoside equivalent determined by spectrophotometric method, Total Phenol= total phenolics (µmol/g DW) as Gallic acid equivalent, ORAC = Oxygen radical absorbance capacity (µmol Trolox Eq/g DW.

Table 3. Mean separation of ORAC, total anthocyanin, phenolics, and individual anthocyanins by species in 2010 and 2011.

2010 ORAC (µmol Trolox Eq/g DW)		2011 ORAC (µmol Trolox Eq/g DW)	
Taxon	**Mean**	**Taxon**	**Mean**
2n melanocarpa	881.4 a	2n melanocarpa	713.8 a
4n melanocarpa	764.0 b	4n melanocarpa	673.8 ab
prunifolia	710.3 bc	prunifolia	652.9 ab
mitschurinii	621.8 c	mitschurinii	574.6 b
2010 Total Anthocyanin (mol/g DW)		**2011 Total Anthocyanin (mol/g DW)**	
Taxon	**Mean**	**Taxon**	**Mean**
2n melanocarpa	69.6 a	mitschurinii	43.0 a
mitschurinii	51.5 b	2n melanocarpa	42.2 a
4n melanocarpa	49.0 b	4n melanocarpa	38.2 ab
prunifolia	25.4 c	arbutifolia	21.3 bc
		prunifolia	13.0 c
2010 Total Phenolics (mol/g DW)		**2011Total Phenolics (mol/g DW)**	
Taxon	**Mean**	**Taxon**	**Mean**
prunifolia	813.2 a	arbutifolia	661.1 a
4n melanocarpa	732.1 ab	4n melanocarpa	648.8 ab
2n melanocarpa	726.2 ab	prunifolia	641.4 ab
mitschurinii	626.7 b	mitschurinii	558.4 ab
		2n melanocarpa	521.3 b
2010 Water Content (%)		**2011 Water Content (%)**	
Taxon	**Mean**	**Taxon**	**Mean**
mitschurinii	78.4 a	mitschurinii	77.1 a
2n melanocarpa	74.2 b	arbutifolia	73.5 b
4n melanocarpa	73.1 b	4n melanocarpa	71.9 bc
prunifolia	69.7 c	prunifolia	70.7 bc
		2n melanocarpa	69.4 c

Table 3 continued. Mean separation of ORAC, total anthocyanin, phenolics, and individual anthocyanins by species in 2010 and 2011.

2010 Cyanidin-3-O-Glucoside (mol/g DW)	
Taxon	**Mean**
mitschurinii	1.8 a
2n melanocarpa	1.2 b
4n melanocarpa	1.0 b
prunifolia	0.6 c

2011 Cyanidin-3-O-Glucoside (mol/g DW)	
Taxon	**Mean**
mitschurinii	1.5 a
4n melanocarpa	0.8 b
2n melanocarpa	0.7 b
prunifolia	0.5 b
arbutifolia	0.4 b

2010 Cyanidin-3-arabinoside (mol/g DW)	
Taxon	**Mean**
2n melanocarpa	18.9 a
4n melanocarpa	14.3 a
mitschurinii	13.9 a
prunifolia	7.8 b

2011 Cyanidin-3-arabinoside (mol/g DW)	
Taxon	**Mean**
2n melanocarpa	12.6 a
mitschurinii	12.3 a
4n melanocarpa	11.8 ab
arbutifolia	7.1 ab
prunifolia	5.4 b

2010 Cyanidin-3-O-Galactoside (mol/g DW)	
Taxon	**Mean**
2n melanocarpa	47.9 a
mitschurinii	34.2 b
4n melanocarpa	32.5 b
prunifolia	16.2 c

2011 Cyanidin-3-O-Galactoside (mol/g DW)	
Taxon	**Mean**
2n melanocarpa	28.0 a
mitschurinii	27.9 a
4n melanocarpa	24.6 ab
prunifolia	14.7 b

2010 Cyanidin-3-xyloside (mol/g DW)	
Taxon	**Mean**
mitschurinii	1.6 a
2n melanocarpa	1.6 a
4n melanocarpa	1.2 ab
prunifolia	0.7 b

2011 Cyanidin-3-xyloside (mol/g DW)	
Taxon	**Mean**
mitschurinii	1.4 a
4n melanocarpa	1.0 ab
2n melanocarpa	0.9 ab
arbutifolia	0.6 b
prunifolia	0.4 b

Table 4. Summary of anthocyanin content in units of (µmol/g DW) as Cyanidin 3-O-glucoside equivalents determined by the HPLC method, and other fruit characteristics means ± standard error for *Aronia* accessions in 2010 first and second harvests.

		Cy3Gal†		Cy3Glu		Cy3A		CyX		Sum	
		2010 1st	2010 2nd	2010 1st	2010 2nd	2010 1st	2010 2nd	2010 1st	2010 2nd	20101st	2010 2nd
Accession	Sp.*	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean
PI545682	me4n	n/a±n/a	38.9±1.2	0.5±n/a	0.8±0.0	8.4±n/a	14.6±0.4	0.5±n/a	0.9±0.1	30.7±n/a	55.1±1.5
UC002	mit	35.2±3.6	60.1±6.2	1.6±0.2	3.4±0.2	14.4±2.2	22.0±2.2	1.6±0.3	3.8±0.3	52.9±6.2	89.2±9.0
UC003	mit	32.8±4.4	54.6±6.5	2.1±0.3	2.7±0.8	14.9±1.8	22.06±1.7	2.1±0.3	3.2±0.8	51.9±6.8	82.5±9.7
UC009	me2n	58.9±1.3	90.0±2.2	0.8±0.0	1.3±0.0	1.1±0.01	1.3±0.0	0	0	60.7±1.3	92.6±2.2
UC023	me2n	42.5±7.3	69.0±3.7	1.3±0.3	1.6±0.1	18.0±3.2	30.4±1.2	1.4±0.2	2.5±0.1	63.1±10.6	103.5±5.0

		H_2O(%)		Total Anthocyanins		Total Phenol		ORAC	
		2010 1st	2010 2nd	2010 1st	2010 2nd	2010 1st	2010 2nd	2010 1st	2010 2nd
PI545682	me4n	68.3±n/a	72.0±1.3	5.6± n/a	8.5±0.3	811.7±n/a	670.3±59.3	893.7±n/a	861.7±23.7
UC002	mit	79.1±1.0	77.0±0.7	10.6±0.9	15.1±1.9	657.0±21.5	642.9±30.9	718.0±34.7	684.8±26.3
UC003	mit	70.3±1.8	75.0±1.5	9.1±1.1	15.7±0.7	468.3±32.8	532.0±7.8	587.3±21.0	837.3±48.7
UC009	me2n	77.7±0.7	71.0±0.6	10.9±0.3	15.0±0.4	657.0±15.8	618.0±41.3	823.1±59.0	829.6±33.1
UC023	me2n	78.0±0.3	72.8±0.2	11.1±1.4	16.9±1.5	757.9±67.5	660.4±23.2	1001.8±29.3	1067.6±38.9

*Sp. = species, mit = *A. mitschurinii*, me4 = *A. melanocarpa* tetraploid, arbu = *A. arbutifolia*, prun = *A. prunifolia*, me2n = *A. melanocarpa* diploid.

†Cy3Gal = cyanidin-3-galactoside, Cy3Glu = cyanidin-3-glucoside, Cy3A = cyanidin-3-arabinoside, CyX = cyanidin-3-xyloside, Sum = sum of anthocyanins, H2O(%) = water in percent, Total Anthocyanins = (µmol/g DW) as Cyanidin 3-O-glucoside equivalent determined by spectrophotometric method, Total Phenol= total phenolics (µmol/g DW) as Gallic acid equivalent, ORAC = Oxygen radical absorbance capacity (µmol Trolox Eq/g DW).

Chapter 3

Interspecific and Intergeneric Hybridization as Pathways to Improved *Aronia* Cultivars.

Introduction

Agricultural production based on one or a few genotypes is vulnerable to major outbreaks of pests and disease. Southern Leaf Blight of Corn in the 1970's (Tatum, 1971), and The Irish Potato Famine (Gliessman, 2007), were demonstrations of problems created from a narrow genetic base for a crop.

Movement of disease and pest tolerances from a wild relative to a cultivated crop has been done countless times by plant breeders, for example, resistance to 12 different diseases in cultivated tomato originate from wild *Lycopersicon* (now considered part of the genus *Solanum*) species. Wild crop relatives have been sources of pest resistance in barley, cassava, sweet potato, sunflower, grapes, tobacco, cacao, sugarcane, potato, and wheat (Eigenbrode, 2011; Flanders at al., 1992) to name a few. Wild relatives of pumpkins and squash have also been extremely important to the creation of disease resistance cultivars (Provvidenti et al., 1978; Cohen, 2003).

Lack of genetic diversity in a crop also limits its use under differing environmental conditions and in a variety of climatic regions. Creating a diverse gene-pool and understanding what species are available for hybridization are therefore of the utmost importance to a crop's future development. In addition to disease resistance and climatic tolerance, diversity in a crop gene-pool may yield different maturation times, fruit quality differences, varying plant habits, and unique ornamental traits.

Aronia is generally described as having few pests, though problems with ring spot virus and lacebugs are known (Bremer, 1984; Dirr, 2000). Cultivation of *Aronia* in North America, the native range of the genus, may be particularly problematic and may need a diverse array of genotypes due to of the presence of natural pests and diseases that have evolved to use *Aronia* as a host. Cultivation of *Aronia* on this continent could be viewed as the inverse of the invasive plant escape hypothesis, which posits that a plant species is more successful when it is grown outside of its native range because it is removed from its pests and diseases (Wolfe, 2002; Mitchell and Power, 2003; Van der Putten 2005). Here *Aronia* is being cultivated in increasing densities within its natural range.

Aronia as a juice crop has very low genetic diversity. The main black chokeberry cultivar for juice production is 'Viking', a plant with larger, rounder leaves and larger fruit than wild genotypes. It has long been thought to be a product of an intergeneric cross of *Sorbus aucuparia* and *A. melanocarpa* (Kask, 1987), which Leonard (2011) confirmed using amplified fragment length polymorphisms (AFLPs). These cultivated *Aronia* plants have been given their own specific epithet, *A. mitschurinii*. Other cultivar names exist, such as 'Aron' and 'Nero,' though random amplification of polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) studies revealed that these other cultivars were genetically very similar or nearly identical to 'Viking' (Milosz et al., 2011; Persson et al., 2004). This improved form is tetraploid and seedlings are all apogamous products of the same genotype (Persson et al., 2001; Persson et al. 2004).

Aronia grown as an ornamental is also very genetically depauperate with only three commonly grown cultivars. There are two ornamental *A. melanocarpa* cultivars, 'Autumn Magic' and 'Morton' (Iroquois Beauty^{TM}), and one red chokeberry cultivar,

'Brilliantissima' (Dirr, 2009). All of these ornamental cultivars are tetraploid, and appear to be apogamous based on our observation of seedling progeny. Tetraploids, even when pollinated by a diploid, do not appear to produce sexual offspring. A tetraploid can only produce sexual offspring is when its pollen is used on a diploid maternal plant, creating triploid offspring that would likely also be apogamous. Since so few cultivars are available in the landscape and fruit industries, there is great potential for disease and pest problems in the future. Additionally, the available genotypes are apogamous and do not produce variable progeny, so they cannot be used to develop new cultivars.

Fortunately, there are paths to increase the genetic diversity of *Aronia*. First, diploid *Aronia melanocarpa* that can reproduce sexually from seed can be found in the wild in eastern North America and there is one diploid *A. melanocarpa* cultivar 'Professor Ed'. (Persson et al., 2004; Connolly and Brand unpublished). These diploid plants are variable and display morphological traits that may be useful in the landscape and fruit industries. There is good potential to select cultivars from these diploid plants and to combine traits from differing accessions by cross pollination and subsequent variable sexually produced progeny.

Second, within Pyrinae, the subtribe of Rosaceae in which *Aronia* is included, hybridization between species within genera is common (Campbell, 2007; Persson et al., 2004). Apomictic accessions or cultivars of *A. mitschurinii, A. arbutifolia,* and tetraploid A. *melanocarpa* could be used as pollen parents in crosses with diploid *A. melanocarpa* maternal plants. These hybridizations would yield variable, but triploid progeny. The triploids themselves may be suitable as cultivars or possibly they could be used in further crosses. Triploids often have reduced sexual reproductive capacity, but in several taxa

they are known to retain some viable pollen and ovule production e.g. *Miscanthus sinensis* (Rounsaville et al., 2011). Triploids have been shown to produce diploid and tetraploid progeny in some cases (Burton and Husband, 2001).

Beyond the genus *Aronia* itself intergeneric hybridization is another strategy to improving or breeding new berry crop and ornamental cultivars. Several hybrids between genera occur in the Pyrinae. Sixteen genera are reported to be involved with intergeneric crosses in this subtribe of the Rosaceae (Postman, 2011). Some are known from the wild, such as ×*Sorbaronia* C.K. Schneid (*Sorbus* × *Aronia*) and ×*Amelasorbus* Rehder (*Amelanchier* × *Sorbus*), (Connolly, 2009; Haines and Vining, 1998; Haines, 2011; Postman, 2011), while many others are only known from cultivation, e.g. ×*Sorbopyrus* (*Sorbus* × *Pyrus*), ×*Sorbocotoneaster* Pojark. (*Sorbus* × *Cotoneaster*), and ×*Sorbocrataegus* (*Sorbus* × *Crataegus*) (Facciola, 1990; Postman, 2011; Sax, 1929).

Hybridization between species of *Sorbus* and *Aronia* are relatively common both in the wild and in horticulture (Fig.1). In New England and the Canadian Maritime Provinces, natural intergeneric hybrids of *Aronia* sp. and *S. americana* (American mountain ash), *S. decora* (showy mountain ash), and *S. aucuparia* (European mountain ash) are recorded (Connolly, 2009; Fernald, 1950; Haines and Vining, 1998; Hyland and Steinmetz, 1944; USDA, 2009). This compatibility of *Aronia* and *Sorbus* seems to be full of breeding possibilities, especially since improved lines, *A. mitschurinii*, have already been developed from this intergeneric combination. There are several known or probable intergeneric crosses between *Aronia* and *Sorbus* (Fig.2).

The purpose of this chapter is to understand the ploidy structure of *Aronia* to elucidate the potential that exists for crossing this genus with Pyrinae intergenerics,

Sorbus species, *Pyrus communis* (common pear), and to attempt crosses between intergenerics involving *A. melanocarpa*. The hope is that this ploidy data will shed light on the role chromosome number plays in the formation of wide crosses between *Aronia* and other Pyrinae. The desired outcome is to create a broader gene-pool for *Aronia* and increase the possibilities for plant improvement. It is hypothesized that hybrids with diploid maternal plants should produce new crosses as a product of true sexual reproduction.

Methods

Flow Cytometry

Flow cytometry is an automated way to evaluate plant ploidy. A modified version of the protocol in Arumuganathan and Earle (1991) summarized in Lehrer et al. (2008) was followed. Briefly, the analysis depends on using a standard plant with a known cytotype (e.g., a diploid is compared with an unknown of the same or a closely related species). Two to three newly emerged leaves were macerated using a fresh razor blade in nuclei suspending solution in a 55mm petri dish on a freeze pack. The methods were then modified in accordance with Meng and Finn (1999) by adding 2 g of PVP-10 per 50 ml of extraction buffer and fluorescently staining released nuclei after filtering with propidium iodine, instead of during maceration. Relative fluorescence of total DNA was measured using a Becton-Dickson FACS Calibur Dual Laser Flow Cytometer (Becton, Dickson and Co., Franklin Lakes, NJ) at the Flow Cytometry and Confocal Imaging Facility at the University of Connecticut in Storrs, CT. The cytometer was equipped with an Argon ion laser emitting radiation at 488 nm. For each sample, 10,000-20,000

particles were measured. Data were logged and displayed in histograms by BD Cellquest ™ software (Becton, Dickson and Co., Franklin Lakes, NJ). Standard tetraploid and diploid sample histogram peaks were compared to samples of unknown ploidy. Peaks of the unknowns could be compared to the standards and categorized as diploid or tetraploid. If fluorescent peaks were intermediate in value, the sample was determined to be triploid. Any plants with ambiguous peaks were run multiple times and compared to several knowns until a clear ploidy level could be determined.

Branches of *Sorbus* species and intergeneric hybrids (×*Sorbaronia sorbifolia*, ×*S. alpina*, ×*S. dippelii*, *Sorbus domestica*, and ×*Sorbocotoneaster pozdnjakovii*) were cut from accessions at Harvard University's Arnold Arboretum, in Jamaica Plain, MA, and tested for ploidy determination. Wild collected accession of ×*Sorbaronia fallax* from Ashburnham, MA (UC 140), and Mansfield Center, CT (UC 078 and UC079) were also tested using flow cytometry. Flow cytometry was performed on cultivated ×*Sorbaronia fallax* 'Ivan's Beauty' and ×*Sorbocrataegus* 'Ivan's Belle'.

Cross Pollination

Aronia is protogynous (Hardin, 1973; Leonard, 2011); anthers are pink prior to pollen dehiscence, yellow post dehiscence, and turn from yellow to brown as they wither and pollen is no longer available. Pollinations were done in a pollinator-free greenhouse. Previously opened flowers that were already shedding pollen were removed from the cyme. Flowers intended for crosses on the maternal plants were emasculated when flowers still had pink anthers, while fresh yellow pollen-coated stamens were removed from the paternal plants and pollen deposited on the emasculated maternal flower

stigmas. Pedicels of pollinated flowers were marked with paper tags. Any remaining buds on the cyme were removed to reduce resource competition. Fruits were allowed to mature and were harvested when they reached ripeness.

In spring of 2010, 2011, and 2012, 700 hybridization attempts were made between *Aronia*, *Sorbus*, and intergeneric hybrids (Tables 1&2). For cross pollinations involving *S.* ×*arnoldiana* × *alnifolia* (UC169), ×*S. sorbifolia* (UC127), *S. domestica* (UC170), ×*Sorbocotoneaster pozdnjakovii* (UC167), ×*S. alpina* (UC168), and ×*S. dippelii* (UC123) small branches with flower buds of were cut from accessions at the Harvard University's Arnold Arboretum, in Jamaica Plain, MA. Cut branches were immediately put in plastic bags with moist paper towels, chilled in a cooler, and transported to the University of Connecticut. Cut stems were placed in water in a warm greenhouse or at room temperature under lights until they flowered. Flowering shoots of ×*Sorbopyrus* (UC171, UC172, and UC173) were shipped from the USDA-ARS National Clonal Germplasm Repository in Corvallis, Oregon.

One hundred and fourteen *Aronia* × *Aronia* crosses were made and the diploid accession (UC012) was used extensively as a maternal plant. This accession was used due to it's extremely dwarf stature, a desired characteristic for the creation of new ornamental and fruit production cultivars. Additionally, UC012 was used previously to make interspecific hybrids with *A. arbutifolia* (Brand unpublished), and therefore it was hypothesized that this accession may have wide pollen compatibility. Diploids (UC012, UC023, and UC063) were pollinated by polyploid *A. mitschurinii* (UC002/UC003), and *A. prunifolia* (UC113).

Fifty *Aronia* × *Pyrus* hybridization attempts were made. Local *Pyrus communis* (UC165 and UC166) flowers were harvested from trees near the University of Connecticut campus and used to pollinate flowers of diploid *A. melanocarpa* (UC012); the pears could not be identified to cultivar. *Aronia* × *Sorbus* crosses were attempted 42 times using diploid *A. melanocarpa* (UC012) as the maternal parent. Branches from three *S. aucuparia* accessions (UC162, UC163, and UC164) were collected and placed in a beaker with water until they flowered. Additionally, *S.* ×*arnoldiana* × *alnifolia* (UC169) and *S. domestica* (UC170) from the Arnold Arboretum were used as pollen parents. *Aronia* diploids (UC012 and UC034) were pollinated by intergeneric hybrids 185 times. The intergenerics used to pollinate UC012 and UC034 were ×*S. sorbifolia* (UC127), triploid ×*S.* fallax 'Ivan's Beauty' (UC137), ×*Sorbocotoneaster pozdnjakovii* (UC167), diploid ×*S. fallax* (UC140), and ×*Sorbopyrus* (UC171, UC172, and UC173). Two hundred and eight intergeneric × intergeneric crosses were made using diploid ×*S. fallax* accessions (UC078 and UC140) as the female parents. The intergeneric pollen parent for these crosses were ×*S. dippelii* (UC123), ×*S. sorbifolia* (UC127), ×*S. alpina* (UC168), ×*Sorbopyrus* (UC171, UC172, and UC173). Fifty diploid ×*S. fallax* (UC140) flowers were pollinated using *Pyrus* (UC165 and UC166) pollen. Diploid ×*S. fallax* (UC140) was backcrossed 36 times to *S. aucuparia* (UC162, UC163, and UC164). Fifteen hybridization attempts were made between diploid *Sorbus alnifolia* (UC124) with pollen from *A. arbutifolia* var. *purpurea* (UC013) and an *A. mitschurinii* type (UC003).

To test if *Sorbus aucuparia* could be used as a maternal parent, open pollinated fruits of *S. aucuparia* known to be in the vicinity of either cultivated or wild *Aronia* spp. were collected from three maternal plants (UC162, UC163, and UC164). Seeds were

extracted and stratified. One hundred open pollinated seeds from each of the three trees were planted and examined for ×*Sorbaronia* progeny. Additionally, triploid ×*S. fallax* 'Ivan's Beauty' (UC137), triploid ×*Sorbocrataegus* 'Ivan's Belle' (UC139), and diploid ×*S. fallax* (UC140) were allowed to be open pollinated in proximity to each other and to *A. melanocarpa*, *A. arbutifolia*, and *A. prunifolia*. Seeds of these intergenerics were planted to test for apogamy, fertility, and hybridization by counting and examining their progeny.

Seeds of the above crosses were extracted and from the fruits. Germination methods followed Leonard (2010) where seeds were cold stratified in moist sand for 90 days in 50ml conical centrifuge tubes or polyethylene bags at 5ºC. Seeds were germinated in 32 oz clear plastic salad trays with dome lids filled with potting medium that had been sifted using hardware cloth screen with .5 cm square openings. The component ration for the medium was 5:3:1 composted pine bark mulch, peat, and sand. Seeds were lightly covered with approximately 2 mm of media. The environmental conditions for germination were 24ºC ± 2ºC with 12 hour per day white fluorescent light (40 µmol·m-2·s-1). Seedlings were transferred to standard 50 cells per flat plug trays filled with the 5:3:1 composted pine bark mulch, peat, and sand and place in a heated (18ºC ±9ºC) glass greenhouse. Sixty days after planting the first true leaves of the seedling where examined and compared to maternal and paternal plants. If paternal morphological characteristics were present the seedling was considered a true cross and not an accidental self.

Results

Flow Cytometry

The taxa from the Arnold Arboretum including ×*S. sorbifolia* (UC127), *S. domestica* (UC170), ×*S. pozdnjakovii* (UC167), and ×*S. dippelii* (UC168) were found to be diploid (Fig, 2). The intergeneric hybrid ×*S. alpina* (UC168) was shown to be triploid. Wild collected accessions of ×*S. fallax* from Ashburnham, MA (UC 140), and Mansfield Center, CT (UC078 and UC079) were found to be all diploid. Flow cytometry revealed that cultivated ×*Sorbaronia fallax* 'Ivan's Beauty' (UC137) and ×*Sorbocrataegus* 'Ivan's Belle' (UC139) were triploid.

Cross Pollination

The results of the cross pollinations varied depending on the taxa involved (Table 2). Diploid *A. melanocarpa* (UC012, UC023, and UC063) × *A. mitschurinii* (UC002, UC003) pollinations were attempted 99 times, but only one seedling appears to have the correct morphology, this seedling had more orbiculate leaves than diploid *A. melanocarpa* and appeared to be semi-dwarf in stature (Fig. 3). Other *Aronia* × *Aronia* crosses produced a few weak seedlings that did not survive. Unexpectedly, diploid *A. melanocarpa* (UC012) produced 5 seedlings when pollinated by *Pyrus communis* (UC165 and UC166) though they soon died after germinating. *Aronia melanocarpa* (UC012) × *S. aucuparia* (UC162, UC163, and UC164) yielded 4 seedlings from 22 crosses, the seedlings had deeply lobed leaves and are multi-stemmed. Two plants grew from the cross of diploid *A. melanocarpa* (UC012) and *S.*× *arnoldiana* × *alnifolia*

UC169, but only one has survived, this progeny had very irregularly lobed serrated leaves and a semi-dwarf growth habit (Fig. 4). Crosses of diploid *A. melanocarpa* (UC012) with *S. domestica* (UC170) were unsuccessful. *Aronia* crosses with intergenerics were possible; diploid *A. melanocarpa* (UC034) set seed when diploid ×*S. fallax* (UC140) pollen was used on its flowers, bearing five seedlings, these plants resemble diploid *A. melanocarpa* but have deeper leaf margin serrations. A single offspring grew from the diploid *A. melanocarpa* (UC012) ×*S. fallax* 'Ivan's Beauty' UC137 combination, but perished. Intergeneric crosses with each other were fairly successful. Diploid ×*S. fallax* (UC140) and diploid ×*S. dippelii* (UC123) produced 40 seedlings, that often had pale green leaves ranging from entire to lobed, with white pubescence on the leaf undersides and twigs (Fig. 5). While diploid ×*S. fallax* (UC140) and diploid ×*S. sorbifolia* (UC127) yielded 20 offspring, with shiny green leaves that also ranged from entire to lobed, which has reddish fall color (Fig. 6). Triploid ×*Sorbaronia alpina* (UC168) was the only ×*Sorbaronia* nothospecies that did not induce seed set on diploid ×*S. fallax* (UC140). Diploid ×*S. fallax* was easily backcrossed to *S. aucuparia* (UC162, UC163, UC164) producing nine seedlings with leaves similar to *S. aucuparia* but with some fusion of the terminal leaflets (Fig. 7). Diploid ×*S. fallax* (UC078) produce fruit and seeds when pollinated by ×*Sorbopyrus* (UC171, UC172, and UC173), the resulting seeds will be planted in 2013. Crosses of ×*S. fallax* (UC140) and *P. communis* (UC165 and UC166) did not set fruit. *Aronia mitschurinii* (UC002/UC003) and *A. arbutifolia* (UC013) used as paternal parents with *S. alnifolia* (UC124) did not induce fruit set. Open pollinated ×*S. fallax* (UC140) had 63% germination and produced abundant seedlings with a multitude

of leaf shape variations (Fig. 8).The 300 seeds of *S. aucuparia* (UC162, UC163, UC164) yielded a single seedling with ×*Sorbaronia* morphology.

Discussion

The future of *Aronia* breeding has much potential but also has many pitfalls. Crosses with polyploids, especially triploids, were generally not successful. ×*Sorbaronia alpina* which was shown to be triploid and therefore likely apogamous cannot be used as a maternal parent. However, even when used as a pollen parent with diploid ×*S. fallax*, no seeds were produced. This taxon likely has low pollen viability, although it was not directly tested. *Aronia mitschurinii*, the common species used as a fruit crop, is essentially reproductively isolated from diploid *A. melanocarpa*, as demonstrated by the production of only one surviving seedlings resulting from 99 controlled pollinations. It seems likely that *A. mitschurinii* produces mostly non-viable pollen.

The easiest crosses and most fertile hybridizations appear to be those between genera or with intergeneric hybrids. These productive intergeneric crosses, or backcrosses, were all between diploids entities, and have not previous been documented. One pathway to producing new *Aronia* type varieties appears to be by using diploid accessions of ×*S. fallax*, ×*S. sorbifolia*, and ×*S. dippelii* in crosses with each other or with diploid *A. melanocarpa*.

The genus *Sorbus* is estimated to have 250 species (Aldasoro et al., 2004**).** There are likely many other *Sorbus* species that diploid *A. melanocarpa* can be hybridized with. Unfortunately, branches of several other *Sorbus* species that were collected from the Arnold Arboretum did not force well and failed to survive long enough to produce pollen.

Sorbus ×*arnoldiana* × *alnifolia* was one *Sorbus* taxon that easily produced pollen and was used successfully on *A. melanocarpa* flowers. Interestingly, *S.* ×*arnoldiana,* itself is a hybrid of *S. aucuparia* × *S. discolor* (Reider, 1920) leading to a quadrispecific intergeneric combination of genetics from *A. melanocarpa*, *S. aucuparia*, *S. alnifolia* and *S. discolor*. Two trispecific intergeneric hybrids were also successful. One involved *A. melanocarpa*, *S. aucuparia*, and *S. americana*, and the second involved *A. melanocarpa*, *S. aucuparia* and *S. aria*. We were able to obtain ×*Sorbaronia fallax* in crosses with *Sorbus* as the pollen parent and *Aronia* as the maternal parent. Additionally, the reciprocally cross seems possible, ×*Sorbaronia fallax* was obtained while growing out seed collected from open pollinated maternal plants of *S. aucuparia* that must have been pollinated by *Aronia* sp. growing nearby.

Diploid ×*S. fallax* produced abundant seedlings when open pollinated. These plants are highly polymorphic with leaves ranging between simple slight serrations to nearly compound. Genetic marker testing may be useful in determining if these plants are the product of self pollination or pollination with *A. melanocarpa*, *A. arbutifolia*, *A. prunifolia* or all of the above. Crosses or progeny involving the diploid ×*S. fallax* appear vigorous and have a variety of interesting leaf forms that may be useful in an ornamental cultivar. The fruit of the F1 diploid ×*S. fallax* (UC 140) and triploid 'Ivan's Beauty' (UC 137) were maroon, and more persistent and crack resistant than *A. melanocarpa*. Fruit cracking can be a problem in *Aronia* (Jeppsson, 2000).This resistance to cracking is advantageous for berry harvesting, and may be a trait that *A. mitschurinii* inherited from its progenitor ×*S. fallax*. Seedlings generated from all crosses are being grown on the

University of Connecticut Plant Science Research and Educational facility and will be evaluated for pomological and ornamental traits.

Seedlings of diploid *A. melanocarpa* × *P. communis* were produced, but were unexpected, due to the genetic differences between the two genera. Only 5 seedlings germinated and soon died even when moved to an agar growth medium. These seedling developed radicals and cotyledons but appeared to be lacking shoot apical meristems. After transferred to tissue culture media the seedlings expired when stores in their cotyledons were exhausted, since no true leaves were produced. This result, though not immediately useful, is encouraging to the development of an *Aronia* type plant with much larger fruit. Potentially, embryo rescue could be used, followed by induction of callus tissue formation. Shoot apices have been successfully induced from callus tissue in apple (*Malus*) a closely related genus (Caboni et al., 2000). We have obtained scion wood from the National Clonal Germplasm Repository USDA Agricultural Research Service Corvallis, Oregon, the next steps in this breeding program will be to use these additional accessions of ×*Sorbopyrus* to pollinate diploid *A. melanocarpa* and diploid ×*S. fallax* (Fig. 9).

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Table1. Description of *Aronia* and related taxa used for experimental cross pollinations. *2n? ploidy was not tested.

UConn #	Species	Cultivar/other ID	Source/Nursery	Town	State	latitude	longitude	n
UC 002	*Aronia mitschurinii*	Nero	Spring Meadow	Grand Haven	MI	n/a	n/a	4n
UC 003	*Aronia mitschurinii*	Viking	Spring Meadow	Grand Haven	MI	n/a	n/a	4n
UC 012	*Aronia melanocarpa*	Birch Pt. Beach dwarf	wild	S. Thomaston	ME	44°02'18"N	69°05'43"W	2n
UC 013	*Aronia arbutifolia*	Purpurea strain	Colvos Creek	Vashon Island	WA	n/a	n/a	4n
UC 023	*Aronia melanocarpa*	Professor Ed	wild	n/a	NH	n/a	n/a	2n
UC 034	*Aronia melanocarpa*	Steuben, ME	wild	Steuben	ME	44°27'25"N	67°55'45"W	2n
UC 063	*Aronia melanocarpa*	S. Worcester Co. Black	wild	Brookfield	MA	42°10'19"N	72°06'54"W	2n
UC 078	×*Sorbaronia fallax*	old cars, fine textured	wild	Mansfield	CT	41°46'11"N	72°11'41"W	2n
UC 113	*Aronia prunifolia*	Windham Cedar Bog	wild	Windham	CT	41°44'36"N	72°09'44"W	4n
UC 123	×*Sorbaronia dippelii*	759-78	Arnold Arbo	Boston	MA	n/a	n/a	2n
UC 124	*Sorbus alnifolia*	n/a	ForestFarm	Williams	OR	n/a	n/a	2n
UC 127	×*Sorbaronia sorbifolia*	1239-85-A	Arnold Arbo	Boston	MA	n/a	n/a	2n
UC 137	×*Sorbaronia fallax*	Ivan's Beauty	Jung's Seed	Randolph	WI	n/a	n/a	3n
UC 139	×*Sorbocrataegus*	Ivan's Belle	Jung's Seed	Randolph	WI	n/a	n/a	3n
UC 140	×*Sorbaronia fallax*	coarse textured	wild	Ashburnham	MA	42°38'42"N	71°53'54"W	2n
UC 162	*Sorbus aucuparia*	Town Hall	specimen tree	Mansfield	CT	41°48'05"N	72°14'30"W	2n
UC 163	*Sorbus aucuparia*	Maple Rd.	specimen tree	Mansfield	CT	41°47'26"N	72°15'03"W	2n
UC 164	*Sorbus aucuparia*	Baker rd.	specimen tree	Mansfield	CT	41°46'44"N	72°11'20"W	2n
UC 165	*Pyrus communis*	Red barn	specimen tree	Mansfield	CT	41°49'05"N	72°15'19"W	2n
UC 166	*Pyrus communis*	Community Garden	specimen tree	Mansfield	CT	41°49'20"N	72°15'37"W	2n
UC 167	×*Sorbocotoneaster*	89-68-A	Arnold Arbo	Boston	MA	n/a	n/a	2n
UC 168	×*Sorbaronia alpina*	675-55-A	Arnold Arbo	Boston	MA	n/a	n/a	3n
UC 169	*Sorbus alnifolia* ×*arnoldia*	434-88	Arnold Arbo	Boston	MA	n/a	n/a	2n?*
UC 170	*Sorbus domestica*	1043-64-A	Arnold Arbo	Boston	MA	n/a	n/a	2n
UC 171	×*Sorbopyrus*	Sorbopyrus 'Baciu 1'	USDA/ARS	Corvalis	OR	n/a	n/a	2n?
UC 172	×*Sorbopyrus*	Sorbopyrus 'Baciu 2'	USDA/ARS	Corvalis	OR	n/a	n/a	2n?
UC 173	×*Sorbopyrus auricularis*	Washington Park Arbo	USDA/ARS	Corvallis	OR	n/a	n/a	2n?

Table 2. Results of experimental cross pollination with *Aronia* and related taxa.*have not been planted † ×*Sorbaronia* seedlings only.

Experimental cross pollinations 2010-2012

Maternal	paternal	crosses	fruit	fruit set(%)	seeds	seeds/fruit	seedlings	germination(%)
Aronia* × *Aronia								
UC012	UC002/003	75	27	36.0	86	3.2	1	1.2
UC012	UC113	15	6	40.0	14	2.3	1	7.1
UC023	UC002/003	10	7	70.0	20	2.9	2	10.0
UC063	UC002/003	14	0	0.0	0	0.0	0	0.0
Aronia* × *Pyrus								
UC012	UC165/166	50	13	26.0	32	2.5	5	15.6
Aronia* × *Sorbus								
UC012	UC162/3/4	22	n/a	n/a	n/a	n/a	4	n/a
UC012	UC169	10	4	40.0	12	3.0	2	16.7
UC012	UC170	10	0	0.0	0	0.0	0	0.0
***Aronia* × Intergeneric**								
UC012	UC127	40	3	7.5	6	2.0	1	16.7
UC012	UC137	40	4	10.0	7	1.8	1	14.3
UC012	UC167	15	0	0.0	0	0.0	0	0.0
UC034	UC140	15	3	20.0	14	4.7	5	35.7
UC034	UC171/2/3	75	0	0.0	0	0.0	0	0.0
Intergeneric × Intergeneric								
UC078	UC171/2/3	25	2	8.0	5	2.5	?*	0.0
UC140	UC123	36	31	86.1	94	3.0	40	42.6
UC140	UC127	27	21	77.8	66	3.1	20	30.3
UC140	UC168	20	1	5.0	0	0.0	0	0.0
UC140	UC171/2/3	100	0	0.0	0	0.0	0	0.0
Intergeneric x *Pyrus*								
UC140	UC165/6	50	0	0.0	0	0.0	0	0.0
Intergeneric × *Sorbus*								
UC140	UC162/3/4	36	10	27.8	39	3.9	9	23.1
Intergeneric Open pollinated								
UC137	OPEN	n/a	n/a	n/a	78	n/a	14	18.0
UC139	OPEN	n/a	n/a	n/a	99	n/a	6	6.1
UC140	OPEN	n/a	n/a	n/a	100	n/a	63	63.0
Sorbus* × *Aronia								
UC124	UC002/3	9	0	0.0	0	0.0	0	0.0
UC124	UC013	6	0	0.0	0	0.0	0	0.0
***Sorbus* Open pollinated**								
UC162	OPEN	n/a	n/a	n/a	100	n/a	0†	n/a
UC163	OPEN	n/a	n/a	n/a	100	n/a	1	n/a
UC164	OPEN	n/a	n/a	n/a	100	n/a	0	n/a

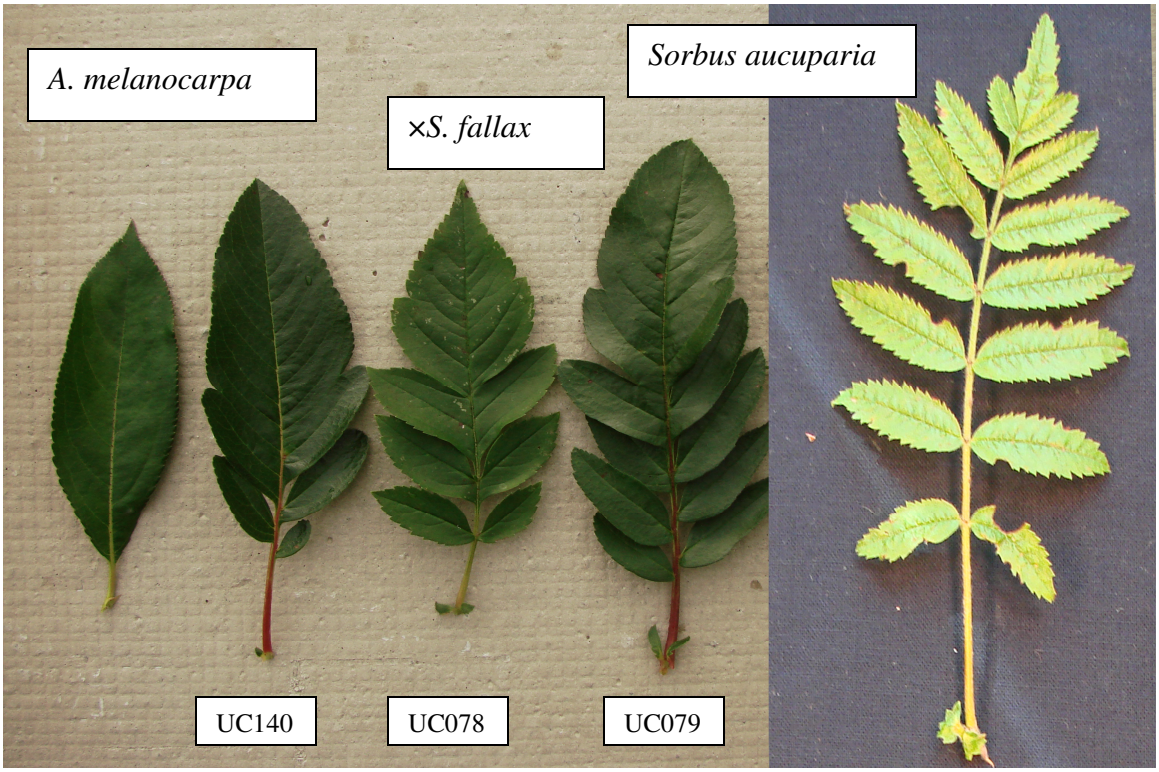

Fig. 1. Leaves of *A. melanocarpa* and *Sorbus aucuparia* with three different genetic individuals of ×*Sorbaronia fallax*.

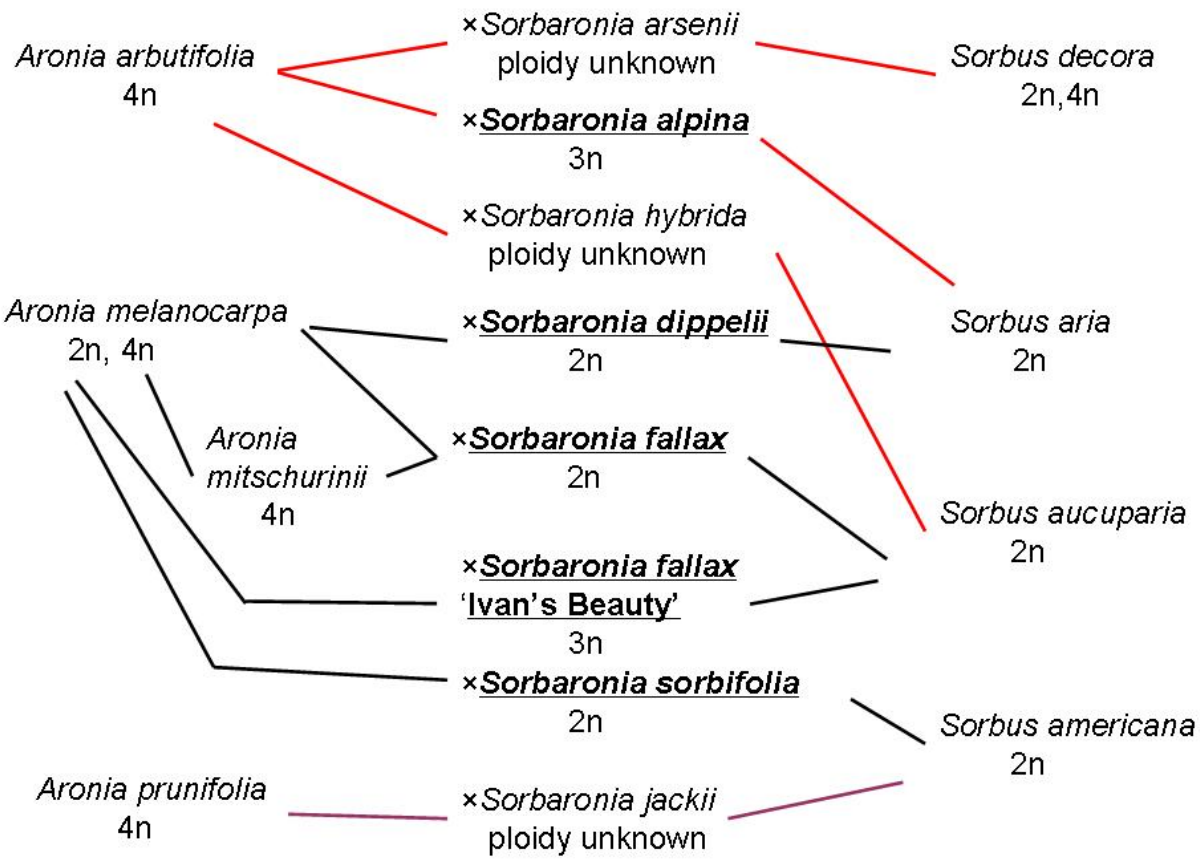

Fig. 2. Known intergeneric hybridization between *Aronia* and *Sorbus*. Underlined and bolded ×*Sorbaronia* nothospecies were represented by live collections at the Arnold Arboretum or the University of Connecticut and used for cross pollinations in this study.

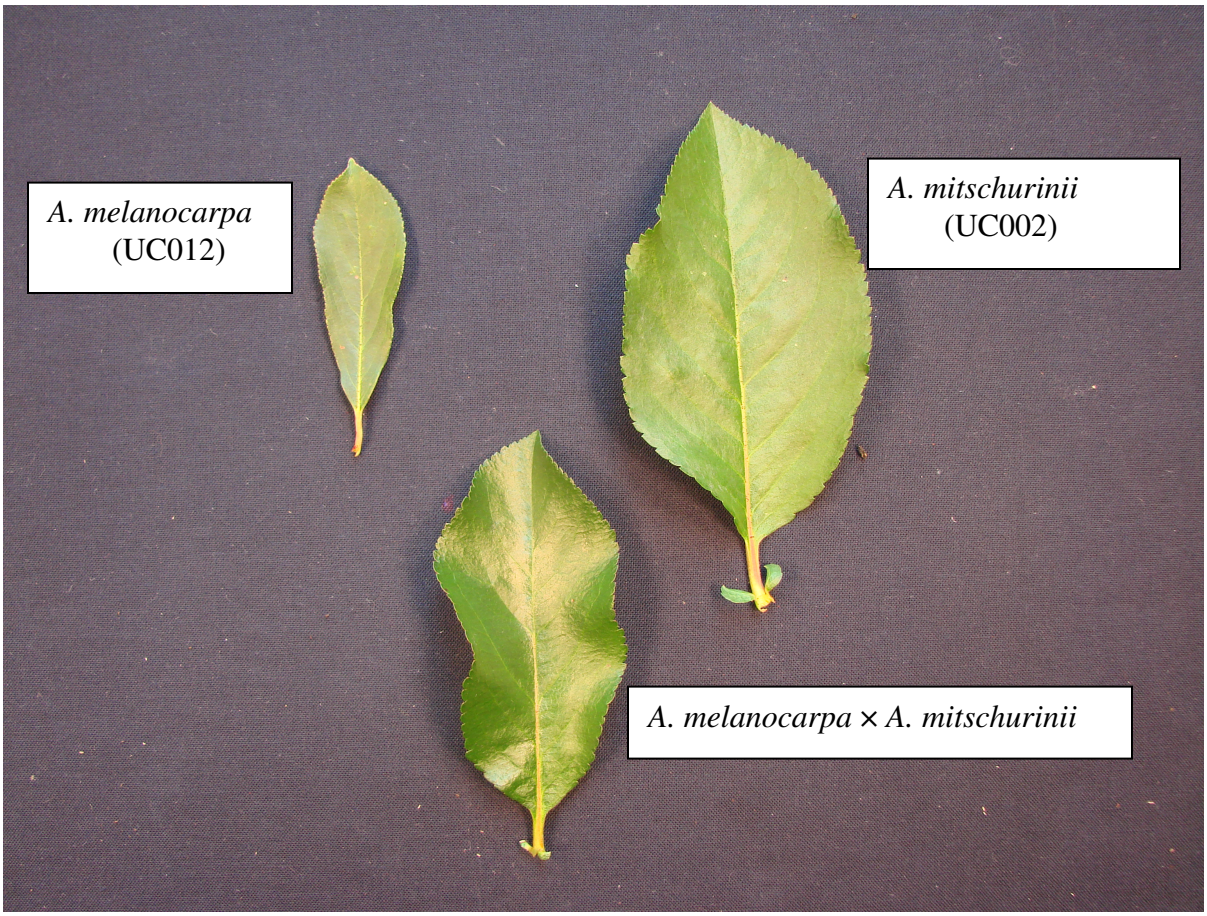

Fig. 3. Leaves of *Aronia melanocarpa* (UC012), *A. mitschurinii* (UC002) and the resulting hybrid.

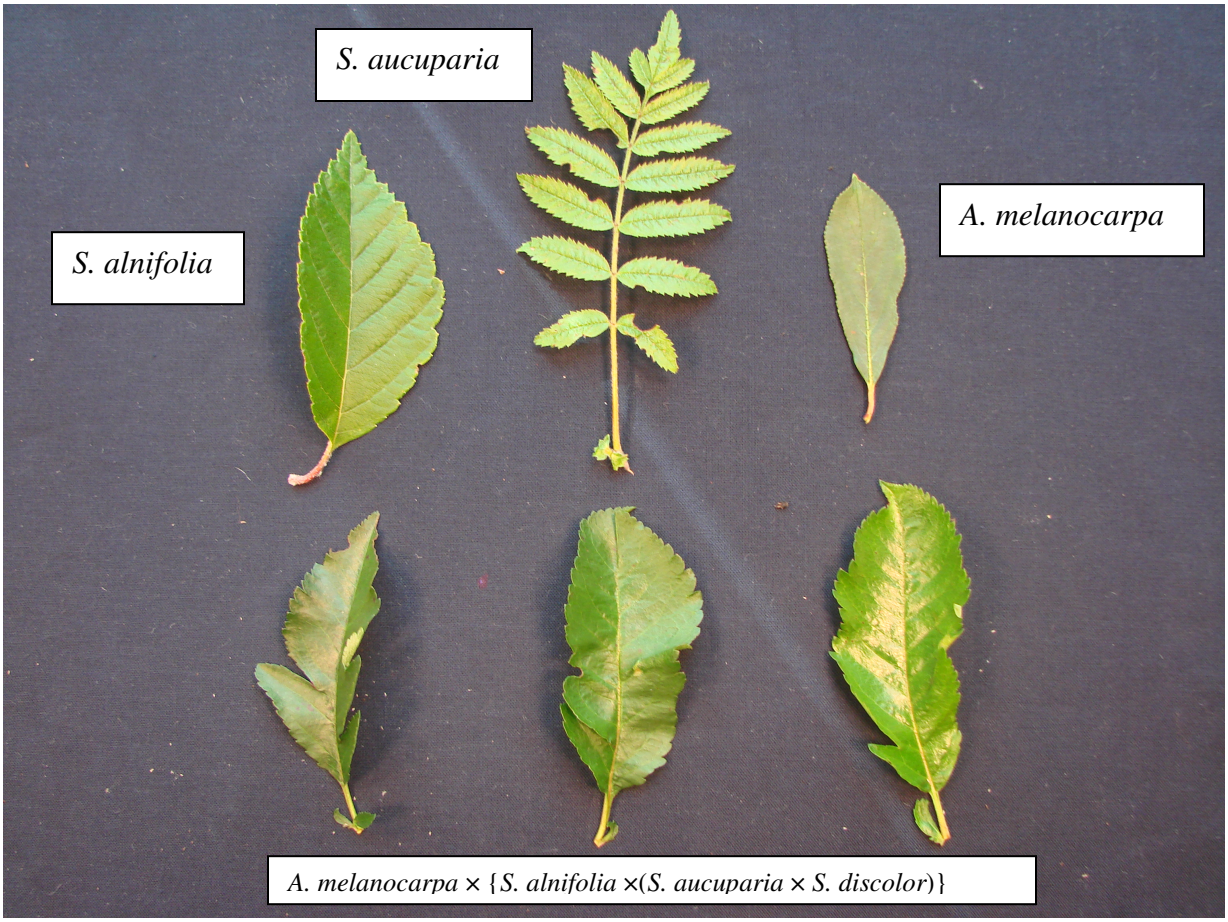

Fig. 4. Leaves of the quadrispecific hybrid *Aronia melanocarpa* (UC012) ×{*Sorbus* alnifolia ×(S. aucuparia × S. discolor)} UC169, and three of its parental species.

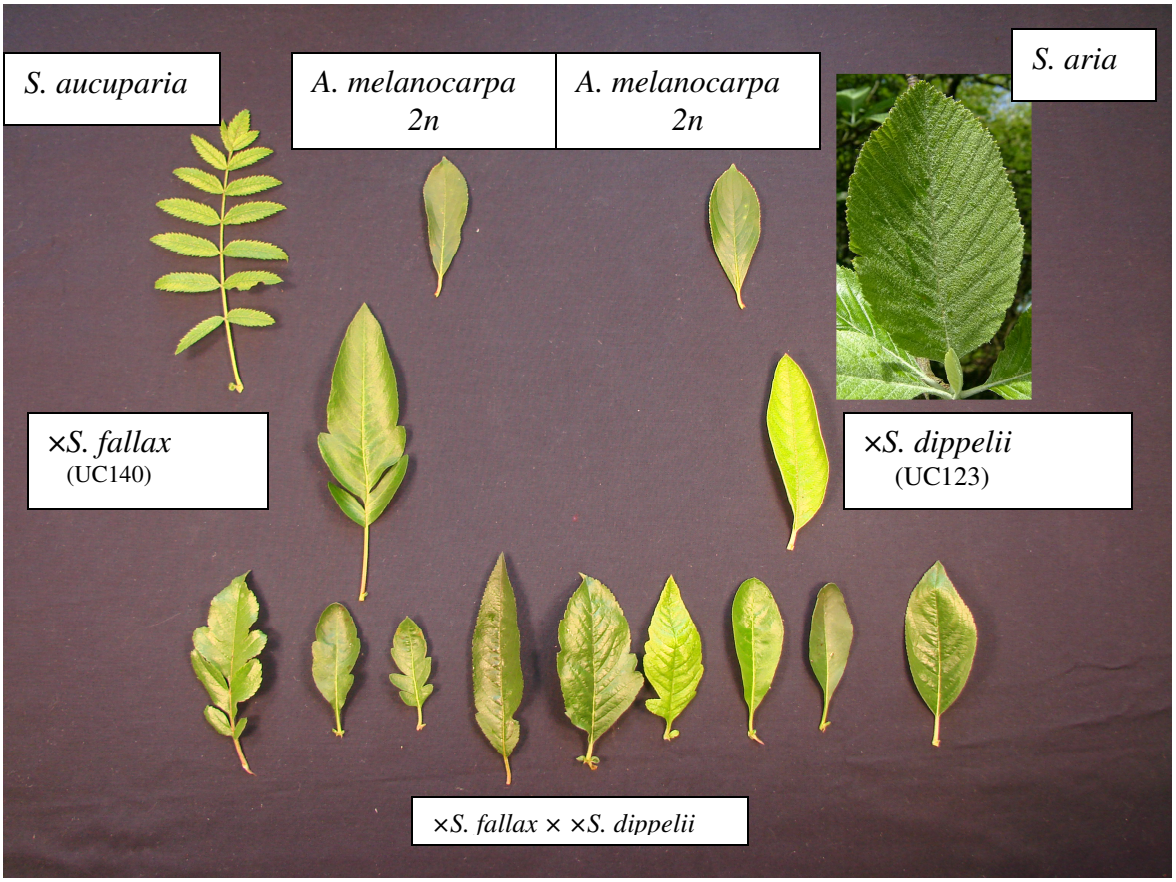

Fig. 5. Leaves of *Sorbus aucuparia*, *Aronia melanocarpa* and *S. aria*, their hybrid offspring ×*Sorbaronia fallax* (UC140) and ×*S. dippelii* (UC123), and the resulting tri-specific hybrids.

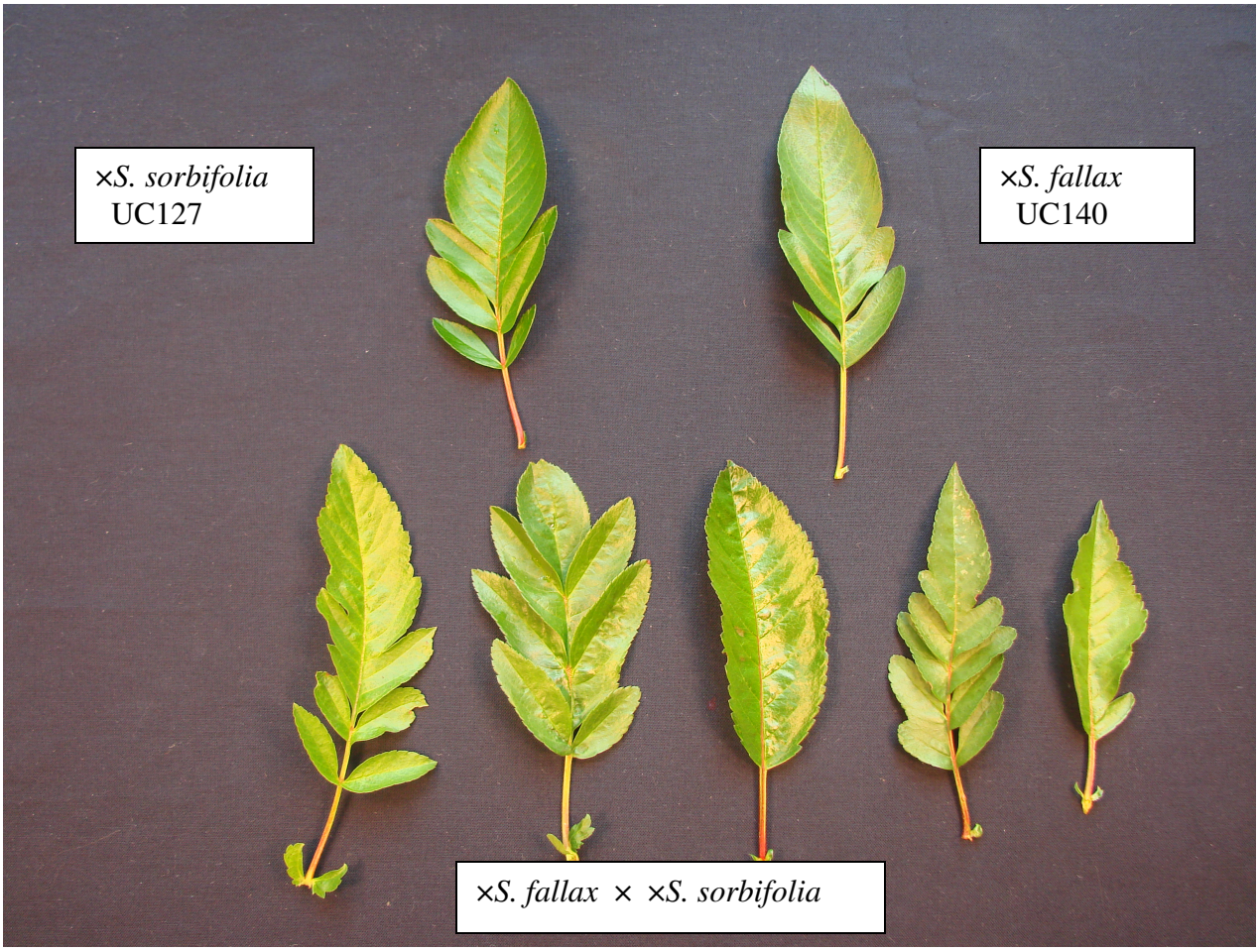

Fig. 6. Leaves of ×*Sorbaronia sorbifolia* (UC127) and ×*Sorbaronia fallax* (UC140) and the resulting tri-specific hybrids.

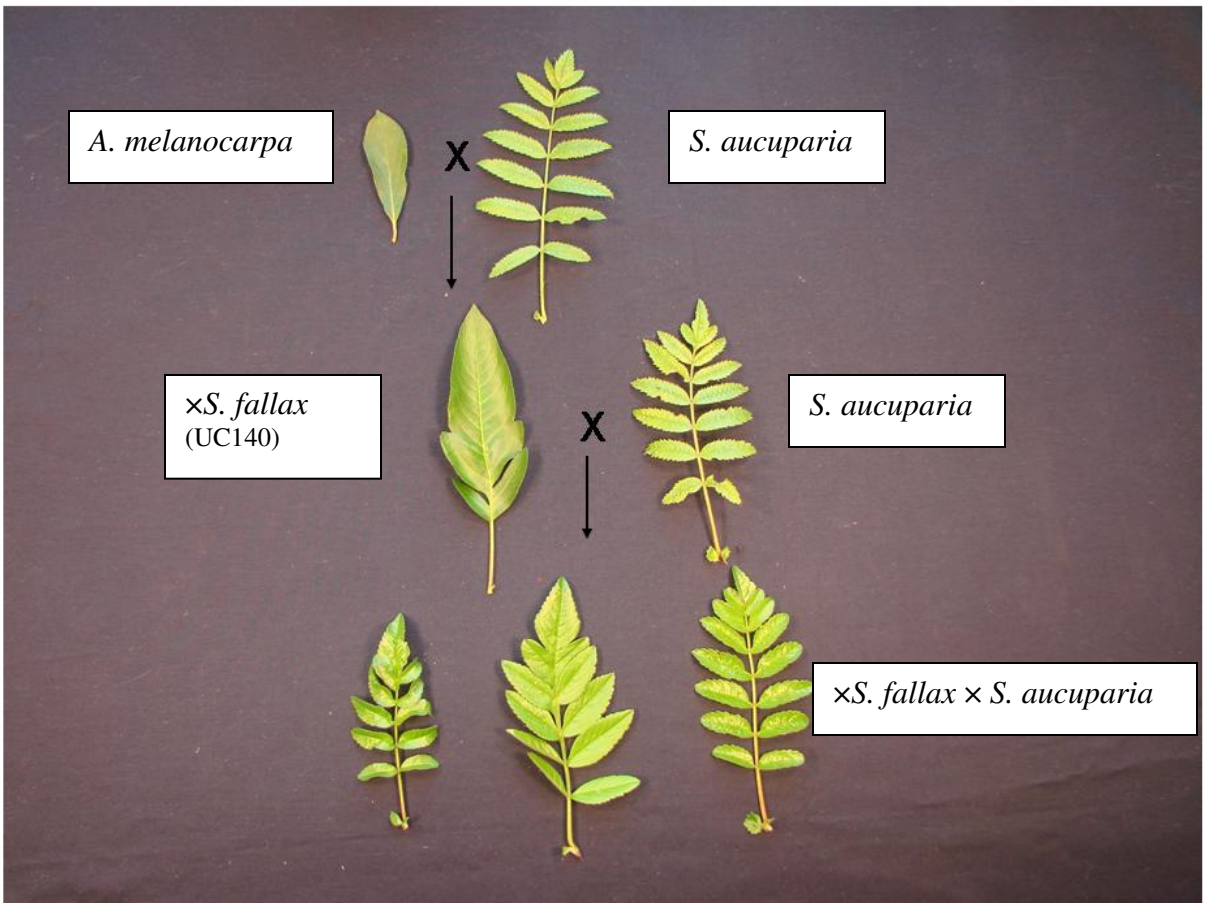

Fig. 7. Leaves of *Aronia melanocarpa*, *Sorbus aucuparia*, ×*Sorbaronia fallax* (UC140) and the resulting backcross of ×*S. fallax* (UC140) and *S. aucuparia* (UC162, UC163, UC164).

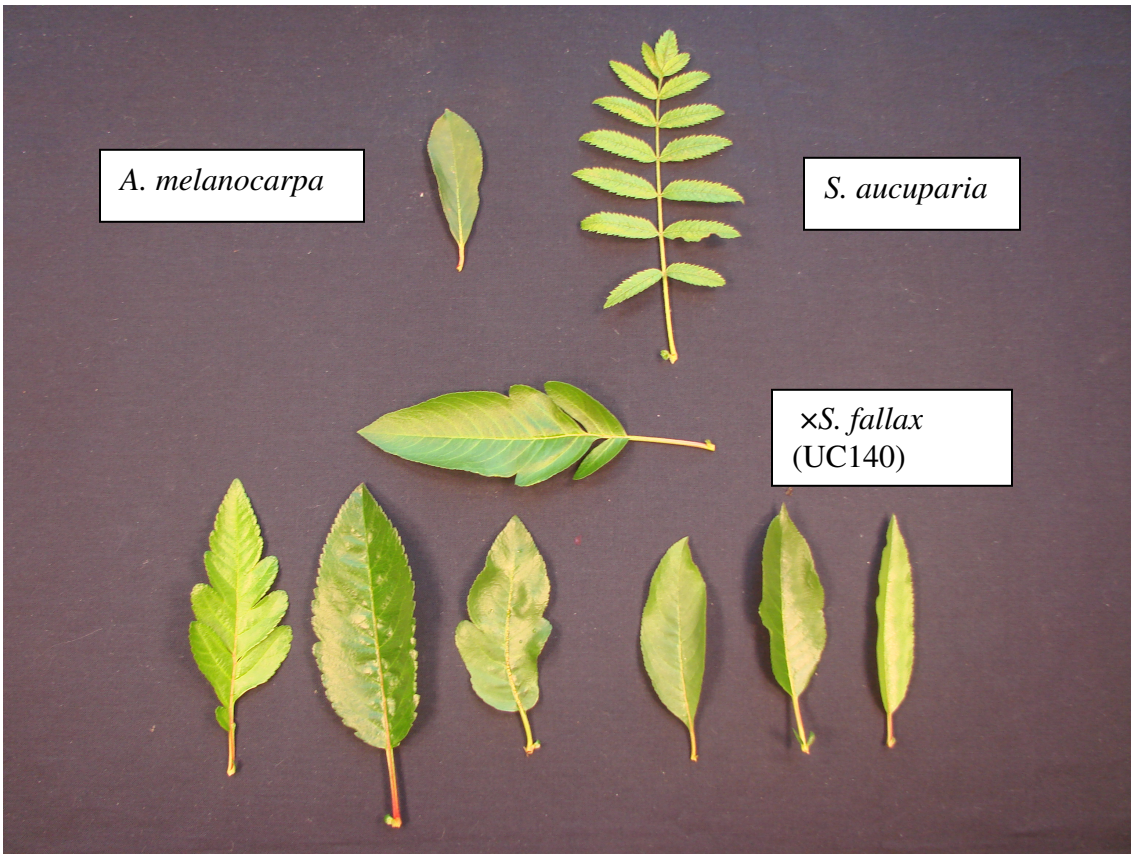

Fig. 8. Leaves of *Aronia melanocarpa*, *Sorbus aucuparia* their hybrid offspring ×*Sorbaronia fallax* (UC140) and the resulting open pollinated seedlings.

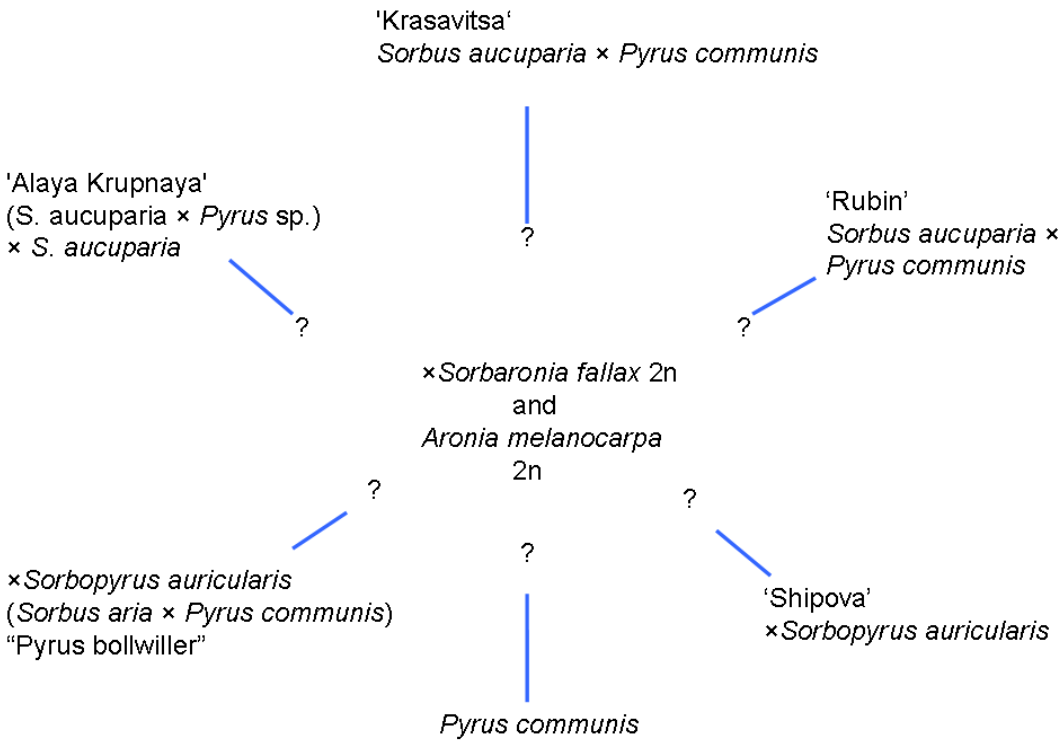

Fig. 9. Future hybridization to be done between diploid ×*Sorbaronia fallax*, ×*Sorbopyrus,* and *Pyrus communis.*