Sex and the single apomict

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Rosaceae

Origin of Maleae from $x = 9$ ancestor

- Potter et al. (2007); Campbell et al. (2007); Xiang et al. (2017); Zhang et al. (2017)
- Amygdaloideae
  - $(x = 8, 9)$
  - 9 tribes...
- Rosoideae ($x = 7$)
- Dryadoideae ($x = 9$)

Dryadoideae

- This small Nitrogen-fixing clade (4 genera, approx. 10 species) consists exclusively of diploids ($2n = 2x = 18$).
Rosoideae

• Potentilleae – “Reliable evidence for apomixis is restricted to two evolutionary lineages of Potentilleae: the *Potentilla* core group and *Alchemilla/Aphanes*.” Dobeš et al. (2015) [NB Christoph Dobeš’ talk, this symposium, and the symposium posters]

• Rubeae – *Rubus* ... “We found 100 % sexuality of diploid species, whereas triploid species had obligate unreduced embryo sac development. In contrast, tetraploid plants had varying degrees of sexuality. Additionally, we discovered that *R. bifrons* has the ability to undergo a reproduction mode switch as a reaction to environmental conditions.” Šarhanová et al. (2012)

• Sanguisorbeae – *Polylepis* ... “However, the hard-shelled nutlets and the apparently short-lived endosperm of *Polylepis* seeds have so far hampered assessments of apomixis by flow cytometry (M. Kessler, personal observation).” Kessler et al. (2014)
Amygdaloideae

- Tribe Amygdaleae; data on polyploidy in this tribe come from *Prunus* L. Although *Prunus* is economically important, and the breakdown of gametophytic SI in polyploids is well-studied in the genus (Hauck et al. 2006), polyploidization apparently interpreted as the result of fertilization of unreduced gametes only by Tavaud et al. (2004), and then without reference to the occurrence of gametophytic apomixis. Allopolyploidization inferred in *Prunus* nrITS phylogeny by Chin et al. (2014)

- Tribe Maleae (“Maloideae”); WGD origin from within the subfamily

- Genera from fleshy-fruited Rosaceae in subtribe Malinae (apples; notably also hawthorns, mountain ashes, whitebeams, and serviceberries) are useful as models with which to explore the evolutionary implications of asexual reproduction.
Fig. 5 in Whole genome comparisons of *Fragaria, Prunus* and *Malus* reveal different modes of evolution between Rosaceous subfamilies. Jung et al. (2012) BMC Genomics 13:129
https://doi.org/10.1186/1471-2164-13-129

Fig. 3 in The genome of the domesticated apple (*Malus × domestica* Borkh.). Velasco et al. (2010) Nature Genetics 42, 833–839.

Origin of Maleae (x = 15, 17) from x = 9 ancestor.
Rosaceae tribe Maleae

Fig. 1. Summary trees from maximum likelihood (ML) analyses of (a) combined chloroplast DNA sequence data, and (b) nuclear ribosomal ITS DNA sequence data for Pyreae.
What’s special about hawthorns (*Crataegus*)?

- Some hawthorn species grown for fruit (China, Mediterranean basin, Mexico, SE U.S.A.)
- Traditional herbal medicine
- Documented therapeutic use in treatment of heart disease (Cochrane Review; Diane et al. 2016)
- But taxonomically complex
**Crataegus**
Unspecialized flowers;
Zoochorous; fleshy fruits
Stamen number polymorphism

• appears to have spread together with apomixis
• C. subg. Americanae (North Americ only)
  – C. series Crus-galli; A\textsubscript{10} 4x C. crus-galli
  – C. series Punctatae; A\textsubscript{20} 2x C. punctata
  – Studied in Ontario (Dickinson & Phipps 1986)
  – Also A\textsubscript{10} 4x C. chrysocarpa, A\textsubscript{10} 4x C. macracantha, studied in western North America and Ontario

• North American C. subg. Sanguineae
  – C. series Cerrones (A\textsubscript{20} 2x; A\textsubscript{10} 4x)
  – C. series Dougiasianae (A\textsubscript{20} 2x, 3x; A\textsubscript{10} 4x)
  – C. series Purpureofructae (A\textsubscript{10} 4x only)
  – Studied in western North America and Ontario (Dickinson et al. 1996, 2008)
Crataegus fruits vary in color from yellow through orange, red, and purple to black

Crataegus douglasii - approx. 10 stamens per flower

Crataegus suksdorfii - approx. 20 stamens per flower

Crataegus flowers vary in calyx lobe margination in a manner correlated with fruit color (red, black)

Photo: K. Morse
Pollen and self-compatibility

- Pollen appears to be meiotically reduced in both diploid and tetraploid *Crataegus*
- Rosaceae exhibit gametophytic self-incompatibility
- Diploid *Crataegus* are self-sterile (Dickinson & Phipps 1986; Dickinson et al. 1996)
- GSI breaks down in polyploids; tetraploid *Crataegus* are self-fertile (triploids have reduced pollen fertility)
- Pollen-ovule ratios >> 1,000 regardless of ploidy level (A$_{20}$ diploids, 2—10K; A$_{10}$ tetraploids, 1—9K)
Gametophytic apomixis

- Apospory well-documented cytologically (Muniyamma & Phipps 1979 and subsequently; Dickinson & Phipps 1986; Ptak 1989; Dickinson et al. 1996)
- Correlated with morphological variability (Dickinson & Phipps 1985)
- Documented with flow cytometry (Talent & Dickinson 2007)
- Occurrence documented analytically, using SSR data (Lo et al. 2010a)
- Pollination experiments demonstrate pseudogamy
Crataegus punctata (2x) reproduces sexually, while *C. crus-galli* (4x) and *C. tenax* (3x) both produce seeds clonally; do local populations arise from offspring of only one or a few parents?

All three species are native to Ontario.

**Morphological variability (1985)**

![Diagram showing morphological variability](image)

Fig. 4. Disposition of the 160 OTU sample in the space of the first and second principal components of the correlation matrix for the 11 flower and fruit descriptors (table 1). These axes account for 43% and 20% of the trace of the correlation matrix, respectively. Correlations of the descriptors with the components are indicated by the vector diagram. Symbols as described in figure 2; see table 4 for additional details.
Gametophytic apomixis in *C. crus-galli* sensu lato can explain its topodeme structure.

Genetic data (single-seed SSRs)

Lo et al. (2010a)
**Table 3.** Estimates of outcrossing, selfing, and apomixis rates among *Crataegus punctata* and *C. crus-galli* progenies, including both multilocus and single-locus estimates from microsatellite data (loci as given in Table 2). Standard deviations are obtained from 1000 bootstraps with families as resampling units.

<table>
<thead>
<tr>
<th></th>
<th><em>C. punctata</em></th>
<th><em>C. crus-galli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Multilocus outcrossing rate ($t_m$)</td>
<td>0.96 ± 0.03</td>
<td>0.3 ± 0.051</td>
</tr>
<tr>
<td>Average single locus outcrossing rate ($t_s$)</td>
<td>0.85 ± 0.035</td>
<td>0.18 ± 0.053</td>
</tr>
<tr>
<td>CH01F02-based outcrossing rate</td>
<td>0.67 ± 0.076</td>
<td>0.13 ± 0.054</td>
</tr>
<tr>
<td>CH03A02-based outcrossing rate</td>
<td>0.90 ± 0.094</td>
<td>0.25 ± 0.067</td>
</tr>
<tr>
<td>CH04F06-based outcrossing rate</td>
<td>0.69 ± 0.069</td>
<td>0.16 ± 0.064</td>
</tr>
<tr>
<td>CH04G04-based outcrossing rate</td>
<td>0.71 ± 0.13</td>
<td>0.31 ± 0.016</td>
</tr>
<tr>
<td>CH05D04-based outcrossing rate</td>
<td>0.96 ± 0.075</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>Biparental inbreeding ($t_m - t_s$)</td>
<td>0.12 ± 0.035</td>
<td>0.11 ± 0.028</td>
</tr>
<tr>
<td>Rate of selfing ($s$)</td>
<td>0.036</td>
<td>0.17</td>
</tr>
<tr>
<td>Rate of apomixis ($a$)</td>
<td>—</td>
<td>0.47 ± 0.099</td>
</tr>
<tr>
<td>Fixation coefficient ($F$)</td>
<td>0.07 ± 0.061</td>
<td>0.3 ± 0.006</td>
</tr>
</tbody>
</table>
Flow cytometry

• Flow cytometry of (fresh) leaf tissue has been critical for efficiently surveying variation in ploidy level in *Crataegus* (Talent & Dickinson 2005)
• Flow cytometry of seeds (Talent & Dickinson 2007a, b, c; Talent 2009) demonstrates the multiplicity of fertilization events possible for unreduced female gametes in *Crataegus*
• Flow cytometry of seeds has also provided a basis for modeling the evolution of gametophytic apomixis in *Crataegus* and the Rosaceae (Talent 2009; http://nadiatalent.github.io/).
• Flow cytometry of seeds also potential advantages of pseudogamous apomixis (Talent 2009; cf. Haskell 1960 and Šarhanová et al. 2017, both re *Rubus*)
Most North American black-fruit hawthorns belong to *Crataegus* L. subg. *Sanguineae*
Flow cytometric seed scan

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

- Magisterial statements about the infrequency of hybridization based on few if any data (e.g. Phipps 2005).
- Most studies have been made on hybridization between diploid species; almost all, in fact, on hybrids involving the Eurasian species *C. monogyna*, both in Europe (Christensen 1982; Christensen 1992, 1996), and in North America where the species has become naturalized (Wells & Phipps).
- Thus until recently, no studies of allopolyplloidization in *Crataegus*, i.e. until the work of Lo et al. (Lo et al. 2007; Lo et al. 2009; Lo et al. 2010) and Zarrei et al. (2014).
Phenological isolation may be absent when exotic species become naturalized.

Overlapping flowering period

Hybrid described as C. ×ninae-celottiae (Christensen et al. 2014)

Frequency of co-occurrence (# of sites)

Assembled from data collected by P.F.Ulf-Hansen and P.G.Smith from specimens at UWO
Hybridization in *Crataegus*

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• Thus, until recently, no studies of allopolyploidization in *Crataegus*, i.e. until the work of Lo et al. (Lo et al. 2007; Lo et al. 2009; Lo et al. 2010) and Zarrei et al. (2014).
Hybridization in North America between sexual diploids (Christensen et al. 2014)

In western Oregon, *Crataegus suksdorfii* is a native diploid (2x); *C. monogyna* (also 2x) is introduced and naturalized; their hybrid is *C. ×cogswellii*.

In southern Ontario, *Crataegus punctata* is a native diploid (2x); *C. monogyna* (also 2x) is introduced and naturalized there as well; their hybrid is *C. ×ninae-celottiae* (Christensen et al. 2014).
Hybridization in *Crataegus*

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- Thus until recently, no studies of allopolyploidization in *Crataegus*, i.e. until the work of Lo et al. (Lo et al. 2007; Lo et al. 2009; Lo et al. 2010) and Zarrei et al. (2014).
Identifying hybrids:

Distribution of ITS2 ribotypes within cytotypes and taxa (based on 369 bp per accession):

Zarrei et al. (2014) Reticulate Evolution in North American black-fruiting Hawthorns (Crataegus Section Douglasia; Rosaceae): Evidence from Nuclear ITS2 and Plastid Sequences. Ann. Bot. 114: 253-269. The number of clones sequenced per accession was proportional to the ploidy level of the accession (in parentheses, the number of accessions per taxon).
Next-generation DNA sequencing

• Hyb-Seq approach
• targeted on low copy-number nuclear genes from apple genome
• a small, structured data set...
• genomic libraries were constructed for 24 Crataegus accessions representing 12 diploid species and four tetraploid ones, and all seven major lineages within the genus
Diploid (2x) species

Crataegus

Sample for current study placed on Crataegus phylogeny based on 14 cpDNA loci (Zarre et al. 2015 AoB Plants 7: plv045, Fig. 1)

infrageneric classification:
Section – Series

x = 17 (Rosaceae Tribe Maleae)

cpDNA tree based on 10,570 bp from 14 chloroplast loci, for total of 44 Crataegus accessions
Diploid and tetraploid species

Pacific Northwest, Cypress Hills, upper Great Lakes basin

Rocky Mountains

subgenera

Sanguineae

Americanae

Crataegus

transcontinental

Sample for current study placed on *Crataegus* phylogeny based on 14 cpDNA loci (Zarrei et al. 2015 AoB Plants 7: plv045, Fig. 1)

Infrageneric classification:
Section – Series

$x = 17$ (Rosaceae Tribe Maleae)
Sample Preparation

- target enrichment baits designed for 1254 exons from 257 nuclear genes, using the published apple genome as a reference
- solution hybridization with MycroArray RNA baits (24-plex)
- obtained total of 314,000 bp sequence data
Data from chloroplast genome

Chloroplast genome transmitted maternally (i.e. not via pollen)

Crataegus chloroplast genome data comprise 129,580 bp

These results suggest subg. Sanguineae maternal parentage of the tetraploids
Phylogenetic Analysis

These results corroborate subg. *Sanguineae* parentage of the tetraploids.

62 *gene trees* (of 167) have a similar topology

gene tree 10 (of 167)

•maximum likelihood (RAxML)
Phylogenetic Analysis

17 gene trees (of 167) have a similar topology

These results suggest subg. Americanae parentage of the tetraploids

• maximum likelihood (RAxML)
Phylogenetic Analysis

167 genes (314,000 bp)

- network (SplitsTree/NeighborNet)
NGS Conclusions

• Next-generation DNA sequence data (a LOT!) corroborate what we thought we knew about hybridization in *Crataegus* based on a MUCH smaller datasets

• Our results suggest that in *Crataegus* the smaller datasets achievable with older DNA sequencing methods (using strategically chosen loci) can also yield data useful in inferring relationships within and between species groups

• Our results are instructive for workers seeking to resolve phylogenetic relationships in subgenera *Americanae* (eastern N. America) and *Crataegus* (western Eurasia)

• More info available online at [http://labs.eeb.utoronto.ca/dickinson/NABFH-I/](http://labs.eeb.utoronto.ca/dickinson/NABFH-I/)
NGS Conclusions

• Despite the results obtained, the Hyb-seq approach used here (begun in 2013!) may be suboptimal when attempting to resolve allelic relationships between allopolyploids and their parents

• Use of the PacBio platform and the PURC bioinformatics pipeline by Rothfels, Pryer & Li (2017) shown to be highly successful for resolving allopolyploid phylogenies

• Future work on Maleae phylogeny and reticulation would almost certainly benefit from this approach
Geographic parthenogenesis

• In *C. sect. Douglasia* (Pacific Northwest *C. subg. Sanguineae*)
  • Diploid $A_{20}$ *C. suksdorfii* has a narrow geographic range (Oregon W of the Cascades, adjacent California and Washington) and a narrow climatic niche
  • Autotriploid $A_{20}$ *C. gaylussacia* has most narrow range, climatic niche
  • Allotriploid $A_{20}$ *C. suksdorfii* has wider range, climatic niche
  • Allotetraploid $A_{10}$ *C. douglasii* has widest range, climatic niche
• Diploid-tetraploid contrast in relative allocations to dispersal versus establishment (Coughlan et al. 2016)
Geographic distribution of herbarium records representing 590 independent sites, from which at least one individual tree has been collected for either C. subg. *Americanae* (n=214) or C. subg. *Sanguineae* (n=376).

Geographic ranges

Coughlan et al. in press

both C. subg. *Americanae*

C. subg. *Sanguineae*
Geographic distribution of herbarium records representing 590 independent sites, from which at least one individual tree has been collected for either C. subg. Americanae (n=214) or C. subg. Sanguineae (n=376).

Geographic ranges

diploid $A_{20}$
autotriploid $A_{20}$
C. gaylussacia
all C. subg. Sanguineae

Coughlan et al. in press
Principal Components Analysis of 19 WorldClim variables for herbarium records representing 590 independent sites, from which at least one individual tree has been collected for either C. subg. Americanae (n=214) or C. subg. Sanguineae (n=376).

Climate niches

both C. subg. Americanae

C. subg. Sanguineae
Principal Components Analysis of 19 WorldClim variables for herbarium records representing 590 independent sites, from which at least one individual tree has been collected for either *C. subg. Americanae* (n=214) or *C. subg. Sanguineae* (n=376).

**Climate niches**

- Autotriploid $A_{20}^{C. gaylussacia}$
- Diploid $A_{20}$
- All *C. subg. Sanguineae*
Proportional fruit pulp and seed masses and Geographic Parthenogenesis

$2x$ fruit pulp mass $< 4x$ fruit pulp mass
$2x$ seed mass $> 4x$ seed mass

Coughlan et al. (2016)
Geographic parthenogenesis

• Similar patterns observed in C. ser. Cerrones (Rocky Mountains C. subg. Sanguineae)
• Diploid $A_{20}$ C. saligna has narrowest geographic range (W Colorado, NE Utah) and climatic niche
• Allotetraploid $A_{10}$ C. rivularis, C. erythropoda occupy wider geographic ranges, broader climatic niches
• Climatic niches of C. ser. Cerrones and subg. Americanae all overlap.
• Nevertheless, only C. saligna exhibits more densely arranged secondary and tertiary veins that have been suggested to be adaptations for resistance to xylem cavitation due to drought and (or) freezing temperatures
Rocky Mountain hawthorns (*Cerrones*)

- Are there morphological differences associated with the niche specialization?

In earlier work on C. series *Douglasianae* we observed variation in vein density associated with differences in habitat factors (Fujs 2013; elevation, temperature regime, moisture regime).

In C. series *Cerrones* from the Rocky Mountains we cleared and stained leaves, made digital images, and quantified vein density and other features.
Log in as “2423” with password “reticulation”
Geographic Parthenogenesis

- Polyploidy and/or hybridity condition wider climate niche
- Associated with greater relative allocation of biomass to seeds of **diploids** (= establishment)
- Associated with greater relative allocation of biomass to fruit pulp of **tetraploids** (= dispersal; hybridization effect)
- Associated with self-compatibility and pseudogamous gametophytic apomixis in **tetraploids** (hybridization effect)
- **Not** associated with greater density of leaf venation (drought/freezing tolerance) found in (some) diploids
Hybridization happens

• Allotetraploid *C. douglasii* and *C. rivularis* share some character states with one or the other parent
• Other character states in allotetraploid *C. douglasii* and *C. rivularis* are intermediate between those of the parents
• These suggest the role of hybridization
• **Only the breakdown of GSI** in allotetraploid *C. douglasii* (and *C. rivularis* presumably) appears to be due to polyploidization per se
Hybridization happens

<table>
<thead>
<tr>
<th></th>
<th>4x C. subg. Americanae</th>
<th>3-4x C. subg. Americanae</th>
<th>2x C. subg. Sanguineae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>C. chrysocarpa</strong> and/or <strong>C. macracanthes</strong></td>
<td><strong>C. douglasii</strong> C. rivularis</td>
<td><strong>C. suksdorfii</strong> C. saligna</td>
</tr>
<tr>
<td>Leaves, venation</td>
<td>not dense</td>
<td>not dense</td>
<td>dense in C. saligna</td>
</tr>
<tr>
<td>Thorn length</td>
<td>longest</td>
<td>longer</td>
<td>short, shortish</td>
</tr>
<tr>
<td>Fruit color</td>
<td>red</td>
<td>black</td>
<td>black</td>
</tr>
<tr>
<td>Fruit size</td>
<td>large</td>
<td>(large)</td>
<td>small</td>
</tr>
<tr>
<td>Relative seed size</td>
<td>small</td>
<td>small</td>
<td>large, (large)</td>
</tr>
<tr>
<td>Compatibility</td>
<td>SC</td>
<td>SC</td>
<td>GSI</td>
</tr>
<tr>
<td>Pseudogamous apospory</td>
<td>present</td>
<td>present</td>
<td>absent</td>
</tr>
<tr>
<td>Geographic range</td>
<td>continental</td>
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<td>narrow</td>
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<tr>
<td>Ecological niche</td>
<td>widest</td>
<td>wide, offset</td>
<td>narrow, offset</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wide, overlapping</td>
<td>overlapping</td>
</tr>
</tbody>
</table>
“Sorbus” sensu lato

• Early work by Liljefors
• Dismembered on the basis of molecular phylogeny (Lo & Donoghue 2012) by Sennikov & Kurto (2017)
  • Robertson et al. (2010) and other British workers
  • Sosa et al. (2014) on breeding system variation in Canarian and Spanish Aria
  • Hajrudinović et al. (2015) seed FC of 2x, 3x, and 4x Aria demonstrates 2x sexuality, 3x apomixis, and 4x mixed mating systems
Fig. 1 The current hypotheses for the origins of Sorbus taxa in the Avon Gorge, according to Rich & Jeremy (1998), and Nelson-Jones et al. (2002) excluding recently described taxa. Members of the nothosubgenus Tormaria and nothosubgenus Soraria are thought to represent allopolyploids, with Tormaria taxa having genomes inherited from both S. torminalis and a member of the subgenus Aria and Soraria taxa having genomes inherited from both S. aucuparia and a member of subgenus Aria.
• RAPD profiles demonstrate clonality in tetraploid Cotoneaster (Nybom & Bartish 2007)

• FC and sequence analyses of nuclear and cpDNA loci of an East Asian agamic complex; hypothesis that allotetraploid formation involved an unreduced egg from diploid parent (Li et al. 2017)
Dickson (1992) introduced the use of flow cytometry to work on Maleae, documented ploidy level variation in Rosaceae, notably *Malus coronaria*

- Apomixis and fertilization of unreduced gametes inferred from seedling anthocyanin presence (Sampson 1969)
- Flow cytometric seed screen (Ranney et al. 2004)
- Potential introgression of domestic apple into *M. coronaria* (Kron & Husband 2009)
Amelanchier

- Gametophytic apomixis, polyploidy, and hybridization shown by C. S. Campbell and students
- Documentation of triploid sexuality and its potential role in the origin of tetraploids via a triploid bridge (Burgess et al. 2014)
- Limited role for geographic parthenogenesis in tetraploids
All these genera suggest...

- Gametophytic apomixis common in the two large Rosaceae subfamilies.
- Malinae ($x = 17$) are part of a lineage that originated through whole genome duplication from ancestral $x = 9$ Amygdaloideae.
- Strongly suggests fertilization of unreduced female gametes key to origin of Malineae.
- Evidence for spread of apomixis via reduced pollen of tetraploids as part of the radiation of large genera.
- Pseudogamy seems to be critically associated with apospory (Talent 2009).
- Gametophytic apomixis makes possible a dynamic breeding system responsible for more than just clonal perpetuation of apomictic genotypes.
Sex and the single apomict

It works!
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