A Multi-Dimensional Approach to Comparative Genomics in *Fragaria* (Rosaceae)

Thomas M. Davis
Melanie E. Shields
Bo Liu
Lise B. Mahoney
Qian Zhang
Department of Biological Sciences, University of New Hampshire, USA

Kim E. Hummer
Nahla V. Bassil
USDA-ARS, NCGR, Corvallis, Oregon, USA

Wambui Njuguna
Department of Horticulture, Oregon State University, Corvallis, Oregon, USA

Magnus Lundberg
Torsten Eriksson
Department of Botany, Stockholm University, Stockholm, Sweden

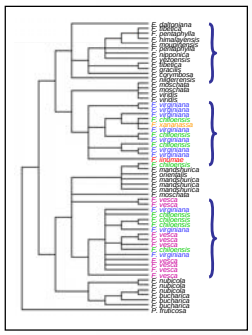
Background & Context

Species: The genus *Fragaria* (the strawberries) comprises about 22 species, including the octoploid cultivated strawberry, *Fragaria ×ananassa* and its immediate octoploid ancestors, *F. chiloensis* and *F. virginiana*. Each of the latter have four distinct subspecies.

Ploidy: Ploidies in *Fragaria* range from diploid (2n = 2x = 14) to decaploid (2n = 10x = 70).

Genome size: The size of the basic (x = 7) *Fragaria* genome is about 200 Mb.

Previous phylogenies & octoploid ancestry: Phylogenetic studies of three protein-encoding genes (Davis & DiMeglio, 2004; Rousseau-Gueutin et al., 2009) identified *F. iinumae*, and *F. vesca* and/or *F. mandshurica*, as likely diploid allele donors to the octoploids



Asian diploids and tetraploids – no octoploid alleles.

Octoploid alleles clustered with *F. iinumae* allele.

Octoploid alleles clustered with *F. vesca* and *F. mandshurica* alleles.

Phylogeny based on ADH intron sequence (Davis & DiMeglio, 2004)

Octoploid subgenome composition models

Patterned after the subgenome composition model of bread wheat (AABBDD), three subgenome composition models have been proposed for the octoploid *Fragaria* species, based upon observations of meiotic pairing:

Model 1: AAAABBCC (Fedorova, 1946)

Model 2: AAA'A'BBBB (Senanayake & Bringhurst, 1967)

Model 3: AAA'A'BBB'B' (Bringhurst, 1990)

Hypothesis & Objectives

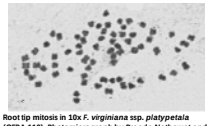
We hypothesize that octoploid subgenome composition is more complex and variable than has been previously recognized.

Objective and approaches: Survey octoploid subgenome compositions, *using 1) molecular cytogenetic assays, and 2) targeted allele sampling.*

Results

Ploidy determinations:

Ploidy levels were inferred from flow-cytometric measurements of nuclear DNA content, as confirmed by chromosome counts.



Root tip mitosis in 10x *F. virginiana* ssp. *platypetala* (CFRA 110). Photomicrograph by Preeda Nathewet and Tomohiro Yanagi, Kagawa University, Kagawa, Japan.

Ploidy	DNA ratio*
Diploids	0.23 - 0.29
Tetraploids	0.46 - 0.50
Hexaploid	0.67
Octoploids	0.74 - 0.83
Decaploids	1.0 - 1.16
F. virginiana ssp. *platypetala (CFRA 110)*	1.0
F. iturupensis	1.16

*Relative to standard = *Ilex crenata* 'Fastigiata'.

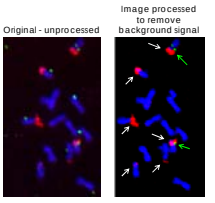
Nuclear DNA content measurements were performed using leaf tissue samples. The service provider was Plant Cytometry Services, Schijndel – The Netherlands.

KEY FINDINGS:

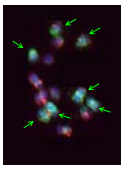
- Octoploid's genomes < 4 times as large as diploid's.
- Decaploidy exists within a subspecies (*F. virginiana* ssp. *platypetala*) previously thought to be exclusively octoploid.
- Genome size variability among both octoploids and decaploids suggests variability in both 8x and 10x subgenome compositions.

Fluorescent in situ hybridization:

FISH probes targeted the 25S (Cy3 = red) and 5S (fluorescein = green) rRNA loci (A), while GISH probes were constructed using total genomic DNA from various pairs of Fragaria species (B).



A) FISH: Root tip mitosis in 2x *F. vesca* 'Hawaii'. White and green arrows indicate 25S and 5S loci, respectively.



B) GISH: Root tip mitosis in diploid hybrid - F. vesca x F. nilgerrensis. F. vesca probe = Cy3 (red); F. nilgerrensis probe = fluorescein (green - arrows)

KEY FINDINGS:

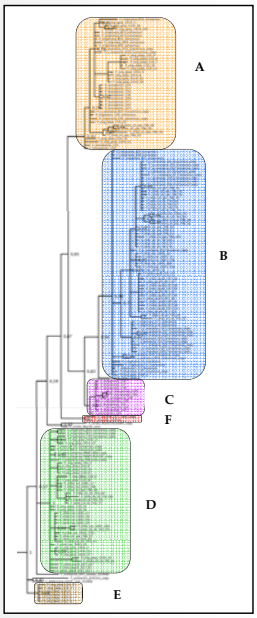
- In diploid *F. vesca* "Hawaii", the current subject of a whole genome sequencing project, three chromosome pairs have 25S loci, of which one pair also has a 5S locus. Using additional site-specific probes, each chromosome will be differentiable.
- *Fragaria* species and subgenomes differ sufficiently to allow differentiation by GISH in hybrids and allopolyploids.

Subgenomic allele signatures:

This analysis was based upon 600-750 bp sequence reads from the RGA-Subtilase gene pair locus.

KEY FINDINGS:

- Alleles of 8x and 10x accessions fell into five clades (A-E), and were differentially distributed among taxa.
- Clade D comprised alleles from 2x *F. vesca* and *F. mandshurica*, and many 8x and 10x accessions, but not from 10x *F. iturupensis*.
- Clades C and E were comprised of alleles only from, respectively, *F. iturupensis* and newly discovered 10x forms of *F. virginiana* ssp. *platypetala*. These unique alleles may come from unknown diploids that participated in the hybrid origins of the 10x forms.
- No 2x alleles clustered with the 8x and 10x alleles in clades A and B. These alleles may trace to undiscovered 2x ancestors.
- No 8x- or 10x- derived alleles clustered with those of *F. iinumae* (Clade F), a presumed ancestral diploid!



Conclusions

The surprising discovery of a new 10x form of *F. virginiana*, the finding that genome size and allele-representation vary at both the 8x and 10x levels, and the finding that 8x and 10x accessions harbor RGA-subtilase alleles that cannot be traced to any known diploid, indicate that current knowledge of *Fragaria* species diversity and subgenome composition remains far from complete. FISH and GISH techniques show great promise as a means for examining subgenome composition and structure in *Fragaria*.

References:

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