



# **Natural Health Products and *Crataegus* of the Pacific Northwest**

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Paul Shipley, Jeanette Lee,  
Jenn Coughlan, Mehdi Zarrei

## NHPs and *Crataegus* of the Pacific Northwest

- Well-documented use of hawthorns in traditional treatments for heart disease
- Results of recent meta-analyses demonstrating statistically significant therapeutic benefits from hawthorn preparations
- We have made
  - vouchered collections of leaves, flowers, and fruits for chemical analyses and animal trials
- so as to document
  - taxonomic diversity
  - breeding behaviour and ploidy level variation
  - geographic parthenogenesis
  - diversification due to intersectional hybridization of the *Crataegus* flora of the Pacific Northwest
  - unique compositional signatures of phenolics in several species
  - effects of a limited number of extracts on an animal model of human metabolic syndrome

## NHPs and *Crataegus* of the Pacific Northwest

- Work on Pacific Northwest hawthorns has been spurred by interest in using native hawthorn species to develop Natural Health Products





## NHPs and *Crataegus* of the Pacific Northwest

- This interest takes advantage of the well-documented use of hawthorns in traditional treatments for heart disease



Commercially available products rely almost exclusively on Eurasian species, and to a great extent on imported raw materials.



- ...and recent results of meta-analyses demonstrating statistically significant therapeutic benefits from hawthorn preparations.

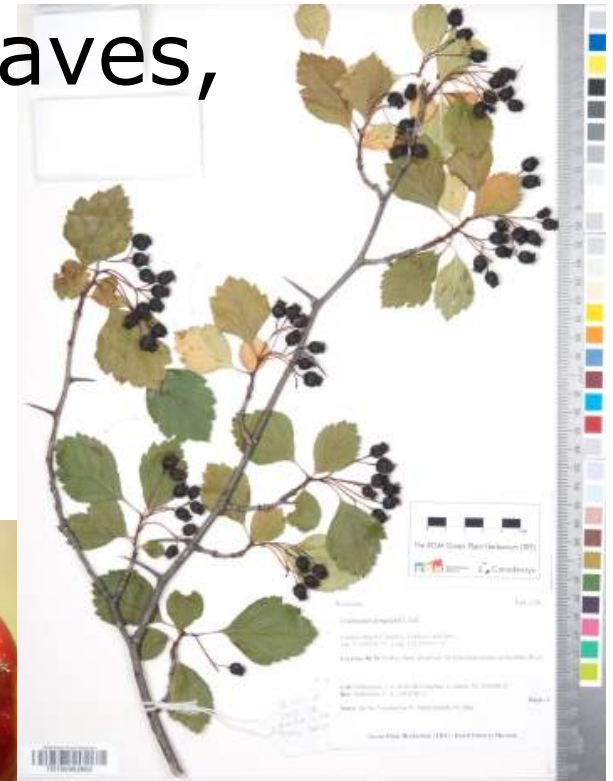
Hawthorn extract (made from the dried leaves, flowers and fruits of the hawthorn bush) may be used as an oral treatment option for chronic heart failure. In this review, 14 double-blind, placebo controlled randomised clinical trials (RCTs) were found. They did not all measure the same outcomes and several did not explain what other heart failure treatments patients were receiving. Those trials that could be included in a meta-analysis showed improvements in heart failure symptoms and in the function of the heart. **The results, therefore, are suggestive of a benefit from hawthorn extract used in addition to conventional treatments for chronic heart failure.**

- NSERC Canada Strategic Research Project funding was obtained to
  - develop methods with which to authenticate NHPs derived from hawthorns
  - develop biology-based processing methods with which to derive valuable NHPs from these renewable bio-resources
  - confirm the heart and cardiovascular protective properties in an established rodent model
  - If possible, identify the functional components of hawthorn NHPs using appropriate methods



## NHPs and *Crataegus* of the Pacific Northwest

- This and other funding let us make vouchered collections of leaves, flowers, and fruits...



- ...so as to provide tissue samples from these collections to the Canadian Center for DNA Barcoding, and...

Species: *Crataegus* (Family: Rosaceae) [1.2]

Sequence ID: TDC006-10-00-01 | GenBank Accession: [KJ251348](#)

Last Updated: 2014-04-20 | Genome: Chloroplast

Genus: *Crataegus*  
Nucleotide: 514 bp

```

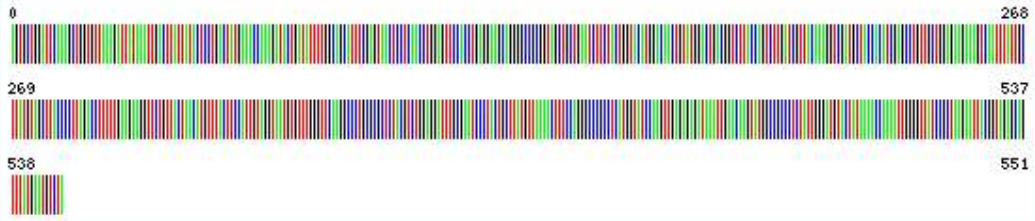
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Amino Acids:

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DKLNKYGRPLLGCITIKPLGLSAKNYGRAVYECL
    
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Illustrative Barcode:



0 268  
269 537  
538 551

[Add Tags & Comments](#) | Comments: 0 | Associated Tags: No Tags

**ELECTROPHEROGRAM TRACE FILES:**

Length	Pcr Primers	Seq Primer	Read	Status	Run Date
514	rbcLa-F / rbcLa-R	rbcLa-R	Reverse	high qual	2010-12-06
523	rbcLa-F / rbcLa-R	rbcLa-F	Forward	high qual	2010-12-06



## NHPs and *Crataegus* of the Pacific Northwest

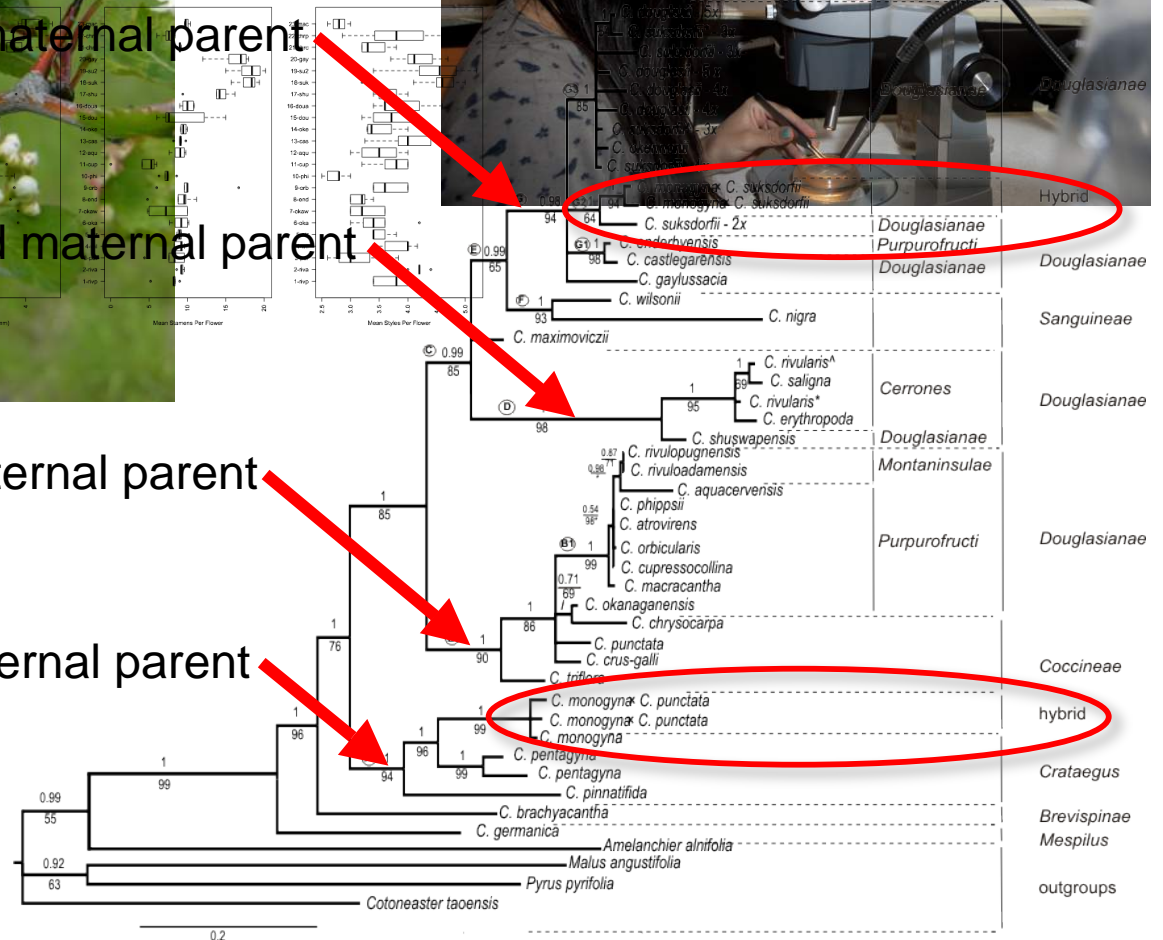
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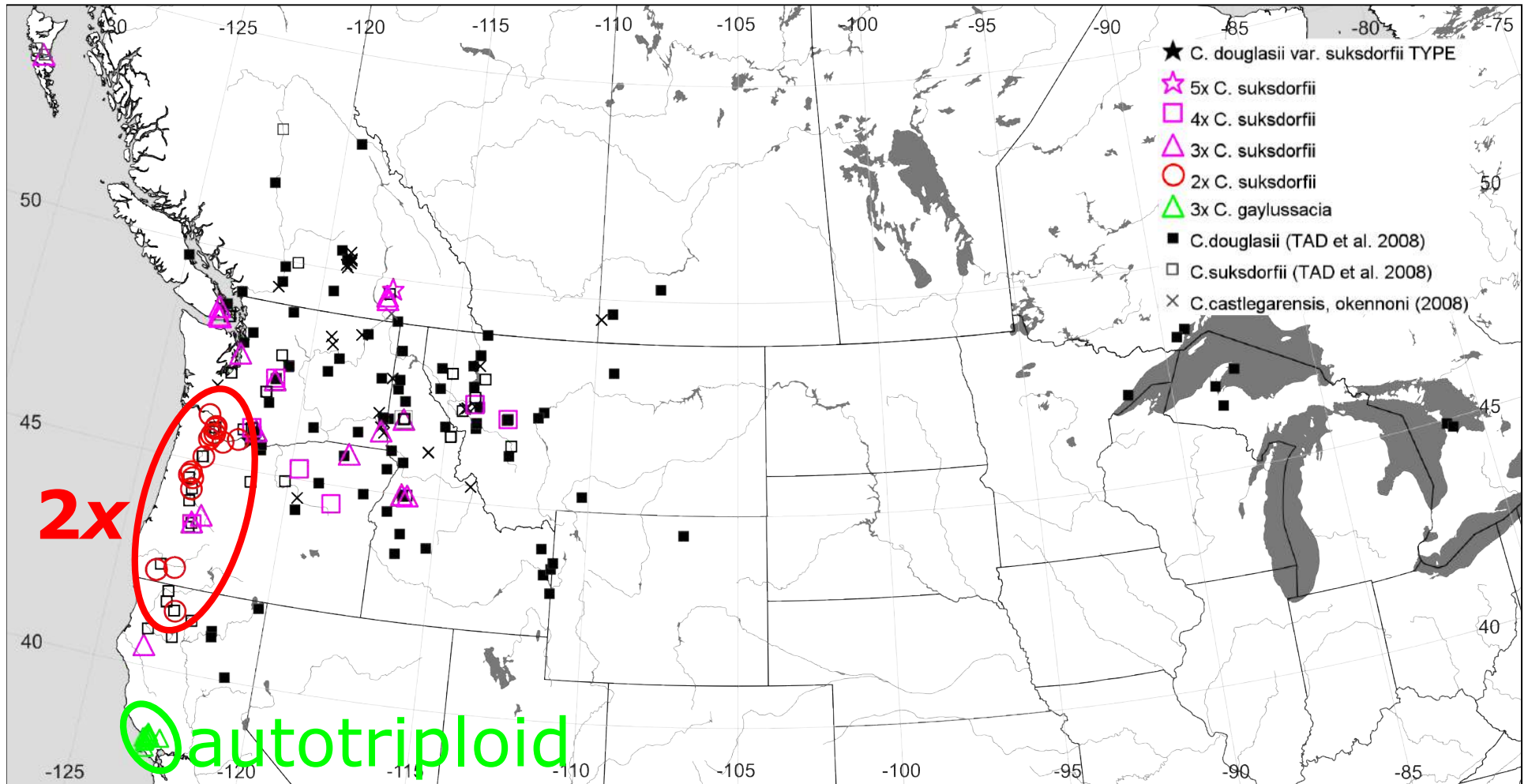
Shery Han



Kathleen Buck



# NHPs and *Crataegus* of the Pacific Northwest



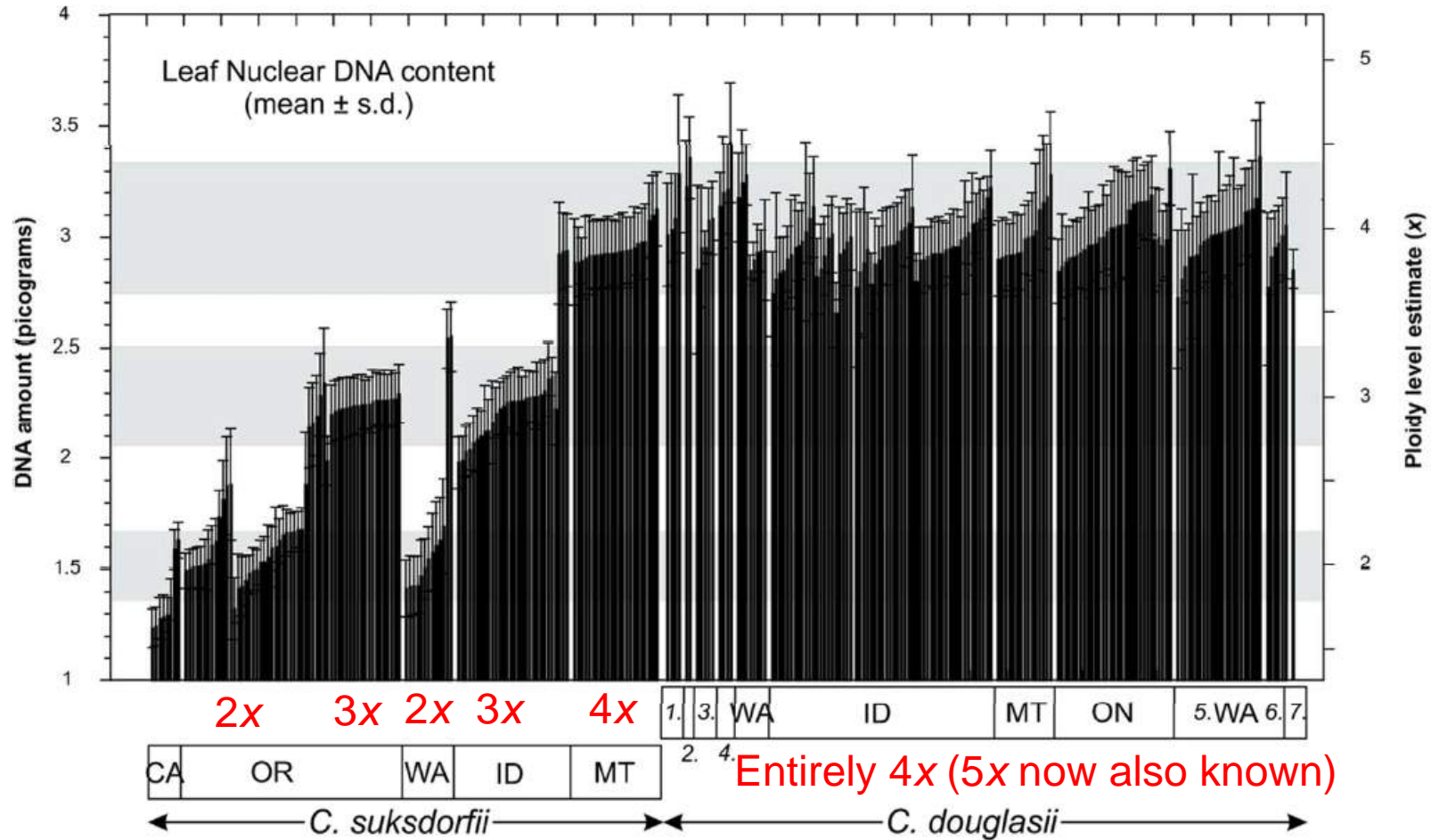
SimpleMappr plus Inkscape

Geographic parthenogenesis in *Crataegus* series *Douglasiana*



# NHPs and *Crataegus* of the Pacific Northwest

## Flow cytometry

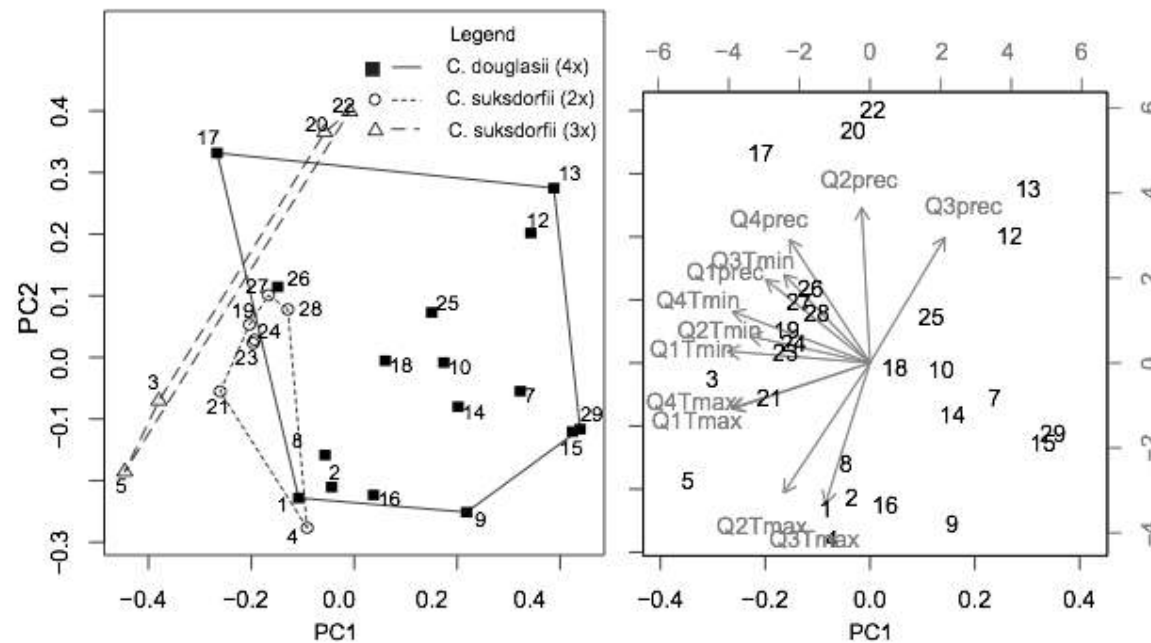


Section *Douglasianae* comprises diploids and polyploids as does section *Coccineae*.

19:18 Lo et al. (2013) Geographical parthenogenesis in Pacific Northwest hawthorns (*Crataegus*; Rosaceae). *Botany* 91: 107–116; Talent unpubl. data.

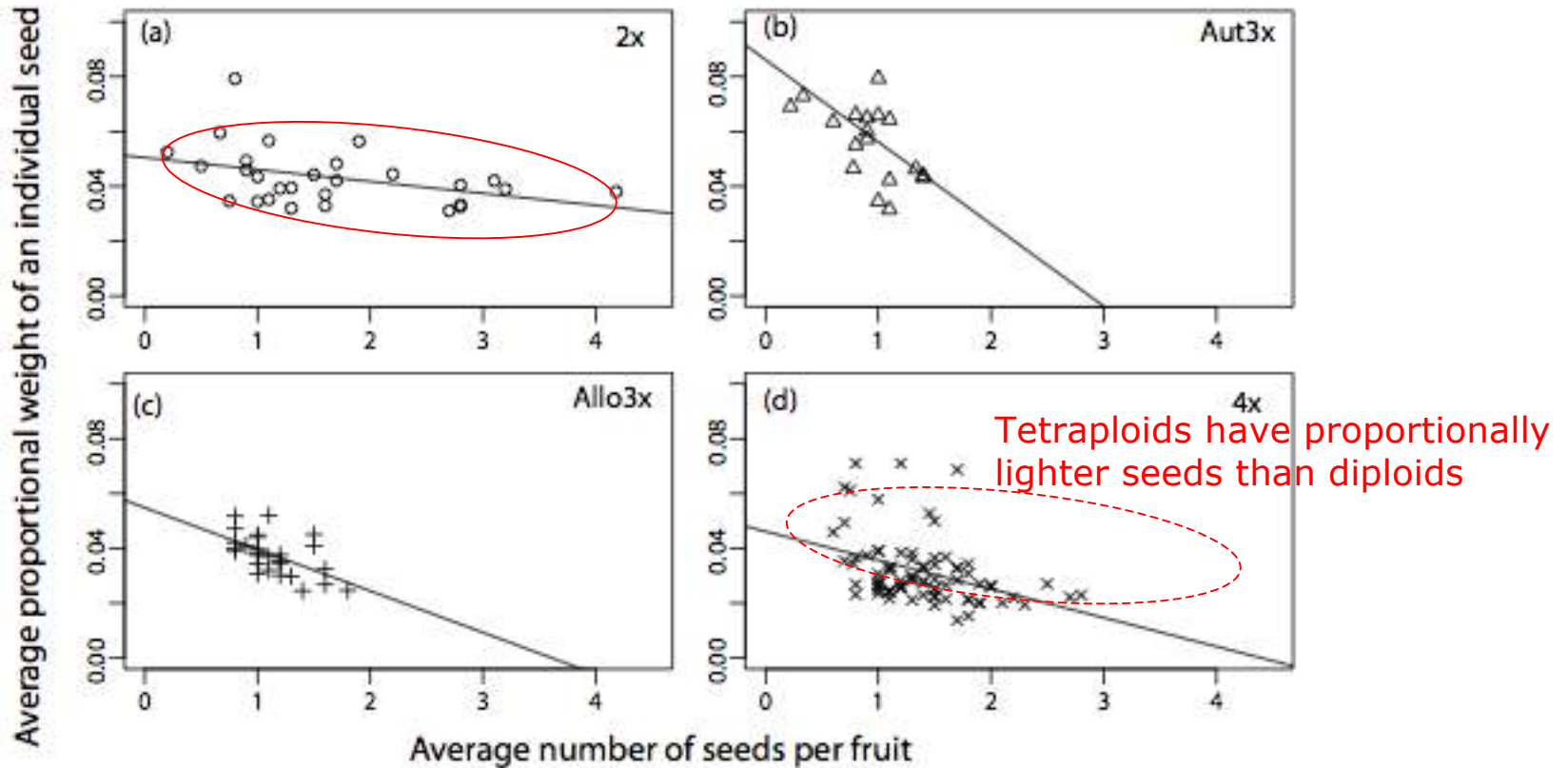
# NHPs and *Crataegus* of the Pacific Northwest

Quarterly mean values,  $T_{min}$ ,  $T_{max}$ , and Precipitation



McGoey et al. (2014) Stomata size in relation to ploidy level in North American hawthorns (*Crataegus*, Rosaceae). *Madroño* 61(2): 177-193.

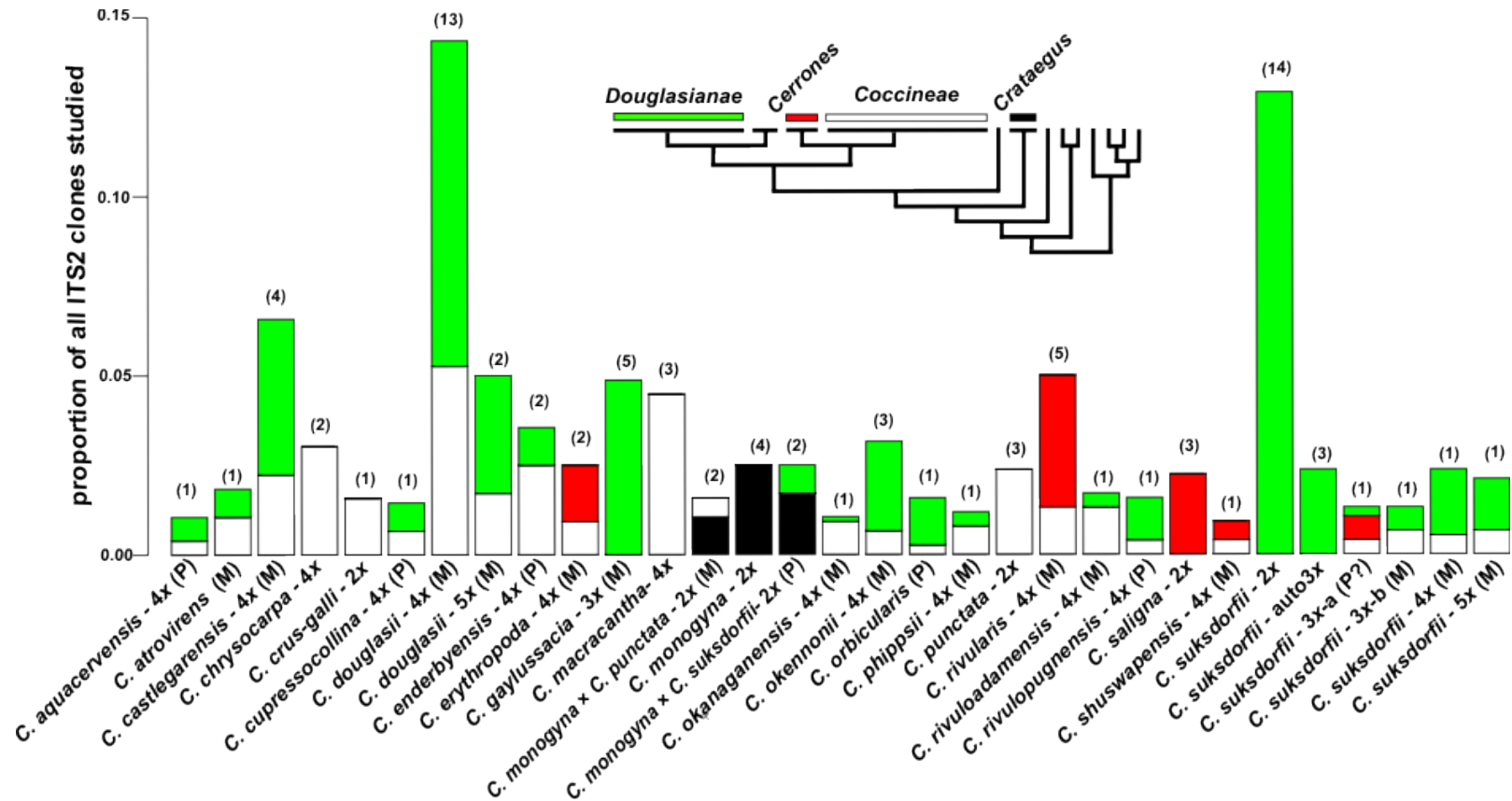
# NHPs and *Crataegus* of the Pacific Northwest



Coughlan et al. (2014) Stomata size in relation to ploidy level in North American hawthorns (*Crataegus*, Rosaceae). *J. Biogeography* in press.



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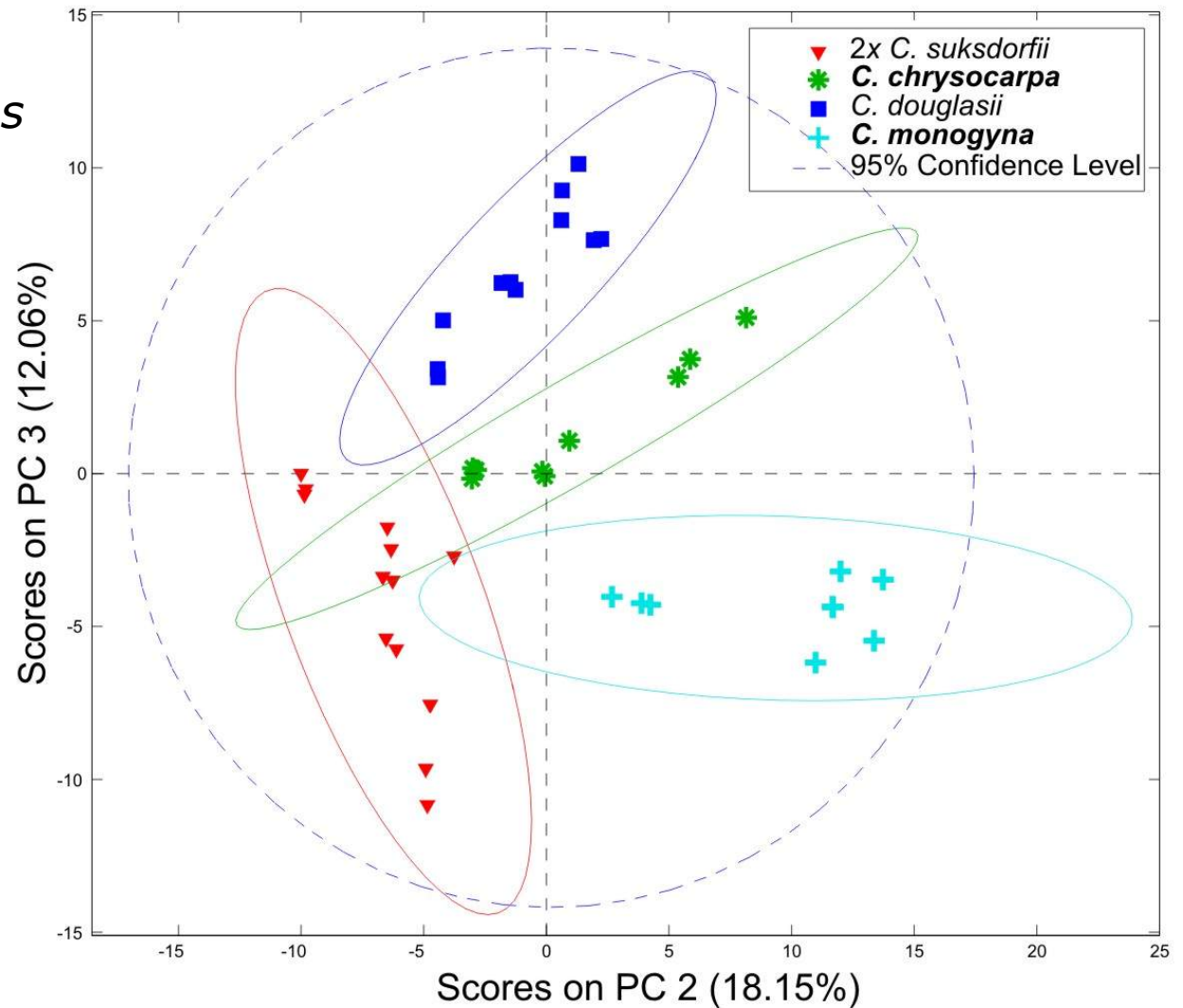
Zarrei et al. (2014) Reticulate Evolution in North American black-fruited Hawthorns (*Crataegus* Section *Douglasia*; Rosaceae): Evidence From Nuclear ITS2 and Plastid Sequences. *Ann. Bot.* in press.

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## NMR spectroscopy-based metabolomics of *Crataegus* leaf phenolics

Because phenolic compounds were targeted, the chemical shift range of 6-8 ppm was used. After import into Solo, the classes were defined for each sample according to its species identification. Before multivariate analysis, Pareto scaling and mean centering were applied to the data set. Following this, either a principal components analysis (PCA) model or partial least squares discriminant analysis (PLS-DA) model was generated.



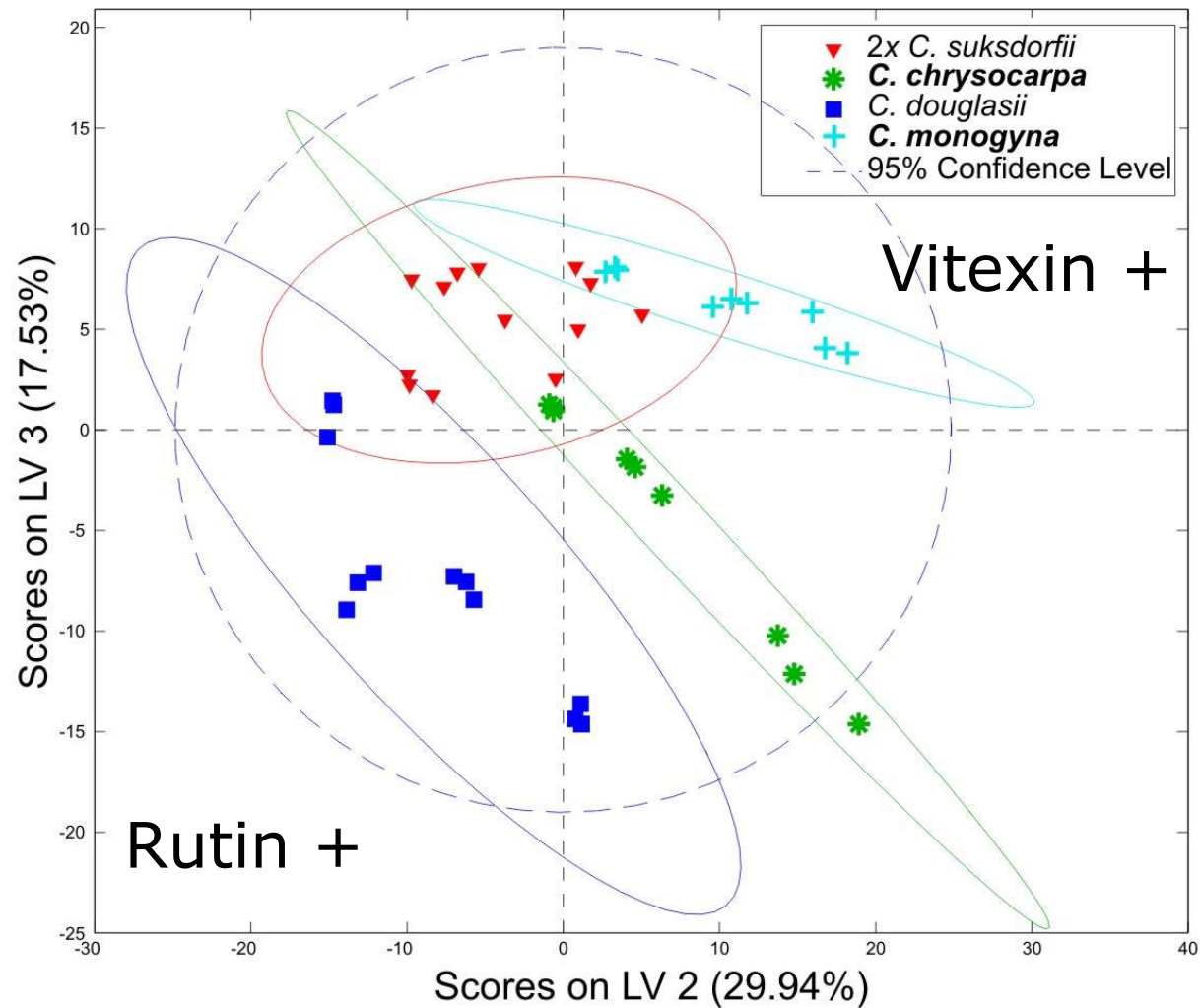


## NHPs and *Crataegus* of the Pacific Northwest

Calculated concentrations per g leaf material of three cardioprotective compounds in North American *Crataegus*

	Hyperoside (mg/g)	Chlorogenic Acid (mg/g)	Vitexin 2''-O-rhamnoside (mg/g)
<i>C. suksdorfii</i>	2.7 ± 1.1	7.3 ± 3.7	N.D.
<i>C. douglasii</i>	7.7 ± 1.8	19.0 ± 6.0	N.D.
<b><i>C. chrysocarpa</i></b>	3.3 ± 1.6	27.3 ± 8.5	N.D.
<b><i>C. monogyna</i></b>	7.9 ± 1.8	17.2 ± 4.3	6.5 ± 2.1

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- In vivo tests of hawthorn preparations were carried out using two sources of plant material (leaf and flowers, and fruit)
- These tests compared two hawthorn species (introduced *C. monogyna*, native *C. chrysoarpa*)
- These tests employed an animal model of human metabolic syndrome



## *Crataegus* of the Pacific Northwest

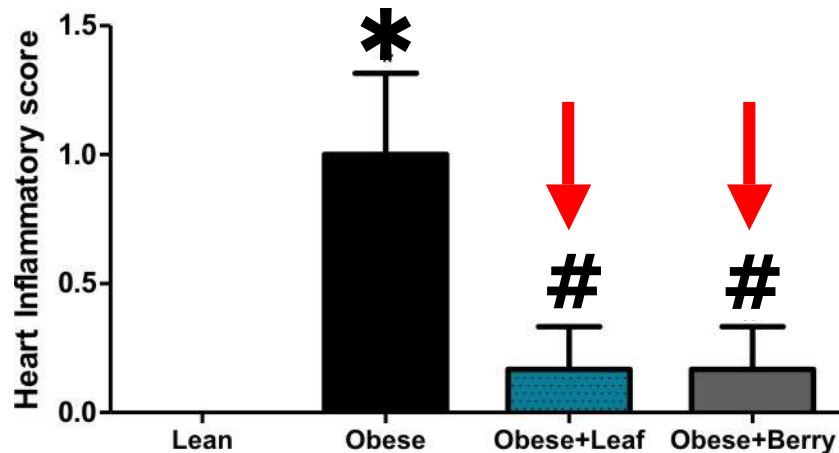
- The JCR:LA-cp rat:
  - one of a number of strains that carry the mutant autosomal recessive *cp* gene.
  - Animals, of all strains, that are homozygous, for the gene (*cp/cp*) become **obese**, insulin resistant, and hypertriglyceridemic.
  - Normal rats (+/+) are **lean** and metabolically normal.

## *Crataegus* of the Pacific Northwest

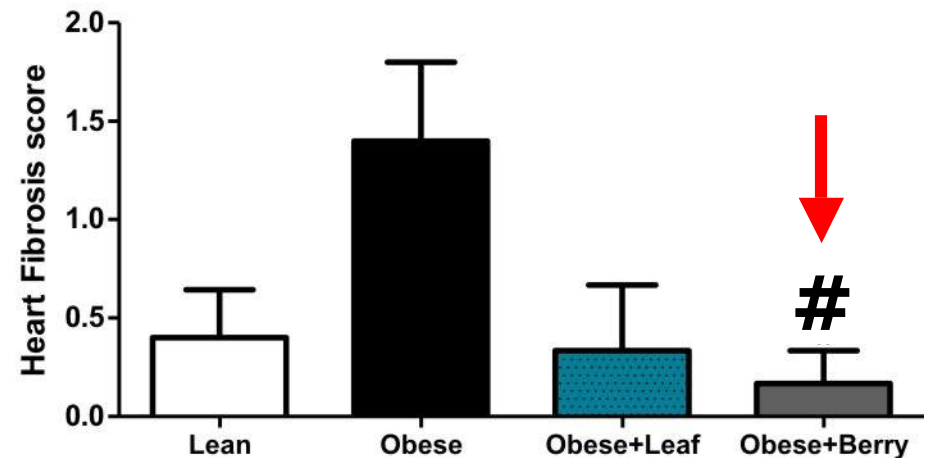
- Four treatments (groups of rats):
  - **lean** and metabolically normal (+/+), fed a normal diet (lean control)
  - **obese** (*cp/cp*), fed a normal diet (obese control)
  - **obese** (*cp/cp*), fed a normal diet *plus* hawthorn leaf supplement (*C. monogyna*)
  - **obese** (*cp/cp*), fed a normal diet *plus* hawthorn fruit supplement (*C. chrysoarpa*)

- Selected results, Proctor et al. in prep.

8a. Heart Inflammatory Score

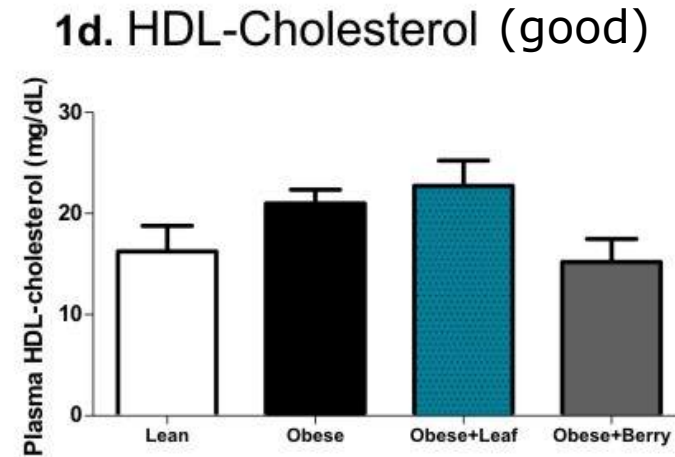
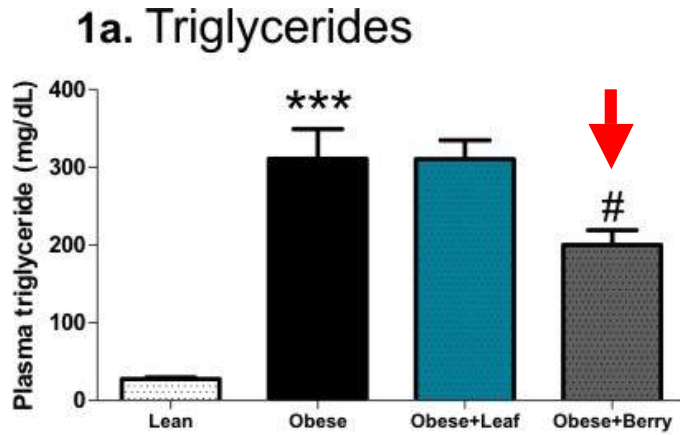


8b. Heart Fibrosis Score



- Both leaf and fruit extracts provided significant improvements in heart condition, and in blood lipids...

# NHPs and *Crataegus* of the Pacific Northwest





- Conclusion: the chemicals in hawthorn preparations, regardless of plant part used (leaf and flowers, fruit) or species (introduced *C. monogyna*, native *C. chrysocarpa*), provided significant improvements in heart symptoms in these animal trials.

- Conclusion: further analyses of the results from these experiments may also suggest possible mechanisms for the action of these hawthorn preparations

- Acknowledgments
  - HerbPro Co-op (Jeanette Lee\*)
  - U. of Alberta (Spencer Proctor\*)
  - UBC Okanagan (Paul Shipley\*)
  - Paula Brown\*, BCIT
  - Nadia Talent, ROM Departmental Associate
  - Saša Stefanović\*, UofT Mississauga
  - Masha Kuzmina, Smithsonian Institution
  - Student research assistants
  - NSERC Canada Strategic Project Grant and Discovery Grants (TAD, SS)
  - ROM Governors Peer Review Grants

<sup>19:18\*</sup> participants in the NSERC Strategic Grant proposal