

The Canadian Sweet Chestnut

-Newsletter of the Canadian Chestnut Council-

Issue # 85

September 2022



<http://www.canadianchestnutcouncil.ca>

Council Mission - to help restore the American Chestnut to the areas of Canada it once occupied.

Current Priorities

- 1) Breeding resistance
- 2) Breaking Isolation / Establishing Gene pool Nodes
- 3) DNA Analysis
- 4) Survey of existing Chestnuts in the wild

In this issue:

- Notice of Annual Meeting – **NOTE DATE and TIME CHANGE**
- American Chestnut Trees at the Royal Botanical Gardens (Hamilton, Ontario)
- Annual Membership dues.

Canadian Chestnut Council Annual Meeting – 2022

Because of scheduling conflicts, the date and time of the meeting has been changed. Mark your calendar. The annual meeting will take place Sunday, October 23rd at 1:30pm. The meeting will be held both in person and virtually.

Registration -1:00pm Meeting – 1:30pm

Tim Horton Children's Foundation – Onondaga Farms
Glen Morris Road, St. George Ontario

The meeting will include the Chair's annual update on the initiatives of the Canadian Chestnut Council. Guest Speaker's Dr. Brian Husband and Sophia Stoltz researchers from the University of Guelph will be presenting "*Reflections on the Recovery of American Chestnut in Canada: Research Progress and Priorities in the Husband Lab*". This talk will focus on their DNA findings and analysis.

American Chestnut Trees at the Royal Botanical Gardens – Sarah Richer

This article and photos were provided by Sarah Richer. Sarah is an RBG Species at Risk Biologist, Natural Lands Team. Sarah is also an Interim Director of the Canadian Chestnut Council)

So as not to bury the lead, I'll start with "The Good News" - a total of EIGHT pure American Chestnut (*Castanea dentata*) are currently known (as of September 2022) to exist in the wild at Royal Botanical Gardens' natural areas.

APPLAUSE!

I will also provide this teaser to "The Fun Nerdy News" - if you think you can use leaves and twigs, with 100% accuracy, to identify a native chestnut and differentiate it from a non-native chestnut or hybrid, the reference material that says you can do so seems to need some updating, or possibly be thrown out.

First, details on “The Good News”:

There are records of a total of 17 separate *Castanea* specimens in the Royal Botanical Gardens’ natural areas within the last 20 years. I hope you find it as encouraging as I do to know that three of those 17 were discovered by accident between 2015 and 2019, so it is likely other extant specimens await discovery here. RBG continues to participate in the Canadian Chestnut Council’s Breaking Isolation project, beginning in 2016 with 19 *C. dentata* saplings planted into the natural areas and an additional two accessioned into the gardens’ formal plant collections. As of spring 2022, only six of these saplings remain, largely having succumbed to various factors, including blight. The fact these comparatively privileged specimens that benefitted from added protections against deer browse and spongy moth defoliation showed this survival rate (only six out of 21 still living after six years) highlights the relative miracle of any wild *C. dentata* specimens that did not have their initial survival subsidized still remaining on the landscape. Unfortunately, most of our wild pure specimens are in decline, including what used to be our largest, most robust tree. Interestingly, a small handful of viable nuts were retrieved from this tree; they produced seedlings that also tested as pure *C. dentata*, despite there being no other known mature specimens in the area – a feat for a tree species generally considered to be self-infertile.



Breaking Isolation project; pictured are (left) Lauren Craig, the 2022 Species at Risk Intern; (right), is Serina Tourangeau, 2022 summer student. This is one of the tallest specimens from that project that we’ve got!

Visits to RBG's 17 wild specimens between 2019 and 2022 confirmed sad news - just 12 of those 17 were still alive; and not all of those 17 got to live long enough to learn their DNA test results. One tree had been lost due to damage sustained from a dead tree falling on it; others had succumbed to various causes, including blight. DNA tests were freshly conducted in 2021 and 2022 by some of our endangered Chestnuts' guardian angels, namely Sophia Stoltz and her colleague Paul Kron out of Dr. Brian Husband's lab at the University of Guelph. Sophia's work confirmed that at least three (likely four, pending confirmation) of these specimens were non-native horticultural specimens or hybrids - likely deliberately installed in the natural areas by ill-informed horticulturalists decades ago, under the mistaken assumption that placing non-native species into natural areas would benefit biodiversity (of course it's now widely accepted in the conservation world that doing so actually threatens native biodiversity). More importantly, Sophia's work confirmed that RBG's natural lands house at least eight wild specimens of pure *C. dentata* - one residing in Hendrie Valley, two occurring on the north side of Cootes Paradise, and the remainder in the woods south of Cootes Paradise.



May 17th, 2022; right is Paul Kron and middle is Sophia Stoltz from the University of Guelph, they work under Dr. Brian Husband; left is Lauren Craig (RBG SAR Intern) using a telescoping pole clipper to collect a leaf sample for a DNA test from what is now the largest (dbh-wise) of our still-healthy American Chestnuts (this one's test showed it was pure *Castanea dentata*).

Now comes the details on “The Fun Nerdy News”:

My botanist colleagues in RBG’s Science Department had consulted dichotomous keys in reference literature to visually key out voucher samples from several of our chestnut specimens – I was dismayed by their preliminary results that indicated all were hybrids except for one. The sample of what was possibly RBG’s only pure *C. dentata* (RBG #80) exhibited features that matched what the published reference material considered to be *C. dentata* traits: leaves with hooked teeth; small and numerous lenticels; upper leaf mid-veins nearly glabrous; buds nearly/entirely glabrous and not parallel to stem; brown glands on the leaves; and hairless spines on the fruit. The other specimens, however, were apparently more likely to be hybrids - they sported some *C. dentata* features, but also had characteristics the publications associated with *C. sativa* and/or *C. mollissima*: yellow glands between veins; leaf teeth not hooked; leaf upper and lower mid-veins sparsely pubescent to pubescent, some even with long hairs; buds sparsely pubescent and parallel to stem; leaves somewhat broad in relation to length. I accepted their visual ID findings with their provided caveat - that there is inherent uncertainty with both old herbarium specimens and dichotomous keys to visually identify species, especially rare ones that are known to readily hybridize. For example, could we be certain the printed materials and voucher samples are based on correctly identified pure *C. dentata* specimens in the first place, or could they involve ones that might be affected by the occasional back-crossed hybrid specimen, resulting in some features that appear non-uniformly across a specimen? To add to the in-field identification frustrations, it's recommended to visually key out chestnuts using a full combination of fruit, husk, buds, twigs, and leaves from both sunny AND shaded areas of a tree - but that is not always achievable from branches within reach of even long-handled leaf-sampling tools, and not all specimens reliably bear nuts. We were eager to compare the botanists’ notes against the DNA results.

Sophia Stoltz did not disappoint -

The DNA results confirming that the specimens visually keyed as hybrids were in fact pure *C. dentata* was an immense relief. Her DNA analysis of RBG’s *C. dentata* specimens resulted in very intriguing data; included here below for you to enjoy:

From A December 2021 email from Sophia Stoltz to Sarah Richer:

“The reference C. dentata [for the DNA tests] are from the Connecticut Agricultural Research Station in New Haven Connecticut...If you remember from my presentation for the Canadian Chestnut Council annual general meeting, I had

*shown a figure of population structure outlining differences among Canadian and American populations. I demonstrated that there seem to be two clusters of American chestnut trees, the samples in Canada cluster more closely with each other than they do with the US samples. It is unlikely that the clustering patterns indicate any adaptive differences between the US and Canada, but that samples that are members of the same 'cluster' simply share more alleles/background genetic diversity. Here's the weird part: The RBG samples I tested cluster more closely with the US samples than they do with Canadian samples, except for RBG-080 which aligns best with the Canadian cluster. The reason I ask, is that there are morphological differences between US and Canadian leaves and twigs. A former MSc student in our lab had noted among other differences, that "leaves from Canadian populations had shorter petioles, fewer marginal teeth/cm, a less acute leaf base, were less hairy on the adaxial [upper side] midvein and hairier on the abaxial [underside] midvein than their American counterparts". Basically, in his thesis, he found that the use of molecular markers was the only way to be 100% accurate when deciphering *Castanea* individuals at the species-/hybrid-level if you only have leaves and twigs for reference. I am inclined to agree that the keys we have are too limiting and narrow to appreciate the array of morphological differences among some specimens...I find it interesting that those classified as possible hybrids cluster more closely with US populations I tested and the one classified as likely pure *C. dentata* aligns more closely with the Canadian populations I have sampled. I agree that this seems to be a neat opportunity for some follow-up work."*

These findings have stirred my curiosity even more. Are there any differences in blight resistance between trees that match the Canadian-type samples vs those that match the US-type samples, or of their hybrids, if hybrids between the two occur? What type do any specimens known throughout southwestern Ontario belong to - is RBG the only spot with specimens matching the US-type cluster? If so, could these US-type plants have arrived here from historic trade of edible nuts between First Nations groups and/or Europeans along Lake Ontario? Or could RBG's Cootes Paradise be the hub where the US-type genetic cluster reached across the Niagara Peninsula and met with the Canadian-type?

It would be interesting to compare the visual ID features of those DNA reference specimens against both our printed reference material and RBG's herbarium specimens. Clearly, the parameters for visually identifying American Chestnuts in-field need to be broadened beyond what is currently in published literature. At minimum, in an ideal world, to further aid the accuracy of in-field identification, a pictorial guide could be developed that displays in detail the range of visual features that occur among the fruit, husk, buds, twigs, and leaves from both sunny and shaded areas of DNA-test-confirmed *C. dentata* specimens of both the

Canadian-type cluster and US-type cluster. In the meantime, for any chestnut trees I find in future, I'll be submitting leaf samples for DNA analysis to be 100% certain I focus our limited capacity for protection efforts towards pure American Chestnuts.

Membership Dues (Terry Anderson) –**Don't forget to renew your 2022/2023 Membership!**
The membership year runs from October 1 to September 30.

It is the Foundation's policy to remove members from the mailing list after three years in arrears. The fee is \$25.00 and your cheque should be mailed to Terry Anderson. Membership fees and donations are tax deductible.

Make cheques payable to: the Canadian Chestnut Council.

Please send to:

Dr. Terry Anderson
261 Sandy Brook Way,
Kingsville, ON. N9Y 0A4

To apply for new membership, please complete and mail the **Membership Form** to Terry Anderson.

Want more information:

Website - www.canadianchestnutcouncil.ca

Contact - Mr. Ron Casier Phone - 519-631-5279 Email - ronjcasier@gmail.com	Membership Secretary – Dr. Terry Anderson Address - 261 Sandy Brook Way, Kingsville, ON. N9Y 0A4 Phone - 519-733-3796 Email - andersonterry419@gmail.com
---	---

Council Directors – Chuck Beach, Ron Casier, Gordon Chinnick, Heather Dover, Neil Dunning, Doug Fagan, John Hill, Ken MacGillivray, Nathan Munn, Stephen Penney, Christine Vey.
Interim Director – Sara Richer