

Title: Identification and investigation of genetic diversity in American chestnut (*Castanea dentata*) planted beyond the historical range in western North America

Summary: Efforts to restore American chestnut to its formerly historic range are centered on the generation of blight-resistant trees and the introgression of blight-resistant genotypes into remnant populations. However few trees in the historic range reach sexual maturity, limiting genetic diversity among a potential restored population. American chestnut planted in western North America prior to the spread of blight represent a potential genetic diversity repository. We aim to locate, map, and demographically characterize American chestnut planted throughout the greater Pacific Northwest, and to initiate population and landscape genetic analyses to better understand genetic variation and geographic origins of western trees.

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Duration of Project: 1 year (Winter 2023-Winter 2024)

Total Amount Requested: \$9,982, student summer stipends to complement funding from institutional sources and the M.J. Murdock Charitable Trust

Short and Long Term Goals: The goals of the proposed project are to (1) test whether American chestnut planted throughout the greater Northwest (Idaho, Oregon, Washington, northern California, British Columbia) exhibit the same, or greater, genetic variation than trees throughout the historical distribution, (2) determine whether western American chestnut exhibits genetic variants not observed among extant eastern populations, and (3) to determine whether western American chestnut trace genetic ancestry from a single region of the historical range or whether they were derived from throughout the historical distribution. Our long-term goal is to establish a collaboration with other research teams to develop additional genetic tools for further investigation, and to help inform restoration efforts by characterizing underutilized sources of genetic variation that should be introgressed into blight-resistant lineages.

Narrative: American chestnut (*Castanea dentata*) was one of the most common tree species of eastern North America prior to the advent of the chestnut blight (*Cryphonectria parasitica*) in the early 20th century (Ziegler 1920; Smith 2000; Jacobs et al. 2013; Dalglish et al. 2016). Being prized for its durable and rot-resistant wood, annual production of edible seeds, and supporting other forest species, American chestnut occupies a special place in the cultural history of North America stretching back to pre-colonial periods (Jaynes and Graves 1963; Lutts 2004; Diamond et al. 2000). The rapid functional extinction of *C. dentata* occurred within the lived memory for many residents of eastern North America, representing a significant cultural loss, but also the loss or decline of co-evolved species (e.g., *Curculio caryatrypes* Boheman 1843, Anderson, 2017; *Andrena rehni* Viereck 1907, Swatt 2023). However, some American chestnut populations avoided blight exposure due to occurring in isolated locations (Diller 1965), while others persist today as small individuals resprouting from persistent living root crowns throughout much of the historical distribution (e.g., Stephens and Waggoner 1980; Paillet 1982, 1988, 2002; Stephenson et al. 1991; Schwadron 1995; Tindall et al. 2004; Van Drunen et al. 2017; Laport 2020; Laport et al. 2020). These remnant trees have been the focus of intense study, with many being included in

breeding and other genetic modification efforts to generate blight-resistant trees.

While breeding and transgenic efforts have been successful in generating resistant plants, successful restoration of *C. dentata* to eastern forests requires the establishment of self-sustaining blight resistant populations of trees. Hybrid and transgenic trees generated so far exhibit limited genetic diversity due to intensive breeding efforts and genetic modification with a limited number of trees (Powell et al. 2019; Newhouse 2020), and the genetic legacies of such strong population bottlenecks (strong reductions in effective population size) can have lingering negative demographic consequences (Hoelzel et al. 1993; Hoelzel 1999). Planned restoration strategies with generated resistant trees include broadening genetic diversity by promoting, or manually, outcrossing resistant trees to reproductive remnant individuals within hybrid trial orchards and throughout the native range (Steiner et al. 2017). However, relatively few reproductive trees remain in the native range, especially in parts of the historical distribution that were heavily affected by *C. parasitica*, and it is likely that some genetic diversity important for local adaptation was lost during the spread of *C. parasitica* over the last ~100 years (Sandercock et al. 2022).

American chestnut growing throughout western North America represent an opportunity to recapture pre-blight genetic variation via introgression with naturally-occurring trees throughout the historical distribution (Saielli et al. 2014) as well as newly generated blight resistant trees. Human migrations from eastern to western North America over the last ~200 years resulted in the transport of potentially hundreds or thousands of American chestnuts that were planted on homesteads, in town squares, and cemeteries (Diller 1965). As a favorite forest species of people originating throughout the greater Appalachian and surrounding regions of eastern North America, these trees may have represented a connection to a former life and home (Fraser 1864; Williams 1873; Lutts 2004). The transport of commensal, agricultural, and supplemental food species is common throughout the history of global human migrations and can have significant consequences on the genetic diversity of transported species (e.g., inbreeding, bottlenecks, founder effects, etc.; Angert et al. 2008). Founder populations may contain unique genetic variation and/or locally adapted genetic variants that have gone extinct in the core distribution of the species because transported individuals were not randomly selected from the core distribution. Therefore, transported individuals could be exceptionally valuable as a source of novel genetic variation for restoration efforts (Schemske et al. 1994, Jacobs et al. 2013).

American chestnut planted or grown in western North America might represent an important source of genetic variation for restoration efforts with mature remnant and blight-resistant trees in eastern North America. Many of the extant western specimens were planted beyond the range of *C. parasitica* and have so far escaped blight with some individuals persisting as reproductive trees for ~100-150 years (Diller 1965). Some of these trees may also represent locally-adapted genetic variants that were extirpated from the historical distribution, and would therefore be highly desirable to reintroduce to the blight-resistant populations (Sandercock et al. 2022). However, the geographic origins of the trees planted throughout western North America have largely been lost to time with oral and written accountings being imperfect. Therefore, it remains unclear whether the *C. dentata* growing beyond the historical distribution contains genetic variation from throughout the historical distribution, or whether the western *C. dentata* have a geographically constrained origin representing only a subset of the range-wide genetic diversity recently described by genomic analyses throughout the historical distribution (Sandercock et al. 2022).

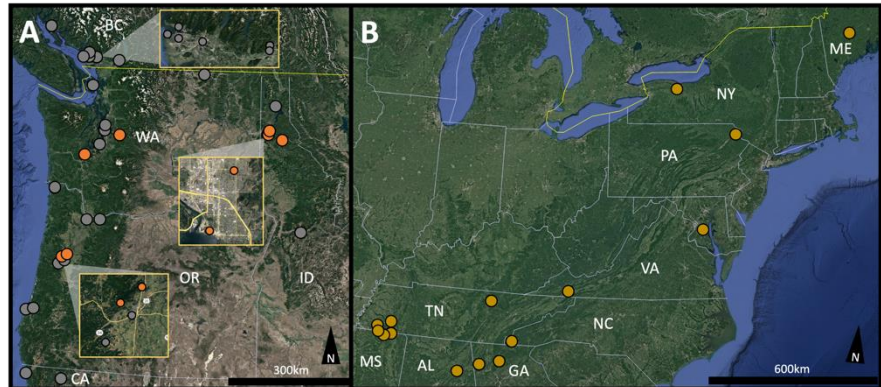
Here, we aim to locate, collect material from, and genetically characterize *C. dentata* throughout the greater Pacific Northwest to potentially aid restoration efforts by characterizing a source of new genetic variation that could help alleviate the genetic bottlenecks of blight, breeding efforts, and genetic modification. We aim to address three questions:

(1) Are levels of genetic variation among western American chestnut greater than among extant American chestnut from the historical distribution?

(2) Are western American chestnut characterized by unique genetic variation not observed among extant eastern populations of American chestnut?

(3) Are western American chestnut derived from throughout the historical distribution in eastern North America or do they trace genetic ancestry from a single region (e.g., the southern Appalachians)?

Fig. 1. A) Locations of American chestnut in the greater Pacific Northwest. Orange dots indicate collected samples for preliminary molecular work. Additional collections (at grey sites) are necessary to enhance evaluations of genetic diversity in comparison to American chestnut in the historical range. B) American chestnut in the historical range for which we have so far obtained cpDNA and/or ITS sequences.



Experimental Plan: We have identified at least 28 locations throughout the greater Pacific Northwest (Washington, Oregon, Idaho, northern California, British Columbia) where *C. dentata*, either singly or in small numbers, have been reported to grow (Fig. 1A). American chestnut localities were identified using searches of citizen-scientist (e.g., iNaturalist.org) and biodiversity information repositories (e.g., Global Biodiversity Information Facility; gbif.org), herbarium records (e.g., Consortium of Pacific Northwest Herbaria; <https://pnwherbaria.org>), and conversations with local naturalists and land managers. Following prior protocols (Shaw et al. 2012) we have already collected relatively young, undamaged leaves for DNA extraction from a subset of identified western American chestnut (seven locations; orange dots in Fig. 1A). Preliminary analyses of population genetic and landscape genetic variation are underway using five chloroplast DNA (cpDNA) intergenic spacers (psbA-rpl2, rpl16F-rpl16R, rpoB-trnC, rpl32-trnL, and trnCF-ycf6) and the internal transcribed spacer of the nuclear ribosomal DNA (ITS) utilized in previous analyses of landscape genetic variation in American chestnut (Fig. 2; Shaw et al. 2012). However, additional collections of western American chestnut from locations throughout the greater Pacific Northwest are needed to better characterize genetic diversity relative to populations from the historical distribution.

1) Are levels of genetic variation among western American chestnut greater than among extant American chestnut from the historical distribution? Many American chestnut were planted in western North America prior to the rapid spread of chestnut blight and may have

connectivity between western and historical distribution populations, we will generate a cpDNA haplotype network from the aligned sequences using PopART. Additionally, we will examine demographic histories using Tajima's D (Tajima 1989) and haplotype mismatch distributions to compute parameters of demographic size variation (Slatkin and Hudson 1991; Rogers and Harpending 1992), providing clues about whether the western trees have experienced a genetic bottleneck—and how severe it may have been—relative to American chestnut in the historical distribution.

3) Are western American chestnut derived from throughout the historical distribution in eastern North America or do they trace genetic ancestry from a single region? People migrated to western North America along several different routes (e.g., “Oregon Trail,” “California Trail,” “Around the Horn,” etc.), with some forced migration west of the Mississippi River by people with deep cultural ties to American chestnut (i.e., “Trail of Tears”). Thus, it is likely that trees growing in western North America derive from throughout the historical distribution (i.e., northern, mid-Atlantic/Appalachian, and southwestern historical range). However, the mid-Atlantic/Appalachians may represent a “center of dispersal” given patterns of westward migration (America's Great Migration Project, <https://depts.washington.edu/moving1/Oregon.shtml>), but it remains unclear whether this is reflected in the diversity of extant western trees. Along with available oral histories for western trees and our DNA network analysis (above), we will use phylogenetic inference using Bayesian (Mr. Bayes; Huelsenbeck and Ronquist 2001) and Maximum Likelihood (RAxML; Stamatakis 2014) approaches to examine the evolutionary relationships between sampled western and eastern trees. Supplementing our network and phylogenetic analyses, we will employ Bayesian clustering analyses using STRUCTURE (Pritchard et al. 2000) on our combined western-eastern dataset to help investigate the potential geographic origins of western *C. dentata* relative to the DNA sequences of eastern *C. dentata*.

Anticipated results and interpretation: The transport of American chestnut to western North America was fortuitous in that it may have resulted in the creation of an accidental pre-blight genetic diversity repository. Our investigations of genetic diversity in western American chestnut will allow us to determine whether genetic diversity is higher in western *C. dentata* than in eastern *C. dentata*. Despite a smaller population size, western American chestnut might exhibit higher haplotype diversity than eastern populations if they were collected from a pre-blight population with higher overall genetic diversity than observed among extant populations and transported west prior to the spread of blight. Similarly, our investigations may uncover unique haplotypic diversity among western *C. dentata*. The spread of *C. parasitica* resulted in the death of millions of trees (Diller 1965), and likely caused the extinction of many unique and locally-adapted genetic variants. Discovery of unique genetic variants may be potentially valuable for ongoing investigations into restoration of blight-resistant trees throughout a historical distribution spanning a wide range of climatic, edaphic, and biotic conditions (Barnes and Delborne 2019). In addition, identifying the regional origins of the western American chestnut—whether from a single part of the historical distribution or from throughout the range—will help with reintroduction and restoration planning in the face of suitable habitat shifts due to climate change (Barnes and Delborne 2019). Uncovering the geographic and genetic origins of western American chestnut will also provide context for understanding the story of human migration to western North America through the 1800s and early 1900s through our love

of a foundational forest species.

Several prior investigations of *C. dentata* population and landscape genetic structure employing cpDNA and ITS sequences have revealed crucial insights into the population genetic structure and biogeographic history of *C. dentata* throughout the historical distribution (e.g., higher genetic diversity in the southwestern portion of the historical range; e.g., Huang et al. 1998; Dane 2009; Shaw et al. 2012). Many of the western *C. dentata* are likely remnants of populations with (presumably) higher pre-blight genetic diversity, and our proposed cpDNA and ITS analyses are likely powerful enough to characterize major phylogeographic and landscape-level genetic structure. These methods also represent a robust, relatively inexpensive, first characterization of genetic variation that is directly comparable to prior cpDNA and ITS studies. At the same time, relatively slow-evolving and maternally-inherited plastid markers, may leave some questions unresolved about landscape-level patterns of genetic diversity relevant for informing reintroduction of blight-resistant trees and the restoration of locally-adapted populations. The insights gained from these studies, however, can be used as a springboard for future collaborations with The American Chestnut Foundation and associated research teams to conduct a deeper analysis of genetic diversity in western American chestnut employing DNA sequencing approaches and analyses similar to those recently employed for historical range-wide analyses (Sandercock et al. 2022).

Timeline for completion of goals: Preliminary collection of leaf tissue for DNA extraction, PCR, and sequencing of plastid markers began during summer 2023. Undergraduate student researchers have become proficient with lab protocols, and initial DNA extractions, PCR, and sequencing reactions have been successful (Fig. 2). Preliminary population and landscape genetic analyses will be conducted during winter 2023/early spring 2024. A two-week collecting trip is being planned for spring/early summer 2024 to obtain silica-preserved leaf tissue, demographic information, and voucher specimens for trees at additional localities (Fig. 1). During summer 2024, we will extract DNA from new collections for PCR and sequencing of cpDNA and ITS regions we previously tested. Completion of population and landscape genetic analyses will occur in fall 2024 and winter/spring 2025 (Fig. 3).

2023				2024									
Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Collection trip: ID	Preliminary sequence editing; Population & landscape analyses						Order field collection, DNA extraction, and PCR supplies	Collection trip: OR, WA, BC, ID	DNA extractions, PCR, DNA sequencing			Population & landscape analyses; draft manuscript	
Collection trip planning		Manuscript draft w/preliminary findings											

Fig. 3. Proposed timeline for initiation and completion of western North American chestnut collecting and molecular analyses.

Assessment of Outcomes and Dissemination of Results: Successful completion of the proposed research will result in a manuscript for submission to an academic journal (e.g., *Castanea*) reporting on the genetic diversity of western American chestnut relative to extant eastern trees. To reach a broader less-technical audience, we will prepare a popular press article submission to The American Chestnut Foundation’s journal, *Chestnut*. Research outcomes will also be shared widely with the broader scientific and primarily undergraduate institution research community through student presentations at professional meetings (Botanical Society of

America, Ecological Society of America, etc.), the 2023 and 2024 Murdock College Science Research Conferences, and the 2024 and 2025 College of Idaho Student Research Conference and Research Colloquium.

Budget Breakdown and Justification: The College of Idaho is a small liberal arts college (~1,100 students) emphasizing undergraduate education and primary research experiences. Our budget request is for field and lab work conducted by undergraduate students (Christina Riddle, Kelvin Sakyi, Jordan Steele, Chessa-Grace Foreman) during spring and summer 2024. Student research stipends will be funded by a mix of institutional fellowships, the M.J. Murdock Charitable Trust, and The American Chestnut Foundation award. Primary expenditures will be on travel to field locations for leaf tissue collections, and lab materials for DNA extractions, PCR, and DNA sequencing, and student stipends.

Expenses	Amount requested
Lab expenses	
DNA extraction and PCR supplies (extraction kits, Taq, dNTPs, cleanup, consumables, etc.)	\$900.00
DNA sequencing	\$1,600.00
Field expenses	
Fuel (2673 miles @ 25 miles/gallon = 107 gallons @ \$3.90/gallon)	\$417.00
Car rental (14 days)	\$1,450.00
Hotel rooms (14 days @ \$110.00/night)	\$1540.00
Collecting supplies (zip bags, coin envelopes, silica desiccant)	\$75.00
Student stipend (10 weeks @ \$10/hr)	\$4,000.00
Total	\$9,982.00

The authors declare no known Conflict of Interests or Commitment.

References:

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PROFESSIONAL APPOINTMENTS:

Assistant Professor of Biology and Director of the H.M. Tucker Herbarium, College of Idaho 2022-present
Assistant Professor of Biology and Curator of the Herbarium, Rhodes College 2018-2022

EDUCATION:

PhD, Ecology & Evolutionary Biology, University of Rochester 2013
MS, Ecology & Evolutionary Biology, University of Rochester 2009
BS, Biology, Summa Cum Laude, Oregon State University 2003

RECENT PEER REVIEWED PUBLICATIONS (*mentored undergraduate co-author):

- Gerstner, B., M. Mann, **R. G. Laport**, and K. D. Whitney. 2023. Differentiation of rhizosphere fungal communities by host ploidy level in mixed-ploidy *Larrea tridentata* populations. *Oikos*. [dx.doi.org/10.1111/oik.09856](https://doi.org/10.1111/oik.09856).
- Laport, R. G.**, Z. Brookover*, B. Christman*, J. Ng, K. Phillely, and J. H. Craddock. 2022. Environmental niche and demographic modeling of American chestnut near its southwestern range limit. *The American Midland Naturalist* 188: 137-176. [dx.doi.org/10.1674/0003-0031-188.2.137](https://doi.org/10.1674/0003-0031-188.2.137).
- Laport, R. G.**, R. L. Minckley, and D. Pilson. 2021. Pollinator assemblage and pollen load differences on sympatric diploid and tetraploid cytotypes of the desert-dominant *Larrea tridentata*. *American Journal of Botany* 108(2): 297-308. [dx.doi.org/10.1002/ajb2.1605](https://doi.org/10.1002/ajb2.1605).
- Weaver, W. N.* , J. Ng, and **R. G. Laport**. 2020. LeafMachine: machine learning software for autonomous phenotypic trait extraction from digitized herbarium specimens and digital plant images. *Applications in Plant Sciences*. 8(6): e11367. [dx.doi.org/10.1002/aps3.11367](https://doi.org/10.1002/aps3.11367).
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RECENT GRANTS, FELLOWSHIPS, & AWARDS (*mentored undergraduate awardee):

- 2023 National Science Foundation, Collaborative Research: CAPACITY Collections Program: SAPLING: Tree Species of the Americas: A Project Leveraging Informatics, Natural History, and Geography (PI, A. Hipp and consortium, Morton Arboretum; Senior personnel, R.G. Laport) **\$1,106,374 (revised and re-submitted)**
- 2022 National Science Foundation, Collaborative Research: CAPACITY Collections Program: SAPLING: Tree Species of the Americas: A Project Leveraging Informatics, Natural History, and Geography (PI, A. Hipp and consortium, Morton Arboretum; Senior personnel, R.G. Laport) **\$1,106,374 (not funded)**

2021	National Science Foundation, DBI - Major Research Instrumentation: Acquisition of a fluorescence activated cell sorter (FACS) supporting multi-disciplinary approaches to exploring biological responses. (PI, M.E. Miller; Senior Personnel, R. G. Laport) \$357,741
2021	Rhodes College Hill Fund for Curricular Development and Pedagogical Innovation. Creating an Inclusive Summer Session Field Biology Institute (PIs: P.T. Kelly, R.G. Laport) \$8,000
2020	National Science Foundation, Collaborative research: Digitization TCN: TreeVRE Consortium: a virtual research environment for investigating trait diversity of trees of the Americas. (PI, A. Hipp and consortium, Morton Arboretum; Senior personnel, R.G. Laport) \$684,232 (not funded)
2020	Rhodes College Faculty Early Leave Research Award \$30,000
2019-2022	AI for Earth Microsoft Azure Compute Grant to develop LeafMachine: Autonomous Trait Data Extraction from Digitized Plant Specimens using Machine Learning. (PIs: W. Weaver*, J. Ng, and R.G. Laport) \$10,000
2016-2021	National Science Foundation, DEB Ecological and Evolutionary Processes (1556371). Is pollinator discrimination among populations of the southwestern desert creosote bush differing in chromosome number promoting speciation? (PI, R. G. Laport; Co-PI, D. Pilson) \$149,999.
2016-2019	National Science Foundation, Early Concept Grants for Exploratory Research (1550813). Disentangling the roles of ecological and historical processes in community structure: A continental-scale approach. (PI, J. Ng; Co-PI, R. G. Laport) \$299,647.
2016	Research Experience for Undergraduates (REU) Supplement to NSF-EF 1550813: Disentangling the roles of ecological and historical processes in community structure: A continental-scale approach. Support for Vivianna Sanchez* (Mount Saint Mary's Univ.) and Michelle Gaynor*(Univ. of Central Florida) \$16,090.

GRADUATE STUDENT ADVISORY COMMITTEES

Benjamin Gerstner, University of New Mexico, PhD, Ecology & Evolutionary Biology	2021-2023
Michelle Gaynor, University of Florida, PhD, Ecology & Evolutionary Biology	2019-
Darlene Coppe, Black Hills State University, MSc, Integrative Genomics	2021-2022
Benjamin Shreves, Black Hills State University, MSc, Integrative Genomics	2021-

SUPERVISION OF UNDERGRADUATE RESEARCHERS AND INDEPENDENT RESEARCH:

College of Idaho

Christina Riddle; DNA extractions, PCR, sequencing, greenhouse plant care and pollination	2022-
Kelvin Sakyi; DNA extractions, PCR, sequencing, greenhouse plant care, microscopy	2023-
Chessa-Grace Foreman; DNA extractions, PCR, sequencing, greenhouse plant care	2023-
Jordan Steele; DNA extractions, PCR, sequencing, greenhouse plant care and pollination	2023-
Kelly Golden; herbarium specimen mounting, database entry, georeferencing	2022-
Emmanuela Ibiejugba; herbarium specimen mounting, database entry, georeferencing	2022-
Margo Harness; greenhouse plant maintenance	2022-2023

Rhodes College

Aidan Kron; Microscopy, stomatal measurements, plant care, forest field work, pollinations	2021-2022
Katherine Cruse; Microscopy, stomatal measurements, plant care, forest field work, pollinations	2021-2022
Helen Pennington; Demographic modeling	2021-2022
Braxton Jeffcoat; Microscopy, stomatal measurements, plant care, forest field work, PCR	2020-2022
Jacob Spears; Microscopy, stomatal measurements, plant care, forest field work, pollinations	2020-2022
Nowreen Sarwar; Microscopy, stomatal measurements	2020-2022
Zoe Brookover; Microscopy, demographic modeling, plant care, herbarium specimens, PCR	2019-2021
Brian Christman; Microscopy, demographic modeling, plant care, herbarium specimens	2019-2021
Ellinor Aronson; Microscopy, stomatal measurements, plant care, herbarium specimens, PCR	2019-2020
Erin Dempsey; Microscopy, GIS & ecological niche modeling, plant care, herbarium specimen	2019-2020
Abby Ellingwood; microscopy, leaf stomatal impression measurements	2019
Ali Campbell; Microscopy, stomatal measurements, plant care, herbarium specimens	2019

Wendy Harvey

The College of Idaho, Department of Biology
2112 Cleveland Blvd., Caldwell, ID 83605
email: wendy_harvey@hotmail.com; phone: 208-284-8434

PROFESSIONAL APPOINTMENTS:

Lecturer of Biology, College of Idaho	2020 - present
Visiting Instructor, College of Idaho	2017 - 2020
Biology Adjunct, College of Western Idaho	2019 - 2020

EDUCATION:

2014 Masters in Biology, Boise State University
2003 Bachelors of Science, College of Idaho

PEER REVIEWED PUBLICATIONS (*mentored undergraduate co-author):

Otero, Claire E.; Noeker Jacob A.; Brown Mary M.; Wavreil Florence D.M.; **Harvey Wendy A.**; Mitchel Kristen A.; Heggland, Sara J. 2019. Electronic cigarette liquid exposure induces flavor-dependent osteotoxicity and increases expression of a key bone marker, collagen type I. *J Appl Toxicol*; 1–11. <https://doi.org/10.1002/jat.3777>

Harvey W.A., Jurgensen K., Pu X., Lamb C.L., Cornell K.A., Clark R.J*, Klocke C*, Mitchell K.A. 2016. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) increases human hepatic stellate cell activation. *Toxicology* 344-346: 26-33.

Arbon K.S*, Christensen C.M*, **Harvey W.A.**, Heggland S.J. 2012. Cadmium exposure activates theERK signaling pathway leading to altered osteoblast gene expression and apoptotic death in Saos-2 cells. *Food Chem Toxicol* 50(2):198-205.

Smith S.S*, Rodriguez Reyes J*, Arbon K.S., **Harvey W.A.**, Hunt L.M., Heggland S.J. 2009. Cadmium induced decrease in RUNX2 mRNA expression and recovery by the antioxidant N-acetylcysteine (NAC) in the human osteoblast-like cell line, Saos-2. *Toxicol in Vitro* 23:60-66.

Harvey W.A., Frost S.T*, Machynia K.T*, Gerdes M*, Heggland S.J. 2008. Differential cell sensitivity to cadmium exposure in RTgill-W1, RTG-2, and RTL-W1 rainbow trout (*Oncorhynchus mykiss*) cell lines: An in vitro cell line model to study cadmium-induced cytotoxicity. *J Idaho Acad Sci* 44:19-29.

Zhukalin M., Blanksma M.K.*, Silva T.D., Sychira S.W., **Harvey W.A.**, Heggland S.J., Craig P.R. 2007. Characterization and in vitro cytotoxicity of ethanolamine-derived cadmium chelating agents. *Biometals* 20:61-72.

SUPERVISION OF UNDERGRADUATE RESEARCHERS AND INDEPENDENT RESEARCH: College of Idaho

Kelvin Sakyi; DNA extractions, PCR, sequencing, greenhouse plant care, microscopy 2023- present

Alexander Leblanc; Idaho INBRE Scholars CRISPR Cas, cell culture, microscopy 2023

Daniella Manirakiza; Idaho INBRE Scholars CRISPR Cas, cell culture, microscopy 2023

Colton Troxel; Idaho INBRE Scholars CRISPR Cas, cell culture, microscopy 2023

Maura Sweeney; Idaho INBRE Scholars CRISPR Cas, cell culture, microscopy 2023

Kelvin Sakyi, Idaho INBRE Scholars CRISPR Cas, microbiology, PCR 2022

Tiffany Thompson, Idaho INBRE Scholars CRISPR Cas, microbiology, PCR 2022

Maella Djoube Fodop, Idaho INBRE Scholars CRISPR Cas, microbiology, PCR 2022

Undergraduate Conference Presentations

“Utilizing CRISPR/Cas9 to Knockout the Matrix Metalloproteinase 9 Gene in Human Lung Fibroblast Cells”, Idaho INBRE Conference, Moscow ID 2023 – *student poster presentation, 2nd place Scholar Poster Award*

“Calcium and Collagen III Expression in Transfected Human Lung Fibroblast Cells”, Idaho INBRE Conference, Moscow ID 2023 – *student poster presentation*

“CRISPR-Cas Deactivation and Reactivation of LacZ Gene in E. Coli via Blue-White Screening and PCR Verification”, Murdock College Science Research Conference, Vancouver, WA 2022 – *student poster presentation*

“CRISPR-Cas Transformation Efficiency of LacZ Gene in E. Coli Utilizing Heat Shock and Electroporation”, Murdock College Science Research Conference, Vancouver, WA 2022 – *student poster presentation*

“CRISPR-Cas Deactivation and Reactivation of LacZ Gene in E. Coli via Blue-White Screening and PCR Verification”, Idaho INBRE Conference, Moscow ID 2022 – *student poster presentation, 2nd place Scholar Poster Award*

“CRISPR-Cas Transformation Efficiency of LacZ Gene in E. Coli Utilizing Heat Shock and Electroporation”, Idaho INBRE Conference, Moscow ID 2022 – *student poster presentation, 2nd place Scholar Poster Award*