The Final Oral Examination
for the Degree of

DOCTOR OF PHILOSOPHY
(Department of Biology)

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2008 Trinity Western University; B.Sc. (Biology)

“Molecular Characterization of Shikimate and Quinate Biosynthesis in Populus trichocarpa: Functional Diversification of the Dehydroquinate Dehydratase/Shikimate (Quinate) Dehydrogenase (DQD/SDH/QDH) Superfamily via Gene Duplication”

Wednesday, December, 11th, 2013
11:00 AM
Cunningham Bldg, Room 146

Supervisory Committee:
Dr. Jürgen Ehlting, Department of Biology, UVic (Supervisor)
Dr. C. Peter Constabel, Department of Biology, UVic (Member)
Dr. John Taylor, Department of Biology, UVic (Member)
Dr. Caren Helbing, Department of Biochemistry and Microbiology, UVic (Outside Member)

External Examiner:
Dr. Jorg Bohlmann, Department of Botany, UBC

Chair of Oral Examination:
Dr. Alison Chapman, Department of English, UVic
Abstract

The shikimate pathway connects primary metabolism with the biosynthesis of the three aromatic amino acids (phenylalanine, tyrosine, and tryptophan), which are essential protein building blocks. This pathway also provides precursors for a wide array of plant secondary metabolites with adaptive functions in plant development and defense. The third and fourth steps of the shikimate pathway (the conversion of shikimate from 3-dehydroshikimate via 3-dehydroquinate) are catalyzed by a bifunctional enzyme called 3-dehydroquinate dehydratase/shikimate dehydrogenase (DQD/SDH). DQD/SDHs have been molecularly characterized in a few plant species including *Arabidopsis thaliana*, *Solanum lycopersicum* and *Nicotiana tabacum*. The embryo-lethal phenotype of Arabidopsis null mutants lacking DQD/SDH highlights a critical role of shikimate in primary metabolism. Quinate shares high structural similarity with shikimate and is a typical secondary metabolite present in many plant species. Quinate and its derivatives (e.g. chlorogenic acid) serve important functions in plant defense due to their astringent and antimicrobial properties. Quinate can be derived from 3-dehydroquinate, and this reaction is catalyzed by quinate dehydrogenase (QDH), the reaction mechanism of which resembles that of SDH. With a functional genomics approach, I demonstrated that two of the five poplar putative DQD/SDHs (Poptr1 and Poptr5, poplar DQD/SDH1 and 2) have exclusive specificity for shikimate, while the other three (Poptr2 to 4, poplar QDH1 to 3) are involved in quinate biosynthesis. Phylogenetic reconstruction of the DQD/SDH/QDH superfamily has identified two distinct clades in seed plants that may act preferentially on either shikimate or quinate whereas lineages, that have diverged prior to the angiosperm/gymnosperm split, only have a single copy DQD/SDH. An evolutionary analysis was carried out using the established phylogeny, and the sequence of the immediate pre-duplication ancestral DQD/SDH (>300MYA) was reconstructed. Protein structure modelling and *in vitro* biochemical characterization of the ancestral recombinant protein was
performed along with some extant members of this family (pre-duplication representatives: *Rhodopirellula baltica* (Rhoba), *Chlamydomonas reinhardtii* (Chlre), *Physcomitrella patens* (Phypa) and *Selaginella moellendorffii* (Selmo); after-duplication species: *Pinus taeda* (Pintal & Pinta2) and *Populus trichocarpa* (Poptr1 & Poptr3)). Together, the results indicate that quinate biosynthetic activity was gained prior to duplication and remained low until it became beneficial and favored by selection. Optimization of quinate biosynthetic activity was at the expense of losing some primary shikimate biosynthetic function (pleiotropic conflict). This can only be solved by gene duplication and further specialization leading to genes encoding specialized enzymes (either SDH or QDH). Diversification of the DQD/SDH/QDH superfamily likely occurred through sub-functionalization via a mechanism described as “Escape from Adaptive Conflict.”

**Awards, Scholarships, Fellowships**

2013  *George H. Duff Student and Post-doc Travel Grant* for 2013 Canadian Society of Plant Biologists (CSPB) Annual Meeting.


2010  *Amelia Leigh Memorial Fellowship* at UVic.

2008  B.Sc. (Honour) Degree in Biology with distinction awarded by TWU.

2007  *TWU Dean’s List Award.*

2005-2008  *TWU Academic Scholarship* for four consecutive school years.

2003  *TWU ESLI Outstanding Students Award.*
Presentations


3. Guo, J. “Molecular Evolution of Shikimate/Quinate Metabolism in Plants.” 22nd Annual Biology Graduate Symposium, University of Victoria, Victoria, BC, Canada. Nov. 2010 (oral)


Publications