

BIOGRAPHICAL SKETCH

DAVID EDWARD SALT, B.Sc. (Hons), M.Sc., Ph.D.

2004 – present **Scientific Director, Bindley Bioscience Center for Genomic Research and Technology,**
Discovery Park, Purdue University, West Lafayette, IN

2005 – present **Full Professor, Horticulture and Landscape Architecture,** Purdue University, West Lafayette,
IN

II. Research, Scholarly Activity, and Creative Endeavor Effectiveness:

1. Research Activity (Funded)

Title: The Genetic Basis of Natural Ionomic Variation

Abstract: Evolutionary forces are known to drive the fixation of locally adaptive genetic variation within populations, and such variation is thought to be involved in the differential response to disease and pharmacological agents observed in human populations. Natural variation is well documented in plants, and plants provide an excellent model for investigations into its genetic origins, maintenance and adaptive significance. We aim to take advantage of the natural genetic variation that occurs between populations of the genetic model plant *Arabidopsis thaliana* (*Arabidopsis*) to identify gene functions that vary between these natural populations. Such a set of polymorphic genes and gene functions will provide the essential tools required in the future to uncover the genetic mechanisms that drive the fixation of locally adaptive genetic variation within a population. To link natural genetic variation to function, we will apply our high-throughput Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS) based elemental-profiling platform, in combination with powerful genetic mapping tools, to a genotyped panel of 1000 *Arabidopsis* accessions and Recombinant Inbred Lines (RILs). Such a system will allow the identification of loci that drive the natural variation we observe in the plant's elemental-profile or “ionome” including P, Ca, K, Mg (macronutrients); Cu, Fe, Zn, Mn, Co, Ni, Se, Mo, I (micronutrients of significance to plant and human health); Na, As, and Cd (minerals causing agricultural or environmental problems). We have demonstrated the feasibility of such studies by identifying naturally occurring alleles of several genes in *Arabidopsis* that function to control shoot levels of various elements, including Na, Co and Mo. Sequence analysis of the haplotypes of such loci across the large panel of *Arabidopsis* populations will identify which of these genes have been under recent directional selection due to local adaptation. Such information will help shed light on the evolutionary processes involved in adaptation of organisms to local environments, to understanding ion homeostasis networks and will have applications to improving the mineral content of food crops for improved human health. After all, plants provide the major source of nutrition for a large portion of the world's population.

Title: Ionomics

Abstract: Uptake and translocation of mineral nutrients in plants is essential for plant growth, human nutrition and health. In spite of recent advances in identifying genes involved in nutrient transport, the systems that control acquisition of individual nutrients remain largely unknown. In this project we are using inductively-coupled plasma mass spectrometry (ICP-MS) to help identify gene networks that control uptake and accumulation of a wide array of plant mineral nutrients and toxic metals. The approach involves the quantification of 18 elements including Li, Na, P, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd and Pb 100-500 mutagenized *Arabidopsis thaliana* plants per day. Over the last 4-years we have applied this technology to perform both forward and reverse genetic screens. To date we have analyzed approximately 60,000 plants, including fast neutron mutagenized plants and T-DNA insertional mutants in ~900 putative ion-transporters and regulators. We have also analyzed ~ 100 *Arabidopsis* accessions and 4 recombinant inbred populations. Using map-based cloning, DNA micro array-based bulk segregant analysis and deletion mapping, and reverse genetics we have identified several genes to date that regulate the ionome, including ferroportin-like (*FPT2*), dirigent-like (*DIR10*), and *HKT1*. An online searchable database containing ion-profile data on over 60,000 plants is now available (www.purdue.edu/dp/ionomics) and will be routinely updated over the next 2-years. This project is part of a larger collaborative effort funded initially by the NSF Plant Functional Genomics program and now by the NSF 2010 program. More details about the collaborators and project can be found at <http://plantst.sdsc.edu/> and <http://www.cbs.umn.edu/arabidopsis/ionome/>.

Title: Tools for differential metabolomics

Abstract: This is a highly interdisciplinary effort by a team of chemists, biologists, engineers and information scientists collaborating to develop a set of tools for characterizing and recognizing temporal changes in the metabolome of model organisms. Three sets of tools will be developed; those that enhance the quantification of changes in the metabolome, instrumentation that augments throughput by either massively parallel analysis or differential analysis, and separations materials and techniques that improve sampling and resolution of metabolites. Dr Salt's role in the project is to develop high-throughput ICP-MS systems for the ionic profiling of all 5,000 strains in the yeast (*Saccharomyces cerevisiae*) knockout collection to define all the genes in this model organism involved in regulating the ionome. Further, Dr Salt will develop laser-ablation ICP-MS (LA-ICP-MS) to enhance throughput by reducing sample preparation. Using LA-ICP-MS Dr Salt will also develop spatially resolved ionic imaging to allow the quantification of ionic profiles across defined tissues and organs.

Title: Acquisition of Metabolic Profiling Instrumentation at Purdue

Abstract: This project has established the Purdue University Metabolic Profiling Facility (MPF). Not only is metabolomics a rapidly growing area, but few institutions have established the infrastructure required to support this new field of research. Purdue University, with its combination of expertise in mass spectrometry, genomics, agriculture, pharmacology, and other areas of metabolism-related research has a unique opportunity to establish itself at the forefront of this field. Purdue University has demonstrated its commitment to founding the MPF through the purchase of a Micromass LCT premier LC-MS, and by renovating 1500 square feet of space for the facility in the Horticulture Building (R302). Further instruments including GC-TOF and LC-MS and LC-MS/MS have also been purchased and installed in the facility, and a technician has been hired to run the facility. The MPF will provide important new capabilities to many research programs on the Purdue campus. PI Clint Chapple, co-PI's David Rhodes, David Salt and Barry Wanner. A recent grant from the NIH (PI Fred Regnier; co-PI's Jiri Adamec, Graham Cook, Jo Davisson, John Morgan, Dan Raftery, David Salt, Barry Wanner) will support the purchase and development of a laser ablation ICP-MS platform for the development of spatially resolved ionomics. This instrumentation will also be developed as part of the Metabolic Profiling Facility (MPF) at Purdue. The MPF is now established in HORT 302 and is being managed by the Bindley Bioscience Center.

Title: Genomics Revolution Uncloaked

Abstract: This project focuses on developing the Informal Science Education exhibit "*Genomics Revolution Uncloaked*". The majority of the design, development and building of the exhibit is being supported by a grant from the NSF 2010 program (IBN 0419695). Funds from the ASPB Education Foundation are being used to provide a dynamic and visually appealing "portal" through which visitors associate and learn about plant functional genomics. The ASPB Education Foundation award will leverage funds previously obtained from the NSF, and be used towards the implementation of computer-based, interactive software, that will simulate a plant cell undergoing abiotic and biotic stresses, and allow the visitor to control the fate of such cells by reacting to the changing conditions. This simulation will then be used to enrich the physical exhibit. Much like other simulation gaming architecture programs, the interactive *cellular voyager* software will allow the user to navigate the intra- and extra-cellular environment to identify regions that are being afflicted by the corresponding stress stimulus. This design will utilize the Cellscape Explorer™ technology developed by Dr. Jeremy Friedberg from Vive Technologies Inc (<http://www.vivetechologies.com/>). Dr. Friedberg has a doctorate degree in molecular genetics and plant biotechnology. This expertise will be essential for the development of the interactive, simulated scenarios (see letter of support). Additionally, the program will incorporate simple, yet effective, genomic, transcriptomic, metabolomic and ionic profile panels that will allow the user to monitor the cellular changes that occur during the different scenarios. By learning how to interpret these profiles while navigating through the cell, the visitor will learn about functional genomics in a graphic and fun manner. The gaming architecture platform of cellular models is an effective way to attract and educate visitors of all ages. Funds from the ASPB Education Foundation will also be used to support the additions of special effects into the exhibit, to allow for the dramatic illustration of concepts presented in the virtual world to be mirrored in the physical exhibit. The enhancement of the *cellular voyager* through these interactive elements will educate in a stimulating and dynamic way, so that the visitor will form a long-term connection between plants and their impact on human society. Ultimately, this will spark greater interest in plant science in general and plant functional genomics as it relates to agriculture, human health and environmental preservation.

2. Developing Research Activity (Grants pending)

Title: PSCIC Preliminary Proposal: Collaborative for Cyber-Enabled Plant Science

PI: David E Salt (Purdue University).

Co-PI: Joe Pekny (Purdue University), Gerhard Klimeck (Purdue University), Michael McLennan (Purdue University), Beverly Sypher (Purdue University).

Senior Personnel: Gary Bertoline (Purdue University), Scott Brandt (Purdue University), Ahmed Elmagarmid (Purdue University), Michael Gribskov (Purdue University), Victor Raskin (Purdue University).

Proposal Status: NSF Cyberinfrastructure for the Biological Science: Plant Science Cyberinfrastructure Collaborative Preproposal declined.

Abstract To meet the Grand Challenges in Plant Science it is critical that we gain the scientific benefits that high-density data integration offers. However, to achieve this we need to address the need for an enabling **Plant Science Cyber Infrastructure** (PSCI), to underpin this transformative process. Three key components are required for the success of a PSCI: **Technology** - *Development of and open access to protocols and policies defining the PSCI*, **Organization** - *Development of robust operations and management protocols*, **Community** - *The formation of a broad community of users working together to create and evolve the PSCI*. Furthermore, all such activities need to be embedded within the Plant Science community through various management mechanisms. The PSCI will be developed within a **Collaborative for Cyber-Enabled Plant Science (CCEPS)**, from herein know as the **Collaborative**, whose expertise and knowledge-base will be layered and distributed among and within the Technology, Organization and Community cores. The capabilities of the PSCI will include the creation and deployment of a **Futures Development Laboratory** to bring cutting-edge information technology to the discovery workflows of plant scientists; novel **Knowledge Generation Environments** for data-integration, synthesis and visualization; installation of **high-performance on-ramps** for seamless access to the TeraGrid for rendering and computation; data curation, archiving for **interoperability**; development of **metadata standards** and information management systems to **formalize high-throughput data acquisition**, through unique **Cyber-assisted outreach and education** activities. The majority of the PSCI functionalities will be delivered through an intuitive, flexible and interactive web portal the, **PlantScienceHUB**, enabling intellectual synthesis and collaboration. To ensure successful implementation of the PSCI through targeted workflows and community involvement, a unique organizational structure will be established around two synergistic components. A **Steering Committee**, whose authority will be balanced by a community-based organization called the **Plant Science Cyber Infrastructure Society** (PSCIS). The centralizing force of the Steering Committee will provide a strong organizing principle to set strategic goals and priorities, while the decentralizing principles of the community-based PSCIS will provide strong community oversight through elected representation on the Steering Committee. Community core of the Collaborative will be responsible for preparing the next generation of plant scientists, and taking the lead in outreach using novel approaches in Cyber-assisted learning. A high school or middle school student could utilize data from the **PlantScienceHUB** in classroom projects, participate in simulations that explain the scientific properties of plants, access remote instrumentation, take virtual field trips, collaborate in experiments with college students and faculty, and be mentored by other members of the Collaborative. Teacher training across levels will be an integral part of the PSCI as we take on the challenge of educating the next generation of plant scientists. The PSCI will make possible multiple reality learning environments for upgrading teachers in ways that will harness the imagination of this generation of learners. The PSCI will provide access to the significant computational power necessary to build multi-sensory and engaging learning environments that complement contemporary students' lifestyles. By providing a complementary convergence of pedagogy, science and the PSCI the Collaborative will educate the community of stakeholders to stimulate future teaching, learning, collaboration, and innovation in Plant Science.

Title: **From Ionome to Genome: Mapping the Gene Networks Controlling Nutrient Content in Rice Grain**

PI: David Salt (Purdue University)

CoPIs: Mary Lou Guerinot (Dartmouth College); Shannon Pinson (Texas A&M University)

Proposal Status: NSF Plant Genome Research Program proposal pending.

Abstract: Rice provides the major source of nutrition for a large proportion of the world's population. Mineral nutrients such as Ca, Fe, and Zn play critical roles in human health, with over 3 billion people suffering from Fe and Zn deficiencies. Unfortunately for those who rely on rice for subsistence, rice grain is not a good source of these nutrients and can contain toxic elements such as As and Cd. As such, alterations in the mineral content of rice grain to either increase or decrease levels of various elements would impact human health. In this project, we will take advantage of reverse genetics and mine our existing ionomic data sets on 4385 yeast, and 1054 Arabidopsis knockout lines to identify genes that potentially function to control the rice grain ionome. T-DNA, *tos17* and *Ds* insertional alleles in these candidate genes will then be identified. We also propose to use the tools of high-throughput elemental analysis, genetics and DNA microarray-based mapping to identify loci that regulate mineral

nutrient accumulation in rice grain. A core set of rice accessions selected from the USDA National Small Grains Collection (NSGC) (1,790 accessions) will be screened using ICP-MS for variation in accumulation of B, Na, Mg, P, K, Ca, Mn, Fe, Co, Cu, Zn, As, Se, Mo, Cd and I. We will also screen rice grain from the genotyped Lemont x TeQing (F16) RIL population which our preliminary studies have already shown to be segregating QTLs for a number of important elements including Fe and Zn. The availability of TeQing-into-Lemont introgression lines (TILs) will be used for finer mapping of these QTLs. The integration of high-throughput phenotyping and genotyping will allow the rapid identification of genes and linked markers for application to breeding and transgenic improvements in the mineral nutrient content of rice grain.

2. Publications

A. Refereed Papers:

Year 2006

Rus A, Baxter I, Muthukumar B, Gustin J, Lahner B, Yakubova E and **Salt DE** (2006) Natural variants of AtHKT1 enhance Na⁺ accumulation in two wild populations of Arabidopsis. *PLoS Genetics* 2(12): e210.

[doi:10.1371/journal.pgen.0020210](https://doi.org/10.1371/journal.pgen.0020210)

Ellis DR, Gumaelius L, Indriolo E, Pickering IJ, Banks JA, **Salt DE** (2006) Isolation and characterization of a unique arsenate reductase from the arsenic hyperaccumulating fern *Pteris vittata*. *Plant Physiology* 141:1544-1554.

Yoo CY, Miura K, Jin JB, Lee J, Park HC, **Salt DE**, Yun DJ, Bressan RA, Hasegawa PM (2006) SIZ1 SUMO E3 Ligase Facilitates Basal Thermotolerance in Arabidopsis Independent of Salicylic Acid. *Plant Physiology* 143 1548-1558.

Waters BM, Chu HH, Didonato RJ, Roberts LA, Easley RB, Lahner B, **Salt DE**, Walker EL (2006) Mutations in Arabidopsis yellow stripe-like1 and yellow stripe-like3 reveal their roles in metal ion homeostasis and loading of metal ions in seeds. *Plant Physiology* 141: 1446-1458.

Pickering IJ, Gumaelius L, Harris HH, Prince RC, Hirsch G, Banks JA, **Salt DE**, George GN (2006) Localizing the biochemical transformations of arsenate in a hyperaccumulating fern. *Environmental Science and Technology* 40: 5010-5014.

Peer WA, Mahmoudian M, Freeman JL, Lahner B, Richards EL, Reeves RD, Murphy AS, **Salt DE** (2006) Identifying model hyperaccumulating plants II: Germplasm analysis of 27 Brassicaceae accessions from a wide geographic area. *New Phytologist* 172: 248-260.

Rampey RA, Woodward AW, Hobbs BN, Tierney NP, Lahner B, **Salt DE**, and Bartel B (2006) An Arabidopsis basic helix-loop-helix leucine zipper protein modulates metal homeostasis and auxin conjugate responsiveness. *Genetics* 174 1841-1857.

B. Non-Refereed Papers in magazines

C. Book Chapter

D. Patents, Provisional Patents and Invention Disclosures

3. A. Invited Lectures

Year 2006

Dartmouth College, Hanover, Oct 2006.

Sulfur-containing defense compounds: Pivotal players in plant stress tolerance. Heidelberg Institute of Plant Sciences, Heidelberg, Germany, Oct 2006.

Annual Meeting of the Max Planck Institute for Molecular Plant Physiology, Golm, Germany, June 2006.

6th International Symposium on Speciation of Elements in Biological, Environmental and Toxicological Sciences, Bialowieza National Park, Poland, June 2006.

Pan American Plant Membrane Biology, South Padre, TX, May 2006.

4. A. Major Professor Graduate Students

H&LA Ph.D. student – Tommy Sors, *Council for Biotechnology Information, Young Scientist Scholarship Award (3 awarded nationwide)* (2003 – present)

Plant Biology Ph.D. student – Gun-Nam Na (2002 – present).

Botany and Plant Pathology Ph.D. student – Luke Gumaelius (2002 – 2006), Graduated Spring 2006. Co-supervise with Dr Jody Banks.

Plant Biology Ph.D. student – Jeff Gustin, *NSF IGERT fellow* (2002 – present).

B. Graduate Student Committee member

Matheus Bennati, H&LA, 2004 - present

Srinivas N Makam, Purdue, H&LA, 2002 – present.

C. Postdoctoral students

Dr Muthukumar Balasubramaniam (2003 – present), Ivan Baxter (2004 – present), Dr Bakhtiyor Yakubov (2001 – 2007), Hyeong Cheol Park (2005 – present), John Danku (2006 - present).

D. Technicians

Brett Lahner, (2000 – present), Lena Yakubov, (2004 – present), Tina Graham (2004 – present), Marina Tikhonova (2005 – present)

E. Undergraduate Research

Ben Hartwig (visiting undergraduate from Germany)

F. Visiting Scientist

5. Research grants and scholarly awards

A. Active grants

Awarded Year 2006

Title: The Genetic basis of natural ionomic variation

PI's: David E Salt

coPI's Mary Lou Guerinot (Dartmouth College), Magnus Nordborg (University of Southern California)

Award: National Institutes of Health, NIGMS 03/31/2007 – 03/30/2010, \$1,086,788

Account: HORT – Salt

Title: Tools for differential metabolomics

PI's: Fred Regnier

coPI's Jiri Adamec, Graham Cook, Jo Davisson, John Morgan, Dan Raftery, David Salt, Barry Wanner

Award: National Institute of Health, 10/01/04 - 09/30/07, \$4,719,117 (Salt budget \$740,859)

Account: CHEM – Regnier, minor account HORT – Salt

Title: Acquisition of Metabolic Profiling Instrumentation at Purdue

PI's: Clint Chapple

coPI's David Rhodes, David Salt and Barry Wanner

Award: National Science Foundation, MRI, 10/01/04 - 09/30/07, \$839,427

Account: BIOCHEM – Chapple

Title: Ionomics

PI's: Mary Lou Guerinot, Dartmouth College

Jeffrey F. Harper, The Scripps Research Institute

David E. Salt, Purdue University

Julian I. Schroeder, University of California at San Diego

John Ward, University of Minnesota.

Award: National Science Foundation, 2010 Project, 9/1/2004 to 8/31/2008, \$3,490,000 (Salt subcontract \$1,321,016).

Account: HORT - Salt

Title: Center for Phytoremediation Research and Development

PI's: David E Salt & Kathy Banks

Award: Indiana 21st Century Fund for Research and Technology, 07/01/2003 to 06/30/2007, \$2,000,000.

Account: HORT - Salt

Title: Genomics Revolution Unlocked

PI's: David E Salt

Award: American Society of Plant Biologists Education Foundation 08/01/2005 – 07/31/2007, **\$30,000**

Account: HORT - Salt

D. Scholarly Awards

Purdue University Faculty Scholar 2003 – 2008

Nominated by Nature Biotechnology (March 2006) as one of the “thought leaders and technology pioneers” in biotechnology in the past 10 years (Nature Biotech **24**: 291)

E. Miscellaneous

University Trask Technology Innovation Awards review committee.

University Grievance Committee.

7. Evidence of regional or national recognition.

Reviewing Activity

Reviewed manuscripts 2006 Plant Cell, Plant Physiology, Plant Journal, New Phytologist, Environmental Science and Technology, Annals of Botany, Plant Molecular Biology

Reviewed grants 2006 USDA, NSF Scientific Programme of Indonesia – Netherlands.

Other service activities

Member of the American Society of Plant Biologists Education Committee, 2004 – 2009.

Monitoring Editor Plant Physiology, 2005 – 2010.

Editorial Board Member, International Journal of Phytoremediation, 2003 – present.

8. Collaborative and interdisciplinary efforts in research and scholarship.

Purdue University

Discovery Park, e-Enterprise Center/Cyber Center/Bindley Bioscience Center Development of the Purdue Ionomics Information Management Systems (PiiMS).

Dr Fred Regnier, Tools for differential metabolomics

Dr Kathy Banks, Civil Engineering, Joint PI's on 21st Fund grant (\$2,000,000) “Center for Phytoremediation Research and Development”

Dr Lazlo Csonka, Biology Department, Identification of genes involved in metal accumulation and resistance (Center for Phytoremediation Research and Development)

Dr Jody Banks, Botany and Plant Biology, joint PI's on DOE grant (\$450,000) “Molecular dissection of arsenic hyperaccumulation in the fern *Pteris vittata*”

Dr Jo Davison, Discovery Park, establishment of a core metabolic profiling facility and “omics” cyberinfrastructure.

Membership in the Purdue Discovery Park Bindley Bioscience Center, Cyber Center and Oncology Center

Nationally

Dr Mike Mason/Dr Eric Schaller, Dartmouth College, Characterization of ionic changes in *arr* and other two-component system mutants.

Dr Elsbeth Walker, University of Massachusetts, Characterization of ionic changes in *ysl* mutants

Dr Erin Connolly, University of South Carolina, Characterization of ionic changes in *fro* mutants

Dr Kendal Hirschi, Baylor College of Medicine, Characterization of ionic changes in *cax* mutants

Dr Ingrid Pickering, Stanford Synchrotron Radiation Laboratory, Use of X-ray absorption spectroscopy to characterize the in vivo molecular environment of metals, metalloids and nonmetals in plants.

Dr. Mary Lou Guerinot, Dartmouth College, Gene Discovery in Aid of Plant Nutrition, Human Health and Environmental Remediation.

Dr. Jeffrey F. Harper, The Scripps Research Institute, Gene Discovery in Aid of Plant Nutrition, Human Health and Environmental Remediation.

Dr. Julian I. Schroeder, University of California at San Diego, Gene Discovery in Aid of Plant Nutrition, Human Health and Environmental Remediation.

Dr. Bonnie Bartel, Rice University, Characterization of the role of the ILR3/bHLH105 transcription factor in regulating the ionome.

Dr. John Ward, University of Minnesota, Characterization of ionic mutants.

Internationally

Dr Roger Reeves, Massey University, Genome-wide hunt for metal hyperaccumulation genes.

Dr Yongguan Zhu, Research Center for Eco-environmental Science, Chinese Academy of Science. China. Natural variation in the rice grain ionome.

Dr Moo Young Eun, National Institute of Agricultural Biotechnology, Rural Development Administration, Korea. Natural variation in the rice grain ionome.

9. New Plans for 2007

Title: **Evolutions Footprints: Adaptation of the Arabidopsis Genome to Contrasting Environments**

PI: David E Salt (Purdue University)

Co-PI: Ivan Baxter (Purdue University), Justin Borevitz (University of Chicago), Diane Byers (Illinois State University), Joy Bergelson (University of Chicago)

Senior Personnel: Gary Bertoline (Purdue University), Ahmed Elmagarmid (Purdue University), Cynthia Moore (Illinois State University), Phillip Owens (Purdue University), Philip Scarpino (Indiana University-Purdue University Indianapolis), Kurt Spokas (ARS, North Central Soil Conservation Lab)

Proposal Status: NSF FIBR program, preproposal Dec 2007

Summary of Scientific Objectives and Approaches: Extensive genotypic variation is known to exist in many different organisms. However, examples of how this natural genetic variation has been molded by evolutionary forces to generate phenotypes with adaptive significance are very limited, even though it is central to neo-Darwinian thinking. The arrival of settlers, and the plant *Arabidopsis thaliana* (Thale cress), in the upper Midwest 200 years ago initiated a natural experiment in evolutionary genetics in which the settlers altered the environment through the agricultural practices they introduced, and *A. thaliana* adapted to survive in this changing landscape. The results of this experiment are now accessible to us through the recent emergence of genome-wide genotyping tools for *A. thaliana*. These tools provide us with the unique ability to decipher the story of how this small weed's genome has been manipulated by the selective pressures it has encountered in the upper Midwest over the last 200 years. Using county agricultural records and oral histories tracing land usage, and data on weather patterns and soil properties, we will develop a spatial and temporal map tracking changes in the environment across multiple sites in the Midwest (IN, IL and MI) where *A. thaliana* is known to grow in both disturbed and natural locations. Genome-wide genotyping will be performed and correlations between population structure and environmental factors determined. Broad assays of developmental and physiological traits underlying fitness will be scored on the wild populations, loci driving these traits cloned, and near isogenic and transgenic plants used in the laboratory and field to establish the impact of these loci on fitness. The collection of *A. thaliana* populations and associated habitat information will form the core of the *Midwestern Arabidopsis Ecological and Evolutionary Observatory* (MAEEO) upon which all our studies will be founded. To manage the observatory, and communicate its findings to the broader community, we will develop the web-based *Virtual Evolutionary Observatory* (VEO).

Expected Broader Impacts of the Proposed Research: Our broader impacts are expected to be in education at all levels (graduate, undergraduate, high school and life-long learners). Our broad-based outreach and education

program will focus on the incorporation of research-based skills into classroom curriculum at the high school and college level, as well as in the laboratory. These skills will incorporate not only experimental design and data collection, but also analysis of large data sets available through the ^YEO. We will engage student and community volunteers in the process of identifying and characterizing sites across IN, IL and MI where *A. thaliana* grows. An educational portal, the “Evolution Café”, will be established within the ^YEO. This will be a virtual space for learning activities centered on adaptation and evolutionary processes. We will develop a series of educational activities at both high school and college levels, investigating a broad range of topics associated with this project including the evolutionary significance of environmental adaptation. Each of these lessons will be field-tested in various Illinois State University courses as appropriate. We plan to disseminate all completed activity materials through ^YEO, workshops, and professional meetings. We also anticipate developing a traveling Informal Science Education exhibit that will present information relating to plant adaptation and evolution in response to a changing environment at State Park venues. Incorporating a diverse group of students is important to the success of the project. We will link the MAEEO with the MARC/AIM Summer Research Program, the Minority Student Academic Center, and the Illinois Louis Stokes Alliance for Minority Participation.

Title: Whole-Genome Shotgun Sequencing of the Metal Hyperaccumulating Plant *Thlaspi caerulescens*

PI: David E Salt (Purdue University)

Proposal Status: DOE Joint Genome Initiative, Community Sequencing Project, competitive preproposal chosen for submission of full proposal Mar 12, 2007.

Community Representatives: Mark Aarts (Wageningen University, Netherlands), Alan Baker (University of Melbourne, Australia), Martin Broadley (University of Nottingham, UK), Rufus Chaney (USDA Beltsville Agricultural Research Service, USA), Pierre Czernic (Université Montpellier, France), Leon Kochian (Cornell University, USA), Ute Kraemer (Max Planck Institute for Molecular Plant Physiology, Germany), Steve McGrath (Rothamsted Research, UK), David E Salt (Purdue University, USA) and Henk Schat (Vrije Universiteit Amsterdam, Netherlands)

Description: We wish to obtain draft sequencing by whole-genome shotgun sequencing to 8X depth of *Thlaspi caerulescens*, a plant that can hyperaccumulate in its native habitat over 1% of its shoot dry weight as cadmium, nickel or zinc. We also request draft assembly and annotation.

Justification: Southern France populations of *Thlaspi caerulescens* have an extraordinary capacity to hyperaccumulate shoot concentrations of cadmium (Cd), nickel (Ni) and zinc (Zn) that are 100-fold higher than other plants growing in the same habitat. This hyperaccumulation is also accompanied by hypertolerance to these potentially toxic trace metals. For example, *T. caerulescens* can accumulate 1 – 3% (10,000 – 30,000 mg kg⁻¹) of its shoot dry weight as Cd, Ni or Zn without exhibiting symptoms of toxicity; levels of accumulation that would kill most plants. With its relatively compact growth habit, self-compatibility, relatively small genome (with approximately 200 Mb, less than double *Arabidopsis thaliana*), close relationship to *A. thaliana* (87 – 89% identity in coding regions), and ability to be easily genetically transformed, *T. caerulescens* is rapidly becoming the model organism of choice for the study of metal hyperaccumulation in plants (Assunção et al., 2003; Peer et al., 2003, 2006). There is a high level of apparently uncorrelated variation in the degree and specificity of metal accumulation and tolerance between different *T. caerulescens* accessions permitting genetic and associated biochemical and physiological studies of these traits. The genome of *T. caerulescens* contains genes that are not present in *A. thaliana*, and the expression regulation of a number of genes thought to be involved in metal hyperaccumulation in *T. caerulescens* is very different from *A. thaliana* and other related non-accumulators. These results suggest that *T. caerulescens* has evolved its ability to hyperaccumulate Cd, Ni and Zn independently of *Arabidopsis* and that it will be an invaluable source of metal hyperaccumulation determinants for development of plants ideally suited to phytoextraction applications.

Utilization: The availability of a whole-genome sequence for *T. caerulescens* would allow rapid progress to be made in identifying the genetic loci that contribute to the hyperaccumulation and tolerance of Cd, Ni and Zn in this plant. In general, the availability of an additional Brassicaceae genome sequence next to *A. thaliana* and the newly sequenced very close relative's *A. lyrata* and *Capsella rubella* will be instrumental to further understand genome evolution and identify evolutionary target sequences. Furthermore, comparison of the genomes of *T. caerulescens* and the newly sequenced halophyte *Thellungiella halophila* (Brassicaceae) will allow the identification of underlying commonalities in the genomic mechanisms that drive the evolution of adaptations to extreme abiotic stresses in plants.

Community Interest: No other plant species has shown such high promise for practical phytoremediation of Cd from contaminated soils which cause risk to humans from Cd accumulation into food crops. This practical application of *T. caerulescens* has directly led to numerous laboratories throughout the world being interested in the

mechanism of metal hyperaccumulation in *T. caerulescens* and other related *Thlaspi* species. These groups are working on many different aspects of this problem from questions of plant/soil interface, through genetics, biochemistry, physiology and ecological aspects. A whole-genome sequence for *T. caerulescens* would enable the majority of these groups to move their research forward and help to progress the science from being purely descriptive to providing mechanistic answers to the questions being asked.

DOE Relevance: The goal of the proposed research is relevant to the JGI/DOE mission by proposing to develop the underlying science needed for the generation of plant-based solutions to the sustainable and long-term stewardship of sites contaminated with inorganic legacy wastes. Identification of the genetic loci responsible for Cd, Ni and Zn hyperaccumulation will provide the community with tools to genetically manipulate the ability of plants to hyperaccumulate these metal for the development of plants ideally suited for their removal from the environment, in a process called phytoextraction. Furthermore, a better understanding of the mechanisms that define the metal specificity of accumulation and tolerance will allow further improvements in the development not only of phytoremediation crops, but also in the nutritional improvement of food crops with reduced Cd and elevated concentrations of the essential micronutrient Zn.

Title: Research Coordination Network to Foster Ionic Discovery

PI: David E Salt

CoPI: Mary Lou Guerinot

Program Status: NSF Research Coordination Networks in Biological Science, proposal in the early stages of development – deadline June 26, 2007.

III. General Contributions: (last 5 years)

2. University committees

TRASK Innovation Committee, 2003 – 2009.

University Grievance Committee 2005 – present.

Member of the Bindley Bioscience Executive Committee 2004 – present.