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Plenary Symposia

PS-1

Biotic Responses to Climate Change in the Antarctic Dry Valleys. D. H. WALL[1] and B. J. Adams[2]. [1]School of Global Environmental Sustainability and Department of Biology, Colorado State University, Fort Collins, CO 80523 and[2]Department of Biology, and Evolutionary Ecology Laboratories, Brigham Young University, Provo, UT 84602. Email: diana.wall@colostate.edu

Antarctica is a cold desert with less than 2% of the continent ice-free. The largest ice free areas on the continent, the McMurdo Dry Valleys, are composed of soils, glaciers, lakes and meltstreams. This terrestrial ecosystem has no vascular plants or animals above ground, but within different soil habitats are invertebrates – rotifers, tardigrades, nematodes, and microarthropods. The responses of organisms in these ecosystems to alterations in temperature and moisture can inform future scenarios of climate change. We examined the factors affecting the response of invertebrates such as climate, habitat suitability (i.e., soil chemical and physical properties), and the stress response mechanisms affecting their activity and functioning. Experimental manipulations of temperature and moisture were imposed to measure potential responses of individuals, species, and entire soil communities to climate change. Results from these experiments and observed warming events in the cold desert indicate that climate change in extreme desert ecosystems alters soil habitat suitability, significantly impacts the few soil species present, and potentially their role in ecosystem functioning

PS-2

Coral Cryopreservation Offsetting Climate Change on Reefs. MARY HAGEDORN. Smithsonian Conservation Biology Institute, Washington, D.C. 20008. Email: hagedornm@si.edu

Coral reefs are some of the oldest, most beautiful ecosystems on our planet responsible for harboring over ¼ of animals in our oceans. But they are in dire trouble around the world from both local and global causes, such as increased sedimentation and over-fishing on reefs, as well as, the increased warming and acidification of our seas. Regardless of this ominous future, our laboratory holds out hope for these amazing places on earth by creating germplasm banks for many its inhabitants. To date, we have banked the sperm and embryonic stem cells of over 9 species of Australian, Caribbean and Hawaiian coral. We have thawed and created new coral from these frozen cells. This banked material can now be used to diversify areas where coral populations are shrinking. In addition to coral, however, we are establishing preservation protocols for many other reef inhabitants. Recently, we cryopreserved the symbiotic algae living inside coral. These algal cells may be an important key for understanding and helping coral survive stress. We are also cryopreserving the testicular cells of reef fish, such as gobies. Certain small species of fish are critical for reducing algal overgrowth on reefs. Fish testicular cells can completely resurrect a species even if the species is extinct, because these frozen and thawed cells can be injected into sterile host who will then develop ovaries or testes and grow up new males and females of the extinct group. Finally, we are developing robust preservation methods for tropical urchin sperm and embryos. These organisms help graze reefs, protecting them against algal overgrowth and deleterious, long-term phase shifts from coral- to algae-dominated reefs. Studies on assisted evolution will depend upon these extensive genetic libraries to produce strains better adapted to living in our changing oceans. The creation of these frozen repositories are currently the only process offsetting the damaging effects of climate change on our reefs.

PS-3

Cryopreservation as a Tool for Securing the Future of Plant Biodiversity. VALERIE PENCE. Cincinnati Zoo and Botanical Garden, Center for Conservation and Research of Endangered Wildlife, 3400 Vine Street, Cincinnati, OH 45220. Email: valerie.pence@cincinnatizoo.org

It is estimated that of the more than 350,000 species of higher plants in the world, at least 10-25% are of conservation concern. Loss of habitat, invasive species, and climate change

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are among the most important current and potential threats, and botanical institutions and organizations have joined in a worldwide effort to counter these threats, outlined in the Global Strategy for Plant Conservation. One of the Targets of the GSPC focuses on the ex situ conservation and subsequent restoration of conserved species, an effort that is most efficiently addressed by seed banking. However, there is a subset of species for which seed banking is not workable, either because the seeds are recalcitrant, are short-lived in storage, or the species are producing few or no seeds. For these *exceptional* species, cryopreservation-based conservation methods are needed, using seeds, embryos, dormant buds, and shoot tips as propagules for storage. The challenges of dealing with exceptional species include the lack of a list of such species, scientific and technical challenges, and the resulting challenge of costs. A recent workshop focusing on exceptional species has resulted in a statement of need and initiated an international effort to develop a strategy for dealing with these challenges. Efforts have begun to create a working list of exceptional species. Research using statistical approaches shows promise for improving in vitro and cryopreservation methods. Evaluation of the survival of some of the oldest wild materials stored in liquid nitrogen is underway at CREW, providing insight into the effectiveness of liquid nitrogen storage. These efforts, combined with international and cross-institutional collaborations should improve efficiency and thus reduce costs of preserving exceptional species. As these challenges are addressed, the ability of cryobanks to su pplement traditional seed banking efforts will increase, ensuring that all threatened taxa are preserved, as a resource for the restoration and potential translocation needs of the future.

PS-4

Molecular Mechanisms of Cellular Stress Responses: MicroRNA Regulation in Heat Shocked Cells. R. Roufayel, R. L. Rumney, D. S. Johnston, and D. D. MOSSER. Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, CANADA. Email: rmosser@uoguelph.ca

Exposure of organisms to environmental stress can adversely affect cell structure and function, but can also induce adaptive responses that provide enhanced resistance to these stressful conditions. Hyperthermic exposure causes protein misfolding and aggregation, which if prolonged or severe can trigger apoptosis as a means to eliminate irreversibly damaged cells. However, prior exposure to a mild heat stress induces the synthesis of molecular chaperone proteins known as heat shock proteins that perform vital cellular roles in protein homeostasis. The major heat-induced molecular chaperone protein HSP70 is a potent anti-apoptotic factor. The focus of our research is to determine how hyperthermia triggers apoptosis and how this is prevented by HSP70. Stress-induced apoptosis is regulated by interactions between pro-apoptotic and anti-apoptotic members of the Bcl-2 family of proteins. Regulation of individual Bcl-2 family members can occur through transcriptional, translational and post-translational mechanisms. We have found that exposure of human cells to hyperthermia changes the transcript levels of distinct pro-apoptotic Bcl-2 family members and that this is regulated by altered expression of specific miRNAs that target these transcripts. As well, HSP70 overexpression prevents pro-apoptotic Bcl-2 protein expression by regulating miRNA levels in heat-stressed cells. These results suggest that miRNA expression represents a critical heat-sensitive target regulating cell survival in heat-stressed cells.

PS-5

Climate Change Mitigation and Adaptation: Continued Innovation in Agriculture is Essential. MICHAEL A. HALL and David Gustafson. Monsanto Company, 700 Chesterfield Parkway West, Chesterfield, MO 63017. Email: Mike.hall@monsanto.com

Multiple lines of evidence confirm that climate change represents a major challenge for agricultural systems to successfully meet accelerating global demand for safe, nutritious, and affordable food. These trends will likely induce geographic shifts in production regions, and will likely force farmers to choose different varieties or perhaps even different crops. A recent report from the IPCC highlights the threat that climate change represents to global food security. Monsanto and other companies are innovating to improve the ability of farmers to meet these challenges. It is essential for continued innovations in agriculture in order to meet growing crop demand in a way that uses as few resources as possible – including land, water, and energy. Monsanto has a long history of innovating in agriculture and its inventions in agronomics, breeding, and biotechnology have made it possible for dramatic increases in both productivity and conservation of these resources to occur. The company has recently announced new areas of research & development investments in ag biologicals and information technology (The Climate Corporation). These both hold the promise of accelerated innovation, in order to help farmers continue to provide the nutritious food that the world needs – and doing so in a way that preserves the planet for future generations.

PS-6

Mechanisms of Resilience to Environmental Stressors within and Among Populations of Killfish. FERNANDO GALVEZ. Louisiana State University, Dept. Biological Sciences, 216

Life Sciences Building, Baton Rouge, LA 70803. Email: galvezf@lsu.edu

Maintenance of physiological homeostasis is critical in enabling persistence in fluctuating environments, especially in this era of rapid environmental change and increased variation in environmental extremes. A general result of climate change and global warming are changes in precipitation patterns that could result in salinity changes in estuaries. Estuaries are highly dynamic and variable habitats, where physiological plasticity and resilience to variable salinity are highly advantageous phenotypes that enable the initial stages of diversification across disparate osmotic niches. As such, salinity is arguably one of the most important barriers restricting the distribution of aquatic animals in the environment. Some species can change their phenotype to compensate for broad changes in environmental salinity, whereas other species have narrow salinity tolerance ranges. This presentation will describe the physiological responses and the genomic underpinnings of osmotic stress in natural populations of fish from the genus Fundulus. Utilizing a comparative and integrative approach, the mechanisms of acclimation to hypoosmotic and hyperosmotic challenges will be explored, describing differences in the compensatory responses to osmotic challenge between closely related fish taxa. The presentation will conclude with recent research investigating the role of polyamines in epithelial remodeling during osmotic stress, mechanisms of paracellular ion regulation ion transporting epithelia, and work to develop *in vitro* models to study ion transport in the gill epithelium of *Fundulus* species.

PS-7

Rapid Targeted Genome Modification in Mice, Rats and Rabbits. EDWARD J WEINSTEIN, Andrew J Brown, Evguenia Kouranova, Aaron McCoy, Kevin Forbes, Yumei Wu, Rachel Henry, Diana Ji, Andre Chambers, Joe Warren, and Xiaoxia Cui. SAGE Labs, St. Louis, MO. Email: edward.weinstein@sageresearchlabs.com

The rat, rabbit, zebrafish, and pig have long been important experimental models in multiple fields of study. Unlike the mouse, efficient gene targeting in these species has remained a near impossibility with researchers forced to rely on random methods of mutagenesis. Using the zinc finger nuclease and the CRISPR/Cas9 technologies we have circumvented the need for embryonic stems cells and directly targeted genes in the fertilized embryo. Data will be presented on the creation and characterization of genetically engineered mice, rats, and rabbits where key genes have been removed from the genome. Characterization will be shown for a p53 knockout rat, a panel of drug transporter knockout rats, and GE rats that model Parkinson's Disease. Furthermore, data will be presented on the addition ("knock-in") of genes, in a targeted manner, into the rodent genomes as well as the creation of conditional knockouts and humanized models.

Disclosure: Author was or is employed by SAGE Labs.

PS-8

Precise Engineering of Genomes with Sequence Specific Nucleases. DAN VOYTAS. Dept. of Genetics, Cell Biology & Development and Center for Genome Engineering, University of Minnesota, Minneapolis, MN 55455. Email: voytas@umn.edu

Methods for precisely altering DNA sequences in living cells enable detailed functional analysis of genes and genetic pathways. In plants, targeted genome modification has applications ranging from understanding plant gene function to developing crop plants with new traits of value. Our group has enabled efficient methods for targeted genome modification of plants using sequence-specific nucleases. With zinc finger nucleases (ZFNs), TAL effector nucleases (TALENs), and the CRISPR/Cas9 system, we have achieved targeted gene knockouts, replacements and insertions in a variety of plant species. Current work is focused on optimizing delivery of nucleases and donor DNA molecules to plant cells to more efficiently achieve targeted genetic alterations.

Disclosure: Author is Board Member of University of MInnesota.

PS-9

RTDS™ - an Oligonucleotide-Directed Mutagenesis for Trait Development. PETER R. BEETHAM, Christian Schöpke, and Greg F.W. Gocal Cibus US LLC, 6455 Nancy Ridge Road, Suite 100, San Diego, CA 92121. Email: pbeetham@cibusllc.com

The Rapid Trait Development System™ (*RTDS*) is a form of directed mutagenesis capable of altering any base within an entire genome. This technology has been advanced at Cibus and is being deployed to develop useful traits in crop and microbial systems. With the advent of large-scale DNA sequencing of populations and the detailed structural data on genes that was subsequently generated, we are able to deploy this technology accurately to a plethora of genes to provide many commercially valuable traits. The *RTDS* technology is more precise than random mutagenesis and other recent gene editing technologies and harnesses the cell's natural DNA

repair system to correct and change specific targeted bases within the genome of a cell. The Gene Repair OligoNucleotides (GRONs) are chemical compounds including a synthetic oligonucleotide containing modified 5' and 3' termini and are designed to create at least one mismatched base-pair. This mismatched base-pair(s) structure within the GRON acts as a signal to attract the cell's repair system to the mismatch site and to correct (replace, insert or delete) the designated base(s) within the target region. Once the correction process is complete the GRON molecule is degraded. The now-modified or repaired gene retains its normal pattern of expression. This technique has been successfully applied in bacterial, fungal, mammalian systems, and to develop traits in crop plants. The current status of our work in plants and microbial systems will be discussed, emphasizing the opportunities and challenges of this technology.

Disclosure: Author was or is employed by Cibus US, LLC.

PS-10

Integrated Metabolomics, Gene Expression, and GWAS Identify New Saponin Biosynthetic Genes in *Medicago truncatula*. LLOYD W. SUMNER[1], Bonnie S. Watson[1], Zhentian Lei[1], DongSik Yang[1], Yuhong Tang[1], Derek Nedveck[2], Peter Tiffin[2], and Nevin Young[2]. [1]The Samuel Roberts Noble Foundation, Ardmore, OK 73401 and [2]University of Minnesota, St. Paul, MN 55108. Email: lwsumner@noble.org

Triterpene saponins are structurally diverse secondary metabolites found in many plant families, including the Leguminosae. They possess a broad spectrum of bioactivities ranging from allelopathy and anticancer activities to antifungal, antibacterial, anti-insect and anti-nutritive properties. In spite of their functional importance, the biosynthetic pathways for saponins remain largely uncharacterized. We are using an integrated metabolomics, correlated gene expression profiling and genome wide association studies (GWAS) for the discovery, prioritization, and characterization of novel saponin biosynthetic genes in the model legume *Medicago truncatula* which is known to accumulate a large variety of differentially glycosylated saponins. In this project, saponins from aerial and root tissues of close to 200 accessions in the Medicago Hapmap collection were profiled by UPLC- qTofMS. It was determined that the collection contained ecotypes with highly varied accumulation of saponins, both within the different tissues as well as between accessions. Eight lines with differential saponin accumulation were chosen for further characterization, including correlated gene expression analyses using RNAseq and genome wide association (GWAS) relative to the differential saponin accumulation. The correlated gene expression and GWAS results guided the selection and prioritization of gene candidates for subsequent cloning and pathway characterization. In vitro biochemical assays confirmed the activity of saponin biosynthetic enzymes. Additional molecular genetic confirmation was performed through the analysis of Tnt1 insertional mutantations within the targeted saponin genes and through the analysis of plants stably transformed with known and putative saponin genes. This presentation will describe the integrated technologies and approaches used, and provide examples of novel gene discoveries.

PS-11

Using Quantitative Phosphoproteomics to Connect Genotype to Phenotype. FOREST M WHITE. Koch Institute for Integrative Cancer Research, MIT, 77 Massachusetts Ave, Cambridge, MA 02139. Email: fwhite@mit.edu

Over the past 5+ years, there have been extensive efforts by multiple groups to characterize genetic aberrations in human cancers and other common diseases. While these studies have led to very large scale datasets and have identified many common mutations, in most cases the mechanistic connection between genetic aberration and disease phenotype is poorly understood. To uncover the mechanisms by which genetic alterations drive oncogenic phenotypes, we have developed a quantitative mass spectrometry based approach to map signaling networks in a broad variety of biological samples, from cell lines to mammalian tissues. To effectively monitor protein phosphorylation events governing signaling cascades, our approach enables the simultaneous quantification of tyrosine phosphorylation of specific residues on dozens of proteins at multiple time points under a variety of perturbations. We have applied this technique to identify key signaling nodes regulating EGFR, Insulin Receptor, and T Cell Receptor signaling network response to stimulation. Using this technology, we have performed an in-depth characterization of signaling networks alterations resulting from RAS mutations in colorectal cancer cell lines and have begun to probe the relationship between genetic lesions and signaling network alterations in glioblastoma tumors resected from human patients and genetically engineered murine models.

PS-12

Western Corn Rootworm Transcriptome Assembly and Genomic Analysis of Population Structure. LEX FLAGEL[1], Raman Bansal[2], Randall A. Kerstetter[1], Mao Chen[1], Matthew Carroll[1], Ronald Flannagan[1], Thomas Clark[1], Barry S. Goldman[1], and Andy P. Michel[1]. [1]Monsanto Company and [2]Dept. of Entomology, Ohio Agricultural Research and Development Center. Email: lex.e.flagel@monsanto.com

Western corn rootworm (*Diabrotica virgifera virgifera*) is a beetle that is native to North America and the dominant maize pest in the US Corn Belt. It is estimated that each year Western Corn Rootworm (WCR) costs US farmers at least 1 billion dollars. WCR's impact on maize production has been exacerbated by the fact that it has proved difficult to control. We have worked to develop a genetic toolkit for WCR to better understanding it's biology and the genetic basis of insecticide resistance. This toolkit includes an EST assembly and a large number of SNP markers. Using these resources we can infer the evolutionary history of WCR and begin to identify candidate genes for insecticide resistance.