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UFGI Strategic Plan

The discovery of the three-dimensional double helix architecture of DNA in 1953 was not only a defining moment for biology, but arguably one of the most significant scientific discoveries of all time. It fundamentally and permanently changed the course of biology and genetics. The unraveling of DNA's structure, combined with its elegant mechanism for self-replication and the existence of a universal genetic code for all living beings, have together provided the basis for the understanding of fundamental cellular processes, mutation and genetic repair, genetic variation, the origin of life and evolution of species, and the structure/function/regulation of genes. The double helix is also proving to be of immense significance to advances in agriculture, medicine and such other diverse fields as anthropology, criminology, computer science, engineering, immunology, nanotechnology, etc. It was the study of DNA that led to the development of tools that brought about the biotechnology revolution, the cloning of genes, and the sequencing of entire genomes. Yet, most knowledgeable people agree that what has been achieved in DNA science thus far is only the beginning. Bigger and better applications, which will impact directly on the quality of human life and sustainability of life on earth, are yet to come. In order to attain these objectives, the digital nature of DNA and its complementarity are beginning to be exploited for the development of biology as an information-based science. Indeed, a paradigm shift is already taking place in our view of biology, in which the natural, physical, engineering and environmental sciences are becoming unified into a grand alliance for systems biology. Indeed, biology in the 21st century will be surely dominated by this expanded vision. The Genetics Institute is committed to fostering excellence in teaching and research, and in promoting cross-campus interdisciplinary interactions and collaborations. In the pursuit of these objectives, it offers a graduate program in genetics, and has identified the following four key areas for teaching, research and development: Bioinformatics, Comparative Genomics, Population and Statistical Genetics, and Epigenetics.

Florida Genetics 2007 Schedule

Wednesday, November 7, 2007

Noon - 6:15 p.m.: Check-in at UF's Cancer & Genetics Research Complex

1:30-1:45 p.m., Opening Remarks: Indra K. Vasil, Ph.D., and Kenneth I. Berns, M.D., Ph.D.

Session I — Developmental Genetics

Chair: Brian Harfe, Ph.D.

1:45-2:30 p.m.

Michael Snyder, Ph.D.

Director, Yale Center for Genomics and Proteomics

Analyze this and that: Genomes and proteomes

2:30-3 p.m.

Martin Cohn, Ph.D.

Associate Professor, Department of Zoology, University of Florida

Molecular development of the external genitalia

3-3:45 p.m.

Vicki Rosen, Ph.D.

Professor and Chair, Department of Developmental Biology, Harvard School of Dental Medicine

BMPs link skeletal development and bone regeneration

3:45-4:15 p.m.

Brian Harfe, Ph.D.

Assistant Professor, Department of Molecular Genetics and Microbiology, University of Florida

Patterning a vertebrate embryo

4:15-6:15 p.m.: Poster Session and Reception

Thursday, November 8, 2007

8 a.m.-3:15 p.m.: Check-in at UF's Cancer & Genetics Research Complex
8-8:30 a.m.: Coffee

Session II — Plant Genetics and Evolution

Chair: Matias Kirst, Ph.D.

8:30-9:15 a.m.

Elizabeth Kellogg, Ph.D.

E. Desmond Lee and Family Professor of Botanical Studies,
Department of Biology, University of Missouri – St. Louis

Genes and morphology in diversification of the cereals and their relatives

9:15-9:45 a.m.

Matias Kirst, Ph.D.

Assistant Professor, School of Forest Resources and Conservation, University of Florida

Evolution of gene expression in flowering plants

9:45 a.m.-10:15 a.m.

David Oppenheimer, Ph.D.

Associate Professor, Department of Botany, University of Florida

RAPs: Novel regulators of actin organization in land plants

10:15-11 a.m.

Thomas G. Whitham, Ph.D.

Regents' Professor of Biology, Department of Ecology and Evolution

Director, Merriam-Powell Center for Environmental Research

Northern Arizona University

The genetic components of community structure and ecosystem processes, and their conservation implications

11 a.m.-1:30 p.m.: Poster Session and Lunch

Session III: Evolutionary Genetics

Chairs: Connie Mulligan, Ph.D., and Marta Wayne, Ph.D.

1:30-2:15 p.m.

Henry Harpending, M.D., Ph.D.

Distinguished Professor, Department of Anthropology, University of Utah

Humans are evolving rapidly and the rate is accelerating

2:15-2:45 p.m.:

Connie Mulligan, Ph.D.

Associate Professor, Department of Anthropology, University of Florida

Reconstructing human migrations: Projects from the Americas and from Africa

2:45-3:15 p.m.

Marta Wayne, Ph.D.

Associate Professor, Department of Zoology

Director of the Graduate Program in Genetics and Genomics, University of Florida

Genetical genomics and the X chromosome

Presentation Abstracts

Analyze this and that: Genomes and proteomes

Snyder M

Department of Molecular and Cellular Biology, Yale University, New Haven, CT

A variety of approaches have been used to integrate the functional elements of genomes of yeast and humans. Tiling arrays have been used to map new transcribed regions and transcriptional regulatory elements. This information has been used to construct regulatory networks and identify key components in networks. We have also examined network evolution in related yeasts. In addition, we have constructed and used proteome chips in yeast and humans to probe for novel biochemical activities, protein modification, and human disease. Integration of diverse data types reveals common regulatory modules used by eukaryotes.

Biography of Michael Snyder, Ph.D.

Dr. Michael Snyder is the Lewis B. Cullman Professor of Molecular and Cellular Biology and Professor of Molecular Biophysics and Biochemistry at Yale University. He is also the Director of the Yale Center of Genomics and Proteomics. Dr. Snyder received his Ph.D. training in the laboratory of Dr. Norman Davidson at the California Institute of Technology and carried out postdoctoral training in Dr. Ronald Davis's laboratory at Stanford University. He is a leader in the field of functional genomics and proteomics. His laboratory study was the first to perform a large-scale functional genomics project in any organism, and currently carries out a variety of projects in the areas of genomics and proteomics both in yeast and humans. These include the large-scale analysis of proteins using protein microarrays and the global mapping of the binding sites of chromosomal proteins. His laboratory built the first proteome chip for any organism and the first high resolution tiling array for the entire human genome. Dr. Snyder has published over 200 manuscripts and is editor of a number of journals including *Functional and Integrative Genomics*, *Molecular and Cellular Proteomics*, *Proteomics*, *Drug Discovery Today*, *PLoS Genetics* and *Genes and Development*. He sits on many international advisory boards and was a co-founder of Protometrix Inc., a protein microarray company that was purchased by Invitrogen in 2004, and a new company, Affomix Inc.

BMPs link skeletal development and bone regeneration

Rosen V

Department of Developmental Biology, Harvard School of Dental Medicine, Boston, MA

Bone morphogenetic proteins (BMPs), members of the transforming growth factor- β (TGF- β) super family, were originally identified based on their unique ability to induce bone formation at extraskeletal sites. Their presence in bone matrix and their osteoinductive nature suggested that BMPs would be likely to influence bone repair, and true to that prediction, several recombinant human BMP proteins are currently approved for human use as bone regeneration agents. While we know quite a lot about the pharmacological actions of BMPs, our understanding of how BMPs act as endogenous mediators of skeletogenesis has been hampered by the fact that BMP signaling mediates many facets of embryonic development, so that global knockouts of individual BMPs may lead to early embryonic lethality (BMPs 2, and 4), perinatal lethality (BMP7), or no phenotype (BMP6), in an unpredictable manner. To get around this problem, we constructed a series of mice deficient singly or in combination in the ability to produce BMP2, BMP4 and BMP7 in the early limb. Results of these studies allowed us to conclude that there is a threshold level of BMP signaling required for proper skeletal development, with loss of individual BMP genes compensated for by other BMPs, and that postnatal limb bone growth, maintenance of bone mass, and ability to initiate fracture repair are dependent only on the presence of BMP2. Why BMP2 is central to bone homeostasis is a current focus in the lab.

Biography of Vicki Rosen, Ph.D.

After receiving a Ph.D. in cell biology/physiology and spending several years as a postdoc, Dr. Vicki Rosen started her career as an independent investigator as a scientist at a fledgling biotech company, Genetics Institute, in the fall of 1984. Her project was to identify the factors present in bone that were responsible for bone formation. This idea, named bone morphogenetic protein (BMP) by Dr. Marshall Urist in 1965, had remained an ill-defined concept for many years. As part of a research team that combined protein biochemistry, molecular cloning and cell biology, Dr. Rosen and her colleagues were able to isolate the first BMPs genes and report on their activities in 1988. It was at this time that she became interested in the physiological roles that BMPs have in the skeleton, and the signaling pathways used by BMP proteins to exert these effects. Dr. Rosen continued to work on these questions at Genetics Institute until 2001 when she moved to Harvard School of Dental Medicine, shifting her lab from an industrial to an academic setting. She is currently professor and chair, Department of Developmental Biology. Her research remains focused on BMPs and the roles they play in musculoskeletal tissues.

Genes and morphology in diversification of the cereals and their relatives

Kellogg EA

Department of Biology, University of Missouri-St. Louis, St. Louis, MO

Plant systematists have made great progress in recent years in describing phylogenetic relationships among plants, and these phylogenies are commonly used to describe patterns of morphological change through time. The morphological changes must be caused by changes in genes; identifying those genes is the challenge of evolutionary developmental genetics ("evo-devo"). By focusing on a single plant family, the grasses, which contains excellent genetic and genomic resources in addition to considerable systematic knowledge, we have been able to identify genes underlying multiple morphological changes in the family. For example, the shift from tropical to temperate habitats that preceded the diversification of the cool-season grasses (subfamily Pooideae) correlates with development of a vernalization requirement and recruitment of a floral meristem identity gene (FUL1) as a component of the vernalization signaling pathway. In another example, we have found that production of floral clusters (spikelets) ending in a flower correlates with restricted expression of a different meristem identity gene (LHS1). In a third example, production of sterile branches, an evolutionary innovation of a group of serious weeds, involves loss of meristem identity genes and truncation of an otherwise-conserved developmental program. Somewhat unexpectedly, comparative studies have allowed us to test hypotheses of gene function and developmental role. Inference of gene function from model systems alone may be limited by available mutant alleles or reverse genetic tools, whereas evolution has produced a rich array of genetic variation that can add to the toolkit of the developmental geneticist.

Biography of Elizabeth A. Kellogg, Ph.D.

Dr. Elizabeth Kellogg is the E. Desmond Lee and Family Professor of Botanical Studies at the University of Missouri-St. Louis, and an Associate of the Missouri Botanical Garden. She has spent her career studying cereal crops and their wild relatives in the grass family, plants on which all of civilization depends. She has published well over 100 papers, and is co-author of a major textbook. In addition to her Ph.D. degree from Harvard University, she has been awarded an honorary doctorate from the University of Cordoba in Argentina. She has recently received a Guggenheim Fellowship, as well as the Chancellor's Award for Research Excellence at the University of Missouri-St. Louis. She has been elected a Fellow of the American Academy for the Advancement of Science (AAAS) and has served as President of the American Society of Plant Taxonomists, and of the Society for Systematic Biology.

The genetic components of community structure and ecosystem processes, and their conservation implications

Whitham TG

Department of Biological Sciences and the Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ

Because different genotypes of cottonwoods support different communities of arthropods and microbes, and affect ecosystem processes such as decomposition and nutrient cycling, these predictable effects are termed community and ecosystem phenotypes. These phenotypes are especially important to evaluate when they are expressed in foundation tree species as they are the drivers of community structure and ecosystem processes. Experimental forest studies show that these phenotypes exhibit broad-sense heritability. The existence of these heritable phenotypes has broad implications. First, genetic diversity in cottonwoods positively affects biodiversity. For example, the genetic diversity in stands of cottonwoods explains about 60% of the variation in the diversity of an arthropod community composed of 207 arthropod species. Thus, the loss of genetic diversity in a common species could result in the extinction of species dependent upon those genotypes for their survival. Second, there are genetic components to ecosystem services that explain about 50% of the variation in carbon storage, water cycles, and nutrient fluxes. Because the field of ecosystem science is largely genetics-free, it is important to understand how tree genetics affects carbon storage and other ecosystem services, which are important to the climate change debate. Third, the effects of climate change on the genetic structure of foundation species, is likely to alter their community and ecosystem phenotypes to affect a much larger community of organisms. Our studies with climate sensitive insect resistant and susceptible pines suggest that ~1000 species from microbes to vertebrates have been affected by the recent record drought in the southwest United States. Fourth, because the phenotypes of genetically modified organisms are likely to have community and ecosystem phenotypes, it is important to evaluate these higher order phenotypes before their release is approved. These and other findings are based upon the combined efforts of many colleagues and students who have been supported by an NSF FIBR grant. A key paper that illustrates the collaborative nature of our approach is: Whitham TG, Bailey JK, Schweitzer JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf E, Allan GJ, DiFazio SP, Potts BM, Fischer DG, Gehring CA, Lindroth RL, Marks J, Hart SC, Wimp GM, and Wooley SC: A framework for community and ecosystem genetics: From genes to ecosystems. (*Nature Reviews Genetics* 7:510-523, 2006).

Biography of Thomas Whitham, Ph.D.

Dr. Thomas Whitham grew up in a wholesale nursery in Iowa that developed new varieties of trees and shrubs that were derived from clones of somatic mutants found in the field. Observations of clonal variation in common trees and an interest in the insects found on these trees greatly influenced his education and research interests. After discovering that the distribution of insects was highly sensitive to plant genotype, he began to explore how other factors such as plant development, interactions with other species, and climate change could interact with tree genotype to affect community structure and ecosystem processes. To explore these interactions, large scale experiments were required. This led to collaborations with conservation agencies such as the Bureau of Reclamation, the Ogden Nature Center, and the Utah Department of Natural Resources in which experimental forests were planted as part of riparian habitat restoration. Because the underlying genetic makeup and/or pedigree of all trees used in these restoration efforts had been quantified, these landscape-level experiments allowed the cottonwood research group to map ecologically important traits and quantify the heritability of higher order traits such as biodiversity, community structure, stability and ecosystem processes. This leveraging of restoration and basic research resulted in the planting of costly landscape-level experiments that otherwise would have been difficult to establish. These forests emphasize both restoration and a genes-to-ecosystem approach that has contributed to the emerging field of community and ecosystem genetics. This research is largely funded by an NSF Frontiers in Integrative Biological Research (FIBR) grant to study the community and ecosystem genetics of poplars in the United States and parallel studies in Australia with eucalypts. Dr. Whitham has an undergraduate degree in Horticulture and Plant Pathology from Iowa State University, an M.S. in Zoology from Ohio State University, and a Ph.D. in Biology from the University of Utah. He is the recipient of the George Mercer Award from the Ecological Society of America and is a Regents' Professor at Northern Arizona University where he is the Executive Director of the Merriam-Powell Center for Environmental Research.

Humans are evolving rapidly and the rate is accelerating

Harpending HC

Department of Anthropology, University of Utah, Salt Lake City, UT

Many of us have harbored an implicit assumption that modern humans appeared about 50,000 years ago and have not changed much since. Recent evidence from large genome surveys shows that a substantial portion of our genome is undergoing strong selection and that the rate of change is increasing. We are not the same as our ancestors even one or several thousand years ago. Since the spread of agriculture at the end of the Pleistocene human population sizes have increased manyfold and the chance of any favorable mutation occurring is proportional to population size; hence the recent speedup is probably a consequence of the size of our species. Since the great diaspora of modern humans from Africa, large scale gene flow has been restricted among major human groups so new advantageous variants are geographically restricted. This means that continental populations are becoming more different from each other.

Biography of Henry C. Harpending, Ph.D.

Dr. Henry Harpending is a distinguished professor and the George Thomas presidential endowed chair of anthropology at the University of Utah, with current interests in pre-industrial populations, the history of modern humans, and the evolution of human social life. His most recent work includes genetic diversity within and between human populations where some genetic markers, such as mitochondrial DNA, microsatellites, and quantitative traits, evolve rapidly enough to have information about size and isolation of subpopulations in the recent past. This work has found evidence that our species had only a few thousand members during the last interglacial and that there were several subsequent demographic expansions, the earliest among the ancestors of contemporary sub-Saharan Africans. In addition, he has concentrated on family demographic histories with particular interests on the effects of infectious infertility on population structure, consequences of preferential treatment of children by sex for mortality and for growth and development, and the relationships among wealth, family organization, and individual reproductive outcomes. His current interests are the search for archaic human (e.g. Neanderthal) DNA in our species and the social consequences of human genetic diversity. Dr. Harpending is on the editorial boards of *Human Biology*, *The American Journal of Human Biology*, and *HOMO: Journal of Comparative Human Biology*. He is a faculty scholar medal awardee from Penn State University, a Fellow at the American Association for the Advancement of Science, and a member of the National Academy of Sciences. He has also been on the faculty of the University of New Mexico and Pennsylvania State University.

Molecular development of the external genitalia

Cohn MJ*

Department of Zoology and Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL

Genitourinary malformations are among the most common birth defects in humans. Development of external genitalia requires tight coordination of proximodistal outgrowth, three-dimensional patterning and tubular morphogenesis. Congenital malformations of the penis arise when these processes are disrupted. The most common penile anomaly is hypospadias, which is characterized by failure of urethral tube closure, and is often accompanied by abnormal dorsal-ventral patterning. Affected children can have mislocalized, multiple or oversized urethral openings, and males with severe hypospadias are born with ambiguous genitalia. In the United States, the frequency of hypospadias doubled, without explanation, from 1968 to 1993, and now affects 1:250 live births. Despite this, there is a relatively poor understanding of the cell types that give rise to, and the molecular mechanisms that control morphogenesis of, external genitalia. A major focus of our lab is the identification of the signals that control both outgrowth of the external genitalia and urethral tube formation. We have identified a new signaling region, the urethral epithelium, and found that its signaling activity is mediated by the Sonic hedgehog (Shh) protein. Shh has multiple roles in external genital development, and disruption of Shh function at different stages results in different classes of genital defect. In addition, our experiments are uncovering a complex gene network that functions to coordinate outgrowth and patterning of the external genitalia. The results have implications for our understanding of the developmental basis of hypospadias and will be pertinent to tissue engineering and regeneration of urologic organs.

Patterning a vertebrate embryo

Harfe BD*

Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL

How an individual cell decides what type of cell it will become has been a fundamental problem in biology for decades. The improper specification of just a single cell can have catastrophic consequences for the developing embryo. One of the goals of my laboratory's research is to elucidate how digits form in the developing limb using both the mouse and chick model systems. Two classes of molecules, the bone morphogenetic proteins (Bmps) and Sonic Hedgehog (Shh), are thought to play a role in this process. Using limb-specific mouse knockouts of these molecules we have uncovered novel roles for these proteins in limb development. My laboratory is also interested in the role microRNAs play in development. Using a conditional allele of Dicer we constructed, we removed Dicer in a tissue-specific manner during mouse development. In these animals, numerous defects were observed demonstrating the important role microRNAs play in vertebrate development. The laboratory has also begun to examine the molecular pathways responsible for intervertebral disk development using the mouse model system.

* = UF Genetics Institute faculty

Evolution of gene expression in flowering plants

Kirst M*

School of Forest Resources and Conservation, University of Florida, Gainesville, FL

The sequencing of the *Populus trichocarpa* genome creates the first opportunity to describe the complete transcriptome of a woody perennial species and compare it to the herbaceous model plant *Arabidopsis thaliana*. We characterized gene expression in a broad range of organs from the *P. trichocarpa* sequenced genotype Nisqually-1 and identified extensive transcription diversification in paralogues, suggesting neofunctionalization, particularly in woody organs. A comparison to *A. thaliana* revealed very limited conservation in the expression pattern of orthologs, indicating rapid evolution of gene function measured by gene expression variation. However, the expression of a small set of orthologs has remained surprisingly conserved in the two species, despite separation of lineages for over 100 million years. These include genes implicated in essential plant mechanisms such as photosynthesis, as well as genes expressed in a broad range of plant organs in both species. For these genes there appears to be strong selection against diversification in transcription regulation.

RAPs: Novel regulators of actin organization in land plants

Oppenheimer DG*

Department of Botany and the Graduate Program in Plant Molecular and Cellular Biology, University of Florida, Gainesville, FL

Control of actin dynamics underlies many processes basic to life including cellular movement and membrane trafficking. In plants, the actin cytoskeleton plays an important role in controlling the direction of cell growth in expanding cells. To further understand how regulation of actin dynamics controls plant cell expansion, we screened for mutants that affected the expansion of epidermal hairs (trichomes) on *Arabidopsis* plants, and examined these mutants for changes in actin organization by immunofluorescence and confocal microscopy. One of these mutants, *irregular trichome branch 3 (itb3)*, showed dramatic changes in actin organization during trichome development including an overabundance of actin cables and the formation of actin rings. Cloning of the ITB3 gene revealed that it encoded a novel plant-specific protein of unknown function that is conserved in land plants but not found in algae, fungi or animals. To identify proteins that interact with ITB3, we conducted a yeast two-hybrid screen using ITB3 as bait. We identified a plant homolog of actin depolymerizing factor/cofilin (ADF) as a potential binding partner of ITB3. The interaction of ITB3 with ADF was confirmed in vitro. We named this family of proteins RAPs for Regulators of ADF in Plants. RAPs comprise a large gene family in plants; *Arabidopsis* has 22 genes encoding RAPs, and rice has 33 RAP family members. ADF genes are also found in multiple copies in plant genomes with 12 genes present in *Arabidopsis*. We examined insertional mutation resources of *Arabidopsis* for mutants representing other members of the RAP and ADF families. Trichome phenotypes of these mutants suggest that specific RAPs interact with specific ADF family members to regulate particular aspects of trichome cell morphogenesis. This work was supported by a grant from NSF (IOB 0352916)

Reconstructing human migrations: Projects from the Americas and from Africa

Mulligan CA*

Department of Anthropology, University of Florida, Gainesville, FL

Anatomically modern humans have colonized all corners of the globe. These colonization events represent a wide range of processes from relatively recent and simple, such as the single expansion into the Americas ~15,000 years ago, to ancient and highly complex, such as the initial migration out of Africa ~50,000-100,000 years ago followed by multiple back-migrations and expansions throughout the Horn of Africa and Arabia. Our research on the Americas suggests there was a long period (~20,000 years) of population equilibrium and genetic diversification in greater Beringia prior to a rapid expansion into the Americas. On the other side of the planet, our research on Middle Eastern populations reveals that Bedouin, Palestinian, and Yemeni groups are genetically intermediate between African and other Old World populations. However, preliminary results suggest that these populations may not be equivalently intermediate, thus we are performing simulation analyses to distinguish among ancient isolation by distance, pre-historic admixture, and recent admixture. Investigation of two divergent colonization scenarios informs our understanding of migrations in the study areas as well as migration processes in general.

Genetical genomics and the X chromosome

Wayne M*

Department of Zoology, University of Florida, Gainesville, FL

The maintenance of genetic variation remains a challenge throughout evolutionary genetics, but especially for the genetic variation of gene expression. Ironically, the complication likely stems from the fact that we can actually describe the mechanism of phenotypic variation at the molecular level for gene expression, in contrast to higher order phenotypic traits. Variation in gene expression must result from *cis*, *trans*, and *cis x trans* effects; and each of these parameters may have its own genetic architecture, for each gene. The mechanisms and inheritance of sex differences in gene expression are of particular interest, because sexual dimorphism arises in large part from sex-specific transcription, given that females and males share all genes except those on the Y chromosome. However, in flies and humans, males are hemizygous for the X, while females are not. This constrains the mode of inheritance for gene expression both for X-linked genes, and for autosomal genes with major X-linked transcription factors. The evolutionary implications of sex-specific mode of inheritance for expression include a molecular mechanism for frequent observations of faster evolution in males.

Posters

* = UF Genetics Institute faculty

1. Phylogeny and diversification of rosids inferred from the plastid inverted repeat

Alexandre R², Brockington SF¹, Moore MJ¹, Soltis PS^{2,*}, Soltis DS^{1,*}

¹Department of Botany, University of Florida, Gainesville, FL

²Florida Museum of Natural History, University of Florida, Gainesville, FL

Previous molecular phylogenetic analyses of the angiosperm clade, the rosids, employing approximately ~ 5000 base pairs, recovered a phylogeny in which many of the deeper level relationships were poorly supported. To clarify and provide support for interfamilial relationships within the rosids, we amplified and sequenced the entire chloroplast inverted repeat (~26,000 bp) for 32 taxa. The inverted repeat was sequenced using the PCR-based method ASAP. This was combined with a dataset from published chloroplast sequences to create a total dataset of 60 taxa. Various phylogenetic methods were used to analyze the data matrix including Parsimony and Maximum Likelihood. These analyses revealed that Vitaceae are sister to all other rosids; the remainder form two large clades. Many poorly supported lineages received increased support as indicated by higher bootstrap values. The slower evolving plastid genes associated with the inverted repeat of the chloroplast region proved useful in providing support and resolution at deeper levels within this clade.

2. Emerging trends in the evolution of primary endosymbiotic bacteria

Allen JA^{1,2}, Light JE², Perotti MA³, Braig HR³, Reed DL²

¹Department of Zoology, University of Florida, Gainesville, FL

²Florida Museum of Natural History, University of Florida, Gainesville, FL

³School of Biological Sciences, University of Wales, Bangor

Primary endosymbiotic bacteria are thought to have increased the diversity of insects by supplementing their diet and enabling them to radiate into nutrient poor niches. Primary endosymbionts (p-endosymbionts) live their entire life cycle within the insect and are transmitted to the next generation of hosts through the maternal lineage. P-endosymbionts also evolve faster (i.e., accumulate nucleotide substitutions faster) than their insect hosts and other closely related but free-living bacteria. This faster rate has traditionally been attributed to small population sizes, lack of recombination, genetic drift, and, more recently, reduced efficacy of selection. Based on original studies of the aphid p-endosymbiont (*Buchnera*), it has traditionally been thought that 16S rRNA of p-endosymbionts evolves at a rate of roughly 1-2% per 50 million years. More recently, other host/endosymbiont assemblages have exhibited rates of evolution that greatly exceed this range, such as *Blochmannia* (ant endosymbiont), which is evolving at a rate 14 times faster than previously examined p-endosymbionts. We have sequenced the 16S rRNA gene from *Riesia*, the p-endosymbiont in primate sucking lice, and found that it is evolving over 30 times faster than the traditional rate. When comparing rates of evolution among all characterized insect p-endosymbionts (including endosymbionts from aphids, cockroaches, tsetse flies, whiteflies, psyllids, ants and lice), we find that the younger assemblages are evolving at a much faster rate than the host/endosymbiont assemblages that have older, longer term, associations. These results suggest an initial rate increase at the beginning of p-endosymbiont symbiosis with its insect host and a stabilization of the rate as the association ages.

3. Proteomics of redox regulated proteins in methyl jasmonate treated plants

Alvarez S, Wilson G, Zhu M, Chen S*

Department of Botany, University of Florida, Gainesville, FL

Methyl jasmonate (MeJA) is a signaling molecule that plays a key role in the regulation of metabolic processes, reproduction and defense against insects and pathogens. It has been shown that MeJA induces accumulation of reactive oxygen species (ROS) by regulating the activities of antioxidant enzymes. ROS such as hydrogen peroxide may act through redox regulated proteins to activate or inactivate different signaling pathways as well as transcription factors, which in turn regulate gene expression and cell-cycle processes. In this study, we aim to investigate the redox regulated proteins in methyl jasmonate treated plants using proteomic approaches. The proteins identified act downstream of MeJA and are possibly involved in plant defense processes. Here we report a proteomic approach used to identify redox regulated proteins in *Arabidopsis* leaves in response to a 24h treatment with MeJA. Several proteins involved in ROS detoxification were identified. The implications of the changes of these proteins were discussed. In addition, we were able to map the disulfide bonds in several proteins using the monobromobimane labeling technology.

4. Is collagen present in *Limulus polyphemus*?

Arce TR¹, Zhang GJ¹ and Cohn MJ^{1,2,*}

¹Department of Zoology, University of Florida, Gainesville, FL

²Department of Anatomy and Cell Biology, University of Florida, Gainesville, FL

In 2006, Zhang and Cohn found type II collagen in lamprey. Their follow-up finding of type II collagen in hagfish, and amphiColA in lancelets suggested that the duplication and divergence of clade A collagen led to the origin of vertebrate chondrocytes, and thus a means for cartilage development in these stem vertebrates. The main focus, thus far, has been on collagenous cartilage in vertebrate lineages. Cartilage, or cartilage-like tissue, has been found in many invertebrate phyla as well, including Cnidaria, Annelida, Inarticulata, Mollusca, and Arthropoda. An arthropod, *Limulus polyphemus*, has been reported to have non-collagenous based cartilage. In this study we attempt to determine the presence of clade A fibrillar collagen and SoxE genes in the horseshoe crab (*Limulus polyphemus*). We performed Mason Trichrome staining, proteomic analysis of protein extract to screen for collagen, and initial immunohistochemical experiments on limulus. Our initial results have shown what appear to be chondrocytes in limulus gill tissue. Proteomic analysis, as well as preliminary immunohistochemical study, has not confirmed the presence of collagen in limulus tissue extract. In an attempt to identify additional non-vertebrate organisms that possess fibrillar collagen we utilized Mason Trichrome tissue analysis on the channeled apple snail (*Pomacea paludosa*). We will present our results and future studies that will be needed to further explore the presence of collagen in invertebrates.

5. Conservation genetics of the endangered Okaloosa darter

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Molecular genetics play an important role in the management of threatened and endangered species. The endangered Okaloosa darter (*Etheostoma okaloosae*) occurs in only six streams that drain into two Choctawhatchee Bay bayous in northwest Florida. The Okaloosa darter was listed in 1973 due to its limited distribution, habitat degradation and loss, and concerns over competition with the common and widespread brown darter (*E. edwin*). Our research incorporates mitochondrial and nuclear markers to examine demographic parameters and historic versus contemporary connectivity among creek and drainage populations. We present initial mtDNA results (complete cytochrome b gene sequences) focusing on 1) the population history of brown darters within the Okaloosa darter range and 2) the population structure and level of isolation among the six Okaloosa darter creeks. Preliminary results based on mtDNA sequence data incorporating coalescent and phylogenetic approaches, suggests that significant population structure exists among streams. Further, brown darters found in the lower reaches of the Okaloosa native streams are likely not introduced as was assumed when the darter was first listed.

6. An epistatic model for mapping phenotypic plasticity of a count trait

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Different expression of a given genotype in morphology, physiology, and anatomy over changing environments is called phenotypic plasticity. The understanding of the genetic basis of phenotypic plasticity has been one of the most important challenges facing modern biology. The formation of phenotypic plasticity may be due to higher homozygosity at certain genes (homeostasis hypothesis), environment-dependent expression of the loci (allelic sensitivity hypothesis), or epistatic interactions between plasticity genes and trait genes (gene regulation hypothesis). Testing these three hypotheses requires different genetic designs and statistical models. In this study, we develop a statistical model for detecting specific quantitative trait loci (QTLs) that stimulate the phenotypic plasticity of a count trait through genetic regulations. The model was derived with a bivariate Poisson distribution with one variable for phenotypic means over different environments and the other for phenotypic differences between the same pair of environments. A multi-QTL model was implemented into a general mixture model framework, allowing the tests of how the same QTL affects the means and differences and whether the epistasis between different QTLs contribute to phenotypic plasticity. A real example for the number of sylleptic branches on the main stems of poplar hybrids was used to elucidate the interpretation and utility of our model. The new model will provide a useful tool for dictating the picture of genome by environment interactions that determine the final phenotypes of complex count traits.

7. Evolution and diversity of invertase genes in *Populus trichocarpa*

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Invertase plays a key role in carbon utilization as it catalyzes the irreversible hydrolysis of sucrose into glucose and fructose. The invertase family in plants is composed of two sub-families thought to have distinct evolutionary origins. These sub-families are distinguished by their pH optima for activity: acid invertases apparently originated in eubacteria and are targeted to the cell wall and vacuole, while neutral/alkaline invertases apparently originated in cyanobacteria and function in the cytosol. The recently sequenced genome of *Populus trichocarpa* allows us to identify the genes encoding invertase in an economically important plant system, in order to understand both the evolutionary development of this family as well as its role in carbon allocation and partitioning in forest trees. Here we describe the identification of eight acid invertase genes; three of which belong to the vacuolar targeted group (PtVIN1-3), and five of which belong to the cell wall targeted group (PtCIN1-5). Similarly, we report the identification of 16 neutral/alkaline invertase genes (PtNIN1-16). An examination of the micro-syntenic regions surrounding the poplar invertase genes reveals extensive colinearity with *Arabidopsis* invertases. Expression analysis using both whole genome microarrays as well as quantitative RT-PCR reveals a general conservation of expression profiles of genes encoding acid and neutral invertase in response to auxin, nitrogen, and wounding treatments, and during development. We also find evidence for expression of a novel intronless vacuolar invertase (PtVIN1), which apparently arose through reverse transcription of a processed PtVIN2 transcript that subsequently inserted in the genome. To our knowledge this is the first intronless invertase found in plants.

8. Sonic Hedgehog signaling in the apical ectodermal ridge is essential for proper formation of the vertebrate limb

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The vertebrate limb develops along three axes: proximo-distal, dorso-ventral and antero-posterior. Proximo-distal patterning is regulated by the apical ectodermal ridge (AER); antero-posterior patterning is controlled by the zone of polarizing activity (ZPA); and the dorso-ventral axis is determined by signals from the non-AER ectoderm. Sonic Hedgehog (SHH), a protein secreted from the ZPA, has been shown to be both necessary and sufficient for ZPA function. Through the analysis of Shh target genes, for example *Ptc1* and *Gli1*, it has become clear that the Shh signaling cascade is active in the limb mesoderm. Recently, array-based experiments have found that a number of target genes of the Shh signaling pathway were also present in the AER. For Shh signaling to occur in the limb AER, SHH protein must also be detectable in this region. Immunohistochemistry using an anti-SHH antibody revealed the presence of SHH protein in the limb bud ectoderm. In addition, we have found that exceptionally low levels of at least one Shh target gene can be detected in the AER by methods not based on array technology. The potential role Shh signaling may play in the limb AER is unknown. To determine if Shh signaling within the AER plays a role in limb patterning, we used a conditional knockout approach to remove Shh signaling specifically from this region of the limb. Removal of Shh signaling from the AER resulted in the production of an extra digit, indicating that Shh signaling within the AER is required for proper formation of the vertebrate limb. We are currently performing additional transgenic manipulations and using whole mount *in situ* hybridizations to elucidate the function of Shh signaling in the AER.

9. Comparison of the chondrogenic potential of BMP-4, BMP-7, IHH, Sox-9, and CTGF when delivered to mesenchymal stem cells as transgenes

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Articular cartilage is a highly specialized tissue that protects the bones of diarthrodial joints from forces associated with load bearing, friction, and impact; however, there is limited capacity for regeneration in response to injury or disease. Adult mesenchymal progenitor cells (mesenchymal stem cells; MSCs) are readily available from many tissue sources and are capable of differentiating along multiple lineages; thus, they are well-suited for cell-based therapies for cartilage repair. Gene transfer is a viable method of achieving sustained, local expression of specific protein factors, and it may be useful for inducing chondrogenic differentiation of MSCs *in vivo*. Potent chondroinduction of MSCs in high density aggregate culture was previously observed following delivery of cDNAs for TGF- β 1 and BMP-2. In this study, we have expanded these analyses to include cDNAs whose protein products are associated with chondrogenic differentiation during development, which include BMP-4, BMP-7, Indian hedgehog (Ihh), Sox-9, and connective tissue growth factor (CTGF). Using a high-density aggregate culture system, we performed a series of assays to examine the capacity of these candidate transgenes to induce chondrogenesis of bovine MSCs. We infected individual flasks of early passage bovine MSCs with adenoviral vectors carrying the cDNAs for each transgene at doses ranging from 100 vp/cell to 10,000 vp/cell. At 24 hours post-infection, 2.0×10^5 cells were pelleted by centrifugation, and the resulting cell aggregates were cultured for 21 days in serum-free chondrogenic medium. Chondrogenesis was evaluated by examining aggregate morphology, histological staining for proteoglycans and by immunohistochemical analysis for collagen types I and II. The greatest biological responses for each transgene were observed in the dose range of 100-1000 vp/cell; higher viral doses appeared to inhibit chondrogenesis. Robust chondrogenesis was observed following delivery of BMP-4, BMP-7, Ihh, and Sox-9; however, qualitative differences were evident among the treatment groups.

10. Spatial and temporal patterns in the floral transcriptome of the basal angiosperm, *Persea americana* (avocado): an exploration of ancient whorls

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The first transcriptional profiling of flowers in a basal angiosperm, *Persea americana* (avocado), has revealed a number of potentially fundamental elements as well as the novelties we expect of separate evolutionary "experiments" in flower development. Using custom microarrays we have identified 1081 genes with at least two-fold up-regulation in particular floral organs and/or developmental stages relative to leaves. Hierarchical clustering suggests that these assemble into four regulatory units that accommodate homologs of AG, AP3 and PI, AGL6, and AP1, respectively. Members of the AG cluster are expressed predominantly in stamens and carpels, often also in fruit, conceivably contributing to C-function in an evolutionarily conserved reproductive developmental program. B-function may be provided by members of the AP3/PI cluster, most of which are expressed primarily in stamens, many also in the perianth, or in all the floral organs. Also present are homologs of components of the SCF^{UFO} complex, suggesting conservation in the promotion of AP3 and PI expression across angiosperms. The genetic regulators of perianth development may be found among the members of the AGL6 cluster. The transcriptional profiles of the two, morphologically alike, tepal whorls are nearly identical, and correlate strongly with the stamen transcriptome, conceivably, perhaps, harboring regulatory networks inherited from a stamen developmental program. If so, *Persea* tepals provide an unprecedented opportunity to examine the evolutionary genetics of andropetaloidy; perianth evolution through stamen sterilization. All *Persea* genes expressed primarily in inflorescence bud were assigned to the AP1 cluster, indicating potential evolutionary conservation in role of AP1 in floral meristem specification, but not A-function. Comparisons with floral microarray data for *Arabidopsis* found few instances of potential evolutionary conservation in the downstream regulation of floral organ development, mostly relating to stamen and carpel developmental genetics, but in *Persea*, expression often extends to the tepals.

11. Genetic analysis of BMP2 and BMP4 in the limb apical ectoderm ridge

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During early limb development the apical ectodermal ridge (AER) expresses a number of signaling proteins including bone morphogenetic proteins (BMPs) 2, 4 and 7. BMPs in the limb are expressed in both the mesoderm and the ectoderm and have been proposed to play a role in anteroposterior digit patterning. To date, it has not been possible to directly examine the role Bmp ligands play in limb development since mice deficient in BMP2 or BMP4 die before digit condensation occurs. Previous studies have circumvented this problem by conditionally removing a BMP receptor (BMPR-IA) in the limb mesoderm or ectoderm or expressing a BMP antagonist (noggin) in the AER. The functions of each individual BMP remains unknown because each of these previous studies blocked all BMP signaling. Our studies take advantage of an *Msx2-cre* ectoderm-specific transgene to delete *Bmp2* and 4 in the AER. Double knockout of *Bmp2* and *Bmp4* in the ectoderm results in limb polydactyly, syndactyly and interdigital webbing due to decreased programmed cell death (PCD) and to increased cell proliferation. Using RNA *in situ* hybridization we showed that the expression domains of *Fgf4* and *Fgf8* in the AER were expanded spatially and temporally in double mutants. Further RNA *in situ* analysis showed the expression domains of 5' Hox genes were expanded anteriorly in the limb mesenchyme. *Wnt7a* and *Lmx1b* expression were normal in dorsal ectoderm and mesoderm, respectively. However, *Engrailed 1* (*En1*) expression was absent in the ventral half of the AER although there was *En1* expression in ventral ectoderm. Thus, *En1* expression in the AER is not responsible for dorsoventral patterning. These results demonstrate that ectodermal BMP2 and 4 are partially functionally redundant in the limb ectoderm, function in the cell autonomously, and play an important role in interdigital PCD and anteroposterior patterning of the developing limb.

12. Are you taking full advantage of services offered by the GAL?

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The ICBR Genetic Analysis Laboratory (GAL) is known for its ability to do high quality DNA extractions and PCR reactions. DNA is extracted from many different sources (blood, tissue, bones, teeth, etc.) and amplified using PCR for sequence analyses of mitochondrial and nuclear DNA and for fragment analyses. The lab designs new and optimizes or adapts published protocols to meet our client's needs. A very powerful tool that we offer to our clients is a microsatellite library enriched for di-, tri-, and tetranucleotide repeats for their organism of interest. Microsatellites are a class of DNA that consists of repeated DNA, i.e., CACAn or AGTAGTn. Since these repeats are biparentally inherited, they can be used to determine gene flow, fine scale population structure, individual identity, and have been associated with some diseases such as Huntington's disease. A microsatellite library incorporates a series of molecular techniques that must all be successfully completed to find loci that can be shown to be polymorphic. The individual services offered at the GAL, along with enriched microsatellite library production, include DNA isolations, PCR reactions (small and large scale), PCR cleanups for cycle sequencing, and fragment analysis of microsatellites, T-cell response, AFLPs, etc. In this poster, we will describe some of the more common services offered to clients and illustrate their value by highlighting their use in some projects.

13. Expression of Sle1a is required for observed T cell-intrinsic phenotypes

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Patients suffering from Systemic Lupus Erythematosus present with several manifestations, one of which being a decrease in the number of circulating regulatory T cells (Tregs). However, it has been shown that the suppressive capacity of the remaining population is maintained. Loss of tolerance to nuclear antigens is associated with major lupus susceptibility locus Sle1 in the NZM2410 lupus model, comprising three independent loci (Sle1a, Sle1b, and Sle1c). Sle1-expressing T cells exhibit increased levels of activation associated with increased levels of proliferation and cytokine production. The Sle1 locus is associated with a decreased level of Tregs as well as decreased expression of the transcription factor Foxp3 among this population preceding autoantibody production. This phenotype is accounted for by Sle1a. However, it was necessary to assess whether expression of Sle1a in T cells is required to produce the observed phenotype. While expression of the Sle1a interval results in decreased Treg levels as well as Foxp3 expression among this population, with a truncated region of Sle1a (Sle1a(15)) presenting intermediate levels, these cells maintain their suppressive capacity and show no difference regarding expression of other markers associated with the regulatory phenotype as compared to B6. Flow cytometric data from bone-marrow mixed chimeras only show enhanced proliferation and activation of the Sle1a-expressing T cells, as well as a decrease in Treg levels of B6.Sle1a origin. These data indicate that expression of Sle1a results in T cell intrinsic activation. In addition, the observed decrease only in Tregs expressing Sle1a suggests that Sle1a regulates Treg levels in a T cell specific manner. By examining Sle1a(15), the observed intermediate phenotype suggests the presence of two genes within the Sle1a region contributing to the break in self-tolerance, and that this may be associated with decreased levels of Tregs.

14. A genetic model for predicting the incidence of cancer with age

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Cancer incidence increases with age. This epidemiological pattern of cancer incidence can be attributed to molecular and cellular processes for individual subjects. As an inherited disease, genes are thought to play a central role in shaping the incidence of cancer with age. In this article, we derived a dynamic statistical model for explaining the epidemiological pattern of cancer incidence based on individual genes that regulate cancer formation and progression. We incorporate the mathematical equations of age-specific cancer incidence into a framework for functional mapping aimed to identify QTLs for dynamic changes of a complex trait. The mathematical parameters that specify differences in the curve of cancer incidence among QTL genotypes are estimated within the context of maximum likelihood. We provide a series of testable quantitative hypotheses about the initiation and duration of genetic expression for QTLs involved in cancer progression. Computer simulation was used to examine the statistical behavior of the model. The new model proposed will provide a powerful tool to detect genes for cancer progression and a general framework for explaining the epidemiological pattern of cancer incidence.

15. Suppressor mutants of NPR1 restore salicylic acid tolerance and pathogen resistance in *Arabidopsis thaliana*

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In plants, pathogen recognition triggers activation of defenses, resulting in resistance. Signal transduction of the pathogen signal is essential for transcriptional reprogramming and defense activation. NPR1 is a key component in several of these signaling pathways, and is essential for pathogen resistance. The small molecule salicylic acid (SA) is another essential component of plant defense signaling, and activates NPR1 following pathogen recognition. NPR1 in turn controls SA synthesis and is essential for alleviating SA-related cytotoxic effects, though how NPR1 accomplishes this is unknown. In this study we took advantage of the toxic effects of SA on *npr1* seedlings and isolated several suppressors of this mutation. Suppressor mutants displayed several phenotypes including restoration of SA tolerance and pathogen resistance. Efforts to identify these mutations by map-based cloning are underway. Identification of new signaling components through this approach will enhance our understanding of plant defense activation, and contribute to reducing harmful effects of pathogens on important crops.

16. Whole genome sequence analysis of the bacterial endophyte, *Enterobacter cloacae* P101

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Enterobacter cloacae P101 is a bacterial endophyte. Endophytes, which are microorganisms that colonize plants, can provide beneficial effects to their hosts such as increased plant growth and mineral absorption. The P101 strain was first isolated from a biomass crop known as *Panicum virgatum* L, and plants inoculated with P101 demonstrate increased growth versus wild type plants. To date, no *Enterobacter* or endophyte genome has been published and unlocking the genetic information will lead to an increased understanding of how endophytes can provide benefits to their hosts. The *Enterobacter cloacae* P101 genomic DNA was isolated and sequenced with the Genome Sequencer 20 from 454 Life Sciences at the University of Florida Interdisciplinary Center for Biotechnology Research. Several sequencing runs generated 2 million individual sequence reads that total to 141 Mb of raw sequence data. Using the GS Paired End adaptors technology, 371,000 paired end reads were added to the sequence data to streamline genome assembly. The assembly software, Newbler, assembled the reads into 520 contigs. The paired end read data was used by Newbler to assemble the contigs into 37 scaffolds. The average scaffold size is 143 kb and the scaffolds total 5.3 Mbp in length. The P101 genome size is estimated to be 5.2- 5.4 Mbp in length. The assembled contigs and scaffolds will be verified with alternative genome assemblers: PCAP and SeqMan from DNASTAR. Genome closure is in progress, and a variety of methods including PCR and Sanger sequencing are being used to close the gaps between scaffolds. Initial annotation of scaffolds by FGENESB identified 5,856 open reading frames. The sequence of the P101 genome will provide a valuable resource for comparative genomics and gene organization as a model for other *Enterobacter* species.

17. Resolving gene families, nearly-identical paralogs, and novel transcripts using 454-based 3'UTR profiling

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Differences in gene expression underlie central questions in eukaryotic biology extending from gene function to evolutionary mechanisms and quantitative traits. However, resolving expression of closely-related genes (e.g. alleles and gene family members) is challenging on a genome-wide scale due to extensive sequence similarity and frequently incomplete genomic sequence data. We present a new expression-profiling strategy that utilizes long-read, high-throughput sequencing to capture the information-rich 3'-untranslated region (UTR) of mRNAs. Resulting sequences resolve gene-specific transcripts independent of a sequenced genome. Analysis of ~229,000 3'-anchored sequences from maize (*Zea mays* L) ovaries identified 14,822 unique transcripts represented by ≥ 2 sequence reads (consensus sequences). Total RNA from ovaries of drought-stressed wild-type and *viviparous-1* mutant plants was used to construct a multiplex cDNA library. Each sample was labeled by incorporating one of 16 unique three-base key codes into the 3'-cDNA fragments, and combined samples were sequenced using a GS 20 454 instrument. Transcript abundance was quantified by frequency of sequences identifying each unique mRNA. The 3'UTR profile resolved 12 unique transcripts representing nine *Cellulose Synthase (CesA)* gene family members in maize ovaries. In addition, this method distinguished nearly-identical paralogs, as illustrated by two *Auxin Repressed Dormancy Associated* transcripts, which showed reciprocal mRNA abundance in wild-type and mutant samples. We further evaluated 454-based 3'UTR profiling for global transcriptome analysis in parallel with a microarray approach. A multiplexed library of 12 samples representing four sequential stages of maize ovary development generated ~578,000 good-quality 3'-anchored sequences (including ~23,000 unique consensus sequences) using the FLX 454 technology. Resolution of nearly-identical paralogs, identification of 3'-RNA processing variants, and quantitative profiles of transcriptional changes over a dynamic range of expression provided an invaluable complement to microarray analyses. In addition, recovery of newly-encountered, non-arrayed genes enabled flexibility for gene discovery in a non-sequenced genome.

18. A putative role for RNA splicing in maize endosperm-embryo developmental interactions

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Endosperm-embryo interactions are an important but poorly understood aspect of seed development. The Rough endosperm3 (Rgh3) locus is involved in these interactions at a developmental level. Rgh3 seed mosaics marked with the pr1 anthocyanin gene indicate that Rgh3 is required in the endosperm for the normal development of the embryo. Rgh3 also has an autonomous function in the embryo and is required for seedling viability. We identified a tightly-linked transposon-tag from the rgh3-70 allele. This transposon insertion disrupts a predicted splicing factor with a U2 snRNP auxiliary factor homology motif (UHM). UHMs are RNA recognition motif (RRM)-like domains that function in protein-protein interactions. Analysis of Rgh3 cDNAs and genomic sequences suggest alternative spliced transcripts of Rgh3. Maize ESTs indicated that Rgh3 is expressed in endosperm tissue. Consistent with this expression pattern, the rgh3 phenotype is characterized by an overproliferation of the aleurone cells and aberrant development of the basal endosperm transfer cell layer. An analysis with endosperm cell type specific markers in mutant rgh3 seeds suggests that the basal endosperm transfer cell defect occurs after cell type specification. Rgh3 endosperm cultures grew forming homozygous mutant callus. This data further supports Rgh3 possible role in cellular differentiation. Rgh3 maps to the long arm of chromosome 5. Complementation tests with other seed mutants mapped to the same region indicated that Rgh3 is a novel locus. Our data suggest that RNA splicing may have an important role in endosperm-embryo developmental interactions.

19. Discovery, the structure of haplotype blocks, and cladistic analyses of polymorphisms of natriuretic peptide receptor A (NPR1) gene

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Genetic variation is increasingly recognized as an important contributor to complex diseases. The variation of NPR1 gene has been suggested to be a potential risk factor with hypertension and cardiac hypertrophy. Systematically characterizing the variation of NPR1 leads to localization of the susceptibility disease sites with two approaches. An approach focuses on one marker at a time to test potentially causal variants directly; another one types several markers to locate causal loci via association between loci. The second approach utilizes the property of linkage disequilibrium and the structure of haplotype blocks. Haplotypic methods play a powerful role at detecting casual variants, but the larger number of halotypes, the less power of locating the haplotypes having disease susceptibility sites. However, a cladistic approach can group haplotypes in nested clades, therefore to increase the power identifying clades containing potential candidate disease susceptibility sites, consequentially, to pinpoint the causal site in those clades. In our study, we identified and characterized 26 SNPs in the 5' UTR, exons, intron, and 3' UTR of NPR1 using DHPLC in combination with big-dye terminator chemistry-based direct sequencing from four populations including Caucasian, African American, Chinese and Native American. The results of functional analysis show that four non-synonymous SNPs and one synonymous SNP have medium to high risk levels of developing disease. Based on imperfect phylogeny, four blocks were identified in the combined population. The cladistic approach has shown the advantage to group the haplotypes into subgroups. The statistical significant analysis was also applied to test the significant difference of LD between populations. A lack of linkage disequilibrium might discourage us to apply haplotype analysis in association study. However, our study may present a novel way to detect the causal variants.

20. Ovariole number and biochemistry in *Drosophila melanogaster*

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An important goal in evolutionary genetics is to determine how genetic variation is maintained in natural populations given that selection works to counteract it. It is known that ovariole number in *Drosophila melanogaster* is positively correlated with fecundity and is under strong selection. Therefore, we would expect little variation for ovariole number; however, this is not the case. In addition, the progeny of starved females have more ovarioles than the progeny of unstarved females but decreased early fecundity, possibly promoting more efficient dispersal for longer periods. Moreover, differences between sexes within genotypes for flight ability exist, such that genotypes with the most efficient male flight have the least efficient female flight. There may be a tradeoff between flight ability and investment in fecundity within females because more fecund females are less streamlined. Thus, energy-rich genotypes might produce fast flying males, but slow flying and fecund females. One objective of this experiment is to quantify the amounts of glucose/glycogen, triacylglycerides, and protein in *D. melanogaster* with the purpose of examining the tradeoffs in energy between female flies of differing genotypes for ovariole number and differences in energy content between sexes for each genotype. Another objective is to quantify differences in energy content for each biological macromolecule between starved and unstarved *D. melanogaster* females and males with the purpose of investigating trade-offs between investments in progeny and dispersal in the environment.

21. UF Genetics.com: Enhancing the public understanding of genetics

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Currently, much of the public receives their information about genetics from the news media. Unfortunately, newspapers and television reports tend to simplify scientific research and may omit important information. Researchers have cautioned journalists and other science educators to be mindful of the interpretation of genetics they offer both students and the general public. Accurate science education focusing on genetics can help the next generation become more informed citizens. To address this concern, the Scientific Thinking and Educational Partnership (STEP) program at the University of Florida developed the ufGENETICS.com Web site to introduce educators, students and media professionals to genetics research conducted at UF. The goal of the ufGENETICS.com project is to offer information to the public about genetics research that is accurate and relevant to everyday life. The Web site contains monthly genetic features on a variety of topics including biotechnology, gene therapy, conservation ecology, and genetic markers. The Web site is geared towards educators and media professionals, groups that currently inform students and the public about science. The materials were developed with an entertainment-education approach that involves a strong interest approach with an educational message. Each monthly feature contains the following components:

- Series of three to five videos that run approximately three minutes each.
- News feature story and sidebar story highlighting a particular aspect related to genetics, with accompanying high-resolution photos.
- Lesson plans that integrate the videos, news feature stories, and other resources into high school science curricula.
- Downloadable print materials for use in classrooms.

To date faculty featured in the videos and accompanying materials represent the following departments: pharmacy, veterinary medicine, microbiology, agronomy, and entomology.

22. Comparative phylogeography and population genetics of Lake Wales Ridge, FL, endemics

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The Lake Wales Ridge of Florida is one of the oldest ecosystems in North America and only 14% of it remains today. The age and uniqueness of the ecosystem has resulted in many endemic species of both plants and animals, most of which are threatened or endangered. I propose a comparative phylogeographic analysis of both plant and animal taxa endemic to the ridge comparing origin (based on the range of the closest relative) and age of divergence. Common patterns can shed light on the origins of the high endemism on the ridge as well as mechanisms of speciation. Additionally, focusing on threatened plants, I will use nuclear loci to look at their genetic diversity in terms of global diversity and geographical hotspots in order to better understand and conserve this system. This population genetic study will also enable me to try and relate genetic diversity to other factors such as nearest congener, time since speciation or extent of past bottlenecks. This will advance our understanding of genetic diversity, its origin and true meaning for conservation purposes beyond the species of focus or even this system.

23. Hydrilla genetic diversity survey in Florida

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Hydrilla verticillata is a serious aquatic invasive plant in Florida. This weed is found throughout Florida in freshwaters mainly in the southern and central regions. It is also found in all of the gulf states including Georgia and from Maryland to California. Hydrilla is capable of out-competing native aquatic vegetation. It grows very rapidly asexually from rootstocks, subterranean turions, vegetative buds (turions), and vegetative nodes. Dense surface mats associated with hydrilla infestations cause problems because they hinder navigation and flood control, interfere with recreational activities, and reduce the biodiversity in aquatic ecosystems. According to the Florida Department of Environmental Protection, between 1980 and 2004, approximately \$158 million in government funds was spent managing hydrilla in Florida public waters with non-biological control methods. With the recent discovery of herbicide resistance in hydrilla, there is renewed interest in biological control. Scientists at the University of Florida are undertaking an international effort to search for hydrilla biological control agents. The native range of hydrilla includes large parts of Asia, northern Australia and a few lakes in east/central Africa. Historical evidence suggests that hydrilla arrived in Florida through the aquarium plant trade in the 1950s, and probably came from Sri Lanka. The University of Florida Research & Extension, in collaboration with the University of Miami, is conducting a study of the population genetics of hydrilla, which is believed will help to better determine the origin of Florida hydrilla. Identification of the native origin of Florida hydrilla will be useful for targeting surveys for biological control agents. The purpose of this presentation is to discuss the Extension Agent's role in this genetic survey. The presenter will also describe sampling methodology and the use of genetic sampling in the global search for viable hydrilla biological controls. Preliminary results will be shared.

24. Deletions within the HSV-1 LAT promoter or enhancer that impair reactivation result in increased histone H3 K4 dimethylation

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During latency the HSV-1 lytic genes are shut down and only the latency-associated transcript (LAT) region is abundantly transcribed. Both the LAT promoter and the LAT 5' exon/enhancer are hyperacetylated relative to lytic genes at histone H3 lysines 9 and 14 (K9, K14) when examined in latently-infected murine dorsal root ganglia (DRG). This type of histone modification is generally associated with transcriptionally permissive chromatin, while hypoacetylation is observed in less transcriptionally permissive regions. Furthermore, the LAT region displays deacetylation of the LAT enhancer and a decrease in LAT abundance preceding an increase in ICPO acetylation during early explant, a model of molecular reactivation. It has previously been shown that the latent genomes of LAT promoter mutants are altered in histone modifications during latency in the mouse (Wang *et al.*, *PNAS* 102:16055, 2005). To examine whether differences in histone modifications in LAT mutants are responsible for their decreased ability to reactivate, we assayed the chromatin profiles of two reactivation-negative mutants, 17ΔPst and 17ΔSty, during latency and reactivation. 17ΔPst contains a 202 bp deletion of the core LAT promoter and does not reactivate. 17ΔSty contains a 371 bp deletion in the LAT 5' exon, and while able to transcribe LAT, does not reactivate in the rabbit. Trigeminal ganglia (TG) from rabbits latently-infected with HSV-1 strain 17syn+, 17ΔPst, or 17ΔSty were assessed for dimethylated H3 K4, a marker of transcriptional permissiveness. Interestingly, 17ΔPst demonstrated a dramatic increase in enrichment of dimethyl H3 K4 over wild-type upstream of the deletion. In 17ΔSty the LAT promoter region and the 5' exon were also more enriched in H3 K4 dimethylation than wild-type. These increases in H3 K4 methylation suggest that deletion of either the LAT promoter or the 5' exon causes a loss of a repressor or other chromatin regulatory element. Contrary to observations made following explant of murine

DRG, following epinephrine induction of rabbit eyes, there was no remodeling of histone modifications associated with the LAT or ICP0 promoters on the latent HSV-1 genomes within the rabbit TG within four hours post-induction. Unlike the changes in the chromatin induced by explant of murine DRG, epinephrine induction of latently-infected rabbits did not display chromatin-level changes of the LAT or ICP0 promoters.

25. Longitudinal evolution of HIV-1 in breast milk and plasma

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International guidelines currently advise HIV-1 positive women in resource-poor settings to exclusively breastfeed their infants for several months followed by an abrupt weaning to minimize overall infant mortality, despite the risk of transmitting the virus. These recommendations are based on the results of observational studies, although the benefits of abrupt weaning remain inconclusive. However, no studies have been published to date describing the molecular evolution of the virus in breast milk. We analyzed HIV-1 gp120 sequences from plasma and breast milk sampled over a two-year period from a Zambian individual infected with a subtype C virus. Numerous recombinant sequences were identified with breakpoints primarily localized within the C2 region of the env gene. The majority of the recombinant sequences were found in the breast milk. An alignment of non-recombinant sequences spanning the 3' end of the env region (C2-V5 domains) was analyzed using Bayesian and maximum likelihood phylogenetic methods. At the initial time point corresponding with delivery, three well-supported viral sub-populations (virodemes) were present in the breast milk distinct from a fourth virodeme in the plasma. By the fourth month after delivery, breast milk and plasma viruses appeared as a single panmictic population. Finally, by the twelfth to the twenty-fourth month after delivery, viral sequences from plasma and from breast milk separated again into two well-supported monophyletic clades. Furthermore, the later virus in the breast milk apparently shared a most recent common ancestor with the viruses circulating in both tissues four months after delivery. The loss of HIV-1 compartmentalization between breast milk and plasma four months after delivery may be the result of intense viral gene flow between the two tissues. In contrast the re-emergence of distinct virodemes by month twelve suggests a reduced flow with consequent genetic drift during the time interval separating the samples. Understanding the complex population dynamic underlying HIV evolution within these tissues may be essential to uncover the molecular mechanisms responsible for the differential risk of transmission associated with duration and exclusivity of breastfeeding.

26. FoxA1 and FoxA2 are required during septation and development of the anogenital system

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Congenital malformations of the anogenital system occur at a high frequency among newborn humans. Despite clinical descriptions of these birth defects, the basic developmental genetics underlying such malformations remains poorly understood. FoxA genes are transcription factors that play a critical role in the establishment of the definitive endoderm, and are required during development of several gut-derived structures such as the liver and the prostate. Embryos null for FoxA1 die two weeks after birth and embryos lacking FoxA2 die between embryonic days 10-11, with severe defects in all three germ layers, including the absence of a notochord and failure to form a gut tube. Given their importance during gut development, we examined the role of FoxA genes during development of the anogenital system. In order to circumvent the early lethality of FoxA2 mice, we utilized a tamoxifen-inducible cre driven by Shh (ShhCreERT2) to delete a conditional FoxA2 allele on various FoxA1 null backgrounds. Here we show that loss of both FoxA1 and FoxA2 results in persistent cloaca. Additionally, we find that both alleles of FoxA1 are necessary to compensate for a loss of FoxA2. FoxA1 and FoxA2 are co-expressed with Shh throughout the gut endoderm. As FoxA1 and FoxA2 have been shown to be both regulators of and regulated by Shh, we further investigated whether loss of FoxA1 and FoxA2 results in a loss of Shh signaling.

27. Differential expression of A, B, and C chains of C1q in external genital and limb development of the mouse

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Apoptosis plays a key role in external genitalia and limb development. C1q is a target recognition protein of a classical complement pathway crucial for the clearance of pathogens and apoptotic cells. It is involved in a number of immunological processes such as phagocytosis of bacteria, neutralization of retroviruses, cell adhesion, maintenance of immune tolerance via clearance of apoptotic cells and modulation of dendritic cells, B cells and fibroblasts. C1q has a hexameric structure containing an N-terminal collagen-like region and a C-terminal globular (gC1q) domain. The gC1q domain, which is the main ligand recognition domain of C1q, has a heterotrimeric structure composed of the C-terminal halves of the A, B and C chains. Little is known about the expression or function of C1q genes during embryonic development. We have examined the expression patterns of the A, B and C chains of C1q at different stages of mouse development using *in situ* hybridization. Tissue specific patterns of expression were found in both the external genitalia and the developing limbs. These patterns are similar to those of macrophage expressed gene 1 (Mpeg1). Results suggest that C1q genes function to sculpt the genitalia and the digits during embryonic development.

28. Functional characterization of 3-isopropylmalate dehydrogenase in *Arabidopsis*

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Glucosinolates are a class of plant secondary metabolites that function in the defense against herbivores and microorganisms. Upon tissue damage, glucosinolates are hydrolyzed by endogenous thioglucosidases called myrosinases to produce several biologically active compounds, typically isothiocyanates, thiocyanates and nitriles. Depending on their precursor amino acids, glucosinolate can be grouped into aliphatic, aromatic and indole glucosinolate, which derived from methionine, tryptophan and phenylalanine, respectively. Methionine-derived aliphatic glucosinolates are produced through a three-step chain-elongation cycle, which can add a single methylene group each time, leading to different side-chain structures. The enzyme involved in the formation of 3-malate derivatives has not been characterized. Here we use bioinformatics, biochemistry and reverse genetics to characterize the enzyme in *Arabidopsis*. We identified a candidate 3-isopropylmalate dehydrogenase, which probably functions as a heterodimer with another protein. They catalyze the isomerization and decarboxylation reactions to produce 3-malate derivatives. This was supported by analysis of glucosinolate profiles in a knockout mutant of the enzyme. Compared to wild type controls, all the long-chain aliphatic glucosinolates were decreased in the mutant. Future work includes studying the tissue-specific expression, subcellular localization and enzyme complex formation involving the enzyme.

29. Histone demethylase LSD1 regulates hematopoietic differentiation by association with lineage-specific transcription factor SCL

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The lineage specific basic helix-loop-helix transcription factor SCL (Stem Cell Leukemia) is critical for the formation of hematopoietic lineages during normal hematopoiesis and is aberrantly activated in up to 60% of patients with T-cell acute lymphoblastic leukemia (T-ALL). To understand the mechanisms underlying the control of SCL function, we have purified SCL-containing multiprotein complexes and identified the interacting polypeptides. We found that SCL forms a complex with histone demethylase LSD1, corepressor CoREST, and HDAC1/2, which exhibits histone demethylase and deacetylase activities. Furthermore, LSD1 enhances SCL-dependent transcriptional repression, and the demethylase activity of LSD1 is essential for the repressive activity. More importantly, SCL association with LSD1 and CoREST complex declines upon DMSO-induced differentiation of erythroid cells. Consistent with the decrease of the SCL and LSD1 interaction during erythropoiesis, enforced expression of LSD1 not only stimulates MEL cell growth, but also perturbs DMSO-induced differentiation of MEL cells. The results demonstrate that chromatin demethylase LSD1 and cofactor CoREST regulate SCL transcriptional function during erythropoiesis, and suggest that chromatin modifying enzyme may affect hematopoiesis and leukemogenesis through association with the lineage-specific transcription factor SCL.

30. Using mechanistic growth models to map quantitative traits loci for developmental timing and duration

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A number of statistical methods have been proposed to map quantitative trait loci (QTLs) underlying complex phenotypes primarily based on the goodness of fit to observational data rather than on any biological mechanism. We have framed a new statistical strategy for the genetic mapping of QTLs that regulate developmental timing and duration through the incorporation of specific biological laws behind the phenotypic expression of complex traits. The new strategy displays great potential in improving the precision, power and resolution of QTL mapping and generates a number of quantitative testable hypotheses applicable in any kind of organism. The model was used to analyze a real data set from a soybean genome project, leading to the identification of several QTLs that control the timing and duration of maximal growth rate for plant height. The new strategy can ask, answer and disseminate many more biologically interesting questions, and thus allow QTL mapping to interface more directly with genetics, development and evolution.

31. N-terminal acetylation, methylation, and phosphorylation of *Haloferox volcanii* 20S proteasomes

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20S proteasomes are large, energy-dependent proteases that associate as four heptameric rings of α and β subunits ($\alpha_7\beta_7\beta_7\alpha_7$). The haloarcheon *Haloferox volcanii* synthesizes two α -type subunits (α_1 and α_2) and a single β . Two 20S proteasomes, the $\alpha_1\text{-}\beta$ and $\alpha_1\text{-}\alpha_2\text{-}\beta$, have been identified during stationary phase. An acidic isoform of the α_1 subunit is also present in exponential growth, but absent in stationary phase. This study aims at identifying post-translational modifications of the α and β subunits of these 20S proteasomes. Multiple isoforms of the three subunits (α_1 , α_2 and β) have been identified using two-dimensional gel electrophoresis. Tandem mass spectrometry experiments have revealed that both the α_1 and α_2 subunits are N-terminally acetylated and the α_2 and β subunits are phosphorylated (at T13 and S129, respectively). In addition to acetylation and phosphorylation, several methylesterification sites have been identified on the α_1 subunit. Functions associated with the phosphorylation and methylesterification events have yet to be determined. There are two detectable forms of the α_1 N-terminus by mass spectrometry, the N-terminally acetylated form and an unacetylated form where the methionine has been removed. The two forms of the protein exist in a wild-type cell at a 100 to 1 ratio favoring the acetylated form. Amino acid substitutions in the penultimate position of the α_1 influence this ratio. For example, Q2A is acetylated after the methionine is removed, several other substitutions such as Q2T, Q2D, and Q2P exist as a mixture of unmodified, acetylated on M1, and methionine cleaved forms. These substitutions affect protein levels and peptidase activity of 20S proteasomes. A list of putative N-terminal acetyltransferases has been identified by BLAST and phylogenetic analysis. Targeted chromosomal knock-outs have been generated in several of these candidate genes using the *pyrE2* system. Many of these knock-outs affect the ratio of acetylated to unacetylated forms of the protein as well as protein levels.

32. The MAML1 co-activator: A novel regulator for NF- κ B signaling and its potential role in hepatocyte survival

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NF- κ B signaling regulates diverse biological processes including immune and inflammatory responses, apoptosis, proliferation and differentiation. Deregulation of NF- κ B signaling is associated with a variety of disorders such as autoimmune diseases and cancer. In this study, we identified a novel regulator, the MAML1 co-activator in mediating constitutive NF- κ B activation. We showed that MAML1 influences the basal activity of NF- κ B via two novel mechanisms. First, MAML1 interacts with I κ B (the inhibitor of NF- κ B), and leads to its degradation. Second, MAML1 facilitates the nuclear import of NF- κ B subunit p65 and enhances the transcriptional activation of NF- κ B responsive promoters. In cultured cancer cells, we found that MAML1 expression level directly influences the strength and duration of TNF α -induced NF- κ B signaling. Importantly, mice that are deficient of the *Maml1* gene display hepatocyte necrosis, indicating that Maml1 might be required for hepatocyte survival. Therefore, our overall data suggest that MAML1 might regulate hepatocyte survival through its role in NF- κ B signaling.

33. Producing transgenic Tifton-85 and TifEagle grasses using *Agrobacterium* vacuum infiltration of stolon nodes

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In the genetic transformation of monocotyledonous species, callus culture is usually an unavoidable step. The initiation and maintenance of embryogenic callus is genotype-dependent, time consuming and laborious. We describe a protocol that expedites a straightforward and callus-free transformation procedure for two Bermuda grass cultivars, Tifton-85 and TifEagle. An efficient regeneration system was established for both cultivars using different concentrations of kinetin and auxins. Stolon nodes were infected and co-cultivated with *Agrobacterium tumefaciens* strains GV3101 or AGLO containing the Cry 1Fa gene for insect resistance. Green shoots were directly produced from infected stolon nodes four to six weeks after hygromycin selection. Rooted putative transgenic plantlets were then obtained seven to eight weeks later. To date, 72 plants have been obtained using this procedure. The established plants are currently being screened for the transgene by PCR and the transgenic nature of five plants already has been demonstrated by Southern blot hybridization. Further research is focused on increasing the efficiency of transformation and evaluating expression of the transgene.

34. Molecular genetics of mitochondrial function in maize

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The mitochondrial genome encodes proteins essential for mitochondrial respiration and ATP synthesis. Nuclear gene products are, however, required for the expression of mitochondrial genes and the elaboration of functional mitochondrial protein complexes. To better understand the roles of nuclear genes in plant mitochondrial function, we developed a unique collection of nuclear mutations that disrupt mitochondrial functions in maize. These mutations restore male fertility to plants with the mitochondrial-encoded cytoplasmic male sterility type S (CMS-S) trait. Although restorer mutations rescue CMS-S pollen, many are homozygous lethal for maize kernel development and may, therefore, disrupt essential mitochondrial functions. These functions were investigated through studies of mitochondrial gene expression in CMS-S pollen restored to male fertility and in normal N pollen controls. Two mechanisms of fertility restoration are suggested by the results of these assays: Five unlinked restorer mutations conditioned global loss of mitochondrial gene products through post-transcriptional mechanisms. These restorer mutations are hypothesized to effect fertility by also conditioning the loss of the mitochondrial gene product responsible for CMS-S. These phenotypes indicate that maize pollen development can succeed in the absence of mitochondrial respiration and validate CMS-S maize as a system for functional genomic analysis of mitochondrial biogenesis in plants. A second group of restorer mutations had no obvious effects on mitochondrial gene expression. These are hypothesized to disrupt mitochondrial-signaled cell death events that are associated with pollen collapse but necessary for maize seed development.

35. A broader role for E2F6 in the repression of meiosis-specific genes

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E2F6, a member of the E2F family of transcription factors, was recently shown to play an essential role in the repression of six germ-cell-specific genes in somatic cells. Here we report that E2F6 is required for the repression of another germ-cell-specific gene, *Ant4*. This discovery prompted us to investigate whether additional germ-cell-specific genes require E2F6 for their repression. In particular, we focused on a subpopulation of germ-cell-specific genes whose expression is restricted to meiotic cells. We compiled a list of 24 meiosis-specific genes and observed that 19 of them (79.2%) have the core E2F6-binding element (TCCCGC) within 200bp upstream of their transcription initiation sites. The high frequency of binding element appearance relative to non-meiotic gene populations suggests that this site is of biological relevance. Using embryonic stem cells, we demonstrate that E2F6 binds to the promoters and represses the transcription of these meiosis-specific genes. These data suggest a broader role for E2F6 in the repression of meiosis-specific genes. However, the observation that E2F6 is not required for somatic cell repression of most of these genes supports the possibility that a functional redundancy exists whereby other safeguards are in place to ensure the repression of most meiosis-specific genes in somatic cells.

36. ICBR bacterial genome finishing programme

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The ICBR is a well-known hub of research support for the University of Florida and other research partners. As the past 10 years have witnessed a dramatic increase in the number of sequencing projects, the ICBR sequencing group has gradually evolved as a high throughput-sequencing centre with state of the art technology including pyrosequencing using 454 Life Sciences Corporation technology. In ICBR, 454 sequencers have been in operation for nearly two years. With the introduction of paired end reads and improved assembly software, 454 makes bacterial genome sequencing possible. Not only does the 454 sequencing method provide better coverage but also helps eliminate cloning bias. The ICBR is also acquiring the SOLiD System from Applied Biosystems, which will exponentially extend sequencing capability. This machine can produce up to one GB of sequence data from single run and will be installed shortly. Utilizing advancements in technology and software, the ICBR community-sequencing program now also includes the publication quality finishing and detailed analysis of the genome of different representatives of the microbial world. Complete genome information is a vital starting point for genome annotation, comparative analysis, deletion analysis, microarray, etc. The finished genome is essential for analyzing previously unknown and difficult to cultivate microbes, for rearrangement analysis or for the creation of useful microbial strains. Thus, in order to take full advantage of an organism it is necessary to fully sequence the genome. Our pipeline encompasses all phases of a complete sequencing effort, from library construction through genome assembly and annotation. The finishing process includes activities of informatics as well as wet lab experiments. It consists of manual editing through the use of both commercial and proprietary software for semi-automated assembly, including Phred, PHRAP and Consed. Paired end DNA sequence information is also used to resolve repeat sequences and maximize the level of contig ordering. Gaps are then filled by different methods such as, primer walking, alternative reaction chemistries, and/or direct PCR sequencing. Sequence data is finally inspected for quality, discrepancies, coverage and contiguity error rate less than one in 10,000 bases.

37. The enhancer-blocking and silencing activities of a chromatin insulator element in the HSV-1 LAT region are physically separable

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A chromatin insulator-like element was previously identified in the repeat long region of the HSV-1 genome and shown to bind the cellular insulator protein CTCF during latency in murine sensory ganglia (Amelio *et al.*, *J. Virol.* 80:2358, 2006). This insulator element (CTRL2) is located between the LAT enhancer and ICP0 and demonstrates both enhancer-blocking and silencing activities. In the present study, we sought to determine whether the enhancer-blocking and silencing activities map to overlapping or distinct portions of the 1.3-kb insulator and whether these activities were dependent on CTCF binding. Transient transfection assays performed with luciferase constructs containing subfragments of the insulator region have demonstrated that: 1) the enhancer-blocking activity of the insulator is located exclusively within the 5' half of the 1.3-kb fragment, 2) the silencing activity is located exclusively within the 3' half of the insulator element, and 3) deletion of the CTCF-binding motifs from either the larger 1.3-kb fragment or a 3' 750 bp subfragment abrogates the silencing activity but not the enhancer-blocking property of this insulator. Since the enhancer-blocking and silencing activities of this element appear to be cell-type specific, work is currently under way using a yeast-one hybrid screen to identify important cellular factors that may play key roles in regulating transcription of ICP0 and LAT during latency and reactivation.

38. Genome-wide *in silico* identification, comparative analysis and functional validation of human putative CSL-regulated genes

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The discovery of direct downstream targets of transcription factors (TFs) is essential for understanding the molecular mechanisms underlying complex and highly regulated biological processes. Notch receptor-mediated signaling is an evolutionarily conserved pathway regulating diverse developmental processes and its mis-regulation is implicated in a number of developmental disorders and cancers. Currently, with the limited identified targets, it is difficult to understand the diverse responses of Notch receptor activation. In order to understand the genetic mechanisms underlying Notch signaling-regulated complex processes, we conducted a genome-wide search for novel putative target genes of CSL, a key transcription factor controlling the Notch signaling pathway. First, the CSL binding elements from those known Notch target genes were used to construct a position weight matrix (PWM) for the CSL protein. This PWM was then used for genome-wide screening to search for the potential binding elements in the entire collections of non-redundant human, mouse and rat promoter sequences. The conservation of the potential binding sites was assessed by comparing the promoter sequences from the orthologs of these three species. This analysis identified a set of genes containing several previously identified Notch target genes as well as many potential novel targets, indicating that such analysis is valid for identifying Notch targets. Several novel putative genes were selected for functional validations. Our initial analysis reveals several genes as novel direct targets of CSL, thus increasing the spectrum of Notch targets that will allow us understand the varied functional consequences of Notch signaling. Moreover, our results demonstrate that the integrated strategy of bioinformatics and functional genomics is a powerful approach to identify direct downstream targets for transcription factors genome-wide.

39. The maize 6-phosphogluconate dehydrogenase 3 gene plays an important role during seed development

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The oxidative pentose phosphate pathway (OPPP) is a major source of reducing power and metabolic intermediates in central carbon metabolism. In this pathway, 6-phosphogluconate dehydrogenase (6PGDH) decarboxylates 6-phosphogluconate to provide NADPH and pentose sugars for metabolism. Recent metabolic labeling studies of starch biosynthesis in the maize endosperm suggest that the OPPP plays a central role in starch accumulation. We have identified a transposon insertion in the maize *Pgd3* locus. *Pgd3* is predicted to encode the chloroplast-localized isoform of 6PGDH. Co-segregation analysis showed that the *pgd3-umu1* mutant is tightly linked to a rough endosperm (*rgl*), reduced grain-fill phenotype. In addition, a reverse genetics screen of other *rgl* mutants identified a second mutant allele, *pgd3-umu2*. Reciprocal crosses between these two alleles fail to complement the *rgl* mutant phenotype indicating that mutations in *Pgd3* cause defective seed phenotype. Native-PAGE enzyme assays show that both mutant alleles cause an enzymatic knockout of the chloroplast-localized 6PGDH isozyme in maize. Combined, these data suggest that the chloroplast-localized OPPP is likely to have a central role in seed development and may be a target for yield improvement in maize.

40. Functional mapping: How to study genotype by environment interactions for growth curves

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Functional mapping has proven to be powerful in mapping quantitative trait loci (QTLs) that control biological processes. Functional mapping incorporates mathematical aspects of biological processes into a general QTL mapping framework, and a number of testable hypotheses about the genetic control of development can be made. In this study, we extend the idea of functional mapping to study genotype by environment interactions for growth curves. With this new model, we can characterize the dynamic patterns of the genetic effects of QTLs governing growth curves and estimate the global effects of underlying QTLs during the course of growth and development. In a real example with soybean data, our model has successfully detected several QTLs that cause significant genotype by environment interactions for the plant height growth process. The model provides a basis for deciphering the genetic architecture of trait expression adjusted to different biotic and abiotic environments for any organism.

41. Histone methyltransferase PRMT1 is important for USF1-mediated transcriptional activation of beta-globin gene

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USF1 is a ubiquitously expressed helix-loop-helix transcription factor that binds to E-box elements. We showed earlier that USF1 mediates the barrier activity at the endogenous 5'HS4 of the chicken β -globin locus by recruiting histone modifying enzymes, including the histone H4R3-specific methyltransferase PRMT1, that maintain a local active chromatin structure. However, in the mouse β -globin locus, USF1 interacts with conserved E-box elements within HS2 of the locus control region (LCR) and the β -major promoter. It has been shown that USF1 plays a critical role in regulating the developmental stage-specific transcription of the β -major globin gene. To further understand the diverse roles of USF1 protein, we investigated whether PRMT1 plays an important role in USF1-mediated

transcriptional activation of the β -globin gene. We report that USF1 and PRMT1 are colocalized to the endogenous HS2 and the β -major promoter of the mouse β -globin locus in erythroid cells. Consistent with recruitment of PRMT1 by USF1, H4R3 methylation at these two sites is increased during differentiation of erythroid cells. Furthermore, knockdown of PRMT1 expression by shRNA leads to a loss of H4R3 methylation, as well as histone acetylation at these two sites. Accompanied with the loss of histone modifications, the expression of the β -major globin is significantly inhibited in the PRMT1 kd ES cells. Taken together, these results show that H4R3 methylation by PRMT1 plays an important role in transcriptional activation of the β -globin gene and suggest that H4R3 methylation by PRMT1 appears to be essential for the transcription of the β -globin gene by establishing and maintaining an active 'open' chromatin structure.

42. A statistical model for characterizing cis- and trans-acting regulation by eQTL

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Two different types of eQTL regulation have been thought to contribute to genetic variation in gene expression. For the first type, cis-regulating, the DNA variants of a gene directly influence the transcript levels of that gene and, therefore, the underlying eQTL can be expected to map to the structural gene producing the transcript. For the second type, trans-regulating, the DNA variants of a gene exert an indirect effect on its expression, in which the underlying eQTL should co-localize to the structuring gene. In this study, we propose a general statistical model based on linkage analysis to discriminate between cis- and trans-regulating eQTL and estimating their additive, dominant and epistatic effects, for the purpose of the identification of positional candidates in QTL studies. This model provides a useful tool for studying the genomic architecture of complex traits.

43. Regulation of locus control region mediated transcription complex recruitment to the beta-globin gene locus by transcription factor USF

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The human beta-globin genes are expressed exclusively in erythroid cells and regulated by a locus control region (LCR). The LCR is composed of several DNaseI hypersensitive (HS) sites and located upstream of the globin genes. Our previous work demonstrated that transcription complexes are recruited to individual LCR HS sites prior to appearance at the globin gene promoters during erythroid differentiation of embryonic stem cells. We also demonstrated that RNA polymerase II (RNA Pol II) can be transferred from immobilized LCR templates to globin gene promoters in an *in vitro* system. We found that the ubiquitously expressed transcription factor USF is required for efficient recruitment of RNA polymerase II to the locus control region and to the beta-globin gene. We now generated transgenic mice expressing a dominant negative mutant of USF exclusively or predominantly in erythroid cells. The transgenic mice exhibit a reduction in RNA polymerase II recruitment to the LCR and to the adult beta-globin gene. Expression of the adult beta-globin gene is decreased in transgenic mice; however, expression of the embryonic/fetal betaH1 gene is elevated, supporting the hypothesis that the genes are competitively regulated by the LCR. Furthermore, we present evidence demonstrating that USF is subject to proteolytic degradation in undifferentiated erythroid cells. The data suggest that transcription in the globin locus is regulated by tissue and stage-specific transcription factors that cooperate with ubiquitously expressed proteins. We propose that tissue- or cell-type specific activities increase chromatin accessibility in the globin locus and that proteins like USF subsequently mediate the recruitment of transcription complexes.

44. TaxaSorter: A solution to metagenomic projects

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A metagenomic project generates a large collection of heterogeneous sequences. Assigning the correct taxonomic origin for each and every sequence is the key to answer all questions metagenomic projects try to address. We have developed a program, TaxaSorter that classifies sequences into a hierarchical taxonomy tree based on sequence similarities. The algorithm is based on BLAST search. However, instead of simply taking the taxonomic origin of the first hit, probability values of all taxonomic origins from the top 100 hits are calculated for every query sequence. If only one taxonomic origin is assumed for each sequence, the one with the highest probability is selected. Otherwise, all possible origins are retained with their corresponding probability values. Then, all sequences are sorted through a hierarchical taxonomy tree where each taxon node is associated with the total count of linked and sub-linked sequences. TaxaSorter also includes functions to compare two taxonomy trees on all taxon nodes to identify nodes that are significantly over-represented or under-represented. Simulations were conducted to estimate the sensitivity and specificity. TaxaSorter was applied on several metagenomic data sets, including both traditional Sanger sequences and 454 sequences. The sample result from a soil metagenomic project was presented. TaxaSorter is implemented as a module in BlastQuest, available at UF/ICBR genomics server.

45. The role of histone deacetylase activity on GATA-1 mediated hematopoiesis and leukemogenesis

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Hematopoietic specific transcription factor GATA-1 is essential for hematopoietic development. Misregulation of GATA-1 is linked to hematologic diseases including leukemia. GATA-1 activity is required for erythroid and megakaryocytic cell differentiation. However, recent discoveries indicate that GATA-1 is associated with HDAC1 containing corepressor complexes throughout differentiation of erythroid cells. We hypothesize that GATA-1 associated deacetylase activity is attenuated during erythroid differentiation. We previously found that HDAC1 can be acetylated *in vivo* and acetylated HDAC1 completely lost deacetylase activity (Qiu *et al.*, *Molecular Cell* 22:669-679, 2006). In this study, we investigate the role of HDAC1 in erythroid differentiation. Our results indicate that during erythroid differentiation, GATA-1 associated deacetylase activity is significantly decreased and further diminished at day 5 of DMSO induction in MEL cells. More interestingly, acetylated form of HDAC1 within the GATA-1 complex increases during erythroid differentiation. Overexpression of HDAC1 mutant mimicking the acetylated HDAC1 promotes erythroid differentiation. These observations suggest a novel but rather general regulation mechanism of histone deacetylase containing complexes. Further studies will allow us to understand the molecular basis of the regulation of deacetylation of HDACs and their roles in hematopoiesis and general transcription regulation.

46. Preliminary genetic analysis of the Florida and Belize manatee populations

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The endangered West Indian manatee contains two subspecies: the Florida manatee (*Trichechus manatus latirostris*) and the Antillean manatee (*T.m. manatus*), represented in this study by the Belize population. Morphological, ecological and biological data support the subspecies distinction and endangered status, but do not address current relatedness among populations. Integrative approaches are needed to identify and sustain ecological processes and evolutionary lineages of threatened species. Therefore, in addition to traditional conservation studies, molecular analyses are used to aid in the assessment and management of the West Indian manatee. Previous mitochondrial DNA studies investigated historical genetic relationships within manatee populations. Low genetic diversity was observed with three haplotypes in Belize and only one haplotype in Florida. Correspondingly, nuclear microsatellite DNA markers revealed little contemporary structure and high migration rates within Florida. In this study, microsatellite markers are used to examine the Belize population structure and assess relatedness and migration between the Florida and Belize populations. The genotypes of 91 Belize and 96 Florida manatees at 15 polymorphic microsatellite loci were investigated. A Bayesian phylogenetic inference analysis was performed using the program STRUCTURE 2.2 to identify putative ancestral source populations for extant manatees. Additionally, neighbor-joining trees were constructed to illustrate genetic distances between individuals and populations. The genetic analyses detected structuring within the Belize population and considerable diversity between the two subspecies. Results from this study should benefit management and conservation decisions.

47. Proteomics: Advance technology for the analysis of cellular process

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In recent years, genomics and proteomics have emerged as key technologies in biological and biomedical research. Proteomics can be defined as the qualitative and quantitative comparison of proteomes under different conditions to further unravel biological processes. By studying at the protein level, proteomics allows researchers to investigate how proteins affect cell processes and how cell processes and the environment may affect proteins. Proteomics includes the determination of localization, modifications, interactions, and activities of proteins and ultimately how these are related to their function. Proteomics developed initially from the study of comprehensive protein visualization on two-dimensional electrophoresis gels and has been expanded by mass spectrometry and the growth in searchable sequence databases providing sensitive methods of characterizing and analyzing both small molecules and proteins. Currently, by use of more sophisticated technology such as a combination of multidimensional chromatography and mass spectrometry, thousands of proteins can automatically be identified. Using mass spectrometric based techniques, quantitative information on differential-protein expression can be obtained using isotope labeling methods such as iTRAQ™, ICAT™ or SILAC. Along with our capacity of separating thousands of proteins and characterizing differential protein expression using 2D-DIGE technology, we have a suite of state-of-the-art mass spectrometers available for life sciences and advanced technology research, including a tandem time-of-flight (4700 Proteomics Analyzer, AB), a hybrid quadrupole/time-of-flight (QSTAR XL, AB), and hybrid quadrupole-linear ion-trap (4000 QTRAP, AB). These instruments are mainly used for protein identification, post-translational modifications characterization and differential protein expression using iTRAQ™ reagents. Proteomics also provides the tools to expand into more sophisticated biochemical approaches, such as the study of protein interactions that can be determined directly by performing a pull-down assay with a bait protein followed by mass spectrometric identification of the bound proteins. Proteomics is useful for both large-scale surveys of proteins and detailed studies of the functional relationships among the proteins of interest.

48. Effects of metabolic rate on protein evolution

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Since the Modern Evolutionary Synthesis was first proposed early in the 20th century, attention has focused on assessing the relative importance of mutation versus natural selection to protein evolution. Here we test a model that yields general, quantitative predictions on rates of protein evolution by combining principles of individual energetics with Kimura's Neutral Theory. The model successfully predicts much of the heterogeneity in rates of protein evolution for diverse eukaryotes (i.e. fish, amphibians, reptiles, birds, mammals) from different thermal environments. Data also show that the ratio of non-synonymous to synonymous substitution is independent of body size, and thus presumably of effective population size. Together, these findings indicate that rates of protein evolution are largely controlled by mutation rates, which in turn are strongly influenced by individual metabolic rate.

49. Statistical properties of open reading frame distributions: A broad scale comparison of genome architecture

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A better understanding of the size and abundance of open reading frames (ORFs) in whole genomes may shed light on the factors that control genome complexity. Here we examine the statistical distributions of open reading frames (i.e. distribution of start and stop codons) in the fully sequenced genomes of DNA viruses, prokaryotes, and unicellular and multi-cellular eukaryotes. Results show that the size-frequency distributions for ORFs are strikingly similar across all genomes, and highly correlated with GC content. In addition, these results indicate that a large fraction of these distributions can be described by random processes.

50. Dominance and additive variation contributions to the maintenance of new mutations of ovariole number in *Drosophila melanogaster*

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Mutation is the ultimate source of genetic variation. Genetic variation has multiple components, including dominance variation and additive variation. Both types of variance are subjected to selection, which works to rid a population of mutation, but have very different evolutionary dynamics under the same strength of selection. Further, short-term response to selection is contributed almost completely by additive variation. How then is genetic variation maintained in natural populations? The maintenance of genetic variation is partially attributed to long-term selection response of dominance meaning dominance is affecting the fixation time of new mutations. For ovariole number of *Drosophila melanogaster* in natural population, additive variation makes up a much larger proportion of the genetic variation than dominance variation. Is there a mutational bias for additive variation, or is dominance variation being eliminated by selection? Here we estimate the relative contributions of additive and dominance variation to new mutations for ovariole number by crossing mutation accumulation lines from two genetic backgrounds to a reference line. The resulting F1 progeny were dissected and an epistatic interaction was found within one of the backgrounds, making it impossible to estimate additivity and dominance. Therefore, the experiment was repeated with a different reference line where there is no epistatic interaction. The dominance to additive ratio will be calculated to determine if new mutations for ovariole number are overdominant, completely dominant, mostly dominant, or mostly additive. The results could provide insight to the methods in which selection is acting and genetic variation is maintained.

51. Effects of genetic variation of ovariole number in *Drosophila melanogaster* on flight dispersal in the field

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Trade-offs between life-history traits help maintain genetic variation, and play a central role in evolution. The examination of such trade-offs may contribute to the understanding of the continued coexistence of the opposing forces, natural selection and genetic variation. Trade-offs between reproduction and fitness are important for survival of many energy-limited species, such as *Drosophila melanogaster*, in which resource allocation influences reproduction, body size, and flight ability. In this present study, the trade-off between ovariole number, which is positively correlated to female fecundity (Bouletreau-Merle *et al.*, *Oecologia* 53:323-329, 1982) and flight dispersal in *D. melanogaster* was examined. The first release and recapture experiment demonstrated that females with a high ovariole number were recaptured more frequently than females with a low/medium ovariole number. In contrast, males from the high ovariole number genotype were recaptured less frequently than the males from the low and medium ovariole number genotypes. The opposing trend between females and males within genotypes illustrates a genotype by sex interaction, which may contribute to maintenance of genetic variation and hence the high heritability of ovariole number. Results from a second release and recapture experiment were consistent with the first.

52. High-throughput genotyping using Illumina bead arrays

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An integrated, accurate and scalable high-throughput genotyping system is now available to UF researchers at the ICBR Core facilities (First floor, South wing, CGRC Building). The system (Illumina) is based on a random array of 3-micron glass beads lodged on 2-micron deep micro-wells that have been etched on either glass slides or the tips of fiber optic bundles. An array is composed of many bead-types, each represented at a 20-30 fold redundancy. Each bead-type contains 700-800 thousand copies of a two-part probe. One probe segment serves as a bead identifier (bead code) and another segment is designed to hybridize uniquely to a SNP locus. Detection is accomplished through allele-specific, fluorescent based assays and scanning in a high-resolution (0.8 microns) scanner (BeadStation GX). Image data analysis is done using Illumina's BeadStudio software in conjunction with bead decoding files provided with each array. The system has proven to be highly robust and accurate. When DNA quality is not compromised, call rates of 99% and higher are easily accomplished. Depending on the nature of the project, researchers can choose from two different assay formats. First, the GoldenGate assay allows the genotyping of a maximum of 1536 SNP sites on 96 different samples per array (SAM, sentrix array matrix). Second, the Infinium II assay is used for beadchips capable of genome-wide genotyping of up to one million SNP loci. Although Illumina currently offers only human, mouse and rat genotyping arrays, the availability of a more diverse set of products is expected as the popularity of this platform increases.

53. Ancient origin and functional differentiation of chaperonin-like genes involved in Bardet-Biedl syndrome

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Chaperonins are oligomeric complexes that play important roles in *de novo* protein folding and renaturation and in protecting the cells from the deleterious effects of various forms of stress. They are divided into two structural classes, one typically found in Bacteria and eukaryotic organelles (Group I) and one found in Archaea and in the

eukaryotic cytosol (Group II). In eukaryotes, the Group II chaperonin complex is a double-ringed structure called CCT/TriC complex, which is composed of 16 monomers coded by genes belonging to eight anciently-diverged paralogous families (cct1 to cct8). The Bardet-Biedl syndrome (BBS) is a human pathological condition that has been associated to mutations in twelve different genes (BBS1-BBS12). Among these, BBS6, BBS10 and BBS12 have been recently characterized as a group of fast evolving genes related to eukaryotic Group-II chaperonin genes that are only found in vertebrates. By thorough analysis of several complete genomes representative of all major eukaryotic lineages, we have been able to determine that BBS6, BBS10 and BBS12 are members of a family of genes found in phylogenetic groups as diverse as Opisthokonts (including animals and fungi), Amoebozoa (including *Dictyostelium*) and Heterokonts (including diatoms), placing their origin much earlier in eukaryotic history than previously suggested, as a distinct new gene family of chaperonin genes. This family of genes is characterized by rapid evolution and many of its members appear to be lost or are unrecognizable in various eukaryotic lineages. Synonymous vs. non-synonymous nucleotide substitution patterns suggest that the members of this gene family have been subject to intense processes of functional differentiation. Homology modeling and sequence conservation analysis provide insights into the possible roles of these proteins in substrate-binding and participation to the chaperonin complex.

54. High-throughput annotation of genomic datasets

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We describe the initial implementation of Genephony, an online tool for the creation and manipulation of very large genomic datasets. Genephony allows the user to easily create “sets” containing biological entities (e.g. genes, SNPs, pathways, etc) and to combine them in a variety of ways in order to generate new sets. Sets are stored in a dynamic workspace through which the user can freely navigate. Relying on an extensive underlying database of genomic information, the system makes it easy to integrate, annotate and interpret the results of high-throughput experiments, providing automated operations that would be otherwise impractical if performed manually. Genephony will soon be made publicly available; our expectation is that it will become a useful tool for translational research, high-throughput biology, and for all knowledge-intensive data manipulation tasks in computational biology.

55. Custom gene expression microarray design pipeline

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Transcriptome libraries sequenced using either traditional Sanger technology or pyrosequencing technology (454 Life Science, Inc.) or both can be quickly transformed into a custom gene expression microarray through our data processing pipeline. This pipeline is comprised of data cleaning, assembly (hybrid-assembly if both technologies were involved), annotation, coding region prediction, redundancy reduction, orientation determination, probe design and array layout design. As a routine practice, a total of 250 probes designed from reverse complement strands of genes from five different organisms, namely, human, *Arabidopsis*, rice, zebrafish and yeast are included as negative controls. Another 1000 – 2000 probes were included for QC/QA purposes. This pipeline employs several 3rd party programs such as Paracel TranscriptAssembler, Newbler, BLAST and Agilent eArray system, and in-house developed programs such as BlastQuest and AssemblyFilter. Available formats include 244K, 2x105K, 4x44K and 8x15K. To date, eight custom Agilent microarrays were designed according to this protocol. And all of them have been used in gene expression research projects. Here, as an example, we presented a custom array design for a new non-model organism.

56. Clade A fibrillar collagen duplication in zebrafish

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In order for a diverse and complex number of organisms to arise over evolutionary time, genetic variation within populations was required for natural selection to act upon. A process by which loci can be replicated in an organism from ancestral generations to future generations is known as gene duplication. During gene duplication, loci are replicated and genes on those loci are either maintained, deleted, or beneficial mutations can overwrite previous genes. This can result in subfunctionalization (both copies necessary to complement each other), neofunctionalization (locus with a new function), or nonfunctionalization (non-functioning duplicate). Since it is well established that the teleost genome has undergone an extra round of genome duplication, we test the hypothesis that the clade A fibrillar collagen genes experienced gene duplication in zebrafish. We cloned and isolated six zebrafish clade A fibrillar collagen duplicates. Phylogenetic analysis confirmed that gene duplication had taken place. We are currently investigating their expression patterns via *in situ* hybridization. The results will have implications for understanding the relationship between genome and skeletal evolution, and may account for the unusual complexity of teleost connective tissues.

57. Noise in the quorum-sensing mechanism of the marine bacterium *V. fischeri*

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Vibrio fischeri is a luminescent marine bacterium that naturally colonizes several marine macroscopic life forms. It is mostly known for colonizing the light organ of the squid *Euprymna scolopes* in a symbiotic relationship. The bacteria start emitting light when they reach a critical cell density inside the light organ of the host. They sense cell density by means of the LuxIR quorum-sensing mechanism that controls light production. Quorum sensing by this bacterium is of interest for its network has the same architecture as the ones possessed by a large number of other proteobacteria, including pathogens to humans. The objective of our research is to see how noise in the gene regulatory system affects the quorum sensing communication. Quorum sensing in *V. fischeri* has been extensively studied in bulk cultures, but little is known about how it is affected by gene noise, or the intercell variability in gene expression. Recent observations in other systems have shown that noise can drive switching between different "modes" of gene expression. It has been suggested, though not experimentally proved, that this may occur in the LuxIR system. We are measuring the light output of individual cells in time and under different conditions. We are using dark field microscopy imaging to find and focus the cells, and an intensified CCD (iCCD) to collect the bioluminescence light of individual cells (or small clusters) in time. The analysis of the individual cell luminescence signal and its noise can give important information about time scales and the properties of the network; and eventually lead to a mathematical model for noise properties of the quorum sensing system.

58. Genetic dissection of the lupus susceptibility locus, *Sle1c*

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Systemic lupus erythematosus (SLE) is an autoimmune disease in which numerous genetic loci epistatically contribute, along with environmental and stochastic events, to a break in self tolerance. SLE is characterized by high titers of autoantibodies, especially those that bind nuclear antigens such as dsDNA, histones/chromatin, and small nuclear ribonucleoproteins (snRNPs). The high levels of immune complexes that are formed by these anti-nuclear autoantibodies (ANA) bound to autoantigens are the main culprits in the pathogenesis of the SLE. These

immune complexes can cause disease resulting in dermatitis, neuropathy, arthritis, cardiomyopathy, anemia, and nephritis. The genetic variables that contribute to SLE and other autoimmune diseases are numerous and complex, making it difficult to link disease related phenotypes to their corresponding alleles. In order to simplify this task, we have adopted a technique known as "Genetic Dissection" to study a murine model of SLE. Using this approach, we performed linkage analyses for various SLE phenotypes on F2 crosses between NZM2410 (NZM), a strain known to succumb spontaneously to a severe lupus-like disease, and C57BL/6 (B6), a healthy control strain. Three NZM-derived lupus susceptibility loci were identified and named Sle1, Sle2, and Sle3 on chromosomes 1, 3 and 7, respectively. These individual loci were then congenically bred onto the B6 background to yield the new strains B6.Sle1, B6.Sle2, and B6.Sle3 and were each shown to exhibit unique phenotypes found in the NZM mouse, but did not result in disease. Importantly, when the three loci were combined on the B6 background to generate a triple congenic strain, B6.Sle1Sle2Sle3, the severe disease was reconstituted. Unfortunately, it was found that these loci were, themselves, complex, and it was necessary to generate subcongenic strains. Here we focus on Sle1c and its subcongenics. Using these subcongenic strains, we were able to narrow the range for candidate genes to ~1Mb.

59. Tmem16a is required for murine lung development

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A recent screen in our lab compared gene expression between cells from the mouse zone of polarizing activity (ZPA) and cells from the rest of the limb using a combination of fluorescence activated cell sorting and the Affymetrix microarray platform. We have focused our efforts on characterizing one of the genes that this screen identified as upregulated in the ZPA. *Tmem16a* is a member of the TMEM16 family of proteins. Members of this family have eight predicted transmembrane domains and a highly conserved 3' domain of unknown function. RNA *in situ* hybridization has demonstrated expression of *Tmem16a* in a variety of tissues during mouse development and human TMEM16A is overexpressed in many cancers. We have generated a null allele of *Tmem16a* by homologous recombination in mouse embryonic stem cells. Mice homozygous for this deletion have no apparent limb defects, but die shortly after birth. We are currently investigating a severe lung defect in mutants that results from a failure of alveolar septation.

60. Conservation genetics of the endangered Miami blue butterfly

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The Miami blue butterfly, *Cyclargus thomasi bethunebakeri* (Comstock and Huntington) (Lepidoptera: Lycaenidae) is state-endangered in Florida and a candidate for federal listing. Extant colonies exist in a metapopulation structure and are limited to a single location, Bahia Honda State Park in the lower Florida Keys. A captive breeding colony was initiated from Bahia Honda stock to safeguard the taxon and to provide organisms for reintroduction. Wing fragment samples from both wild and captive colony specimens were collected for DNA extraction and microsatellite analyses. These genetic data were used to infer population structure, overall genetic variability, and gene flow within the extant metapopulation for 2005 and 2006. Genetic variability was also assessed for several generations of the captive colony. Comparisons of microsatellite data between captive colony and wild-caught individuals reveal differences in allelic diversity and overall genetic variability. The conservation implications of using captive-bred individuals for reintroduction are discussed.

61. A new fast and dependable algorithm demonstrates the high rate of HIV-1 intra-patient recombination

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Despite several studies demonstrating the occurrence of human immunodeficiency virus type 1 (HIV-1) intra-patient recombination, no general method exists to identify the most likely recombinants in a data set of HIV-1 aligned sequences. Traditional phylogenetic based techniques such as bootscanning lack power and require the *a priori* identification of parental strains. We describe a simple algorithmic procedure based on split-decomposition networks and the PHI test, a robust statistical test for recombination. This new algorithm allows detection of HIV-1 intra-patient recombinant sequences in a fast and reliable way. We re-analyzed sequence data longitudinally sampled from PBMC in four patients, as well as sequence data sampled from multiple tissues in two patients. Our results demonstrate that intra-patient recombination was significantly underestimated in these studies, and that up to one third of the V1-V3 sequences longitudinally sampled from a given subject can be of recombinant origin. Since intra-patient recombination leads to the creation of mosaic genomes violating the tree-like assumption of evolution, results and conclusions based on phylogenetic analysis of HIV-1 data sets including recombinants can be severely misleading. The results of our analysis not only indicate that the algorithm could be a valuable tool for quick detection of recombinant sequences before proceeding to phylogenetic analysis, but also suggest that HIV-1 recombination *in vivo* is far more frequent and significant than previously thought.

62. Standing genetic variance reflects mutational variance for fitness and body size in two species of *Caenorhabditis*

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Over the past several years our lab has been investigating the properties of new mutations in a model nematode system. Mutations have been allowed to accumulate in the (relative) absence of natural selection, thus allowing us to estimate the genetic variance introduced by new mutation (VM) for two species of Rhabditid nematodes, *Caenorhabditis elegans* and *C. briggsae*. However this begs the question, what is the relationship of VM to the standing genetic variance (VG) and what can this relationship tell us about the nature and magnitude of selection acting on these species? To complement our previous studies and generate estimates of VG, we assayed wild strains of both, *C. elegans* and *C. briggsae*, for fecundity at 20°C and adult body size. Comparisons of VM to VG between our mutation accumulation lines and the natural isolates allow us to infer the magnitude and pattern of constraint on phenotypic evolution, as well as give insights into the forces responsible for genetic diversity within and between these species. Our results suggest that the standing genetic variance in *C. briggsae* is much greater than in *C. elegans*, with *C. briggsae* displaying approximately twice the variance of *C. elegans* for both fitness and body size. The data also provides evidence for Mutation Selection-Balance acting on the life history trait of fitness, with a VG to VM ratio much smaller than 4Ne. Also, the persistence time of a new mutation was found to be longer in *C. briggsae* than in *C. elegans*.

63. Molecular characterization and risk assessment of apomictic, transgenic bahiagrass (*Paspalum notatum* Flugge)

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Bahiagrass is a predominant forage and low-input turf grass in the southeastern United States. Bahiagrass has apomictic tetraploid (2n=40) and sexual diploid (2n=20) genotypes, both of which have commercially important cultivars. Apomictic tetraploid cultivar 'Argentine' was chosen for genetic transformation. Its apomictic mode of reproduction should allow production of uniform seed progeny and might reduce the risk of unintended gene dispersal by pollen. To further investigate this, we co-transformed minimal unlinked nptII and bar expression constructs (MC's) into bahiagrass callus by biolistic gene transfer. The vector backbone was removed prior gene transfer to enhance co-expression and expression stability and eliminate the prokaryotic antibiotic resistance expression cassette. Twenty-one nptII expressing plants were confirmed by ELISA following selection of 300 bombarded calli on paramomycin containing media. Relative simple integration patterns for the nptII gene, and higher copy numbers and more complex integration patterns for the non-selected bar gene were detected by Southern blot analysis. Consistent with earlier reports the co-integration and co-expression frequency of the unlinked MC's was higher than 90%. Integration of quantitative ELISA-, Southern blot- and herbicide resistance data under greenhouse and field conditions indicated that MC's support high level expression of the bar gene and resistance to high glufosinate application rates. Molecular analysis of the seed progeny of the transgenic lines, will also be presented. Glufosinate resistance in apomictic 'Argentine' bahiagrass was used as a marker to study pollen-mediated intraspecific gene flow from transgenic apomictic 'Argentine' bahiagrass to wild-type diploids and wild-type tetraploids under field and greenhouse conditions. Data on herbicide resistance of seed progeny, intraspecific gene transfer frequencies, ploidy, fertility and transgene integration patterns of gene transfer events will be presented.

64. Multiphasic role of Shh during external genital development and cloacal septation

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Loss of Sonic hedgehog (Shh) function during embryonic development leads to a suite of congenital malformations, including holoprosencephaly, limb reduction, persistent cloaca, and agenesis of the external genitalia. During normal development of the urogenital system, Shh is expressed throughout the hindgut endoderm and has been proposed to act as an organizing signal during development of the external genitalia. The early and severe anogenital phenotype in Shh null mice has precluded an examination of Shh function during later stages of anogenital development. In order to examine the temporal role of Shh signaling during anogenital development, we employed a conditional knockout system that allows for the spatially- and temporally-controlled removal of Shh signaling. We utilized mice with a tamoxifen-inducible form of cre-recombinase knocked into the Shh locus (ShhcreErT2) and crossed these to mice homozygous for a conditional allele of Shh (ShhC/C). Injection of pregnant dams before, during, and after initiation of genital tubercle outgrowth allowed for an examination of Shh function over the course of external genital and anorectal formation. Our results indicate that Shh is required during the first two phases of genital tubercle development (early outgrowth and dorsoventral patterning of the phallus), but is not required during sexual differentiation of the penis and clitoris. Early removal of Shh function also results in improper partitioning of the cloaca. Failure of these embryos to form a bilaminar urethral plate is a secondary consequence of a disruption to cloacal morphogenesis. Removal of Shh at later time points leads to severe ventral hypospadias, uncovering a later role for Shh during urethral tube formation and in dorsoventral patterning of the genital tubercle. These results implicate Shh signaling in a wide range of urogenital defects and reveal a role for Shh as an integrative mechanism involved in associative complexes of the urogenital and anorectal systems.

65. Genetic and biochemical characterization of a dihydroxyacetone kinase-linked phosphoenolpyruvate:protein transferase system in *Haloferax volcanii*

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Dihydroxyacetone (DHA) kinases are a family of widespread, sequence-conserved enzymes that phosphorylate DHA and other short-chain ketoses and aldoses. Two groups of DHA kinases have been well characterized based on the source of the high-energy phosphate utilized: ATP-dependent (homodimeric, two-domain proteins found in eukaryotes and eubacteria) and PEP-dependent (tripartite proteins found in eubacteria). While the biochemical properties of DHA kinases have been elucidated for eubacteria, little is known of their function and regulatory mechanisms in archaea. The long-term goals of this study are to determine the metabolic function of a putative dihydroxyacetone kinase phosphotransferase system (DHAK-PTS) in the haloarchaeon *Haloferax volcanii* and establish whether this system is regulated at the transcriptional and/or post-transcriptional level. Using the pop-in/pop-out, markerless gene deletion method, the gene encoding a DhaL homolog was deleted from the genome of *H. volcanii* H26. In *E. coli*, DhaL serves as the nucleotide-binding subunit of DHA kinase and the co-activator of DhaR, an enhancer binding protein. The resulting *H. volcanii* H26 Δ dhaL mutant exhibited growth under microaerobic conditions on minimal medium supplemented with 20 mM DHA, reaching a maximum O.D. (600 nm) of 1.7 and displaying a doubling time of 6.9 h. The sole catabolic product was 2,3-butanediol, detected via HPLC using a BioRad HPX-87H column. Parent strain H26 did not exhibit growth on minimal medium supplemented with DHA at any tested concentration. Both H26 and H26 Δ dhaL exhibited similar growth phenotypes on minimal medium supplemented with 20 mM glycerol or 20 mM pyruvate, reaching a maximum O.D. (600 nm) of 1.6-1.7, suggesting that the H26 chromosomal *dhaL* knockout specifically effects DHA metabolism. Cells grown on pyruvate produced 2,3-butanediol as the sole catabolic product, while cells grown on glycerol generated oxidative intermediates as well as 2,3-butanediol. Future endeavors include: performing growth complementation assays using the abovementioned growth media for an H26 Δ dhaL strain complement (pJAM811) encoding DhaL; generating chromosomal gene knockouts in other components of this system and performing subsequent growth curve assays; performing enzyme activity assays; and determining whether this system is controlled at the transcriptional level and/or post-transcriptional level, particularly focusing on proteasomal control.

66. The roles of E2F6 and DNA methylation in the regulation of gene expression during germ cell development

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Mammalian primordial germ cells (PGCs) are specified in the extraembryonic mesoderm, migrate to the developing genital ridge and subsequently differentiate into gonocytes. Colonization of the gonad coincides with a widespread DNA demethylation event in the germ cell genome, leading to the upregulation of a subset of gonocyte-specific genes. DNA methylation contributes to silencing these gonocyte genes in migratory PGCs and in somatic tissues. Additionally, recent studies suggest a role for the transcription factor E2f6 in silencing germ cell-specific genes in somatic tissue. A subset of germ cell-specific genes, including *Tuba3*, *Stag3* and *Smc1B*, is ectopically expressed in somatic tissues lacking *E2f6* and DNA hypomethylation is found at the promoter region of at least one of these genes. These observations have led us to investigate a potential relationship between E2f6 and DNA methylation in the temporal regulation of gonocyte-specific gene expression in the developing germ line. Here we provide evidence that:

- 1) Members of the E2f family, including E2f6, are expressed in PGCs
- 2) E2f6-regulated genes are not activated as a result of the genome-wide DNA demethylation event coinciding with gonocyte differentiation
- 3) The *Tuba3* promoter is highly methylated in PGCs. Remarkably, unlike other known genes, *Tuba3* DNA methylation appears to be stable through major stages of early embryogenesis and germ cell development. Current efforts are focused on creating an *E2f6* gene trap mouse line to precisely determine the expression pattern of *E2f6* in early development and its contribution to stability of DNA methylation in the germ line.

67. Conservation genetics of *Crotalaria avonensis* (Fabaceae), an endangered Lake Wales Ridge, FL plant

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The Lake Wales Ridge consists of sandy xeric uplands dominated by pines, shrubby oaks, and hickory at the center of the Florida peninsula. Periodically isolated from North America by rising sea levels during recent ice ages, many of these dune plants and animals remained restricted to the Ridge. Thus, the Lake Wales Ridge has one of the highest concentrations in North America of endemic species. One such endemic, *Crotalaria avonensis* (Fabaceae), is a federally listed endangered species. We have used microsatellite loci to investigate the genetics of *C. avonensis* populations. Genetic data can provide insight into distribution diversity, mating patterns and population substructuring. Additionally our data indicate that *C. avonensis* is tetraploid. Implications of these data for conservation planning will be discussed.

68. Estimating microbial population densities based on genomic signatures

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We consider the problem of estimating microbial compositions of environmental samples for applications such as environmental monitoring and microbial disease diagnosis. Identifying the members of a community and their respective proportion is a challenging problem due to the fact that the members are often dependent upon each other and thus cannot be isolated and cultivated as pure cultures in a laboratory. The available computational methods to estimate species richness and abundance are limited. We propose a simple but effective method to overcome the limitations of the existing methods based on the concept of genomic signature. A simulation study is performed and the results demonstrate the effectiveness of the new algorithm.

69. Are head lice and clothing lice genetically distinct? A coalescent approach

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Humans are parasitized by two types of lice in the genus *Pediculus*: head lice, which live on the scalp, and clothing lice, which live in the clothing. Pesticide-resistant head lice are common among schoolchildren in developed countries as well as among the poor in both developed and undeveloped countries. Clothing lice are less prevalent, and occur mainly on those living in poor conditions. Precisely how and when clothing lice emerged from a head louse ancestor is not well known, but is clearly related to the habitual use of clothing among humans. Furthermore, it is unclear whether head and clothing lice have diverged genetically to the point that taxonomists would classify them as two distinct species. Phylogenetic analyses of mtDNA have consistently shown that clothing lice are nested within a single clade of head lice, however, Leo *et al.*, (*Heredity* 95:34-40, 2005) recently used microsatellite data from 11 double infestations to demonstrate a lack of gene flow between head and clothing lice. To determine if head and body lice are genetically distinct, we analyzed publicly available data from three nuclear genes (18S, EF1-alpha, and RPII) and one mitochondrial gene (COI) in a coalescent framework to measure migration using the software packages MDIV and IM. Preliminary results indicate that head and clothing lice have diverged, and only a small proportion of head lice initially colonized clothing. Our preliminary analyses also indicate that migration is unidirectional from head to clothing.

70. A statistical strategy for mapping imprinted quantitative trait loci: Implications for genetic mapping in mice

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The role of genetic imprinting in shaping development has been comprehensively investigated in plants, animals and humans. However, a statistical method that can detect and estimate the effects of imprinted quantitative trait loci (iQTLs) throughout the entire genome has not been extensively developed. In this study, we propose a general strategy for mapping and estimating the distribution and effects of iQTLs that are expressed from only one of the parental chromosomes. The experimental design used is based on four F2 populations, initiated with a pair of reciprocal crosses between two inbred lines, so that the inheritance of parent-specific alleles could be traced. The computing model and algorithm were implemented with the maximum likelihood approach. The new strategy presented was applied to study the mode of inheritance for iQTLs that control survival in mice. Monte Carlo simulation studies were performed to investigate the statistical properties of the new model with the data simulated under different imprinting degrees. The F2-based design and a series of analytical testing strategies provide a standard procedure for testing iQTL involvement in the genetic control of complex traits.

71. Development of a system to assess *in situ* switching in the *Babesia bovis* locus of active *ves* transcription (LAT)

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Babesia bovis is an intraerythrocytic protozoal parasite that maintains persistent infection of its vertebrate host by at least two mechanisms: cytoadhesion and antigenic variation. Identification of the *ves* multigene family encoding the "variant erythrocyte surface antigen-1" (VESA1) and the genomic locus from which *ves* genes are actively transcribed (LAT) enabled the demonstration of segmental gene conversion as one mechanism of antigenic variation. Previous observations revealed a similar organization and the presence of a highly conserved intergenic region structure among all the *ves* genes identified, including both the actively transcribed *ves* genes at LAT site and transcriptionally silent sequence donor genes. In this study, we are attempting to determine whether antigenic variation may also occur by *in situ* switching of transcriptional activity from the current LAT to another previously silent gene pair. We propose to induce transcriptional switching of the LAT by selecting for a switch from the current active *ves1a* gene to another LAT-like locus. The activation of another *ves* locus will be characterized to clarify if mutually exclusive *ves* gene expression is occurring and controlled by *in situ* switching of the LAT. Toward the goals we have initiated the development of a transfection system for *B. bovis* with the employment of a transfection vector carrying selectable markers. We have determined that *B. bovis* parasites are adequately susceptible to puromycin, blasticidin-s and pyrimethamine, providing the possibility of positive selection and maintenance of transfected parasites. Transfection vectors have been constructed to allow identification *in vitro* and *in vivo* of suitable promoter and terminator sequences for transfection. The characteristics of DNA uptake and its stability within bovine erythrocytes have been determined. Preliminary results revealed strong promoter activity of *ves* intergenic region. These data will provide crucial groundwork for the development of transfection in *B. bovis*. Supported by NIAID grant #R01 AI055864.

72. A model of arthrofibrosis using intra-articular gene delivery of TGF- β 1

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Idiopathic adhesive capsulitis (IAC) of the shoulder is a disease of unknown etiology characterized by painful, chronic fibrotic expansion of the synovium and joint capsule, which gradually leads to loss of joint motion. Although IAC affects approximately 3% of the population, little is known about its pathogenesis. Similar to other fibrotic conditions, it is likely that the harmful effects of IAC are mediated by transforming growth factor- β 1 (TGF- β 1). In an effort to establish an animal model of this disease and develop an understanding of the cellular and molecular events contributing to arthrofibrosis, we used an adenovirus to over-express TGF- β 1 cDNA in the knees of athymic nude rats. Various doses of Ad.TGF- β 1 were delivered into groups of rats which were then sacrificed periodically. The joint tissues were examined macroscopically, histologically and for patterns of expression of extracellular matrix-associated genes. At days 5 and 10, TGF- β 1 stimulated a dramatic fibrotic event, causing a rapid increase in knee diameter and the complete encasement of the joints in dense scar-like tissue, locking the joints at 90° of flexion. Histologically, massive proliferation of resident synovial fibroblasts was seen followed by their differentiation into myofibroblasts. The fibrotic expansion was observed to overrun and displace the normal architecture of the joint capsule and fuse with the articular cartilage. The expression profiles showed exceptionally high levels of MMP production, as well as tenascin C, osteopontin and thrombospondins 1 and 2. By day 30 the predominant phenotype of the expanded tissue had changed to articular cartilage, indicated by cellular morphology, the matrix composition of the tissue, and 100 and 300 fold increases in link protein and collagen type II expression, respectively. These results suggest that cellular and molecular events leading to IAC share many aspects with tumorigenesis and metastasis. They also demonstrate the proliferative potential and plasticity of fully differentiated cells in adult tissues.

73. Mapping quantitative trait loci of complex traits based on zygotic linkage disequilibrium

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Linkage disequilibrium-based mapping that capitalizes on historical recombinant events has proven to be powerful for detecting quantitative trait loci (QTLs) that control a complex trait in a natural population. This approach, founded on the non-random association between markers and QTL at the gametic level, requires the population mapped to be in Hardy-Weinberg equilibrium (HWE), which may not be a case for many genetically informative isolated populations. Here, we present a new QTL mapping approach based on linkage disequilibria at the genotypic or zygotic level by accommodating the deviation from HWE. This approach allows joint or separate estimation of Hardy-Weinberg disequilibrium at individual loci, gametic and non-gametic linkage disequilibria, trigenic, and quadrigenic linkage disequilibria between the markers and QTLs. By testing these different types of disequilibria, we generalized framework for inferring the existence of the underlying QTL for a complex trait. We performed simulation studies and real data analyses to investigate the statistical properties of this approach and validate its utilization. This approach will open a general gateway for studying the detailed picture of the genetic architecture of quantitative variation in natural populations.

74. Bahiagrass with expression of HvWRKY38 transcription activator displays improved drought tolerance

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Bahiagrass (*Paspalum notatum* Flugge) is an important turf and forage grass in the south-eastern US and in subtropical regions around the world. The productivity and persistence of bahiagrass is limited by environmental stresses like drought, freezing and in salt affected areas. We isolated several transcription activators of genes involved in abiotic stress response and will present data on over-expression of the HvWRKY38 transcription activator in bahiagrass. Transcription factors, like HvWRKY38 are capable of activating the expression of multiple genes involved in protection against environmental stresses. A constitutive HvWRKY38 expression cassette was successfully introduced into bahiagrass cv. Argentine via biolistic gene transfer as indicated by Southern blot or PCR analysis. Over-expression of this transcription factor was confirmed by real-time RT-PCR. We will present physiological data on dehydration tolerance including biomass production and stress symptoms following severe dehydration, and photosynthetic efficiency during and after stress.

75. Deficiency in a cytosolic ribose-5-phosphate isomerase causes chloroplast dysfunction, cell death, and disease susceptibility in *Arabidopsis*

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The oxidative pentose phosphate pathway (oxPPP) is part of the central metabolism, which not only produces the reducing equivalent NADPH for biosynthesis and maintaining the redox potential in the cell, but also generates erythrose-4-phosphate, a precursor of the shikimate pathway, leading to the biosynthesis of the antimicrobial compounds called phytoalexins and the defense signal molecule salicylic acid (SA). It has been shown that the oxPPP is induced upon pathogen attack and elicitation, which leads to the hypothesis that the oxPPP may play a role in plant defense responses. However, thus far no evidence is available to demonstrate that a direct link between the oxPPP and plant defense responses exists. Here we report identification and characterization of *Arabidopsis* T-DNA knockout mutants of the RPI2 gene (At2g01290), which encodes a cytosolic RPI that catalyzes the reversible interconversion of ribulose-5-phosphate and ribose-5-phosphate in the nonoxidative stage of the oxPPP. Knockout of RPI2 interferes with the chloroplast function and causes premature cell death. The *rpi2* mutants accumulate less starch in the leaves and flower significantly later than wild type when grown under short-day conditions. More interestingly, knockout of RPI2 also partially compromises SA accumulation following pathogen infection and leads to enhanced susceptibility to the bacterial pathogen *Pseudomonas syringae* pv. *maculicola* ES4326. The *rpi2* mutant is the first plant oxPPP mutant that exhibits enhanced disease susceptibility, which supports the existence of a link between the oxPPP and plant defense responses. Thus, further investigation of mutants of the genes that encode the oxPPP enzymes will allow us to identify the missing link between the oxPPP and plant defense responses, and gain insight into the role of the oxPPP in plant's response to other environmental stresses.

76. Nonparametric covariance estimation in functional mapping of quantitative trait loci

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Estimation of the covariance structure of longitudinal processes is a fundamental prerequisite for the practical deployment of functional mapping designed to study the genetic regulation and network of quantitative variation in dynamic complex traits. We present a nonparametric approach to global estimation of the longitudinal covariance structure of a quantitative trait measured repeatedly at times. This approach is formulated by regressing the trait variable on its predecessors, allowing for the estimation of a sequence of varying-coefficient and varying-order regression models. This approach is embedded within the framework for functional mapping to genomewide scan for the existence of quantitative trait loci underlying a dynamic trait, leading to enhance the breadth of use of this mapping method while preserving its biological relevance. A live example from a mouse genome project is analyzed to illustrate the methodology. Extensive simulation studies are performed to reveal the statistical properties and advantages of the nonparametric covariance modeling for functional mapping.

77. Building a skeleton: The origin of vertebrate cartilage development

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The phylogenetic relationships of the vertebrates were established largely based on anatomical characters, particularly those of the skeleton. Skeletal development has been well studied in higher vertebrates (e.g., mouse and chicken), however, little is known about the developmental mechanisms responsible for evolutionary origin of the vertebrate skeleton. Here we investigate cartilage development in two jawless vertebrates, hagfishes and lampreys, and in a sister group to the vertebrates, lancelets (amphioxus). We report that both lamprey and hagfishes have Col2a1-based cartilage, suggesting that this type of cartilage was present in common ancestor of all crown vertebrates. Our analysis of lancelets revealed the presence of an ancestral clade A fibrillar collagen (ColA) gene that is expressed in the notochord. Thus, during the chordate-vertebrate transition, an ancestral clade A fibrillar collagen gene underwent duplication and diversification, and this process may underlie the evolutionary origin of vertebrate skeletal tissues.

78. Quantitative PCR: A very useful tool for genomics research

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Real-time quantitative PCR remains one of the most sensitive and quantitative tools for genomics research used today. ICBR offers both custom and pre-optimized QPCR assays for an assortment of specific applications. We manage all aspects of the project from assay design to data analysis. This service is advantageous to researchers conducting the following studies: microRNA quantification assays, SiRNA knockdown assay, gene expression-primary validation/quantification, gene expression-conformation of microarray data, pathogen (viral/bacterial) detection/quantification, allelic discrimination/SNP genotyping analysis, transgene detection/quantification and biological diversity/contamination.

79. Genomic analysis of tissue compartmentalization during development of the mouse external genitalia

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Development of external genitalia in mammalian embryos is a well-organized morphogenetic process involving outgrowth, proximodistal and dorsoventral patterning, and epithelial tubulogenesis. External genitalia development in males and females begin with formation of a genital tubercle, an outgrowth of lateral plate mesoderm, surface ectoderm, and endodermal epithelium derived from the urogenital sinus. The molecular mechanisms that regulate morphogenesis of the mammalian external genitalia are poorly understood. Previous studies in our laboratory revealed that the urethral epithelium acts as a signaling center of the genital tubercle and Sonic hedgehog from the urethral epithelium is required for outgrowth, patterning, and cell survival in the developing external genitalia. In order to determine the transcriptional profile of this newly-defined signaling region, we separated by flow cytometry the urethral epithelium and mesenchymal cells of E12.5. *shhg*^{cre} mouse embryos and performed microarray analysis. We identified 88 genes that are differentially expressed in the urethral epithelium and genital mesenchyme. Of these, 14.7% are cytoskeleton/structure proteins, 13.6% are membrane-associated proteins with multiple activities, 11.4% are signal transduction proteins, 8% are transcription factors, 9% are cell growth and proliferation regulators, 11.4% are metabolic enzymes, 15% belong to other identified protein classes and 25% are unknown proteins. Eighty-two genes were found more highly expressed in urethra and six genes more highly expressed in mesenchyme. More than 60 urethra expressed genes were confirmed using both whole mount and section *in situ* hybridization. Interestingly, most of urethral expressed genes were also found to be strongly expressed in gut, suggesting a close relationship between the molecular mechanisms of gut and urethral patterning.