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Coevolution driving speciation? Evidence from a Joshua tree hybrid zone

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The idea that biotic interactions foster diversification is supported by substantial circumstantial evidence, but is challenging to test. We use a uniquely suitable system to ask whether putative coevolution in a plant-pollinator interaction is contributing to speciation. Two subspecies of Joshua tree (*Yucca brevifolia brevifolia* and *Y. b. jaegeriana*) have reciprocally obligate relationships with their pollinators, two sister species of yucca moth (*Tegeticula synthetica* and *T. antithetica*). The plant-pollinator pairs exhibit trait matching suggestive of coevolution in key characters involved in the interaction – style length in the trees, ovipositor length in the moths. Although speciation in the moths is complete, the trees hybridize in a narrow contact zone in southern Nevada. We hypothesize that coevolution is contributing to reproductive isolation in the trees. If this is true, we expect to find disruptive selection on style length in and around the hybrid zone. To test this prediction, we used RAD sequencing to identify 9516 SNPs in the Joshua tree genome. We then performed a GWAS to find loci associated with variation in style length. Fst-outlier analysis identified 1797 loci putatively under selection. Finally, we performed Bayesian Genomic Clines analyses to better characterize the nature of the selection acting within the hybrid zone. Together, these analyses suggest that loci associated with style length are overrepresented among those under differential selection in the two subspecies, and are also likely to experience disruptive selection in the hybrid zone. These results support the Joshua tree-pollinator interaction as a source of reproductive isolation, potentially contributing to speciation in this group.

Adaptive Ecotypic Variation and Genetic Divergence of a Widespread Grass *Andropogon gerardii* across a Great Plains' Climate Gradient

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Local adaptation is a fundamental phenomenon in evolutionary, conservation, and global climate change biology, with applications for restoration and climate adaptation. Local adaptation is defined as ecotypes from local populations outperforming non-local transplants. Our research deals with

adaptive variation in drought tolerance and local adaptation of an ecologically dominant grass big bluestem *Andropogon gerardii* across the Great Plains' grasslands. This grass represents as much as 70% of prairie biomass and is widely used in conservation plantings over ~ 5 million acres across the Great Plains. Because of the wide geographic distribution across a precipitation gradient (500-1200 mm/yr from western KS to IL, we expect intraspecific variation in *A. gerardii* in response to precipitation. Objectives are to 1) use reciprocal gardens to investigate ecotype differences in vegetative and reproductive traits, 2) characterize genetic divergence among ecotypes, and 3) identify outlier loci and potential candidate genes responsible for divergence. Three ecotypes (central KS, eastern KS, and Illinois) were reciprocally planted in Colby, Hays, and Manhattan, KS, and Carbondale, IL. We evaluated ecotypic differences in vegetative and reproductive features across sites, predicting ecotypes would perform best at "home". Canopy area and height increased from west to east, with no evidence for ecotype differences in Colby and Hays. In Carbondale, the IL ecotype showed an increase indicating local adaptation. In Carbondale and Manhattan, the CKS ecotype flowered 20 days earlier than other ecotypes with greater probability of seed in western sites relative to other ecotypes. IL ecotype produced greatest seed in its home site. CKS ecotype shows traits consistent with drought tolerance—reduced canopy, short stature, and early flowering. PCA shows trait variation sorting along a longitudinal precipitation gradient. Morphology was correlated with seasonal mean precipitation and temperature, elevation, and size of precipitation events. To characterize genetic divergence, we used Genotyping-by-Sequencing to identify 4,641 SNPs. STRUCTURE showed evidence for K=3. The IL ecotype existed as a distinct genetic group while the Kansas ecotypes showed admixture. Outlier analysis in Bayescan identified 373 outlier SNPs showing a signature of divergent selection. Of the top 1% of outliers identified in a Bayenv-climate association analysis, SNPs were primarily associated with seasonal diurnal temperature variation and seasonal precipitation. We mapped our GBS tags to the genome of *Sorghum bicolor*, the closest sequenced relative of bluestem. Using TASSEL, we identified SNPs associated with adaptive traits: flowering (1), blade width (1), canopy area (10), leaf nitrogen content (1) and height (22). Key candidate genes of interest that were both outliers and identified in our genotype-phenotype association include: nitrogen content-glutamate synthase (nitrogen assimilation), height-GA1 (internode length), and emergence-WUSCHEL transcription factor (development). Further characterization of these candidate genes may corroborate observed functional differences among ecotypes in nitrogen economy, height and biomass, and development. These results combining phenotypic reciprocal gardens and genetic studies provide insight into plasticity of adaptive traits and genetic divergence and may help inform sourcing for grassland restoration in future drier climates.

Uncovering the genes driving sex determination in a self-fertilizing hermaphroditic fish, *Kryptolebias marmoratus*

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The mangrove rivulus fish, *Kryptolebias marmoratus*, is the only known self-fertilizing hermaphroditic vertebrate. Due to the preferential self-fertilization, naturally homozygous lineages arise after several generations of selfing. Self-fertilization plus rivulus' diverse behavioral repertoire, unique population genetic structure, and its extraordinary sexual plasticity, whereby genetically identical animals can exist as either hermaphrodite or male, makes it an emerging model vertebrate system with potential to address fundamentally important questions using genomics approaches. Rivulus exist predominantly as hermaphrodites but in some populations males reach frequencies up to 25%. Sex determination is temperature dependent; water temperatures below 20°C yield higher percentages of individuals developing as male. These features provide a unique opportunity to study patterns of gene expression underlying developmental sexual plasticity. Here, we describe the assembly of the 980

megabase genome and leverage the generation of a high quality draft assembly to study gene expression in embryos in response to temperature treatments. To do this, we performed RNAseq on embryos from three isogenic lineages exposed either to cold (20°C) or warm (25°C) water temperatures throughout development. We also collected embryos before and after the critical period during which sexual phenotype is temperature sensitive. This experimental design allowed us to examine gene expression before and after sex has been established. Since colder water temperatures govern maleness, we predicted increased expression differences in the cold, pre-critical group versus all other treatment groups. We find large suites of genes changing in response to cold temperature. This experiment is an important first step towards understanding the genes that govern sex determination in vertebrates.

Ecological field studies and copy number variation (CNV) analyses shed light on the selective mechanisms maintaining North American white clover cyanogenesis clines

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White clover (*Trifolium repens*) is a widespread, perennial legume that is native to Europe and was introduced to North America within the last 500 years, where it has repeatedly evolved climate-associated clines in cyanogenesis, the ability to produce hydrogen cyanide upon tissue damage. In these clines, higher proportions of cyanogenic plants occur in warmer and more arid climates. Two genes, *Ac* and *Li*, encode the two biochemical components required for cyanogenesis, and both loci have evolved gene deletion alleles, thus creating four possible cyanotypes: *Ac+Li+* (cyanogenic), and *Ac+li-*, *ac-Li+*, and *ac-li-* (acyanogenic). Additionally, both *Ac* and *Li* exhibit copy number variation (CNV; from 0-7 copies). We used field experiments, wild population sampling, and genetic analyses to assess the selective and genetic factors that shape cyanogenesis cline evolution. Field experiments were conducted at Washington University's Tyson Research Center, located near St. Louis, MO. In a germination experiment utilizing 218 wild samples of seeds collected from 20 sites across North America, we found that a higher proportion of cyanogenic seedlings survived in a field plot at Tyson vs. greenhouse facilities, resulting in increased frequency of the *Li+* allele in the field at early life stages. Using replicate cuttings from 161 adult genotypes that were sampled across 15 sites from Florida to British Columbia and grown from seed in the Washington University greenhouse facilities, we performed a common garden experiment at Tyson; plants were assessed for herbivory and fitness traits throughout the 2015 field season. We found that cyanogenic plants experienced significantly less herbivore leaf damage than acyanogenic plants. Fitness measurements for these 161 genotypes began on April 11 and are ongoing. Once completed, we will assess relationships between herbivory, fitness, CNVs, and seasonal climatic fluctuations. Lastly, we collected ~50-60 wild samples from each of four climatically distinct North American locations (Gainesville, FL; Ardmore, OK; St. Louis, MO; and Duluth, MN). We found a positive correlation between *Ac* CNV and aridity, as well as a positive correlation between *Li* CNV and minimum winter temperature; these results suggest local climatic adaptation through selection on CNVs. Future work will utilize genome-wide SNP data, population genomics with more extensive wild sampling across North America, and QTL mapping of fitness traits in reciprocal common gardens to identify SNPs associated with local adaptation and to further assess the adaptive role of CNVs in North American white clover.

The interaction between *Caenorhabditis elegans* and the bacterial pathogen *Stenotrophomonas maltophilia*.

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Nematodes play an important role in various habitats where numerous factors serve to shape their communities. One such factor is the potentially pathogenic nematode-prey interaction. This project is focused on the elucidation of the genes that the bacterivorous nematode *Caenorhabditis elegans* employs to respond to the emerging nosocomial bacterial pathogen *Stenotrophomonas maltophilia*. The study of this interaction has ecological and medical relevance as *S. maltophilia* is found in association with *C. elegans* and other *Rhabditids* in the wild and, has been isolated from various clinical sources. A virulent *S. maltophilia* strain JCMS requires the action of several *C. elegans* conserved innate immune pathways that serve to protect the nematode from other pathogenic bacteria. However, insulin-like DAF-2/16 signaling pathway mutants that are typically pathogen resistant are susceptible to JCMS, and several DAF-2/16 regulated genes are not significantly differentially expressed between JCMS and avirulent *E. coli* OP50. We have identified mRNA transcripts expressed under different bacterial treatments to find genes that might explain how JCMS evades the DAF-2/16 pathway. The identified set includes 438 differentially expressed transcripts, representing 395 different genes, among pairwise comparisons of wild-type nematodes fed OP50, JCMS or avirulent *S. maltophilia* K279a. Candidate genes were nominated from this list of differentially expressed genes using a probabilistic functional connection model. Six of seven genes that were highly connected within a gene network generated from this model showed a significant effect on nematode survival by mutation. Of these genes, *C48B4.1*, *mpk-2*, *cpr-4*, *clec-67* and *lys-6* are needed for combating JCMS, while *dod-22* was solely involved in response to K279a. Only *dod-22* had a documented role in innate immunity, which demonstrates the merit of our approach in the identification of gene candidates. To a lesser extent, we have also focused on the identification of virulence factors and the mode of action employed by *S. maltophilia*. JCMS virulence requires *rpfF*, *xps* and involves living bacteria that accumulate in the intestinal lumen. Additionally, the bacterial secretion encoding genes *cs*, *p773*, *p1176*, *pi1y1* and *xdi* are involved in JCMS evasion of *daf-2*. In summary, we have discovered a novel host-pathogen interaction between *C. elegans* and *S. maltophilia* JCMS, revealing genes that are involved on each side of the interaction.

Balancing selection and convergent evolution in an antimicrobial peptide

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Conventional thinking has been that antibacterial peptides (AMPs) are functionally redundant and evolutionarily dispensable at the individual gene level. In *Drosophila*, this inference has been drawn from observations that antibacterial peptide genes show low rates of amino acid divergence between species and high rates of genomic duplication and deletion, and that genetic variation in individual AMP genes makes little or no contribution to organism-level defense phenotypes. In a recent genetic mapping project, however, we identified a serine/arginine polymorphism in the *Diptericin A* gene of *Drosophila melanogaster* that is highly predictive of resistance to specific bacterial infections. The same amino acid polymorphism is segregating in the *Diptericin A* gene of the sister species *D. simulans*, having arisen convergently by independent mutation of the homologous codon. The serine allele confers resistance to bacterial infection in both *D. simulans* and *D. melanogaster*. Examination of the larger *Drosophila* phylogeny reveals that the arginine mutation has arisen independently at least 5 times in the genus. We have additionally found multiple *Diptericin A* loss-of-function mutations segregating at high frequencies in *D. melanogaster* and *D. simulans* and individuals bearing null alleles are highly susceptible to infection. These observations have led us to

reevaluate the supposed mode of evolution of *Diptericin* and other antibacterial peptide genes. We now favor the hypothesis that AMP genes evolve under a trench warfare model, where the selection pressure favoring alternative amino acid states fluctuates over time and space depending on pathogen pressure. Previous screens for molecular adaptation have explicitly tested for adaptive divergence and would fail to detect convergent or balanced mutations. The frequent incidence of loss-of-function alleles in nature suggests that AMP function in immune defense is balanced by deleterious consequences in the absence of infection, and serial pseudogenization and duplication-subfunctionalization may explain the rapid gene family dynamics.

A genome wide scan for incompatibilities in hybrid fish populations

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A fundamental question in Evolutionary Biology is the question of how species form and remain distinct. According to the Biological Species Concept, species are groups of interbreeding natural populations that are reproductively isolated from other such groups and interbreeding between species is prevented by a variety of isolating mechanisms. Defining speciation in terms of the evolution of reproductive isolation allowed Dobzhansky and Muller (and before them Bateman) to propose the so called “BDM incompatibility” model to explain what has been called “Darwin’s Dilemma” -- that is, how reproductive isolation could evolve without traversing a fitness valley. The answer according to this model is epistasis – notably, derived substitutions can accumulate along two lineages, by whatever mechanism, and it is only when hybrids are formed that derived (or ancestral) substitutions will be exposed to each other for the first time, potentially causing a reduction in the fitness of hybrids. Negative epistatic interactions between genes from different parental genomes decrease the fitness of hybrids and can limit gene flow between species. However, little is known about the number and genome-wide distribution of genetic incompatibilities separating closely-related species. To detect interacting genes, we perform a high-resolution genome scan for linkage disequilibrium between unlinked genomic regions in naturally occurring hybrid populations of swordtail fish. We estimate that hundreds of pairs of genomic regions contribute to reproductive isolation between these species, despite them being recently diverged. Many of these incompatibilities are likely the result of natural or sexual selection on hybrids, since intrinsic isolation is known to be weak. Moreover, patterns of genomic divergence at these regions imply that genetic incompatibilities play a significant role in limiting gene flow even in young species.

Age-related change in cold stress tolerance in *Drosophila melanogaster*

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Organisms occur in environments that vary spatially and temporally throughout their lifespans, and the genetic architecture of ecologically important traits plays a critical role in the change in trait expression through the aging process. Resistance to cold stress is one important fitness trait that is expected to decline through ontogeny; however this general expectation is based on the response of a small number of *Drosophila* isogenic lines. To further characterize this change in cold stress resistance, we performed rapid acclimation experiments (i.e., rapid-cold hardening or RCH) on 100 isogenic lines of the *Drosophila melanogaster* in the *Drosophila* Genetic Reference Panel (DGRP) at early (one-week-old) and late (four-weeks-old) age. Consistent with previous investigations of RCH and the DGRP lines, we observed significant variation among lines at both early and late age points. In addition, cold stress resistance differs significantly ($p < 0.05$) between early and late aged flies. However, the direction of the change in expression of cold stress resistance varies among lines, with several lines increasing in cold stress resistance as they age. The genetic architecture underlying

the response to cold stress also changes with age, with several unique SNPs at each age. Analyses of genetic variance indicate that aging in these lines is most likely caused by mutation accumulation (the accumulation of deleterious alleles in a population that are expressed late in life), and association mapping further supports this hypothesis. Further investigation of fitness consequences associated with cold tolerance suggests that the RCH response is positively correlated with courtship efficiency ($p < 0.05$), while basal levels of cold tolerance are negatively correlated with courtship efficiency ($p < 0.1$).

Selection scans identify genomic regions associated with adaptation to environment in *Populus balsamifera* (L.)

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Populus balsamifera has a large geographic range and local populations occupy distinct locations along strong environmental gradients of climate and photoperiod. Local adaptation to environment is widespread in forest trees, and here, we seek to understand the genomic basis of adaptation to environment across the range of this boreal forest tree. We analyzed 437 individuals collected from 63 populations, and obtained genome-wide SNP data from >150K loci using genotype-by-sequencing at 48-plex. Population structure was estimated using maximum likelihood clustering (ADMIXTURE) and discriminant analysis of principal components (DAPC). Tests for local adaptation manifest as F_{ST} outliers and SNP-environmental associations were estimated with Bayescan and LFMM. We identified population divergence between eastern North American, central, and western North American populations. In order to minimize the effect of neutral allelic variation on selection scans, we subset our data and focus on core populations in the central range, and search for parallel adaptation in two longitudinal samples. We find genomic regions suggesting novel, locally adapted loci to longer growing seasons and warm, dry environments which may be useful for integrating into poplar breeding programs under future climates.

Short term adult plasticity in *Drosophila melanogaster* and its role in climatic adaptation

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Gene expression shows plasticity in response to environmental heterogeneity. These patterns of expression may have been shaped and maintained by natural selection. To test this hypothesis, we investigated two geographically distinct populations of *Drosophila melanogaster* from eastern North America and determined their gene expression patterns in response to certain environmental variables. The populations were given a short-term exposure to unique temperature and photoperiod regimes, after which differential patterns of expression were identified. We observed a differential response of gene expression between the Northern and Southern populations where the Southern population showed increased levels of plasticity in response to both temperature and photoperiod treatments. A differential expression response was also apparent in how the populations responded to specific temperature (Hot and Cold) and photoperiod (Long and Short Day) conditions, providing further evidence for spatially varying selection as the agent driving patterns of gene expression. Functional analysis revealed similar gene ontology categories enriched in both populations. This indicates a parallel mechanism of response at the categorical level and suggests that differences in response plasticity may be attributed to differences in individual genes within a category. We investigated this hypothesis by comparing our dataset with a complimentary dataset detailing the genomic differentiation in the same Northern and Southern populations. As predicted, we found significant enrichment of SNPs in the upstream region of the differentially expressed genes, indicating that the expression variation is driven by cis-regulatory modulation. This study provides evidence for differential expression response to environmental variables based on the geographic origin of the populations, which is consistent with patterns of local adaptation based on selection due to climatic variables.

Morph-specific daily cycles of gene expression in a wing-dimorphic cricket: Functional genomics meets chronobiology

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The role of circadian rhythms in organismal adaptation is a poorly understood topic in evolutionary biology. Increasingly, studies are comparing differences in gene expression between ecologically important phenotypes to understand the molecular basis of adaptation. Yet, these studies typically ignore the potential importance of adaptive daily fluctuations in gene expression. The wing-dimorphic cricket, *Gryllus firmus*, has been extensively studied with regard to physiological, endocrine and biochemical specializations for flight versus reproduction, and their trade-off. In a previous study, the long-winged-dispersing morph (LW), that delays egg production, exhibited a dramatic daily cycle (>10-fold change) in the blood level of juvenile hormone (JH), a key metabolic and reproductive hormone. By contrast, the short-winged, flightless morph (SW), which exhibits enhanced egg production, showed a much smaller cycle (ca. 1-fold change). Because JH potentially regulates the expression of numerous traits, we used RNA-Seq and qPCR to assess the extent of corresponding morph-specific differences in daily rhythms of global gene expression. Transcript abundance of the same 7,500 genes was quantified in the fat body (major organ of intermediary metabolism) of the LW and SW morphs of *G. firmus* under a 16 h light: 8 h dark photoregime in the laboratory. Samples were taken early in the photophase (JH titer low and equivalent in both morphs) and late in the photophase (JH titer much higher in the LW than SW morph). The LW and SW morphs differed significantly in gene expression during both early and late in the photophase. Importantly, the LW morph exhibited a significantly greater daily change in global gene expression (6.9%) compared to the SW morph (1.9%; $P < 0.001$). Indeed, the magnitude of daily change in gene expression in the LW morph was greater than the difference in gene expression between the LW and SW morphs at the same time of day ($P < 0.001$). qPCR of selected genes verified that morph-specific differences in gene expression measured at two time points on one day reflect cyclic changes over multiple days. Genes exhibiting morph-specific cycles of gene expression included those encoding components of hormonal or intracellular signaling (e.g., insulin-like peptide; angiotensinogen converting enzyme, MAP kinase), components of the circadian clock (timeless), and metabolic enzymes (glucose oxidase, phosphofructokinase-fructose biphosphatase). These morph specific changes in JH level and gene expression may be important components of morph-specific adaptation for circadian flight: only the LW morph flies, and only at night. Given the prevalence of circadian rhythms, many ecologically-important polymorphisms likely contain morphs that differ with regard to daily cycles of organismal function, behavior, and life history. Adaptive circadian cycling in gene expression that underlie cyclic changes in these traits is potentially an important but virtually uninvestigated aspect of organismal adaptation.

Reproductive isolation and environmental adaptation shape the phylogeography of Mountain Pine Beetle (*Dendroctonus ponderosae*)

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The Mountain Pine Beetle (MPB) is an eruptive pest of various pine species native to western North America, reaching from northern Mexico to Canada along the Rocky Mountain and Sierra Nevada ranges. Current MPB ranges are expanding as a result of climate change and infestations cause extensive damage to susceptible forests. Applying genome-wide RAD sequencing to over 700 individuals from 37 populations, we are examining phylogeographic structure, gene flow, and adaptation across the range. Strong population structuring occurs between geographic regions with extensive admixing occurring in the sky island populations residing in the Great Basin. However results also

suggest that there are distinct genetic boundaries between some geographically proximate regions. Experimental crossing experiments have previously found postzygotic isolation between geographically distant populations. Moreover crosses between the geographically close populations of Oregon and Idaho also showed a reduction in hybrid male fitness despite their proximity. Population structuring of the autosomes and sex chromosomes show that these populations are permeable to gene flow at autosomal sites but Y chromosome sites show clear disjunction between regions. Population structure suggests that separate genetic lineages occur across similar latitudes in the Rockies and Sierra Nevada. Leveraging a complementary QTL study of development time, a crucial trait in seasonal synchronization, we also discuss tests for parallel adaptation across lineages.