

ECOLOGICAL GENOMICS

Symposium

Poster Abstracts

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Undergraduate Posters

1

Bacterial Motility Experimentally Evolves in Response to Chemoattractant Selection and Phage Predation

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Bacteria are ubiquitous in the biosphere, and bacterial motility plays a vital adaptive role in numerous contexts, including ecological adaptation and pathogenicity.^{1,2} Bacterial motility in response to chemical stimuli — chemotaxis — can be positive or negative, if the bacteria move towards or away from higher stimuli concentrations, respectively. Despite the importance of chemotaxis, little is known about the effects of common selective pressures on this process, including selection for motility towards nutrients or away from bacteria-specific viruses. We selected for motility towards nutrients in *Escherichia coli*, where positive chemotaxis is known to occur for the amino acids Serine and Aspartate.³ 10,000 generations of experimental evolution for increased taxis towards one of the amino acids and away from the other were performed. Compared to the ancestral strain, evolved isolates showed increased chemotactic responses either specifically to the selected-for amino acid or non-specifically, but all isolates demonstrated highly concentration specific responses. We selected for motility away from bacteria-specific viruses in *Pseudomonas aeruginosa*, where positive and negative chemotaxis exists for a wide variety of compounds.⁴ Bacteria and lytic bacterial viruses were inoculated into a soft agar plate spatially separated and allowed to spread before transferring a series of equidistant points to new media. Over time, the bacteria-virus coevolution led to a stable spatial structure: resistance to the virus decreased with distance from the initial viral inoculation point, while motility increased with distance. Future work will determine if there exists a fitness tradeoff between resistance and motility.

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2. Josenhans, C. & Suerbaum, S. The role of motility as a virulence factor in bacteria. *Int. J. Med. Microbiol.* 291, 605–614 (2002).
3. Mesibov, R. & Adler, J. Chemotaxis toward amino-acids in *Escherichia coli*. *J. Bacteriol.* 112, 315–& (1972).
4. Sampedro, I., Parales, R. E., Krell, T. & Hill, J. E. *Pseudomonas* chemotaxis. *FEMS Microbiol. Rev.* 39, 17–46 (2015).

2

Host cues establish selective conditions favoring the experimental evolution of avirulent agrobacterial cheaters

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Cooperation often involves the production of a public good, wherein individuals contribute to the costly production of a resource that is widely available. The cooperative pathogenesis of the generalist plant pathogen *Agrobacterium tumefaciens* is conferred by genes encoded on the Ti virulence plasmid. Infection by *A. tumefaciens* involves genetic transformation of plant host cells and causes the plant to release nutritious opines. Catabolism of these opines requires another set of genes carried on the Ti plasmid. However, public goods provide an opportunity for cheaters who do not pay the cost yet receive the benefits of cooperative action. To better understand the genetic mechanisms and environmental conditions allowing for the origin of cheating in this system, we experimentally evolved populations of *A. tumefaciens* with or without vir-gene induction by a plant cue, and with or without opine nutrients. Pathogenesis gene expression consistently and rapidly declined over time among all populations evolving in environments in which vir-genes were induced, yet opine catabolic function was only rarely lost. These results suggest that de novo mutation can readily introduce cheating phenotypes that successfully invade initially-cooperative populations in environments where plant cues are present and that selection efficiently eliminated costly gene expression while maintaining beneficial functions.

3

Genetic architecture of multi-trait adaptation to geothermal soils in yellow monkeyflowers of Yellowstone National Park

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Microgeographic adaptation provides a fertile context for understanding the genetic processes that shape natural variation and contribute to biological diversity. In particular, soil heterogeneity can provide sufficient selective pressure for divergence in the face of gene flow, leading to major life history transitions and novel trait evolution. *Mimulus* (monkeyflowers) are an emerging model genus for ecological genomics, due to tremendous diversity, experimental tractability, and a wealth of genomic resources. In Yellowstone National Park, *Mimulus guttatus* occurs in both geothermal soils and nearby nonthermal bogs; thermal populations have evolved a distinct suite of traits, including both novel adaptations (e.g., dwarfism) and shifts parallel to independent transitions elsewhere in the species complex (e.g., annuality, selfing). Using a combination of replicated PoolSeq and targeted genetic mapping, we have identified a 24-gene region on linkage group 6 that explains 29% of variation in rhizome count among F2 individuals (N=364). This major QTL is distinct from the widespread chromosomal inversion (on LG8) that explains life-history differentiation elsewhere in the range. This locus also explains 22% of variation in internode length, and likely plays a major role in the evolution of both dwarfism and annuality in Yellowstone *M. guttatus*. This finding contributes to our understanding of adaptation to extreme habitats, as well as the genetics of parallel trait evolution.

4

Polyploidy and diversity in Phlox: unusual patterns of genome size in *P. nana* Nutt. (*Polemoniaceae*)

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Polyploidy is prominent in the genus *Phlox*, and some species exhibit variation in ploidy levels, or cytotypic variation. *Phlox nana* is of particular interest because diploid, tetraploid and hexaploid populations occur across parts of the species distribution in the desert southwest. A recent study highlighted two areas wherein inference of cytotypic patterns was challenging: the Davis Mountains region of West Texas, and a site on the Pecos Plains of New Mexico ("Caprock"). Study of plants in these areas yielded unusual genome size measurements that did not enable clear inference of ploidy levels, potentially due to presence of multiple ploidy levels, variation in genome size at homoploid levels, and/or presence of B chromosomes. This study intensively sampled plants in spring of 2015 and 2016, including nine sites in the Davis Mountains (generally five individuals per population) and 13 individuals at the Caprock site. Flow cytometry was used to estimate the genome sizes of samples, and chromosome counts were conducted on individuals from both areas. Both diploid and tetraploid populations occur in the Davis Mountains, sometimes in close geographic proximity, while evidence indicates that Caprock individuals are tetraploid. Interestingly, the tetraploid genome size varies across these areas. Most chromosome counts from the Davis Mountains do not reveal the presence of B chromosomes, but B chromosomes are common in the Caprock population. Overall, this study clarifies patterns of cytotypic diversity in *P. nana*, and points to factors that must be considered in geographical studies of cytotypic variation. It also contributes to a framework for ongoing study examining genetic, ecological, and morphometric diversity relative to polyploidy in this system.

5

Differential enzyme and physiological response to heavy metal stress in mine and old field populations of *Andropogon virginicus*

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High levels of heavy metals in soil and water induce stress in plants, resulting in physiological and enzymatic response. Heavy metals interrupt photosynthesis and create oxidative stress, causing an increase in production of antioxidant enzymes such as ascorbate peroxidase and catalase. Large heavy metal mine wastes in the Tar Creek, OK Superfund site, an abandoned Lead and Zinc mine, provide a putative source of selective pressure on plants to genetically adapt to physiological and oxidative stress imposed by heavy metals. Native old field grass *Andropogon virginicus*, grows widely in mine and old field (uncontaminated) sites and was studied to assess physiological and enzymatic response under zinc stress and whether these responses differed between mine and old field populations due to adaptive genetic response. We predicted that *A. virginicus* populations from Tar Creek would show a differential response to heavy metals such that the plants from the metal population would be less affected by heavy metals compared to control old field plants, signaling an adaptive genetic response. *A. virginicus* seeds were harvested from metal contaminated Tar Creek sites and old fields in KS and used in a greenhouse study. Fifty plants, comprised of plants from 5 mine and 5 old field populations (total 150 plants), received 20 mL of either 0, 60, or 100 ppm zinc solution daily for ten days as zinc nitrate. Physiological data were collected to assess chlorophyll absorbance and photosynthetic yield and harvested tissue samples were spectrophotometrically assayed for antioxidant enzyme levels. After zinc treatment, chlorophyll absorbance and photosynthetic yield decreased in both mine and old field plants, without any difference between mine and old field source populations. Surprisingly, levels of the antioxidant enzymes ascorbate

peroxidase and catalase did not change with zinc treatment. However, plants from mine populations had lower levels of enzymatic activity for both enzymes at all treatment levels suggesting differentiation on mine and old field populations in key stress enzymes. Zinc conditions induce physiological stress, but not differential levels of ascorbate peroxidase and catalase activity. Further study is needed to assess other antioxidant enzyme pathways and their potential role in the response of *A. virginicus* to heavy metal stress.

6

A comparison of the effects of continuous versus patchy geographic distributions on the structuring of genetic variation in two bat-pollinated, wind dispersed *Agave* species (*Agavaceae*)

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Many factors may affect the structuring of genetic variation in plants, including their pollinators, seed dispersers, and the continuity of their geographic ranges. In this study we examined the relative effects of these three factors in structuring genetic variation *Agave palmeri* (diploid) and *Agave parryi* (autotetraploid), which share the same pollinator and seed dispersal mechanism, but differ in the continuity of their geographic distributions. Both of these species are thought to be predominantly bat pollinated and wind dispersed, which because they allow long-distance migration are generally thought to have the effect of reducing population differentiation. However, the two species differ in their distributions, which may have contrasting effects on the structuring of genetic variation; *A. palmeri* has a widespread distribution throughout lower elevation areas in northern Mexico, southern Arizona, and New Mexico, whereas *A. parryi* is more restricted in its distribution and is found only in higher elevations on sky islands in central Arizona, southwestern New Mexico, and the highlands of Mexico. In this study, we examined whether the patchy distribution of *A. parryi* has caused stronger structuring of genetic variation relative to *A. palmeri*, or whether pollinators and seed dispersal reduces these effects. We sampled 12 populations of each of the two *Agave* species from Arizona and New Mexico, genotyped individuals at 9 microsatellite loci, and analyzed patterns of genetic diversity and structure. Results show genetic structure in populations of *A. parryi*, forming 3 distinct clusters that correspond to geographic locations. Since genetic variation is structured only in *A. parryi*, we can conclude that the patchy distribution limits gene flow in *A. parryi* even though pollen and seeds should be able to allow genetic material to travel long distances.

All Other Posters

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The Effect of Entomophagous Pathogens *Beauveria bassiana* and *Metarhizium anisopliae* on *Helicoverpa zea* larval gene expression

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Helicoverpa zea is attacked by variety entomophagous pathogens such as bacteria, virus or fungi. We tested pathogenicity of potential fungal pathogens such as *Beauveria bassiana* or *Metarhizium anisopliae* on *H. zea* growth and survival. Third instar caterpillars feeding on artificial diet inoculated with either of these two fungal suspensions were compared to caterpillars feeding on artificial diet without either suspension as our control treatment. In addition, two environmental conditions were tested in separate experiments. In the first experiment the incubator temperature was set to 37 C° without extra humidity added to the diet cups containing the caterpillar. After four days of exposure we determined no mortality and little difference in growth among the treatments. The second experiment was repeated in the same manner, however the incubator was set to 25 C° and filter paper was treated with autoclaved distilled water that was added to the diet cups to increase humidity. In this experiment after four days of exposure there was dramatic growth reduction and mortality for the caterpillars that fed on *B. bassiana* than *M. anisopliae* and the control treatment. We then looked at gene expression of caterpillars such as *B. bassiana* in comparison to the control treatment. Caterpillars after being weighed were flash frozen and ground up in Liquid Nitrogen to produce a fine powder for total RNA purification for quantitative real-time PCR analysis. The gene expression analysis revealed that caterpillars treated with *B. bassiana* resulted in a general reduction in several digestive related genes such as aminopeptidases, trypsin-like serine proteases, and lipases in comparison to the control treatment. In additions, genes related to immunity and particularly detoxification genes such as cytochrome P450 and glutathione S-transferase were down-regulated as a result of exposure to the fungi. This study demonstrated that *H. zea* feeding on *B. bassiana* has more mortality than when feeding on *M. anisopliae*. We saw wide spread down regulation of genes related to digestion and metabolism and further demonstrates *B. bassiana* potential as biological control agent for *H. zea*.

8

Differential Gene Expression of *Helicoverpa zea* (tomato fruitworm) in Response to Feeding on Different Types of Tomato (*Solanum lycopersicum*) Plant Tissues

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Plants can evolve defenses to caterpillar feeding. As a response to these defenses, the herbivore evolves counter-defenses. Most previous studies have focused on the leaves and/or fruits of the tomato plant (*Solanum lycopersicum*). Here I am investigating the response of *Helicoverpa zea* (tomato fruitworm) after feeding on different types of tomato plant tissues. We are looking at the gene expression of caterpillars fed on tomato leaves or fruits compared to caterpillars fed on artificial diet. Caterpillars were weighed before and after herbivory. Average caterpillars weight gains were significantly higher for the artificial diet, closely followed by caterpillars that fed on the tomato fruits. Caterpillars that fed on tomato leaves were dramatically smaller. Microarray analysis showed 4,008 genes were significantly stimulated. The transcriptomic expression for compensatory gene expression for caterpillars that fed on leaves in comparison to fruits was particularly dramatic for digestive genes such as proteases, lipases and stress related genes. This is likely due to tomato leaves having higher levels of plant defenses and lower well nutritive available than fruits. All in all, gene expression analysis gives us a better understanding of caterpillar counter-defenses in relationship to its host.

Predicting Phenotypic and genotypic response to the dominant prairie grass *Andropogon gerardii* to climate change in the Central US grasslands

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Kansas State University, Division of Biology (MK); Southern Illinois University (SB); Missouri Botanical
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Andropogon gerardii is an ecologically dominant grass in the Midwest. With its wide distribution across climate gradients, it becomes urgent to understand phenotypic and genetic variation to predict response to current and future climates. We characterized phenotypes and genotypes of populations across precipitation (40-119 cm/yr) and temperature gradients (5-15°C) and incorporated intraspecific variation into species distribution models of current and predicted response under climate change. We grew plants from seed in the greenhouse and measured blade width, height, biomass, and chlorophyll absorbance, and genotyped to assess genetic diversity and divergence. We used phenotypes as input into distribution models to predict current and future phenotypes under climate change and investigated genetic divergence and outlier SNPs. Preliminary analysis shows a main effect among population phenotypes (height, width, biomass, and chlorophyll absorbance $P < 0.001$). The phenotypic cline across populations was mainly explained by rainfall (PC1 and PC2 explained 78.8% of variability. PC1 was associated with height, blade width and biomass, and PC2 associated with chlorophyll absorbance). The distribution model for 2070 predicts that short-statured, dwarfed phenotypes found in the present-day dry shortgrass prairies of the west will become favored ~800 km eastward while robust, tallgrass phenotypes of current core will become favored ~700 km northeastward of their current location. We identified 7,318 SNPs and population structure suggests four genetic groups (Western Plains, Ohio Valley, Upper Midwest, Northern Plains). The greatest genetic diversity currently occurs in the Central Great Plains where genetic groups converge. Bayenv2 analysis shows the top 1% (73) of SNPs are associated with various aspects of precipitation. These results suggest that sourcing of plant material for grassland and rangeland restoration should anticipate future climates.

10

Effect of Plant Defense Hormones Jasmonate and Salicylate on Caterpillar Gene Expression

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Jasmonate and salicylate are important and ubiquitous plant defense hormones that stimulate a wide range of plant defenses. Jasmonate is stimulated by wounding, particularly caterpillars, and results in the stimulation of anti-nutritive defenses that can reduce the growth of herbivores. Salicylate is primarily associated with plant pathogens and smaller piercing-sucking herbivores such as aphids and whiteflies. While it is well known that these plant hormones stimulate signal cascade that synthesis of allelochemicals that alter caterpillar gene expression it is not clear whether these plant hormones directly effect caterpillar gene expression. In this study, we determined the effect of jasmonate and salicylate on the caterpillar's gene expression. We chose a generalist herbivore, corn earworm (*Helicoverpa zea*) as neonates and allowed them to feed on an artificial diet containing either jasmonate or salicylate for seven days. The control was a diet free of these added hormones. Caterpillars weights were reduced after feeding on the jasmonate treated diet in comparison to control or salicylate treated diet. We then flash froze and purified total RNA from the caterpillar to measure gene expression for those caterpillars with qPCR. Digestive genes such as trypsin, lipase and glucose oxidase were particularly up-regulated for caterpillars that fed on the jasmonate treated diet in comparison to salicylate and the control diet. Additional gene expression will be discussed, but it appears the caterpillars are able to respond to jasmonate priming for defenses that are normally associated with the plant hormone. However, in a diet void of this, plant defenses are maladaptive.

11

What Perceptions and Contexts Underpin Ghana's BioEthical Challenges in the 21 Century?

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Gaps which often hinder the prosecution of the biotechnology agenda in developing economies as Ghana are broadly technological, organisational and orientational in nature. More important than these gaps are the entrenched perceptions which strongly underpin these gaps. As whimsical and diverse as these perceptions may be across key stakeholders (government, private investors, professionals and NGOs), they sure play an important role in the narrowing of gaps in developing agro-based economies as Ghana's. The earlier these perceptions are concertedly and unanimously identified and addressed through consensus building and dialogue, the quicker Ghana can be brought to the path to narrowing or closing yawning gaps in its biotechnology agenda for the timely meeting of its sustainable development goals.

12

Oxygen transport in hydrogen sulfide-rich environments: myoglobin and hemoglobin gene expression and evolution

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Hydrogen sulfide (H₂S) is a potent toxicant that creates extreme environmental conditions in deep sea hydrothermal vents and freshwater sulfide springs. H₂S binds cytochrome c oxidase (COX), a complex of the electron transport chain, and this reaction results in the inhibition of aerobic ATP production. Organisms have adapted to H₂S environments through a variety of mechanisms, including increased efficiency of detoxification mechanisms and modification of toxicity targets in the electron transport chain. However, H₂S interacts with many biologically important proteins, and it is unknown whether additional targets of H₂S toxicity have been modified to maintain function under these conditions. H₂S readily binds to the heme group of the oxygen transport proteins hemoglobin and myoglobin, ultimately reducing oxygen binding and transport efficiency. This effect is important in an ecological context, as H₂S-rich environments are also characterized by hypoxia. We investigated eleven evolutionary independent lineages of fish of the family Poeciliidae that inhabit sulfide springs, including the genera *Gambusia*, *Limia*, *Poecilia*, *Pseudoxiphophorus*, and *Xiphophorus*. Using RNA-sequencing data, we compared expression levels of hemoglobin and myoglobin genes between sulfide spring populations and closely related non-sulfidic reference populations. Our results show upregulation in some, but not all, lineages of sulfide spring fishes. Additionally, we examined hemoglobin and myoglobin genes for evidence of positive selection. In conjunction with previous work conducted in this system, our study suggests that adaptation to H₂S-rich environments involves modification of multiple physiological pathways.

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Comparative analysis of generalist *Helicoverpa zea* expression and growth on diverse array of host plants

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Millions of dollars a year are spent annually attempting to reduce crop damage from *Helicoverpa zea*, commonly known as the 'corn earworm' or 'tomato fruitworm'. This research aims to understand how *H. zea* larvae compensates on a variety of plants with different defense compounds and tissue types. A series of growth assays were performed with *H. zea* on several economically important plants: artificial diet, leaves from soybeans (*Glycine max*), tobacco (*Nicotiana tabacum*), corn (*Zea mays*), and leaves or fruit of tomato plants (*Solanum lycopersicum*). Additional testing was performed with tomato plants treated with plant defensive hormones jasmonate or salicylate. Third instar caterpillars were weighed after 72 hours of herbivory on their respective plant tissues. The bioassays demonstrated caterpillars grew greatest on artificial diet and tomato fruit in relation to the variety plant leaves. Among the plant leaves caterpillars grew best on tomato leaves followed by tobacco, soybean, and corn leaves respectively. Gene expression was determined with real-time qPCR to measure a variety of compensatory digestive genes, growth, and stress related genes. Like gene expression correlated well to these bioassays where the caterpillars stimulated higher levels of digestive gene expression in aminopeptidase, trypsin, lipase, and glucose oxidase when feeding on plant tissues where growth was lowest. In addition, caterpillar growth related genes such as juvenile hormone inducible protein and ecdysone oxidase correlated to bioassay. Likewise, *H. zea* immune system response genes attacin and gallerimycin were found to be stimulated based on the type of plant. This study shows how gene expression is altered in generalist caterpillar to a wide range of host plants.

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Asymmetry matters: genomic assessment of directional bias in gene flow excludes the apparent influence of exogenous selection for the maintenance of a spruce hybrid zone

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Assessing directional bias in interspecific gene flow might be important in determining the evolutionary trajectory of closely-related species pairs. Using a set of 300 SNPs having variable propensity to cross species boundary, we evaluated the genomic extent and direction of interspecific gene flow in a progenitor-derivative spruce species pair (black spruce and red spruce). A higher rate of gene flow was found from black spruce towards red spruce purebreds than *vice versa*. This asymmetry could reflect the historical gene flow between the two taxa at the time of species inception and during postglacial colonization. A clear asymmetry in introgression was depicted by a greater gene flow between red spruce and hybrids than between black spruce and hybrids. While backcrossing towards red spruce was invariably high across the genome, the actual species boundary is between hybrids and black spruce where gene flow is impeded at those genomic regions impermeable to introgression. Associations between hybrid index and climatic variables (total annual precipitation and mean annual temperature) were tested, as these might indicate a role for exogenous selection in maintaining the species boundary. While an apparent association was found between the hybrid index and precipitation, it collapsed when considered in light of the directional bias in interspecific gene flow. Hence, considering asymmetrical patterns of introgression allowed us to falsify an apparent role for exogenous selection. Although this was not formerly tested here, we suggested that this pattern could result from asymmetrical endogenous selection, a contention that deserves further investigations.

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Gene Expression Observed from the interaction between Corn Earworm (*Helicoverpa zea*) caterpillars and Corn (*Zea mays*) tissues

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Helicoverpa zea, Corn Earworm caterpillars are generalists that can feed on a wide range of plants and their tissues. This experiment aimed to observe the changes to gene expression in by caterpillars caused by feeding on a variety of plant tissues such as the corn fruit, leaves, husk, and silk. Initially, a bioassay was performed where third instar caterpillars fed on these corn tissues in comparison to the artificial diet as an optimal control. From the bioassay we found that caterpillars grew the greatest on the corn fruit in relation to the other corn tissues. Caterpillars were then flash frozen in liquid nitrogen for total RNA purification for caterpillar gene expression measurements with real-time qualitative PCR. A variety of digestive, growth, immunity and detoxification genes were analyzed. Our results showed that caterpillar digestion and detoxification gene expression was highest for the caterpillar treatments with slowest growth - such as leaves and husk suggesting that there was a metabolic cost to the caterpillars overcoming these less suitable plant tissues. Likewise, the gene expression was lowest in caterpillar treatments that were allowed to feed on the corn fruit and the artificial diet suggesting there was very little metabolic costs in consuming these plant tissues. By understanding the gene expression of caterpillars in relation to host plant tissues researchers may develop a better appreciation of the co-evolution of plant herbivore interactions.

16

Exploring Leaf Associated fungal Communities of Common Grassland Plants

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Plant leaves and roots are colonized by large and diverse communities of fungi that can impact important physiological processes of their hosts. These communities have previously been challenging to characterize due to their microscopic scale and difficulty of isolation into pure culture. This project uses next generation sequencing to characterize leaf-associated fungal communities of four dominant C4 grasses of the Great Plains: *Andropogon gerardii*, *Schizachyrium scoparium*, *Sorghastrum nutans*, and *Panicum virgatum*. Leaves were sampled at four sites along a precipitation gradient across an approximately 670km longitudinal transect from central Missouri to northwest Kansas to evaluate the relative effects of host species and collection site on fungal community composition. Repeated sampling in 2015 and 2016 at these sites will allow exploration of inter-annual variation. Total DNA was extracted from leaf samples, fungal barcode markers (Internal Transcribed Spacer – ITS7 and ITS4) PCR-amplified, and generated amplicons Illumina Miseq sequenced. The acquired paired-end sequence data were analyzed using a modified Mothur standard operating protocol. Community richness, diversity and evenness were estimated and compared using nested analysis of variance (ANOVA). Compositional attributes of the communities were visualized using Non-metric Multidimensional Scaling (NMS) and compared with permutational multivariate Analyses of Variance (perMANOVA). Preliminary analyses suggest that diversity is highest at the driest, westernmost site for all included hosts, whereas other sites do not differ. Conversely, evenness is highest at an intermediate site at the more mesic end of the gradient and decreased westward. Initial NMS ordinations indicate that these leaf associated fungal communities are more distinct within sampling site than within host species. This indicates these communities are likely determined by local climatic or soil factors rather than by host species.

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Dynamic shifts in the genetic architecture with age and across traits: Implications for evolutionary theories of aging

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The evolution of aging is a complex process influenced by genetic and environmental variation. Aging is a highly pleiotropic phenotype, affecting not only lifespan but also the ability of an organism to tolerate environmental stress. Although many studies have focused on the evolution of lifespan, few have addressed the shift in environmental stress response phenotypes across age. To understand how aging evolves, we measured environmental stress response phenotypes across ages at both the phenotypic and genetic level in the *Drosophila melanogaster* Genetic Reference Panel (DGRP). Specifically, we measured age-related changes in cold stress survival, cold stress plasticity, starvation resistance, and mortality in young and old flies within the DGRP. We tested the Mutation Accumulation (MA) and Antagonistic Pleiotropy (AP) evolutionary theories of aging and found that MA predictions were broadly supported by the presence of age-related change in the genetic architecture underlying each phenotype between young and old age. Consistent with MA, age-specific associated SNPs for each phenotype were non-overlapping across age, and genetic correlations were either non-significant or significantly positive, demonstrating independence of the effects of age-specific SNPs. Together, these results indicate that the age-related decline in individual stress and fitness phenotypes is primarily driven by age-specific genetic architectures underlying each phenotype in young and old flies. However, when multiple phenotypes were compared across ages, several significant negative genetic correlations were detected between starvation resistance and cold tolerance, and associated SNPs had antagonistic effects between traits across ages. These patterns are consistent with AP predictions, and suggest that AP may have an important influence on aging through trade-offs between phenotypes. Considering all possible phenotypic

combinations in young and old flies, we detected 4 examples of patterns consistent with AP predictions among phenotypes across age while MA predictions were consistently upheld when considering each of the 4 phenotypes across age as well as positively phenotypically correlated phenotypes in 2 cases, suggesting that both completely shifting genetic architectures (MA) and changing genetic effects (AP) explain the variation in stress response phenotypes across age. Subsequent analysis of mutant *D. melanogaster* lines to test the antagonistic patterns detected confirmed our results, suggesting that the evolution of aging is influenced by a combination of MA and AP within and among phenotypes. These patterns of age-related change in SNP effects within and among phenotypes point to a new test for assessing the role of MA and AP which both clarifies previous tests for these mechanisms and provides new insight into the role of natural selection and correlated phenotypes on the evolution of aging.

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Experimental Natural Selection of Big Bluestem Ecotypes Across the Great Plains: Evaluating Local Adaptation Under Realistic Conditions

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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. Our research deals with 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, is the major forage grass for cattle, and is widely used in conservation plantings of ~ 5 million acres across the Great Plains. Because of its wide geographic distribution across a precipitation gradient (500-1200 mm/yr from western KS to IL), we hypothesized for intraspecific variation in *A. gerardii* in response to precipitation as well as strong adaptation of bluestem to home climate conditions. Three big bluestem ecotypes (central Kansas (CKS), eastern Kansas (EKS), and Southern Illinois (SIL)) were reciprocally planted in Colby, Hays, and Manhattan, KS, and Carbondale, IL. Plantings consist of single ecotype plots (seeded with other prairie plants to simulate a natural prairie) and plots with all three ecotypes mixed together. Mixed plots, the focal point of this study, allow for competition among ecotypes and allows for one of the first rigorous tests of local adaptation under natural ecological conditions in which ecotypes compete against each other in different climates in the same competitive plot. Objectives were to 1) evaluate cover and local adaptation in single ecotype community plots, and 2) quantify the outcome of competition between ecotypes in mixed plots. Cover of big bluestem in single ecotype plots show evidence of local adaptation of the xeric CKS ecotype to western Kansas and of the mesicSIL ecotype to Illinois. We genotyped plants from single ecotype plots (i.e. known ecotype) to be used to train a random forest classifier to assign ecotype composition in mixed plots. For the single ecotype plots, we genotyped 348 plants for 1,156 SNPs. For mixed plots of unknown composition, we genotyped 92 plants from 4 replicate blocks in each planting site. After training the random forest classifier showed a cross-validation accuracy of 76.7%. We expected that, if there was no selection due to competition, all three ecotypes would be represented equally in mixed plots across sites. Instead, the random forest model indicated that the xeric CKS ecotype dominated Colby (65.9%) and Hays (68.1%) planting sites while the SIL ecotype dominated Manhattan (55.1%) and Illinois (67.4%) planting sites. These results suggest near competitive exclusion of mesic ecotypes in the xeric sites and vice versa. Taken together, results from single ecotype and mixed plots support evidence for local adaptation of distinct xeric and mesic ecotypes. This is one of the first studies of local adaptation and selection conducted under realistic ecological conditions. Ultimately, these results will provide recommendations on climate-adapted source populations for restoration planting in future warmer and drier climates.

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OXPHOS evolution and ecological speciation in toxic sulfide springs

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Incompatibility between gene products encoded by mitochondrial and nuclear genomes (mitonuclear incompatibilities) is a mechanism of hybrid breakdown hypothesized to serve as a potential contributor to speciation. Oxidative phosphorylation (OXPHOS) consists of genes encoded by both genomes and is responsible for aerobic ATP production, a fundamental cellular process in eukaryotes. Consequently, OXPHOS is a key candidate for the emergence of mitonuclear incompatibilities, because hybridization between divergent populations or species can lead to the breakup of co-adapted mitonuclear gene products. Hydrogen sulfide (H₂S) rich habitats and their inhabitants provide an ideal natural setting for testing hypotheses about the evolution of mitonuclear incompatibility. H₂S is extremely toxic to most organisms due to its inhibition of OXPHOS and consequently results in direct selection on components of the pathway. Direct selection on OXPHOS in replicated lineages of Poeciliid fishes inhabiting sulfidic environments could lead to the coevolution of mitonuclear interacting proteins that do not function when combined with subunits originating from closely related non-sulfidic populations, resulting in hybrid incompatibilities and contributing to ecological speciation. Using transcriptome data and protein structure modeling, we are investigating patterns of molecular evolution and mitonuclear coevolution in the OXPHOS pathways of sulfidic populations. Analyses of molecular evolution consistently indicate relaxed selection on OXPHOS genes in sulfidic populations. Furthermore, some lineages of sulfide spring poeciliids exhibit signatures of strong positive selection on OXPHOS genes.

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Transcriptomic Expression in Tomato Plants in Response to Arbuscular Mycorrhizal Symbioses and Caterpillar Herbivory

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Plants have established a mutualistic relation with Arbuscular mycorrhiza forming fungi (AMF) for more than 400 million years and it is estimated that more than 80% of plant species are able to maintain this symbiosis. AMF are obligate biotrophs which require a plant species for their nutrition and proliferation. In exchange for this symbiosis, the fungi appear to increase plant defenses to both abiotic and biotic stresses that are found in the environment. Also in nature, a tri-trophic interaction occurs commonly between these fungi, its host plants and caterpillar herbivores. Previous evidence supports that AMF fungi stimulates defenses in tomato plants against caterpillars. In our study, I measured the transcriptomic expression of tomato plants (*Solanum lycopersicum*) that were grown with or without AMF in growth chambers under greenhouse conditions with or without a third instar *Manduca sexta* (Lepidoptera: Sphingidae) caterpillars. After 24hrs of herbivory the leaves were frozen in liquid nitrogen, homogenized, and total RNA was isolated from each sample for qPCR analysis. We then measured many of the more well known plant defense, and stress genes. In tomato leaves increased anti-nutritive defense genes were stimulated primarily due to caterpillar feeding such as the protease inhibitors, polyphenol oxidase, and lipoxygenase and appeared to be modulated by AMF, in some cases stimulating and in other cases suppressing. Additional genes will be discussed, our findings demonstrate the complexity of tri-trophic interactions and their alteration of plant defenses. These findings may also lead to novel methods of pest control that can result in the less use of pesticides.

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Range genetics of eastern black cherry (*Prunus serotina* Ehrh. Subsp. *Serotina*) and its relationship to the rare Alabama cherry (*Prunus alabamensis* Mohr.)

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Black Cherry (*Prunus serotina* Ehrh.) (Rosacea) is a forest tree species widespread in eastern North America and found sporadically in the southwestern United States, Mexico, and Guatemala. This tree is both commercially important as timber for furniture and ecologically important as a source of nutrition for local wildlife. This taxon is morphologically variable across its large range, and numerous subspecies have been recognized in portions of its distribution. Dense sampling across this range was made possible by genotyping microsatellite loci in hundreds of herbarium samples from all portions of the range of this taxon. With this large set of samples in hand, we tested basic hypotheses regarding gene flow and isolation by distance, a genetic pattern in which populations geographically closer to one another are more genetically similar to each other than populations which are further apart. This pattern has been studied in relatively few North American plant taxa, and no study has rigorously sampled across the range of such a widespread species. The second aim of this study is to determine whether or not the sympatric, but morphologically distinct subspecies *Prunus serotina* subsp. *alabamensis* (*Prunus alabamensis*), corresponds to a genetically distinct group, and whether any distinction that exists is due to environmental and/or landscape variables.

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Species range limits, recombination rate variation, and chromosome inversions shape deleterious load in wild barley

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Deleterious mutations that can persist in populations owing to the limits of natural (purifying) selection. The potential for purifying selection to remove deleterious variants from populations is highly dependent on effective population size (N_e). The distribution of deleterious mutations across the genome of individuals and across a species range is likely affected by N_e at multiple scales. For example, individuals in peripheral populations may be more prone to the accumulation of deleterious variants than centrally located populations because of frequent population founding and loss, and limited, unidirectional migration. Regions with a low effective recombination rate also experience reduced N_e , resulting in a higher prevalence of deleterious variants. This effect could be amplified within structural variants, such as inversions, which can result in dramatically suppressed recombination and high linkage disequilibrium (LD). We examine these effects in wild barley (*Hordeum vulgare* L. ssp. *spontaneum*), the progenitor of cultivated barley (*H. vulgare* L. ssp. *Vulgare*). Using a combination of SNP genotyping and exome capture resequencing we make use of the draft barley reference genome to identify both the geographic and genomic distribution of deleterious mutations in 186 wild barley accessions.

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Genomic regulation of abiotic stress response in the soil nematode *Oscheius tipulae*

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Nematodes play an important role in soil health as they cycle nutrients bound in the biomass of their prey. One square meter of soil can hold millions of individuals, and they can be found in every ecosystem on the planet. Ecosystems vary seasonally in their abiotic factors, and nematodes survive this wide variety of abiotic stresses by altering their behavior and producing molecular compounds that protect membrane integrity. The effector genes responsible for abiotic stress tolerance are relatively well-known, but the initial regulatory pathways are not. Because there are many overlaps between the responses, there are many different regulatory processes that could be in control. My research looks to explain how the free-living soil nematode *Oscheius tipulae* regulates its phenotypic responses to freezing, desiccation, and heat stress on a genomic level. The first step is to sequence the *O. tipulae* genome through high-throughput sequencing using Illumina HiSeq. Illumina RNA-Seq has also been used to analyze differential gene expression under heat, freezing, and desiccation stresses. A DNA methylation ELISA assay has provided evidence DNA methylation at the 5 position of cytosine is present in *O. tipulae*, a process that is not found in *Caenorhabditis elegans* despite the two species belonging to the same taxonomic family. Further testing will shed light on the role DNA methylation may have in possible *O. tipulae* epigenetics. This project can expand existing knowledge about nematode genomics, as well as provide more information on general genetic regulatory processes that are not fully understood at this time.

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High-throughput screening of microbial interactions with microwell arrays

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Highly diverse microbial communities reside on plant root systems. Microbial interactions both help shape these communities and are a critical factor in determining plant health. We are developing a new adaptable tool for the discovery of interactions occurring within root-associated bacterial communities. Traditional approaches test only a few interactions at a time, leaving many bacterial communities poorly characterized. By simultaneously testing thousands of different bacterial interactions, our approach will greatly accelerate the pace of discovery. The high-throughput screening platform will use a microwell array to create thousands of unique pairings between the focal species and different bacterial species within a microbiome. Cell pairs will be trapped within their respective wells using a polymer membrane and then monitored with a fluorescent microscope for effects on the focal species. Wells showing enhancement or suppression of focal species function will be extracted and the antagonizing or promoting species will be sequenced for identification. We will validate this technology platform with two screens of *Helianthus annuus* microbiomes sampled from sites under different land use management regimes at the Konza Long-Term Ecological Research (LTER) site. The first screen will identify bacteria that antagonize or promote the growth of the generalist plant pathogen *Agrobacterium tumefaciens*. To target specific mechanisms of microbial interaction, the second screen will identify microbiome members that either positively or negatively influence induction of *A. tumefaciens*' quorum sensing system.

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Genome Size Evolution in the Wild Sunflower *Genus Helianthus*

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Genome size varies greatly across flowering plants, ranging over three orders of magnitude. Considerable variation in genome size also can be found among species that are evolutionarily closely related. Elucidating the mechanisms that have given rise to genome size variation is important for understanding the organization and evolution of plant genomes. The objective of the current work is 1) to generate a database of genome size estimates for all species in the sunflower genus *Helianthus*; and 2) to characterize patterns of genome size evolution in a phylogenetic comparative framework. *Helianthus* consists of 49 species native to North America that differ in ploidy level, geographic range, and life history. Estimates of genome size are being determined by flow cytometry for 2-3 populations per species with biological replication within populations. To date, genome size estimates have been determined for 40 species: 31 diploids, 4 tetraploids, and 5 hexaploids. Genome size variation among diploid species ranges from $2C=6.42$ to 22.93 pg, variation among tetraploid species ranges from $2C=18.75$ to 24.41 pg, and variation among hexaploid species ranges from $2C=19.62$ to 23.32 pg. Phylogenetic comparative analysis of diploid species reveals both clade-specific and species-specific patterns of genome size evolution. Clade-specific patterns show genome size increases, decreases and relative stasis when compared to an ancestrally reconstructed genome size estimate. A single annual diploid species, *H. agrestis*, has undergone a massive genome size increase, with a current genome size 2.58 fold higher than the ancestrally reconstructed estimate and 1.70-3.57 fold higher than any other diploid species.

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Gene expression of bacteria-inoculated soybean glycine max in response to heat stress

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Bacterial inoculants are beneficial microorganisms known to promote plant growth and enhance plant defense by inducing systemic resistance in infected host. *Rhizobium* and *Bacillus* species are commonly used in plant fertilizers due to their mutualistic relationship with a variety of plant species. To determine the effects of bacterial inoculants on soybean (*Glycine max*) gene expression exposed to thermal stress, sterilized seeds were inoculated with *Rhizobium* and *Bacillus* species and allowed to germinate for six days at room temperature. We applied six different biological treatments--Defensor, Rhizoboost, *Rhizobium*, combined Defensor and *Rhizobium*, combined Rhizoboost and *Rhizobium*, or uninoculated control. All inoculum contained beneficial soil microorganisms. Thereafter, germinated seedlings were incubated at 25°C or 35°C for 16 hours. Gene expression levels of various plant defense and stress response genes were examined by real-time quantitative polymerase chain reaction. The expression of many of the known and important defense and stress genes were altered by heat treatment and type of microbial inoculant used. Although some information is available on the effects of these fertilizers at the organismal level, this project provides information on the effects of bacterial inoculants in response to abiotic stress at the genetic level.

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Transcriptomic Expression in Tobacco Plants in response to Arbuscular Mycorrhizal Symbioses and Caterpillar Herbivory

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Arbuscular mycorrhiza is fungi that forms symbiotic relationship with plants. *Mycorrhiza* are obligate biotrophs which requires a plant species for their nutrition and survival. While doing so the fungi appears to increase plant defenses to herbivores. Our previous evidence supports *Mycorrhiza* fungi stimulates defenses in tobacco plants against caterpillars. I investigated the gene expression of wild tobacco plants (*Nicotiana attenuata*) that were grown with or without AMF in a greenhouse with or without a third instar *Manduca sexta* (Lepidoptera: Sphingidae) caterpillars. The caterpillars fed for 48hrs and then the leaves were analyzed for gene expression analysis by qPCR for a variety of tobacco plant defense and stress genes. Most of these defense genes were stimulated primarily due to caterpillar feeding, but appeared to be altered in part to the fungi. These findings may better help us to understand the interaction of the fungi and host plant to its environment.

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Rapid adaptation in a contaminated environment: Evolutionary adaptive response of old field grass *Andropogon virginicus* to heavy metals in an abandoned lead and zinc mine

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Soil and climate factors, historically important drivers of speciation, have been substantially altered by human activities, placing new selective pressures on plant populations. Rapid adaptation to changing environmental conditions has been shown in plants exposed to copper and coal mines and other extreme environments. This research investigates potential adaptive variation in the perennial grass *Andropogon virginicus* in response to contaminated soils at the Tar Creek Superfund Site, an abandoned Lead and Zinc mine that spans 12,600 acres across Kansas, Oklahoma, and Missouri. Using plants from in and outside this mine, we can compare genotype, phenotype, and gene expression between populations to investigate the basis of metal tolerance. Through a greenhouse reciprocal transplant using field soils and a Lead addition experiment, we seek to answer the following questions: 1) Are there genetically differentiated ecotypes of *A. virginicus* adapted to extreme mine site conditions? 2) What is the basis of these differences in DNA sequence variation? 3) Do mine site and old field populations differ in gene expression? We hypothesized that mine populations would show genetic differentiation and local adaptation to mine conditions.

Seeds collected from field populations of *A. virginicus* were germinated in the greenhouse. DNA was extracted from plants representing 20 populations to compare between and within population variation, awaiting sequencing results. Plants from 12 populations (6 in mine areas, 6 in uncontaminated sites) were transplanted into soils collected from the same 12 locations in a greenhouse soil reciprocal transplant. Each combination of plant population and soil was replicated twice ($12 \times 12 \times 2 = 288$ plants). Physiological data (chlorophyll fluorescence, height, plant stress) was periodically collected to compare the effects of soil treatment and source population. 15 plants from the same 12 populations were transplanted into greenhouse soils for the Lead addition experiment (180 plants total). Plants from each population were randomly assigned to the "high" metal addition treatment (10 ppb $\text{Pb}(\text{NO}_3)_2$), "low" metal addition treatment (5 ppb $\text{Pb}(\text{NO}_3)_2$), and control treatment (KNO_3). Physiological data (chlorophyll fluorescence, height, plant stress) will be periodically collected to compare the effects of treatment and source population. Changes in plant biomass before and after metal addition will be compared using

ImageJ, and tissue samples will be assayed for Ascorbate Peroxidase (APX), Catalase (CAT), and Glutathione Reductase (GR).

Initial physiological assessment data from the soil reciprocal transplant indicate higher fitness of all populations in old field soil under experimental conditions. These preliminary results are inconclusive, but may indicate that mine populations acquire metal tolerance through phenotypic plasticity during early development or metal tolerant mycorrhizae. Elucidating the mechanisms of metal tolerance in *A. virginicus* could beneficially guide the use of tolerant ecotypes in restoration efforts, which have cost over \$300 million since 1983. This species is a strong candidate for phytostabilization, which could help reduce erosion, windblown dust, and run-off from contaminated soils. The results of this research will be communicated to the US EPA and the USFWS to help guide seed sourcing decisions for restoration in this and other abandoned mines in the US and worldwide.

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Identifying QTLs underlying phosphorus use in a resurrected *Daphnia* mapping population

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Resurrecting diapausing (resting) eggs of the aquatic keystone herbivore, *Daphnia*, preserved in lake sediments for centuries has revealed striking shifts in nutrient use physiology that are correlated with nutrient enrichment (i.e., cultural eutrophication). As such, this system allows us to address key issues in ecological genomics with a unique temporal approach that is not possible with more common space-for-time substitutions (i.e., comparing populations to infer evolutionary trajectories). Previous work illuminated transcriptome-wide variation underlying such major changes in phosphorus kinetics between ancient and modern genotypes. Here, we use quantitative trait loci (QTL) mapping to identify genomic regions underlying P-use. An F2 mapping population has been constructed by crossing a ~5-10-yr old (dam) clone with a ~40-50-yr-old (sire) clone that differ in P-use. F2 genotypes exhibit substantial physiological variation in P-use. These genotypes are being sequenced to identify loci underlying differences in P-use. We plan to use these markers to query extant *D. pulicaria* inhabiting lakes differing in trophic status to understand the genomic basis of adaptation to cultural eutrophication.

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The genetics of visual attraction

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The genetics of assortative mating has long fascinated biologists, however identifying genes responsible for variation in mate selection has proven difficult. Here we take advantage of next generation sequencing and variation in male preference for a visual trait in *Heliconius cydno* butterflies to identify genes associated with visual attraction. We identify SNPs perfectly associated with a visual attractant, a white or yellow forewing band. We then identify SNPs associated with life-long male preference and initial male preference for white- or yellow- banded females. Life-long preference is polygenic and influenced by genes associated with neurodevelopment. Initial attraction is associated with a different, small set of genes that influence neurodevelopment and gene expression. Our results suggest that variation in initial attraction can be driven by a small number of genes with large effect, and that the genes influencing evolutionarily important behaviors such as mate selection may change throughout an individual's lifetime.