



# **Phenotypic Plasticity: Evolution at the Intersection of Ecology, Genetics, and Development**

**The Arnold Arboretum of Harvard University  
Boston, MA  
May 1-3, 2015**

A workshop sponsored by microMORPH,  
a NSF Research Coordination Network

Co-Organizers:  
Pamela Diggle  
Dave Des Marais  
William (Ned) Friedman

**Faculty Participants**

Sally Assmann	Penn State University, <i>Biology Department</i>	sma3@psu.edu
Ben Blackman	University of Virginia, <i>Department of Biology</i>	bkb2f@virginia.edu
Dave Des Marais	Harvard University, <i>Arnold Arboretum</i>	desmarais@fas.harvard.edu
Lisa Donovan	University of Georgia, <i>Department of Plant Biology</i>	donovan@plantbio.uga.edu
Andrew Doust	Oklahoma State University, <i>Botany Department</i>	andrew.doust@okstate.edu
Mark van Kleunen	Universität Konstanz, <i>Ecology, Department of Biology</i>	mark.vankleunen@uni-konstanz.de
John Stinchcombe	University of Toronto, <i>Ecology and Evolutionary Biology</i>	john.stinchcombe@utoronto.ca
Sonia Sultan	Wesleyan University, <i>Department of Biology</i>	sesultan@wesleyan.edu

**Graduate Student and Post-Doctoral Participants**

Melis Akman	University of California, Davis, <i>Department of Plant Sciences</i>	akmanmelis@gmail.com
Robert Baker	University of Wyoming, <i>Botany Department</i>	robert.baker@uwyo.edu
Jack Colicchio	University of Kansas, <i>Department of Ecology and Evolutionary Biology</i>	Colicchio@ku.edu
Eric Goolsby	University of Georgia, <i>Department of Plant Biology</i>	cire514@uga.edu
Alannie-Grace Grant	University of Pittsburgh, <i>Department of Biological Sciences</i>	agg18@pitt.edu
Jacob Herman	Wesleyan University, <i>Department of Biology</i>	jherman@wesleyan.edu
Andrea Hodgins-Davis	University of Michigan, <i>Department of Ecology and Evolutionary Biology</i>	andrea@d.umich.edu
Grey Monroe	Colorado State University, <i>Department of Bioagricultural Science and Pest Management</i>	greymonroe@gmail.com
Rajendhran Rajakumar	University of Florida, <i>Department of Molecular Genetics and Microbiology</i>	rajakumar@ufl.edu
Elizabeth Spriggs	Yale University, <i>Department of Ecology and Evolutionary Biology</i>	elizabeth.spriggs@yale.edu

**Attending microMORPH Steering Committee Members**

Pamela Diggle	University of Connecticut, <i>Ecology and Evolutionary Biology</i>	pamela.diggle@uconn.edu
William (Ned) Friedman	Harvard University, <i>Arnold Arboretum and Department of Ecology and Evolutionary Biology</i>	ned@oeb.harvard.edu
Michael Donoghue	Yale University, <i>Department of Ecology and Evolutionary Biology</i>	michael.donoghue@yale.edu
Cynthia Jones	University of Connecticut <i>Department of Ecology and Evolutionary Biology</i>	cynthia.s.jones@uconn.edu

**Other Participants**

Hamish Spencer	University of Otago, <i>Department of Zoology</i>	hamish.spencer@otago.ac.nz
----------------	--	----------------------------

**Friday, May 1<sup>st</sup>**

8:00 AM	Meet bus to Weld Hill (meet outside of hotel lobby)
8:30 AM - 9:00 AM	Breakfast
9:00 AM - 9:15 AM	Introductions
9:15 AM - 9:45 AM	<b>Pam Diggle</b> <i>Overview of microMORPH and workshop goals</i>
9:45 AM - 10:25 AM	<b>Mark van Kleunen</b> <i>The benefits of plasticity?</i>
10:25 AM - 10:45 AM	Discussion
10:45 AM - 11:15 AM	Break
11:15 AM - 11:30 AM	<b>Alannie-Grace Grant</b> <i>Does plasticity in mating system affect niche breadth?</i>
11:30 AM - 11:45 AM	Discussion
11:45 AM - 12:00 PM	<b>Grey Monroe</b> <i>The evolution of phenotypic plasticity in response to water stress</i>
12:00 PM - 12:15 PM	Discussion
12:15 PM - 1:30 PM	Lunch
1:30 PM - 2:10 PM	<b>Lisa Donovan</b> <i>How does phenotypic plasticity fit into eco-evo-physiological perspectives on plant growth strategies and associated traits?</i>
2:10 PM - 2:30 PM	Discussion
2:30 PM - 3:10 PM	<b>John Stinchcombe</b> <i>Plasticity in plant growth: evolutionary lines of greatest and least resistance</i>
3:10 PM - 3:30 PM	Discussion
3:30 PM - 4:00 PM	Break
4:00 PM - 4:15 PM	<b>Rob Baker</b> <i>Function valued trait mapping: Modeling leaf development reveals independent genetic modules for shape and size</i>
4:15 PM - 4:30 PM	Discussion
4:30 PM - 5:10 PM	<b>Ben Blackman</b> <i>Shifting thresholds: the evolution of discontinuous reaction norms in Mimulus</i>
5:10 PM - 5:30 PM	Discussion
5:30 PM - 6:00 PM	General Discussion
6:00 PM - 6:30 PM	Free Time - Explore Arboretum
6:30 PM - 8:30 PM	Closing Remarks and Dinner
8:30 PM	Meet Bus to Hotel (meet outside of Weld Hill)

**Saturday, May 2<sup>nd</sup>**

8:00 AM	Meet bus to Weld Hill (meet outside of hotel lobby)
8:30 AM - 9:15 AM	Breakfast
9:15 AM - 9:30 AM	<b>Eric Goolsby</b> <i>Phylogenetic comparative methods for studying function-valued traits</i>
9:30 AM - 9:45 AM	Discussion
9:45 AM - 10:00 AM	<b>Beth Spriggs</b> <i>Patterns of leaf shape variation in Viburnum</i>
10:00 AM - 10:15 AM	Discussion
10:15 AM - 10:45 AM	Break
10:45 AM - 11:25 AM	<b>Andrew Doust</b> <i>Phenotypic plasticity in Setaria viridis (Poaceae)</i>
11:25 AM - 11:45 AM	Discussion
11:45 AM - 12:00 PM	General Discussion
12:00 PM - 1:15 PM	Lunch
1:15 PM - 1:55 PM	<b>Sonia Sultan</b> <i>Trans-generational plasticity in Polygonum: environmental inheritance and adaptive diversity</i>
1:55 PM - 2:15 PM	Discussion
2:15 PM - 2:30 PM	<b>Jack Colicchio</b> <i>Phenotypically plastic, epigenetically informative, and evolutionarily intriguing: Mimulus guttatus as a transgenerational inheritance model system</i>
2:30 PM - 2:45 PM	Discussion
2:45 PM - 3:00 PM	<b>Rajendhran Rajakumar</b> <i>The developmental basis of phenotypic evolution in ants: hormones, genes, and epigenetics</i>
3:00 PM - 3:15 PM	Discussion
3:15 PM - 3:45 PM	Break
3:45 PM - 4:00 PM	<b>Jacob Herman</b> <i>Experimental reduction of DNA methylation levels inhibits expression of adaptive transgenerational plasticity to drought in Polygonum persicaria</i>
4:00 PM - 4:15 PM	Discussion
4:15 PM - 4:55 PM	<b>Sally Assmann</b> <i>What does RNA structure have to do with plasticity?</i>
4:55 PM - 5:15 PM	Discussion
5:15 PM - 5:45 PM	General Discussion
5:45 PM - 6:30 PM	Free Time - Explore Arboretum
6:30 PM - 8:30 PM	Closing Remarks and Dinner
8:30 PM	Meet Bus to Hotel (meet outside of Weld Hill)

**Sunday, May 3<sup>rd</sup>**

8:00 AM	Meet bus to Weld Hill (meet outside of hotel lobby)
8:30 AM - 9:00 AM	Breakfast
9:00 AM - 9:40 AM	<b>David Des Marais</b> <i>A population genomic perspective on the evolution of phenotypic plasticity</i>
9:40 AM – 10:00 AM	Discussion
10:00 AM -10:15 AM	<b>Andrea Hodgins-Davis</b> <i>Disentangling the role of environment as agent of selection and as an agent of development</i>
10:15 AM - 10:30 AM	Discussion
10:30 AM -10:45 AM	Break
10:45 AM - 11:00 AM	<b>Melis Akman</b> <i>Transcriptome sequencing reveals signatures of local adaptations linked to functional traits and environmental gradients in <i>Protea repens</i></i>
11:00 AM - 11:15 AM	Discussion
11:15 PM -12:00 PM	General Discussion and Wrap-up
12:00 PM -1:30 PM	Lunch
1:30 PM	Taxis to Airport Begin

**Abstracts:**

	Page		Page
Melis Akman	5	Alannie-Grace Grant	10
Sally Assmann	6	Jacob Herman	11
Robert Baker	6	Andrea Hodgins-Davis	12
Ben Blackman	7	Mark van Kleunen	12
Jack Colicchio	7	Grey Monroe	13
Dave Des Marais	8	Rajendhran Rajakumar	13
Lisa Donovan	8	Elizabeth Spriggs	14
Andrew Doust	9	John Stinchcombe	15
Eric Goolsby	9	Sonia Sultan	15

**Melis Akman**

(University of California, Davis)

Co-authors: J.E. Carlson, K.E. Holsinger and A.M. Latimer

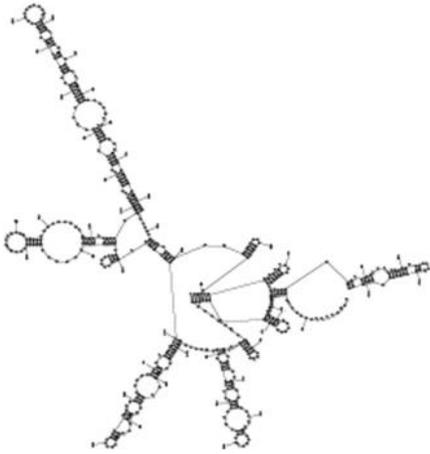
**Transcriptome sequencing reveals signatures of local adaptations linked to functional traits and environmental gradients in *Protea repens***

Understanding both environmental and genetic mechanisms underlying locally adaptive trait variation, including plasticity variation, across a species' range is critical for predicting how species will respond to changing climate. We performed a common garden experiment with a South African shrub, *Protea repens*, displaying diverse phenotypes along strong environmental gradients. We grew plants from seeds collected at 19 populations spanning the species' range, and sequenced the transcriptomes of these plants to reveal gene pathways associated with potentially adaptive trait variation. We showed that gene networks enriched for growth related pathways correlated strongly with source site minimum winter temperature and with leaf area, stem diameter and maternal plant specific leaf area. Our results strongly suggest that there is heritable control of gene expression in the common garden shaped by source population site climate, and reflected in traits both in the common garden seedlings and mother plants in the wild. In our second experiment, we subjected select populations of sugar bush to drought stress, in order to detect plastic responses of these populations in terms of gene expression, physiological and morphological traits and growth patterns. We found that there are varying levels of plasticity in these populations to drought stress, reflected in their photosynthetic rates and growth patterns. The transcriptome and physiological data from this study will also be presented.

## Sarah M. (Sally) Assmann

(Penn State University)

### What does RNA structure have to do with plasticity?



We often think of plasticity as a macroscopic or whole plant trait; however, there is also plasticity at the cellular and subcellular levels. Within the living cell, RNAs fold into complex structures (see the figure for one example) and we have recently developed a method, structure-seq, which allows transcriptome-wide assessment of RNA secondary structure *in vivo*. Our dataset on the structures of over 10,000 Arabidopsis mRNAs has led us to hypothesize that there is plasticity in the structures that mRNAs take on, and that this plasticity may be related to the functions of the encoded proteins and to the prevailing environmental conditions. These ideas will be developed for discussion.

## Robert Baker

(University of Wyoming)

Co-authors: W.F. Leong, S. Welch, and C. Weinig

### Functional trait mapping: Modeling leaf development reveals independent genetic modules for shape and size

We examined the morphological development of leaves throughout ontogeny in an experimental segregating population of *B. rapa* (RILs). RILs were grown in crowded and uncrowded conditions over multiple years, and leaf length and width data were collected throughout ontogeny. We generated Bayesian models of leaf development and use parameters of these models in quantitative-genetic analyses to demonstrate plastic responses in leaf development to crowding, and to explore patterns of correlation with physiology, plant architecture (as measured by spectroradiometric readings), circadian rhythms, and fitness. To examine the g\*e interactions, model parameters describing leaf development were mapped to a high-density linkage map of *B. rapa*. In addition to uncovering significant g\*e, this study highlights the importance of an ontogenetic approach for understanding the genetic and environmental components of leaf development, because different QTL underlie growth rate and final leaf size. Furthermore, we demonstrate that leaf development itself is under selection. When predicting phenotypes based on genotypes, final measures of leaf size do not reveal important aspects of leaf morphology, especially growth dynamics during early development when leaf-based photosynthesis is particularly critical for carbon assimilation.

## Ben Blackman

(University of Virginia)

### **Shifting thresholds: the evolution of discontinuous reaction norms in *Mimulus***

Threshold traits are often characterized by discontinuous reaction norms in that expression of a trait or continuation of developmental progress can be obligately contingent on environmental cues. The genetics of how such forms of developmental plasticity evolve has been poorly explored and may be subject to fundamentally distinct considerations relative to continuous reaction norms. We have investigated the geographic diversity of photoperiodic flowering and its genetic basis in annual populations of the common monkey flower *Mimulus guttatus*, an obligate long-day plant. Although the critical minimum photoperiod necessary for flowering is strongly correlated with elevation and growth season start date, flowering time in inductive conditions is correlated with season duration and its relation to elevation changes with latitude. That these two aspects of a discontinuous reaction norm can evolve separately not only indicates that different agents of selection drive their evolution, but also predicts that unique genetic architectures govern divergence of each trait. Genetic mapping by multiplex shotgun genotyping in multiple high x low elevation crosses confirms this prediction: critical photoperiod loci do not co-localize with flowering time loci. Surprisingly, the loci contributing to clinal divergence in critical photoperiod are nearly all transect-specific. That convergent evolution at the genetic level is largely unobserved for this trait is contrary to a classic prediction of how plasticity may evolve. These findings also raise the question of whether developmental flexibility or selective constraint is the primary force shaping the genetic architecture of threshold trait evolution across the species range.

## Jack Colicchio

(University of Kansas)

### **Phenotypically plastic, epigenetically informative, and evolutionarily intriguing: *Mimulus guttatus* as a transgenerational inheritance model system**

Transience and reversibility are both often used to describe phenotypic plasticity, suggesting that this phenomenon is anything but “stable”, yet increasing evidence suggests that in some cases it is just that. The transmission, and some cases stability, of plastic phenotypes across generations suggests that a molecule besides DNA may be heritable and contribute to progeny phenotype. First, I show that post bisulfate amplification (PBAT) followed by whole genome methylome sequencing has utility in this system and that a high level of variance in gene expression can be explained by modeling on patterns of methylation and gene structure. Second, I present our findings that over 900 hundred genes are differentially expressed in the progeny of wounded *M. guttatus* plants. These genes tend to be involved in stress response pathways and cell wall metabolism, and cluster in ways that suggest there are a small number of heritable signals. Finally, I present recent data that suggests trichomes limit herbivore damage in nature, and discuss common garden experiments to examine the fitness effects of transgenerational phenotypic plasticity. A versatile base of ecological, evolutionary, developmental, and genetic exploration of *M. guttatus*, along with recent discoveries into its remarkable plasticity and

informative methylome, primes it to be of great value in the growth of the field of transgenerational phenotypic plasticity.

## **Dave Des Marais**

(Harvard University)

### **A population genomic perspective on the evolution of phenotypic plasticity**

Heritable natural variation in environmental response is the substrate for the evolution of phenotypic plasticity. This variation in response, or Gene by Environment interaction (GxE), is readily observed in cellular, physiological, developmental, and whole-organism characters. Considerable work in model plant systems has begun to describe the molecular components involved in environmental response; it remains unknown which of these mechanisms vary in natural populations and drive evolutionary change in plasticity. I will describe our work identifying which molecular and physiological aspects of plant response traits are conserved within species, and which vary, and describe how natural selection acts differently on these two classes of traits. I finish by considering the evolution of plasticity in the context of molecular signaling networks and describe some preliminary results demonstrating that network topology may allow us to predict how natural selection might act on plastic traits.

## **Lisa Donovan**

(University of Georgia)

### **How does phenotypic plasticity fit into eco-evo-physiological perspectives on plant growth strategies and associated traits?**

The worldwide leaf economic spectrum (WLES) is multivariate axis that describes co-variation in leaf traits across many species. It widely interpreted as reflecting a spectrum of plant ecological and adaptive strategies ranging from fast growing resource acquisitive plants to slow growing resource conservative plants. The mechanistic basis of the WLES trait pattern is generally argued as resulting from selection and genetic constraints, but this doesn't address the inclusion of phenotypic plasticity expressed as ontogenetically and environmentally driven variation. We use trait data from a diverse herbaceous genus, *Helianthus*, to illustrate how ecological and evolutionary interpretations of trait variation and co-variation for WLES and associated traits could benefit from greater consideration of multiple scales and sources, including the role of phenotypic plasticity. A compilation of *Helianthus* data from naturally occurring and experimental plants demonstrates a remarkably broad range of leaf N and LMA variation in this herbaceous genus when compared to the WLES. "Common garden" experiments that minimize environmental and ontogenetic variation are used assess the magnitude and patterns of putatively adaptive traits. In these comparisons, the range of species values is again surprisingly large in comparison to the WLES and phylogenetically informed analyses demonstrate substantial evolutionary lability. Differences among populations sourced from native habitats and between artificially selected populations are much smaller, as expected. Additional experiments, however, demonstrate that environmentally induced and ontogenetic based variation can be remarkably large and have the potential to overwhelm the ability to detect trait adaptive differentiation and correlated trait evolution.

Although these patterns might call into question the value of interpreting ecophysiological trait patterns assessed in just one environment as adaptive, it should be possible to make headway by incorporating expectations of plasticity from older plant strategy paradigms and deciding on key stages and environments for informative assessments. We provide examples of plasticity in leaf and root traits to illustrate possibilities for incorporating plasticity into investigations of adaptive differentiation.

## **Andrew Doust**

(Oklahoma State University)

### **Phenotypic plasticity in *Setaria viridis* (Poaceae)**

Foxtail millet (*Setaria italica*) and its wild progenitor green foxtail (*S. viridis*) are native to Northern China, with green foxtail now being one of the world's most widespread weeds. We are using the *Setaria* system as a model to examine the genetic regulation of tillering (basal branching) in panicoid grasses, such as switchgrass, sorghum, and maize. Although we aim to uncover basic mechanisms of branching regulation, we are also investigating how genetics and development interact with environment to allow the plant to respond to variation in parameters such as light intensity, light quality, water availability, and plant density. Much of our work has been on one genotype of *S. viridis*, accession A10.1 (recently sequenced by the DOE Joint Genome Institute), both in growth trials to determine the limits of plasticity and in mapping experiments using recombinant inbred lines derived from a cross between A10.1 and an accession of foxtail millet to identify quantitative trait loci (QTL) that control phenotypic traits. We find remarkable plasticity in growth form of *S. viridis* in different environmental conditions, along with suggestive evidence that variability increases when plants are grown in suboptimal conditions. Much of the plasticity seen in plant architecture appears to be driven by changes in maturation time controlled by photoperiod and temperature. Quantitative trait loci (QTL) analyses reveal a surprising degree of co-localization of QTL across trials for maturation time but much variation for plant architecture, especially branching. However, comparative genomic analyses suggest that the underlying genetic architecture of branching has conserved elements across the grass family, supported by the observation that wild grasses all exhibit plasticity in branching phenotypes.

## **Eric Goolsby**

(University of Georgia)

### **Phylogenetic comparative methods for studying function-valued traits**

Phylogenetic comparative methods, such as ancestral state reconstruction, phylogenetically independent contrasts, and methods for estimating phylogenetic signal, have transformed the field of comparative biology. Although these methods offer substantial flexibility in studying trait evolution, many shortcomings limit their utility for applications relating to phenotypic plasticity and environmentally responsive traits. Namely, the inherent assumption of phylogenetic comparative methods that species means are fixed is clearly violated with phenotypically plastic traits. More recently developed comparative methods allow for the incorporation of within-species variation, but these methods still rely on the assumption that variation is distributed around a fixed species mean. Few

methods account for environmentally or temporally driven variation, such as phenotypic responses to abiotic stress or developmental trajectories. Such traits, are sometimes referred to as reaction norms, infinite-dimensional, or function-valued, are more appropriately expressed as mathematical functions linking independent predictor variables to the trait of interest. Here, I introduce an extension of phylogenetic generalized least squares (PGLS) methods for reconstructing ancestral function-valued traits. The method is fully compatible with other PGLS-based methods, which can be used to test for phylogenetic signal, assess correlated trait evolution, perform phylogenetic ANOVA, and compare evolutionary rates, all in a function-valued context. These methods are explored empirically using the evolution of salt tolerance and phenotypic responses to soil salinity in wild *Helianthus* (the sunflower genus). The evolutionary history of salt tolerance and correlated functional trait evolution in *Helianthus* is assessed using both traditional univariate phylogenetic comparative methods and the function-valued methods presented here, and the strengths, weaknesses, challenges, and future directions of the study of function-valued trait evolution in a phylogenetic context are discussed.

### **Alannie-Grace Grant**

(University of Pittsburgh)

#### **Does plasticity in mating system affect niche breadth?**

For my talk, I discuss my research results so far, demonstrating significant niche breadth differences in sister species. In brief, I used selfing-outcrossing seven sister species pairs in *Colinsia* and *Tonella*. Individual species niches +/- 95% confidence intervals were generated using 100 bootstrap replicates with the MaxEnt niche modeling program. For each species' niche model replicate, the niche breadth was calculated via Levin' B. Sister species niche breadth differences were assessed using an unpaired t-test. For the majority of pairs assessed, sister species niche breadths were significantly different, with selfers having wider niche breadths. Selfing species niches had fewer areas of high habitat suitability than outcrossers, which suggest potentially recent range expansions for selfers. Area-under-the-curve values (a metric of MaxEnt model performance) tended to be lower for selfers. This suggests that other unmeasured factors (environmental or biotic) may be affecting the niche of selfers. Together, these results support the hypothesis that selfing may affect species niche breadth, potentially by increasing the likelihood of colonization success. Important future directions for this research is to determine if local adaptation or phenotypic plasticity in selfing rate play an important role in the niche breadth differences of sister species. In future experiments, I will quantify the extent to which greater levels of phenotypic plasticity (in mating system and other traits) are driving the patterns of habitat diversity and range.

### **Jacob Herman**

(Wesleyan University)

#### **Experimental reduction of DNA methylation levels inhibits expression of adaptive transgenerational plasticity to drought in *Polygonum persicaria***

Environments experienced by parent plants often have profound effects on offspring phenotypes. In some cases, such transgenerational plasticity enhances offspring performance under the

same conditions experienced by the parent(s). Despite much interest in these adaptive transgenerational effects, the mechanisms responsible for their transmission across generations have received relatively little attention. Recent studies suggest that environmentally introduced, heritable changes in DNA methylation may be a mechanism for this form of plasticity.

Previous studies of the generalist annual *Polygonum persicaria* found that drought-induced transgenerational effects on offspring root length and extension rate were associated with greater survival when seedlings were grown in very dry soil. To test for a causal role of DNA methylation in the expression of these inherited effects, we experimentally demethylated seedling offspring of well-watered and drought-stressed *P. persica* plants from 12 inbred genetic lines by germinating seeds on plates of agar containing zebularine, a DNA methyltransferase inhibitor. We then transplanted these offspring into a dry-soil environment in a growth chamber, where we compared the effects of parental drought on development of zebularine-treated vs. control seedlings. Reducing methylation levels did not have an overall specifically negative effect on plant growth and development; instead, reducing methylation levels specifically altered the effect of parental drought. When offspring were germinated in control conditions, seedlings of droughted parents had on average 20% longer root systems compared to seedlings of well-watered parents from the same genetic lines, a result that confirmed previous findings. This increase in root length provided plants with greater access to limited soil moisture, which was reflected in the 16% greater biomass attained by offspring of droughted parents compared to offspring of well-watered parents. Reduction of DNA methylation levels removed these adaptive transgenerational effects of drought: zebularine-treated seedlings of droughted parents had 23% shorter root systems and 27% lower biomass compared to seedlings of droughted parents germinated in control conditions. Underlying these average effects was considerable genetic variation for the effect of zebularine treatment on expression of the parental drought effect. A repeated-measures analysis of root extension over time also found that zebularine treatment altered the effect of parental drought, again in a manner that varied by genetic line. These results show that expression of adaptive transgenerational plasticity to drought depends on the state of the offspring DNA methylome. The existence of genetic variation for these methylation-mediated effects suggests that variation in plant phenotypes may be caused by a complex interaction between environmental variation, genetic variation, and epigenetic variation.

## Andrea Hodgins-Davis

(University of Michigan)

### Disentangling the role of environment as agent of selection and as an agent of development

“The environment is not the only agent of selection, in the sense of being the arena where different phenotypes achieve different degrees of survival and reproductive success. It is also an agent of development, which importantly influences the range of phenotypes that will be produced by a given genotype.” –Mary Jane West-Eberhard 1989 *Annu. Rev. Ecol. Syst.* 20:249-78

One of the central challenges in understanding the evolution of phenotypic plasticity is that variation in the environment both induces and selects upon new variants. To understand the extent of plasticity available to selection, then, requires disentangling the role of environment as agent of selection and as an agent of development. Here, I present ongoing work designed to disentangle these roles by systematically characterizing the effects of novel mutation on gene expression phenotypes across a range of ecologically relevant environments. Two complementary approaches using budding yeast as model provide insight into the range of mutational variation available for gene expression: a) RNA sequencing of mutation accumulation lines of budding yeast and their ancestral genotype across a gradient of copper concentrations provides a genome-wide perspective on mutational variance in plasticity for gene expression, and b) a high-throughput screen for variation in expression of a fluorescent reporter driven by the promoter regions of single genes permits a thorough characterization of the distribution of mutational effects and the correlation among effects of new mutants across environments. Preliminary results from RNA sequencing of the MA lines suggest that stressful environments exacerbate mutational effects. I will present work currently in progress to contrast mutational variation for gene expression plasticity with that present natural strains in order to infer the degree of evolutionary constraint exerted on expression plasticity.

## Mark van Kleunen

(Universität Konstanz)

### The benefits of plasticity?

It is frequently assumed that phenotypic plasticity is beneficial, because it should increase environmental tolerance. Consequently, it is frequently suggested that organisms that are more plastic are more successful. However, is this really the case? My group uses large multi-species comparative experiments, in-depth single-species studies, and meta-analytical studies to test whether more successful species (or genotypes) are phenotypically more plastic, and whether this results in a higher environmental tolerance. We found evidence that in heterogeneous environments, plasticity in root morphology (i.e. root foraging behavior) is beneficial, and that these plastic changes are more pronounced in invasive alien than in non-invasive alien species. However, in many other systems, the benefits of plasticity were not evident. For example, typical plastic responses to shading such as increased leaf length and specific leaf area were not higher in invasive than in non-invasive alien plants. Furthermore, preliminary results of a meta-analysis suggest that the magnitude of plastic changes in specific leaf area is not related to the maintenance of a high performance in shade environments.

Therefore, I argue that many of the plastic changes that we think are adaptive might simply reflect passive changes to the environment, or result as by-products of other plastic responses.

## Grey Monroe

(Colorado State University)

### **The evolution of phenotypic plasticity in response to water stress**

In 1968 Cook and Johnson found that, when grown in terrestrial and aquatic environments, individuals of *Ranunculus flammula* displayed greater phenotypic plasticity for the development of leaf morphology if they originated from sites that frequently experienced both terrestrial and aquatic conditions. Alternatively, individuals from sites where only one condition was experienced displayed canalization of leaf morphology across environmental treatments. Now, almost 50 years later, the importance of temporal environmental heterogeneity on the evolution of phenotypic plasticity remains a topic of great interest. By analyzing remote sensing data collected over the last 34 years, I have found that the historic frequency of drought varies greatly between populations across the natural geographic range of *Arabidopsis thaliana*. This variation in selection histories may have resulted in local adaptation and affected the evolution of phenotypic plasticity in response to water stress within these populations. To address this, I am currently exploring how the distribution of *A. thaliana* in Eurasia relates to the historic frequency of drought. Additionally, I plan to conduct hypothesis driven experiments on population to measure phenotypic plasticity for developmental and physiological traits under different levels of water availability. I predict that populations whose histories are characterized by greater variation in the frequency of drought will display greater phenotypic plasticity in response to changes in water availability. I also predict that genetic polymorphisms differentiating populations along an axis of drought frequency will be associated with genes affecting plasticity to water availability. My talk will discuss the theoretical framework, design, progress and future directions of this research project.

## Rajendhran Rajakumar

(University of Florida)

### **The developmental basis of phenotypic evolution in ants: hormones, genes, and epigenetics**

Ants are social insects that exhibit an incredible degree of phenotypic plasticity. During development, different environmental perturbations can permit a single genotype to give rise to an array of alternative phenotypes related to dramatic differences in morphology, longevity, reproduction and behavior. This environmental sensitivity is the basis for the diversity of complex ant caste systems. As a graduate student in the Abouheif lab at McGill University, I used ant development of the hyperdiverse genera *Camponotus* and *Pheidole* as models to investigate the hormonal and epigenetic mechanisms that facilitate the environmental generation of variation within and between castes and the evolution of novel castes. First, using the ant genus *Camponotus*, we investigated the mechanisms underlying genotype-by-environment interaction that facilitate the generation of quantitative variation. Specifically, we found that DNA methylation is involved in the generation of continuous sizing within the worker caste. We functionally demonstrated that in the carpenter ant *Camponotus floridanus*, global

microMORPH Workshop 2015

Phenotypic Plasticity: Evolution at the Intersection of Ecology, Genetics, and Development

(genome-wide) DNA methylation indirectly regulates quantitative methylation of the highly conserved cell-signaling gene *Epidermal Growth Factor Receptor (Egfr)* to generate the continuous size variation of workers. Therefore, naturally occurring inter-individual differences in a quantitative trait can be generated by environmental variation through quantitative inter-individual differences in epigenetic regulation of a gene. This mechanism, alongside genetic variation, may determine the phenotypic possibilities of loci for generating quantitative trait variation in natural populations. Second, using the genus *Pheidole*, we investigated the hormonal and developmental genetic basis underlying the evolution of novel worker castes. This genus is composed of over 1000 species, all of which comprise a complex worker caste system of minor workers and soldiers. In a handful of these species, there exists an additional novel worker caste: the supersoldier. Through phylogenetic and developmental genetic analysis, we determined that this caste has evolved in parallel in different species. We then discovered through field observations and hormonal manipulations that there exists an ancestral developmental potential in this group: all *Pheidole* species have a hidden capacity to produce supersoldiers through environmental induction, the recurrence of which can lead to their evolution through a process known as genetic accommodation. More generally, the recurrent induction of ancestral developmental potential may facilitate the adaptive and parallel evolution of phenotypes. Collectively, these results from *Camponotus* and *Pheidole* ants show how the environment acts on development through the integration of hormones, genes and epigenetic mechanisms to generate both quantitative and qualitative phenotypic variation for selection to act on.

## Elizabeth Spriggs

(Yale University)

### Patterns of leaf shape variation in *Viburnum*

Correlations between temperature and leaf teeth are so ubiquitous that leaf margin analysis is frequently used to predict paleoclimates. Recently, leaf shape associations with climate have also been documented, and phylogenetic analysis has shown that these traits can evolve rapidly as lineages move into new habitats. The adaptive significance of leaf teeth and leaf shape, however, is not well understood, and there is substantial variation both within species and within individual plants. We show that leaf variation within individuals is highly regular in five diverse *Viburnum* species and identify a set of rules that governs trait variation across diverse leaf morphologies, various growth architectures, and over 50 Ma of evolutionary divergence. Specifically, we find consistent patterns in leaf shape and leaf margins traits along branches in the course of a single season (heteroblasty). Among populations, we find that the known leaf form trends across species are also evident within two widespread *Viburnum* species complexes. Southern individuals are more ‘tropical’ while Northern individuals are more ‘temperate’ in their leaf characteristics. The heteroblasty we have documented in *Viburnum* is similar to patterns found in *Populus*, *Acer*, *Liquidambar*, and *Crataegus*, indicating that this is a widespread phenomenon in temperate woody plants. Although the relative contributions of genetic and plastic variation are still unknown, our results suggest that changes in phenology can lead to a ‘frame shift’ in the heteroblastic leaf sequence either toward or away from temperate traits like leaf teeth.

Developmental plasticity, and specifically plasticity in phenology, would alter leaf shape and could be a first step toward evolving new leaf forms.

## John Stinchcombe

(University of Toronto)

### **Plasticity in plant growth: evolutionary lines of greatest and least resistance**

Plant growth and development is spectacularly sensitive to the external environment, including water conditions, shade or sun, and the presence of inter- and intraspecific competitors. A host of developmental rates and transitions, including overall growth, allocation of meristems, and the transition to flowering are sensitive to ecological cues. These sensitivities allow plants to plastically alter their growth and development in response to ecological variation. Understanding the evolution and genetics of plasticity in growth and development patterns, therefore, provides a window into how ecological, evolutionary, developmental, and genetic process interact to shape complex phenotypes. Using plant growth curves as an exemplar trait, I will explore the evolution of complex plastic phenotypes in response to intraspecific density, two ecological variables that have pronounced effects on the plant growth and development. However, rather than analyzing a few landmark ages, I will present analyses on the entire growth curve, using the curve itself as the unit of analysis. In particular, I will explore newly developed methods for finding and describing evolutionary lines of greatest resistance—and evolutionary lines of least resistance—for traits that are best described as curves. By comparing between different intraspecific density environments, I will explore how intraspecific competition and the shade avoidance response affect evolutionary lines of greatest resistance, and least resistance. By comparing between different light environments, I will explore how external abiotic environments and resource availability affect these key evolutionary trajectories. Collectively, these comparisons will inform how key ecological variables affect the evolutionary dynamics of key developmentally plastic traits.

## Sonia Sultan

(Wesleyan University)

### **Trans-generational plasticity in *Polygonum*: environmental inheritance and adaptive diversity.**

An intriguing aspect of plasticity is the capacity for environmentally stressed parental (generally maternal) individuals to alter traits of their offspring in specific ways that enhance offspring success under those same stresses. This transgenerational aspect of plasticity results in inherited adaptations that are not based on genetic variants. A case study examines transgenerational effects of ecologically realistic stresses, such as drought and shade, based on multi-generation norm of reaction experiments with naturally evolved genotypes. Drought-stressed parent plants of the annual ecological generalist *Polygonum persicaria* produced seedling offspring with longer, more rapidly extending root systems and significantly greater seedling growth in dry soil, compared with the offspring of genetically identical parents that had been given ample water. Further studies with *P. persicaria* revealed that adaptive transgenerational drought plasticity persisted over two generations. When both parent and

grandparent plants were drought-stressed, offspring size and root extension increased significantly. These seedlings also had lower mortality in very dry soil than genetically identical seedlings whose parent and/or grandparent had been amply watered, confirming the fitness impact of these inherited environmental effects. Expression of adaptive transgenerational effects varied considerably among genotypes (i.e., genotype x parental or grandparental environment interaction). At a higher level of diversity, closely related taxa may have different repertoires of transgenerational plasticity. The closely related, ecologically restricted species *P. hydropiper* expressed maladaptive rather than adaptive effects of parental drought stress on offspring: in this species, drought-stressed parents simply produced smaller seedlings with correspondingly slower-extending root systems. Since the survival of seedlings is a critical factor in the ability of species to establish populations, such species' differences in transgenerational plasticity could influence relative success in establishing populations in resource-poor or variable sites, and hence shape ecological distribution. Indeed, this appears to be the case in *Polygonum*: although *P. persicaria* occurs in a broad range of moisture conditions in the field, *P. hydropiper* does not establish populations in either dry or variably dry sites. In addition to genotypic and species-level differences, architectural position on the maternal plant can alter transgenerational environmental effects on offspring development. These inherited effects are likely mediated by both provisioning and epigenetic modifications; they contribute to fitness-related variation in natural populations but show different dynamics than variation based on allelic diversity.