



The Developmental Basis of Evolutionary Innovation

**The Arnold Arboretum of Harvard University
Boston, MA
March 3-5th, 2017**

A workshop sponsored by microMORPH,
a NSF Research Coordination Network

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Friday, March 3rd

8:45 AM	Meet bus to Weld Hill (outside of Taylor House)
8:30 AM - 9:00 AM	Breakfast (at Weld Hill, for participants staying at the arboretum apartments)
9:00 AM - 9:15 AM	Introductions
9:15 AM - 9:45 AM	William (Ned) Friedman and Pamela Diggle <i>Welcome to the Arnold Arboretum & overview of microMORPH and workshop goals</i>
9:45 AM -10:25 AM	Spencer Barrett <i>Some unresolved questions in plant reproductive biology</i>
10:25 AM - 10:45 AM	Discussion
10:45 AM -11:00 AM	Break
11:00 AM - 11:40 AM	Carolyn Wessinger <i>Genetic and developmental features of repeated floral evolution in Penstemon</i>
11:40 AM - 12:00 AM	Discussion
12:00 PM -1:30 PM	Lunch
1:30 PM - 1:50 PM	Irene Liao <i>How a flower lost its SWEETness: insights into nectar reduction</i>
1:50 PM - 2:00 PM	Discussion
2:00 PM – 2:40 PM	Robin Hopkins <i>The evolution and development of mate recognition in Phlox</i>
2:40 PM - 3:00 PM	Discussion
3:00 PM – 3:30 PM	Break
3:30 PM – 3:50 PM	Annika Smith <i>An integrated approach to exploring floral evolution in Tropaeolum, the nasturtiums</i>
3:50 PM - 4:00 PM	Discussion
4:00 PM – 4:20 PM	Adam Roddy <i>Macroevolution of floral hydraulic traits and strategies for maintaining turgor</i>
4:20 PM - 4:30 PM	Discussion
4:30 PM - 5:00 PM	General Discussion
5:00 PM - 6:30 PM	Free Time - Explore Arboretum
6:30 PM -8:30 PM	Dinner
8:30 PM	Meet Bus to Hotel (meet outside of Weld Hill)

Saturday, March 4th

8:45 AM	Meet bus to Weld Hill (outside of Taylor House)
8:30 AM - 9:00 AM	Breakfast (at Weld Hill, for participants staying at the arboretum apartments)
9:15 AM - 9:30 AM	Morgan Moeglein <i>The leaf developmental trajectory in Viburnum: connecting intra-individual variation to evolutionary diversification in leaf form</i>
9:30 AM - 9:45 AM	Discussion
9:45 AM -10:00 AM	Emily Chan <i>The genetics and ecology of variation in shoot architecture</i>
10:00 AM - 10:15 AM	Discussion
10:15 AM -10:45 AM	Break
10:45 AM - 11:25 AM	Jannice Friedman <i>Evolution of phenology and allocation in annual and perennial life history strategies</i>
11:25 AM - 11:45 AM	Discussion
11:45 AM -12:00 PM	General Discussion
12:00 PM -1:30 PM	Lunch
1:30 PM – 2:10 PM	Elizabeth (Toby) Kellogg <i>The spikelet pair and its role in diversification</i>
2:10 PM – 2:30 PM	Discussion
2:30 PM - 2:50 PM	Daniel Woods <i>Evolution of flowering time with a focus in the grass family</i>
2:50 PM – 3:00 PM	Discussion
3:00 PM – 3:30 PM	Break
3:30 PM – 3:50 PM	Annis Richardson <i>Boundary gene regulatory networks in grasses</i>
3:50 PM – 4:00 PM	Discussion
4:00 PM – 4:40 PM	Günter Theißen <i>On the origin of novelties: FQCs link plant development and evolution</i>
4:40 PM - 5:00 PM	Discussion
5:00 PM – 5:30 PM	General Discussion
5:30 PM - 6:30 PM	Free Time - Explore Arboretum
6:30 PM -8:30 PM	Dinner
8:30 PM	Meet Bus to Hotel (meet outside of Weld Hill)

Sunday, March 5th

8:45 AM	Meet bus to Weld Hill (outside of Taylor House)
8:30 AM – 9:00 AM	Breakfast (at Weld Hill, for participants staying at the arboretum apartments)
9:00 AM - 9:40 AM	Leonie Moyle <i>Phylogenomic, genetic, (and developmental) approaches to understanding trait evolution in two recent plant radiations</i>
9:40 AM – 10:00 AM	Discussion
10:00 AM -10:20 AM	Ray Watson <i>Gene expression evolution during sunflower domestication</i>
10:20 AM - 10:30 AM	Discussion
10:30 AM -10:45 AM	Break
10:45 AM – 11:25 AM	Jeff Doyle <i>'Curiouser and Curiouser': Down the rabbit-hole to the nucleome, the interleaved genome, and other mysteries</i>
11:25 AM - 11:45 AM	Discussion
11:45 PM -12:00 PM	Concluding Remarks
12:00 PM -1:30 PM	Lunch
	Taxis to Airport Begin

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Spencer C.H. Barrett*(University of Toronto)***Some unresolved questions in plant reproductive biology**

My talk will be unorthodox; instead of focusing on a single question or study system, I will present short vignettes on projects with many unresolved questions. The main emphasis will be on what we do not know, rather than what we know. The projects range in scope from evolutionary ecology to evolutionary genomics, and were not conceived to specifically embrace developmental questions. As a result, I am hoping participants at the workshop will make connections that I fail to see.

My interests largely concern the evolution of mating systems. Mating in seed plants arises from interactions between plant traits and the environmental context in which individuals reside. These interactions commonly cause non-random mating, including selfing and promiscuous outcrossing within local neighborhoods. Extrinsic ecological factors influence all stages of the mating process – pollination, pollen-tube growth, ovule fertilization – as well as seed development, determining offspring quantity and quality.

Our work focuses on the three major transitions in plant reproductive systems – the evolution of selfing from outcrossing, separates sexes from combined sexes, and wind- from animal pollination. Below are some questions I will address.

- *Evolutionary breakdown of heterostyly to selfing* – Why is the inheritance of distyly and tristylly similar given their phylogenetically divergent origins? Do supergenes exist in heterostylous species? What is the genetic and developmental basis of selfing variants that replace outcrossing morphs?
- *Gender strategies, dioecy and biased sex ratios* – When is the gender of a flower determined? What are the mechanisms causing biased sex ratios? How can male- versus female-determining pollen tubes be distinguished to test hypotheses on certation and sex ratio bias?
- *Evolution of wind pollination from animal pollination* – What determines whether lineages evolve selfing versus anemophily through reproductive assurance? What floral traits predispose lineages to evolve anemophily? Is ambophily an intermediate stage in the evolution of anemophily?

Emily Chan

(Duke University)

The genetics and ecology of variation in shoot architecture

Investigating the environmental factors that select for developmental variations is important for understanding how organisms evolve. Plant branching architecture provides a novel way to study how developmental variation leads to evolution. The indeterminate growth of plants through meristems allows individuals to develop branches throughout their lifetimes. However, not all meristems develop into branches. Some species experience complete apical dominance where a single shoot grows from the apex producing axillary meristems along the shoot that remain repressed unless damage occurs to the apical meristem. Other species do not repress the growth of all axillary meristems and exhibit as more bush-like architecture. Species like the perennial Rocky Mountain wild flower, *Boechea stricta*, contains a range from some axillary meristem growth to complete apical dominance within the species. The most basic architectural form of *B. stricta* follows apical dominance. It is a single rosette in which the apical meristem of the rosette elongates to produce a stalk-like inflorescence in the summer. The architecture varies from producing one to eight rosettes and inflorescences within its first year in the greenhouse. A genome-wide association study (GWAS) of inflorescence number on over 300 sequenced accessions has identified four correlated SNPs. In addition, this architectural variation is correlated with the latitude and elevation of accession origin, which suggests local adaptation of the trait. This variation with genetic control and environmental correlations suggests that architectural variation is evolving within a species via natural selection.

Jeff Doyle

(Cornell University)

‘Curiouser and Curiouser’: Down the rabbit-hole to the nucleome, the interleaved genome, and other mysteries

Two questions dominate my interests: 1) What is polyploidy? and 2) How did nodulation (symbiotic nitrogen fixation) arise, particularly in the legume family? The two questions are related, as polyploidy has been implicated in the evolution of nodulation in legumes. Moreover, both questions involve homoplasmy. Polyploidy is a massive parallelism, arising independently in many eukaryotic lineages; does it have emergent properties? Nodulation has also arisen multiple times, perhaps even within legumes, involving parallel recruitment of pre-existing gene regulatory networks from other symbioses, leading to deep homologies. The two phenomena also converge on such issues as the biology of single cell types, the complex internal structure of the nucleus, and the nature of transcriptional regulation. It has been assumed, since at least the 1970s, that polyploidy can fundamentally alter basic cellular parameters due to increased sizes of nuclei and cells. However, it turns out that it is unknown what controls nucleus or cell size. Different cell types—the building blocks of tissues and organs—have different characteristic size parameters ... but what, exactly, is a “cell type” and how does cell type evolve? Several symbioses involve the root hair, a cell type whose evolutionary origins are unknown, and that presumably originated or recruited new functions for nodulation to evolve. Recruitment of gene regulatory modules is thought to be involved in cell type evolution; in the “nucleome”, spatial distribution of chromatin in TADs (“territorially associated domains”) may affect gene regulation and create functional groupings of genes; the rules for gene regulatory elements remain obscure, and some recent theories hold that the entire genome is transcriptionally “interleaved”, but

the implications for modular function and recruitment are unknown; little is known about conserved noncoding sequences (CNS) in plants ... there are many open questions concerning the molecular evolutionary underpinnings of form and function!

Jannice Friedman

(Syracuse University)

Evolution of phenology and allocation in annual and perennial life history strategies

The timing of individuals' growth, reproduction, and death are fundamental to their fitness and represent striking adaptive differentiation. In flowering plants, one of the main differences between annuals and perennials is the switch from vegetative growth to flowering. In annuals this transition occurs once and is followed by death, while perennials cycle repeatedly through vegetative and reproductive phases. Using a combination of field and controlled growth experiments we are identifying the key genetic and ecological differences between annuals and perennials within a single species, *Mimulus guttatus*. Using naturally occurring genetic variation, we are asking how seasonal variation in temperature and photoperiod contribute to the timing of germination, growth and flowering, and allocation to sexual and clonal reproduction. Our research investigates differences in germination timing, and flowering responses to vernalization and photoperiod. Using QTL mapping and quantitative genetic analyses, we have identified shared genetic pathways, and constraints on adaptive evolution due to genetic correlations between fitness components. Furthermore, we are using field experiments with recombinant families to assess fitness differences and identify QTLs in the native environment between annuals and perennials, and perennials from different parts of the native range. Our research sheds light on how selection has shaped allocation strategies in annual and perennial populations, and how plants may continue to evolve as climates shift and alter phenology, and the relative benefits of sexual and clonal growth.

Robin Hopkins

(Harvard University)

The evolution and development of mate recognition in *Phlox*

Plants cannot move to seek out appropriate mates but they can still be agents of choice. Two of the most important mate choices are whether or not a plant will self-pollinate and whether or not a plant will reject pollen from another species. For both of these choices some plants have evolved molecular signaling between pistils and pollen to identify and reject inappropriate mates. I will present a case study of how these two mate choices are mechanistically and evolutionarily linked to each other in the Texas wildflower species *Phlox drummondii*. Within this species we find a strong correlation between self-incompatibility and unilateral incompatibility with a closely related species *P. cuspidata*. The correlated variation in incompatibility is associated with differential ability of both self and interspecific pollen to bind on the stigma of *P. drummondii* plants. Self-compatible plants are also more compatible with *P. cuspidata* pollen than are self-incompatible plants. Our results suggest a mechanistic link between self-incompatibility and unilateral incompatibility. Furthermore, we find significantly higher self-compatibility in allopatric regions of *P. drummondii* range compared to sympatric areas where the two *Phlox* species grow in close proximity. This geographic distribution of incompatibility suggests that the evolution of self-compatibility within *P. drummondii* may be influenced by interactions with interspecific compatibility.

Elizabeth A. Kellogg

(Donald Danforth Plant Science Center)

The spikelet pair and its role in diversification

The spikelet pair is a synapomorphy for the grass tribe Andropogoneae, a group that includes maize, sorghum, and many ecologically dominant grassland species. The spikelet pair is controlled by novel proteins and protein domains, and cannot be explained simply by cis-regulatory changes in existing gene networks. The 1200 species of the tribe are the result of a rapid radiation in the late Miocene, coincident with the spread of the major C4 grasslands of the world. The spikelet pair originated well before the major radiation, suggesting either that it is not an important innovation or that it was followed by a time lag. However, the radiation coincides with a shift in the position of the abscission zone from below the spikelet to below the spikelet pair, such that the pair is shed as a unit. At the same time, the fates of the two spikelets of the pair become decoupled, such that they may differ in size, extent of development, sex expression, presence or absence of hairs, and awns. Combinatorial variation of these characters easily can account for the large number of species in the tribe. The spikelet pair clearly controls pollen presentation, the timing of anthesis, and breeding system, and we presume is under selection for this function. In addition, the morphology of the spikelet pair, particularly the structure and position of hairs and awns, has a demonstrated function in dispersal. Finally, the two spikelets of the pair differ in their role in carbon capture. Experiments with isotopes of carbon show that the seed-bearing spikelet and its awn are carbon sinks, whereas the pedicellate spikelet is a source of photosynthate for the developing seed. Thus the spikelet pair functions at pollination, seed-filling, and dispersal. The correlation of each of these with particular environments can be tested in the context of a well-resolved phylogeny of the tribe.

Irene Liao

(Duke University)

How a flower lost its SWEETness: insights into nectar reduction

In flowering plants, the shift from outcrossing to selfing is one of the most common evolutionary transitions. This transition is associated with changes in several floral characters, collectively known as the selfing syndrome. While one study demonstrates that natural selection drives reduced flower size in selfers, evidence for other syndrome traits is lacking. I am examining this issue by focusing on reduced nectar production in *Ipomoea lacunosa* (Convolvulaceae). This morning glory has a 95% selfing rate and exhibits selfing-syndrome characters (reduced floral size, pigment loss, little nectar) compared to its outcrossing sister species, *I. cordatotriloba*. As a first step, I am determining whether reduced nectar production has evolved independently of other selfing-syndrome traits. I measured the nectar volume, nectar sugar concentration, total nectar sugar amount, and corolla length in an F3 population created from a cross between *I. cordatotriloba* and *I. lacunosa*. I determined that nectar volume is correlated with all three traits. With my collaborators, we are in the process of identifying QTLs for these traits from data generated from double-digest restriction-site associated DNA sequencing. From literature searches, I have identified several genes associated with nectar production. One of the genes co-segregates with nectar volume differences between the two species, which is a potential QTL. Overall, this will lay the groundwork for molecular experiments to examine the evolutionary forces driving the reduction of nectar production in the evolution of the selfing syndrome.

Morgan Moeglein

(Brown University)

The leaf developmental trajectory in *Viburnum*: connecting intra-individual variation to evolutionary diversification in leaf form

How have leaf traits been shaped by selection over evolutionary time? I am using leaf trait variation in *Viburnum* as a model for studying the evolution of leaf form. Temperate *Viburnum* often exhibit strong within-individual variation in leaf shape between preformed leaves that overwinter in buds and neoformed leaves that form during the growing season without a resting bud stage. The preformed leaves are often more circular in shape with toothed margins, while the neoformed leaves tend to be more elongate fewer teeth or even entire margins. Differences between temperate and tropical members of the clade recapitulate this contrast, with temperate members generally being rounder and toothier and tropical members being more elongate and entire. How different are the developmental trajectories of preformed and neoformed leaves and how might these differences relate to the repeated shifts in leaf form during tropical and temperate transitions? I am combining a detailed microscopy analysis of leaf development with RNA sequencing and flow cytometry to identify key developmental stages in leaf development, and in particular to understand how repeated transitions to bud packing in temperate *Viburnum* altered the early developmental trajectory of leaves, producing the great diversity of leaf form that we see today.

Leonie C. Moyle

(Indiana University)

Phylogenomic, genetic, (and developmental) approaches to understanding trait evolution in two recent plant radiations

For decades, studies of species radiations—instances of rapid successive lineage splitting events—have provided insight into the causes of diversification, including the genetic and developmental changes involved in the origin of novel traits. Recently, these analyses have been revolutionized by the availability of next-generation sequencing, which enable a detailed characterization of clade-wide variation across hundreds to millions of genomic sites. These data can be used to illuminate phylogenetic relationships among populations and species and, when combined with trait and developmental data, to uncover the physiological and genetic mechanisms responsible for trait diversification. We have been examining these processes in two closely related, ecologically diverse, clades in the Solanaceae—*Solanum* sect. *Lycopersicum* (wild tomatoes) and *Jaltomata* (the sister clade to *Solanum*). Using clade-wide comparative transcriptomic data, we observe several common features of genome-level diversification in both clades, most notably: evidence for abundant shared ancestral variation across whole clades; rampant gene tree discordance due to incomplete lineage sorting



Floral diversity among 10 species
from the genus *Jaltomata*

(ILS); and, discrete post-speciation introgression events between lineages. These complex patterns of genetic differentiation and sharing appear to be characteristic of species radiations in a diverse range of organisms. Despite these complexities, we show that (when carefully handled) phylogenomic data can still be used to identify loci that are evolving via divergent selection. Loci with selected changes that occur on branches associated with the evolution of novel trait variation are candidate loci contributing to rapid diversification. By combining these data with trait variation, genetic (including QTL mapping), and developmental data, we have begun to identify the specific genetic and developmental mechanisms of trait diversification in these two groups. This includes genetic and developmental mechanisms of lineage-specific adaptation associated with ecological differentiation in *Solanum*, and rapid floral evolution in *Jaltomata*.

Annis Richardson

(University of California, Berkeley)

Boundary gene regulatory networks in grasses

Every plant requires the formation of correct boundaries to develop, defining organ separation, influencing growth rate patterns, polarity, and final organ shape. Boundaries are also often the site of novel outgrowths or shape modifications. For example, in the grasses the ligule (an adaxial outgrowth of tissue) forms at the boundary between the lower (sheath) and the upper (blade) leaf regions, and in the dicots stipules form between the petiole and lamina blade. These boundary structures can be characteristic of whole families, indicating possible evolutionary adaptations. Boundaries are defined by the activity of gene regulatory networks (GRN) which are composed of nodes (e.g. genes) and their interactions (e.g. transcriptional regulation or protein-protein interactions). Despite the importance of boundaries, the GRNs responsible for plant boundary formation (particularly with respect to boundaries within organs) are largely unknown, especially in the grasses. Less is known about how boundary GRNs have been modified during evolution to generate new morphologies.

We are using maize as a grass model to identify boundary GRN components and explore how they are modified during development and evolution to generate distinct morphologies. To this end we are taking two approaches. The first is to identify a new node, by mapping and cloning a new leaf specific boundary mutant, fused leaf 1 (*fsl1*). The second is to identify new interactions for the key boundary regulatory node, *LIGULELESS 2* (*LG2*), which has a role in both leaf and tassel development. We are using a combination of Maize SNP50 bulk segregant analysis, RNAseq, ChIPseq, genetics, protein analytical techniques (e.g. yeast 2 Hybrid), 3D imaging and molecular biology (e.g. in situ hybridisation), to map, clone and functionally characterize *fsl1* and to identify *LG2* interactions. We will incorporate this new information into existing maize boundary GRNs and compare the maize GRN elements with other grass species, like *Setaria* and barley, to identify any conserved or modified elements. We will also compare the role of *LG2* in the leaf and tassel to identify key regulatory changes in different tissues and help shed light on why *LG2* has been recruited for functions in different developmental contexts. Important elements of these GRNs will then be tested using transgenics (e.g. using CRISPR to knock out genes of interest). Overall, we hope that this will contribute to grass boundary GRN knowledge and help us understand how boundary GRNs have been modulated during grass evolution to generate novel morphologies.

Adam Roddy

(Yale University)

Macroevolution of floral hydraulic traits and strategies for maintaining turgor

During the Cretaceous, early angiosperm leaf evolution was dominated by decreases in cell size that facilitated increases in vein and stomatal densities. Together these traits enabled leaves to more efficiently transport water and thereby increase photosynthetic assimilation rates. At the same time, flowers were rapidly diversifying morphologically in association with their animal pollinators. Due to their novel developmental pathways, floral anatomical and physiological traits are predicted to have evolved independently from those in leaves. Because flowers are heterotrophic, selection may have favored reduced water transport capacity in flowers and reliance on other physiological strategies to maintain turgor. Furthermore, with increasing aridity threatening plants globally, determining the physiological function of flowers is critically important to our understanding and predicting biotic responses to climate change.

I will present data from a series of studies employing a water balance framework to characterize the macroevolutionary patterns in floral hydraulic traits and physiological function. Variation in hydraulic traits more among extant species suggests that developmental programs may constrain hydraulic traits in early divergent angiosperm flowers to be more leaf-like, but that monocot and eudicot flowers have traits divergent from those in leaves. Additionally, selection on morphological traits important to pollination is tightly linked to physiological traits, suggesting that there are developmental constraints on these traits. Comparing traits in leaves and flowers shows that while early angiosperm flowers may have had traits resembling leaves, floral diversification is coupled to relaxation of correlations between leaf and floral traits. Indeed, leaves and flowers of monocots and eudicot exhibit divergent trait evolution, suggesting that the one key to floral diversification has been the evolution of greater modularity in the plant bauplan. Untethered by high physiological costs, morphological traits in monocot and eudicot flowers may more rapidly and directly track pollinator preference.

Annika Smith

(University of Florida)

An integrated approach to exploring floral evolution in *Tropaeolum*, the nasturtiums

The ability to successfully address the complex, multidimensional process of plant character evolution requires approaches that integrate across domains: genetics, evolution, development, and ecology. Likewise, in order to understand the patterns of plant character evolution across a broad phylogenetic scale, we must continue to extend beyond current model organisms and identify new candidate genes implicated in phenotypic evolution. I will explore the potential for an approach that synthesizes systematics, evo-devo, and bioinformatics to generate candidate gene hypotheses in non-model plants. Drawing on successful approaches from vertebrate systems, I propose a data-driven approach using ontologies to link the phenotypes and developmental processes of non-model plant clades to underlying candidate genes identified from the model plant *Arabidopsis*. I will discuss the application of this approach in the context of non-model *Tropaeolum*, commonly known as nasturtiums, to explore the genetic and developmental basis of phenotypic convergence and constraint in floral evolution. The placement of *Tropaeolum* within Brassicales, the same clade in which *Arabidopsis* occurs, provides the possibility of employing an ontological approach to develop candidate gene hypotheses for floral phenotypes. Additionally, more distantly related plant clades with convergent phenotypes could also be systematically queried through ontologies to explore the molecular and morphological basis for

convergence and to suggest additional candidate genes. Both the challenges and potential inherent in ontological approaches will be discussed, as well as the integral role that development must play in the process of ontology design. Comparative genomic, phylogenetic, and gene expression studies are downstream methods that could be used in the interpretation of the candidate genes suggested by the ontologies. Ultimately, I hope to use this approach to elucidate the mechanisms behind the patterns of variation in floral form, especially as they relate to pollination, within *Tropaeolum*.

Günter Theißen

(Friedrich Schiller University Jena)

On the origin of novelties: FQCs link plant development and evolution

The expansion and diversification of MIKC-type MADS-domain transcription factors (MIKC TFs) is strongly correlated and appears to be causally linked to the origin of major novelties during the evolution of land plants, such as ovules/seeds, flowers and fruits. MIKC TFs are a synapomorphy of streptophytes (charophyte green algae + land plants). Based on the presence of a keratin-like K domain they have the capacity to constitute different dimeric and tetrameric complexes in a combinatorial way. By binding to specific cis-regulatory elements on DNA (CArG boxes) they constitute floral quartet-like complexes (FQCs) that control diverse sets of target genes and hence developmental processes in land plants. Since the protein constituents of FQCs are all paralogous MIKC TFs, events such as gene duplication, sub- and neofunctionalisation, paralog interference and escape from adaptive conflict all may have played important roles during the evolution of MIKC TFs in land plants. To better understand the proximate mechanisms that generated major novelties in land plants, my group studies FQC formation in a phylogenetic framework throughout the streptophytes. I will report about recent insights concerning the origin of FQCs during evolution and the molecular basis of FQC formation and function. We hypothesize that the unique phylogenetic trajectory of paralogous MIKC TFs interacting in FQCs facilitated the diversification of efficient genetic switches controlling development and hence the origin of novelties in land plants. This way FQCs provide a link between plant development and evolution.



< **Figure 1. Example of an FQC: the floral quartet specifying stamen identity in angiosperms.** The looped DNA is shown in blue, with two protein binding sites (CArG boxes) in yellow. The four circles represent MIKC TFs (red, class B proteins; blue, class C protein; green, class E protein; with B, C, E according to the ABCDE model of flower development). Figure courtesy of Florian Rümpler.

Key References:

- 2) Theißen, G., Melzer, R., Rümpler, F. (2016). MADS-domain transcription factors and the floral quartet model of flower development: linking plant development and evolution. *Development* 143, 3259-3271.
- 2) Gramzow, L. et al. (2014). MADS goes genomic in conifers: towards determining the ancestral set of MADS-box genes in seed plants. *Ann. Bot.* 114, 1407-1429.

Ray Watson

(University of California, Berkeley & University of Virginia)

Gene expression evolution during sunflower domestication

The developmental and historical context of genetic changes underlying phenotypic evolution provide insight into the process of adaptation. Domestication offers an ideal case study for adaptive evolution by providing a circumscribed timeline over which many traits rapidly evolve under selection. Over the last 5000 years of domestication, the common sunflower, *Helianthus annuus*, underwent selection for dramatic phenotypic evolution including the loss of seed shattering, seed dormancy and self-incompatibility; an increase in size and weight of fruits and inflorescences; and reduced branching. To reveal changes in transcription potentially contributing to this morphological divergence, we tested for differential gene expression between wild and domesticated sunflowers by RNA-sequencing. Wild and domesticated plants were grown in controlled conditions, and we sampled 9 tissues across five developmental stages relevant to domestication traits. Reads were aligned to the sunflower reference genome and significant differential expression was determined by consensus of several statistical packages. To link expression divergence with signatures of selection during domestication, we examined the intersection of differentially expressed genes with loci highlighted by selective sweep analyses comparing sequence divergence between wild and domesticated sunflowers. By connecting sweeps to developmental stage or tissue specific expression patterns, we advance inferences about which loci contributed to the morphological evolution of specific sunflower domestication traits. Our candidate domestication genes will be targeted for ancient DNA sequencing from archeological samples to build a timeline of adaptive evolution during domestication and study the rate and scope of sunflower evolution.

Carolyn Wessinger

(University of Kansas)

Genetic and developmental features of repeated floral evolution in *Penstemon*



In *Penstemon*, flowers adapted to hummingbird pollination have repeatedly evolved through coordinated evolutionary transitions in a complex of distinct floral traits. This evolutionary transition is (partially) in alignment with floral trait correlations present in a population that display the ancestral bee pollination syndrome. Therefore it is possible that pre-existing correlations among floral traits facilitate rapid adaptation to hummingbird pollination. Genetic and morphological data suggest that predictable changes to underlying developmental pathways underlie independent origins of hummingbird-adapted flowers in this group. The genetic basis of flower color change suggests a potential genetic constraint on evolutionary reversal from flowers attractive to hummingbirds to those attractive to bees.

Daniel Woods*(University of Wisconsin, Madison)***Evolution of flowering time with a focus in the grass family**

Proper timing of flowering is essential for reproductive success in plants and is a major determinant of plant form. A key adaptation to seasonal variation in temperate climates is the evolution of a vernalization requirement. Vernalization is the process by which competence to flower is achieved after prolonged exposure to winter cold. In addition to vernalization prolonged exposure to noninductive short days (SD) followed by a shift into inductive long days (LD) has the ability to substitute for the vernalization response in a range of flowering plant families. A vernalization and SD vernalization requirement is found in a range of flowering plant families, yet little is known about the molecular nature of this phenomenon outside the eudicot model *Arabidopsis thaliana* and nothing is known about SD vernalization. We report the characterization of the vernalization and SD vernalization response across diverse *Brachypodium* accessions, and assess flowering time from crosses between accessions that vary in their vernalization requirement. There is considerable variation in flowering responses across the *Brachypodium* accessions tested and natural variation studies suggest rich genetic diversity underlying this trait. Additionally, we have screened for flowering time mutants, which we are now characterizing. Relative to the large genomes, long generation times, and domesticated backgrounds of cultivated cereals, *Brachypodium* offers a powerful opportunity to understand flowering time at a deeper molecular level in grasses.