My research program examines the role of environmental variation in the maintenance of functional genetic variation within species. We address this basic problem of balancing selection using two model systems, *Drosophila* and *Daphnia.* Due to their rapid development time, these assemblages of broadly distributed species have multiple generations per year (~10), enabling us to study their evolutionary dynamics in real-time and across a varied spatial landscape. Our work on *Drosophila* focuses on the genetic basis of local adaptation to seasonal and latitudinal variation in climate. Our work on *Daphnia* examines the evolutionary forces that drive spatially structured and seasonally variable genetic variation in anti-predator defenses and sexual-specialization. In our research, we combine field-work, genomic analysis, large-scale phenotyping efforts, and molecular genetics with computational and experimental tools that we develop to gain insight into the basic evolutionary forces that maintain diversity.

***Drosophila***. *D. melanogaster* living in orchards throughout the world experience seasonal fluctuations in selection pressure driven by changes in temperature and resource level. These wild populations of flies harbor an abundance of functional genetic variation, wherein some individuals are genetically more likely to survive winter and others are better equipped to exploit the favorable growing season. Seasonal variation in phenotype is presumably generated by polymorphisms that fluctuate in frequency between seasons. Ongoing work in my lab seeks to (1) further document genetic variation across time and space in *Drosophila* and to use this genomic data to infer the rapid evolutionary dynamics of natural populations; and, (2) to uncover the genetic architecture, molecular function, and evolutionary history of natural polymorphisms that underlie selectively maintained phenotypic variation.

We have taken two broad approaches to advance our *Drosophila* work. First, we are involved in several large-scale collection and re-sequencing efforts. I am a participant of, and leader in, a global collaboration to generate estimates of allele frequencies genome-wide for populations sampled among seasons, across decadal time-scales, and over multiple continents. The current incarnation of this collaboration is the joint effort of two consortium that I am involved with, *DrosRTEC* and *DrosEU*. Using a smaller version of this data-set, we demonstrated that seasonal adaptation occurs in multiple populations and show that the direction of allele-frequency changes across the genome is predictable with knowledge of temperature in the weeks prior to fly sampling (Machado\*, Bergland\*, *et al.*, in revision - *eLife*).

In another independent collection effort, we are characterizing seasonal changes in aspects of population structure at a local orchard, coupling individual sequencing with estimates of allele frequencies from pooled samples of flies collected every two weeks over a three year period. This work, carried out by Alyssa Black (PhD student) will allow us estimate basic parameters of local population structure and population size as they change through the growing season. Estimates of these parameters are important for building explicit models of allele frequency change and formally testing alternate hypotheses about the nature and extent of seasonally varying adaptation.

Our second approach to studying local adaptation in flies is to link genotypes to the phenotypes that adaptively vary across time and space. We are mapping natural genetic variation in temperature dependent diapause in *D. melanogaster* (Priscilla Erickson, Jane Coffins Childs Postdoctoral Fellow). To conduct this work, we (Cory Weller, PhD student) have developed a novel mapping strategy which uses synthetic hybrid populations derived from pre-sequenced inbred lines coupled with ultra-shallow sequencing of individuals to accurately (>99.9%) reconstruction phased genomes (Weller & Bergland, in prep – *Genetics*). For our diapause work, thousands of hybrid individuals representing genetic variation sampled across East Coast were exposed to a range of temperatures (10-15°C) across four photoperiods, enabling us to assess the interaction of multiple environmental factors on diapause incidence and map natural variation in environment dependent diapause (i.e., ‘GWAS’).

By layering GWAS results on top of our rich knowledge of allele frequencies across time and space, we have been able to pinpoint extremely promising candidate loci. One such locus is a putative regulatory polymorphism upstream of a highly conserved micro-RNA (*mir-184*). This particular SNP is the sixth most seasonally variable SNP in the genome and in the top 0.1% of our GWAS hits, a pattern unlikely by chance. Mutations in *mir-*184 are known to have ovarian phenotypes, possibly through its predicted regulatory effects on *dilp8*, a gene that shows strong differences in expression between diapausing and non-diapausing ovaries of wild-type flies. We are working to validate the effects of this SNP through line-crosses of inbred wild-type strains, quantitative complementation, transcriptional profiling, and molecular genetic analysis. Using field based assays at our experimental orchard, we are testing whether this polymorphism affects fitness in the wild, and are relating it to seasonal changes in diapause.

To complement our work on specific traits, we are examining signals of local adaptation across time and space at loci controlling expression variation (i.e., eQTL) identified in the lab and the field (Yang Yu, PhD student). By examining signals of local adaptation among large number of putatively functional polymorphisms, we will be able to make quantitative statements about the predictability of local adaptation and further examine the extent to which rapid adaptation across time and space promotes neutral and fitness related genetic variation genome-wide.

***Daphnia***. *Daphnia pulex,* a small aquatic crustacean living in ponds and lakes across the temperate zone, is a cyclic parthenogen and an important keystone species through its role as grazer and prey. Clonal isolates of *D. pulex* sampled within and among ponds and across seasons show extensive genetic variation in life-history traits, predator defense capacity, and sexual dynamics. Our work in Daphnia examines the molecular evolution of loci controlling variation in these traits and works to identifying the evolutionary forces maintaining dramatic phenotypic variation among clones.

Our Daphnia work is primarily situated around a set of interconnected ponds in southern England. We have established a large collection (~300) of isofemale lines from these ponds. Each of these lines has been fully resequenced and mapped to a new, high quality reference genome that we have generated (Karen Barnard-Kubow, NRSA Postdoctoral Fellow; Dörthe Becker, Marie Curie Horizons 2020 Postdoctoral Fellow). We discovered that one of our focal ponds was dominated by two clonal lineages that differ in sexual proclivities. One clonal lineage produces a high percentage of male offspring and sexual (recombinant) offspring, whereas the other tends to produce a large number of asexually produced offspring. In a down-stream pond no set of clones dominate and clonal isolates are recombinant offspring of the dominant lineages in the first pond (or their close relatives) enabling us to map the genetic basis of the tradeoff between reproductive investment strategies. We are currently characterizing fitness differences between these two dominant clonal lineages in the lab through mesocosm and life-table experiments and examining their changes in frequency and sex in the field through seasonal sampling.

We are situating this work in a broader context by examining within- and between population variation in the environmental determinants of sexual-specialization in ponds sampled across England and using whole genome data to study the recent evolutionary history of this dynamic and wide-spread polymorphism. This work is important because will allow us to test if sexual-specialization evolves in response to aspects of the local environment or if it is rather governed by frequency dependent processes.

We have also characterized variation within and among isofemale lines of Daphnia for their predator defense. When juvenile *D. pulex* sense the presence of predators they grow a thickened ridge along the dorsal edge of their carapace and often develop a several small spikes. These defenses increase the handling time of predators, thereby decreasing the risk of predation. To accurately characterize induced morphological defenses among our collection of clones we have developed a semi-supervised image analysis tool that produces standardized ‘traces’ of the dorsal edge (Becker *et al*, in prep – Methods in Ecology and Evolution). The goal of this work is to characterize the nature of genetic variation in predator defense within and among clonal lineages and to link observed variation of induced responses to variation in predator abundance across time and space. This work is important because it allows us to test models of the persistence time of ecologically balanced polymorphism in response to dynamic inter-specific interactions.