1) <u>Major activities.</u> In the second year of this MIRA grant, my lab has made progress in studying the genetic basis of rapid adaptation in Drosophila and Daphnia.

Drosophila: Our work on Drosophila has focused on identifying the genetic basis of natural variation in fitness related traits that are subject to seasonally and spatially varying selection pressures. To accomplish this goal, we are performing high-resolution QTL mapping using transient, multi-parental populations (number of founders: 32 - 200). We have developed and validated a pipeline that accurately reconstructs genomes of hybrid individuals from sparse sequence data (0.5X/individual; PhD student Cory Weller).

This work has been focused on understanding the overwintering biology of Drosophila. We are currently undertaking experiments that examining seasonally variable gene expression (PhD candidate Yang Yu), starvation tolerance (Weller), diapause (post-doc Priscilla Erickson), and cold-tolerance & sensation (undergraduate Helen Stone + Erickson). These experiments use combinations of lab- and field-assays for mapping.

Our work on diapause is the most advanced. We have phenotyped ovarian status and reconstructed fully phased genomes of ~3000 flies exposed to a variety of temperature and photoperiod conditions. We have found that diapause in *D. melanogaster* is strongly temperature dependent and only weakly affected by photoperiod. Initial GWAS results show promising and strong associations with promising candidate genes (e.g., the cold sensor, *brivido-1*). Intriguingly, we find evidence of seasonally variable selection at these GWAS loci using allele frequency estimates from ~25 populations sampled throughout North America and Europe. We have established over-wintering experiments at our orchard at Morven Estates. In the upcoming year, we will begin a rigorous validation and analysis of the molecular & physiological mechanisms of the most promising candidate genes. We are also examining the role of other known thermosensory proteins in the temperature-dependent induction of diapause.

We also study the basic population genetics of Drosophila. Currently, we are examining meta-population dynamics. For this work (PhD student Alyssa Black), we have collected and sequenced individually sampled flies across the growing season. Our analyses so far have suggested significant departures from Hardy-Weinberg expectations, suggesting the presence of population structure. We are also actively engaged in several large, international collaborative efforts (DrosRETC & DrosEU) to sample and sequence Drosophila collected over time and space. Through analysis of these data, we have discovered that temperature is a strong predictor of seasonally adaptive allele frequency changes across 25 broadly distributed population.

Daphnia: Our work on Daphnia has focused on several basic projects. First, we are studying the evolutionary genetics of predator defenses. We have established a collection of ~200 fully sequenced Daphnia clones and a new reference genome

(10X & Dovetail) from a meta-population in southern England (post-docs Dörthe Becker & Karen Barnard-Kubow). We have phenotyped these clones for morphological predation defenses. We are developing an automated image analysis tools for this work (tech Austin Edwards), and have a complete pipeline established to process raw image files. Our analysis of these phenotypic and genomic data are underway.

We have also been studying basic population genetic questions using the genomic data that we gathered. We have found that these Daphnia populations are dominated by clones which frequency recombine and rapidly change in frequency. We are working to continue our examining of the population genomics of Daphnia through continued sampling and population genomic analyses. This work will allow us to test specific hypotheses about the role of cyclic parthenogenesis in the maintenance and turnover of lineages through time.

2) <u>Specific Objectives</u>. During the second year of this MIRA, we have generated large quantities of phenotypic and genomic data. We are actively analyzing these data in order to gain insight into the genetic basis of rapid adaptation in two organisms, Daphnia and Drosophila. Over the course of the next year, we will continue to analyze these data; we will validate GWAS results in our Drosophila work; we will examine phenotype-genotype relationships in Daphnia; we will continue with field sampling for both organisms.

3) <u>Significant results</u>:
   • Through simulation approaches, we have shown that association mapping using the hybrid swam approaches, used for example in our diapause work, have high power and increased specificity compared to alternative approaches (e.g., DGRP).
   • Quantitative genetic variation in temperature dependent diapause is subject to temporally and spatially varying selection.
   • Daphnia populations exhibit signs of rapid adaptation.

4) <u>Key Outcomes</u>. We have continued our work examining the genetics basis of rapid adaptation in two systems. This work is focused on studying the genetics of environmentally sensitive traits and seeks to understand how these traits evolve in response to changes in selection pressure through time and space. We are conducting this work in two model systems and contributing to existing genomic resources in both systems.