1. Background

All organisms live in environments that vary through time and space. As a consequence of environmental heterogeneity, populations of most species exhibit patterns of local adaptation in life-history, stress tolerance, and morphology. Local adaptation, a form of balancing selection, promotes functional genetic diversity within a species and can sometimes maintain it at intermediate frequencies for long periods of time. The overarching goal of my research program is to understand the genetic and physiological architecture of local adaptation in fitness related traits, to examine the long- and short-term evolutionary history of loci underlying local adaptation, and to further our understanding the nature of standing genetic variation in general.

I study the genetic and physiological architecture of local adaptation in two complimentary systems. The primary system I work on is *Drosophila melanogaster* which has served for decades as an important model for understanding local adaptation[1]. *D. melanogaster* originated in central Africa[2] and colonized the world in the wake of human migration over the last ca. 100-10,000 years[3]. Fly populations arrayed along latitudinal[4] and altitudinal[5] clines exhibit patterns of genetically based phenotypic differentiation consistent with local adaptation to temperate environments. Flies from poleward or higher elevation locales tend to invest resources more heavily in somatic maintenance whereas those from equatorial or lower elevation locales tend to invest more heavily in reproductive output. This trade-off is consistent with the basic resource allocation model of life-history evolution.

Genetically based phenotypic differentiation in fitness related traits also occurs among seasons[4]. Notably, flies sampled in spring - the recent descendants of individuals that survived winter - tend to be more hardy whereas those sampled during fall - the descendants of those individuals that prospered during summer - tend to invest resources more into reproduction. Because of the short generation time of *D. melanogaster*, rapid adaptive change among seasons occurs in fewer than 10-15 generations. We have shown that adaptation over seasonal time scales is driven by dramatic and cyclic changes in allele frequency at hundreds to thousands of loci that have measurable effect on phenotype[6]. These alleles segregate in ancestral populations and are thus likely old, balanced polymorphisms. As I discuss in Recent Progress, understanding the specific genetic architecture of local adaptation is tractable, particularly in organisms like *D. melanogatser*. Moving forward, my lab will pursue a mechanistic, molecular understanding the genetic architecture and physiological processes that underlie adaptation to temperate environments in *Drosophila*.

The second major system that I work on is *Daphnia pulex*. *Daphnia* has long served as a classic model for studying phenotypic plasticity. Individuals in this genus develop defensive morphology (i.e., “neckteeth”) and alter their life-history (growth rates and age-specific fecundity) in response to predator derived chemical cues[7]. *Daphnia* are preyed upon by two main predator groups that impose size selective mortality driving local adaptation and phenotypic plasticity. *Chaoborus* midge larvae tend to prey on small individuals whereas large vertebrate predators such as stickleback fishes tend to prey on large individuals.

Natural ponds vary in predation regime and we have examined adaptive divergence in *Daphnia* collected from ponds with either multiple predators (midge + fish) or a single predator (just midge). We have shown that heterogeneous predation pressure among ponds has driven local adaptation in life-history plasticity. As I discuss in Recent Progress, we have identified hundreds of polymorphisms that predictably vary among ponds with alternate predation regimes. Intriguingly, predation regime varies seasonally and analysis of a limited number of alozymes suggest that the population-genetic structure of *Daphnia* dramatically shifts over seasonal time scales[8]. These observations open the exciting possibility that *Daphnia* exhibits rapid adaptive oscillations in life-histories, similar to what we observe in *Drosophila*. Below, I discuss how we will examine the genetic architecture of local and rapid adaptation by taking advantage of the unique ecological and genetic attributes of *Daphnia*.

Both of the systems that I study are remarkable in that we have found large numbers of loci that show dramatic changes in allele frequency in response to fairly mundane selection pressures (seasons, predation). These, or similar, selection pressures are felt by virtually every species. Flies and *Daphnia* have been exposed to heterogeneity in such selection pressures for long periods of time. This suggests that environmental variation through time and space has maintained a large amount of functional & fitness related genetic variation for eons or has promoted recurrent *de novo* evolution of functional polymorphisms. A strict reading of the Neutral Theory[9] would suggest that such functional variation should be exceedingly rare and, moreover, difficult if not impossible to map to the nucleotide. The empirical fact that we, and others, have had success mapping such polymorphisms to the nucleotide suggests that the Neutral Theory remains incomplete in explaining patterns of genetic variation.

Key Gaps in Understanding and Opportunity for Conceptual Advancement. Recent advances have allowed us to make tremendous strides in understanding the genetic architecture of phenotypic variation and identifying the genomic basis of local adaptation. Many Mendelian traits have now been mapped to the nucleotide[10] and we have developed more nuanced insight into the nature of quantitative traits, even if their exact genetic architecture remains somewhat elusive[11] . We are now able to investigate the genomic basis of local adaptation in virtually every species[12], although our general understanding of how genetic variation maps to phenotypic variation is murky. It has been long suspected that local adaptation in response to environmental heterogeneity through time and space primarily drives phenotypic divergence of highly quantitative fitness traits (e.g., life-histories, morphologies, behaviors, physiology, development). Given the challenges in identifying the genetic architecture of these fitness-related traits there is a key gap in our knowledge of the mechanistic relationship between phenotype and genotype (i.e., 'the architecture') in the context of local adaptation.

The goal of identifying the genetic architecture of adaptation in fitness related phenotypes has recently been criticized[13,14]. For instance, Rockman[13], mirroring earlier sentiments[15], suggests there is an epistemological limit to this pursuit. Notably, he points out empirical support for the 'infinitesimal model' and suggests that measuring the phenotypic effect of common loci with exceedingly small effect size may not be technically feasible. Similar sentiments were recently echoed from a population genetic perspective[16], where a major supposition of the dominant Neutral Theory[9] suggests that the vast majority of common polymorphisms have infinitesimally small effects (i.e., are effectively neutral). Under the infinitesimal model, instances where phenotypic effects of natural alleles are measurable may rather reflect Mendelian loci[13] and thus might not inform our understanding of adaptation of quantitative traits. Yet, as I discuss below the phenotypic effect of loci that contribute to local adaptation in polygenic traits is measurable and strongly affects fitness in the wild. Thus, the dominant models of both evolutionary quantitative genetics and population genetics might not accurately reflect the nature of standing genetic variation.

Because we have a limited knowledge about the genetic architecture of local adaptation, we have a limited ability to address basic questions about their long- and short-term molecular evolutionary history. For instance, with an explicit and mechanistic knowledge of the genetic architecture of local adaptation we will be able to contribute to conceptual advances in evolutionary genetics by addressing the following questions: What fraction of natural polymorphisms contribute to local adaptation - is it a few or many? Is rapid and cyclic polygenic adaptation driven by predictable allele frequency changes at common polymorphisms? How old are ecologically balanced polymorphisms? Does local adaptation promote neutral genetic diversity genome-wide? Rigorously addressing these questions in a systematic and general way requires that we develop an understanding of the genetic architecture of polygenic local adaptation in multiple organisms.

In principal, many of these evolutionary questions can be addressed by identifying loci with significant patterns of differentiation through time and space in the absence of any concrete linkage to phenotypic variation. However, just as in analyses of adaptive evolution from classic molecular evolution and population genetic analysis, such landscape genomic approaches suffer from unknown false positive and negative rates due to historical demography[17]. Thus, without a systematic linkage of genotype to phenotype we have only a limited knowledge of the set of true positive loci with known functional effect. Furthermore, when testing the phenotypic effect of candidate polymorphisms we often focus on those candidates close to well known genes with plausible links to the phenotypes presumed to be under selection. This not only imposes a strong bias[18] but limits opportunities for functional annotation of un-studied genes[19]. One way to systematically link polymorphisms to specific genes in a relatively unbiased fashion is to perform cis-eQTL mapping and layer those functional annotations with estimates of allele frequencies through time and space[20]. Currently we have very little knowledge of the genetic basis of expression variation in *Drosophila* or *Daphnia.* This deficit inhibits us from making clear and testable predictions about the molecular function of polymorphisms we that have already identified from population & ecological genomic inference or addressing the broader questions in evolutionary genetics that I posed above.

2. Recent progress

Drosophila. Classically evidence of adaptation to temperate environments in *D. melanogaster* came from phenotypic, genetic, and genomic analysis along latitudinal gradients, particularly in North America and Australia. Often it was assumed that genetic differentiation along these clinal gradients was generated by spatially varying selection, with demography playing a limited role due to high migration rates among neighboring populations. In contrast, we have now shown that clinal variation in North America and Australia

may have been generated by secondary contact of European and African populations[21]. While adaptation in response to spatially varying selection does occur along these clines, identifying its genetic basis from patterns of allele frequency differentiation through space is difficult because of the confounding effects demography. However, we identified hundreds of loci that repeatedly shift in allele frequency between spring and fall in a single orchard population (Figure 1). Because these short term seasonal fluctuations are independent to the long term demographic history of this species, differentiation at these loci through time is unlikely driven by colonization history and thus likely reflects adaptation[6].

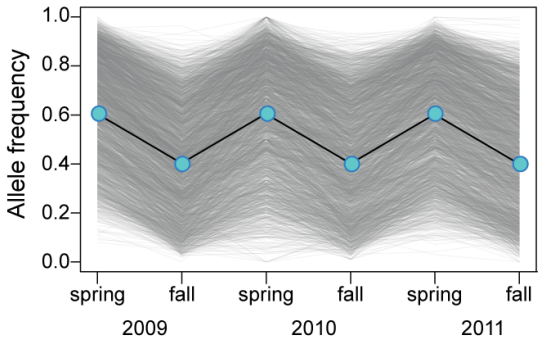

Figure 1. Adaptive oscillations at seasonal SNPs. Each line represents the allele frequency trajectory of a single SNP in a Pennsylvanian orchard population. Bold line shows average change between seasons. Figure taken from Bergland *et al*., 2014.

We sought to test whether adaptive oscillations over seasonal time scales were observable in additional populations throughout North America. To our surprise, large shifts in allele frequency at previously identified 'seasonal SNPs' in these newly sampled populations were only weakly predictable. There are many possible technical reasons for low, but observable, levels of predictability including mis-identification of seasons SNPs due to linkage. However one possibility remains tremendously exciting: **There are many functional polymorphisms that contribute to adaptation among seasons and in any particular phase of the seasonal cycle only some loci change dramatically in allele frequency; under this model, our identified seasonal SNPs only reflect a tiny fraction of functional polymorphisms in the genome that *could* contribute to seasonal adaptation.** Sporadic oscillations of seasonal SNPs might reflect different selective pressures from year to year or population to population. Alternatively, theory we are developing suggests that that weakly predicable changes in allele frequency among independent replicate populations exposed to identical selection pressures is not unexpected expected when populations undergo dramatic polygenic adaptation with per locus s ~ 10%.

Several lines of evidence suggest that seasonal SNPs are likely functional despite only moderate predictability of allele frequency change between spring and fall. First, we find that seasonal SNPs tend to vary among populations in ways that are consistent with adaptation to temperate environments: winter favored alleles are more common in northern locales than summer favored alleles and these patterns are found among our newly collected samples. Second, we have characterized the phenotypic effect of several seasonal SNPs. In one example[22], we examined seasonal SNPs in *Insulin Receptor* (*InR*) and showed that these naturally segregating polymorphisms have measurable affects on highly polygenic life-history and morphological traits. Intriguingly, these SNPs showed large adaptive oscillations in only a subset of years[22] despite adequate power to detect large shifts in allele frequency among seasons. This pattern is reminiscent of the moderate predictability adaptive oscillations among populations. We have begun a more systematic analysis of the phenotypic effect of seasonal SNPs by contrasting patterns of allele frequency change among seasons with SNPs associated with traits known to vary seasonally: generally, winter favored alleles are more likely to be associated with increased stress tolerance[6].

The other line of evidence that many seasonal SNPs are functional comes from population genetic inference. We calculated genetic diversity surrounding seasonal SNPs to test the idea that fluctuating selection pressures (a form of balancing selection) maintains genetic diversity at linked neutral SNPs. Theory suggests that an accumulation of genetic diversity is expected, but its decay rate will depend on the number of functional SNPs in the region. Because of the high recombination rate in Drosophila, we expect that such peaks of diversity should decay by ~500bp around a single balanced polymorphism. Yet, on average across all seasonal SNPs, we see elevated diversity for upwards of 50Kb. One model that can explain our elevated regions of diversity is that there are genes with multiple, independent seasonal SNPs that are maintained for long time-periods as a consequence of environmental heterogeneity through time and space.

To begin to rigorously test this polygenic model of adaptation we need to understand the phenotypic effect of seasonal SNPs identified through population/ecological genetic inference. Thus, as I discuss below, my initial research projects will be focused on identifying regulatory function of seasonal SNPs via cis-eQTL mapping. These projects will utilize resources that I currently have in hand. Such resources include a new set of ~250 inbred and fully resequenced strains of *D. melanogaster* we collected in the fall in Maine or during the spring and fall Pennsylvania. These lines can be used in conjunction with other resequenced populations

such as the DGRP population from North Carolina[23]. Analytic methods I will use in the proposed mapping experiments will be based on a pilot project that uses a similar experimental design but was conducted indoors. In addition, I have generated allele specific mRNA-seq and FAIRE-seq libraries from F1 hybrids between spring and fall inbred lines that were exposed to different environmental conditions; these data can be used to vet and verify the proposed cis-eQTL experiments. Finally, this work will complement the ongoing efforts of a consortium of fly biologists world-wide that I am co-organizing whose goal is to generate genome-wide estimates of allele frequencies through time and space over decadal scales and among multiple continents. Our consortium's work will be an incredible population genomic resource for the community and will enable research in computational and statistical methods to identify loci underlying adaptation to temperate environments. Understanding the functional role of these polymorphisms in a systematic manner will therefore be of great interest and utility to the community and represents a major innovation of this grant proposal.

Daphnia. Our work on *Daphnia* is at a different stage than our *Drosophila* work but I anticipate it will follow a similar trajectory. Here, I describe the state of our *Daphnia* research which focuses on adaptive differentiation of life-history traits in response to differences in predator regime.

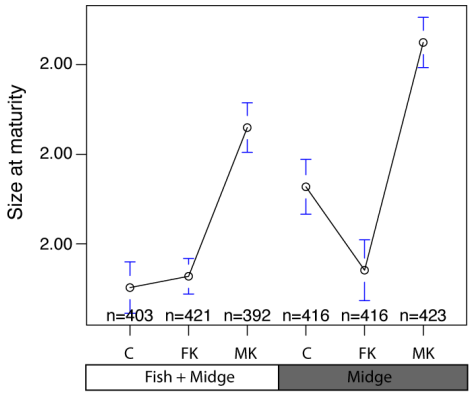

Figure 2. Adaptive reaction norms of Daphnia in response to fish (FK) kairamones, midge (MK) kairamones, or none (C) from clones collected in two different predation regimes, either fish and midge or just midge. Points represent average size at maturity among independent clones from eight ponds.

Daphnia, a keystone species group in aquatic ecosystems, are herbivorous grazers that are critical to the prevention of eutrophication and an important prey for larger planktonic predators. *Chaoborus* midge larvae, a gape-limited predator, tend to prey on smaller Daphnia individuals whereas larger vertebrate predators, such as sticklebacks and salamanders, tend to prey on larger individuals. These predation pressures impose strong size selective mortality in *Daphnia* populations.

Daphnia alter their morphology and life-history in response to these predators. In response to midge kairomones, Daphnia grow characteristic neck-teeth which prevent predation. When exposed to these same kairomones, *Daphnia* also tend to sexually mature later and a larger size. Conversely, when exposed to fish cues *Daphnia* tend to mature early and at a smaller size (Figure 1). We have shown that *Daphnia* from ponds with only midge predation (M) or midge and fish predation (MF) have adaptive differences in the plastic life-history response to these predators. These shifts in the age and size of reproduction are predicted by life-history theory[24].

We resequenced pooled samples of *Daphnia* clones from these ponds to estimate allele frequencies genome-wide. These data have identified several hundred SNPs that change in allele frequency among predation regimes. These loci are enriched near endocrine genes with known effects on invertebrate life-histories suggesting that they directly underlie adaptive divergence in life-history traits. Intriguingly, population genomic analyses of these loci suggests that many have undergone recent selective sweeps independently in each pond. The high rate of recurrent adaptation at these loci is possibly consistent with the life-cycle of Daphnia or the colonization history of the focal ponds.

There is much work to be done on this *Daphnia* system and it is ripe for investigation and innovation. The Daphnia genome has been sequenced and annotated and others are actively developing population genomic resources for this model system[25]. Data we generate under this proposed research will therefore be of interest and value to the *Daphnia* community.

3. Research plan.

Herein, I propose a series of projects in *Drosophila* and *Daphnia* to functionally characterize cis-eQTL in the context of ecologically relevant, environmental variation. Identification of environment dependent cis-eQTL will aid in the biological interpretation of polymorphisms identified to underlie local adaptation through time and space. By merging functional data that I will generate with existing and new allele frequency data from natural populations we will be able to make substantial progress in understanding the genetic and physiological architecture of local adaptation. Doing so will enable us to address the broader questions in evolutionary genetics that I highlighted above at the end of *Key Gaps*. By addressing this basic question of the genetic architecture in two species that differ in sexual dynamics (obligate versus facultative out-crossers) living in different ecosystems (terrestrial versus aquatic) and exposed to different classes of

selection pressure (abiotic versus biotic) we will be able to make generalized statements about the long- and short-term evolutionary history of loci that underlie polygenic local adaptation.

Drosophila. My lab will advance our knowledge of the genetic architecture of local adaptation in *Drosophila* by functionally characterizing cis-eQTL and their natural, season specific effects. Identifying cis-eQTL in the context of natural environmental change is important for several reasons. Notably, we know that the laboratory is not the wild and that the physiological pressures of lab life will be different from a fly in nature. For instance, circadian rhythms vary between flies in the lab and those kept in semi-natural conditions; microbiota do as well[26] and these commensals are now understood to dramatically shape organismal physiology[27]. Because physiology directly affects life-history, developmental, and behavioral traits, GWA mapping performed in the lab may identify a limited set of functional loci and possibly reflect idiosyncratic or cryptic associations. Thus, it is crucial to measure phenotypic effects of naturally segregating polymorphisms in response to ecologically relevant, environmental variation.

To further understand the genetic architecture of rapid adaptation to temperate environments over seasonal time scales we will identify season dependent cis-eQTL. To do so, we will utilize four sets of fully resequenced and inbred *D. melanogaster* strains. These four sets of fly strains were derived from collections made in Maine (fall), during the spring and fall in Pennsylvania, and in North Carolina (the DGRP); each set contains 80-200 strains. We will inter-mate strains within population sets for a small number of generations (~4) and use these flies to seed large cages (~9 m^3) maintained outside at an experimental orchard in Charlottesville, VA. We shall rear flies outdoors for one generation, maintaining them on semi-natural food made from a standardized apple-based medium (e.g., cornmeal-molasses medium plus cored, homogenized apples). We will then harvest outdoor reared flies and transfer them directly to RNA-later for down-stream library-prep. We will perform this experiment at four time points from early spring to late fall. At each sampling time-point we will harvest 96 3-5 day old flies per sex per replicate cage (2 reps) per population set (96 flies x 2 sexes x 2 reps x 4 populations x 4 time points = ~6000 flies/year). We will replicate this experiment over the course of three years, thereby generating one of the largest and most highly powered cis-eQTL mapping experiments performed to date in any species. Furthermore, this outbreeding scheme will potentially ameliorate some of the problems of GWA mapping that arise when performed in highly inbred lines[28]. This experimental design is sufficiently flexible and efficient to allow for modifications: e.g., collecting flies at different ages, modifying larval or adult resource level or substrate.

We will subsequently extract DNA and RNA simultaneously from single flies in a high-throughput fashion using an automated liquid-handling robot; individually indexed sequencing libraries will be generated for each fly. We will use a low-cost modified version of the Nextera library preparation method[29] for both gDNA and cDNA; preliminary experiments we have conducted demonstrate that there is sufficient gDNA and mRNA obtained from individual flies to generate these libraries. We will utilize custom synthesized indexing primers[30], enabling a dual-indexing strategy that enables us to multiplex ~6000 individuals per.

In addition to affording us the ability to perform a highly replicated cis-eQTL mapping experiment among seasons and years, the use of fully resequenced inbred strains as the founders of our outbred population cages allows us to infer the full genome of each individual fly with high accuracy even with very shallow coverage per fly (~0.0001-0.001X). We will use existing analytic methods to perform this genome inference[31]. mRNA-seq libraries will be sequenced at higher coverage per fly than gDNA but still relatively low coverage compared to traditional mRNA-seq experiments. Low per-fly mRNA-seq coverage will prohibit accurate estimation of gene expression on an individual basis. But, because we will be sequencing so many individuals we will be able to combine gene expression estimates across individuals with the same allelic state at putative cis-eQTL. *in silico* pooling of individuals will therefore allow us to identify statistically significant cis-eQTL that affect total gene expression and allele-specific expression. Finally, because we will be performing this experiment across seasons we will also assess seasonal changes in gene expression.

One possible complication to our experimental design is that we will be extracting mRNA from whole flies. By averaging signal across so many tissues, signals of differential gene expression in many genes may be attenuated. To ameliorate this potential problem, we will design target capture probes to enrich our samples for genes found to be differentially expression through the season as well as endocrine genes associated with life-histories (e.g., insulin signaling genes, catecholamine synthesis genes, Toll/Imd signaling). In addition, we will design capture probes for the most highly expressed genes (e.g., Actin) and un-enrich our samples for these housekeeping genes. These approaches have been validated[32] and will allow us to

estimate more accurate gene expression levels for those genes that are weakly expressed but may nonetheless contribute to the physiological mechanisms of adaptation to temperate environments.

To follow up on these caged population experiments, we will also collect wild *D. melanogaster* throughout the Virginia area for gene expression & metapopulation structure analysis. The purpose of these experiments with wild flies is to: (1) verify that the seasonal expression measurements we obtain from the caged flies match those of flies in the wild; and (2) assess basic aspects of fine-scale meta-population structure that occurs over meters to kilometers and possibly affects the dynamics of temporally variable selection.

Daphnia. Similar to our experiments with Drosophila, we will identify cis-eQTL in *D. pulex* and asses how these loci are associated with local adaptation to predation pressure. To perform these experiments, we will first establish a new set of Daphnia clones from the ponds in which we based our initial assessment of adaptive differentiation in morphological and life-history plasticity. We will isolate 96 independent clones from each of these eight ponds and fully re-sequence their genomes (8 ponds x 96 clones = 768 genomes). The *Daphnia* genome is relatively small (~200 Mb[33]) and thus we will be able to obtain high quality (~10X) genome sequences for each clone with a modest sequencing effort.

These fully resequenced clones will form the basis of our cis-eQTL and phenotypic mapping efforts. First, we shall re-asses morphological and life-history plasticity in response to a range of either midge and fish kairomones (4 exposure levels/kairomones) following established protocols[7]. Next, we will sample ~100 individuals per clone during the 2nd instar, the developmental period when neckteeth growth begins, for mRNA-seq. Note, this experimental design differs from our Drosophila work in that we will be using multiple individuals per clone/treatment; this approach is justified because *Daphnia* are facultative parthenogens and thus clonal lineages do not suffer the same sort of inbreeding effects as isogenic *Drosophila* strains. We will generate shallow mRNA-seq profiles per clone and kairomones treatment (768 clones x 4 exposure levels x 2 kairomones types = ~6000 samples) using an automated liquid handling robot coupled with an inexpensive Nextera library preparation method and highly multiplexed dual-indexed sequencing adaptors.

Genomic, transcriptomic, and phenotypic data from these clones will allow us to map loci that modulate the plastic response to predation pressure. One possible complication in this experiment is that we will be utilizing clones from distinct populations and thus population structure must be carefully accounted for while performing genome-wide association mapping. However, we can resolve this technical problem by utilizing existing statistical methods designed to perform GWA among structured populations[34]. Furthermore, as I described above for our fly work, one possibility is that we will have limited resolution to identify differentially expressed genes or allele specific expression for weakly expressed transcripts; to ameliorate this problem, we will employ a similar enrichment/un-enrichment strategy using target capture probes.

Future Directions. The work proposed above will provide a solid foundation for addressing my ultimate goal of understanding the genetic architecture of local adaptation. The tools and analytic methods we develop over the course of this proposal will aide in future work which I will briefly describe here.

Drosophila: We are very interested in resolving whether the adaptive oscillations we observe over seasonal time scales are driven exclusively by abiotic factors such as climate or also result from intrinsic cycles that are a consequence of populations overshooting carrying capacity[35]. In flies, parental environments have been shown to modulate offspring physiology[36,37], gene expression[38], and thus likely affects life-history. Thus parental environments plausibly plays a role in cyclic adaptation over seasonal time-scales. The methods and experimental approaches we develop during the course of the proposed research will aide in studying these trans-generational effects.

Daphnia: Adaptive evolution of Daphnia in response to predator pressure likely drives adaptive evolution of phytoplankton that *Daphnia* consume. These phytoplankton (e.g., Chlamydomonas) form multi-cellular clumping phenotypes as a physiological response to *Daphnia* and also show signs of rapid and cyclic adaptive evolution in response to planktonic grazers[39]. Thus, adaptive evolution of *Daphnia* in response to predator heterogeneity through time and space may drive adaptive evolution of the phytoplankton they consume leading to an ‘ Adaptive Cascade’ (similar to a Tropic Cascade[40]). In the future, we shall test this model using sequenced clones that we develop during the course of this proposed research.

1. Adrion, J. R., Hahn, M. W. & Cooper, B. S. Revisiting classic clines in Drosophila melanogaster in the age of genomics. *Trends Genet.* (2015). doi:10.1016/j.tig.2015.05.006. PMID: 26072452
2. Pool, J. E. *et al.* Population genomics of Sub-Saharan *Drosophila melanogaster*: African diversity and Non-African admixture. *PLoS Genet* **8,** e1003080 (2012). PMID: 23284287
3. David, J. & Capy, P. Genetic variation of *Drosophila melanogaster* natural populations. *Trends in Genetics* **4,** 106–111 (1988). PMID: 3149056
4. Schmidt, P. S., Matzkin, L., Ippolito, M. & Eanes, W. F. Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. *Evolution* **59,** 1721–1732 (2005). PMID: 16331839
5. Rajpurohit, S., Parkash, R., Ramniwas, S. & Singh, S. Variations in body melanisation, ovariole number and fecundity in highland and lowland populations of *Drosophila melanogaster* from the Indian subcontinent. *Insect Science* **15,** 553–561 (2008). PMID: NA.
6. Bergland, A. O., Behrman, E. L., O'Brien, K. R., Schmidt, P. S. & Petrov, D. A. Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*. *PLoS Genet* **10,** e1004775 (2014). PMID: 25375361
7. Beckerman, A. P., Rodgers, G. M. & Dennis, S. R. The reaction norm of size and age at maturity under multiple predator risk. *Journal of Animal Ecology* **79,** 1069–1076 (2010).
8. Lynch, M. Ecological Genetics of Daphnia pulex. *Evolution* **37,** 358 (1983). PMID: 20522144
9. Kimura, M. *The neutral theory of molecular evolution*. (1984). PMID: NA
10. Linnen, C. R. *et al.* Adaptive Evolution of Multiple Traits Through Multiple Mutations at a Single Gene. *Science* **339,** 1312–1316 (2013). PMID: 23493712
11. Wood, A. R. *et al.* Defining the role of common variation in the genomic and biological architecture of adult human height. *Nature Genetics* **46,** 1173–1186 (2014). PMID: 25282103
12. Savolainen, O., Lascoux, M. & Merilä, J. Ecological genomics of local adaptation. *Nature Reviews Genetics* **14,** 807–820 (2013). PMID: 24136507
13. Rockman, M. V. The QTN program and the alleles that matter for evolution: all that's gold does not glitter. *Evolution* **66,** 1–17 (2012). PMID: 22220860
14. Rausher, M. D. & Delph, L. F. Commentary: When does understanding phenotypic evolution require identification of the underlying genes? *Evolution* **69,** 1655–1664 (2015). PMID: 25973520
15. Lewontin, R. C. *The Genetic Basis of Evolutionary Change*. (1974). PMID: NA
16. Stephan, W. Signatures of positive selection: from selective sweeps at individual loci to subtle allele frequency changes in polygenic adaptation. *Molecular Ecology* n/a–n/a (2015). doi:10.1111/mec.13288. PMID: 26108992
17. Bergland, A. O., Tobler, R., González, J., Schmidt, P. S. & Petrov, D. A. Secondary contact has contributed to genome-wide patterns of clinal variation in North American and Australian populations of *Drosophila melanogaster*. bioRxiv: 009084. PMID: NA
18. Pavlidis, P., Jensen, J. D., Stephan, W. & Stamatakis, A. A critical assessment of storytelling: gene ontology categories and the importance of validating genomic scans. *Mol Biol Evol* **29,** 3237–3248 (2012). PMID: 22617950
19. Bergland, A. O., Chae, H.-S., Kim, Y.-J. & Tatar, M. Fine-scale mapping of natural variation in fly fecundity identifies neuronal domain of expression and function of an aquaporin. *PLoS Genet* **8,** e1002631 (2012). PMID: 22509142
20. Fraser, H. B. Gene expression drives local adaptation in humans. *Genome Res.* **23,** 1089–1096 (2013). PMID: 23539138
21. Bergland, A. O., Tobler, R., González, J., Schmidt, P. & Petrov, D. Secondary contact and local adaptation contribute to genome-wide patterns of clinal variation in Drosophila

melanogaster. *bioRxiv* 009084 PMID : NA
22. Paaby, A. B., Bergland, A. O., Behrman, E. L. & Schmidt, P. S. A highly pleiotropic amino acid polymorphism in the Drosophila insulin receptor contributes to life-history adaptation. *Evolution* **68,** 3395–3409 (2014). PMID: 25319083
23. Mackay, T. F. C. *et al.* The *Drosophila melanogaster* Genetic Reference Panel. *Nature* **482,** 173–178 (2012). PMID: 22318601
24. Stearns, S. C. *The Evolution of Life Histories*. (Oxford University Press on Demand, 1992). PMID: NA
25. Xu, Sen *et al.* A Male-Specific Genetic Map of the Microcrustacean Daphnia pulex Based on Single Sperm Whole-Genome Sequencing. *Genetics* genetics.115.179028 (2015). doi:10.1534/genetics.115.179028. PMID: 26116153
26. Staubach, F., Baines, J. F., Künzel, S., Bik, E. M. & Petrov, D. A. Host species and environmental effects on bacterial communities associated with Drosophila in the laboratory and in the natural environment. *PLoS ONE* **8,** e70749 (2013). PMID: 23967097
27. Dobson, A. J. *et al.* Host genetic determinants of microbiota-dependent nutrition revealed by genome-wide analysis of Drosophila melanogaster. *Nature Communications* **6,** 6312 (2015). PMID: 25692519
28. Mueller, L. D. & Cabral, L. G. DOES PHENOTYPIC PLASTICITY FOR ADULT SIZE VERSUS FOOD LEVEL IN DROSOPHILA MELANOGASTER EVOLVE IN RESPONSE TO ADAPTATION TO DIFFERENT REARING DENSITIES? *Evolution* **66**, 263–271 (2012). PMID: 22220880
29. Kryazhimskiy S, Rice DP, Jerison ER, Desai MM. Microbial evolution. Global epistasis makes adaptation predictable despite sequence-level stochasticity. *Science*. **344(6191):**1519-22. (2014) doi: 10.1126/science.1250939. PMID: 24970088
30. Meyer M, Kircher M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb Protoc*. **(6)**: doi: 10.1101/pdb.prot5448. PMID: 20516186
31 Kessner D, Turner TL, Novembre J. Maximum likelihood estimation of frequencies of known haplotypes from pooled sequence data. Mol Biol Evol. **30**(5):1145-58. (2013) doi: 10.1093/molbev/mst016. PMID: 23364324
32. Ueno, T. et al. High-throughput resequencing of target-captured cDNA in cancer cells. Cancer Science **103**, 131–135 (2012). PMID: 21929543
33. Colbourne, J. K. et al. The ecoresponsive genome of Daphnia pulex. Science 331, 555–561 (2011). PMID: 21292972
34. Price, A. L., Zaitlen, N.A., Reich, D. & Patterson, N. New approaches to population stratification in genome-wide association studies. Nature Reviews Genetics 11:459-463 (2010). PMID: 20548291.
35. Ginzburg, L. Ecological Orbits : How Planets *Move and Populations Grow*. (Oxford University Press, USA, 2004). PMID: NA
36. Matzkin, L. M., Johnson, S., Paight, C. & Markow, T. A. Preadult Parental Diet Affects Offspring Development and Metabolism in Drosophila melanogaster. *PLoS ONE* **8,** e59530 (2013). PMID: 23555695
37. Giesel, J. T., Lanciani, C. A. & Anderson, J. F. Effects of Parental Photoperiod on Metabolic Rate in Drosophila melanogaster. *The Florida Entomologist* **72,** 499 (1989). PMID: NA
38. Shiota, H., Goudarzi, A., Rousseaux, S. & Khochbin, S. Transgenerational inheritance of chromatin states.. *Epigenomics* **5,** 121–122 (2013). PMID: 23566089
39. Jones, L. E. *et al.* Rapid contemporary evolution and clonal food web dynamics. *Phil. Trans. R. Soc. B* **364,** 1579–1591 (2009). PMID: 19414472

40. Altieri, A. H., Bertness, M. D., Coverdale, T. C. & Herrmann, N. C. A trophic cascade triggers collapse of a salt-marsh ecosystem with intensive recreational fishing. *Ecology* (2012). PMID: 22834380

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Bergland, Alan Olav

eRA COMMONS USER NAME (credential, e.g., agency login): bergland.alan

POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Oregon	B.S.	06/2004	Philosophy
University of Oregon	B.S.	06/2004	Biology (with Honors)
Brown University	Ph.D.	06/2010	Ecology and Evolution
Stanford University	Postdoctoral	04/2014	Biological Sciences
Stanford University	Research Assoc	12/2015	Biological Sciences

A. Personal Statement

My research program seeks to understand the adaptive evolution and genetic basis of life-history traits in response to ecologically relevant environmental variation. This integrative work uses a mixture of tools from field ecology, quantitative-, molecular-, and population-genetics, combined with rigorous statistical analyses. My initial training as an undergraduate was in field ecology, where I studied the role of larval nutrition and overwintering thermal stress on adult fitness components in the pitcher-plant mosquito, *Wyeomyia smithii*. Inspired by this work, as a Ph.D. student I investigated the genetic and physiological basis of phenotypic plasticity in female fecundity of *Drosophila melanogaster.* My PhD work entailed several very large phenotyping efforts (thousands of flies assayed and over a million eggs counted by eye) and identified one locus controlling natural variation in fecundity. While successful I nonetheless wanted to understand the evolutionary forces acting on many loci controlling natural variation in life-history traits. Accordingly, as a post-doc I studied the genetic basis of adaptive differentiation in *D. melanogster* along latitudinal clines and among seasons. In 2016, I will being a faculty position at the University of Virginia where my research program will utilize my diverse skill set to study the evolutionary history and genetic/physiological basis of natural variation in fitness related traits in a generalized and high-throughput way. Below, I list four publications that demonstrate my experience and qualifications for the proposed research.

As a post-doc I have developed independent collaborations with scientists throughout the world that complimented my main research projects. While this work is still in progress, I will briefly describe it here. In collaboration with Chris Balakrishnan (East Carolina University), Elaina Tuttle (Indiana State University), and others I have played a leadership role in examining the evolutionary history of a remarkable, stably balanced 100Mb inversion polymorphism in the White-Throated Sparrow. We show that this inversion, which is maintained at intermediate frequencies by near perfect disassorative mating, arose via adaptive introgression and has subsequently evolved much as neo-sex chromosomes do. I will continue to play a small role in this project as it continues to develop. A second independent research program I have become involved in is

studying the genetic basis of adaptive differentiation of phenotypic plasticity in *Daphnia pulex.* This work is in collaboration with Andrew Beckerman (Univ. of Sheffield, UK). In my research proposal, I describe how I will continue to work on this system in collaboration with Dr. Beckerman. Finally, in collaboration with Dmitri Petrov (Stanford) and Paul Schmidt (U Penn) I have spearheaded a large consortium of fly biologists throughout the US to collect flies on a seasonal basis near their home institutions and subsequently use these collections for whole-genome resequencing. This consortium met in 2012 at NESCent and in the subsequent years we have grown to over a dozen labs throughout the US. Our work has spurred similar efforts in Europe and I serve as a go-between for the US and European groups. Moving forward, I will continue to play a major role in this international collaboration.

I have been actively involved in mentoring students and I am committed to continuing this practice. At Stanford, I mentored >10 undergraduate students, post-grad research technicians, and graduate students. Many of these students completed honors theses or similarly in-depth, semi-independent research projects leading to one published MS and several others in preparation. The majority of these students have been minorities, women, or first-generation college students and I am committed to promoting gender, ethnic, cultural, and intellectual diversity in the sciences through continued mentorship. Three of these students have now entered PhD programs in Ecology/Evolution/Genetics at Harvard, UC Berkeley, and UC Davis.

Relevant Publications:

Bergland AO, E Behrman, K O'Brien, P Schmidt & D Petrov, 2014. Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in Drosophila. *PLoS Genetics* 10(11): e1004775.

Paaby AB, **Bergland AO**, Behrman EL, Schmidt PS, 2014. An amino acid polymorphism in the Drosophila Insulin Receptor demonstrates pleiotropic and adaptive function in life-history traits. *Evolution* (68): 3395-3409

Bergland AO, Chae HS, Kim YJ & Tatar M, 2012. Fine scale mapping of natural variation in fly fecundity identifies neuronal domain of expression and function of an aquaporin. *PLoS Genetics* 8(4): e1002631

Bergland AO, Genissel A, Nuzhdin SV & Tatar M, 2008. Quantitative trait loci affecting phenotypic plasticity and the allometric relationship of ovariole number and thorax length in *Drosophila melanogaster. Genetics* 180: 576-582

B. Positions and Honors

Positions and Employment

2014 - 2015 Research Associate, Dept. of Biology, Stanford University, Stanford, CA
2016 - Assistant Professor, Dept. of Biology, University of Virginia, Charlottesville, VA

Other Experience and Professional Memberships

2004 - Member, Genetics Society of America (GSA)
2014, 2015 NSF DEB grant review panelist
2014, 2015 *Ad hoc* grant reviewer for BBSRC, INR, Austrian Science Foundation
2014 Post-doc representative for the GSA Awards Committee
2015 GSA 100-year Anniversary Committee

Honors

2007-2008 Oliver Cromwell Gorton Arnold Biological Fellow, Brown Univ.

C. Contribution to Science

1. Demonstrating the presence of polygenic adaptive oscillations over seasonal time scales. It is well known that adaptive evolution of highly quantitative traits can proceed rapidly in response to natural selection pressures. The genetic basis for such adaptive evolution has remained elusive and it has often been assumed the difficulty in identifying the genetic targets of natural selection is due in part to the genetic architecture of traits under selection. One standard model is that that rapid adaptive evolution of

quantitative traits is driven by small changes in allele frequency at a large of number of loci with individually small effects. This is assumption is referred to as the 'infinitesimal model' as is a foundational model in quantitative-genetics. This model plays a major role in shaping the Neutral Theory, the dominant framework of population-genetics. One consequence of this model is that identifying specific loci that contribute to rapid adaptive evolution of quantitative traits may be difficult, if not impossible.

Drosophila melanogaster undergoes rapid adaptive evolution in quantitative life-history traits over seasonal time scales. We sought to identify loci that underly this adaptive process by sampling flies over the course of three years in a temperate orchard during the spring and fall. Surprisingly, we identified hundreds of loci that shift dramatically in allele frequency between seasons, on average between 40 and 60% (Bergland *et al*, 2014). We have shown that these loci are enriched for functional genomic elements, respond predictably to an acute frost event, vary in a predictable way among populations arrayed along latitudinal clines, and are associated with measurable differences in stress tolerance phenotypes. We also showed that these so called "seasonal SNPs" are old. The vast majority of them predate the migration of flies out of Africa and the are more likely than expected by chance to be polymorphic in *D. melanogaster*'s sister species, *D. simulans*. This latter result suggests that seasonal SNPs may be millions of years old and possibly maintained at intermediate frequencies in the species by some balancing selection caused by environmental heterogeneity through time and space.

Importantly, this work has shown that many loci of large phenotypic and fitness effect do contribute to rapid adaptation in quantitative traits over very short time scales. We have also begun to examine the phenotypic effect of selected seasonal SNPs and have shown that their effects are measurable and highly pleiotropic (Paaby et al, 2014). Therefore, our work suggests that the infinitesimal model may not hold.

My work on rapid adaptation over seasonal time scales occurred while I was a post-doc and was performed in collaboration with Paul Schmidt (U Penn) and Dmitri Petrov (Stanford). I prepared sequencing libraries, analyzed all the high-throughput sequencing data, and developed forward simulations to demonstrate the plausibility of rapid adaptive evolution at hundreds of loci.

In addition to conceptually advancing our understanding of rapid adaptive evolution, this work fostered two technical advances in high-throughput sequencing. The first was the demonstration that pooled-resequencing is an accurate way to assess allele frequencies genome-wide (Zhu *et al.* 2012). The second was the development of software to estimate patterns of linkage disequilibrium from pooled resequencing data (Feder et al 2012).

Bergland AO, E Behrman, K O'Brien, P Schmidt & D Petrov, 2014. Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in Drosophila. *PLoS Genetics* 10(11): e1004775.

Paaby AB, **Bergland AO**, Behrman EL, Schmidt PS, 2014. An amino acid polymorphism in the Drosophila Insulin Receptor demonstrates pleiotropic and adaptive function in life-history traits. *Evolution* (68): 3395-3409

Feder A, Petrov D & **Bergland AO**, 2012. LDx: Estimation of linkage disequilibrium from high-throughput pooled resequencing data. PLoS ONE 7(11): e48588

Zhu Y, **Bergland AO**, Gonzalez-Perez J & Petrov D, 2012. Empirical validation of pooled whole genome population re-sequencing in *Drosophila melanogaster*. PLoS ONE 7(7): e41901

2. <u>Uncovering the role of historical admixture in generating clinal variation in *D. melanogaster*.</u>

D. melanogaster has long served as an important model for studying local adaptation. Largely, work on local adaptation in this system has focused on examining patterns of genetically based phenotypic variation along latitudinal clines. For historical reasons, this work has primarily focused on latitudinal clines in North America and Australia. Genetically based phenotypic clines and many clines in allele frequency are parallel between these two continents which were colonized by *D. melanogaster* in the last several hundred years. Parallel clinality has been taken as strong evidence of local adaptation.

By examining genome-wide estimates of allele frequencies in a world-wide sample of flies, I showed that these parallel clines were likely generated by the colonization history of flies and not necessarily spatially varying selection. Notably, my work showed that both North America and Australia likely represent secondary contact zones between European and African lineages of flies and that this process generated clinal variation across a large fraction of the genome. This finding, therefore, dramatically shifts our understanding of a well studied and classic system in evolutionary biology.

My work on clinal variation in *D. melanogaster* occurred while I was a post-doc. I collected fly samples, generated sequencing libraries, performed all the statistical analysis.

Bergland AO, Tobler R, González J, Schmidt P & Petrov D. Secondary contact and local adaptation contribute to genome-wide patterns of clinal variation in Drosophila melanogaster. In review, Molecular Ecology; preprint available at bioRxiv: 009084

3. Identifying the physiological and genetic basis for natural variation in life-history traits.
The life-history of an individual - its age-specific patterns of reproduction and survival - determine population growth rate and demographic fitness. Thus, life-history traits are likely to be subject to strong selective pressures in the wild (Bergland 2011). Despite their central importance in evolutionary biology, the genetic basis for natural variation in life-history traits has remained elusive.

During my Ph.D. I performed two extensive QTL mapping experiments seeking to identify the genetic basis of life history traits in *D. melanogaster* One broader study described a complex genetic architecture underlying natural variation in ovary- and body-size (Bergland et al 2008). In a second study, I mapped natural variation in fecundity to a single gene *Drip* (Bergland et al 2012). This gene encodes for an aquaporin that allows for efficient transport of water and (possibly) small solutes across cell membranes. Aquaporins are highly expressed in the malpighian tubules, the insect equivalent of the kidney. Surprisingly, I found that *Drip* was differentially expressed between high- and low- fecundity strains in ~12 neurons in the brain and modulate fecundity through an endocrine pathway involving both dopamine and corazonin. Ultimately, this work identified a new gene that affects fecundity and linked it to a physiological pathway. The limitation of this work was that I had no idea whether this mutation was segregating at intermediate frequencies due to balancing selection or if it was a rare mutation that was unconditionally deleterious. The drive to answer this question led to my post-doc work which I described above.

Bergland AO, Chae HS, Kim YJ & Tatar M, 2012. Fine scale mapping of natural variation in fly fecundity identifies neuronal domain of expression and function of an aquaporin. PLoS Genetics 8(4): e1002631

Bergland AO. Mechanisms and ecological genetics of reproduction in Dipteran insects, 2011. In *Molecular mechanisms of life history evolution*, eds. Flatt, T. & A. Heyland. Oxford University Press, Oxford, UK

Bergland AO, Genissel A, Nuzhdin SV & Tatar M, 2008. Quantitative trait loci affecting phenotypic plasticity and the allometric relationship of ovariole number and thorax length in *Drosophila melanogaster. Genetics* 180: 576-582

My publication list can be found here: http://www.ncbi.nlm.nih.gov/pubmed/?term=bergland+ao

D. Research Support

Ongoing Research Support
N/A

Completed Research Support

F32 GM097837-01 Bergland (PI) 04/01/2011-03/31/2014
Genomics of Natural Populations
The goal of this project was to (1) assess the accuracy of population based pooled resequencing on genome-wide estimates of allele frequencies and to apply this technique to investigate the evolutionary forces shaping

patterns of genetic variation among populations of D. melanogaster (2) sampled along latitudinal clines and (3) among seasons.

Stanford CEHG trainee research grant Bergland (PI) 09/01/2013-08/31/2015
Physiological Mechanisms Underlying Rapid Adaptive Evolution
The goal of this project was to test the hypothesis that hormonal genes underlie the tradeoff between somatic maintenance and reproductive output and are differentially expressed between winter- and summer- adapted flies.

NESCent Catalysis Grant Bergland, Petrov, Schmidt (multi-PI) 04/2012
Tracking the Biotic Response to Global Climate Change Through Genomic Analysis
The main goal of meeting grant was to initiate a collaboration amongst a diverse set of scientists to study the evolutionary genomics of Drosophila spp. in response to global climate change

SUMMARY STATEMENT

PROGRAM CONTACT:
DANIEL JANES
(301) 594-0943
daniel.janes@nih.gov

(Privileged Communication)

Release Date: **03/28/2016 08:50 AM**

Revised Date:

Application Number: **1 R35 GM119686-01**

Principal Investigator

BERGLAND, ALAN OLAV

Applicant Organization: UNIVERSITY OF VIRGINIA

Review Group: **ZRG1 CB-E (50)**
Center for Scientific Review Special Emphasis Panel
RFA-GM-16-003: Maximizing Investigators' Research Award for New and Early Stage Investigators (R35)

Meeting Date: **03/14/2016**
Council: **MAY 2016**
Requested Start: **07/01/2016**

RFA/PA: **GM16-003**
PCC: **G126DJ**

Project Title: **The genetic and physiological architecture of rapid and cyclic adaptation**

SRG Action: **Impact Score:42**
Next Steps: **Visit http://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
1	250,000	389,200
2	250,000	389,200
3	250,000	389,200
4	250,000	389,200
5	250,000	389,200
TOTAL	1,250,000	1,946,000

1R35GM119686-01 Bergland, Alan

RESUME AND SUMMARY OF DISCUSSION: The goal of this program is to investigate the consequences of genetic polymorphisms that rapidly and cyclically change over short periods. The review group agreed that the applicant is well-trained, had a very good record of productivity as a trainee preceding his independent research career which just started, has demonstrated his ability to serve as a mentor, and has a solid research environment and institutional support. Ultimately, the panel felt that short-term, cyclic changes in allele frequencies has important implications for evolutionary theory and interpretation of allele frequency change, and most of the panel expected the applicant's research program to have an above average, but not the highest, impact on the field. The *Drosophila* research was thought to have a solid foundation of prior work and resources. However, there was uncertainty among the committee members as to the likely success of the work looking at *Daphnia* response to predation and concern that longer-term directions for the research were not articulated well.

DESCRIPTION (provided by applicant):
Dramatic shifts in aspects of the natural environment can act as strong selective forces in the wild and often drive rapid adaptive evolution. The genetic basis of this adaptive evolutionary change has largely remained elusive, particularly when the traits under selection are complex and influenced by a large number of genes. The purpose of this grant is to unravel the genetic basis of rapid adaptive evolutionary change in two distinct species that are subject to different types of selection pressures in the wild. We will focus on rapid adaptive evolution of D. melanogaster to seasonal fluctuations in selection pressure as well as adaptive evolution of Daphnia pulex to variation in predation pressures. By studying a similar set of questions in two species, we will be able to make generalized statements about the genetic basis of adaptation to environmental heterogeneity and the evolutionary history of alleles that contribute to rapid adaptation. For both systems, we have already identified genetic polymorphisms that significantly change in frequency among seasons or predation regime and likely contribute to rapid adaptation in response to these selection pressures. Often these polymorphisms fall outside of easily defined gene coding sequences. Therefore many likely affect gene expression yet we do not know which gene they affect. Thus, we will perform a series of experiments to systematically link these putatively adaptive polymorphisms to expression variation in nearby genes. We will perform these experiments by exposing experimental populations of flies and water-fleas to ecologically relevant environmental variation in semi-natural enclosures and use novel methodologies that rely on high-throughput sequencing to link genotype to phenotype. This work will provide valuable information for the broader genetics community by identifying functional and evolutionary relevant polymorphisms in two model genetic systems. This work will also conceptually advance evolutionary genetics by affording us the opportunity to study the long- and short-term evolutionary history of the loci that underlie rapid adaptation in response to subtle shifts in selection pressure.

PUBLIC HEALTH RELEVANCE
Using the model organisms Drosophila melanogaster and Daphnia pulex, this projects seeks to identify the functional significance of natural polymorphisms previously identified to be subject to temporally or spatially variable selection. Analytic tools and conceptual advancements made here will help us understand the nature of genetic variation which is a critical step in understanding the nature of disease.

CRITIQUE 1

Overall Impact:

The PI, although new to a faculty position, has established himself as a productive, creative and independent researcher. His proposed work addresses a key topic in evolutionary biology, concerning

the adaptation of populations to “local” selective pressures that act over short time scales or distances. His important postdoctoral work on seasonal variation in Drosophila polymorphisms forms the basis for an ambitious research program that promises to yield, for some time to come, major findings about life-history and gene-expression evolution. The expansion of the PI’s scope to the Daphnia system is well reasoned and further ensures that there will be many interesting results and questions to sustain this research program into the future. The PI does not have as much experience working with Daphnia as he does with Drosophila, but a strong collaboration for the Daphnia work is in place. The PI’s institution provides a rich environment for evolution and genomics, and the department is committed to his success. There is therefore high confidence that the proposed research program will have major, long-term impact.

– **1. Significance:**

Strengths

- The proposed research program addresses an essential topic in evolutionary biology with important practical implications: how populations adapt “locally” (i.e., to selective pressures that vary over short time scales or distances). This is a topic about which little is known and that should produce important questions for some time to come. It is therefore very appropriate for long-term pursuit, as envisioned by the MIRA funding mechanism.
- Although the proposed research in the short term is focused on cis-eQTL mapping, there is high potential to expand the scope of the work to many life-history traits (and the PI has an excellent training background for doing so).
- The PI developed the Drosophila project as a postdoc but his proposal to expand the work to Daphnia (for which he makes a good case as a study system) will help him to establish his independence.

Weaknesses

- The proposal talks about making “generalized statements” about local adaptation by virtue of studying two distinct taxa (Drosophila and Daphnia). It is not clear how much generalization can be done from a sample of two taxa. But given the major gaps in understanding local adaptation, and the good reasons for studying Daphnia in its own right, this over-optimism is not a big weakness of the proposal.

– **2. Investigator(s):**

Strengths

- The PI has excellent training in Drosophila physiology, molecular genetics and evolutionary genetics, as well as a background in insect field ecology. He is therefore very well suited to lead the proposed research program.
- The PI has a very good record of productivity and impact. His Ph.D. work with Marc Tatar produced several papers, including an important one in *PLoS Genetics* that reported the mapping of natural variation in *Drosophila* fecundity to differential expression in a small set of neurons of an aquaporin-encoding gene. His postdoctoral research with Dmitri Petrov also produced several papers, most notably a groundbreaking *PLoS Genetics* paper that identified hundreds of polymorphisms that oscillate in frequency seasonally, consistent with adaptive evolution over short time scales.
- The PI has shown independence and initiative in establishing collaborations, including the collaboration with Andrew Beckerman that is the basis of proposed work on *Daphnia*.
- The PI’s faculty position starts in 2016, so he does not yet have a track record of running his own lab, but all signs point to a level of independence and productivity that make it likely he will be successful in doing so.

- As a postdoctoral researcher, the PI mentored >10 undergraduates, technicians and graduate students in the lab. These mentoring experiences appear to have been substantial, with several leading to honors theses and/or papers, and several mentees have gone on to top graduate programs. The PI has experience with, and a commitment to, mentoring members of under-represented groups.

Weaknesses

- The PI does not have much experience working with *Daphnia* (although this is unlikely to be a limitation because of the strong collaboration with Andrew Beckerman).

– **3. Innovation:**

Strengths

- The focus on seasonal variation in Drosophila polymorphisms is new and exciting.
- The PI's past work, including his Ph.D. work on life-history variation and his postdoc work on seasonal variation, shows a knack for pursuing interesting problems at the frontier of knowledge. The PI has also contributed to technical advances, especially in pooled sequencing. This bodes well for his ability to continue doing cutting-edge work.
- The identification of Daphnia as a promising study system for work similar to the Drosophila work, because of pool-specific differences in predation of Daphnia, shows creativity and foresight.

Weaknesses

– **4. Approach:**

Strengths

- The PI's prior work demonstrating a large number of seasonally oscillating polymorphisms in Drosophila, forms a strong foundation for the proposed next experiments, involving cis-eQTL mapping (to begin to get at the links between seasonally changing allele frequencies and their functional consequences),
- The PI's very strong track record of studying life-history traits using genetic tools suggests that the research program will be carried out with care, rigor and creativity.
- Using high-throughput sequencing to get genotype and expression data from thousands of individual flies is ambitious and clever, in particular the idea for how low-coverage RNA-seq data can be used for eQTL analysis by pooling within allele classes.
- The breadth of the proposed research program is increased through collaboration on the Daphnia work, and a set of common genomic approaches will be used for the Drosophila and the Daphnia work, suggesting that this MIRA-funded research will make efficient and cost-effective use of resources.

Weaknesses

- The PI acknowledges the potential drawbacks of isolating mRNA from whole flies for transcriptomic analyses (e.g., swamping out tissue-specific differential expression signals). One potential solution that is proposed is to de-enrich samples for highly expressed transcripts from housekeeping genes (e.g., encoding Actin). A better approach, especially in the long term, might be to dissect particular tissues relevant to life history traits (e.g., endocrine tissues, reproductive tissues) or even single cell types for expression analysis.

– **5. Environment:**

Strengths

- The PI's department has shown a commitment to his success, providing the startup funds and facilities that he will need to be successful.
- The PI joins a strong evolution group and will have access to excellent genomics resources.
- The chair and a senior colleague in evolutionary biology will provide mentoring.

Weaknesses

Protections for Human Subjects:

Inclusion of Women, Minorities and Children:

Vertebrate Animals:

Is the proposed research involving vertebrate animals scientifically appropriate, including the justification for animal usage and protections for research animals described in the Vertebrate Animals section?

Biohazards:

Select Agents:

Resource Sharing Plans:

Budget and Period of Support:

- Recommend as requested.

Additional Comments to Applicant: (Optional)

CRITIQUE 2

Overall Impact:

The proposed program would examine the potential for adaptive maintenance of genetic variation in natural populations as a function of fluctuating environments. Maintenance of polymorphism by fluctuating environments over time and space is often hypothesized but rarely demonstrated. This could be the substrate for a major and substantive research program in evolutionary genetics, but the presently proposed program stops short of deep investigation of process and instead rests on extensive survey sampling. The PI has a strong background in Drosophila evolutionary genetics, including a publication track record closely linked to the proposed experiments. However, he has little background in Daphnia and those experiments may prove more daunting than anticipated. There is much to like about this program in concept, but the research foci need sharpening and the program needs greater depth in testing or challenging existing population genetics theory.

- **1. Significance:**

Strengths

- The genetics of adaptation to variable environments is very poorly understood, despite the fact that environmental variation might quite plausibly result in the adaptive maintenance of

polymorphism in populations. There are few appropriate theoretical models so empirical observation is critical. The lack of theoretical expectation, however, necessitates development of models under which the empirical data can be interpreted.

- Drosophila melanogaster and Daphnia pulex are both important model systems for evolutionary genetics research.

Weaknesses

– **2. Investigator(s):**

Strengths

- PI has good training in empirical evolutionary genetics
- PI is one of the most prominent current proponents of models where functional genetic variation is adaptively maintained by variable environments.
- Recent publication rate is good, although the PI has a central authorship position on many of them. It should be noted the PI has not yet been in position as assistant professor, so all publications are from postdoctoral and graduate work. There has been no opportunity for an independent publication record to manifest.

Weaknesses

- PI does not have apparent extensive experience working with Daphnia, and laboratory experiments with Daphnia may prove more challenging than anticipated.

– **3. Innovation:**

Strengths

- Maintenance of Drosophila populations outside in semi-natural cage settings is innovative. Any such experiment is obviously subject to caveat and will require great care in experimental design and execution, but these experiments could be a major advance over conventional Drosophila experiments intended to test adaptation to environmental conditions but conducted entirely in the lab.

Weaknesses

– **4. Approach:**

Strengths

- Experimental objectives are innovative and at the forefront of empirical ecological and population genomics.
- The Future Directions suggest a strong set of experiments that could be a stronger focus in near-term work.

Weaknesses

- eQTL experiment will be labor intensive but is largely exploratory. There is no clear model being tested or developed, either with respect to the pattern of seasonal variation or in the specific genes expected to cycle. Little explanation is given as to how seasonal variation in eQTL frequency will be translated into organism-level adaptation to cycling environment.
- As with all field studies, the Drosophila cage experiment will suffer if field conditions are unexpectedly outside the annual norm. A good example is this El Nino winter, which has brought warmth and almost no precipitation to the northeast US and a huge amount of precipitation to the US west coast. This would be a bad year to study seasonal ecology of any organism unless there were very many additional years to buffer against occasional anomalies.

- The Daphnia experiments may prove to be much more difficult than anticipated. The Daphnia will need to be harvested, crossed, and reared in the lab. Collection should be straightforward, but crossing and maintenance may be more difficult. How much developmental plasticity is there as a function of rearing condition independent of kairomones?

– **5. Environment:**

Strengths

- UVA is an adequate environment for the proposed research

Weaknesses

Protections for Human Subjects:

Inclusion of Women, Minorities and Children:

Vertebrate Animals:

Is the proposed research involving vertebrate animals scientifically appropriate, including the justification for animal usage and protections for research animals described in the Vertebrate Animals section?

Biohazards:

Select Agents:

Resource Sharing Plans:

Budget and Period of Support:

Additional Comments to Applicant: (Optional)

CRITIQUE 3

Overall Impact:

This research program focuses on the genetic basis for local adaptation, using Drosophila and Daphnia as model systems. The key hypothesis in the field is about how standing variation is leveraged to adapt to new conditions – does selection favor many genes with very small effects, or are there fewer loci with larger effects? During postdoctoral work, the PI found ancient alleles that contribute to seasonal adaptation in Drosophila populations, arguing for the latter hypothesis. Here, the PI proposes to extend this work by sequencing new populations of Drosophila and Daphnia under different environmental conditions (seasonal variation for Drosophila and predator variation for Daphnia) and perform eQTL analysis. However, the questions that the PI outlined at the beginning of the proposal regarding the nature of standing variation are not directly addressed by this eQTL analysis. Instead, these experiments are a first step to explain how adaptive loci contribute to phenotype. The proposal would benefit from more discussion of the value of this long-term goal and other strategies to investigate it. Instead, the PI proposes very similar experiments in two model systems, without giving a clear picture of the next phase of research.

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 R35 GM119686-01; PI Name: Bergland, Alan Olav

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

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Center for Scientific Review Special Emphasis Panel

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ZRG1 CB-E (50)
03/14/2016 - 03/15/2016

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Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.

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