**Justification for increase in funding.**

I am requesting a 10% increase in funding from the previous reporting period. This increase is to cover increased salary and overhead. In addition, this increase is important because start-up funds will expire soon, making grant funding the sole source of funding.

**Senior Personnel.**

**Alan O. Bergland, Ph.D.** (51% effort). Principal Investigator will be responsible for the overall administration and direction of the project. Summer support is being requested for 3 months per year.

**Personnel at University of Virginia**

**Karen Barnard-Kubow, PhD. Research Scientist** (100% effort). Will be responsible for overseeing Daphnia research, coordinating sampling, conducting crosses to determine the genetic architecture of sexual investment, generating and analyzing sequencing data.

**Joaquin Nunez, PhD. Post-doctoral researcher** (100% effort). Will be responsible for experimental Drosophila research associated with overwintering experiments and analyzing patterns of allele frequency differentiation from pre-existing estimates and new samples collected across time and space.

**Connor Murray, Graduate Research Assistant,** (100% effort for 3 summer months). Will be responsible for modeling dynamics of Daphnia populations*,* inference of demographic history of Daphnia populations, mesocosm fitness experiments.

**Robert Porter, Graduate Research Assistant,** (100% effort for 3 summer months). Will be responsible for ephippial bet-hedging experiments in Daphnia.

**Benefits**

Benefits are calculated at 6.3% for the PI, 28.3% for the Post-Doc, 38.1% for the Lab Technician, and 6% for the Graduate Research Assistants.

**Equipment**

We are not requesting any major equipment purchases.

**Materials and Supplies (**

DNA / RNA extraction and library prep. Currently, we can perform DNA extraction and library preparation using a modified Nextera protocol at a cost of ~$6-7 per library; RNA extraction and library will cost more ~$10-15 sample. The experiments that we conduct will likely be on the order of 1000 individuals for DNA extraction, or 500 individuals for RNA extraction, and thus our **annual costs for library prep is < $10000 / year**.

Costs include services performed by the University of Virginia Genomics Core and utilize our prior investment in custom synthesized barcodes enabling multiplexing of ~4000 individuals.

High-throughput sequencing services. We typically outsource all sequencing to service providers and typically utilize the medium- to large-capacity Illumina runs. For many of our experiments (both Drosophila and Daphnia) we can utilize very low-coverage sequencing (<0.05X, $0.60/sample) because we can reconstruct phased genomes using parental haplotypes as priors. For these experiments we can multiplex about ~2000 individuals per HiSeq X lane (~$1300/lane). For higher coverage individual based sequencing, we can get ~10X coverage for 2x96 individuals for either Drosophila or Daphnia using a single lane of NovaSeq (~$7,500); for pooled sequencing, we try to obtain ~150X coverage and can multiplex ~12-14 samples on a single lane of NovaSeq. **We will keep sequencing costs at <$15,000 per year and believe that this will be sufficient to generate sequencing of natural populations, plus experimental crosses.**

Fly maintenance & husbandry. We maintain about a hundred inbred lines collected through the world in the lab; these lines are used for creating Hybrid Swarm populations. It costs ~$50/year for us to maintain a line (total costs ~$5K/year). Hybrid swarm populations are inexpensive to maintain in the lab (total costs ~$500/year); outdoor population cages, fed fruit substrate are a bit more expensive (~$500/month). **In general, the total cost of our Drosophila work is ~$7500 per year.**

Daphnia maintenance & husbandry. **We are able to run our Daphnia lab for about $15,000** **a year**. This cost includes salary for 3 undergraduates working 8 hrs/week, algae, and disposables (transfer pipettes, calcium filters for our water-still, chemicals for water solutions).

General lab supplies. **We purchase ~$5K/year in general lab supplies**, including chemicals, computer and electronic parts, hardware to upgrade and maintain our environmental chambers.

**Travel**

We request $5K/year for domestic travel and $5K/year for foreign travel. Domestic travel is for lab members to attend conferences, and incorporates conference registration fees. Foreign travel is for lab members to travel to England for field sampling of Daphnia. On average, a sampling trip costs ~$1000 (airfare); $300 (transit); $300 (food / lodging).

**Publication costs.**

We request $3000/year to cover publication costs in open access journals.