

**SUMMARY STATEMENT**

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

**( Privileged Communication )**

**Release Date:** 03/28/2016  
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**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

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**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](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.

<b>Project Year</b>	<b>Direct Costs Requested</b>	<b>Estimated Total Cost</b>
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
<b>TOTAL</b>	<b>1,250,000</b>	<b>1,946,000</b>

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**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.

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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](http://grants.nih.gov/grants/peer_review_process.htm#scoring).



MEETING ROSTER  
Center for Scientific Review Special Emphasis Panel

CENTER FOR SCIENTIFIC REVIEW  
RFA-GM-16-003: Maximizing Investigators' Research Award for New and Early Stage Investigators (R35)  
ZRG1 CB-E (50)  
03/14/2016 - 03/15/2016

CHAIRPERSON(S)

BEDWELL, DAVID M., PHD  
PROFESSOR  
DEPARTMENT OF MICROBIOLOGY  
UNIVERSITY OF ALABAMA AT BIRMINGHAM  
BIRMINGHAM, AL 35294

TISHKOFF, SARAH ANNE, PHD  
PROFESSOR  
DEPARTMENTS OF GENETICS AND BIOLOGY  
PERELMAN SCHOOL OF MEDICINE  
SCHOOL OF ARTS AND SCIENCES  
UNIVERSITY OF PENNSYLVANIA  
PHILADELPHIA, PA 19104

MEMBERS

AHMAD, KAMRAN, PHD  
STAFF SCIENTIST  
FRED HUTCHINSON CANCER RESEARCH CENTER  
SEATTLE, WA 98109

BASS, BRENDA L., PHD  
PROFESSOR  
DEPARTMENT OF BIOCHEMISTRY  
UNIVERSITY OF UTAH  
SALT LAKE CITY, UT 84132

BURGERS, PETER M, PHD  
MARVIN BRENNECKE PROFESSOR  
DEPARTMENT OF BIOCHEMISTRY  
WASHINGTON UNIVERSITY, SCHOOL OF MEDICINE  
ST. LOUIS, MO 63110

DEPACE, ANGELA H, PHD  
ASSISTANT PROFESSOR  
DEPARTMENT OF SYSTEMS BIOLOGY  
HARVARD UNIVERSITY  
BOSTON, MA 02115

DEVINE, SCOTT E, PHD  
ASSOCIATE PROFESSOR  
INSTITUTE FOR GENOME SCIENCES  
UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE  
BALTIMORE, MD 21201

FAGHIHI, MOHAMMAD ALI, MD, PHD  
ASSISTANT PROFESSOR  
DEPARTMENT OF PSYCHIATRY & BEHAVIORAL SCIENCES  
UNIVERSITY OF MIAMI  
MIAMI, FL 33136

KATJU, VAISHALI, PHD  
ASSOCIATE PROFESSOR  
DEPARTMENT OF VETERINARY INTEGRATIVE BIOSCIENCES  
TEXAS A&M UNIVERSITY  
COLLEGE STATION, TX 77843

KAUFMAN, PAUL D., PHD  
PROFESSOR  
PROGRAM IN GENE FUNCTION AND EXPRESSION  
UNIVERSITY OF MASSACHUSETTS, WORCESTER  
WORCESTER, MA 01605-2324

LAZZARO, BRIAN P, PHD  
ASSOCIATE PROFESSOR  
DEPARTMENT OF ENTOMOLOGY  
CORNELL UNIVERSITY  
ITHACA, NY 14853

LIU, NIANJUN, PHD  
ASSOCIATE PROFESSOR  
DEPARTMENT OF BIostatISTICS  
SCHOOL OF PUBLIC HEALTH  
UNIVERSITY OF ALABAMA AT BIRMINGHAM  
BIRMINGHAM, AL 32594

MILLS, RYAN E, PHD  
ASSISTANT PROFESSOR  
DEPARTMENT OF COMPUTATIONAL MEDICINE  
AND BIOINFORMATICS  
UNIVERSITY OF MICHIGAN  
ANN ARBOR, MI 48109

O'CONNELL, MATTHEW J, PHD  
PROFESSOR  
DEPARTMENT OF ONCOLOGICAL SCIENCES  
ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI  
NEW YORK, NY 10029

PE'ER, ITSHACK G, PHD  
ASSOCIATE PROFESSOR  
DEPARTMENT OF COMPUTER SCIENCE  
COLUMBIA UNIVERSITY MEDICAL CENTER  
NEW YORK , NY 10027

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.

PENALVA, LUIZ OTAVIO, PHD  
ASSOCIATE PROFESSOR  
DEPARTMENT OF CELLULAR  
AND STRUCTURAL BIOLOGY  
UNIVERSITY OF TEXAS HEALTH CENTER OF SAN ANTONIO  
SAN ANTONIO, TX 78229

PETERSON, CRAIG L, PHD  
PROFESSOR  
PROGRAM IN MOLECULAR MEDICINE  
UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL  
WORCESTER, MA 01605

SIEGAL, MARK L, PHD  
ASSOCIATE PROFESSOR  
DEPARTMENT OF BIOLOGY  
CENTER FOR GENOMICS AND SYSTEMS BIOLOGY  
NEW YORK UNIVERSITY  
NEW YORK, NY 10003

SIEPEL, ADAM CHARLES, PHD  
PROFESSOR AND CHAIR  
SIMONS CENTER FOR QUANTITATIVE BIOLOGY  
COLD SPRING HARBOR LABORATORY  
COLD SPRING HARBOR, NY 11724

SILHAVY, THOMAS J., PHD  
PROFESSOR  
DEPARTMENT OF MOLECULAR BIOLOGY  
PRINCETON UNIVERSITY  
PRINCETON, NJ 08544-1014

TRAN, ELIZABETH J, PHD  
ASSISTANT PROFESSOR  
DEPARTMENT OF BIOCHEMISTRY  
PERDUE UNIVERSITY  
WEST LAFAYETTE, IN 47907

WORKMAN, JERRY L, PHD  
INVESTIGATOR  
STOWERS INSTITUTE FOR MEDICAL RESEARCH  
KANSAS CITY, MO 64110

#### SCIENTIFIC REVIEW OFFICER

ADKINS, RONALD, PHD  
SCIENTIFIC REVIEW OFFICER  
CENTER FOR SCIENTIFIC REVIEW  
NATIONAL INSTITUTES OF HEALTH  
BETHESDA, MD 20892

#### EXTRAMURAL SUPPORT ASSISTANT

BONHOMME, CHRISTINE  
TASK LEADER GGG IRG  
GENES, GENOMES, GENETICS IRG  
CENTER FOR SCIENTIFIC REVIEW  
NATIONAL INSTITUTE OF HEALTH  
BETHESDA, MD 20892