OMB Number: 4040-0010
Expiration Date: 12/31/2022

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier
GM119686

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 0653915260000

Legal Name*: UNIVERSITY OF VIRGINIA
Department:
Division:
Street1*: UNIVERSITY OF VIRGINIA
Street2: BOX 400195
City*: CHARLOTTESVILLE
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 229044195

Person to be contacted on matters involving this application
Prefix: First Name*: Angela Middle Name: Last Name*: Behrend Suffix:
Position/Title: Sr. Grants and Contracts Administrator
Street1*: 1001 N. Emmet St.
Street2:
City*: Charlottesville
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 229044195
Phone Number*: 434-924-4270 Fax Number: Email: ospnoa@virginia.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 54-6001796

7. TYPE OF APPLICANT* H: Public/State Controlled Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

❍ New ❍ Resubmission
● Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).
❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
The genetic and physiological architecture of rapid and cyclic adaptation

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2021 03/31/2026

13. CONGRESSIONAL DISTRICTS OF APPLICANT
VA-005

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Alan Middle Name: Olav Last Name*: Bergland Suffix:
Position/Title: Assistant Professor
Organization Name*: UNIVERSITY OF VIRGINIA
Department: Biology
Division:
Street1*: 409 McCormick Rd
Street2:
City*: Charlottesville
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 229040000
Phone Number*: 401-741-6148 Fax Number: Email*: alan.bergland@gmail.com

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$2,187,815.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$2,187,815.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ❍ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

❍ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Stewart Middle Name: P. Last Name*: Craig Suffix:
Position/Title*: Executive Director for Sponsored Programs
Organization Name*: The University of Virginia
Department:
Division: Office of Sponsored Programs
Street1*: 1001 N. Emmet St.
Street2:
City*: Charlottesville
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 229044195
Phone Number*: 434-924-4270 Fax Number: Email*: ospnoa@virginia.edu

Signature of Authorized Representative*
Completed on submission to Grants.gov

Date Signed*
06/22/2020

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 12/31/2022

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	UNIVERSITY OF VIRGINIA
Duns Number:	0653915260000
Street1*:	UNIVERSITY OF VIRGINIA
Street2:	BOX 400195
City*:	CHARLOTTESVILLE
County:	
State*:	VA: Virginia
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	229044195
Project/Performance Site Congressional District*:	VA-005

Additional Location(s) File Name:

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	bergland_abstract_v2.pdf
8. Project Narrative*	bergland_narrative.pdf
9. Bibliography & References Cited	bergland_references.pdf
10.Facilities & Other Resources	bergland_facilities.pdf
11.Equipment	bergland_equipment.pdf

Tracking Number:

Funding Opportunity Number: . Received Date:

Abstract.
Research in my lab works to address the importance of fluctuating selection as a force that generates and maintains genetic variation. We address this basic topic by studying the temporal dynamics of evolution across seasons in two groups of species, *Drosophila* and *Daphnia*. These species have rapid generation times and show phenotypic and genomic signals of cyclic evolution in response to seasonally varying selection pressures. My lab has worked to link genetic variation associated with traits that are subject to seasonal selection with temporal fluctuations in allele frequency. The primary goal for the next five years is to address the questions: What is the strength of fluctuating selection? Does fluctuating selection stabilize polymorphism and promote its persistence? We will address these questions through genomic analysis of time-series data and estimation of the genetic architecture of ecologically-relevant traits, in both the lab and the field. The overall vision of my research program is to study the nature of balancing selection, with a particular focus on the role of balancing selection in maintaining quantitative variation in fitness related life-history traits. By examining this topic in two genetic model systems, we can also gain insight into the basic genetics and biology of overwintering, reproduction, and developmental plasticity.

Narrative. This project seeks to identify the strength and genomic consequences of fluctuating selection in the model organisms *Drosophila melanogaster* and *Daphnia pulex*. This work addresses how environmental variation promotes functional genetic diversity. 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.

Facilities

Much of the physical and computational infrastructure required to perform the proposed work has been developed over the last four years by my lab, or were in place at UVA prior to my arrival. These resources include:

1. Establishment of an experimental orchard for outdoor mesocosm work with Drosophila.
2. Design and production of 48 environmental growth chambers to independently manipulate temperature and photoperiod; these chambers are suitable for experimental work with both Daphnia and Drosophila.
3. Construction of an aquaculture facility for large-scale phenotyping efforts using Daphnia.
4. Development of genomic datasets from populations of Daphnia and Drosophila sampled over multiple years and multiple localities.
5. The Bergland lab utilizes computational resources provided by UVA's high-performance computing cluster (Rivanna). In addition to a well-developed computing environment, Rivanna staff also offer courses and workshops to teach a variety of computational approaches. Members of my lab routinely attend these workshops to improve their skillset in various aspects of bioinformatic analysis.
6. The Biology Department at UVA maintains a Genomic Sequencing Core facility. We utilize the experience and toolset of this facility to generate sequencing libraries. In addition, the director of this facility has worked closely with members of my lab to teach them library construction techniques.

These infrastructural investments are coupled with a rich intellectual environment at the University of Virginia. Key attributes of this environment include:

1. An active and highly collegial network of faculty in ecology, evolution, and genetics. 13 labs associate with the EEB group at UVA. In addition, UVA also has intellectual strengths in cellular and developmental biology (24 labs) and neuroscience/behavior (15 labs). The Bergland lab is primarily situated with the EEB group but maintains collaboration with other labs (primarily Drosophila labs) in the department.
2. The EEB group at UVA holds a weekly seminar series for internal speakers and speakers from the region. This seminar series is coupled with a journal club led by post-docs and graduate students.
3. For the last 3 years, the Bergland lab has hosted "Biology Programming Hour". This is a weekly co-working time for all members of the department to learn programming and data-analysis skills from one-another.

Equipment

Computing resources: The University of Virginia maintains a large shared computing cluster with 8,000 cores and 8PB of various storage. The Bergland lab currently leases 20Tb of backed-up storage. The Bergland lab also maintains a 24-core workstation for smaller scale analyses.

Environment controlled rooms**:** The Bergland lab maintains two 8x8x8 walk-in Environmental Growth Chambers (EGCs). These chambers are capable of temperature, light, and humidity control. One is equipped with 40 small chambers, sufficient to hold 8 1L jars (for daphnia) or 100 fly vials. These small chambers were built and designed by the Bergland lab and offer additional independent control of light and temperature.

Percival growth chambers: The Bergland lab maintains 4 Percival chambers (two double door, and 2 single door). One single door unit is designed for Drosophila use and has phenolic coated coils and humidity control. The others are used for Daphnia culture.

Laminar Flow hood: We maintain a Laminar Flow hood for algae culture work.

Molecular facilities: The Bergland land has access to UVA's Shared Genomics facility which houses PCR thermocyclers and other necessary molecular lab equipment.

Daphnia aquaculture: The Bergland lab maintains an Aquaneering flow-through system for Daphnia culture. Currently, we are utilizing a six shelf stand alone single-sided rack, with the option to expand to 6 double wide racks if necessary.

Outdoor mesocosm facility: The Bergland lab has built and maintains 36 caged peach trees at our experimental orchard at Morven Estates. Cages are built out of UV resistant Luminite, preventing major movement of Drosophila.

UVA Genomics core. The Biology Dept at UVA has an Illumina MiSeq, Roche 454, and ABI capillary sequencer. We will also outsource much of our sequencing needs.

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Alan Middle Name Olav Last Name*: Bergland Suffix:

Position/Title*: Assistant Professor
Organization Name*: UNIVERSITY OF VIRGINIA
Department: Biology
Division:
Street1*: 409 McCormick Rd
Street2:
City*: Charlottesville
County:
State*: VA: Virginia
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 229040000

Phone Number*: 401-741-6148 Fax Number:

E-Mail*: alan.bergland@gmail.com

Credential, e.g., agency login: BERGLAND.ALAN

Project Role*: PD/PI Other Project Role Category:

Degree Type: PHD,BS Degree Year: 2010,2004

Attach Biographical Sketch*: File Name: bergland_biosketch.pdf

Attach Current & Pending Support: File Name: bergland_current_pending_v2.pdf

OMB No. 0925-0001 and 0925-0002 (Rev. 03/2020 Approved Through 02/28/2023)

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

A. Personal Statement

My research program examines the evolutionary dynamics of natural genetic variation in fitness related traits. We ask questions about the provenance and stability of polymorphisms that underlie functional genetic variation, and test how these polymorphisms adaptively track to spatial and temporal variation in selection pressures. This work seeks to assess the nature of balancing selection, and the importance of balancing selection as an evolutionary force which promotes diversity within populations and species. We address these questions using *Drosophila* and *Daphnia.* Due to their rapid development time, these assemblages of broadly distributed species have multiple generations per year (~10-20), enabling us to study their evolutionary dynamics in real-time and across a varied spatial landscape. Our work on *Drosophila* focuses on the genetic basis of local adaptation to seasonal and latitudinal variation in climate and nutrition. Our work on *Daphnia* examines the recurrent seasonal evolution of sexual investment, the mutational variance of predator induced plasticity, and the role of mating systems on the generation and maintenance of fitness-related diversity. In our research, we combine field-work, genomic analysis, and large-scale phenotyping efforts along with computational and experimental tools that we develop to gain insight into the basic evolutionary forces that maintain diversity.

I have always been drawn to the study of the evolutionary genetics of life-history traits. 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 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 the physiological details of this QTL were interesting (Bergland et al 2012), the evolutionary forces acting on it were unclear. Even if discernable, it is also unlikely that the specific strength of these forces at this locus were representative of the many loci controlling natural variation in life-history traits. To gain a more general understanding of the evolutionary forces acting on fitness related traits, as a post-doc I studied the genetic basis of adaptive differentiation in *D. melanogster* along latitudinal clines and among seasons. Using population genomic data, I showed that fly populations living in orchards adaptively evolve over seasonal time-scales (Bergland et al 2014). This work, and subsequent follow-up on additional populations (Machado, Bergland et al 2019), provides evidence that there are many, perhaps thousands, of common polymorphisms in the genome which vary in frequency between seasons, repeatedly across years and across geographic localities. The functional consequence of these adaptive polymorphisms remains elusive, as are the

demographic forces that contribute to seasonal evolution in this species. By understanding these attributes in more detail, we will gain a deeper understanding of the role of temporal variation in selection pressures as a mechanism of balancing selection.

In 2016, I began a faculty position at the University of Virginia. My research group (total: 3 post-docs, 6 PhD students, 3 technicians, 2 undergraduate Honors students, ~20 undergraduate research students), has pursued basic questions about the nature and dynamics of functional variation within species. This work utilizes two organisms, Drosophila and Daphnia, to examine the genetic architecture of standing variation and to map that genetic architecture onto the dynamic changes in allele frequency that we observe through time. The ultimate goal of this work is to characterize the importance of environmental variation in the generation and maintenance of diversity within species. Working on these two organisms simultaneously has been very satisfying: logistically they complement each other and many tools can be shared among them; yet, they offer fruitful intellectual contrast, particularly with respect to the importance of sexual systems in the structuring of genetic variation. For both species, we have established long-term field sites for sample collection, experimental lab- and field-based mesocosms facilities, genomic resources, and experimental and computational to measure the genetic architecture of fitness-related traits across a range of environmental conditions (Erickson *et al* 2020).

I take the training of mentees in my lab very seriously and work closely with people to develop their projects. This training covers aspects of writing and presentation, statistical and computational analysis, and professional development. Mentees from my lab have been awarded fellowships, received small grants from the University and professional societies, and won presentation awards at both the regional and national levels. I have also instilled a training environment where senior members of the lab (at any rank) work to train and mentor more junior people. One post-doc from my lab has gone onto a faculty position (at University of Richmond), one graduate student has gone onto a post-doc (NIH), three undergrads/post-bacs have gone onto Evolutionary Biology PhD programs (UC Berkeley, Yale, UVA).

In addition, I have participated actively as a member of the Drosophila population genetics consortium, DrosEU (http://www.droseu.net). As a member of this consortium, I have been involved in generating a concatenated dataset of allele frequency estimates from >275 populations world-wide. We have developed a flexible pipeline to add new samples easily to this growing dataset and have coupled these samples with weather data prior to sampling, geographic location, and other sampling metadata. The updated version of this data-set is described here: https://github.com/alanbergland/DEST/

For the last 3 years, I have taught a course titled "*Molecular Evolution: diversity, mutants, and the biological myth of race*". This seminar based-course teaches the mathematical foundations of population genetics and also addresses the societal impact that biologists have had in ascribing value (i.e.., superiority and inferiority) to diversity within our own species.

Relevant Publications:

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, 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.

Machado* H, **Bergland AO*,** Taylor R, Tilk S, Behrman E, Dyer K, *et* al. 2019. Broad geographic sampling reveals predictable and pervasive seasonal adaptation in Drosophila. *bioRxiv: 337543v2*

Erickson PA, Weller CA, Song DY, Bangerter-Black A, Schmidt PS, **Bergland AO**. Unique genetic signatures of local adaptation over space and time for diapause, an ecologically relevant complex trait, in *Drosophila melanogaster*. In review. bioRxiv: *2020.05.06.081281v1*

https://www.ncbi.nlm.nih.gov/myncbi/alan.bergland.1/bibliography/public/

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)
2016 - Member, Society for the Study of Evolution (SSE)
2014, 2015, 2016 NSF DEB grant review panelist
2014-2020 *Ad hoc* grant reviewer for BBSRC, INR, Austrian Science Foundation, SNSF, NWR, NSF-DEB
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 University
2020 Dept. of Biology Distinguished Teaching Award, University of Virginia

C. Contributions to Science

1. Local adaptation over seasonal time-scales. Organisms living in temperate environments experience dramatic changes in selection pressure across seasons. For species with many generations per year, rapid adaptive evolution can occur in response to temporal variation in the strength and direction of natural selection. Such adaptive tracking drives heritable phenotypic change through time, in principle caused by temporal changes in allele frequency at functional polymorphisms. Whether adaptive tracking through time is a major evolutionary process occurring within populations has remained an open question, and therefore the importance of temporal variation in selection pressures as a mechanism of balancing selection has remained elusive.

We have demonstrated that *D. melanogaster* adaptively evolves in response to environmental changes that occur across seasons, repeatedly over multiple years, and across multiple localities. Using population-genomic data, we have demonstrated that many loci in the genome change in frequency between seasons. Initial analysis (Bergland et al 2014) focused on a single population in Pennsylvania and identified hundreds of fluctuating polymorphisms; these loci were enriched for various functional annotations and are spread throughout the genome, suggesting polygenicity. We have extended this work (Machado*, Bergland*, et al 2019) to test whether adaptive evolution is a generic feature of fly populations caused by shifts at a common set of loci. Through collaboration with fly biologists throughout North America and Europe, we have examined genome-wide patterns of allele frequency change between seasons at over 20 localities. We demonstrate that there are a common set of loci which change in frequency throughout *D. melanogaster*'s range, and that change in frequency of these loci may be driven by localized variation in climate. We show that these seasonal polymorphisms are old, being generally present in ancestral African populations, and occasionally found to be segregating in the sister species, *D. simulans*.

Fly populations sampled at different times of year often (but not always) show heritable differences in a suite of life-history, stress tolerance, and morphological traits. In general, the view is that selection pressures over the winter select for individuals that allocate more resources into somatic maintenance than reproductive output; *vice-versa* in the summer. In some cases, we have been able to link specific seasonally varying polymorphisms to genetically based phenotypic variation observed among flies sampled in different seasons (Paaby et al 2014). We have argued that polygenic genetic variation along this life-history tradeoff can be stably maintained despite fluctuating selection, and that allele frequency fluctuations underlying this tradeoff can be large, given certain features of the genetic architecture of traits (Wittmann *et al* 2017).

We have extended our analysis of the temporal dynamics of seasonal evolution to a new system, *Daphnia pulex*. This species provides an important contrast to our work on Drosophila because it allows us to assess the role of mating systems and population dynamics on the maintenance of functional variation. Daphnia are

facultative and tend to have small effective population sizes, therefore occupying the other end of the life-history spectrum, relative to Drosophila. Yet, this species is similar to Drosophila in that populations undergo multiple generations per year, and evolve across seasonal time-scales. I discuss our recent advances in studying the temporal dynamics of adaptation in Daphnia in the Research Proposal.

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

Wittmann MJ, **Bergland AO**, MW Feldman, PS Schmidt, DA Petrov. 2017. Segregation lift: A general mechanism for the maintenance of polygenic variation under seasonally fluctuating selection. PNAS. doi: 10.1073/pnas.1702994114

Machado* H, **Bergland AO*,** Taylor R, Tilk S, Behrman E, Dyer K, *et al*. 2019. Broad geographic sampling reveals predictable and pervasive seasonal adaptation in Drosophila. bioRxiv: 337543v2

2. The genetic architecture of natural variation in fitness related traits. All population harbor standing genetic variation in fitness. Whether this variation occurs because of stochastic processes (such as mutation, migration or drift) or by some form of balancing selection remains a fundamental question in evolutionary biology. Addressing this question is important because whether one set of forces are dominant to the other changes out interpretation about the nature of variation and the value of diversity. Assessing the extent of various evolutionary forces on acting on genomes is necessary for accurate interpretation of the evolutionary history of species, and for predicting future evolutionary change.

My work has sought to address this question by mapping natural genetic variation of fitness related traits, and subsequently testing how those polymorphisms change in frequency through time and space. In flies, we have approached this problem using an outbred mapping scheme (Weller and Bergland 2020). This approach utilized a multi-parental population founded by a limited number of genomes. We have developed a pipeline to reconstruct genomes from individual flies using <0.05X coverage, and show that this mapping approach is preferable to some alternatives in a number of important ways. We have applied this approach to study the genetic architecture of temperature-dependent diapause in *D. melanogaster* (Erickson et al 2020). We find that polygenic variation associated with diapause is predictably arrayed across a north-south cline; however, variation between seasons across multiple populations is idiosyncratic. Consistent with Machado, Bergland *et al* (2019), this work suggests that strong and idiosyncratic selection pressures occur between seasons, raising the possibility that much more of the genome is affected by seasonal evolution than previously estimated.

Daphnia provide a unique system to study the genetic architecture of fitness related variation as it evolves over seasons. The small Daphnia populations that we study are effectively wild multi-parental populations, in which recombinant siblings undergo clonal selection across the growing season. This dynamic allows us to perform QTL-mapping in wild populations and directly assess their change through time, in both natural and experimental settings. We are performing such work by focusing on natural polymorphism in sexual investment, and predator induced developmental plasticity. In the Research proposal, I discuss our recent advances studying the genetics of Daphnia populations.

Weller CW & **Bergland AO**. 2020. Accurate, ultra-low coverage genome reconstruction and association studies in Hybrid Swarm mapping populations. In review. bioRxiv: 671925v2

Erickson PA, Weller CA, Song DY, Bangerter-Black A, Schmidt PS, **Bergland AO**. 2020. Unique genetic signatures of local adaptation over space and time for diapause, an ecologically relevant complex trait, in *Drosophila melanogaster*. In review. bioRxiv: *2020.05.06.081281v1*

3. The physiological and molecular basis of life-history variation. Identifying the genetic basis of natural variation is important for identifying novel mutations that provide insight into the basic biology of the organism. Much of my work has focused life-history traits, such as reproductive capacity, or various aspects of stress tolerance as mediated by the nutritional environment. These traits are important to

focus on because they are central to understanding life-history tradeoffs via resource allocation (Bergland 2011).

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.

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

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R35 GM119686 Bergland (PI) 05/01/2016-04/30/2021

The genetic and physiological architecture of rapid and cyclic adaptation
The goal of this project was to examine the genetic basis of rapid adaptive evolutionary change in two distinct species that are subject to different types of selection pressures in the wild. This work sought to identify functional polymorphisms affecting expression and phenotypes, and to study the change in frequency at these loci though time.

Completed Research Support

None in the last 3 years.

Current and Pending Support

Bergland, Alan

Current

NIGMS
R35 GM119686 (PI: Bergland) 8/1/2016 – 5/31/2021
Annual Direct Costs to Laboratory: $250,000
PI Effort - 3.0 summer months
Title of Project - The genetic and physiological architecture of rapid and cyclic adaptation
Description: The goal of this project was to identify loci associated with the traits subject to seasonally varying selection pressure in two species, Drosophila and Daphnia.

Pending

None

Overlap

None

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 0653915260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Alan	Olav	Bergland		PD/PI	0.00			3.0	0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 1**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2021 **End Date*:** 03-31-2022 **Budget Period: 1**

F. Other Direct Costs		Funds Requested ($)*
1. Materials and Supplies		
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8 . Requested Direct Costs		270,937.00
	Total Other Direct Costs	**270,937.00**

G. Direct Costs		Funds Requested ($)*
	Total Direct Costs (A thru F)	**270,937.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	61.5	270,937.00	166,626.00
		Total Indirect Costs	**166,626.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Arif Karim - Director, Cost Allocation Services Tel: 214.767.3600

I. Total Direct and Indirect Costs		Funds Requested ($)*
	Total Direct and Indirect Institutional Costs (G + H)	**437,563.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	437,563.00

L. Budget Justification*	File Name: bergland_budget_justification_v3.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 0653915260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Alan	Olav	Bergland		PD/PI	0.00			3.0	0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 2**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2022 **End Date*:** 03-31-2023 **Budget Period: 2**

F. Other Direct Costs		Funds Requested ($)*
1. Materials and Supplies		
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8 . Requested Direct Costs		270,937.00
	Total Other Direct Costs	**270,937.00**

G. Direct Costs		Funds Requested ($)*
	Total Direct Costs (A thru F)	**270,937.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	61.5	270,937.00	166,626.00
		Total Indirect Costs	**166,626.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Arif Karim - Director, Cost Allocation Services Tel: 214.767.3600

I. Total Direct and Indirect Costs		Funds Requested ($)*
	Total Direct and Indirect Institutional Costs (G + H)	**437,563.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	437,563.00

L. Budget Justification*	File Name: bergland_budget_justification_v3.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 0653915260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Alan	Olav	Bergland		PD/PI	0.00			3.0	0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 3**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2023 **End Date*:** 03-31-2024 **Budget Period: 3**

F. Other Direct Costs		Funds Requested ($)*
1. Materials and Supplies		
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8 . Requested Direct Costs		270,937.00
	Total Other Direct Costs	**270,937.00**

G. Direct Costs		Funds Requested ($)*
	Total Direct Costs (A thru F)	**270,937.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	61.5	270,937.00	166,626.00
		Total Indirect Costs	**166,626.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Arif Karim - Director, Cost Allocation Services Tel: 214.767.3600

I. Total Direct and Indirect Costs		Funds Requested ($)*
	Total Direct and Indirect Institutional Costs (G + H)	**437,563.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	437,563.00

L. Budget Justification*	File Name: bergland_budget_justification_v3.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 0653915260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 4**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Alan	Olav	Bergland		PD/PI	0.00			3.0	0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 4**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2024 **End Date*:** 03-31-2025 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Requested Direct Costs	270,937.00
Total Other Direct Costs	**270,937.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**270,937.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	61.5	270,937.00	166,626.00
		Total Indirect Costs	**166,626.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Arif Karim - Director, Cost Allocation Services Tel: 214.767.3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**437,563.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	437,563.00

L. Budget Justification*	File Name: bergland_budget_justification_v3.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0010
Expiration Date: 12/31/2022

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 0653915260000

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2025 **End Date*:** 03-31-2026 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Alan	Olav	Bergland		PD/PI	0.00			3.0	0.00	0.00	0.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **0.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**0.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2025 **End Date*:** 03-31-2026 **Budget Period: 5**

C. Equipment Description		
List items and dollar amount for each item exceeding $5,000		
Equipment Item		**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file		
	Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 0653915260000
Budget Type*: ● Project ❍ Subaward/Consortium
Organization: UNIVERSITY OF VIRGINIA

Start Date*: 04-01-2025 **End Date*:** 03-31-2026 **Budget Period: 5**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Requested Direct Costs	270,937.00
Total Other Direct Costs	**270,937.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**270,937.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	61.5	270,937.00	166,626.00
		Total Indirect Costs	**166,626.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Arif Karim - Director, Cost Allocation Services Tel: 214.767.3600

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**437,563.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	437,563.00

L. Budget Justification*	File Name: bergland_budget_justification_v3.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

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 lab costs. In addition, this increase is important because start-up funds will expire soon, making grant funding the sole source of funding.

Equipment

I am not requesting any major equipment purchases.

Consortium

I am not requesting any consortium funding.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		0.00
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		0.00
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		1,354,685.00
1. Materials and Supplies	0.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	1,354,685.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		1,354,685.00
Section H, Indirect Costs		833,130.00
Section I, Total Direct and Indirect Costs (G + H)		2,187,815.00
Section J, Fee		0.00
Section K, Total Costs and Fee (I + J)		2,187,815.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 02/28/2023

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	
3. Research Strategy*	bergland_researchStrategy.pdf
4. Progress Report Publication List	bergland_progressReportPublications.pdf
Other Research Plan Section	
5. Vertebrate Animals	
6. Select Agent Research	
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	Bergland-Beckerman-LoS.pdf
10. Resource Sharing Plan(s)	bergland_Data_and_Resource_Sharing.pdf
11. Authentication of Key Biological and/or Chemical Resources	
Appendix	
12. Appendix	

Background. Variation in the strength and direction of natural selection through time is a ubiquitous feature across the tree of life (Bell 2010). In response to temporal fluctuations in selection pressure, populations of many species - particularly those with short generation times - are predicted to adaptively evolve as they track along a moving fitness landscape (Botero *et al.* 2015). Although adaptive tracking is likely a ubiquitous evolutionary process, we have a limited understanding of its prevalence and its effects on patterns of genetic diversity. Genomic signals of adaptive evolution in response to temporally fluctuating selection pressure are limited, particularly for wild populations (Bergland *et al.* 2014; Campbell-Staton *et al.* 2017; Nosil *et al.* 2018), often preventing us from relating empirical and expected patterns of diversity. **To advance our understanding of the temporal dynamics of evolution, my lab studies the genetic signatures and genomic consequences of rapid evolution in response to seasonal fluctuations in selection pressure.**

My research focuses on the seasonal dynamics of evolutionary change in short-lived organisms with multiple generations per year. For such species, the environment changes rapidly from one generation to the next, and populations can cyclically evolve over annual time-scales. **The cyclic nature of seasonal evolution allows us to address a number of basic questions as they unfold**: Is short-term evolution repeatable or predictable at a molecular level? Is temporally fluctuating selection a major evolutionary force that promotes genetic diversity? What are the relative contributions of short-term demographic events versus adaptation in contributing to seasonal changes in the genetic composition of populations? Which traits are subject to seasonally varying selection and what is the genetic architecture of rapid adaptation in the wild? To address these questions, we focus on two study systems, *Drosophila melanogaster* and *Daphnia pulex*.

Populations of *D. melanogaster* living in orchards throughout the world adaptively track in response to seasonal fluctuations in selection pressure (e.g., temperature), over the course of 10-15 generations. These wild populations also harbor extensive genetic variation in an array of ecologically relevant, fitness related traits(Schmidt & Conde 2009; Adrion *et al.* 2015; Mackay & Huang 2018). Genetic variation in starvation tolerance, thermal tolerance, longevity, fecundity, etc., enables some individuals to be more likely to survive winter, and others better able to exploit the favorable growing season (Schmidt *et al.* 2005; Behrman *et al.* 2015; Rajpurohit *et al.* 2017; Behrman *et al.* 2018). Genetically based seasonal variation in phenotype is generated by polymorphisms that fluctuate in frequency between seasons (Cogni *et al.* 2014; Paaby *et al.* 2014). Work in my lab seeks to (1) document genetic variation across time and space in *Drosophila* and to use genomic data to infer evolutionary dynamics of natural populations; and, (2) to uncover the genetic architecture, molecular function, and evolutionary history of polymorphisms that underlie local adaptation.

Daphnia pulex are an ideal system to study the temporal dynamics of evolutionary change. Clonal isolates of *D. pulex* sampled within and among ponds (Reger *et al.* 2018), and across seasons (Pfrender & Lynch 2000), show extensive genetic variation in life-history traits, predator defense capacity, and sexual dynamics. The presence of variation within populations is somewhat surprising, given the seasonal dynamics of *D. pulex* living in small semi-ephemeral ponds. The classic view of such populations is that daphnia hatch in the spring from resting eggs, which are the product of sex the at the end of the previous growing season; these newly hatched daphnia then undergo asexual reproduction and clonal selection, leading to a rapid decline of clonal diversity; sex ensues and the population overwinters as resting eggs (or over-summers in areas where summer drying is the selective agent). In the absence of a diversifying force, such a population would quickly become monomorphic and highly inbred. Contrary to this prediction, the populations that we study are surprisingly diverse at both a molecular and phenotypic level. To assess the importance of different diversifying forces on the maintenance and generation of variation, we study the temporal dynamics of clonal evolution and sex in wild populations, and work to identify the forces that maintain polymorphism.

Key gaps in our understanding. In my view, a major gap in our understanding of evolutionary biology is knowledge about the prevalence of adaptive tracking over short time-scales. Because of this empirical deficiency, we are unable to test basic models that predict the consequences of fluctuating selection and adaptive tracking on patterns of genetic diversity at functional and linked sites (Huerta-Sanchez *et al.* 2008; Cvijović *et al.* 2015; Park & Kim 2019). There are at least two reasons for this major gap: First, as a field we have historically lacked access to temporally sampled genomic datasets of wild populations. Second, quantitative life-history and behavioral traits are likely the primary targets of temporally fluctuating selection (Siepielski *et al.* 2009); therefore, identifying loci underlying adaptive tracking as a means to study evolutionary dynamics over short time-scales is technically challenging. To address these historical and technical challenges, my research program has developed long-term genomic monitoring of natural populations for two species and has developed innovative approaches to mapping quantitative traits in outbred populations.

Is adaptive tracking to fluctuating selection pressures an important phenomenon in general? The answer is yes, but the relative importance likely varies dramatically across taxa. Fundamentally, the importance of

adaptive tracking is a function of grain size (Levins 1968) - the pace of environmental change relative to generation time. Organisms with short generation times are likely to exhibit adaptive tracking; those with longer generation time evolve plasticity (Botero *et al.* 2015). Variation in the relative importance of adaptive tracking across taxanomic groups may contribute to the generation time effect on the rate of adaptive substitution (Cvijović *et al.* 2015; Thomas *et al.* 2010). While it is therefore reasonable to claim that adaptive tracking must affect all species, to some extent, how much of the genome is subject to adaptive tracking, and the strength of fluctuating selection, remain largely unknown (but see (Machado *et al.* 2018; Buffalo & Coop 2020).

Whether adaptive tracking promotes long-lived, balanced polymorphisms is another fundamental mystery. Theoretical models are somewhat conflicted in their predictions (Hedrick 2006), but models that incorporate storage mechanisms such as seed banks (Turelli *et al.* 2001), age-structure (Ellner & Hairston 2015), or aspects of genetic architecture (Wittmann *et al.* 2017; Bertram & Masel 2019), typically show that long-lived balanced polymorphism can exist. If these ecologically relevant polymorphisms segregate at intermediate frequencies for long periods of time, they may have a limited effect on patterns of neutral variation (Charlesworth 2006; Gao *et al.* 2015). These models present a paradox: the stronger the stabilizing force associated with temporally varying selection, the less it contributes to linked polymorphism and, by extension, the less apparent its signal in genome-wide scans. Work proposed here seeks to address this issue through the use of experimental quantitative genetic approaches (Drosophila) and pedigree-based analysis (Daphnia).

Recent Progress. The goal of the previous funding period was to identify loci underlying natural genetic variation in seasonally selected traits. Because these loci are enriched for `true positives`, we can use them to ask basic questions about the temporal dynamics of adaptive tracking, and its consequences on patterns of genomic variation at linked sites. We have pursued this basic question using two species, with the goal of making a more general statement about the extent of balancing selection operating via temporal variation in selection pressures; and to contrast the roles of obligate- and facultative sex on the dynamics and consequences of balancing selection.

Drosophila. To study the evolutionary dynamics of seasonal adaptation in Drosophila, my lab has sought to map natural genetic variation in fitness-related traits, and to apply those data to surveys of allele frequency fluctuations across space and time. Our work has focused on various aspects of overwintering stress tolerance, with particular attention to aspects of thermal- and nutritional-stress (Stone *et al.* 2020). We situate our work in both a laboratory and a field-based context, allowing us to experimentally disentangle environmental and genetic contributions to phenotypic variation.

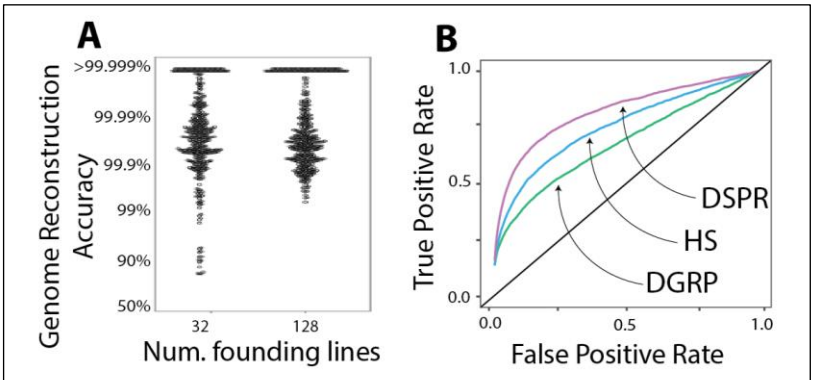

Figure 1. (A) Fully phased genomes can be reconstructed accurately for HybridSwarm (HS) crosses with large numbers of founding lines (results show simulated F5 swarms). (B) Receiver-operator curve for simulate GWAS (10 loci, small effect sizes) using different mapping panels (8-way DSPR; 128 DGRP inbred lines). Inbred panels like the DGRP can exhibit high false positive rate; RILs such as the DSPR have high power, but are limited to the existing founders. The HybridSwarm is an attractive alternative, opening new experimental avenues.

To study the genetic architecture of overwintering traits, we (Weller & Bergland 2019) have developed a mapping protocol that utilizes outbred multi-parental populations. The motivation for this approach is three-fold: (1) it enables mapping across a range of environmental conditions, and (2) it facilitates the generation of mapping-populations with different genetic backgrounds, and (3) it alleviates some issues of high false positive rates which occur when using inbred panels. In this approach, a moderate number of founder lines (ca. 10-100) are intercrossed for ~5 generations as an outbred population, maintained in large population cages (N~50,000). Individuals are then phenotyped and genotyped for subsequent genome-wide association analysis. We developed a pipeline for reconstructing fully phased genomes from ~0.05X sequencing (~$6-7/individual, which includes DNA extraction, library prep, and sequencing). Genome-reconstruction achieves high accuracy (>99.9%, Fig 1A) and is scalable in the number of founders. The practical utility of this approach is that individuals from large, panmictic populations can be distributed across environments, alleviating a substantial amount of vial-effects and the logistical burden of

exposing hundreds- to thousands of lines to a large number of environmental treatments. In addition, mapping populations can be constructed from lines (either inbred or outbred) collected across the geographic range, or through time, enabling mapping experiments to reflect the range of variation within the species.

We evaluated the power and precision of association mapping using this approach (which we call a Hybrid Swarm), relative to other experimental mapping designs (recombinant inbred lines, e.g. DSPR - Long *et al.* 2014; inbred lines, e.g. DGRP - Mackay *et al.* 2012). We find that the Hybrid Swarm approach has a lower false positive rate than inbred lines, likely due to recombination breaking up long-distance linkage-disequilibrium (Nuzhdin & Turner 2013). Like all outbred mapping designs, the Hybrid Swarm suffers a loss of power, at the true locus, relative to inbred lines via the absence of heterozygous intermediates (see Weller & Bergland 2019). However, Hybrid Swarm populations also allow one to examine dominance distributions and to test, for instance, whether dominance values change across environments.

We have applied this mapping approach to the study of diapause and of gene-expression. Our work on diapause (Erickson *et al.* 2020) used replicate 32-way hybrid swarm populations, seeded with inbred lines collected across the East Coast of North America, to map variation in temperature dependent diapause in *D. melanogaster* across ~3000 individuals. We demonstrate that diapause is a highly polygenic trait and further define fine-grained reaction norms using 48 custom built chambers with independent control of light and temperature (10-25°C). Using outdoor mesocosms (36 caged fruit trees with flies fed a standardized mixture of apples and bananas) seeded with advanced Hybrid Swarm populations, we show that there is a genetic shift in diapause propensity coinciding with winter conditions; the magnitude of evolutionary change of diapause was on the order of ~0.5 Haldanes, similar to daphnia size change after predation and beak size change after drought in the Galapagos (Hendry *et al.* 2008). **We demonstrate that standing genetic variation in diapause is polygenic, old (predating colonization of higher latitudes), and shows contrasting patterns of variation across space and time**. Diapause associated SNPs vary across a latitudinal gradient in a predictable manner, i.e., pro-diapause alleles are more common at high latitudes. In contrast, signals of seasonal change at these loci are not apparent when examining seasonal fluctuations identified, jointly, across 20 populations (Machado *et al.* 2018). One possibility is that the lack of concordant allele frequency change at these GWAS hits is due to idiosyncratic shifts in each population. Intriguingly, when examining single population spring-fall pairs, we see signals of fluctuating selection, however the predicted direction and magnitude of selection on these GWAS hits varies from population to population. Similar incongruencies between clinal and seasonal patterns of allele frequency change hold for meta-analysis of eQTL identified from inbred lines (Yang and Bergland, in prep).

This mapping work provides novel insight into our understanding of seasonal adaptation in Drosophila. Using current population genomic data-sets alone, analysis of seasonal fluctuations are biased towards the small (but observable) fraction of the genome which shows consistent shifts between seasons in multiple populations (Machado *et al.* 2018). By combining our mapping studies with population genomic data, our work suggests that an even larger fraction of the genome might be shifting seasonally, in idiosyncratic ways, across multiple populations. Whether these idiosyncratic seasonal shifts reflect adaptive evolution in response to localized shifts in selection pressure remains an open question. More generally, we only have a limited understanding of the influence of sampling bias, meta-populations dynamics, migration, and micro-spatial environmental variation in generating the temporal changes in allele frequency that we observe. We have begun to address some of these possibilities through dense temporal sampling and resequencing of flies collected every two weeks for 3 years at local field sites and experimental mesocosms (work in progress).

Daphnia. To gain a broader perspective on the seasonal dynamics of evolutionary change, my lab has begun working on *D. pulex*. The populations of Daphnia that we study present a number of contrasts to Drosophila, allowing us to take our research in novel directions. The principal distinctions of Daphnia are that it is facultatively sexual, that local populations have small effective population sizes, and that metapopulation dynamics are relatively circumscribed. These features are close to a polar opposite of Drosophila (obligately sexual, large effective population size, high connectivity), allowing us to examine the role of these key life-history and ecological features as they relate to the temporal dynamics of seasonal adaptation.

Our Daphnia research is situated in a series of intermittently connected ponds in southern England (Dorset), where we have been sampling over the last 4 years. We have sequenced ~500 individual field isolates, generated a high-quality reference genome for this population (130Mb, 1 scaffold/chr, BUSCO ~95%; 10X + Dovetail), and sequenced several sympatric and allopatric outgroups (*D. pulicaria, D. obtusa*, *Simocephalus spp*.). We have built a Daphnia facility capable of maintaining ~300 clones, have established lab-based mesocosms facilities which enable competition experiments and are useful for performing crosses, and conducted large multi-environment phenotyping efforts. The populations that we study largely adhere to

the basic model of facultative parthenogenesis (described above), with some notable exceptions (discussed below). One key attribute of these populations is that ponds vary in degree of ephemerality, and thus the frequency of sex. These features provide a unique opportunity to study both competitive dynamics between clones and the accumulation of mutations within clonal lineages, as affected by the degree of ephemerality. Daphnia living in these ponds are closely related, and populations resemble something around a 2- to 8-way intercross between parents who are themselves identical, full- to half-siblings, or cousins (Barnard-Kubow *et al*, in prep). Clonal lineages can persist in populations for multiple years, and analysis of new mutations among clonally related individuals suggests that these clones may be hundreds of generations old. By coupling temporal sampling with genome-sequencing data, we have been able to observe mating dynamics in the wild, and can track these recombinant populations through time. Remarkably, there is abundant heritable genetic variation within these populations making them a natural QTL mapping panel, and a test-bed for studying the dynamics and predictability of recurrent selection on standing genetic variation.

A major project that we have developed examines heritable variation in reproductive allocation among co-existing clonal lineages. As facultative parthenogens, a female daphnid is capable of three modes of reproduction, and she could experience all three at some point in her life: she can produce a brood of ~1-20 clonal female progeny; a brood of ~1-10 male progeny; or she can produce an ephippial case containing up to two embryos ("resting eggs"; note, in Future Directions**,** I discuss a new system to study bet-hedging in vernalization requirements). Diploid resting eggs can be the product of selfing, outcrossing, or can be clonally produced. These reproductive modes are labile, in that a single female will switch back and forth between the production of males, clonal females, or epphipia. Generation time is ~5 days, and successive broods can be produced every 3-4 days. Whether switches between reprodsuctive modes happen stochastically or, alternatively, in response to specific environmental cues is not clear and varies dramatically between populations and closely related species (Smirnov 2017). At least for the populations that we study, ephippia production rate is density dependent, but male production rate is not (neither seem to be photoperiodic).

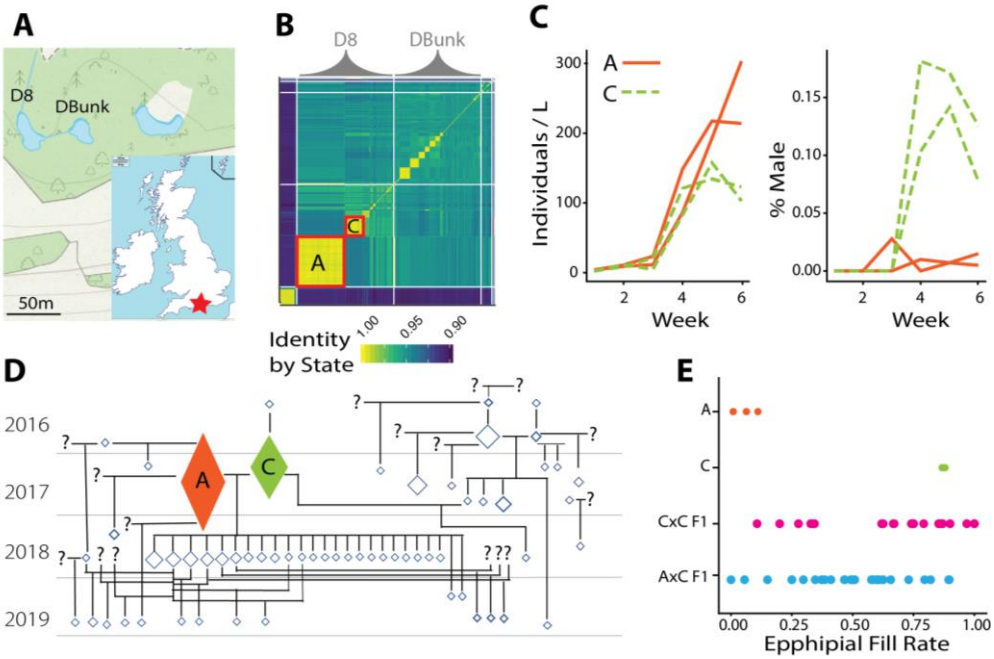

Figure 2. (A) Map of field site. Here, we highlight two ponds D8 and DBunk; we have been sampling from 6 additional ponds in this area (not all shown). (B) Genomic identity by state matrix for sequenced individuals. Large yellow blocks are dominant lineages; A and C are noted. (C) Lineages A and C invest differentially into reproductive mode. Each line is a replicate field isolate of either A or C. (D) Pedigree of D8 individuals inferred from kinship, IBS0, IBS1, and IBS2. Size of diamond is proportional to the number of sampled individuals (smallest diamonds are singletons). Self-fertilization events are represented by a single diamond with line below. (E) There is segregating variation in epphipial fill rate (a proxy for male production) between A and C, as well as amongst selfed-Cs. Each point represents the mean fill rate of a clone. Clones were generated in the lab (CxC; AxC) as well as from field samples (AxC).

Two dominant clonal lineages sampled from one pond in 2017 (Fig 2) exhibit variation in male production rate (~2% vs 15% of total brood), which leads to a significant difference in population growth rates (R_0) as measured in lab mesocosms. These lineages mate readily in the lab and field; additionally, the male-limited "A" lineage is able to produce clonal ephippia, whereas "C" has not been observed to do so. Polymorphisms in sexual allocation have been observed in other daphnid systems (Galimov *et al.* 2011), but a number of

features of the populations we work with differ from previous models, providing us with a unique opportunity to study alternative evolutionary paths of a parallel evolutionary process. Notably, in another system, complete male limitation (i.e., obligate parthenogenesis) arises, recurrently, via introgression from a closely related sister species. Male limitation is therefore primarily driven by variation at one-locus, which behaves in a ZW-like fashion (Ye *et al.* 2019). In the populations that we study, we have identified that variation in male production rate is polygenic, with upwards of ~8 QTL on separate chromosomes segregating between field caught males and females. Neither the dominant lineages, nor these specific QTL, show any evidence of arising via hybridization with another species (although we cannot rule out, at the moment, an unknown 'ghost' lineage). Rather, these dominant lineages appear to be cousins, and we hypothesize that alleles contributing to male limitation arise *de novo* within populations. Ongoing work seeks to narrow the previously identified QTL using field-caught and lab generated AxC recombinant lineages, and to further examine the evolutionary history of these loci. Future work will use the empirical observations of ecology, population dynamics, and genetic architecture that we have generated to parameterize forward-genetic simulations in which to assess the stability and persistence-time of fitness related genetic variation. This work is important because it examines the forces that generate and maintain variation in a system where variation should be quickly lost due to consanguinity and clonal selection.

Overview of Future Research Plans. The work conducted over the last four years has led to the development of genomic, computational, and experimental resources to study the effects of fluctuating selection on the maintenance of variation. **We will build on these advances to directly relate patterns of heritable variation in fitness related traits to the strength of fluctuating selection and the persistence time of balanced polymorphism**. This work will take place across a range of environmental conditions, in both lab and field-based settings, and will continue to layer quantitative genetic insight with population genetic inference.

Drosophila. Despite ample evidence of seasonal adaptation from phenotypic and genomic analysis (discussed above and in Biosketch), many features of this system remain unresolved. Notably, we lack a set of gold-standard true-positive loci that contribute to seasonal adaptation. As a consequence, it remains challenging to assess the consequence of adaptive tracking on patterns of genetic variation at linked sites or to assess the strength and predictability of seasonal adaptation at any given site. More generally, across most taxa, the magnitude and genomic extent of adaptive response to fluctuating selection pressures remains unknown. Technical challenges might limit inference of the strength of selection based on population allele frequencies alone (Buffalo & Coop 2019; Lynch *et al.* 2020) and unknown demographic factors many also affect inference of wild populations. Mapping experiments, as described in *Recent Progress*, can help identify specific loci associated with seasonal adaptation but the success of such edeavors might be restricted to oligogenic traits. Therefore, **experimental quantitative genetic approaches which seek to quantify the heritability and estimate the magnitude of genetic variance components may offer an attractive alternative to study the temporal dynamics of seasonal adaptation**.

To advance our understanding of the strength of selection and the magnitude of adaptive response to seasonally fluctuating environments, we will perform overwintering truncation selection experiments to estimate the strength of selection on heritable variation in gene expression. The experiment utilizes the breeder's equation or its multivariate equivalent (Lande 1979), to calculate the strength of selection [S], with knowledge of heritability [h^2] and the phenotypic response to selection [R]. The basic logic of this experiment is as follows: Using a Hybrid Swarm design, derived from inbred lines collected along the East Coast, we can accurately estimate h^2 of a trait using a genetic relationship matrix (Yang *et al.* 2010) estimated from genome-reconstructions (described above). If we measure the mean phenotype of the population in the generation before and the generation(s) after a selective event, we can estimate the response to selection (R). We can apply this technique to study the strength of selection across the transcriptome to obtain a more generalized and unbiased understanding of the distribution of selection and the magnitude of adaptive response to short term fluctuations in selection pressure. By conducting these truncation selection experiments in the lab (e.g., in response to freezing; Stone *et al.* 2020) and in the field (e.g., overwinter in outdoor mesocosms), **we can experimentally validate that seasonally varying selection is, indeed, operating in these populations and gain insight into the adaptive responses to specific selective events**.

This approach could either examine gene-expression taken across a single whole animal, or a specific tissue type (e.g., the head). We have successfully extracted RNA- and DNA- from single individuals (Weller et al, in prep), and it is even likely that we can reconstruct accurate genomes via RNA seq data alone. Low-cost RNA-seq libraries will be made using BRB-seq (Alpern *et al.* 2019). Note, that because the goal of these experiments is to measure components of variation we will only require, maximally, on the order of 1000-2000

individuals phenotyped and genotyped, as estimation of heritabilities based on GRM approaches are accurate with a limited number of individuals (Visscher & Goddard 2015). Although detecting eQTL is not the main purpose of these experiments, regions of the genome associated with heritable variation in gene expression can be identified using mixed effect modeling (Conomos *et al.*) or sparse linear models (Pérez & de los Campos 2014). This work will identify putative phenotypic targets of natural selection, and will elucidate aspects of the physiological and developmental basis of local adaptation.

Daphnia. Future work in Daphnia will examine the evolutionary outcomes of temporal variation in selection pressure. The goal of this work is to examine how seasonal cycles of clonal growth and sex lead to the evolution of polymorphism and to the evolution of plasticity / bet-hedging. By addressing these topics in Daphnia, we can test basic predictions (Botero *et al.* 2015) about the relationship between the predictability of environmental change on the evolution of these components of phenotypic variance.

First, we will continue our work studying the evolution of polymorphism in sexual investment. We will approach this work using both laboratory experiments and observational studies of natural populations. To experimentally test the evolutionary dynamics of sexual investment, we will conduct crosses within and between clonal lineages and compete these recombinant offspring in mesocosms. We will focus on the two clonal lineages that we have studied extensively which show polymorphism in male production rate (A and C, Figure 2), although we will also generalize this work by expanding analysis to other clones from the Dorset area, or elsewhere, when appropriate. Competition experiments will be seeded with ephippia derived from selfing and outcrossing of these parental clones, and we will track the frequency of recombinant clones through time using a combination of pooled and individual sequencing. These estimates of frequency directly relate to fitness. Note that sequencing effort for this type of experiment can be kept at a reasonable level because we have (or can easily generate) phased genomes for parental clones via trio-phasing (PattersonMurray *et al.* 2015; Choi *et al.* 2018). The distribution of fitness of recombinant offspring offspring will allow us to assess the consequences of inbreeding depression, determine whether fitness is affected by any over-dominant alleles (Charlesworth & Willis 2009), and whether experimental treatments dramatically alter the outcome of the evolutionary process. We will couple these experimental approaches with continued sampling and analysis of population genomic data from the Dorset populations. We will work to examine patterns of molecular evolution as they relate to the inferred pedigree and test whether loci involved in male limitation appear to arisen recently in these populations (via mutation or migration) or whether they have persisted in these populations for long periods of time. This analysis will take advantage of recent advances in ancestral recombination graph analysis (Rasmussen *et al.* 2014) and will couple the analysis of phased genome data with extensive forward simulation (Haller *et al.* 2019), parameterized by known ecological aspects of these ponds. This work is important because it will allow us to experimentally test the role of different ecological forces on the maintenance of genetic variation in this species.

The second major Daphnia project that we will pursue examines apparent bet-hedging in the requirement of vernalization for ephippial hatching. Examination of hatching patterns in lab generated crosses shows that ~30% of ephippia spontaneously hatch without any vernalization cue; the remainder require a combination of cold temperatures and dark, followed by exposure to light and warmth to hatch. In other Daphnia populations, vernalization is required for any hatching (Luu *et al.* 2020), and the appearance of spontaneous hatching likely reflects the adaptation driven by the ecology of the ponds. The ponds in Dorset dry periodically and likely stochastically from year to year, plausibly (Graham *et al.* 2014) leading to local adaptation in bet-hedging (Simons 2009). To advance our understanding of the biology of vernalization, we will first assess phenotypic genetic variation in spontaneous hatching between ponds that vary in ephemerality. We will follow up this work by via bulk segregant analysis of F1 (or more advanced generations, when appropriate) crosses between clones that show variation in vernalization requirement. We will address the prevalence of maternally deposited cues through the use bisulfite sequencing of freshly deposited embryos in order to determine whether maternal modification of methylation state is correlated with the rate of spontaneous hatching (Harris *et al.* 2012). This work will advance our understanding of basic aspects of Daphnia genetics and biology and will also provide insight into the adaptive dynamics of bet-hedging.

Future directions. Future work in Drosophila will study the role of local meta-population dynamics (e.g., seasonal recolonization (Shpak *et al.* 2010)) on the dynamics and patterns of seasonal evolution. This work will allow us to address the role of spatial refugia in the maintenance of genetic variation and the role of founder effects in the outcome of strong selection in quantitative traits. Future work in Daphnia will characterize the molecular genetics of polymorphism in male production, and will utilize RNAi (Hiruta *et al.* 2013) and CRISPR (Hiruta *et al.* 2018)

Progress report publication list:

1. Erickson PA, Weller CA, Song DY†, Bangerter-Black A, Schmidt PS, **Bergland AO**. Unique genetic signatures of local adaptation over space and time for diapause, an ecologically relevant complex trait, in Drosophila melanogaster.
bioRxiv: *https://www.biorxiv.org/content/10.1101/2020.05.06.081281v1*

   • This work was funded by award #61-1673 from the Jane Coffin Childs Memorial Fund for Medical Research PAE. These funds paid for PAE's salary and benefits.

   • This work was supported by NIH R35 GM119686 to AOB. These funds paid for library prep, sequencing, the development of environmental chambers, undergraduate support, fly consumables, development of experimental orchard, and summer support to AOB.

2. Weller CA & **Bergland AO**. Accurate, ultra-low coverage genome reconstruction and association studies in Hybrid Swarm mapping populations.
bioRxiv: *https://www.biorxiv.org/content/10.1101/671925v2*

   • This work was supported by NIH R35 GM119686 to AOB. These funds paid for summer support to AOB, and GRA support for CAW.

3. Machado* H, **Bergland AO*,** Taylor R, Tilk S, Behrman E, Dyer K, *et* al. 2018. Broad geographic sampling reveals predictable and pervasive seasonal adaptation in Drosophila.
bioRxiv: https://www.biorxiv.org/content/10.1101/337543v2

   • This work was supported by NIH R35 GM119686 to AOB. These funds paid for summer support to AOB.

4. Stone HM, Erickson PA, **Bergland AO**. Phenotypic plasticity, but not adaptive tracking, underlies seasonal variation in post-cold hardening freeze tolerance of Drosophila melanogaster. PMCID: PMC6972814.

   • This work was supported by NIH R35 GM119686 to AOB. These funds paid for equipment, fly consumables, and summer support to HMS.

   • This work was funded by award #61-1673 from the Jane Coffin Childs Memorial Fund for Medical Research PAE. These funds paid for PAE's salary and benefits.

   • This work was supported by a University of Virginia Harrison Award to HMS. These funds paid for summer support to HMS as well as some equipment for freeze tolerance assays.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	❍ Yes ● No
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Friday, 26 June 2020

Dear Alan,

I am excited to continue our collaboration studying the evolutionary ecology of Daphnia. This collaboration has led to the development of two main projects; one examining the quantitative genetics of predator induced plasticity and the other the evolutionary genetics of reproductive polymorphism. These collaborative projects are at the core of my research programme on the evolutionary ecology of Daphnia.

Over the last two years, I have assisted in the mentorship of two post-docs, Dr. Karen Barnard-Kubow and Dr. Dörthe Becker, including site visits, research seminars in the UK and time spent in my lab group.

After completing a post-doc with you, Dr. Becker won a prestigious Marie-Curie Independent Research Fellowship based out of my group at the University of Sheffield. The three of us maintain an active collaboration on her project and I look forward to continuing collaboration and co-mentorship.

This collaborative effort has also allowed two UVA undergraduates to work in my lab and do in field work in the Dorset, UK location where we collect the Daphnia and monitor their habitats. These have been very enjoyable and I look forward to hosting addition UVa undergraduate or masters students again.

Importantly, as long as Government regulations permit here, I will be able to assist in collecting Daphnia from Dorset (Southern England) on your behalf if the situation that travel abroad becomes prohibited (e.g., due to Covid-19).

Yours sincerely,

ANDREW P. BECKERMAN
Professor of Evolutionary Ecology
Department of Animal and Plant Sciences
University of Sheffield, UK.

Data and Resource Sharing Plan

Data Sharing Plan. All raw data sequence data will be deposited to the NCBI Short Read Archive upon, or prior to, publication. All phenotypic / experimental data will be deposited onto Data Dryad upon, or prior to, publication. Scripts to analyze the data will be deposited onto GitHub (preferably) and/or DataDryad. Data will be retained on UVA's High-Performance Computer.

Sharing model organisms. All stock lines generated from this proposed work will be available to the broader community. We often send inbred fly lines, as well as clonal Daphnia lines, to other researchers and will continue to do so.

Genomic Data Sharing. As mentioned above, all raw sequencing data will be deposited onto SRA. We will deposit final VCF files for specific publications on DataDryad. We also host data-sharing for population genomic data on a http pass-through site hosted by UVA (http://berglandlab.uvadcos.io). This site is used to share large files with collaborators.