02 INFORMATION ABOUT PRINCIPAL INVESTIGATORS/PROJECT DIRECTORS(PI/PD) and co-PRINCIPAL INVESTIGATORS/co-PROJECT DIRECTORS

Submit only ONE copy of this form **for each PI/PD and co-PI/PD** identified on the proposal. The form(s) should be attached to the original proposal as specified in GPG Section II.C.a. Submission of this information is voluntary and is not a precondition of award. This information will not be disclosed to external peer reviewers. ***DO NOT INCLUDE THIS FORM WITH ANY OF THE OTHER COPIES OF YOUR PROPOSAL AS THIS MAY COMPROMISE THE CONFIDENTIALITY OF THE INFORMATION.***

PI/PD Name: Catherine A Pfister

Gender: ☐ Male ☒ Female

Ethnicity: (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

Race: (Select one or more)
- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Native Hawaiian or Other Pacific Islander
- ☒ White

Disability Status: (Select one or more)
- ☐ Hearing Impairment
- ☐ Visual Impairment
- ☐ Mobility/Orthopedic Impairment
- ☐ Other
- ☐ None

Citizenship: (Choose one) ☒ U.S. Citizen ☐ Permanent Resident ☐ Other non-U.S. Citizen

Check here if you do not wish to provide any or all of the above information (excluding PI/PD name): ☒

REQUIRED: Check here if you are currently serving (or have previously served) as a PI, co-PI or PD on any federally funded project ☒

Ethnicity Definition:
Hispanic or Latino. A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race.
Race Definitions:
American Indian or Alaska Native. A person having origins in any of the original peoples of North and South America (including Central America), and who maintains tribal affiliation or community attachment.
Asian. A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.
Black or African American. A person having origins in any of the black racial groups of Africa.
Native Hawaiian or Other Pacific Islander. A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.
White. A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

WHY THIS INFORMATION IS BEING REQUESTED:

The Federal Government has a continuing commitment to monitor the operation of its review and award processes to identify and address any inequities based on gender, race, ethnicity, or disability of its proposed PIs/PDs. To gather information needed for this important task, the proposer should submit a single copy of this form for each identified PI/PD with each proposal. Submission of the requested information is voluntary and will not affect the organization's eligibility for an award. However, information not submitted will seriously undermine the statistical validity, and therefore the usefulness, of information recieved from others. Any individual not wishing to submit some or all the information should check the box provided for this purpose. (The exceptions are the PI/PD name and the information about prior Federal support, the last question above.)

Collection of this information is authorized by the NSF Act of 1950, as amended, 42 U.S.C. 1861, et seq. Demographic data allows NSF to gauge whether our programs and other opportunities in science and technology are fairly reaching and benefiting everyone regardless of demographic category; to ensure that those in under-represented groups have the same knowledge of and access to programs and other research and educational oppurtunities; and to assess involvement of international investigators in work supported by NSF. The information may be disclosed to government contractors, experts, volunteers and researchers to complete assigned work; and to other government agencies in order to coordinate and assess programs. The information may be added to the Reviewer file and used to select potential candidates to serve as peer reviewers or advisory committee members. See Systems of Records, NSF-50, "Principal Investigator/Proposal File and Associated Records", 63 Federal Register 267 (January 5, 1998), and NSF-51, "Reviewer/Proposal File and Associated Records", 63 Federal Register 268 (January 5, 1998).

02 INFORMATION ABOUT PRINCIPAL INVESTIGATORS/PROJECT DIRECTORS(PI/PD) and co-PRINCIPAL INVESTIGATORS/co-PROJECT DIRECTORS

Submit only ONE copy of this form **for each PI/PD and co-PI/PD** identified on the proposal. The form(s) should be attached to the original proposal as specified in GPG Section II.C.a. Submission of this information is voluntary and is not a precondition of award. This information will not be disclosed to external peer reviewers. ***DO NOT INCLUDE THIS FORM WITH ANY OF THE OTHER COPIES OF YOUR PROPOSAL AS THIS MAY COMPROMISE THE CONFIDENTIALITY OF THE INFORMATION.***

PI/PD Name: Katherine Silliman

Gender: ☐ Male ☒ Female

Ethnicity: (Choose one response) ☐ Hispanic or Latino ☒ Not Hispanic or Latino

Race: (Select one or more)
- ☐ American Indian or Alaska Native
- ☐ Asian
- ☐ Black or African American
- ☐ Native Hawaiian or Other Pacific Islander
- ☒ White

Disability Status: (Select one or more)
- ☐ Hearing Impairment
- ☐ Visual Impairment
- ☐ Mobility/Orthopedic Impairment
- ☐ Other
- ☒ None

Citizenship: (Choose one) ☒ U.S. Citizen ☐ Permanent Resident ☐ Other non-U.S. Citizen

Check here if you do not wish to provide any or all of the above information (excluding PI/PD name): ☐

REQUIRED: Check here if you are currently serving (or have previously served) as a PI, co-PI or PD on any federally funded project ☐

Ethnicity Definition:
Hispanic or Latino. A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race.
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Asian. A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.
Black or African American. A person having origins in any of the black racial groups of Africa.
Native Hawaiian or Other Pacific Islander. A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.
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Collection of this information is authorized by the NSF Act of 1950, as amended, 42 U.S.C. 1861, et seq. Demographic data allows NSF to gauge whether our programs and other opportunities in science and technology are fairly reaching and benefiting everyone regardless of demographic category; to ensure that those in under-represented groups have the same knowledge of and access to programs and other research and educational oppurtunities; and to assess involvement of international investigators in work supported by NSF. The information may be disclosed to government contractors, experts, volunteers and researchers to complete assigned work; and to other government agencies in order to coordinate and assess programs. The information may be added to the Reviewer file and used to select potential candidates to serve as peer reviewers or advisory committee members. See Systems of Records, NSF-50, "Principal Investigator/Proposal File and Associated Records", 63 Federal Register 267 (January 5, 1998), and NSF-51, "Reviewer/Proposal File and Associated Records", 63 Federal Register 268 (January 5, 1998).

List of Suggested Reviewers or Reviewers Not To Include (optional)

SUGGESTED REVIEWERS:
Not Listed

REVIEWERS NOT TO INCLUDE:
Not Listed

COVER SHEET FOR PROPOSAL TO THE NATIONAL SCIENCE FOUNDATION

PROGRAM ANNOUNCEMENT/SOLICITATION NO./CLOSING DATE/if not in response to a program announcement/solicitation enter NSF 15-1

NSF 13-568 **10/08/15**

FOR NSF USE ONLY

NSF PROPOSAL NUMBER

FOR CONSIDERATION BY NSF ORGANIZATION UNIT(S) (Indicate the most specific unit known, i.e. program, division, etc.)

DEB - Evolutionary Genetics

DATE RECEIVED	NUMBER OF COPIES	DIVISION ASSIGNED	FUND CODE	DUNS# (Data Universal Numbering System)	FILE LOCATION
				005421136	

EMPLOYER IDENTIFICATION NUMBER (EIN) OR TAXPAYER IDENTIFICATION NUMBER (TIN)

362177139

SHOW PREVIOUS AWARD NO. IF THIS IS
☐ A RENEWAL
☐ AN ACCOMPLISHMENT-BASED RENEWAL

IS THIS PROPOSAL BEING SUBMITTED TO ANOTHER FEDERAL AGENCY? YES ☐ NO ☒ IF YES, LIST ACRONYM(S)

NAME OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE

University of Chicago

AWARDEE ORGANIZATION CODE (IF KNOWN)

0017749000

ADDRESS OF AWARDEE ORGANIZATION, INCLUDING 9 DIGIT ZIP CODE

5801 South Ellis Avenue
Chicago, IL 60637-5418

NAME OF PRIMARY PLACE OF PERF

University of Chicago

ADDRESS OF PRIMARY PLACE OF PERF, INCLUDING 9 DIGIT ZIP CODE

University of Chicago
1025 E. 57th St
Chicago ,IL ,606375418 ,US.

IS AWARDEE ORGANIZATION (Check All That Apply) (See GPG II.C For Definitions)
☐ SMALL BUSINESS ☐ MINORITY BUSINESS ☐ FOR-PROFIT ORGANIZATION ☐ WOMAN-OWNED BUSINESS

☐ IF THIS IS A PRELIMINARY PROPOSAL THEN CHECK HERE

TITLE OF PROPOSED PROJECT **DISSERTATION RESEARCH: A genomic and experimental characterization of local adaptation**

REQUESTED AMOUNT	PROPOSED DURATION (1-60 MONTHS)	REQUESTED STARTING DATE	SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE
$ **18,529**	**24** months	**06/01/16**	

THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW

☐ BEGINNING INVESTIGATOR (GPG I.G.2)
☐ DISCLOSURE OF LOBBYING ACTIVITIES (GPG II.C.1.e)
☐ PROPRIETARY & PRIVILEGED INFORMATION (GPG I.D, II.C.1.d)
☐ HISTORIC PLACES (GPG II.C.2.j)
☐ VERTEBRATE ANIMALS (GPG II.D.6) IACUC App. Date _____
PHS Animal Welfare Assurance Number _____
☒ FUNDING MECHANISM **Research - other than RAPID or EAGER**

☐ HUMAN SUBJECTS (GPG II.D.7) Human Subjects Assurance Number _____
Exemption Subsection _____ or IRB App. Date _____
☐ INTERNATIONAL ACTIVITIES: COUNTRY/COUNTRIES INVOLVED (GPG II.C.2.j)

☒ COLLABORATIVE STATUS
Not a collaborative proposal

PI/PD DEPARTMENT
Ecology & Evolution

PI/PD POSTAL ADDRESS
1101 E. 57th Street

Chicago, IL 606371404
United States

PI/PD FAX NUMBER
773-702-9740

NAMES (TYPED)	High Degree	Yr of Degree	Telephone Number	Email Address
PI/PD NAME **Catherine A Pfister**	**PhD**	**1993**	**773-834-0071**	**cpfister@uchicago.edu**
CO-PI/PD **Katherine Silliman**	**BS**	**2013**	**773-702-8669**	**ksilliman@uchicago.edu**
CO-PI/PD				
CO-PI/PD				
CO-PI/PD				

CERTIFICATION PAGE

Certification for Authorized Organizational Representative (or Equivalent) or Individual Applicant

By electronically signing and submitting this proposal, the Authorized Organizational Representative (AOR) or Individual Applicant is: (1) certifying that statements made herein are true and complete to the best of his/her knowledge; and (2) agreeing to accept the obligation to comply with NSF award terms and conditions if an award is made as a result of this application. Further, the applicant is hereby providing certifications regarding conflict of interest (when applicable), drug-free workplace, debarment and suspension, lobbying activities (see below), nondiscrimination, flood hazard insurance (when applicable), responsible conduct of research, organizational support, Federal tax obligations, unpaid Federal tax liability, and criminal convictions as set forth in the NSF Proposal & Award Policies & Procedures Guide,Part I: the Grant Proposal Guide (GPG). Willful provision of false information in this application and its supporting documents or in reports required under an ensuing award is a criminal offense (U.S. Code, Title 18, Section 1001).

Certification Regarding Conflict of Interest

The AOR is required to complete certifications stating that the organization has implemented and is enforcing a written policy on conflicts of interest (COI), consistent with the provisions of AAG Chapter IV.A.; that, to the best of his/her knowledge, all financial disclosures required by the conflict of interest policy were made; and that conflicts of interest, if any, were, or prior to the organization's expenditure of any funds under the award, will be, satisfactorily managed, reduced or eliminated in accordance with the organization's conflict of interest policy. Conflicts that cannot be satisfactorily managed, reduced or eliminated and research that proceeds without the imposition of conditions or restrictions when a conflict of interest exists, must be disclosed to NSF via use of the Notifications and Requests Module in FastLane.

Drug Free Work Place Certification

By electronically signing the Certification Pages, the Authorized Organizational Representative (or equivalent), is providing the Drug Free Work Place Certification contained in Exhibit II-3 of the Grant Proposal Guide.

Debarment and Suspension Certification

(If answer "yes", please provide explanation.)

Is the organization or its principals presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency? Yes ☐ No ☒

By electronically signing the Certification Pages, the Authorized Organizational Representative (or equivalent) or Individual Applicant is providing the Debarment and Suspension Certification contained in Exhibit II-4 of the Grant Proposal Guide.

Certification Regarding Lobbying

This certification is required for an award of a Federal contract, grant, or cooperative agreement exceeding $100,000 and for an award of a Federal loan or a commitment providing for the United States to insure or guarantee a loan exceeding $150,000.

Certification for Contracts, Grants, Loans and Cooperative Agreements

The undersigned certifies, to the best of his or her knowledge and belief, that:
(1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.
(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, ''Disclosure of Lobbying Activities,'' in accordance with its instructions.
(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements and that all subrecipients shall certify and disclose accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by section 1352, Title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

Certification Regarding Nondiscrimination

By electronically signing the Certification Pages, the Authorized Organizational Representative (or equivalent) is providing the Certification Regarding Nondiscrimination contained in Exhibit II-6 of the Grant Proposal Guide.

Certification Regarding Flood Hazard Insurance

Two sections of the National Flood Insurance Act of 1968 (42 USC §4012a and §4106) bar Federal agencies from giving financial assistance for acquisition or construction purposes in any area identified by the Federal Emergency Management Agency (FEMA) as having special flood hazards unless the:
(1) community in which that area is located participates in the national flood insurance program; and
(2) building (and any related equipment) is covered by adequate flood insurance.

By electronically signing the Certification Pages, the Authorized Organizational Representative (or equivalent) or Individual Applicant located in FEMA-designated special flood hazard areas is certifying that adequate flood insurance has been or will be obtained in the following situations:
(1) for NSF grants for the construction of a building or facility, regardless of the dollar amount of the grant; and
(2) for other NSF grants when more than $25,000 has been budgeted in the proposal for repair, alteration or improvement (construction) of a building or facility.

Certification Regarding Responsible Conduct of Research (RCR)
(This certification is not applicable to proposals for conferences, symposia, and workshops.)

By electronically signing the Certification Pages, the Authorized Organizational Representative is certifying that, in accordance with the NSF Proposal & Award Policies & Procedures Guide, Part II, Award & Administration Guide (AAG) Chapter IV.B., the institution has a plan in place to provide appropriate training and oversight in the responsible and ethical conduct of research to undergraduates, graduate students and postdoctoral researchers who will be supported by NSF to conduct research. The AOR shall require that the language of this certification be included in any award documents for all subawards at all tiers.

CERTIFICATION PAGE - CONTINUED

Certification Regarding Organizational Support

By electronically signing the Certification Pages, the Authorized Organizational Representative (or equivalent) is certifying that there is organizational support for the proposal as required by Section 526 of the America COMPETES Reauthorization Act of 2010. This support extends to the portion of the proposal developed to satisfy the Broader Impacts Review Criterion as well as the Intellectual Merit Review Criterion, and any additional review criteria specified in the solicitation. Organizational support will be made available, as described in the proposal, in order to address the broader impacts and intellectual merit activities to be undertaken.

Certification Regarding Federal Tax Obligations

When the proposal exceeds $5,000,000, the Authorized Organizational Representative (or equivalent) is required to complete the following certification regarding Federal tax obligations. By electronically signing the Certification pages, the Authorized Organizational Representative is certifying that, to the best of their knowledge and belief, the proposing organization:
(1) has filed all Federal tax returns required during the three years preceding this certification;
(2) has not been convicted of a criminal offense under the Internal Revenue Code of 1986; and
(3) has not, more than 90 days prior to this certification, been notified of any unpaid Federal tax assessment for which the liability remains unsatisfied, unless the assessment is the subject of an installment agreement or offer in compromise that has been approved by the Internal Revenue Service and is not in default, or the assessment is the subject of a non-frivolous administrative or judicial proceeding.

Certification Regarding Unpaid Federal Tax Liability

When the proposing organization is a corporation, the Authorized Organizational Representative (or equivalent) is required to complete the following certification regarding Federal Tax Liability:

By electronically signing the Certification Pages, the Authorized Organizational Representative (or equivalent) is certifying that the corporation has no unpaid Federal tax liability that has been assessed, for which all judicial and administrative remedies have been exhausted or lapsed, and that is not being paid in a timely manner pursuant to an agreement with the authority responsible for collecting the tax liability.

Certification Regarding Criminal Convictions

When the proposing organization is a corporation, the Authorized Organizational Representative (or equivalent) is required to complete the following certification regarding Criminal Convictions:

By electronically signing the Certification Pages, the Authorized Organizational Representative (or equivalent) is certifying that the corporation has not been convicted of a felony criminal violation under any Federal law within the 24 months preceding the date on which the certification is signed.

AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	SIGNATURE	DATE
NAME		

TELEPHONE NUMBER	EMAIL ADDRESS	FAX NUMBER

Direct for Biological Sciences
Division of Environmental Biology
Evolutionary Genetics

Proposal Classification Form
PI: Pfister, Catherine

CATEGORY I: INVESTIGATOR STATUS (Select ONE)

- ☐ Beginning Investigator - No previous Federal support as PI or Co-PI, excluding fellowships, dissertations, planning grants, etc.
- ☐ Prior Federal support only
- ☐ Current Federal support only
- ☑ Current & prior Federal support

CATEGORY II: FIELDS OF SCIENCE OTHER THAN BIOLOGY INVOLVED IN THIS RESEARCH (Select 1 to 3)

- ☐ Astronomy
- ☐ Chemistry
- ☐ Computer Science
- ☐ Geosciences
- ☐ Engineering
- ☐ Mathematics
- ☐ Physics
- ☐ Psychology
- ☐ Social Sciences
- ☑ None of the Above

CATEGORY III: SUBSTANTIVE AREA (Select 1 to 4)

- ☐ BIOGEOGRAPHY
- ☐ Island Biogeography
- ☐ Historical/ Evolutionary Biogeography
- ☐ Phylogeography
- ☐ Methods/Theory
- ☐ CHROMOSOME STUDIES
- ☐ Chromosome Evolution
- ☐ Chromosome Number
- ☐ Mutation
- ☐ Mitosis and Meiosis
- ☐ COMMUNITY ECOLOGY
- ☐ Community Analysis
- ☐ Community Structure
- ☐ Community Stability
- ☐ Succession
- ☐ Experimental Microcosms/ Mesocosms
- ☐ Disturbance
- ☐ Patch Dynamics
- ☐ Food Webs/ Trophic Structure
- ☐ Keystone Species
- ☐ COMPUTATIONAL BIOLOGY
- ☑ CONSERVATION & RESTORATION BIOLOGY
- ☐ DATABASES
- ☐ ECOSYSTEMS LEVEL
- ☐ Physical Structure
- ☐ Decomposition
- ☐ Biogeochemistry
- ☐ Limnology/Hydrology
- ☐ Climate/Microclimate
- ☐ Whole-System Analysis
- ☐ Productivity/Biomass
- ☐ System Energetics
- ☐ Landscape Dynamics
- ☐ Chemical & Biochemical Control
- ☐ Global Change
- ☐ Climate Change
- ☐ Regional Studies
- ☐ Global Studies
- ☐ Forestry
- ☐ Resource Management (Wildlife, Fisheries, Range, Other)
- ☐ Agricultural Ecology
- ☐ EXTREMOPHILES
- ☐ GENOMICS (Genome sequence, organization, function)
  - ☐ Viral
  - ☐ Microbial
  - ☐ Fungal
  - ☐ Plant
  - ☐ Animal
- ☐ MARINE MAMMALS
- ☐ MOLECULAR APPROACHES
- ☐ Molecular Evolution
- ☐ Methodology/Theory
- ☐ Isozymes/ Electrophoresis
- ☑ Nucleic Acid Analysis (general)
  - ☐ Restriction Enzymes
  - ☐ Nucleotide Sequencing
  - ☐ Nuclear DNA
  - ☐ Mitochondrial DNA
  - ☐ Chloroplast DNA
  - ☐ RNA Analysis
  - ☐ DNA Hybridization
  - ☐ Recombinant DNA
- ☐ Amino Acid Sequencing
- ☐ Gene/Genome Mapping
- ☐ Natural Products
- ☐ Serology/Immunology
- ☐ PALEONTOLOGY
- ☐ Floristic
- ☐ Faunistic
- ☐ Paleoecology
- ☐ Biostratigraphy
- ☐ Palynology
- ☐ Micropaleontology
- ☐ Paleoclimatology
- ☐ Archeozoic
- ☐ Paleozoic
- ☐ Mesozoic

- ☐ Cenozoic
- ☐ POPULATION DYNAMICS & LIFE HISTORY
- ☐ Demography/ Life History
- ☐ Population Cycles
- ☐ Distribution/Patchiness/ Marginal Populations
- ☐ Population Regulation
- ☐ Intraspecific Competition
- ☐ Reproductive Strategies
- ☐ Gender Allocation
- ☑ Metapopulations
- ☐ Extinction
- ☐ POPULATION GENETICS & BREEDING SYSTEMS
- ☐ Variation
- ☑ Microevolution
- ☐ Speciation
- ☐ Hybridization
- ☐ Inbreeding/Outbreeding
- ☐ Gene Flow Measurement
- ☐ Inheritance/Heritability
- ☐ Quantitative Genetics/ QTL Analysis
- ☐ Ecological Genetics
- ☐ Gender Ratios
- ☐ Apomixis/ Parthenogenesis
- ☐ Vegetative Reproduction
- ☐ SPECIES INTERACTIONS
- ☐ Predation
- ☐ Herbivory
- ☐ Omnivory
- ☐ Interspecific Competition
- ☐ Niche Relationships/ Resource Partititioning
- ☐ Pollination/ Seed Dispersal
- ☐ Parasitism
- ☐ Mutualism/ Commensalism
- ☐ Plant/Fungal/ Microbial Interactions
- ☐ Mimicry
- ☐ Animal Pathology
- ☐ Plant Pathology
- ☐ Coevolution
- ☐ Biological Control
- ☐ STATISTICS & MODELING
- ☐ Methods/ Instrumentation/ Software
- ☐ Modeling (general)
- ☐ Statistics (general)
  - ☐ Multivariate Methods
  - ☐ Spatial Statistics & Spatial Modeling
  - ☐ Sampling Design & Analysis
  - ☐ Experimental Design & Analysis
- ☐ SYSTEMATICS
- ☐ Taxonomy/Classification
- ☐ Nomenclature
- ☐ Monograph/Revision
- ☐ Phylogenetics
- ☐ Phenetics/Cladistics/ Numerical Taxonomy
- ☐ Macroevolution
- ☐ NONE OF THE ABOVE

CATEGORY IV: INFRASTRUCTURE (Select 1 to 3)

- ☐ COLLECTIONS/STOCK CULTURES
- ☐ Natural History Collections
- ☑ DATABASES
- ☐ FACILITIES
- ☑ Controlled Environment Facilities
- ☐ Field Stations
  - ☐ Field Facility Structure
  - ☐ Field Facility Equipment
- ☐ LTER Site
- ☐ INDUSTRY PARTICIPATION
- ☑ Technique Development
- ☐ TRACKING SYSTEMS
- ☐ Geographic Information Systems
- ☐ Remote Sensing
- ☐ NONE OF THE ABOVE

CATEGORY V: HABITAT (Select 1 to 2)

TERRESTRIAL HABITATS

- ☐ GENERAL TERRESTRIAL
- ☐ TUNDRA
- ☐ BOREAL FOREST
- ☐ TEMPERATE
  - ☐ Deciduous Forest
  - ☐ Coniferous Forest
  - ☐ Rain Forest
  - ☐ Mixed Forest
  - ☐ Prairie/Grasslands
  - ☐ Desert
- ☐ SUBTROPICAL
  - ☐ Rain Forest
  - ☐ Seasonal Forest
  - ☐ Savanna
  - ☐ Thornwoods
  - ☐ Deciduous Forest
  - ☐ Coniferous Forest
  - ☐ Desert
- ☐ TROPICAL
  - ☐ Rain Forest
  - ☐ Seasonal Forest
  - ☐ Savanna
  - ☐ Thornwoods
  - ☐ Deciduous Forest
  - ☐ Coniferous Forest
  - ☐ Desert
- ☐ CHAPPARAL/ SCLEROPHYLL/ SHRUBLANDS
- ☐ ALPINE
- ☐ MONTANE
- ☐ CLOUD FOREST
- ☐ RIPARIAN ZONES
- ☐ ISLANDS (except Barrier Islands)
- ☐ BEACHES/ DUNES/ SHORES/ BARRIER ISLANDS
- ☐ CAVES/ ROCK OUTCROPS/ CLIFFS
- ☐ CROPLANDS/ FALLOW FIELDS/ PASTURES
- ☐ URBAN/SUBURBAN
- ☐ SUBTERRANEAN/ SOIL/ SEDIMENTS
- ☐ EXTREME TERRESTRIAL ENVIRONMENT
- ☐ AERIAL

AQUATIC HABITATS

- ☐ GENERAL AQUATIC
- ☐ FRESHWATER
  - ☐ Wetlands/Bogs/Swamps
  - ☐ Lakes/Ponds
  - ☐ Rivers/Streams
  - ☐ Reservoirs
- ☐ MARINE
  - ☐ Open Ocean/Continental Shelf
  - ☐ Bathyal
  - ☐ Abyssal
  - ☑ Estuarine
  - ☐ Intertidal/Tidal/Coastal
  - ☐ Coral Reef
  - ☐ HYPERSALINE
- ☐ EXTREME AQUATIC ENVIRONMENT
- ☐ CAVES/ ROCK OUTCROPS/ CLIFFS
- ☐ MANGROVES
- ☐ SUBSURFACE WATERS/ SPRINGS
- ☐ EPHEMERAL POOLS & STREAMS
- ☐ MICROPOOLS (Pitcher Plants, Tree Holes, Other)

MAN-MADE ENVIRONMENTS

- ☐ LABORATORY
- ☐ THEORETICAL SYSTEMS
- ☐ OTHER ARTIFICIAL SYSTEMS

NOT APPLICABLE

- ☐ NOT APPLICABLE

CATEGORY VI: GEOGRAPHIC AREA OF THE RESEARCH (Select 1 to 2)

- ☐ WORLDWIDE
- ☐ NORTH AMERICA
  - ☐ United States
    - ☐ Northeast US (CT, MA, ME, NH, NJ, NY, PA, RI, VT)
    - ☐ Northcentral US (IA, IL, IN, MI, MN, ND, NE, OH, SD, WI)
    - ☑ Northwest US (ID, MT, OR, WA, WY)
    - ☐ Southeast US (DC, DE, FL, GA, MD, NC, SC, WV, VA)
    - ☐ Southcentral US (AL, AR, KS, KY, LA, MO, MS, OK, TN, TX)
    - ☐ Southwest US (AZ, CA, CO, NM, NV, UT)
    - ☐ Alaska
    - ☐ Hawaii
    - ☐ Puerto Rico
  - ☑ Canada
  - ☐ Mexico
- ☐ CENTRAL AMERICA (Mainland)
  - ☐ Caribbean Islands
  - ☐ Bermuda/Bahamas
- ☐ SOUTH AMERICA
  - ☐ Eastern South America (Guyana, Fr. Guiana, Suriname, Brazil)
  - ☐ Northern South America (Colombia, Venezuela)
  - ☐ Southern South America (Chile, Argentina, Uruguay, Paraguay)
  - ☐ Western South America (Ecuador, Peru, Bolivia)
- ☐ EUROPE
  - ☐ Eastern Europe
  - ☐ Russia
  - ☐ Scandinavia
  - ☐ Western Europe
- ☐ ASIA
  - ☐ Central Asia
  - ☐ Far East
  - ☐ Middle East
  - ☐ Siberia
  - ☐ South Asia
  - ☐ Southeast Asia
- ☐ AFRICA
  - ☐ North Africa
  - ☐ African South of the Sahara
    - ☐ East Africa
    - ☐ Madagascar
    - ☐ South Africa
    - ☐ West Africa
- ☐ AUSTRALASIA
  - ☐ Australia
  - ☐ New Zealand
  - ☐ Pacific Islands
- ☐ ANTARCTICA
- ☐ ARCTIC
- ☐ ATLANTIC OCEAN
- ☐ PACIFIC OCEAN
- ☐ INDIAN OCEAN
- ☐ OTHER REGIONS (Not defined)
- ☐ NOT APPLICABLE

CATEGORY VII: CLASSIFICATION OF ORGANISMS (Select 1 to 4)

- ☐ VIRUSES
  - ☐ Bacterial
  - ☐ Plant
  - ☐ Animal
- ☐ PROKARYOTES
  - ☐ Archaea
  - ☐ Cyanobacteria
  - ☐ Bacteria
  - ☐ Noncultured Organisms
- ☐ PROTISTA (PROTOZOA)
  - ☐ Amoebae
  - ☐ Apicomplexa
  - ☐ Ciliophora
  - ☐ Flagellates
  - ☐ Foraminifera
  - ☐ Microspora
  - ☐ Radiolaria
- ☐ FUNGI
  - ☐ Ascomycota
  - ☐ Basidiomycota
  - ☐ Chytridiomycota
  - ☐ Mitosporic Fungi
  - ☐ Oomycota
  - ☐ Zygomycota
- ☐ LICHENS
- ☐ SLIME MOLDS
- ☐ ALGAE
  - ☐ Bacillariophyta (Diatoms)
  - ☐ Charophyta
  - ☐ Chlorophyta
  - ☐ Chrysophyta
  - ☐ Dinoflagellata
  - ☐ Euglenoids
  - ☐ Phaeophyta
  - ☐ Rhodophyta
- ☐ PLANTS
  - ☐ NON-VASCULAR PLANTS
    - ☐ BRYOPHYTA
      - ☐ Anthocerotae (Hornworts)
      - ☐ Hepaticae (Liverworts)
      - ☐ Musci (Mosses)
  - ☐ VASCULAR PLANTS
    - ☐ FERNS & FERN ALLIES
    - ☐ GYMNOSPERMS
      - ☐ Coniferales (Conifers)

- ☐ Cycadales (Cycads)
- ☐ Ginkgoales (Ginkgo)
- ☐ Gnetales (Gnetophytes)
- ☐ ANGIOSPERMS
- ☐ Monocots
- ☐ Arecaceae (Palmae)
- ☐ Cyperaceae
- ☐ Liliaceae
- ☐ Orchidaceae
- ☐ Poaceae (Graminae)
- ☐ Dicots
- ☐ Apiaceae (Umbelliferae)
- ☐ Asteraceae (Compositae)
- ☐ Brassicaceae (Cruciferae)
- ☐ Fabaceae (Leguminosae)
- ☐ Lamiaceae (Labiatae)
- ☐ Rosaceae
- ☐ Solanaceae

☐ ANIMALS

- ☐ INVERTEBRATES
- ☐ MESOZOA/PLACOZOA
- ☐ PORIFERA (Sponges)
- ☐ CNIDARIA
- ☐ Hydrozoa (Hydra, etc.)
- ☐ Scyphozoa (Jellyfish)
- ☐ Anthozoa (Corals, Sea Anemones)
- ☐ CTENOPHORA (Comb Jellies)
- ☐ PLATYHELMINTHES (Flatworms)
- ☐ Turbellaria (Planarians)
- ☐ Trematoda (Flukes)
- ☐ Cestoda (Tapeworms)
- ☐ Monogenea (Flukes)
- ☐ GNATHOSTOMULIDA
- ☐ NEMERTINEA (Rynchocoela) (Ribbon Worms)
- ☐ ENTOPROCTA (Bryozoa) (Plant-like Animals)
- ☐ ASCHELMINTHES
- ☐ Gastrotricha
- ☐ Kinorhyncha
- ☐ Loricifera
- ☐ Nematoda (Roundworms)
- ☐ Nematomorpha (Horsehair Worms)
- ☐ Rotifera (Rotatoria)
- ☐ ACANTHOCEPHALA (Spiny-headed Worms)
- ☐ PRIAPULOIDEA
- ☐ BRYOZOA (Ectoprocta) (Plant-like Animals)
- ☐ PHORONIDEA (Lophophorates)
- ☐ BRACHIOPODA (Lamp Shells)
- ☐ MOLLUSCA
- ☐ Monoplacophora
- ☐ Aplacophora (Solenogasters)
- ☐ Polyplacophora (Chitons)
- ☐ Scaphopoda (Tooth Shells)
- ☐ Gastropoda (Snails, Slugs, Limpets)
- ☑ Pelecypoda (Bivalvia) (Clams, Mussels, Oysters, Scallops)
- ☐ Cephalopoda (Squid, Octopus, Nautilus)
- ☐ ANNELIDA (Segmented Worms)
- ☐ Polychaeta (Parapodial Worms)
- ☐ Oligochaeta (Earthworms)
- ☐ Hirudinida (Leeches)
- ☐ POGONOPHORA (Beard Worms)
- ☐ SIPUNCULOIDEA (Peanut Worms)
- ☐ ECHIUROIDEA (Spoon Worms)
- ☐ ARTHROPODA
- ☐ Cheliceriformes
- ☐ Merostomata (Horseshoe Crabs)
- ☐ Pycnogonida (Sea Spiders)
- ☐ Scorpionida (Scorpions)
- ☐ Araneae (True Spiders)
- ☐ Pseudoscorpionida (Pseudoscorpions)
- ☐ Acarina (Free-living Mites)
- ☐ Parasitiformes (Parasitic Ticks & Mites)
- ☐ Crustacea
- ☐ Branchiopoda (Fairy Shrimp, Water Flea)
- ☐ Ostracoda (Sea Lice)
- ☐ Copepoda
- ☐ Cirripedia (Barnacles)
- ☐ Amphipoda (Skeleton Shrimp, Whale Lice, Freshwater Shrimp)
- ☐ Isopoda (Wood Lice, Pillbugs)
- ☐ Decapoda (Lobster, Crayfish, Crabs, Shrimp)
- ☐ Hexapoda (Insecta) (Insects)
- ☐ Apterygota (Springtails, Silverfish, etc.)
- ☐ Odonata (Dragonflies, Damselflies)
- ☐ Ephemeroptera (Mayflies)
- ☐ Orthoptera (Grasshoppers, Crickets)
- ☐ Dictyoptera (Cockroaches, Mantids, Phasmids)
- ☐ Isoptera (Termites)
- ☐ Plecoptera (Stoneflies)
- ☐ Phthiraptera (Mallophaga & Anoplura) (Lice)
- ☐ Hemiptera (including Heteroptera) (True Bugs)
- ☐ Homoptera (Cicadas, Scale Insects, Leafhoppers)
- ☐ Thysanoptera (Thrips)
- ☐ Neuroptera (Lacewings, Dobsonflies, Snakeflies)
- ☐ Trichoptera (Caddisflies)
- ☐ Lepidoptera (Moths, Butterflies)
- ☐ Diptera (Flies, Mosquitoes)
- ☐ Siphonaptera (Fleas)
- ☐ Coleoptera (Beetles)
- ☐ Hymenoptera (Ants, Bees, Wasps, Sawflies)
- ☐ Chilopoda (Centipedes)
- ☐ Diplopoda (Millipedes)
- ☐ Pauropoda
- ☐ Symphyta (Symphyla)
- ☐ PENTASTOMIDA (Linguatulida) (Tongue Worms)
- ☐ TARDIGRADA (Tardigrades, Water Bears)
- ☐ ONYCHOPHORA (Peripatus)
- ☐ CHAETOGNATHA (Arrow Worms)
- ☐ ECHINODERMATA
- ☐ Crinoidea (Sea Lilies, Feather Stars)
- ☐ Asteroidea (Starfish, Sea Stars)
- ☐ Ophiuroidea (Brittle Stars, Serpent Stars)
- ☐ Echinoidea (Sea Urchins, Sand Dollars)
- ☐ Holothuroidea (Sea Cucumbers)
- ☐ HEMICHORDATA (Acorn Worms, Pterobranchs)
- ☐ UROCHORDATA (Tunicata) (Tunicates, Sea Squirts, Salps, Ascideans)
- ☐ CEPHALOCHORDATA (Amphioxus/Lancelet)
- ☐ VERTEBRATES
- ☐ AGNATHA (Hagfish, Lamprey)
- ☐ FISHES
- ☐ Chondrichthyes (Cartilaginous Fishes) (Sharks, Rays, Ratfish)
- ☐ Osteichthyes (Bony Fishes)
- ☐ AMPHIBIA
- ☐ Anura (Frogs, Toads)
- ☐ Urodela (Salamanders, Newts)
- ☐ Gymnophiona (Apoda) (Caecilians)
- ☐ REPTILIA
- ☐ Chelonia (Turtles, Tortoises)
- ☐ Serpentes (Snakes)
- ☐ Sauria (Lizards)
- ☐ Crocodylia (Crocodilians)
- ☐ AVES (Birds)
- ☐ Passeriformes (Passerines)
- ☐ MAMMALIA
- ☐ Monotremata (Platypus, Echidna)
- ☐ Marsupalia (Marsupials)
- ☐ Eutheria (Placentals)
- ☐ Insectivora (Hedgehogs, Moles, Shrews, Tenrec, etc.)
- ☐ Chiroptera (Bats)
- ☐ Primates
- ☐ Humans
- ☐ Rodentia
- ☐ Lagomorphs (Rabbits, Hares, Pikas)
- ☐ Carnivora (Bears, Canids, Felids, Mustelids, Viverrids, Hyena, Procyonids)
- ☐ Perissodactyla (Odd-toed Ungulates) (Horses, Rhinos, Tapirs, etc.)

☐ Artiodactyla (Even-toed Ungulates) (Cattle, Sheep, Deer, Pigs, etc.) ☐ Marine Mammals (Seals, Walrus, Whales, Otters, Dolphins, Porpoises)	☐ TRANSGENIC ORGANISMS ☐ FOSSIL OR EXTINCT ORGANISMS	☐ NO ORGANISMS

CATEGORY VIII: MODEL ORGANISM (Select ONE)		
☑ NO MODEL ORGANISM MODEL ORGANISM (Choose from the list)	☐ Escherichia coli ☐ Mouse-Ear Cress (Arabidopsis thaliana)	☐ Fruitfly (Drosophila melanogaster)

PROJECT SUMMARY

Overview:

Using an ecologically and commercially important estuarine mollusc as a model system, Co-PI Silliman's dissertation aims to determine the spatial scale of adaptive differentiation with gene flow and identify candidate loci under selection. In her recent work, Co-PI Silliman has approached these objectives through population genetic analysis of genotype-by-sequencing (GBS) loci and a common garden experiment with Olympia oyster populations from Puget Sound, WA. If funded, the proposed DDIG research will expand on these studies by testing for adaptive differentiation among demographically distinct populations from California, Oregon, Washington, and British Columbia. Offspring of wild oysters will be raised under common conditions and studied for differences in fitness proxy measurements and response to induced stress. Transcriptomics will be used to characterize cryptic phenotypic variation in gene expression and identify loci under purifying selection in early life stages.

Intellectual Merit :

Like many other coastal species, the Olympia oyster (Ostrea lurida) is found across a wide range of environmental conditions. Whether such tolerances are primarily due to plasticity, local adaptation, or balanced polymorphism is largely unknown. Understanding the processes that cause populations to diverge genetically and phenotypically is crucial to predicting how species will respond to rapid global environmental change. The proposed research will quantify these processes and investigate the interplay between adaptive differentiation and high dispersal. Despite the influx of genomic information across the Tree of Life, functional annotation is still highly dependent on research in disparate model systems. The candidate adaptive loci identified through environmental stress experiments will improve hypotheses of gene function in nonmodel marine invertebrates.

Broader Impacts :

Some of the preliminary results introduced here have already been presented at a professional meeting and all future results will be similarly disseminated and published in peer-reviewed journals. Since the early 20th century, the Olympia oyster has faced extreme commercial exploitation and, more recently, potential risks from ocean acidification. In addition to sharing of results with the academic community, this research thus involves actively working with the conservation community. Co-PI Silliman has developed a network of oyster farmers, government resource managers, and NGOs with stakes in the future success of this species. In collaboration with Puget Sound Restoration Fund (PSRF), a workshop in the spring of 2018 will be taught covering local adaptation and implications of this research for ongoing restoration and management efforts.

The Co-PI has a passion for mentorship and engaging young women in science that has developed through her work with Project Exploration's Sisters4Science and the University of Chicago Multicultural Graduate Community's mentorship program. During the proposed field 2016 season in Manchester, WA, the Co-PI will have an intern through PSRF to participate in experimental methods, data collection, analysis, and manuscript preparation. This opportunity will provide an undergraduate with invaluable research experience and skills.

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For font size and page formatting specifications, see GPG section II.B.2.

*Proposers may select any numbering mechanism for the proposal. The entire proposal however, must be paginated. Complete both columns only if the proposal is numbered consecutively.

DISSERTATION RESEARCH: A genomic and experimental characterization of local adaptation

I. Introduction

A) Background. Comprehending the interplay between neutral and adaptive processes that cause genetic differentiation among populations has long been a major goal of evolutionary biologists. One fundamental concept that arose from early theoretical population genetic models is that diversifying effects of natural selection on different populations can be opposed by homogenizing gene flow (Wakeley 2005, Wright 1930). Historically, strong population structure has been considered unlikely in species with an extended dispersal phase, such as certain seed-dispersing plants or aquatic species with planktonic larvae (Grosberg & Cunningham 2001, Levin 1984). Direct measurement of dispersal distance is challenging or impossible for many such species, leading to the development and use of marker-based gene flow studies (Ashley 2010). Neutral molecular markers in particular have increasingly been used for estimates of demographic population structure. Even as estimates of connectivity have become available for more taxa, the relative role of gene flow as an evolutionary force has remained controversial (Ellestrand 2014). Understanding the processes that cause populations to diverge genetically and phenotypically is crucial to predicting the fate of species in a rapidly changing environment.

While neutral markers can be used to identify the geographic structure of subpopulations and estimate the connectivity between them, they do not give insight into the scale or magnitude of adaptive divergence (Hellberg 2009). Natural environments exhibit spatial heterogeneity in both abiotic and biotic factors, which may lead to populations of a species evolving traits in response to local conditions that give a fitness advantage in their native habitat over foreign genotypes. Whether this adaptive differentiation is due to true local adaptation or balanced polymorphism- where strong purifying selection occurs each generation following dispersal- depends on the scale of dispersal relative to the scale of the selective gradient (Marshall et al. 2010, Schmidt & Rand 2001). For balanced polymorphism, populations connected by high gene flow may only show genetic differentiation at loci under selection. In either case, experimental evidence from common garden and reciprocal transplant experiments indicates that adaptive differentiation can occur at a range of scales, even in some broadcast spawning marine species (Sanford & Kelly 2011, Sotka et al. 2004).

When experimental assessment of local adaptation is not feasible, local adaptation can be detected by comparing allelic differences between populations at a few loci hypothesized to be of adaptive importance (Bonin 2008). Up until recently, identifying candidate adaptive loci has been expensive, time-consuming, and often plagued with inherent biases (Stinchcombe & Hoekstra 2007). The innovation of high throughput sequencing has facilitated genome-wide scans for loci with signatures of selection- such as extreme values of the fixation index (F_{ST}) or elevated values of Tajima's D (Hohenlohe et al. 2010). These approaches can even be applied to nonmodel organisms with limited genomic resources (Galindo et al. 2010). Adaptive loci can also be useful markers in conservation genetics for identifying cryptic structure and characterizing the adaptive potential of a species or population (Eizaguirre et al. 2014). However, tests to detect selection often produce false positives and few studies experimentally follow up on the phenotypic difference between populations or link causal selective pressures (Stapley et al. 2010). I have identified a system where I can couple high throughput sequencing methods with experimental manipulations to investigate selective processes in nature. I thus propose to use reduced-representation genomic and transcriptomic sequencing, bioinformatics, and mesocosm

experiments to determine the spatial scale of adaptive differentiation with gene flow and identify candidate loci under selection.

B) Study System. The native Olympia oyster (*Ostrea lurida*) is an ideal taxon for investigating these topics as it is patchily distributed from California to the central coast of Canada, extending over strong environmental clines and mosaics. Whether this tolerance is primarily due to plasticity, local adaptation, or balanced polymorphism is largely unknown. Recent evidence for local adaptation and population structure in the ecologically similar eastern oyster (*Crassostrea virginicus*) (Burford et al. 2014) supports the hypothesis that adaptive population differentiation in *O. lurida* is also likely. A protandrous hermaphrodite, spawning events are synced between adult males and females based on environmental cues of temperature and seasonality (Coe 1932). Unlike other oysters where both male and females spawn gametes, the females fertilize their eggs with sperm from the water column and brood larvae for 10-12 days. After being released, the weakly swimming larvae are planktonic for about two weeks before setting on a hard substrate. This newly settled "spat" is around 300-340 um and will reach sexual maturity under good growing conditions in about a year.

As ecosystem engineers in estuaries, they provide structured habitat, remove suspended sediments, and limit eutrophication. Since the early 20th century, the Olympia oyster has faced extreme commercial exploitation and, more recently, potential risks from ocean acidification (Hettinger et al. 2013). *O. lurida* thus shares many features with other threatened species, including population declines due to exploitation (Leiva & Castilla 2002), habitat loss (Airoldi et al. 2008), and ongoing environmental stressors (Branch et al. 2013). A 2009 taxonomic study on *Ostrea spp.* using two mitochondrial genes found little to no differentiation among populations, except for a break between Washington and British Columbia (Polson et al. 2009). It is critical that management plans utilize modern techniques to understand the genetic structure of this species and its adaptive potential in the face of climate change.

For my dissertation research, I aim to:
1) characterize the neutral population genetic structure of *O. lurida,*
2) experimentally test for local adaptation between diverged populations, and
3) identify candidate adaptive loci.

Research on the first aim is in progress with all required sequencing data already produced. The funds from this grant will allow me to significantly enhance the investigation of the second and third questions by extending my experimental assessment of adaptation to include demographically distinct populations from across the species' range.

II. Characterizing neutral population genetic structure

How do alleles at neutral and candidate adaptive loci vary geographically? What biotic and abiotic factors help shape this structure?

Before testing hypotheses of adaptation, the background demographic (neutral) model of population structure must be described. By looking at the spatial distribution of neutral genetic variation, one can determine the degree and scale over which subpopulations are on evolutionary independent trajectories. This model could be a smooth continuum of allele frequency shifts consistent with isolation-by-distance (IBD) (Malécot 1968), regional blocks of genetic similarity that correspond to physical barriers (Hare & Avise 1996), or the null model of no significant genetic differentiation across the species' range (Grosberg & Cunningham 2001). I hypothesize that evidence of regional population structure and IBD will be observed across the species' range.

A) Methods. During the summer of 2014, I collected adductor tissue samples from ~25 individuals at 20 sites from Klaskino Inlet, Vancouver Island (50° 17' 55") to San Diego Bay, CA (32° 361' 9"). In the fall of 2014, I began preparing Genotype-by-Sequencing (GBS) libraries. GBS is a reduced-representation genome sequencing technique, where a restriction enzyme digests genomic DNA and unique "barcodes" are ligated to the fragments for identification after multiplexed sequencing. Due to the number of barcodes available to me, up to 96 individuals can be pooled together in a library and sequenced on an Illumina HiSeq lane (Elshire et al. 2011). Raw sequences will then be demultiplexed, quality filtered, clustered to construct consensus sequences, and genotyped to identifying single-nucleotide-polymorphisms (SNPs) through pyRAD (Eaton 2014).

Population genetic summary statistics such as Wright's F-statistics, Nei's theta, and G_{ST} will be performed on the SNPs using the PopGenome R package to look at genetic differentiation among sampled sites and broad geographical regions (Pfeifer et al. 2014). A Mantel test will be performed to investigate the dependence between geographic and genetic distances and test for the presence of an IBD pattern (Jensen et al. 2005). Genetic discontinuities among populations will be identified following the approach described by Manel et al. (2007) and tested for associations with known phylogeographic barriers. Multivariate ordination methods are popular in spatial genetics for exploratory analyses, as they do not make assumptions about the underlying population genetic model (Guillot et al. 2009). For my data, I will use Principle Component Analysis (PCA) as well as Discriminant Analysis of Principal Components (DAPC) using the adegenet package in R (Jombart 2008). Finally, I will use the admixture-based model method Admixture to infer population structure (Alexander et al. 2009).

B) Preliminary work and results. We initially sequenced 90 individuals and 3 technical replicates in March 2015 and demonstrated the efficacy of GBS for *O. lurida*. Although 28 individuals had low sequencing coverage and had to be excluded from subsequent analyses, 224 informative, unlinked SNPs were found in at least 93% of the remaining 62 individuals. Using these SNPs, a Mantel test of F_{ST} and geographic distance between nine regions rejected the hypothesis of panmixia **(Fig. 1).** Subsequent sequencing runs were improved by reducing the number of individuals per lane to maximize sequencing coverage. In total, I have sequenced 228 individuals and 9 technical replicates across 4 lanes of Illumina HiSeq at the University of Chicago Functional Genomics Core, yielding 524 million 100-bp reads. These include 10 samples from *Ostrea conchaphila*, the sister species to *O. lurida* that is found from Mazatlan, Mexico to Panama.

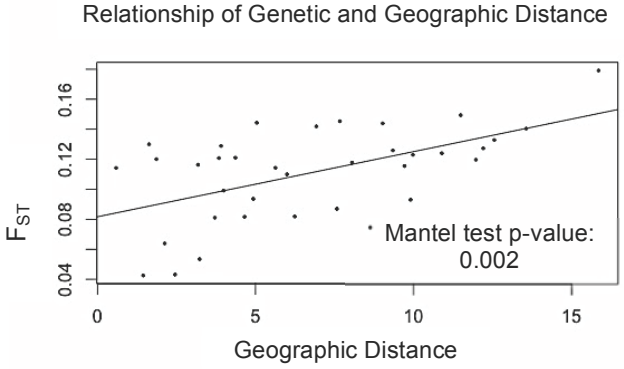

Fig. 1: Plot of Fst (Weir & Cockerham 1984) and Euclidean geographic distances based on 224 unlinked SNPs in 62 *O. lurida* individuals. Missing data were interpolated through the R package adegenet. Sampling sites were grouped into 9 regions, with at least 6 individuals per region. Mantel test p-value based on 999 simulated replicates.

III. Experimental assessment of local adaptation

Do diverged populations exhibit differences in fitness under common, controlled conditions? How does resilience to environmental stress vary among populations?

If genetic variation varies spatially in a species, one might expect to see similar variation in phenotypes as well. However, unlike genetic studies where tissue can be sampled directly from wild populations, phenotypes observed in nature can vary due to environmental, genetic, or genotype x environment interactions. Common garden experiments, where environmental factors of hypothesized selective importance are controlled, are often employed to tease apart genetic contributions to phenotype (Conover et al. 2006). In contrast, reciprocal transplants involve transferring individuals between source locations and do not explicitly test selective pressures (Stelkens et al. 2012). Evidence for transgenerational plasticity and strong maternal effects in marine taxa (Marshall 2008) suggests the ideal experimental design would involve raising and breeding individuals from different populations for at least two generations in a common, laboratory setting (Kawecki & Ebert 2004).

To study the scale of adaptation within a single body of water, my collaborators in the Roberts Lab at the University of Washington conducted a reciprocal transplant experiment with first generation (F1), hatchery-raised Olympia oysters from three distinct populations in Puget Sound (Dabob Bay, Oyster Bay, and Fidalgo Bay). Oysters from each population were outplanted at four Puget Sound sites in June 2013 and survival, growth rates, and reproductive activity were measured until July 2014. These F1 progeny demonstrated variation in fitness metrics among populations and sites. In particular, oysters from Dabob Bay had better survival yet slower growth rates at all sites, indicating a potential adaptive trade-off (Heare et al. 2015 preprint).

A) Preliminary work and results. Even though the Heare et al. (2015) study used F1 oysters spawned from wild broodstock held in common conditions for five months, transgenerational effects from source environments may still have impacted the results. To test if differences in fitness observed in the reciprocal transplant experiment are consistent in F2s raised in controlled, common conditions, I conducted a common garden study in Summer 2015 with ~100 F1 oysters from each population at the K. K. Chew Center for Shellfish Restoration and Research in Manchester, WA. Concerns over transgenerational effects would be mitigated if F2 animals reared in the same environment showed similar interpopulation differences as the F1s. Oysters from each population were spawned in 5 groups of 20 oysters to promote genetic diversity. Newly released larvae were combined based on population and reared in 100 L tanks. After reaching >160 μm in size (as determined by filtering over mesh screens), larvae were moved into a second tank and raised until close to metamorphosis (> 224 μm). Approximately 350 juveniles per population were set on 10 cm x 10 cm PVC tiles before being placed in nearby Clam Bay.

Reproductive output, survival, and growth rate were used as fitness proxies. Reproductive output was estimated by counting the number of spawned larvae from each group. Survival was determined from the number of larvae that grew to > 160 μm and > 224 μm. For these counts, I filtered larvae out over the appropriate screen size, and subsampled to quantify the number of live or dead larvae underneath a microscope, then scaled up by volume. To measure larval growth rate, ~700 one-day old larvae were raised in 1 L beakers for 2 weeks with daily water changes, with 6 replicates per population. Samples of 100 larvae were taken 3 times throughout this period and measured using a microscope and ImageJ. After setting on the PVC tiles, juveniles will be measured every two weeks for two months.

We can further ask whether genetic background affects the response of these animals to relevant stressors through two environmental manipulation experiments at different life stages. The first one, completed in July 2015, investigated variation in salinity tolerance of newly spawned larvae. Larvae were placed in salinities of 30 (ambient), 24, 18, 12, and 6ppt, with three replicates per population and ~700 larvae per replicate. After 24 hours, larvae were returned to

ambient salinity. Water temperature and algae concentration remained constant across treatments. Percent survival and shell length were recorded after one week. To test for resilience to sharp drops in temperature, the outplanted oysters will be returned to Manchester Research Station in December 2015 and a subset will be exposed to a "cold shock" where water temperature will be ramped down by 2degC a day from ambient to 10degC and maintained for 48 hours before ramping back up to ambient. The surviving oysters will be returned to Clam Bay and monitored for survival and changes in growth rate until January 2016. To look for cryptic phenotypic differences in gene expression between populations (Wang et al. 2009, Oshlack et al. 2010), samples will be taken from both treatments at the end of 48 hours to make 6 RNASeq libraries (3 populations x 2 treatments).

In addition to testing for transgenerational plasticity, this common garden study complemented and improved on the previous experiment in Heare et al. (2015) by quantifying early life history fitness proxies, such as larval growth rate, that are otherwise impossible in the field. In a preliminary analysis of protoshell length in 20 7-day-old larvae from each population, size varied significantly among populations and the larvae from Hood Canal were significantly smaller than those from Oyster Bay (pairwise t-test, Bonferonni corr.: $p<0.001$) (**Fig. 2).** While the majority of data analysis is still in progress, this concordant result with the reciprocal transplant indicates that phenotypic differences may indeed be consistent between the two studies.

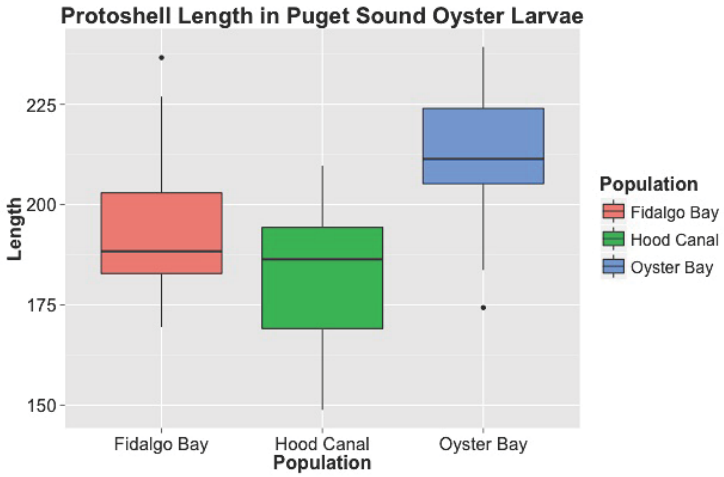

Figure 2: Boxplot of protoshell length in *O. lurida* larvae from three Puget Sound, WA populations, measured 7 days after maternal release. 20 larvae were sampled from each population across three replicates with similar environmental conditions. ANOVA F-statistic: 14.81, p-value < 0.001

B) Proposed work. In order to better understand the spatial scale of adaptation in this species, I propose a common garden experiment with populations of oysters from San Francisco Bay, CA (SFCA), Yaquina Bay, OR (YOR), Hood Canal, WA (HWA), and Ladysmith Harbour, British Columbia (LHBC). Although I am still analyzing the phylogeography of these four sites, my preliminary data suggests they are on separate evolutionary trajectories. Based on available environmental data, these sites also vary significantly in temperature, pH, salinity, and seasonality (IOOS databases). In particular, YOR experiences the greatest frequency of exposure to pH <7.8, levels already below those predicted for 2100. I hypothesize that individuals from YOR will demonstrate the greatest resilience to pH stress experiments. As LHBC experiences the lowest seawater temperatures, I hypothesize that oysters from that population will have the best survival and growth following a cold shock. These populations are all either under consideration for or actively a part of restoration management plans, further prioritizing the need to understand their adaptive characteristics.

Due to risks like disease, transfer of invasive species, and introduction of foreign genotypes, a rangewide reciprocal transplant experiment would be impossible. Instead, this

experiment will be conducted in a quarantine facility available to me at the Manchester Research Station. ~120 adult oysters will be collected by local collaborators from each site in January 2015 and shipped overnight on ice to the facility. Gonad development in *Ostrea spp.* has been linked to temperature (Santos 1993), food availability (Cano 1997), and salinity (Oates 2013). To minimize carryover effects from environmental differences at native sites, the oysters will be kept in flow-through seawater tanks at local, ambient water temperature for 5 months prior to spawning in June 2016. Metrics of reproductive output, survival, and larval growth rate will be collected as described previously. Per quarantine restrictions, juveniles will not be outplanted but instead kept in a flow-through system in the quarantine facility for stressor experiments and measuring growth rate until October 2016.

Two experiments will investigate potential resilience to environmental stress. 1) Three replicates of 10,000 1-day-old larvae from each population will be raised in elevated (900 μatm) pCO_2 until metamorphosis to the benthic, juvenile stage. Based on typical seawater characteristics at the Manchester Research Station, this corresponds to a pH of 7.8 (Robbins et al. 2010). Survival, time until metamorphosis, and growth rate will be compared to control treatments at ambient pCO_2.1,000 larvae will be sampled at Day 1 and 7 from each population/treatment by pooling across replicates. RNASeq libraries for these 16 samples (4 populations x 2 CO_2 levels x 2 time points in development) will be prepared then sequenced on 8 single-end 50 bp Illumina HiSeq lanes through the University of Chicago's Functional Genomics Core. The results of this experiment will integrate well with an ongoing investigation into acidification resilience in Puget Sound Olympia oyster populations (Carolyn Friedman, pers. comm). 2) Three replicates of 100 post-settlement juveniles from each population will be exposed to a "cold shock", where water temperature will be ramped down by 2°C a day from ambient to 10°C and maintained for 48 hours before ramping back up to ambient. This experiment is intended to mirror rapid drops in temperature that occur episodically along the Pacific coast in early winter. Survival and changes in growth rate will be compared to control treatments. 20 juveniles will be sampled across replicates at the end of the 48 hours for each population for RNA extraction and preparation of pooled RNASeq libraries. These 8 libraries (4 populations x 2 treatments) will be sequenced across 4 single-end 50 bp Illumina HiSeq lanes.

Experimental assessments of adaptive, phenotypic variation are valuable tools, although they will not necessarily distinguish between true local adaptation and balanced polymorphism. This distinction is important for understanding the adaptive capacity of a species, as the latter would result in a wide range of phenotypes available to each population every generation and might allow for improved tracking of environmental change (Sanford and Kelly 2011). By examining if the inferred spatial scale of gene flow from **Aim 1** is larger than the scale of observed differentiation from both sets of experiments in **Aim 2,** I can test for true local adaptation.

IV. Identifying candidate adaptive loci through bioinformatics

How do F_{ST} outlier loci vary geographically? What loci are associated with experimentally observed fitness traits? Is mortality at early life stages random, or due to purifying selection on maladapted genotypes?

Typical of other highly fecund species with a dispersal phase, the planktonic larval stage of oysters exhibits a Type III survivorship curve with early mortality (Hedgecock & Pudovkin 2011). Post-settlement mortality is also common for many marine invertebrates, but whether these processes are random with respect to genotype or due to purifying selection is largely unknown. If this mortality were due to selection on maladapted genotypes (termed phenotype-

environment mismatch), then one would expect to see significant shifts in allele frequency across life stages at loci under selection (e.g. Pespeni et al. 2013). Evidence for purifying selection every generation would also lend support for the importance of balanced polymorphism and may explain why genetic structure is observed on surprisingly fine scales in broadcast spawning species (Marshall et al. 2010). Environmental variables that are believed to impact fitness in oysters, such as temperature (Davis 1955), salinity (Bible & Sanford 2013) and pH (Hettinger et al. 2013) can vary significantly even within a single estuary (MacWilliams et al. 2015). I hypothesize that significant allele frequency shifts will be observed between larvae samples from Day 1 and Day 7 of the proposed pCO_2 experiment and between juveniles from the cold shock and control treatments.

While testing every population of interest for response to induced stressors would be informative for developing management contingency plans, it is simply not feasible. A preferred alternative would be to identify genetic markers that are associated with a trait of interest (e.g. resilience to low pH) and screen populations at these loci. F_{ST}-based outlier analyses are increasingly used to provide initial hypotheses of adaptive markers. By applying these tests to the GBS data collected in **Aim 1** and following up with experimental evolution and transcriptomics in **Aim 2**, we can identify robust candidate loci through those that overlap. It should be noted that these methods are unlikely to identify the causal SNPs underlying adaptation, but instead provide strong hypotheses of the gene regions involved and associated markers in linkage disequilibrium.

A) Analysis of outlier GBS loci. F_{ST} outlier tests such as Bayescan and FDIST2 will be used to identify SNPs with extreme values of F_{ST} when compared to a simulated neutral distribution (Foll & Gaggiotti 2008). Another program, Bayenv 2.0, will allow me to investigate whether allele frequencies at an outlier SNP are significantly correlated with environmental variables such as salinity, temperature, and pH (Coop et al. 2010), obtained from public databases (e.g. NOAA). I will identify potential adaptive loci segregating among populations, and outlier loci will be excluded from the previously described analyses of population structure to adhere to assumptions about loci neutrality. Because GBS produces a subset of randomly distributed loci from the genome, some may be in protein-coding regions, regulatory regions, or 'junk' DNA. My collaborators at UWashington have produced a transcriptome for *O. lurida* (Timmins-Schiffman et al. 2013) and are expected to have a sequenced genome by early 2016. These resources, coupled with the BLAST2GO pipeline for gene ontology annotations, will allow me to determine the potential function of outlier loci and characterize their distribution in the genome. The maximum-likelihood estimator of linkage disequilibrium (LD) developed by Maruki and Lynch (2014) for high-throughput sequencing data will be used to map the extent of LD across the genome, which will be vital for integration of SNP data from GBS and RNASeq.

B) Common garden experiments. To test the null hypothesis of random mortality under controlled, "ideal" conditions, larvae samples were taken Day 1 after spawning, at size 160 μm, and at 224 μm during the Puget Sound common garden experiment. GBS libraries are currently being prepared from these pools of larvae, and permutation analyses will determine if there are outlier polymorphisms with respect to allele frequency changes between size classes. Preliminary results indicate size variability among populations is significant and heritable, so individually-barcoded GBS libraries are being prepared for all 300 broodstock adults. These data will be combined with that of my collaborators in the Roberts lab for a genome-wide association study (GWAS) of size in 900 adults using the program SNPTEST (Marchini 2007).

Transcriptomics represent a powerful tool for understanding the adaptive potential of a species, as it provides phenotypic, functional, and genomic information through quantification of gene expression levels, identification of amino acid replacements, and SNP information (Evans 2015). For both the Puget Sound and proposed common gardens, RNASeq data will be analyzed following the De Wit et al. (2012) pipeline to filter, assemble, map, and genotype polymorphisms in RNASeq data. Comparisons of gene expression levels between experimental and control treatments will be done for each population in order to detect candidate genes involved in either low pH or low temperature tolerance. Similar to the analysis of larval GBS libraries, permutation analyses will be used to identify loci with significant allele frequency shifts across treatments. Functional enrichment analysis will test if candidate loci are clustered within specific functional developmental categories. Annotated candidate loci from both GBS and RNASeq data will be compared to robustly identify and characterize gene regions involved in local adaptation and resilience to pH and temperature stress.

V. Intellectual Merit

Marine species have historically been regarded as demographically open, with local adaptation considered rare due to high gene flow. With the growing body of evidence for both population structure and adaptive differentiation, the processes underlying these surprising patterns will be key to determining the scope of their response to climate change. For my dissertation, I have identified an emerging model species with which I can quantify these processes and understand the interplay between adaptive differentiation and high dispersal. The evolutionary patterns found in this study can further be cautiously extrapolated to hundreds of other sessile, broadcast spawning species that experience fluctuating environment.

VI. Broader Impacts of the Proposed Work

The ability of aquatic invertebrates- including some of the most endangered species in the U.S. – to respond to climate change will depend upon their capacity to adapt. In the case of native Olympia oysters, there is political and economic pressure to restore abundance and recover ecosystem function. In addition to dissemination of results to the academic community, my research thus involves actively working with conservation groups. My primary collaborator on this grant, Puget Sound Restoration Fund (PSRF), is a non-profit organization dedicated to restoring marine habitat, water quality, and native species in Puget Sound. Founded in 1997, they are well integrated into the west coast community of oyster farmers, governmental resource managers, and the general public. Through their established community educational programs, I will teach a workshop catered towards oyster farmers and government resource managers in the spring of 2018 covering local adaptation and the implications of my findings for ongoing restoration and management efforts.

PSRF also has an active internship program, with an emphasis on broadening participation in policy and the research. My experiences working with middle-school girls through Sisters4Science and as a mentor in the UChicago Multicultural Graduate Community have motivated my interest in academic mentorship, particularly for those underrepresented in the sciences. During my summer project in 2015, I involved an undergraduate PSRF intern and helped her develop skills in scientific project design, data collection, and statistical analysis. Because of her significant contributions, she will also be involved in manuscript preparation towards her first academic publication. I will similarly involve another PSRF intern for my proposed project next summer.

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BIOGRAPHICAL SKETCH

Catherine A. Pfister
Department of Ecology & Evolution, University of Chicago

Professional Preparation

University of Illinois, Champaign-Urbana	Biology	B. S.	1984
University of North Carolina at Chapel Hill	Marine Sciences	M.S.	1987
University of Washington, Seattle	Zoology	Ph.D.	1993
University of California at Berkeley	Integrative Biology	Postdoc	1993-1995

Appointments

2013 – present	Professor, Department of Ecology & Evolution, Committee on Evolutionary Biology, and The College, University of Chicago
2003 – 2013	Associate Professor, Department of Ecology & Evolution, Committee on Evolutionary Biology, and The College, University of Chicago
1995 – 2003	Assistant Professor, Department of Ecology & Evolution, Committee on Evolutionary Biology, and The College, University of Chicago
1993 – 1995	Miller Institute for Basic Science Postdoctoral Research Fellow, Dept. of Integrative Biology, University of California, Berkeley

Publications

5 Publications Most Closely Related to Proposed Project

1. Pfister CA, Altabet M, Post D. 2014. Animal Regeneration and microbial retention of nitrogen along coastal rocky shores. *Ecology* 95(10):2803–2814.
2. Pfister CA, Gilbert JA, Gibbons SM. 2014.The role of macrobiota in structuring microbial communities along rocky shores. PeerJ 2:e631; DOI 10.7717/peerj.631
3. Wootton, J. T. & C. A. Pfister. 2013. Experimental Separation of Genetic and Demographic Factors on Extinction Risk in Wild Populations. Ecology 94:2117-2123. doi: 10.1890/12-1828.1
4. Pfister, C. A., F. Meyer, D. A. Antonopoulos. 2010. Metagenomic profiling of a microbial assemblage associated with the California mussel, *Mytilus californianus:* a node in networks of carbon and nitrogen cycling. PLoS ONE 5(5): e10518. doi:10.1371/journal.pone.0010518.
5. Wootton JT, Pfister CA, Forester JD. 2008.Dynamical patterns and ecological impacts of changing ocean pH in a high-resolution multi-year dataset. *Proceedings of the National Academy of Sciences* 105(48):18848-18853. PMCID: PMC2596240.

5 Other Significant Publications

1. McCoy SJ, Pfister CA. 2014. Historical comparisons reveal altered competitive interactions in a guild of crustose coralline algae. *Ecology Letters* 17(4):475-483.
2. Pfister CA, Esbaugh AJ, Frieder CA, Baumann H, Bockmon EE, White MM, Carter BR, Benway HM, Blanchette CA, Carrington E, McClintock JB, McCorkle DC, McGillis WR, Mooney TA, Ziveri P. 2014. Detecting the Unexpected: A Research Framework for Ocean Acidification. *Environmental Science and Technology*. 48(17):9982-9994. doi: 10.1021/es501936p.
3. Pfister CA, McCoy SJ, Wootton J T, Martin PA, Colman AS, Archer D. 2011. Rapid environmental change over the past decade revealed by isotopic analysis of the California mussel in the northeast Pacific. *PLoS ONE* 6(10):e25766. PMCID: PMC3185010.
4. Morris WF, Pfister CA, Tuljapurkar S, Haridas CV, Boggs CL, Boyce MS, Bruna EM, Church DR, Coulson T, Doak DF, Forsyth S, Gaillard JM, Horvitz CC, Kalisz S, Kendall BE, Knight TM, Lee CT, Menges ES. 2008. Longevity can buffer plant and animal populations against changing climatic variability. *Ecology* 89(1):19-25.

5. Pfister CA. 1998. Patterns of variance in stage-structured populations: evolutionary predictions and ecological implications. *Proceedings of the National Academy of Sciences*. 95(1):213-218. PMCID: PMC34554.

Synergistic Activities

Principal Investigator, Department of Education Graduate Training Program, Graduate Assistance in Areas of National Need (GAANN), *A Graduate Training Program in Ecology*, Awarded to the University of Chicago, 2004-2007, 2007-2010, 2011-2014, 2015-2018

Associate Editor, *The American Naturalist,* 2003 to 2008, *Proc of the Royal Acad of Sci*, B, 2010-present; Board of Editors, The University of Chicago Press, 2008-2011,

Scientific Advisory Board, 2007-10; Working Group Leader, "Demographic Variation and Climate Change", NCEAS, Santa Barbara, 2004-2006

Participant: NSF Workshop, Evolution and Global Change, May 2010; California Current Acidfication Network, Dec 2011; NSF Postdoctoral Fellow Panelist, 2015

Board Member, Ocean Carbon & Biogeochemistry (OCB; a joint committee formed by NSF, NASA & NOAA), Ocean Acidification Subcommittee, 2012-present

Collaborators and Other Affiliations

Collaborators and Co-Editors: 15

Mark Altabet (University of Massachusetts), Hannes Baumann (Stony Brook University), Heather Benway (Woods Hole Oceanographic Institute), Emily Carrington (University of Washington), Brendan Carter (NOAA), Linda Deegan (Marine Biological Laboratory, Woods Hole), Andrew Esbaugh (University of Texas), Christina Frieder (University of Southern California), Jack Gilbert (Argonne National Laboratory), Bruce Kendall (University of California, Santa Barbara), James McClintock (University of Alabama at Birmingham), Robert Paine (University of Washington), Nicole Phillips (Wellington University of New Zealand), David Post (Yale University), Meredith White (Bowdoin)

Graduate Advisors and Postdoctoral Sponsors: 3

Mark Hay (MS), Robert Paine (PhD), Wayne Sousa (Postdoctoral)

Thesis Advisor and Postgraduate-Scholar Sponsor: 7

Dissertations Directed

Satie Airame, PhD 1999 (UCSB); Karl Polivka, PhD 2002 (U. S. Forest Service); Doug Nutter, PhD 2005 (University of Maryland); Julie Collens, PhD 2009 (Leerink Co); Ole Shelton, PhD 2009 (NOAA/NMFS); Erin Grey, PhD 2009 (Notre Dame University); Sophie McCoy, PhD 2014 (Florida State University); Current graduate students: Simon Lax, Katherine Silliman, Courtney Stepien, Orissa Moulton

Postdoctoral Fellows

None in last 5 years

BIOGRAPHICAL SKETCH

Katherine E. Silliman
Committee on Evolutionary Biology, University of Chicago

(a) Professional Preparation

University of Miami	Coral Gables, FL	Marine Science, Biology Music	B.S. 2013 B.A. 2013
University of Chicago	Chicago, IL	Evolutionary Biology	Ph.D. 2019 (expected)

(b) Appointments

Sept 2013 – present	PhD. Candidate, Committee on Evolutionary Biology University of Chicago
Jan 2013 – May 2013	Teaching Assistant for Chemical Oceanography University of Miami
Aug 2009 – May 2013	Undergraduate Researcher, Hurt Lab Department of Biology, University of Miami

(c) Publications

Hurt C, Silliman K, Anker A, Knowlton N. 2013. Ecological speciation in anemone-associated snapping shrimps (*Alpheus armatus* species complex). *Mol. Ecol.* 22: 4532-4548. doi: 10.1111/mec.12398.

(d) Synergistic Activities

1. Research dissemination: Member of the National Shellfisheries Association (NSA), Association for the Sciences of Limnology and Oceanograhy (ASLO), American Fisheries Society (AFS), Society for Integrative and Comparative Biology (SICB), and Sigma Xi. Oral presentation at the 2015 NSA meeting; poster presentation at the 2013 ASLO meeting.

2. Open science: Maintains a public, online lab notebook and member of the Open Notebook Science Network. All scripts developed for data analysis are available through personal GitHub, and most data are publically available prior to publication.

3. Mentorship: Member of the UChicago Multicultural Graduate Community and participate in the Undergraduate Mentorship Program, where I am mentoring a young woman with a strong career interest in evolutionary biology. Trained and mentored an undergraduate woman through Puget Sound Restoration Funds internship program (June 2015-Aug 2015).

4. Public outreach on evolution: Guest Scientist for Project Exploration's Sisters4Science (Nov 2014-present), teach basic molecular biology to middle-school girls through hands-on extraction of strawberry DNA. Ocean Kids event workshop leader (2010-2012), designed and led activities demonstrating coral diversity at the University of Miami campus for 200 3rd graders from local, high-needs elementary schools.

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors: 10
Arthur Anker (Universidade Federal do Ceará), William Browne (University of Miami), Ryan Crim (Puget Sound Restoration Fund), Doug Eernisse (California State University, Fullerton), Carla Hurt (Tennessee Tech University), Jane Indorf (University of Miami), Nancy Knowlton (Smithsonian National Museum of Natural History), Steven Roberts (University of Washington), Brent Vadopalas (University of Washington), Ryan Walter (California State University, Fullerton).

Graduate Advisors and Postdoctoral Sponsors: 1
Catherine Pfister, Professor, Ecology and Evolution, University of Chicago

Thesis Advisor and Postgraduate-Scholar Sponsor: 0
None

SUMMARY PROPOSAL BUDGET

YEAR 1

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION
University of Chicago

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR
Catherine Pfister

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.	0.00	0.00	0.00		
2.					
3.					
4.					
5.					
6. (**0**) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	**0**	
7. (**1**) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	0.00	**0**	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (**0**) POST DOCTORAL SCHOLARS	0.00	0.00	0.00	**0**	
2. (**0**) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	**0**	
3. (**0**) GRADUATE STUDENTS				**0**	
4. (**0**) UNDERGRADUATE STUDENTS				**0**	
5. (**0**) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				**0**	
6. (**0**) OTHER				**0**	
TOTAL SALARIES AND WAGES (A + B)				**0**	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				**0**	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				**0**	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				**0**	
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				**0**	
2. FOREIGN				**0**	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ **0**					
2. TRAVEL **0**					
3. SUBSISTENCE **0**					
4. OTHER **0**					
TOTAL NUMBER OF PARTICIPANTS (**0**) TOTAL PARTICIPANT COSTS				**0**	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				**7,765**	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				**0**	
3. CONSULTANT SERVICES				**0**	
4. COMPUTER SERVICES				**0**	
5. SUBAWARDS				**0**	
6. OTHER				**4,932**	
TOTAL OTHER DIRECT COSTS				**12,697**	
H. TOTAL DIRECT COSTS (A THROUGH G)				**12,697**	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **Fieldwork (Rate: 26.0000, Base: 6270) (Cont. on Comments Page)**					
TOTAL INDIRECT COSTS (F&A)				**5,358**	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				**18,055**	
K. SMALL BUSINESS FEE				**0**	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				**18,055**	

M. COST SHARING PROPOSED LEVEL $ **0** AGREED LEVEL IF DIFFERENT $

PI/PD NAME
Catherine Pfister

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

SUMMARY PROPOSAL BUDGET COMMENTS - Year 1

**** I- Indirect Costs**
Molecular lab work (Rate: 58.0000, Base 6427)

SUMMARY PROPOSAL BUDGET

YEAR 2

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION
University of Chicago

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR
Catherine Pfister

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.	0.00	0.00	0.00		
2.					
3.					
4.					
5.					
6. (**0**) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	**0**	
7. (**1**) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	0.00	**0**	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (**0**) POST DOCTORAL SCHOLARS	0.00	0.00	0.00	**0**	
2. (**0**) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	**0**	
3. (**0**) GRADUATE STUDENTS				**0**	
4. (**0**) UNDERGRADUATE STUDENTS				**0**	
5. (**0**) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				**0**	
6. (**0**) OTHER				**0**	
TOTAL SALARIES AND WAGES (A + B)				**0**	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				**0**	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				**0**	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				**0**	
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				**300**	
2. FOREIGN				**0**	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ **0**					
2. TRAVEL **0**					
3. SUBSISTENCE **0**					
4. OTHER **0**					
TOTAL NUMBER OF PARTICIPANTS (**0**) TOTAL PARTICIPANT COSTS				**0**	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				**0**	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				**0**	
3. CONSULTANT SERVICES				**0**	
4. COMPUTER SERVICES				**0**	
5. SUBAWARDS				**0**	
6. OTHER				**0**	
TOTAL OTHER DIRECT COSTS				**0**	
H. TOTAL DIRECT COSTS (A THROUGH G)				**300**	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE) **Present at conference (Rate: 58.0000, Base: 300)**					
TOTAL INDIRECT COSTS (F&A)				**174**	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				**474**	
K. SMALL BUSINESS FEE				**0**	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				**474**	

M. COST SHARING PROPOSED LEVEL $ **0** AGREED LEVEL IF DIFFERENT $

PI/PD NAME
Catherine Pfister

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

 ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

SUMMARY PROPOSAL BUDGET

Cumulative

FOR NSF USE ONLY		
PROPOSAL NO.	DURATION (months) Proposed	Granted
AWARD NO.		

ORGANIZATION
University of Chicago

PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR
Catherine Pfister

A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)	NSF Funded Person-months CAL	ACAD	SUMR	Funds Requested By proposer	Funds granted by NSF (if different)
1.	0.00	0.00	0.00		
2.					
3.					
4.					
5.					
6. ( ) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)	0.00	0.00	0.00	**0**	
7. (**0**) TOTAL SENIOR PERSONNEL (1 - 6)	0.00	0.00	0.00	**0**	
B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)					
1. (**0**) POST DOCTORAL SCHOLARS	0.00	0.00	0.00	**0**	
2. (**0**) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)	0.00	0.00	0.00	**0**	
3. (**0**) GRADUATE STUDENTS				**0**	
4. (**0**) UNDERGRADUATE STUDENTS				**0**	
5. (**0**) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)				**0**	
6. (**0**) OTHER				**0**	
TOTAL SALARIES AND WAGES (A + B)				**0**	
C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)				**0**	
TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)				**0**	
D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)					
TOTAL EQUIPMENT				**0**	
E. TRAVEL 1. DOMESTIC (INCL. U.S. POSSESSIONS)				**300**	
2. FOREIGN				**0**	
F. PARTICIPANT SUPPORT COSTS					
1. STIPENDS $ **0**					
2. TRAVEL **0**					
3. SUBSISTENCE **0**					
4. OTHER **0**					
TOTAL NUMBER OF PARTICIPANTS (**0**) TOTAL PARTICIPANT COSTS				**0**	
G. OTHER DIRECT COSTS					
1. MATERIALS AND SUPPLIES				**7,765**	
2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION				**0**	
3. CONSULTANT SERVICES				**0**	
4. COMPUTER SERVICES				**0**	
5. SUBAWARDS				**0**	
6. OTHER				**4,932**	
TOTAL OTHER DIRECT COSTS				**12,697**	
H. TOTAL DIRECT COSTS (A THROUGH G)				**12,997**	
I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)					
TOTAL INDIRECT COSTS (F&A)				**5,532**	
J. TOTAL DIRECT AND INDIRECT COSTS (H + I)				**18,529**	
K. SMALL BUSINESS FEE				**0**	
L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)				**18,529**	

M. COST SHARING PROPOSED LEVEL $ **0** AGREED LEVEL IF DIFFERENT $

PI/PD NAME
Catherine Pfister

ORG. REP. NAME*

FOR NSF USE ONLY		
INDIRECT COST RATE VERIFICATION		
Date Checked	Date Of Rate Sheet	Initials - ORG

C ***ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET**

BUDGET JUSTIFICATION

A. Senior Personnel: $0
B. Other Personnel: $0
C. Fringe Benefits: $0
D. Equipment: $0
E. Travel: $300
Year 1: **$0** *Year 2:* **$300**

Project	**Budget Item**	**Item Cost per Unit**	**Item Total**	**Amount Requested**
Travel Domestic	Chicago, IL to Portland, OR (round trip) for 2017 Evolution conference	$300 (Year 2)	$300	$300
	Lodging in Portland for 2017 Evolution conference	$100 x 5 nights (Year 2)	$500	$0
		Total:	$800	**$300**

The majority of the experiments outlined for this DDIG research would take place from June 10, 2016-October 15, 2016 in Manchester, WA. Due to the extended period of this field season, I will be subletting my housing in Chicago and paying for my travel and accommodations in Seattle, WA out of pocket. This arrangement worked out successfully in the summer of 2015.

In the second year, I am requesting funds for travel to present my results at the Evolution conference to be held in Portland in 2017. To cover accommodations, I will be applying for travel awards through the University of Chicago's graduate school and the Committee on Evolutionary Biology. Flight and hotel estimates were obtained through Expedia.com.

F. Participant Support Costs: $0
G. Other Direct Costs: $12,697

Project	Budget Item	Item Cost per Unit	Item Total	Amount Requested
O. lurida common garden experiments - Fieldwork	Summer facility use fee at K.K.C. Shellfish Research Center in Manchester, WA- includes cost of tank space in quarantine area, live algae cultures for feeding, use of equipment, and utilities	$2,250/month June 8 – Aug. 15 2016 (Year 1)	$4,500	$4,500

	Off-season facility use fee at K.K.C Shellfish Research Center	$750/month Aug. 16 – Oct. 15 2016 (Year 1)	$1,500	$1,500
	RNALater	$270/250 mL (Year 1)	$270	$270
O. lurida common garden experiments - Gene expression	TRI Reagent for RNA Extraction	$170/100 mL x 2 (Year 1)	$340	$340
	NEBNext Ultra Directional RNA Library Prep Kit for Illumina	$1,155/24 samples (Year 1)	$1,155	$1,155
	Agilent 2100 BioAnalyzer verification	$8/library x 24 (Year 1)	$192	$192
	qPCR quantification	$20/HiSeq lane x 12 (Year 1)	$240	$240
	Single-end 50 bp Illumina HiSeq	$750/lane x 12 (Year 1)	$9,000	$4,500
		Total:	$17,197	$12,697

1) Materials and Supplies: $7,765

Fieldwork

For the common garden experiment, we are requesting funding for the facility use fees at the Kenneth K. Chew Center for Shellfish Research and Restoration in Manchester, WA. This facility is involved in a hatchery restoration project with Olympia oysters, and therefore has live algae cultures and equipment tailored specifically to my model species. Their quarantine area is one of the few places in the country that allows experiments with non-commercial, non-indigenous shellfish species. These resources coupled with the staff's expertise make this facility ideal for my proposed project. Facility costs were quoted by Puget Sound Restoration Fund based on research proposal. We are also requesting funding for RNALater, which has successfully been used in prior research for tissue storage.

Gene expression

Funds are requested for supplies to prepare RNASeq libraries from samples of 16 larvae pools and 240 juveniles collected during the temperature and acidification experiments as outlined in the proposal. These samples will be multiplexed to make 24 RNASeq libraries. TRI Reagent will be used for the RNA extraction. To barcode individual samples for

multiplexed sequencing, stocks of NEBNext Multiplex Oligos are available to us through the Pritzker DNA Lab. However, funds are requested for the consumable NEBNext Ultra Directional RNA Library Prep Kit. This kit provides strand information for mRNA transcripts, which will facilitate identification of allele-specific expression. Estimates of cost were obtained through provider catalogs.

6) Other: $4,932

RNASeq Library Sequencing

Prior to sequencing, all libraries must undergo verification and quantification with the Agilent 2100 Bioanalyzer and qPCR. As a well-assembled transcriptome already exists for *O. lurida*, longer reads are not crucial to measuring gene expression. Funds are requested to run 12 RNASeq libraries will be run across 6 single-end 50bp Illumina HiSeq lanes, each of which costs $750. These services will be done through the UChicago Functional Genomics core. The additional $4,500 to sequence the remaining 12 libraries will be obtained through small internal awards such as the Evolutionary Biology Hinds Fund and Institute for Translational Medicine Core Subsidy.

H. Total Direct Costs: *Year 1:* **$12,697** *Year 2:* **$300**

The majority of the requested funds will be needed for fieldwork in Washington, materials, supplies, and 6 runs of sequencing in the first year. The time-intensive, computational analyses associated with sequencing data will take place during the second year, prior to presentation of results at the Evolution 2017 meeting.

I. Indirect Costs: *Year 1:* **$5,358** *Year 2:* **$174**

As of February 5, 2015 the University of Chicago's Federally Negotiated rate is 58% for all modified total direct costs incurred on campus and 26% for all modified total direct costs incurred off campus. $6,270 of the proposed direct costs will be used off campus in Manchester, WA and $6,727 will be used on campus.

J. Total Direct and Indirect Costs: *Year 1:* **$18,055** *Year 2:* **$474**

K. Residual Funds: $0

L. Amount of this Request: $18,529

CURRENT AND PENDING SUPPORT
Cathy Pfister

Current:

Title:	GAANN: Training in Quantitative Ecology
Source:	Department of Education
Total Award Amount:	$885,834
Award Period:	09/01/15-08/31/18
Location of Project:	University of Chicago
Persons-Months Per Year:	1.2 Calendar (no salary recovery)

Title:	DISSERTATION RESEARCH: The Roles of Evolutionary History and Ecological Interactions in the Maintenance of a High-Diversity Algal Assemblage
Source:	NSF
Total Award Amount:	$20,540
Award Period:	05/01/13-04/30/16
Location of Project:	University of Chicago /Tatoosh Island, WA
Persons-Months Per Year:	0.0 Calendar

Pending:

Title:	Dimensions: Collaborative Research: Does parallel functional diversity arise in diverse animal-microbe associations across coastal ocean
Source:	NSF
Total Award Amount:	$529,689
Award Period:	12/01/15-11/30/19
Location of Project:	University of Chicago/Tatoosh Island, WA
Persons-Months Per Year:	1 Calendar

Title:	Kelp forests: their dynamics, services, and fate in a changing climate
Source:	NOAA
Total Award Amount:	$224,257
Award Period:	07/01/16-06/30/18
Location of Project:	University of Chicago/ Washington state coast
Persons-Months Per Year:	1 Calendar

Title:	DISSERTATION RESEARCH: A genomic and experimental characterization of local adaptation (THIS PROPOSAL)
Source:	NSF
Total Award Amount:	$18,529
Award Period:	06/01/16-05/31/18
Location of Project:	University of Chicago/Manchester, WA
Persons-Months Per Year:	0.0 Calendar

CURRENT AND PENDING SUPPORT
Katherine Silliman

Current:

Title:	A Genomic and Experimental Characterization of Local Adaptation
Source:	NSF Graduate Research Fellowship (GRFP)
Total Award Amount:	$138,000
Award Period:	06/01/15-05/31/18
Location of Project:	University of Chicago
Persons-Months Per Year:	0.0 Calendar

Title:	Local Adaptation in the Olympia Oyster
Source:	University of Chicago Hinds Fund
Total Award Amount:	$1,000
Award Period:	06/01/15-05/31/16
Location of Project:	Manchester, WA
Persons-Months Per Year:	2.0 Calendar

Title:	Population Genomics and Phylogeography of *Ostrea lurida*
Source:	National Shellfisheries Association
Total Award Amount:	$1,250
Award Period:	03/15/15-03/14/16
Location of Project:	University of Chicago
Persons-Months Per Year:	4.0 Calendar

Title:	Detecting loci under selection in the Olympia oyster
Source:	University of Chicago, Pritzker Lab Award
Total Award Amount:	$2,987
Award Period:	06/01/15-05/31/16
Location of Project:	University of Chicago
Persons-Months Per Year:	4.0 Calendar

Title:	Conservation Genomics of the Olympia Oyster
Source:	American Fisheries Society, Berkeley Award runner-up
Total Award Amount:	$1,000
Award Period:	08/01/15-07/31/16
Location of Project:	University of Chicago
Persons-Months Per Year:	4.0 Calendar

Title:	Alleviating regulatory impediments to native shellfish aquaculture
Source:	NOAA Sea Grant Aquaculture
Total Award Amount:	$427,371
Award Period:	09/01/12-01/31/16
Location of Project:	Manchester, WA

Persons-Months Per Year: 3.0 Summer

Pending:

Title:	A genomic and experimental characterization of local adaptation
Source:	National Geographic Young Explorers Grant
Total Award Amount:	$5,000
Award Period:	02/20/16 – 02/19/17
Location of Project:	University of Chicago/Manchester, WA
Persons-Months Per Year:	1 Calendar

Title:	DISSERTATION RESEARCH: A genomic and experimental characterization of local adaptation (THIS PROPOSAL)
Source:	NSF
Total Award Amount:	$18,529
Award Period:	06/01/16-05/31/18
Location of Project:	University of Chicago/Manchester, WA
Persons-Months Per Year:	0.0 Calendar

Submission Planned in Near Future

Title:	A genomic and experimental characterization of local adaptation
Source:	University of Chicago, Institute for Translational Medicine
Total Award Amount:	$5,000
Award Period:	01/20/17-01/19/18
Location of Project:	University of Chicago
Persons-Months Per Year:	4.0 Calendar

Facilities, Equipment, and Other Resources

Animal: Animal husbandry and fitness assays with *Ostrea lurida* will be conducted at the Kenneth K. Chew Center for Shellfish Research and Restoration (CSRR) in Manchester, WA. This facility is located at the NOAA Manchester Research Station and cooperatively run by Puget Sound Restoration Fund and NOAA. This facility has a full-time manager who oversees general operations and advises on all hatchery and research projects, a full time phycologist for growing various strains of microalgae, and a full-time technician. Raw and treated (UV, mesh-filtered) seawater is available from nearby Clam Bay and can be temperature controlled with heaters and chillers. A federally and state certified quarantine zone is available on-site for research on non-indigenous shellfish populations or species. Inside the quarantine area is a newly installed pCO_2 manipulation system with sensors that can be accessed remotely to ensure stability throughout experiments.

Laboratory: All RNA extractions and RNASeq library preparation will be done in the Field Museum of Natural History's Pritzker Laboratory for Molecular Systematics and Evolution. This lab is a multi-user facility and has an endowed full-time manager who oversees general lab operation (protocol development, equipment and supply purchasing) as well as two endowed technicians. No user fees are charged for working in the lab. Ample bench space is available as well as all of the equipment required for this type of lab work. The lab was recently renovated to create additional bench space and expose part of the lab as a public exhibit. The CSRR is equipped with a dry laboratory for tissue and seawater sample processing as well as basic microscopic imaging.

Major Equipment:

CSRR

Leica DM1000 Microscope
Beckman Coulter Counter
Orion STAR111 pH Meter
Hach Digital Titrator
Two portable pH probes

DNA Laboratory

BioRad CFX Connect Real-Time PCR system
Three -80°C freezers, Nine -20°C freezers
Thermo Nanodrop for quantifying DNA concentrations
Life Technologies Qubit Fluorometer for quantifying DNA concentrations
Agilent 2100 Bioanalyzer
ABI 3730 Sanger Sequencer
Illumina MiSeq Sequencer
Seven Bio-Rad thermal cyclers and four Eppendorf Mastercycler gradient EP thermal cyclers for polymerase chain reaction

Computing Resources: A large amount of data will be produced during this study and typical personal and desktop computers provide insufficient power for performing these analyses. The Committee on Evolutionary Biology at the University of Chicago provides a Linux server for genomic-scale studies that is frequently used by the Co-PI. With 16 3.0 GHz cores, 128 GB of RAM and 12 TB of storage space, this machine is sufficient for the analyses to be performed for this project. For access to pre-developed RNASeq analysis pipelines or when more computational power is required, the Co-PI and PI also have access to the

University of Chicago's Center for Informatics Research's large memory server with 80 cores and one TB of RAM.
Office: The PI and Co-PI have dedicated office space at the University of Chicago provided by the Department of Ecology and Evolution. Office space and wireless internet are available to visiting researchers at the CSRR.

DATA MANAGEMENT PLAN

Biological and Chemical Data

Data to be generated: Data collected for this project will be biological measurements made on Olympia oyster larvae and juveniles, sequencing data (described further below), and descriptions of physical properties of seawater in the experimental treatments. Biological measurements will include counts of larvae to compare reproductive output, survival, size, and developmental stage. For size measurements of larvae, pictures will be taken through a microscope with a calibrated ocular micrometer. Seawater data will include temperature, salinity, pH, dissolved inorganic carbon, total alkalinity, and CO_2 concentration.

Storage: Biological and chemical measurements will be recorded in waterproof paper notebooks and transferred immediately to Google Sheets datasheets with backups on the Co-PI's personal computer. Pictures taken for size will be stored on both the Co-PI's personal computer and through the online cloud service Dropbox.

Availability: These datasheets, associated metadata (collection methods, daily observations, photo records), and links to Dropbox folder with images will be available through the Co-PI's publicly accessible online notebook (http://marinegenes.com/category/lab-notebook/), which is regularly backed up and archived using the XML export option to a personal hard drive. After completion of experiments and appropriate quality assurances are conducted on chemical measurements, the data will be uploaded to public data repositories including Dryad where they will be permanently stored.

Sequence Data

Data description: Millions of short read genomic and transcriptomic sequences will be generated by the Illumina Hi-Seq platform. The raw sequencing data will be in fastq format where both quality and nucleotide information is included. These files will be analyzed with SNP characterization software (pyRAD), gene expression analyses (GSEA, Bioconductor packages), and downstream statistical packages (python, R). Metadata including file name, data type, taxa, tissue, molecule, platform, length, description, and file locations will be organized in a backed-up Google Sheet on the Co-PI's online notebook.

Storage and Access: Raw data will be transferred and stored on the UChicago Committee on Evolutionary Biology's server with RAID redundancy during the Co-PI's graduate career. Within one month of acquiring raw data it will be uploaded into the NCBI Short Read Archive database for permanent storage and accessibility, along with metadata (description of sample, library preparation, and sequencing method). Data from secondary procedures including mapping and annotation will be available in real-time through the Co-PI's lab notebook. After publication and peer-reviewed quality control, analyzed data will be uploaded to appropriate publicly accessible databases including Gene Expression Omnibus and Dryad. All data will be released once the results are published or no later than three months after the project end date.

Puget Sound
RESTORATION FUND

382 Wyatt Way NE
Bainbridge Island, WA 98110
www.restorationfund.org
(206) 780 – 6947

September 25, 2015

TO: NSF Doctoral Dissertation Improvement Grant in the Directorate for Biological Sciences Program

FROM: Betsy Peabody, Executive Director, Puget Sound Restoration Fund

By signing below (or transmitting electronically), I acknowledge that I am listed as a collaborator on this proposal, entitled "DISSERTATION RESEARCH: A genomic and experimental characterization of local adaptation," with Catherine Pfister as the Principal Investigator. I agree to undertake the tasks assigned to me or my organization, as described in the project description of the proposal, and I commit to provide or make available the resources specified therein.

B Peabody

Signed: _____ Organization: Puget Sound Restoration Fund

Date: 9-25-15

THE UNIVERSITY OF CHICAGO
COMMITTEE ON EVOLUTIONARY BIOLOGY
1025 EAST 57TH STREET • CULVER HALL 401
CHICAGO • ILLINOIS 60637

tx: *773-702-9474*
fx: *773-702-4699*
***e-mail*:** **mcoates@uchicago.edu**

9/4/15

To Whom It May Concern:

Katherine Silliman has advanced to candidacy for a Ph.D. degree.

Sincerely,

Michael I. Coates
Professor and Chair

Context for Improvement

The goal of my research is to study intraspecific adaptation across heterogeneous environments in a system with high potential for gene flow. I am using a combination of genomic and experimental approaches to investigate the spatial scale of both neutral and adaptive differentiation, as well as identify candidate adaptive loci in an ecologically important aquatic invertebrate. To characterize the demographic population structure in *Ostrea lurida*, I sampled 20 sites from San Diego, CA to northwest Vancouver Island, Canada in summer 2014 and have sequenced Genotype-by-Sequencing (GBS) libraries of 228 individuals. In collaboration with the Roberts lab at University of Washington, I conducted common garden experiments with 2nd generation, hatchery-raised oysters from three subpopulations in Puget Sound, WA during summer 2015. In addition to investigating local adaptation on a fine scale, this project provided invaluable experience in animal husbandry and designing environmental stressor experiments.

The proposed DDIG research adds critical breadth to my dissertation by extending my experimental assessments of adaptation to include demographically distinct populations encompassing a wide range of environment factors. These common garden experiments will provide a more comprehensive picture of the spatial scale of adaptation than can be determined from the Puget Sound experiments alone. The transcriptomic data generated from this proposal will also be integrated with the previously assembled GBS data to identify candidate molecular markers for assessing resilience to ocean acidification. Although I only have preliminary results from my previous projects, the nature of grant-writing and fieldwork dictates that I apply for a DDIG at the start of my third year or risk extending my dissertation past the coverage of support from my NSF GRFP.

My advisor, Catherine Pfister, has expertise in population ecology, climate change biology, and has been working on the eastern Pacific coast for over 25 years. Her current research and funding is focused on functional diversity in animal-microbe interactions and the impacts of climate change on kelp forests; therefore, my line of research is significantly distinct from her own. I obtained funding for my 2014 field season and subsequent molecular work through small internal (University of Chicago Hinds Fund, Pritzker Lab Award) and external (National Shellfisheries Association and American Fisheries Society) grants. For my Puget Sound common garden in 2015, I received some funding through the Roberts lab NOAA Sea Grant as my research goals intersected with theirs. However, the NOAA funding will expire in January 2016 and they do not have funding for my proposed project.

Timeline

Oct 2015 –Feb 2016: Finish population genetic analysis of GBS data, including identification of F_{ST}-outlier loci and estimation of linkage disequilibrium. Prepare and submit manuscript.
Nov 2015 – May 2016: Conduct cold shock experiment on Puget Sound oysters, prepare RNASeq and larval GBS libraries, finish analyzing fitness and gene expression data.
June 2016- Oct 2016: Common garden experiments with CA, OR, WA, and BC populations in Manchester, WA.
Nov 2016 – Dec 2016: Prepare RNASeq libraries from pH and temperature experiments.
Jan 2017 – Nov 2017: Analyze fitness and gene expression data from range-wide common garden experiment. Integrate genomic and transcriptomic analyses from both sets of common experiments to identify candidate adaptive loci.
Dec 2017-Mar 2018: Prepare and submit manuscript. Develop workshop on local adaptation and marker-based approaches to restoration management.