DEB - Evolutionary Ecology

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NAME OF ORGANIZATION TO WHICH AWARD SHOULD BE MADE ADDRESS OF Awardee ORGANIZATION, INCLUDING 9 DIGIT ZIP CODE
Duke University 2200 W. Main St, Suite 710 Durham, NC. 277054010

Awardee ORGANIZATION CODE (IF KNOWN)
0029207000

NAME OF PRIMARY PLACE OF PERF ADDRESS OF PRIMARY PLACE OF PERF, INCLUDING 9 DIGIT ZIP CODE
Duke University Durham ,NC ,277054010 ,US.

IS Awardee ORGANIZATION (Check All That Apply)

☐ SMALL BUSINESS ☐ FOR-PROFIT ORGANIZATION ☐ MINORITY BUSINESS ☐ WOMAN-OWNED BUSINESS ☐ IF THIS IS A PRELIMINARY PROPOSAL THEN CHECK HERE

TITLE OF PROPOSED PROJECT DISSERTATION RESEARCH: The Genetics of Adaptation to a Novel Stressful Environment in Mimulus

REQUESTED AMOUNT PROPOSED DURATION (1-60 MONTHS) REQUESTED STARTING DATE SHOW RELATED PRELIMINARY PROPOSAL NO. IF APPLICABLE

$ 14,371 24 months 05/01/12

CHECK APPROPRIATE BOX(ES) IF THIS PROPOSAL INCLUDES ANY OF THE ITEMS LISTED BELOW

☐ BEGINNING INVESTIGATOR (GPG I.G.2) ☐ HUMAN SUBJECTS (GPG II.D.7) Human Subjects Assurance Number _____________
☐ DISCLOSURE OF LOBBYING ACTIVITIES (GPG II.C.1.e) Exemption Subsection ______________ or IRB App. Date ______________
☐ PROPRIETARY & PRIVILEGED INFORMATION (GPG II.D.1) ☐ INTERNATIONAL COOPERATIVE ACTIVITIES: COUNTRY/COUNTRIES INVOLVED
☐ HISTORIC PLACES (GPG II.C.2) ) ☐ HIGH RESOLUTION GRAPHICS/OTHER GRAPHICS WHERE EXACT COLOR
☐ EAGER* (GPG II.D.2) ☐ RAPID** (GPG II.D.1) REPRESENTATION IS REQUIRED FOR PROPER INTERPRETATION (GPG I.G.1)
☐ VERTEBRATE ANIMALS (GPG II.D.6) IACUC App. Date __________________

PHS Animal Welfare Assurance Number _____________

PI/PD DEPARTMENT Department of Biology

PI/PD POSTAL ADDRESS 3314 French Science Center, Science Dr. Box 90338 Durham, NC 277080338 United States

PI/PD FAX NUMBER 919-660-7293

PI/PD NAME

John H Willis PhD 1991 919-660-7340 jwillis@duke.edu

CO-PI/PD

Kathleen Ferris BA 2007 919-684-3030 kgferris@gmail.com

CO-PI/PD

CO-PI/PD

CO-PI/PD
Certification for Authorized Organizational Representative or Individual Applicant:

By signing and submitting this proposal, the Authorized Organizational Representative 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 debarment and suspension, drug-free workplace, lobbying activities (see below), responsible conduct of research, nondiscrimination, and flood hazard insurance (where applicable) as set forth in the NSF Proposal & Award Policies & Procedures Guide, Part I: the Grant Proposal Guide (GPG) (NSF 11-1). 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).

Conflict of Interest Certification

In addition, if the applicant institution employs more than fifty persons, by electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative of the applicant institution is certifying that the institution has implemented a written and enforced conflict of interest policy that is consistent with the provisions of the NSF Proposal & Award Policies & Procedures Guide, Part II, Award & Administration Guide (AAG) Chapter IV.A; that to the best of his/her knowledge, all financial disclosures required by that conflict of interest policy have been made; and that all identified conflicts of interest will have been satisfactorily managed, reduced or eliminated prior to the institution’s expenditure of any funds under the award, in accordance with the institution’s conflict of interest policy. Conflicts which cannot be satisfactorily managed, reduced or eliminated must be disclosed to NSF.

Drug Free Work Place Certification

By electronically signing the NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant is providing the Drug Free Work Place Certification contained in Exhibit II-3 of the Grant Proposal Guide.

Debarment and Suspension Certification

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

Certification Regarding Lobbying

The following 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 loan, 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 NSF Proposal Cover Sheet, the Authorized Organizational Representative or Individual Applicant 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 NSF Proposal Cover Sheet, the Authorized Organizational Representative 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 NSF Proposal Cover Sheet, the Authorized Organizational Representative of the applicant institution 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 undersigned shall require that the language of this certification be included in any award documents for all subawards at all tiers.

<table>
<thead>
<tr>
<th>AUTHORIZED ORGANIZATIONAL REPRESENTATIVE</th>
<th>SIGNATURE</th>
<th>DATE</th>
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<tbody>
<tr>
<td>Karolina Angell</td>
<td>Electronic Signature</td>
<td>Nov 10 2011 4:31PM</td>
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* EAGER - Early-concept Grants for Exploratory Research
** RAPID - Grants for Rapid Response Research
**Directorate for Biological Sciences**  
**Division of Environmental Biology**  
**Evolutionary Ecology**  

**Proposal Classification Form**  
**PI: Willis, John / Proposal Number: 1210755**

### 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
- Earth Science
- 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
  - Biostratigraphhy
  - Palynology
  - Micropaleontology
  - Paleoclimatology
  - Archeozoic
  - Paleozoic
  - Mesozoic
### CATEGORY IV: INFRASTRUCTURE (Select 1 to 3)

<table>
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<tr>
<th>COLLECTIONS/STOCK CULTURES</th>
<th>Field Stations</th>
<th>Technique Development</th>
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<td>Natural History Collections</td>
<td>Field Facility Structure</td>
<td>TRACKING SYSTEMS</td>
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<td>DATABASES</td>
<td>Field Facility Equipment</td>
<td>Geographic Information Systems</td>
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<tr>
<td>FACILITIES</td>
<td>LTER Site</td>
<td>Remote Sensing</td>
</tr>
<tr>
<td>Controlled Environment Facilities</td>
<td>INDUSTRY PARTICIPATION</td>
<td>NONE OF THE ABOVE</td>
</tr>
</tbody>
</table>

### CATEGORY V: HABITAT (Select 1 to 2)

#### TERRESTRIAL HABITATS

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

### 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)
  - Alaska
  - Hawaii
  - Puerto Rico
  - Canada
  - Mexico
- Central America (Mainland)
  - Caribbean Islands
  - Bermuda/Bahamas
- South America

**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**
- Archaeabacteria
- Cyanobacteria
- Eubacteria

**PROTOISTA (PROTOZOA)**
- Amoebae
- Apicomplexa
- Ciliophora
- Flagellates
- Foraminifera
- Microspora

**FUNGI**
- Ascomycota
- Basidiomycota
- Chytridomycota
- Mitosporic Fungi
- Oomycota
- Zygomycota

**ALGAE**
- Bacillariophyta (Diatoms)
- Charophyta
- Chlorophyta
- Chrysophyta

**PLANTS**
- NON-VASCULAR PLANTS
- Bryophyta
- Anthocerota (Hornworts)
- Hepaticae (Liverworts)
- Musci (Mosses)
- VASCULAR PLANTS
- Ferns & Fern Allies
- Gymnosperms
- Coniferales (Conifers)
- Cycadales (Cycads)
<table>
<thead>
<tr>
<th>Ginkgoales (Ginkgo)</th>
<th>Polypolwphora (Chitons)</th>
<th>Coleoaptera (Beetles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnetales (Gnetophytes)</td>
<td>Scaphopoda (Tooth Shells)</td>
<td>Hymenoptera (Ants, Bees, Wasps, Sawflies)</td>
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<tr>
<td>ANGIOSPERMS</td>
<td>Gastropoda (Snails, Slugs, Limpets)</td>
<td>Chilopoda (Centipedes)</td>
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<tr>
<td>Monocots</td>
<td>Pelecypoda (Bivalvia) (Clams, Mussels, Oysters, Scallop)</td>
<td>Diplopoda (Millipedes)</td>
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<tr>
<td>Arecaceae (Palmae)</td>
<td>Cephalopoda (Squid, Octopus, Nautilus)</td>
<td>Pauropoda</td>
</tr>
<tr>
<td>Cyperaceae</td>
<td>ANNELIDA (Segmented Worms)</td>
<td>Symphyta (Symphylla)</td>
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<tr>
<td>Liliaceae</td>
<td>Polychaeta (Parapodial Worms)</td>
<td>PENTASTOMIDA (Lingualulida) (Tongue Worms)</td>
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<tr>
<td>Orchidaceae</td>
<td>Oligochaeta (Earthworms)</td>
<td>TARDIGRADA (Tardigrades, Water Bears)</td>
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<tr>
<td>Poaceae (Graminae)</td>
<td>Hirudinida (Leeches)</td>
<td>ONYCHOPHORA (Peripatus)</td>
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<tr>
<td>Dicots</td>
<td>POLYGONOPHORA (Beard Worms)</td>
<td>CHAETOGNATHA (Arrow Worms)</td>
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<td>Apiaceae (Umbelliferae)</td>
<td>SIPUNCULOIDEA (Peanut Worms)</td>
<td>ECHINODERMATA</td>
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<td>Asteraceae (Compositae)</td>
<td>ECHIUROIDEA (Spoon Worms)</td>
<td>Crinoidea (Sea Lilies, Feather Stars)</td>
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<td>Brassicaceae (Cruciferae)</td>
<td>ARTHROPODA</td>
<td>Asteroida (Starfish, Sea Stars)</td>
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<td>Fabaceae (Leguminosae)</td>
<td>Cheliceriformes</td>
<td>Ophiuroidea (Brittle Stars, Serpent Stars)</td>
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<td>Lamiales (Labiatae)</td>
<td>Merostomata (Horseshoe Crabs)</td>
<td>Echinoida (Sea Urchins, Sand Dollars)</td>
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<tr>
<td>Rosaceae</td>
<td>Pycnogonida (Sea Spiders)</td>
<td>Holothuroidea (Sea Cucumbers)</td>
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<td>Solanaceae</td>
<td>Cirripedia (Barnacles)</td>
<td>HEMICHORDATA (Acorn Worms, Pterobranchs)</td>
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<tr>
<td>ANIMALS</td>
<td>Amphipoda (Skeleton Shrimp, Whale Lice, Freshwater Shrimp)</td>
<td>UROCHORDATA (Tunicata) (Tunicates, Sea Squirts, Salps, Ascidians)</td>
</tr>
<tr>
<td>INVERTEBRATES</td>
<td>Isopoda (Wood Lice, Pillbugs)</td>
<td>CEPHALOCHORDATA (Amphioxus/Lancelet)</td>
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<tr>
<td>MESOZOA/PLACOZOA</td>
<td>Decapoda (Lobster, Crayfish, Crabs, Shrimp)</td>
<td>VERTEBRATES</td>
</tr>
<tr>
<td>PORIFERA (Sponges)</td>
<td>Hexapoda (Insecta) (Insects)</td>
<td>AGNATHA (Hagfish, Lamprey)</td>
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<tr>
<td>CNIDARIA</td>
<td>Apterygota (Springtails, Silverfish, etc.)</td>
<td>FISHES</td>
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<tr>
<td>Hydrozoa (Hydra, etc.)</td>
<td>Odonata (Dragonflies, Damselflies)</td>
<td>Chondrichthyes (Cartilaginous Fishes) (Sharks, Rays, Raffish)</td>
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<tr>
<td>Scyphozoa (Jellyfish)</td>
<td>Anura (Frogs, Toads)</td>
<td>Osteichthyes (Bony Fishes)</td>
</tr>
<tr>
<td>Anthozoa (Corals, Sea Anemones)</td>
<td>Urodela (Salamanders, Newts)</td>
<td>AMPHIBIA</td>
</tr>
<tr>
<td>CTENOPHORA</td>
<td>Gymnophiona (Apoda) (Caecilians)</td>
<td>Anura (Frogs, Toads)</td>
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<tr>
<td>PLANYHELMINTHES (Flatworms)</td>
<td>Amphibia</td>
<td>Urodela (Salamanders, Newts)</td>
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<tr>
<td>Turbellaria (Planarians)</td>
<td>Chelonia (Turtles, Tortoises)</td>
<td>Gymnophiona (Apoda) (Caecilians)</td>
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<tr>
<td>Trematoda (Flukes)</td>
<td>Serpentes (Snakes)</td>
<td>REPTILIA</td>
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<td>Cestoda (Tapeworms)</td>
<td>Sauria (Lizards)</td>
<td>Chelonida (Turtles, Tortoises)</td>
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<td>Monogenea (Flukes)</td>
<td>Crocodilia (Crocodilians)</td>
<td>AVES (Birds)</td>
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<td>GNATHOSTOMULIDA</td>
<td>Monotremata (Platypus, Echidna)</td>
<td>Passeriformes (Passerines)</td>
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<td>NEMERTINEA (Rynchocoela) (Ribbon Worms)</td>
<td>Marsupialia (Marsupials)</td>
<td>MAMMALIA</td>
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<td>ENTOPROCTA (Bryozoa) (Plant-like Animals)</td>
<td>Eutheria (Placentals)</td>
<td>Monotremata (Platypus, Echidna)</td>
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<td>ASCHELMITHES</td>
<td>Insectivora (Hedgehogs, Moles, Shrews, Tenrec, etc.)</td>
<td>Marsupialia (Marsupials)</td>
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<tr>
<td>Gastrotricha</td>
<td>Primates</td>
<td>Eutheria (Placentals)</td>
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<tr>
<td>Kinorhyncha</td>
<td>Humans</td>
<td>Insectiforma (Hedgehogs, Moles, Shrews, Tenrec, etc.)</td>
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<tr>
<td>Loricifera</td>
<td>Rodentia</td>
<td>Chiroptera (Bats)</td>
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<tr>
<td>Nematoda (Roundworms)</td>
<td>Lagomorphs (Rabbits, Hares, Pikas)</td>
<td>Primates</td>
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<tr>
<td>Nematomorpha (Horsehair Worms)</td>
<td>Carnivora (Bears, Canids, Felids, Mustelids, Viverrids, Hyena, Procyonids)</td>
<td>Humans</td>
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<tr>
<td>Rotifera (Rotatoria)</td>
<td>Perissodactyla (Odd-toed Ungulates) (Horses, Rhinos, Tapirs, etc.)</td>
<td>Rodentia</td>
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<tr>
<td>ACANTHOCEPHALA (Spiny-headed Worms)</td>
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<td>Lagomorphs (Rabbits, Hares, Pikas)</td>
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<td>PRIAPULOIDEA</td>
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<td>Carnivora (Bears, Canids, Felids, Mustelids, Viverrids, Hyena, Procyonids)</td>
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<tr>
<td>Artiodactyla (Even-toed Ungulates) (Cattle, Sheep, Deer, Pigs, etc.)</td>
<td>TRANSGENIC ORGANISMS</td>
<td>FOSSIL OR EXTINCT ORGANISMS</td>
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<tr>
<td>---------------------------------------------------------------</td>
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<td>-----------------------------</td>
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<tr>
<td>Marine Mammals (Seals, Walrus, Whales, Otters, Dolphins, Porpoises)</td>
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**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)
**Intellectual Merit**

How do species adapt to new and possibly stressful environments? What genes evolve in response to this novel selection pressure and what traits do they influence? These questions are of great interest to evolutionary biologists since adaptation to a new habitat can maintain genetic variation or eventually lead to ecological speciation. While many studies have demonstrated that different species or populations are adapted to a particular habitat through reciprocal transplant experiments, few studies have elucidated the genes and their causal traits that contribute to this adaptation. This is because within a species or population each locus, and the trait it underlies, is correlated with many others due to demography. Genetic manipulation can disentangle this correlation and elucidate the adaptive significance of an individual locus or trait. The *Mimulus guttatus* species complex is an excellent system in which to do this because species and populations occupy a diversity of habitats, are genetically and morphologically divergent, but largely inter-fertile, and there is a completely sequenced and annotated genome. *Mimulus laciniatus* occurs in a novel exposed, dry granite outcrop habitat while its close round-leaved relative *M. guttatus* occurs in moist seeps and streams. *M. laciniatus* has several life history traits that are widely accepted to be advantageous in this dry and light intensive environment including early flowering under a shorter critical photoperiod than *M. guttatus*, and floral traits associated with self-fertilization. *M. laciniatus* is also the only known species in the entire genus *Mimulus* with a lobed leaf shape which should be adaptive in its dry, exposed granite outcrops. Lobed leaves have a lower boundary layer than round ones and consequently stay closer to air temperature. This can prevent excess water loss by reducing transpiration rates and keep leaves from freezing as they radiate heat to the cold night sky. Studies in other species have been unable to rigorously demonstrate whether these physiological characteristics of lobed leaves are adaptive in nature. The following proposed research is designed to test the adaptive significance of genes underlying leaf shape in *M. laciniatus*. **Research Objective 1: Adaptive Significance of Leaf Shape:** The fitness and physiological affects of leaf shape will be tested by reciprocal transplants in the field using recombinant F3s and Near Isogenic Lines that differ in QTL alleles for leaf shape. **Research Objective 2: Parallel Leaf Shape Evolution:** In order to determine whether lobed leaves are an example of adaptive parallel evolution the genus *Mimulus*, QTLs underlying leaf shape in a recently discovered divergent race of lobe-leaved *Mimulus* will be mapped using bulk segregant analysis and Illumina sequencing.

**Broader Impacts**

This project will significantly contribute to knowledge about the genetic basis of adaptation to different environments. It will also increase scientific knowledge in the local and broader community by providing mentoring opportunities for middle school, high school, and undergraduate students, especially women and minorities, through programs like Women in Math Mentoring and the Summer Research Opportunities Program. This research will be disseminated to the scientific community at large through annual conferences. Being a discreet morphological trait, leaf shape could also serve as an easily accessible example of adaptation for grade school level science classes. Understanding how plants adapt to a harsh environment also has important implications for both agriculture and conservation, especially in light of climate change.
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RESPONSE TO PREVIOUS REVIEWS

Past reviewers expressed 3 main concerns. First some questioned the broader evolutionary significance of leaf shape. I can appreciate these concerns and have clarified the broader aims of my dissertation project, which is to understand how organisms adapt to new environments. I have broadened the scope of my research to include multiple potentially adaptive traits in granite outcrops and identified how my research will contribute to the field of evolutionary biology. Second, there were concerns that I was proposing to construct Near Isogenic Lines (NILs) without knowing how many QTLs controlled leaf shape. I have now mapped three QTLs for leaf shape and am introgressing all three into my NILs. Third the practicality of reciprocal transplant experiments in granite outcrops was questioned. Common garden experiments have previously been performed in *M. laciniatus*’s granite outcrop habitat (see 72). *Mimulus* naturally grows in very dense clumps. I plan to plant seedlings, which are very small and have shallow roots, in early spring when outcrops are still moist from snowmelt, which should minimize transplant shock.

OVERVIEW

Closely related populations or species often occupy ecologically disparate habitats. How do different populations or species adapt to these new and initially stressful environments? What genes evolve in response to this novel selection pressure and what traits do they influence? These are exciting questions since adaptation to a new habitat can maintain genetic variation within species (13, 42, 59, 29, 34) or eventually lead to ecological speciation (49, 50, 65, 17, 64). Adaptation to different habitats at the organismal level can be accomplished through fitness trade-offs at particular loci (antagonistic pleiotropy) or conditional neutrality where a allele has a fitness advantage in its native environment but is neutral in the other (Figure 1). Antagonistic pleiotropy can maintain genetic variation within a species, conditional neutrality will not. To test which of these factors is responsible for differential habitat adaptation it is necessary to test the fitness affects of alleles in their natural environments (1). Many studies have demonstrated that different species or populations are adapted to a particular habitat through reciprocal transplant experiments, but few have elucidated the specific genes and traits that contribute to this adaptation (reviewed in 38 and 35). Conversely, the genetic basis of many potentially adaptive traits have been uncovered in the lab, but the fitness effects of these loci are rarely tested in nature (41, 54, 16, 63, 14, but see 11). Combining QTL mapping, genetic manipulation, and field experiments can disentangle loci from their genetic backgrounds, elucidate the individual loci and traits that contribute to adaptation, and test whether differential habitat adaptation is produced by antagonistic pleiotropy or conditional neutrality.

Plant species are excellent systems for studying the genetics of differential habitat adaptation. Because of their sessile lifestyle, plants often experience strong divergent selection across heterogeneous environments on a small geographical scale (37, 68, 75). Members of the *Mimulus guttatus* species complex occupy a variety of edaphic environments. Most members of the species complex occur in moist seep and streambeds, but some occur in rapidly drying habitats such as serpentine soils, copper mine tailings, and granite outcrops. While adaptation to serpentine and copper has been well studied in *Mimulus*, little is known about adaptation to granite outcrops. Granite outcrops are characterized by shallow rocky soils, high light intensity, and low soil water retention.

The Sierra Nevada native *Mimulus laciniatus* is the most famous granite outcrop endemic in the *M. guttatus* species complex, but we have recently discovered an independent as yet
unnamed taxon, Bald Rock *Mimulus*, that also occurs in a granite outcrop habitat. *M. laciniatus* and Bald Rock possess traits that are likely adaptive in granite outcrops where soils are only a few millimeters deep and dry rapidly once the seasonal snowmelt is gone. These traits are rapid flowering under shorter critical photoperiods (26) and a self-fertilizing mating system. Both taxa also have a lobed leaf shape that distinguishes them from the rest of the genus *Mimulus* and could be an important adaptive trait in these habitats for reasons listed below. The closely related round-leaved *M. guttatus* occurs in deeper, moist seep and streambed soils within meters of these granite outcrops (Figure 2).

My dissertation research addresses how plants adapt to granite outcrop environments. I am dissecting the genetic bases of flowering time, critical photoperiod, floral traits associated with self-fertilization, and leaf shape, and testing the adaptive significance of alleles underlying critical photoperiod and leaf shape in the field. My work on flowering time, critical photoperiod, and floral morphology is supported by another source and is consequently not the subject of this proposal. Here I focus on the adaptive significance of leaf shape. Since lobed leaf shape is unique to granite outcrop *Mimulus* taxa and is seemingly an example of parallel phenotypic evolution it is likely a key trait in adaptation to this habitat. Leaf shape affects the thickness of the leaf boundary layer with lobed leaves having thinner boundary layers than round leaves (30, 69, 57). A thin boundary layer allows a leaf to be heated or cooled more efficiently by convection (30, 57). This can prevent water loss through transpirational cooling or keep leaves above freezing when they radiate heat to a cold night sky which could both be advantageous in *M. laciniatus*’s exposed, dry granite outcrops.

I propose to use QTL mapping, genetic manipulation, reciprocal transplant experiments, and environmental manipulation in the field to test the adaptive significance of leaf shape alleles in *M. laciniatus* and Bald Rock *Mimulus*, and to look for evidence of antagonistic pleiotropy or conditional neutrality at these alleles.

**Research Objectives**

**Objective 1: The adaptive significance of leaf shape in *M. laciniatus***

* A) To determine whether leaf shape is adaptive in *M. laciniatus*’s native habitat and whether leaf shape alleles exhibit antagonistic pleiotropy or conditional neutrality I will plant recombinant F3s and Near Isogenic Lines differing in leaf shape in reciprocal transplants in the field.

* B) To determine how lobed leaf shape is adaptive I will perform manipulative field experiments that test whether leaf shape increases fitness through drought or freeze tolerance.

**Objective 2: Parallel leaf shape evolution in the *M. guttatus* complex***

The occurrence of parallel evolution is strong evidence that a phenotype is adaptive. We have recently discovered a distinct *Mimulus* taxon, Bald Rock (BR), which has very dissected leaves and occurs in *M. laciniatus*-like habitat. To ascertain if BR is an example of parallel evolution at the phenotypic and genotypic levels I will genetically map QTLs underlying this convergent leaf phenotype.

**Background**

**Adaptive Significance of Leaf Shape Variation**

In my dissertation I am studying how plants in the genus *Mimulus* adapt to dry, light intensive granite outcrop environments. Traits that are likely to be adaptive in these habitats include early flowering, self-fertilization, and leaf shape. Early flowering allows plants to reproduce before the
onset of summer drought (39, 25, 47, 33). Self-fertilization is often associated with occupation of drier habitats (39, 47, 51, 80). Small flower size and rapid floral development are correlated with self-fertilization and may reduce floral tissue transpiration costs (28). Research proposed here focuses on the adaptive significance of leaf shape in granite outcrops. Leaf shape varies extensively within and between plant species. Because leaf shape affects the physical properties of a leaf, and leaves are the primary site of gas exchange, water loss and photosynthesis, it should affect plant fitness. Lobed leaves are a striking example of leaf shape variation and have been of particular interest to plant evolutionary biologists (3, 78, 31, 62, 56, 5). Three popular hypotheses about the adaptive significance of lobed leaves are: 1) lobed leaves contribute to drought tolerance (30, 81, 57, 3, 2) lobed leaves contribute to freeze tolerance (19, 57, 4) lobed leaves decrease herbivory (12). Despite interest in the adaptive significance of lobed leaf shape, few have genetically isolated this trait to assess its contribution to fitness (but see 11).

The first and second hypotheses are based on the fact that lobed leaves have a reduced boundary layer in comparison to rounded leaves. The boundary layer is a region of immobile air adjacent to the surface of a leaf. Lobed leaves have smaller boundary layers because every point on the leaf is closer to an edge, or in other words they have a larger perimeter to area ratio (30, 69, Figure 3). A smaller boundary layer allows for more efficient heat transfer by convection between a leaf and the surrounding air which keeps leaf temperatures closer to air temperature (30, 57). This may be adaptive in granite outcrop habitats because in exposed sunny environments leaves are heated above ambient temperature by the absorption of direct solar radiation. High leaf temperatures can impair photosynthesis and endanger protein stability. The primary mechanisms of leaf cooling are radiation, convection, and transpiration. In light intensive environments like granite outcrops, lobed leaves can be cooled more effectively by convection than round leaves (30, 57), and this should lead to less water being lost through evaporative cooling. Lower leaf temperatures and the subsequent lower levels of water loss should give plants with lobed leaves a fitness advantage in dry granite outcrop habitats.

Lobed leaves may also contribute to freeze tolerance because nighttime air temperatures often fall near freezing early in the growing season. On clear nights leaves in exposed, open areas like granite outcrops radiate heat to the cold sky. This heat loss can cause leaf temperatures to fall below freezing even when air temperatures are above 0° C (57), a phenomenon discussed by Darwin (19). Because of their reduced boundary layer lobed leaves should stay closer to air temperature, and thus warmer, at night than round leaves. This could keep lobed leaves from freezing on chilly nights early in the growing season in granite outcrop habitats.

The third adaptive hypothesis suggests that lobed leaves defend against herbivores and pathogens (12, 10). It was proposed that herbivores avoid dissected leaves because of reduced foraging efficiency (12). However, there is scant and contradictory empirical evidence for this hypothesis (61). Given what is known about *M. laciniatus*’s granite habitat, this proposal focuses on testing the drought and freeze tolerance adaptive hypotheses.

**STUDY SYSTEM AND PRELIMINARY DATA**

The *Mimulus guttatus* species complex is an excellent system in which to study the genetics and ecology of adaptation. Species and subspecies in the complex occur across western North America and display a great deal of divergence in morphology, ecology, and mating system, but are still largely inter-fertile (79). *M. guttatus* contains a wealth of genetic resources including an annotated genome sequence, thousands of polymorphic markers, and methods for genetic transformation. Recently our lab has developed highly efficient methods for genotyping and QTL mapping using Illumina full genome re-sequencing (43, 53, 22, 48). Together these facts make this species complex ideal for understanding adaptive divergence at both the phenotypic and genetic levels.
**M. guttatus** is a round-leaved outcrossing species that grows in streambeds and seeps across western North America, occurring only where there is substantial water availability. While *M. guttatus* grows throughout the Sierra Nevada Mountain range it is never found within the granite outcrops that speckle the mountainsides, but always nearby in deeper moist soil. However *M. laciniatus*, a closely related self-fertilizing annual endemic to the central and southern Sierra Nevadas, occurs on thin strips of moss in the middle of these exposed granite outcrops subject to harsh seasonal drought (Figure 2). *M. laciniatus* has a unique lobed leaf shape and occurs from 3000-9000 feet in elevation. A preliminary reciprocal transplant between *M. laciniatus* and *M. guttatus* found that they are adapted to different micro-habitats (71). *M. laciniatus* was thought to be the only member of the genus with a lobed leaf shape but we recently discovered a distinct, currently called Bald Rock *Mimulus*, that has extremely lobed leaves and occupies a granite outcrop habitat.

### The Evolutionary History of M. laciniatus and Bald Rock

To investigate the evolutionary history of *M. laciniatus* I sequenced nuclear loci in and performed phylogenetic analyses with multiple populations of three species within the *M. guttatus* species complex: *M. laciniatus*, *M. guttatus*, and *M. nasutus*. An exciting result of this analysis was the discovery of a divergent *M. laciniatus*-like population, Bald Rock (BR). *M. laciniatus* populations grouped together and were closely related to *M. nasutus*, but BR did not group with *M. laciniatus* populations in neighbor joining trees at 6 out of 7 nuclear loci (22). Bald Rock is over 100 miles away from the nearest known *M. laciniatus* population and has traits that distinguish it from *M. laciniatus* including stem shape, flower size, and extreme leaf lobing (Figure 4, 70, 21). This suggests that Bald Rock may represent an independent origin of lobed leaf shape in the *M. guttatus* species complex.

### Preliminary Field Work

**M. laciniatus** occupies a dry, cold, and light intensive environment

In order to empirically characterize the abiotic granite outcrop environment I measured environmental variables in *M. laciniatus* and *M. guttatus* habitats in the Sierra National Forest. Through measuring soil moisture, soil temperature, ground irradiance, and solar irradiance along transects in both habitats at 7 sites I found that *M. laciniatus*'s environment is significantly drier and more light intensive than *M. guttatus*'s (MANOVA, p-value <0.0001). High light intensity combined with low water availability creates a harsh plant environment since solar radiation raises leaf temperature and water is needed to cool leaves through transpiration. I also found significantly colder ground temperatures in *M. laciniatus*'s habitat than in *M. guttatus*'s (MANOVA, p-value <0.0001).

**M. laciniatus** leaves stay warmer than *M. guttatus* in granite outcrops

I performed a small reciprocal transplant and used thermocouples to measure leaf and air temperatures between adjacent *M. laciniatus* and *M. guttatus* habitats (∼2 meters apart). In the *M. laciniatus* habitat *M. laciniatus* leaves stayed significantly warmer and closer to air temperature throughout the 24 hours than *M. guttatus* leaves (Figure 5). In the *M. guttatus* habitat there was no real temperature difference between *M. laciniatus* and *M. guttatus* leaves (Figure 5) and air temperature was not recorded due to a datalogger malfunction.
Due to their smaller boundary layer, *M. laciniatus*’s lobed leaves can be cooled or warmed more efficiently by convection than the round leaves of *M. guttatus* (57). Cooling *M. laciniatus* leaves by convection may reduce transpiration rates and be adaptive during periods of drought in *M. laciniatus*’s rocky outcrop habitat. In *M. laciniatus*’s high altitude habitat nighttime temperatures often fall near 0°C during the spring and early summer. At night, when *M. laciniatus*’s leaves radiate heat to the cold sky, convective heating may keep them from freezing in open granite outcrops. Thus, lobed leaves may be adaptive in *M. laciniatus*’s open granite habit. My leaf temperature reciprocal transplant lends preliminary support for this hypothesis.

**Genetics of *M. laciniatus* leaves**

The genetics of *M. laciniatus*’s lobed leaf shape were first investigated in the dissertation of Robert Vickery in the 1950’s. Vickery categorized leaf shape phenotypes in an *M. laciniatus* x *M. guttatus* F2 population using a semi-quantitative scale. Based on the phenotypic distribution he hypothesized that lobed leaves had a simple genetic basis (77).

**Leaf Shape QTL Mapping Results**

To QTL map lobed leaf shape I created an F2 mapping population between the *M. laciniatus* inbred line WLF47 and *M. guttatus* inbred line IM62 (n=650). Leaf lobing was quantified by digital analysis of the difference between the area of each leaf’s convex hull and actual leaf area while controlling for size.

I have mapped QTLs underlying leaf shape in *M. laciniatus* using bulk segregant analysis (BSA) and SNP markers generated by Illumina re-sequencing. In BSA F2 hybrids with parental phenotypes are bulked into two separate pools and each pool is genotyped (52, 8, 4, 6, 24, 26). Illumina sequencing can generate millions of Single Nucleotide Polymorphism (SNP) markers in short reads all across the genome (36), and when combined with BSA creates a fast, powerful QTL mapping approach (48).

I found 3 major QTLs for leaf shape (Figure 6). Follow up analyses with genic markers on individual F2s were run to estimate effect size. QTLs on Linkage Group (LG) 4 and LG2 were of large and roughly equal effect, while the QTL on LG11 was of smaller effect (Figure 5). Minor QTLs of very small effect may not have been detected in this analysis, but are not the focus of this study.

Identified QTLs allow Near Isogenic Lines for leaf shape to be created with marker assisted selection.

**Future Candidate Gene Studies**

To determine whether any candidate genes for leaf lobing occurred within my QTLs I BLASTed gene sequences from *Arabidopsis* and tomato against the *M. guttatus* genome. I found that the candidate gene Petroselinum (PTS) occurs within my largest effect QTL on LG4. PTS is a novel KNOTTED1-LIKE HOMEOBOX (KNOX) gene first discovered in the tomato species *Solanum galapagense*, one of the plant species collected by Charles Darwin while he was in the Galapagos. Up-regulation of PTS is responsible for increased leaf dissection between two wild tomato species (40). There was a highly significant, positive association between genotype at an intronic marker tightly linked to PTS and degree of leaf lobing (one-way ANOVA, p <0.0001). This suggests that PTS may be partly responsible for *M. laciniatus*’s lobed leaf phenotype. In future experiments I will be able to

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Figure 5. Leaf Temperature Reciprocal Transplant: solid lines are mean daily temp, dashed are standard errors A) *M. laciniatus* habitat B) *M. guttatus* habitat.
determine the causal mutations and functional differences at this large effect QTL using a candidate gene approach.

**RESEARCH PLAN**

**Objective 1: The Adaptive Significance of Leaf Shape**

**Motivation and Experimental Overview**

To test the adaptive significance of lobed leaves and whether leaf shape loci exhibit antagonistic pleiotropy or conditional neutrality, reciprocal transplant experiments will be performed in the field (15, 9, 1). Year one: leaf shape alleles will be isolated from their native genetic background by making and planting recombinant F3s between *M. laciniatus* and *M. guttatus* in the field. Year two: Near Isogenic Lines (NILs), which take longer to make than F3s, will be ready for field experiments. F3s and NILs will be used to test the fitness effects and function of lobed leaves in reciprocal transplants and manipulative field experiments. If F3s or NILs with lobed leaves have higher fitness than round leaved F3s or NILs in the granite habitat, then it will strongly suggest that leaf shape is adaptive in *M. laciniatus*'s harsh environment. If F3s or NILs with *M. laciniatus* leaf shape alleles have a fitness trade off between habitats then leaf shape loci exhibit antagonistic pleiotropy. If there is no trade-off and *M. laciniatus* alleles are beneficial in their native habitat but neutral in the other then leaf shape loci exhibit conditional neutrality.

**NIL and Recombinant F3 Creation**

In year one I will construct a recombinant F3 population to test the effect of leaf shape alleles on fitness in a randomized genetic background (as in 11). Inbreeding depression is problematic in *M. guttatus* (23) and could have a confounding effect on F3 fitness. To combat this problem I am currently growing two populations of 350 F2s from crosses between two different *M. laciniatus* and *M. guttatus* inbred lines (F2 pop1:WLF47 x YVO6, F2 pop2: WLF68 x YVO27). I will genotype all 700 of these F2s at my three QTL loci using genic markers. I will then intercross F2s from the two populations that are homozygous for the parental alleles at all three loci. This will produce a population of F3 hybrids that are homozygous for either *M. laciniatus* or *M. guttatus* leaf shape alleles, but randomized with respect to the rest of the genome.

In year two I will construct NILs differing in leaf shape that will test the fitness effects of a leaf shape genes in isolation from their genetic background (9). Similar to the F3 crossing design above, two populations of leaf shape NILs are being created from crosses between two separate sets of *M. laciniatus* and *M. guttatus* inbred lines (NIL pop1:WLF47 x YVO6, NIL pop2: WLF68 x YVO27). F2s from each cross have been backcrossed reciprocally for 3 generations using phenotypic selection because I did not have leaf shape QTLs yet. The plants with the most *M. guttatus*-like or *M. laciniatus*-like leaves are selected for backcrossing to both parents in each generation. There will be a total of 5 backcross generations. For the future generations I will genotype each backcross at markers closely linked to each of the three leaf shape QTLs and choose the individuals with the appropriate parental alleles at all three loci. This will produce a population of F3 hybrids that are homozygous for either *M. laciniatus* or *M. guttatus* leaf shape alleles, but randomized with respect to the rest of the genome.
**M. guttatus** leaf shape alleles in *M. laciniatus* background 3) *M. laciniatus* leaf shape alleles with *M. laciniatus* background and 4) *M. laciniatus* leaf shape alleles with *M. guttatus* background. All three major leaf shape loci will be introgressed together into each NIL type.

Additional funding will allow me to construct recombinant F3s and NILs using marker-assisted selection which is more effective than phenotypic selection.

**Reciprocal Transplants: Are lobed leaves adaptive?**

To test whether a trait or locus is adaptive its individual effect upon fitness must be assessed in the field. I will perform two reciprocal transplant experiments over two years between *M. laciniatus*-like granite outcrop habitat and nearby *M. guttatus*-like seep habitat using recombinant F3s in year one and NILs in year two. I will look for fitness trade-offs between leaf shape alleles in order to test whether leaf shape loci exhibit antagonistic pleiotropy or conditional neutrality. All F3s and NILs will be planted in dense blocks at the first true leaf stage in the early spring when outcrops are still moist from snowmelt at two sites in the Sierra National Forest near the town of Shaver Lake, CA.

In year one 1000 recombinant F3s (500 of each homozygote) and 500 of each parental species will be planted in each parental habitat for a total of 2000 F3s at each transplant cite. In year two, 2000 NILs (500 of each type) and 500 of each parental species will be planted in each habitat for a total of 4000 NILs at each site. The Willis Lab has successfully performed reciprocal transplants of this scale before with *M. guttatus* (33, 44).

Plants will occur in the early spring and will be monitored through mid summer. Fitness will be measured by survival to flowering, flower number and seed set. Fitness data will be analyzed using ANOVAs with planned contrasts, one in each habitat, with recombinant F3 genotype as a factor (2 levels) or NIL genotype as a factor (4 levels). The planned contrasts will be between NIL types 2 & 4 and types 1 & 3. If F3s or NILs with lobed leaf alleles have higher fitness in the *M. laciniatus* habitat than those with round leaves then leaf shape is an adaptive trait in *M. laciniatus*. Furthermore, if F3s or NILs with *M. laciniatus*-like leaves have higher fitness than round leaved F3s or NILs in the granite outcrop habitats, but lower fitness in in the moist seep habitat then leaf shape alleles exhibit antagonistic pleiotropy. If F3s or NILs with *M. laciniatus*-like leaves have higher fitness in the granite outcrops, but comparable fitness to round-leaved F3s or NILs in the moist seep habitat then leaf shape alleles exhibit conditional neutrality. However if the fitness difference between leaf shape types is subtle it may not be possible to differentiate between antagonistic pleiotropy and conditional neutrality.

**Manipulative Field Experiments: How are lobed leaves adaptive?**

To test whether lobed leaves increase drought or freeze tolerance I will perform manipulative field experiments during both years on blocks of plants within the reciprocal transplant experiments described above. To relieve selection pressure created by drought I will water two randomly selected blocks of 60 plants in the dry *M. laciniatus* habitat. Lobed leaves clearly increase drought tolerance if F3s and NILs with lobed leaf alleles have increased fitness in the un-watered *M. laciniatus* habitat, but no significant advantage in the watered *M. laciniatus* blocks. To relieve any selection pressure created by leaves losing heat to the cold night sky I will also cover two 60 plant blocks in the *M. laciniatus* habitat with fine mesh every night. Fine mesh should prevent leaves from radiating heat to the sky but allow normal airflow so that the thickness of the leaf boundary layer does not change. Lobed leaves increase freeze tolerance if F3s and NILs with lobed leaf alleles have increased fitness in the uncovered *M. laciniatus* habitat, but no significant fitness advantage in the covered *M. laciniatus* blocks.

Fitness differences between F3 and NIL types in manipulated blocks will be analyzed using ANOVAs, one per experimental treatment, with predictors being F3 type (factor with 2 levels) or NIL
type (factor with 4 levels). I will include planned contrasts between NIL types 2&4 and 3&1 to test for fitness differences between NILs with lobed and un-lobed leaf shape alleles.

Additional funding will allow me to perform two years of reciprocal transplants.

**Objective 2: Parallel Leaf Shape Evolution**

Because the recently discovered Bald Rock *Mimulus* has similar leaf morphology and occupies an *M. laciniatus*-like habitat its lobed leaf shape may be an example of parallel evolution (Figure 4). Parallel evolution occurs when the same phenotype is reached independently by closely related species in similar environments. Parallel genetic evolution occurs when the same genetic changes underlie parallel phenotypic transitions (27, 66). The occurrence of parallel evolution in the *M. guttatus* species complex would be strong evidence that lobed leaves are adaptive in granite outcrops (73, 27, 67, 66). The phylogeny of the *M. guttatus* species complex is unresolved, making it difficult to decipher whether BR is an independent lineage or a long diverged population of *M. laciniatus*. A way to address this ambiguity is to find the genetic basis of BR’s leaf shape. If BR and *M. laciniatus* have different loci underlying their convergent leaf shapes that would be strong evidence of parallel phenotypic evolution and in turn, that lobed leaves are broadly adaptive to granite outcrop environments. If BR and *M. laciniatus* have the same loci underlying leaf shape this could still be an example of parallel evolution that is also parallel at the genetic level. This would add to the growing literature suggesting that in closely related species, parallel phenotypes are often due to the same genetic changes (16, 14). In this case further phylogenetic analysis would be needed.

**The genetics of leaf shape in Bald Rock**

To map the genetic regions of BR’s leaf shape a BR x *M. guttatus* line IM62 F2 mapping population will be generated. Bulk segregant analysis in combination with whole genome Illumina re-sequencing will be used to map QTLs (4, 6, 24, 26, 48). The BR parental line will be sequenced in one lane of Illumina Hi-Seq with single end reads at 40X coverage. F2s with the most Bald Rock-like or *M. guttatus*-like leaves will be bulked into two separate pools. Each pool will be run in one lane of Illumina Hi-Seq (single end reads) giving an average of 40X sequence coverage. Sequence data from both pools will be aligned to *M. guttatus* IM62 genome and the re-sequenced Bald Rock parental line. SNP allele frequency differences will be calculated between pools. A large difference in allele frequency between pools at the same location will indicate association with leaf shape. A PCR based marker screen in all F2s will follow the bulked segregant analysis to confirm QTLs and estimate effect sizes with a one-way ANOVA (predictors: genotype, response: leaf shape). Additional funding will allow me to investigate this potential instance of parallel leaf shape evolution which would significantly expand the scope and impact of my dissertation research.

**Broader Impacts**

Understanding how plants adapt to harsh environments is essential for agricultural improvement and conservation efforts world wide. Climate change makes this task especially urgent. If leaf shape is important for drought tolerance then it could be incorporated into agricultural breeding programs designed for areas of the world subject to drought. I am passionately committed to the advancement of women and minorities in science. Excitingly this project will provide many opportunities to mentor undergraduates and high school students through programs at Duke like the Summer Research Opportunity Program (SROP) for under-represented minorities and the Howard Hughes Medical Institute (HHMI) Summer Research Programs. The past 2 summers I have mentored 2 high school girls in the HHMI program, Connie Wang and Crystal Terry. Crystal’s independent project related directly to my research. I participate in a local organization called Women and Math Mentoring (WAM) which is designed to educate eighth grade girls from the local public schools, most of whom are African American, about career opportunities in math and science. In one of our past activities my three mentees came to the lab for a day and were exposed to concepts and techniques used in my research such as evolution and DNA extraction. All three girls said that coming to the lab and actually taking part in research was their favorite activity and one of them, Quaniesha Jones, volunteered in the lab for a school science project.
References

6. Blackman, B.K. unpublished data
21. Ferris, K.G. personal observation
22. Ferris, K.G. unpublished data
24. Flagel, L.E. unpublished data
26. Friedman, J. unpublished data


42. Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. *The American Naturalist* Vol.87 pp.331-333

43. Lowry D.B. and Cooley A., unpublished data


53. Modliszewski, J. unpublished data


70. Sexton, J. personal observation

71. Sexton, J. and DeMarche, M. unpublished data


82. [http://www.phytozome.net/cgi-bin/gbrowse/mimulus/](http://www.phytozome.net/cgi-bin/gbrowse/mimulus/)
John H. Willis  Phone: (919) 660-7340  
Department of Biology, Box 90338  Fax: (919) 660-7293  
Duke University  Email: jwillis@duke.edu  
Durham, NC 27708-0338

a. PROFESSIONAL PREPARATION.

Brown University; Providence, RI Biology Major A.B. Honors, 1985  
University of Chicago; Chicago, IL Ecology & Evolution Ph.D. 1991  
University of Oregon; Eugene, OR Ecology & Evolution postdoc: 1991-1993

b. APPOINTMENTS.

Professor of Biology (2009 – present); Duke University; Durham, NC  
Associate Professor of Biology (2000 - 2008); Duke University; Durham, NC  
Associate Professor of Biology (1999 - 2000); University of Oregon; Eugene, OR  
Assistant Professor of Biology (1993 - 1999); University of Oregon; Eugene, OR  
Postdoctoral Research Associate (1991 - 1993); University of Oregon; Eugene, OR  
Teaching Assistant (1985 - 1991); University of Chicago; Chicago, IL  
Teaching Assistant (1983 - 1985); Brown University; Providence, RI

c. SELECTED PUBLICATIONS (from those published after 2000).

Five publications related to the proposed project:


Five other publications:


d. EXAMPLES OF SYNERGISTIC ACTIVITIES.

As lead-PI on an NSF FIBR grant and the DOE’s Joint Genome Institute’s effort to sequence the genome of Mimulus guttatus, I am coordinating collaborative efforts to develop genomic resources for plants in the genus Mimulus, that are available to the entire research community <http://www.mimulusevolution.org>. In our lab, we have developed thousands of novel genetic markers (especially microsatellites and gene-based markers) and genetic lines of Mimulus that are being used by other researchers to investigate the ecological and genomic basis of adaption and speciation. I have actively participated in summer research programs designed to encourage the participation of groups underrepresented in science, and have sponsored summer research projects of several students from these groups. I was Director of Graduate Studies for Biology (2004-2008), and Director of the Plant Teaching and Research Facility (2002-2006). I am helping to design a new introductory biology majors course that places new emphasis on genetics and evolution. I have served on many NSF panels and NIH study sections. I have been Associate Editor for Evolution (2001-2005) and The American Naturalist (2001- 2005).

e. COLLABORATORS AND OTHER AFFILIATIONS.

Graduate and Postdoctoral Advisors.

Dr. Douglas W. Schemske (University of Washington), Ph.D. advisor
Dr. Deborah Charlesworth (University of Edinburgh), Ph.D. advisor
Dr. Michael Lynch (University of Oregon), post-doctoral advisor

Thesis Advisor and Postgraduate-Scholar Sponsored.

a. Postdoctorals (11): Alan Kelly (U. of O.), John Kelly (University of Kansas), Lila Fishman (University of Montana), Leonie Moyle (Indiana U.), Andrea Case (Kent State), Amy Bouck (Pioneer Hybrid), Carrie Wu (U. Richmond), Elen Oneal (Duke), Young Wha Lee (Duke), Ben Blackman (Duke), and Jannice Friedman (Duke), Lex Flagel (Duke), Kevin Wright (Duke).
b. PH.D. Students (12): Megan Hall (NYU), Noland Martin (Texas State), Jan Aagaard (U. of Wash.), Andrea Sweigart (U. Rochester), Arielle Cooley (U. Michigan), Young Wha Lee (Duke), David Lowry (Duke), Kevin Wright (Duke), Jennifer Modliszewski (Duke), Kathleen Ferris (Duke), Jessica Selby (Duke), and Laryssa Baldridge (Duke).
Kathleen G. Ferris  
**Address:** Department of Biology  
Box 90338, Duke University,  
Durham NC 27708  
**Phone:** (919) 684-3368  
**Email:** kgf7@duke.edu  

**Professional Preparation**  

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<td>B.A.</td>
<td>The University of Chicago</td>
<td>Biological Sciences</td>
<td>June 2007</td>
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<tr>
<td>B.A.</td>
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<td>June 2007</td>
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<tr>
<td>Ph.D. Candidate</td>
<td>Duke University</td>
<td>Biology</td>
<td>expect. June 2013</td>
</tr>
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</table>

**Appointments**  

**August 2007-present:** (Duke University) Graduate teaching and research assistant, Department of Biology. Advisor: Dr. John H. Willis. Laboratory instructor for the following courses: Experimental Molecular Genetics and Development (1 semester), Principles of Animal Physiology (1 semester), Aids and Emerging Diseases (1 semester), Genetics and Evolution (1 semester), and Infectious Disease (1 semester).  

**March 2005-May 2007:** (The University of Chicago) Undergraduate Research Assistant, Department of Ecology and Evolution. Supervisor: Dr. Joy Bergelson. Assisted in collecting phenotypic and genetic data regarding pathogen resistance genes (R-genes) in *Arabidopsis thaliana*.  

**June 2006-September 2006:** (The Howard Hughes Medical Institute) Undergraduate Research Fellow, Department of Ecology and Evolution. Supervisor: Dr. Joy Bergelson. Conducted an independent research project sequencing pathogeneticity genes in divergent strains of the plant pathogenic bacteria *Pseudomonas viridiflava*.  

**June 2004-September 2004:** (The Missouri Botanical Garden) Research intern, Department of Conservation. Supervisor: Dr. Kimberly McCue, Director of Conservation. Conducted an independent research project on the germination ecology of three native species of *Lesquerella*.  

**Synergistic Activities**  

1. *Mentoring high school and undergraduate students*, June 2009-present. A high school student participating in the 2009 Howard Hughes Summer Research Program was co-supervised by another graduate student in the lab and myself. The project she worked on with me exposed her to the subjects of ecology, evolution, and genetics and she received hands on training in bioinformatics. During the fall semester I assisted another high school student with a science project for her biology class. We have discussed ecology, evolution, and experimental design. This past summer I mentored a young African American woman who was participating in the Howard Hughes Pre-College Research Program. She worked on a project directly stemming from my dissertation work and learned a great deal about leaf physiology and drought tolerance and is now an undergraduate student volunteering in our lab at Duke University. Last year an undergraduate who was in my Evolution and Genetics lab section expressed interest in our lab’s research and began volunteering with my guidance. We have
discussed genetics, adaptation, speciation, and the biophysics of leaf shape over the past year while he has worked on various independent projects related to my dissertation and he is now applying to biology PhD programs.

2. Women and Mathematics Mentoring, November 2008-present. The Women and Mathematics Mentoring program is designed to educate eighth grade girls from local public schools about career opportunities in Math and Science. It is designed to encourage young girls, who are mostly African American, to keep taking math in high school in order to keep their career options open. For the past three years another female graduate student and I mentored three eighth grade girls from the Durham public schools. We took them on science related field trips such as a lecture on Darwin at the NC Museum of Natural Sciences, the Duke Lemur Center, and a day at work in our lab at Duke. The first and third years when the girls came to our lab they extracted DNA from a strawberry and grew bacteria sampled from their hands on Petri dishes. We discussed the biochemistry of the extraction and evolutionary theory, specifically phylogenetics and human evolution. One of the girls, Quaniesha, subsequently volunteered in our lab for a school science project.

3. Women in Biology, August 2007-2010. For the first four years of my graduate career I was one of the leaders of the Women in Biology graduate student group. The goal of this group is to advance the position of women in biology by creating an open environment in our department where women feel comfortable and are treated equitably. The group has hosted lectures by well respected women scientists, had lunch time paper discussions, organized networking events with women ranging from graduate students to tenured faculty within the department, and last year we organized a very successful panel discussion on job flexibility in academia. All our events have been very successful and the feedback from men and women in the department has been overwhelmingly positive.

Publications


K.G. Ferris and J. Sexton. Convergent leaf shape and niche evolution in a cryptic *Mimulus* species. In preparation for submission to *Journal of Evolutionary Biology*

Collaborators and Other Affiliations

Collaborators: Susan Alberts (Duke), Kathleen Donohue (Duke), Lisa Donovan (UGA), Young Wha Lee (Duke), Tom-Mitchell Olds (Duke), Jennifer Modliszwerski (Duke), Mark Rausher (Duke), Jessica Selby (Duke), Jay Sexton (University of Melbourne), Mark McNair (U Edinburgh), Arielle Cooley (Michigan), Carrie Wu (U Richmond), Joy Bergelson (UChicago), Joel Kniskern (UChicago)

Graduate Advisors: John H. Willis (Duke)
<table>
<thead>
<tr>
<th>A. SENIOR PERSONNEL: PI/PD, Co-PI’s, Faculty and Other Senior Associates (List each separately with title, A.7. show number in brackets)</th>
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<td>3. CONSULTANT SERVICES</td>
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| M. COST SHARING PROPOSED LEVEL $ | 0 |
|---|
| AGREED LEVEL IF DIFFERENT $ | 0 |

**FOR NSF USE ONLY**

**PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR**

**John H Willis**

**PI/ PD NAME**

**John H Willis**

**ORG. REP. NAME**

**Karolina Angell**
<table>
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<th>PROPOSAL BUDGET</th>
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<td>PROPOSAL NO.</td>
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<td><strong>PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR</strong></td>
<td>John H Willis</td>
<td>DURATION (months)</td>
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**A. SENIOR PERSONNEL: PI/PD, Co-PI's, Faculty and Other Senior Associates**

(List each separately with title, A.7. show number in brackets)

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1. **John H Willis - none**

2. 

3. 

4. 

5. 

6. (0) OTHERS (LIST INDIVIDUALLY ON BUDGET JUSTIFICATION PAGE)

7. (1) TOTAL SENIOR PERSONNEL (1 - 6)

**B. OTHER PERSONNEL (SHOW NUMBERS IN BRACKETS)**

1. (0) POST DOCTORAL SCHOLARS

2. (0) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.)

3. (0) GRADUATE STUDENTS

4. (0) UNDERGRADUATE STUDENTS

5. (0) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY)

6. (0) OTHER

TOTAL SALARIES AND WAGES (A + B)

**C. FRINGE BENEFITS (IF CHARGED AS DIRECT COSTS)**

TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)

**D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)**

TOTAL EQUIPMENT

0

**E. TRAVEL**

1. DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)

2. FOREIGN

0

**F. PARTICIPANT SUPPORT COSTS**

1. STIPENDS

2. TRAVEL

3. SUBSISTENCE

4. OTHER

TOTAL NUMBER OF PARTICIPANTS (0)

TOTAL PARTICIPANT COSTS

0

**G. OTHER DIRECT COSTS**

1. MATERIALS AND SUPPLIES

2. PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION

3. CONSULTANT SERVICES

4. COMPUTER SERVICES

5. SUBAWARDS

6. OTHER

TOTAL OTHER DIRECT COSTS

4,985

0

**H. TOTAL DIRECT COSTS (A THROUGH G)**

MTDC (Rate: 57.0000, Base: 6262)

TOTAL INDIRECT COSTS (F&A)

3,569

**I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)**

TOTAL DIRECT AND INDIRECT COSTS (H + I)

9,831

**K. RESIDUAL FUNDS**

0

**L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)**

9,831

**M. COST SHARING PROPOSED LEVEL $**

0

AGREED LEVEL IF DIFFERENT $**

**PI/PD NAME**

John H Willis

**FOR NSF USE ONLY**

**INDIRECT COST RATE VERIFICATION**

Karolina Angell

Date Checked | Date Of Rate Sheet | Initials - ORG
|------------|-----------------|----------------
|            |                 |                |

2 *ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET
## SUMMARY PROPOSAL BUDGET

### Duke University

#### PRINCIPAL INVESTIGATOR / PROJECT DIRECTOR

**John H Willis**

<table>
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<th>A. SENIOR PERSONNEL: PI/PD, Co-PI’s, Faculty and Other Senior Associates</th>
<th>NSF Funded Person-months</th>
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| 2. ( 0 ) OTHER PROFESSIONALS (TECHNICIAN, PROGRAMMER, ETC.) | 0.00 | 0.00 | 0.00 |
| 3. ( 0 ) GRADUATE STUDENTS | 0.00 |
| 4. ( 0 ) UNDERGRADUATE STUDENTS | 0.00 |
| 5. ( 0 ) SECRETARIAL - CLERICAL (IF CHARGED DIRECTLY) | 0.00 |
| 6. ( 0 ) OTHER | 0.00 |

**TOTAL SALARIES AND WAGES (A + B)**

**TOTAL SALARIES, WAGES AND FRINGE BENEFITS (A + B + C)**

#### D. EQUIPMENT (LIST ITEM AND DOLLAR AMOUNT FOR EACH ITEM EXCEEDING $5,000.)

- **TOTAL EQUIPMENT**

#### E. TRAVEL

1. **DOMESTIC (INCL. CANADA, MEXICO AND U.S. POSSESSIONS)**

   - 2,804

2. **FOREIGN**

#### F. PARTICIPANT SUPPORT COSTS

1. **STIPENDS**

2. **TRAVEL**

3. **SUBSISTENCE**

4. **OTHER**

**TOTAL NUMBER OF PARTICIPANTS**

**TOTAL PARTICIPANT COSTS**

#### G. OTHER DIRECT COSTS

1. **MATERIALS AND SUPPLIES**

2. **PUBLICATION COSTS/DOCUMENTATION/DISSEMINATION**

3. **CONSULTANT SERVICES**

4. **COMPUTER SERVICES**

5. **SUBAWARDS**

6. **OTHER**

**TOTAL OTHER DIRECT COSTS**

**TOTAL DIRECT COSTS (A THROUGH G)**

**9,154**

#### I. INDIRECT COSTS (F&A)(SPECIFY RATE AND BASE)

**TOTAL INDIRECT COSTS (F&A)**

**5,217**

#### J. TOTAL DIRECT AND INDIRECT COSTS (H + I)

**14,371**

#### K. RESIDUAL FUNDS

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#### L. AMOUNT OF THIS REQUEST (J) OR (J MINUS K)

**14,371**

#### M. COST SHARING PROPOSED LEVEL $ 0

**AGREED LEVEL IF DIFFERENT $**

### FOR NSF USE ONLY

#### PI/PD NAME

**John H Willis**

#### ORG. REP. NAME*

**Karolina Angell**

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*ELECTRONIC SIGNATURES REQUIRED FOR REVISED BUDGET
I will be able to finish my dissertation without additional funding since my studies on adaptive flowering time, photoperiod, and flower size are funded by my advisor’s NSF grant, but the leaf shape portion of my research would suffer. External funding would increase the impact of my dissertation by allowing me to do more field work, disseminate my research at conferences, find leaf shape QTLs in an additional race of *Mimulus* to look at parallel evolution, and construct recombinant F3s and Near-Isogenic-Lines using marker assisted selection (NILs).

**Domestic Travel**

*Field Costs*

With additional funding from NSF I will be able to perform two seasons of field work instead of one. This will allow me to replicate my reciprocal transplant results and will thus improve the reliability of my dissertation research. In both year one and year two I will drive from Durham, NC to the Sierra National Forest, CA in April to plant seedlings for my reciprocal transplant experiments. I will survey for plant physiological parameters, environmental factors, and plant fitness components for the next 6 to 8 weeks.

**Year One**
- Round trip drive to California:  
  \[2690 \times 2 = 5,380 \text{ miles (only claiming 1,400)} \times 0.555 = 777\]

**Year Two**
- Round trip drive to California:  
  \[2690 \times 2 = 5,380 \text{ miles (only claiming 1,400)} \times 0.555 = 777\]

*Conference Travel*

I plan on presenting at the annual conference put on by the Society for the Study of Evolution in both year one and year two. I also plan on presenting at the annual Society for Molecular Biology and Evolution meeting in year one. Having additional funds will increase my ability to disseminate my research findings in the scientific community.

**Year One**
- Evolution Conference Travel: $300  
- SMBE Conference Travel: $450  
- Year One Total: $750

**Year Two**
- Evolution Conference Travel: $500

**Other**

*Sequencing/Genotyping Costs*

NIL and recombinant F3 construction, and Bald Rock leaf shape mapping are currently in need of funding. NIL construction will allow me to test the adaptive significance of leaf shape in the field and in the greenhouse. Without additional funds I could continue to construct
NILs using phenotypic selection. However, this would be a weaker approach because the amount of donor genome left in the final NILs would be much higher and could confound the fitness effects of the leaf shape loci. Without additional funding to construct recombinant F3s I would not be able to look at the effect of leaf shape loci in a randomized genetic background. Therefore having funding to create NILs and recombinant F3s using marker assisted selection would greatly enhance my dissertation. Mapping the genetic regions underlying leaf shape in Bald Rock will allow me to address questions about parallel genetic evolution. This would broaden the scope and increase the impact of my dissertation research.

Year One

Recombinant F3 construction
7 plates of samples, $105 per plate = $735

NIL construction
6 plates of samples, $105 per plate = $630

Year One Total: $1,365

Year Two

BR Leaf Shape Mapping
1 lane of Illumina Hi-Seq single-end sequencing = $1,555
Bald Rock parent ~40X coverage
2 lanes of Illumina Hi-Seq single-end sequencing = $3,010
Each leaf bulk ~40X coverage
F2 screen - 4 plates of samples, $105 per plate = $420

Year Two Total: $4,985

*See sequencing cost section below for more detail*

Total Direct Cost Year One = $2,892
Indirect Cost Year One = $1,648
  Calculated at the current Federally negotiated indirect cost rate

Total Direct Cost Year Two = $6,262
Indirect Cost Year Two = $3,569
  Calculated at the current Federally negotiated indirect cost rate

Total Direct Cost = $9,154
Total Indirect Cost = $5,218
  Calculated at the current Federally negotiated indirect cost rate
**Sequencing Cost Details**
The below section describes how the cost of sequencing and genotyping was calculated using estimated sample sizes and current prices at the Duke IGSP DNA Sequencing Facility.

**Sanger Sequencing**

*Leaf Shape Candidate Gene Sequencing*

1.56 per sample x 20 samples (10 *M. laciniatus* and 10 *M. guttatus*) = $31.20

**Illumina Sequencing**

*Whole Genome Re-sequencing for Bald Rock leaf shape mapping*

*Leaf shape pools:*

100bp single-end reads

1X coverage in *Mimulus* = 430,000,000bp  
1 lane of Illumina Hi Seq = 170,000,000 reads x 100bp/read = 17,000,000,000bp

1 lane of Illumina ~ 40X coverage in *Mimulus*

*Bald Rock parent re-sequencing:*

100bp single-end reads

1 lane of Illumina = 170,000,000 reads x 100bp/read = 17,000,000,000bp

1 lane of Illumina ~40X

<table>
<thead>
<tr>
<th>Project</th>
<th>Coverage</th>
<th>Lanes</th>
<th>Cost/Lane</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>BR parent resequencing</td>
<td>10X</td>
<td>1</td>
<td>$1,555.00</td>
<td>$1,555.00</td>
</tr>
<tr>
<td>BR-leaf bulk</td>
<td>5X</td>
<td>1</td>
<td>$1,555.00</td>
<td>$1,555.00</td>
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<tr>
<td>gutt-leaf bulk</td>
<td>5X</td>
<td>1</td>
<td>$1,555.00</td>
<td>$1,555.00</td>
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<tr>
<td><strong>Total</strong></td>
<td></td>
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<td>$4,665.00</td>
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**EST Genotyping**

1 plate = 96 samples

<table>
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<tr>
<th>Project</th>
<th># Plants</th>
<th># Markers</th>
<th># Samples</th>
<th>~# Plates</th>
<th>Cost/Plate</th>
<th>Cost</th>
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<tbody>
<tr>
<td>Outbred F2s</td>
<td>700</td>
<td>3</td>
<td>2100</td>
<td>7</td>
<td>$105</td>
<td>$735</td>
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<tr>
<td>NIL 3rd-5th BC</td>
<td>200</td>
<td>1</td>
<td>200</td>
<td>2</td>
<td>$105</td>
<td>$630</td>
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<tr>
<td>BR F2 screen</td>
<td>350</td>
<td>6</td>
<td>1050</td>
<td>4</td>
<td>$105</td>
<td>$420</td>
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<tr>
<td><strong>Total</strong></td>
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<td>$1,785</td>
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**Current and Pending Support**

(See GPG Section II.C.2.h for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

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<thead>
<tr>
<th>Investigator: John Willis</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
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<td>Project/Proposal Title:</td>
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<tr>
<td>Person-Months Per Year Committed to the Project.</td>
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<tr>
<td>Project/Proposal Title:</td>
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<tr>
<td>Project/Proposal Title:</td>
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<tr>
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<td>DISSERTATION RESEARCH: The Genetics of Adaptation to a Novel Stressful Environment in <em>Mimulus</em></td>
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<td>Project/Proposal Title:</td>
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*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
**Current and Pending Support**

(See GPG Section II.C.2.h for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
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<th>Investigator: Kathleen Ferris</th>
<th>Support: □ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
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</thead>
<tbody>
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<td>Project/Proposal Title: DISSEPTION RESEARCH: The Genetics of Adaptation to a Novel Stressful Environment in Mimulus</td>
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| Support: □ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support |
| Project/Proposal Title: |
| Source of Support: |
| Total Award Amount: | |
| Total Award Period Covered: |
| Location of Project: |
| Person-Months Per Year Committed to the Project. Cal: | Acad: | Sumr: |

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
FACILITIES, EQUIPMENT & OTHER RESOURCES

FACILITIES: Identify the facilities to be used at each performance site listed and, as appropriate, indicate their capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Use "Other" to describe the facilities at any other performance sites listed and at sites for field studies. Use additional pages as necessary.

Laboratory: The Willis lab has >2,000 sq ft of space equipped for plant ecological and molecular biology work (e.g., microscopy, DNA and RNA research). This lab is contiguous with the Rausher and Mitchell-Olds labs, and all 3 labs share equipment and resources.

Clinical:

Animal:

Computer: The Willis lab has two data servers, six Macs and PCs, networked printers, and ample space on the Biology Department network drives where data are backed up nightly. We also have unlimited access to the extensive high speed computing resources of the University and Department.

Office: There are multiple offices for graduate students and postdocs, and John Willis also has an office.

Other:

MAJOR EQUIPMENT: List the most important items available for this project and, as appropriate, identifying the location and pertinent capabilities of each.

Genogrinder 2000 and Sigma centrifuge for high-throughput DNA preps, and nine 96-well PCR thermocyclers. There are several compound and dissecting microscopes, and access to equipment in the Rausher and Mitchell-Olds labs.

OTHER RESOURCES: Provide any information describing the other resources available for the project. Identify support services such as consultant, secretarial, machine shop, and electronics shop, and the extent to which they will be available for the project. Include an explanation of any consortium/contractual arrangements with other organizations.

The Willis lab has access to the departmental new, very modern, highly controlled research greenhouses and numerous large walkin and smaller reach in growth chambers in the Phytotron and Biology Buildings that are managed by a dedicated staff who provide all plant care. They also have unlimited access to the Genomics Institute's sequencing facility, which has ABI 3730's and one SOLID machine, Illumina Hi-Seq and GAII machines, and 2 Roche 454 machines, as well as Biomek robotics. Duke's Center for
Bioinformatics and Computational Biology provides support services for genomic analysis.
Data Management Plan:

This proposed research will produce phenotypic data from lab and field, genetic mapping data from pedigrees, and DNA marker and sequence data. We will follow community standards [e.g., 1] and recommendations from journals, Dryad, and other community-appropriate sources regarding Best Practices for Data Archiving. We do not anticipate any ethical or privacy issues for these data. All results will be released to the public domain under Creative Commons Zero (CC0), providing an open license so that others can use and build on these data. These data will be of widespread interest to the *Mimulus* research community and others interested in ecological and evolutionary genomics.

Information from experiments will include necessary data and metadata so that other researchers can evaluate and reproduce the research findings. ReadMe files will contain descriptions of experiments and included data, as well as the name and contact information for those who collected the data. Data and metadata will be provided as plain text and tab-delimited text files to ensure access. Tabular data will provide information on units of measurement, symbols, encoding of missing data, abbreviations, and related data in other files or locations. We will use standardized formats and scientific conventions regarding dates, GPS coordinates, and related information. Associated publications will be identified.

During data collection most trait data will be entered directly to computer files. Data collection methods will include hand measurements. Time, data, and location will be reported for each data file. These primary data will archived, along with derived files which are suitable for statistical analyses. Substantial quantities of trait data will be recorded (dozens of measurements for thousands of plants). Individual data files will be named and formatted in order to facilitate automated processing.

Before analysis all trait data will be stored on the Duke University OIT file server, as implemented by the Department of Biology and Duke University Office of Information Technology. This provides a high level data security, backup, and off-site data protection. Field data will be password protected, and stored initially on laptop computers, which have automatic hourly backup at base camp. All field data will be transferred promptly to the Duke University file server for security. Each project has a designated researcher who is responsible for data management and storage, and all researchers report regularly to the PI.

Trait data will be available to the community when papers are accepted for publication. Analysis-ready data will be stored at the journal website or an open access repository such as Dryad.

Finished DNA sequences will be deposited to GenBank. Next generation sequence data will be stored online at www.mimulusevolution.org. Archival storage of next generation sequence reads is currently in a state of flux. NIH has committed funding for the Sequence Read Archive (SRA) and Trace Archive repositories through October 1, 2011, and subsequent plans are unclear. The European Nucleotide Archive (ENA) plans continuous storage for next generation sequence data. Following community best practices, we will deposit archival sequence read data with SRA or ENA, or another appropriate archival location with open access to the research community. High throughput sequence data will be made available six months after the data are checked.
and validated.

Long term data storage will be provided via an open access repository such as Dyrad, RMBL, or through the Duke University Libraries, which provides an institutional repository (DukeSpace) as an archive and access service for digital data. This data repository provides for data redundancy, including off-site storage, and is maintained and supported by the Duke University Libraries according to guidelines established in the “Trustworthy Repositories Audit and Certification” criteria and checklist.

Following Duke University policy, all research records will be archived for a minimum of five years after reporting or publication of a project. The PI will work with the staff of the Duke University Libraries to determine how the data may be kept beyond that period.

New software will be provided through our laboratory web site and at SourceForge.net, a web-based, open source repository for computer code and programs.

Reference Cited:

November 9, 2011

The National Science Foundation
4201 Wilson Boulevard
Arlington, VA 22230

To Whom It May Concern:

Kathleen Ferris has advanced to candidacy for a Ph.D. degree.

Sincerely,

[Signature]

Sonke Johnson
Director of Graduate Studies
Department of Biology
Context for Improvement

My dissertation research focuses on identifying the genes and traits that allow *Mimulus* to live in granite outcrop habitats. Using genetic manipulation, reciprocal transplant studies, and manipulative experiments in the field I will explore the adaptive significance of flowering time, critical photoperiod, self-fertilization, and leaf shape in the two granite outcrop endemics *Mimulus laciniatus* and the recently discovered Bald Rock *Mimulus*. My dissertation research on flowering time, critical photoperiod, and self-fertilization is funded elsewhere. However, my leaf shape research could use additional funding. This research has two primary objectives: 1) to create and use Near Isogenic Lines (NILs) between *M. laciniatus* and *M. guttatus* that differ in leaf shape in reciprocal transplants in the field to determine if and how leaf shape is adaptive and 2) to test whether there has been parallel evolution of lobed leaf shape in *Mimulus* which would be strong evidence that lobed leaves are adaptive in granite outcrop habitats.

I have so far used phylogenetic analysis to discover a seemingly independent origin of lobed leaves in the genus *Mimulus*, Bald Rock. By measuring drought and temperature related environmental variables I have determined that *M. laciniatus*'s granite outcrops are drier, more light intensive, and have colder soil temperatures than *M. guttatus*'s seeps and streams. In a preliminary leaf temperature reciprocal transplant I found evidence that lobed leaves stay closer to air temperature in *M. laciniatus*'s environment. I have mapped QTLs for leaf shape in an F2 population, and I am currently creating F3s and NILs.

Without additional funding from NSF I could make NILs using phenotypic selection. However, this would leave a larger proportion of the donor plant genome in the NILs than marker assisted selection. Marker-assisted selection will improve the quality of my NILs by reducing the number of potentially confounding genetic regions. Also, with DDIG funding I will be able to do two seasons of fieldwork instead of one and this will strengthen any evidence of the adaptive significance of leaf shape. Additional funding will also allow me to investigate a potential instance of parallel leaf shape evolution. Parallel evolution would be further evidence that lobed leaves are adaptive in granite outcrop habitats. While not essential, this project would significantly expand the scope and impact of my dissertation research.

Dr. John Willis’s research focuses on the genetics of plant speciation and local adaptation. Projects underway in the lab include the genomics of adaptation to salinity, copper, serpentine soils, and research into the genomics of polyploid evolution. My research diverges from the rest of the lab in that I am focusing on the adaptive significance of a morphological trait that has undergone parallel evolution. I also primarily use genetics as a tool to illustrate the adaptive significance of lobed leaf shape instead of focusing on finding causal genes and mutations.

So far my research has received support from Dr. Willis’s EnGen, two small departmental grants, and a Sigma Xi. The EnGen has expired. Dr. Willis’s new NSF grant, funding study of the genetic basis of photoperiod and flowering time differences in *Mimulus*, funds part of my dissertation project, but not my leaf shape research. The small departmental grants have mostly been used in preliminary fieldwork. The Sima Xi will only cover the cost of field supplies. Additional funding would greatly increase the efficiency and impact of my dissertation research.