

## **Project Summary**

*Maximum of 1 page*

**Intellectual Merit**

**Broader Impacts**

# Project Description

## 1 Genetic architecture of highland adaptation in Zea mays

### 1.1 Questions

- What is the genetic architecture of highland adaptation?
- How much of the genetic architecture is shared between Mexico and South America?
- How much of the genetic architecture is shared between maize and teosinte?

### 1.2 QTL mapping of highland adaptation traits in high x low mapping populations from Mexico and S. America

- Phenotyping at 4 sites:
  - low Mexico (RS)
  - high Mexico (RS)
  - Missouri (SFG)
  - growth chamber (MBH)
- 960 F2:3 families; 40 checks
- population development (RS, SFG)
- genotyping and mapping (JRI, SFG)
- DNA extraction, genotypes (SFG)
- Sequence parents (JRI)
- Mapping (SFG)

### 1.3 Admixture mapping in mex/parv hybrid zone

- collection trip (MBH)
- 500 individuals from Ahuacatitlan in Mexico (MBH, RS)
- DNA extractions, genotyping (MBH)
- phenotyping (MBH, RS)
- mapping (MBH/GC)

## 1.4 Phenotypes

- macrohairs (SFG, RS, MBH)
- flowering time (SFG, RS, MBH)
- tassel morphology (SFG, RS, MBH)
- plant height every 2 weeks & at flowering (SFG, RS, MBH)
- biomass (SFG, RS, MBH)
- # ears (mz), 50k weight (mz/teo), total seed weight (mz) (SFG, RS, MBH)
- stem/plant color (SFG, RS, MBH)
- germination depth, temp in greenhouse (F2:3 only; MBH)
- roots (inquire with Bloom)
- ionomics (Ivan via letter of support)

## 2 Introgression, admixture, and adaptation

### 2.1 Questions

- Are introgressed loci adaptive?
- Does evidence of introgression and natural selection correspond to QTL?
- Are highland QTL/loci widespread in highland climes?

### 2.2 Introgression and Admixture

- GBS of 30 inds x 10 pops x 2 subspecies (mex & maize) (JRI)
- Popgen on maize/mexicana introgression (JRI, GC)
- GBS Additional 5 admixed parv/mex populations (50 inds. each) (JRI)
- Introgression and adaptation in additional admix pops (JRI, GC)

### 2.3 Global analysis of highland haplotypes

- Occurrence of highland haplotypes/QTL/SNPs in global pops (MBH)
- 500 worldwide accessions GBS (MBH)
- Case study in Chihuahua (ACJ)

### 3 Functional characterization of QTL

#### 3.1 Questions

- What do QTL/selected loci/introgressed loci do?

#### 3.2 Fine map pigmentation

- PT x T43 NIL population (RS)
- GBS genotyping (MBH)

#### 3.3 Allelic series for QTL of interest

- 10 parents:
  - 4 parents F2:3
  - mexicana TIL18
  - Palomero Toluqueo
  - 2 lowland landraces
  - 2 highland landraces
- Cross into 3 parents for phenotyping
  - B73 (SFG)
  - T43 (MBH)
  - CML457 (RS)

#### 3.4 RNAseq

- Time series analysis of plants in field (ACJ):
- 15 Lines
  - 4 F2:3 parents
  - 1 NIL chr4,
  - 1 each mex & parv TIL
  - B73, CML457, T43, PT
  - highland/lowland landraces used in allelic series (MBH to inbreed)
- 12 plants per line per environment (2 pools of 6)
- 4 stages/tissues per plant
- 2 environments (high/low fields)

## Broader Impacts

Also mention online presence? twitter, online dissemination, etc.? slideshare, figshare, preprints, Haldane's sieve?

### 1.5 Exchange Program

- 2 students per year
- 3-6 month stint in lab
- Training: next-gen analysis, crossing & phenotyping, mapping

### 1.6 Phenotyping workshop

- Yearly workshop at UM
- Participants pay to purchase handheld
- 2 day workshop provides software, training on phenotyping in maize

### 1.7 Software

- R code for novel analysis for admixed populations
- Software & pipelines on github

### 1.8 Germplasm resources

- NIL population & F2:3 submitted to stock center

## Results From Prior NSF Support

### Ross-Ibarra: #0922703: Functional Genomics of Maize Centromeres

*Intellectual merit* Centromeres are regions of the genome that organize and regulate chromosome movement, yet the biology of centromeres remains poorly understood. Co-PI Ross-Ibarra's group has focused in particular on the evolutionary genetics of centromeres. This work has demonstrated the remarkable evolutionary lability of centromere tandem repeats, but has shown that there is little evidence in maize for coevolution between centromere sequence and kinetochore proteins. Ongoing work from the Ross-Ibarra lab seeks to characterize kinetochore proteins, assess the phylogenetic evidence for longer-term coevolution, and understand patterns of centromere and genome size variation in natural populations.

*Broader impacts* Co-PI Ross-Ibarra has established and runs an international student exchange program as part of this grant. Data and result of this project have been disseminated via publications and presentations as well as deposited in the maize genetics community database [www.maizegdb.org](http://www.maizegdb.org). Former trainees on the grant include Dr. Matthew Hufford (Co-PI on the current grant).

- Pyhäjärvi T, Hufford MB, Mezouk S, Ross-Ibarra J (2013) *Genome Biol & Evol*: 5:1594-1609
- Hufford MB, Lubinsky P, Pyhäjärvi T, et al. *PLoS Genetics* 9(5): e1003477.
- Melters DP, Bradnam KR, Young HA, et al. (2013) *Genome Biology* 14:R10
- Kanizay LB, Pyhäjärvi T, Lowry E, et al. *Heredity* 110: 570-577.
- Hufford MB, Bilinski P, Pyhäjärvi T, Ross-Ibarra J (2012) *Trends in Genetics* 12:606-615
- Hufford MB, Xun X, van Heerwaarden J, et al. (2012) *Nature Genetics* 44:808-811
- Chia J-M, Song C, Bradbury P, et al. (2012) *Nature Genetics* 44:803-807
- Fang Z, Pyhäjärvi T, Weber AL, et al. (2012) *Genetics* 191:883-894
- Shi J, Wolf S, Burke J, Presting G, et al. (2010) *PLoS Biology* 8: e1000327

**Coop: #1262645: Collaborative Research: ABI Innovation: Visualization And Statistics For Spatial Population Genomic Analysis.**

\$314,260, with an effective date of 05/01/13. Award Duration: 36 months.

*Intellectual merit* We are developing a set of spatial statistics methods based on Gaussian random fields for the analysis of geographic population genomics data. The first method based on this approach has just been published (Bradburd et al. 2013), allowing a sound statistical framework to distinguish the effects of geographic and ecological distance on genetic isolation.

*Broader impacts* The R package of the software has been released: <http://genescape.ucdavis.edu/scripts-and-code/bedassle/>, and has already been used by a many molecular ecologists.

- Bradburd, G., Ralph, P., Coop, G. (2013) *Evolution* 67: 3258-3273

**Flint-Garcia: #0820619: Genetic Architecture of Maize and Teosinte**

\$ 9,823,000. 3/1/2009-2/28/2013. PI Edward Buckler, co-PIs J. Doebley, T. Fulton, S. Flint-Garcia, J. Holland, S. Kresovich, M. McMullen, Qi Sun.

*Intellectual merit* This project extends over more than a decade, and has pioneered the characterization of population genetic and evolutionary parameters of maize diversity, developed resources to connect this genetic diversity to phenotype through both association and joint linkage-association mapping, conducted fine scale analysis of domestication and agronomic QTL, and recently expanded to whole-genome analysis of diversity, evolution, and phenotype. Overall, the maize diversity project has developed a wide range of approaches and broadened understanding of the maize genome, evolution and adaptation, genetic mapping, and the agricultural improvement of maize. Over the last three years, the current iteration of the project (DBI-0820619) has successfully released and analyzed the maize Nested Association Mapping (NAM) population, collaborated on making first and second generation haplotype maps for maize, resolved domestication traits, developed a range of novel statistical approaches for association mapping, and dissected complex traits such as flowering time, kernel composition, disease resistance, height, and inflorescence and leaf morphology. Several positional cloning projects that will facilitate refinement of association mapping approaches are nearing completion. We have also been collaborating with colleagues around the world to resequence more than 100 *Zea* genomes and skim sequence 20,000 maize varieties.

Overall, the project has published 58 scientific papers in the last three years, including 15 in Science, Nature Genetics, and PNAS.

*Broader impacts* The outreach program included a traveling science museum exhibit on maize diversity, evolution and genetics (seen by at least 300,000 people at five venues to date, including the famous Corn Palace in South Dakota), online Teacher Friendly Guide to the Evolution of Maize, seven Genotyping-By-Sequencing (GBS) workshops (held at primarily at Cornell but has also been held in Kenya), and training of postdocs, graduate students and undergraduates, the vast majority of which have continued in scientific careers. Former trainees on this grant include Dr. Ross-Ibarra and Dr. Flint-Garcia (PIs of the current grant).

- Bottoms, C., Flint-Garcia, S. & McMullen, M.D. BMC Bioinformatics 11 Suppl 6, S28 (2010).
- Brown, P.J. et al. PLoS Genetics 7, e1002383 (2011).
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- Cook, J.P. et al. Plant Physiology 158, 824-834 (2012).
- Dubois, P.G. et al. Plant Physiology 154, 173-86 (2010).
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- Hufford, M. et al. Nature Genetics 44: 808-811 (2012).
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- Hung H-Y, Shannon LM, Tian F, Bradbury PJ, Chen C, Flint-Garcia SA, McMullen MD, Ware D, Buckler ES, Doebley JF,
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- Tian, F. et al. Nature Genetics 43, 159-62 (2011).
- van Heerwaarden, J. et al. Molecular Ecology 19, 1162-73 (2010).
- van Heerwaarden, J., van Eeuwijk, F. a & Ross-Ibarra, J. Heredity 104, 28-39 (2010).
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- Zhang, N, A Gur, Y Gibon, R Sulpice, S Flint-Garcia, MD McMullen, M Stitt, ES Buckler.  
PLoS One 5: e9991 (2010)



## References Cited

- [1] Name, *Title of the article*, Journal the article appears in, Year(YYYY).

# Biographical Sketch: Your Name

*Maximum of 2 pages*

## Biographical Sketch

### Professional Preparation

Undergraduate Institution(s)	Major	Degree	Year
Graduate Institution(s)	Major	Degree	Year
Postdoctoral Institution(s)	Area		Year
Year-present	Position, Department, Institution		
Year(s)	Position, Department, Institution		

### Publications

*Five Publications Most Closely Related to the Proposed Project*

1. Author(s): Article Title, *Journal Title* **Volume Number**, Page Numbers, Year of Publication.
- 2.
- 3.
- 4.
- 5.

*Ten Other Significant Publications*

- 1.
- 2.
- 3.
- 4.
- 5.
- 6.
- 7.
- 8.
- 9.

10.

1.

2.

3.

4.

5.

### **Collaborators & Other Affiliations**

*Collaborators:*

*Graduate and Postdoctoral Advisors:*

*Thesis Advisor and Postgraduate-Scholar Sponsor:*

# Budget Justification

*Maximum of 3 pages*

## Personnel

### Other Personnel

### Fringe Benefits

Fringe benefits are applied to personnel salaries using the university approved rates:

- Faculty - % in FYs 2012, 2013, and 2014
- Postdocs - % in FYs 2012, 2013, and 2014
- Graduate students - % in FYs 2012, 2013, and 2014
- Undergraduate students - % in FYs 2012, 2013, and 2014
- Staff - % in FYs 2012, 2013, and 2014
- Part time staff - % in FYs 2012, 2013, and 2014

Additionally, the university applies a risk management charge of 1% on all personnel salaries

## Travel

### Other Direct Costs

*Materials and Supplies:*

*Consultant Services:*

*Graduate Student Tuition*

### Indirect Costs

## **Facilities, Equipment, and Other Resources**

### **Facilities, Equipment & Other Resources**

#### **UC Davis**

Dr. Ross-Ibarra has four standard laboratory benches as part of a shared lab space at UCD. The shared space is the single largest lab space on campus, and provides for seamless interaction between the labs housed there. The space currently houses three other PIs, all working on the genetics and genomics of economically important plant taxa (Dubcovsky, Neale, Dandekar). The lab is equipped with standard equipment and tools for molecular biology, including freezers and refrigeration, a shared liquid handling robot, thermal cyclers, centrifuges, gel rigs, balances, and standard molecular biology supplies. A dedicated low-humidity refrigerator for seed storage is available through the university, and low-humidity storage cabinets for tissues and temporary seed storage are in the laboratory. Dr. Ross-Ibarra occupies half of a large office suite that includes a conference room and cubicle space for 25 people. Both macintosh and PC workstations are available for student and postdoc employees. The PI is a contributing partner in a large computer cluster, giving the lab dedicated access to 192 processors, with the opportunity for use of nearly 800 additional CPU as resources allow. Recent (2013) additions to the cluster have provided it with additional CPU as well as six new shared high-memory (512Gb RAM) nodes, one of which is dedicated to the Ross-Ibarra lab. Dr. Ross-Ibarra is a faculty member of the UC Davis Genome Center, a large facility that includes bioinformatics, genotyping, metabolomics, proteomics, and expression analysis cores able to perform a variety of genomics analyses at cost for UC Davis faculty. The Genome Center also rents time on its equipment, including a bioanalyzer and library preparation robots. As a member of the Genome Center, Dr. Ross-Ibarra also has access to their additional computational facilities. UC Davis has also entered into a recent partnership with BGI (the Beijing Genomics Institute) to provide additional high-throughput sequencing services via a new Sacramento-based sequencing facility.

Dr. Coops dry space is located on the 3rd floor of the Storer building, which houses the Department of Evolution and Ecology. The space is newly renovated space and consists of 3 offices that can seat a total of 8 people, and a conference room. In addition members of the lab have access to an additional conference room and other offices shared with the Begun, Langley, Lott, Kopp and Turelli groups. This group is part of the larger Center and Graduate Group for Population Biology, one of the leading graduate training programs in ecology and evolution in the world. Each current member of Dr. Coops group has a quad-core Mac pro. The computers are loaded with all the necessary software (Word, R, Mathematica etc ) and are connected to the university network as well as to color and black and white printers. The Coop lab has access to the genome center computational facilities: <http://www.genomecenter.ucdavis.edu/core-facilities/>.

#### **Iowa State**

Project components completed in the Hufford Laboratory will include mapping population development, DNA isolation and PCR, and population genetic analysis of genotyping data. Population development will be carried out in field space available at the Curtiss Farm of Iowa State University (ISU). This facility is equipped with irrigation, tractors, tillage equipment, planters, and combines. Seed processing and cold storage facilities are also available on the ISU campus. The Hufford Laboratory has all equipment necessary for DNA isolation and PCR including centrifuges, thermal cyclers, an ultra-low freezer, water baths, a pH meter, balances, and an electrophoresis system. A gel imaging system and a NanoDrop spectrophotometer for DNA quantification are

accessible through the Center for Plant Responses to Environmental Stresses at ISU. The DNA Facility at ISU provides access to cutting-edge genomic technology including HiSeq and MiSeq Illumina sequencing and library preparation for both paired-end and mate-pair approaches. Data analyses will be carried out using the High Performance Computing clusters available at ISU. Dr. Hufford currently has access to the Lightning3 cluster which has a mix of Opteron based servers, consisting of 18 SuperMicro servers with core counts ranging from 32 to 64 and 256 to 512 GB of memory.

**U. Missouri** Dr. Flint-Garcia has 600 sq ft of laboratory space in Curtis Hall, on the University of Missouri campus. The laboratory is fully equipped for molecular genetics, including a chemical hood, a Beckman table top centrifuge with multiple tube buckets, a Tetrad four plate thermalcycler, several freezers, ultra-low freezers and refrigerators, water baths, a pH meter, and balances. In the building, laboratory personnel have ready access to ultracentrifuges and rotors, growth chambers, an autoclave, lyophilizers, a Sorvall high speed preparative centrifuge with four rotors, a shaker-incubator for bacterial cultures, a chromatography cabinet, electrophoresis equipment for DNA, RNA protein and DNA sequence analysis, a plate reading spectrophotometer/flourometer, a pulse-field electrophoresis system, six Thermolyne thermalcyclers, and four Tetrad four plate thermalcyclers. Dr. Flint-Garcia has multiple personal computers, and computing resources including weekly data backups, direct access to a Sun Ultra10 Unix Workstation and NT server for data sharing, and IT support from USDA-ARS. In addition, the co-PI has access to the Lewis bioinformatics cluster (over 180 compute nodes with more than 1200 processor cores and 5400 GB of memory) via the University of Missouri Bioinformatics Core Facility. Dr. Flint-Garcia has 120 sq ft of office space and ample office and desk space for postdocs, technicians and graduate students. Dr. Flint-Garcia shares two ABI 3100 DNA sequencers, an ABI 7900HT RTPCR machine, and a Beckman NxP robot used primarily for DNA extractions with Mel Oliver and Mike McMullen, and other USDA scientists in the unit. Dr. Flint-Garcia has access to greenhouse and field space (with irrigation capability; University of Missouri South Farm and Bradford Research Center), seed processing and cold storage space, and use of winter nursery facilities in Puerto Rico. The co-PI has access to a complete set of field equipment including multiple tractors, tillage equipment, a 4-row plot planter, and a 2-row plot combine.

### **LANGE BIO**

Langebios mandate is to conduct top-ranked research while promoting genomic knowledge for the protection and sustainable use of Mexican biodiversity. Its unique location in the agricultural center of Mexico facilitates field sampling and field experimentation. We have ample experience growing maize in nurseries located on the West Coast (Valle de Banderas, Nayarit), in Central Mexico (Irapuato; Celaya, Guanajuato), and have begun to establish additional sites in the high valleys of Central Mexico (Queretaro; Estado de Mexico). We regularly conduct field expeditions to collect plants in both the dry regions of Northern Mexico (maize collections in Chihuahua, Lamiaceae throughout the Northeast) and the lower valleys of the Eje Volcanico and Costa del Pacifico (Teocintle and maize, Solanaceae, and Cucurbitaceae). Research at Langebio is supported by greenhouse facilities and two service units: Genomics and Mass Spectrometry, both of them equipped with state-of-the-art instrumentation, including several next-generation sequencing machines and diverse mass spectrometry equipments. Other facilities include a computation cluster and a specialized clean room for ancient DNA analysis.

# Supplementary Documentation

## Data Management Plan

*Maximum of 2 pages*

Prior experience – shared data on iPlant, used figshare, slideshare

Sequence data – backed up on drobo at RILab and iPlant, submitted to SRA, genotypes on data dryad or figshare

Germplasm – germplasm sent to stock center, smaller lots kept in database. mysql database accessible by all labs.

**Postdoctoral Researcher Mentoring Plan:** *Maximum of 1 page*

**Documentation of collaborative arrangements** of significance to the proposal through letters of commitment.

**Documentation regarding research involving the use of human subjects**, hazardous materials, vertebrate animals, or endangered species.