University of Nebraska- Omaha

Omaha, Nebraska

Fire Frequency and Genetics of Great Plains Bees

By Courtney Burson

An application for the

Graduate Research and Creative Activity grant.

Presented to the Office of Research and Creative Activity

Courtney Burson's GRACA proposal.

Abstract

Globally, bees are experiencing population declines, which has prompted conservation efforts to afford them some protection. Conservation efforts, however, lack knowledge about effects of land management, such as prescribed burning, and lack of a sound understanding of bees' phylogenetic relationships (i.e. the genetic relationship among bee species), which affects the quantification of bee diversity. To address these issues, 800 bees were collected during 2018 in areas burned less than one year, one year, and two years ago to address the effect of fire frequency on bee diversity. Identification of these specimens is one focus of this proposal. The second component of this proposal is to determine the phylogenetic relationships among selected bee species which will facilitate bee identification for my study while also adding to a data bank important for measuring bee diversity in general. My thesis is designed to add to the bank of bee species genetic signals by sequencing the mitochondrial genomes of four species of bumblebee, *Bombus impatiens, Bombus fraternus, Bombus pensylvanicus*, and *Bombus griseocollis*, which will provide genetic markers for these species for use in future bee identification efforts.

Introduction

There are approximately 4,000 native bees found within the United States, with populations of many currently either declining or being reported as critically endangered (Wilson and Messinger Carril 2016). Bee population declines have been attributed to parasites, agricultural chemicals, and loss of habitat with habit loss considered to have the greatest negative impact (Beismeijer et al. 2006, Cameron et al. 2010, Garibaldi et al. 2011, and Goulson et al 2015). While honey bees (*Apis mellifera*) are frequently used for plant pollination services, there are instances where native bees or other non-honey bees are the primary pollinators (National Research Council 2007). Not only do native bees act as primary pollinators for certain crops, but they can also enhance honey bee pollination services (Greenleaf and Kremen, 2006).

Fire Frequency and Bees.

Studies on the effects of fire frequency on bees have been conducted in Mediterranean scrublands and Argentinian deserts (Peralta et al. 2016, Lazarina et al. 2016) where no effects on diversity were noted four years post-burn, although some differences were noted for individual species. Another study on fire and bees in California found an increased abundance of *Bombus vosnesenskii* in a recently burned area (Mola and Neal 2018). The present study, conducted in the Great Plains, thus is expected to add substantially to what we know about fire and bee populations.

Bee Genetics.

Understanding the genetic relationships among bees provides a more precise assessment of bee biodiversity at a site since it allows for separate identification of similarly appearing bee species based on genotypes. This separation may better explain, for example, the cause of differential resource use at a site, which defines niche separation (i.e. how species differentially use available resources). High levels of biological diversity is believed to be essential for a variety reasons including incorporating enough functional redundancy to give a community resilience in the face of loss of some species (Lévêque and Mounolou 2003). Resilience in the face of such losses may be important in areas, such as native

prairies, that are adapted to historic, natural disturbances such as fire and drought. Understanding phylogeny is also essential to understand a species' potential for adaptation when exposed to disturbances in anthropologically affected areas where native species are under many selection pressures to which they are unaccustomed (Erwin 1991).

Objectives:

The first objective of my proposal is to identify the approximate 800 species collected at Glacier Creek Preserve in summer of 2018 so as to assess bee diversity. The second objective of my proposal is to sequence the mitochondrial genomes of *Bombus impatiens, Bombus fraternus, Bombus pensylvanicus,* and *Bombus griseocollis*. *Bombus impatiens* and *Bombus griseocollis* are common species but *Bombus fraternus* is listed as endangered (Hatfield et al. 2014) and *Bombus pensylvanicus* is listed as vulnerable on IUCN's red list (Hatfield et al. 2015). These identifications will both support accurate identification of bee species affecting calculations of bee diversity and be added to a master bee phylogeny being developed by UN-L's Bee Lab for the region.

Final Product of funding

This project will result in at least one publication on the effect of fire on bee diversity and another paper on sequencing of specific tallgrass prairie bee species, both in peer-reviewed journals, thus contributing to our knowledge about this important group of pollinators. A species list for Glacier Creek Preserve will be created which will also be incorporated in a master list for Nebraska. The results of both objectives will also be presented in suitable scientific venues both on campus, such as the Research and Creative Activity Fair, and off campus, such as the annual meeting of the Entomological Society of America.

Materials and Methods

Study Site: Glacier Creek Preserve, located 20 km northwest of Omaha in eastern Nebraska, is a 172 ha Nature Preserve of which 57 ha were restored to tallgrass prairie in 1970 (Bragg et al. 2018). The restored prairie is managed with spring prescribed burns with a 3-year fire return interval, although only portions of the preserve are burned in any one year. The primary objective of the Preserve is to maintain ecologically sustainable, landscape-level wildlife habitats that focus on tallgrass prairie and related ecosystems. Over 350 species of herbaceous and woody plants occur at the site, many of which rely on bees for pollination.

Experimental Design: The study consists of two phases: (1) collecting and identifying bees at Glacier Creek Preserve and (2) sequencing selected bee species of those collected.

Phase One: Sampling for bees was performed in 2018 on days where temperatures were above 10 C with wind speeds below 24 kph. Sampling consisted of both sweep netting and the use of bee bowls (LeBuhn et al. 2003). Sweep netting was conducted for five minutes in each of four study locations within the Preserve beginning at 10:30 A.M. and ending at 12:30 P.M. each day. Sweep netting was conducted randomly throughout each study area. Bee bowls were placed in transects throughout each study location for 24 hours during each sampling period (LeBuhn et al. 2003).

Specimens from sweep netting were frozen while specimens from bee bowls were stored in containers with 70% alcohol. When the bee bowl specimens were ready to be processed, they were strained from the alcohol, washed with water and soap and dried using a hair dryer (Droege 2009). I conducted a preliminary identification on the bumblebees using The North American Guide to Bumblebees (Williams et al. 2014).

Phase Two: Eight-hundred bees for the fire frequency portion of the study were selected for identification based on their being sufficiently intact to accommodate identification. Since bee identification can only reliably be done by specialists, my preliminary identifications will be verified by Katie Lamke, for which funds are requested in this proposal. Katie Lamke is a graduate student in Dr. Wu-Smart's Bee Lab at the University of Nebraska - Lincoln and is trained in bee identification.

The four bee specimens to be used for the gene sequencing portion of my thesis were separated from plant material and other insects but were not touched to minimize contamination. The specimens have been verified by the Bee Lab and are stored in alcohol prior to preparing them for sequencing. With supplies purchased by funds requested by this proposal, I will prepare these four specimens for sequencing by following established procedures using the ABCAM mitochondrial extraction kit (ABCAM 2012) and an Illumina plate. The kit is used to extract the mtDNA from the sample and the plate is used to secure the mtDNA sample while it is being sequenced. The prepared samples will be sent to the University of Nebraska- Medical center where technicians will sequence the genome, a process that takes approximately two to three months. UNMC will then return the data that describes nucleotide base strands sequenced from each sample. Base strands are portions of a species' full DNA. The next step is the process of determining the species' genome is correctly ordering these DNA strands, a process accomplished by using the software Geneious. The final step in the process is configuration of the product of Geneious (i.e. determination of the species' DNA signature) which is accomplished by using the software DOGMA and which results in the species' unique genetic marker.

Timeline:

February 2019:	Prepare the samples and send to UNMC for sequencing.
February 2019	Receive bees and species list from Katie Lamke.
May - June 2019:	Begin configuring the sequenced samples.
August 2019:	Completion of configuration.

Student and Faculty Roles: The role of the student is to collect specimens, prepare them for sequencing, and configure the DNA once sequenced. The role of the faculty is oversee the project, including budget issues, ensure that procedures are followed correctly and on time, and provide guidance for organizing and editing the final product.

Previous Funding: I received previous funding through UCRCA to purchase materials for collecting bees for a study surveying the effect of fire frequency on bee diversity. Funding, however, neither covered the cost of identification, which is requested in this proposal, nor the cost of gene sequencing.

Budget

Amount requested: $5,000

Item	Estimated Amount
Bee Identification (Katie Lamke) (1)	$1,000
Extraction Kit	$750
Plate for Extraction	$2,500
Sequencing at UNMC (2)	$1,320
Total:	$5,570
Requested Amount	$ 5,000*

*NOTE: I anticipate receiving the remaining $570 from small grants for which I have applied.

1. Cost of bee identification is $20 per hour for an estimated 50-60 hours to identify 800 bees.
2. $330 per sequence for each of four species genomes (4 x $330 = $1,320).

I am currently a teaching assistant at the University of Nebraska- Omaha. I am not requesting a stipend because my research project will be using all $5,000 I am requesting.

Courtney Burson's GRACA proposal.

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Office of Research and Creative Activity

This letter is sent in support of Courtney Burson's application for consideration in the 2019 GRACA competition. As Chair of her Master's Degree Committee, I have worked with Courtney for nearly 2 years in developing and conducting the project for which she is seeking funding support. Courtney's research has two principal objectives: (1) assessing the effect of the number of years between fires on native bee diversity and (2) a genetic analysis of 4 native bee species to accurately identify them. Identification of native bees via genetic characteristics is necessary since many are difficult to identify by appearance alone. Data collected will be added to the Master Bee data bank being developed for the region at the Bee Lab at University of Nebraska - Lincoln. Courtney has completed all administrative requirements needed to date and she continues to make progress in her thesis. The following four subject areas address specific information requested in GRACA guidelines:

Analysis of Viability of Project: Courtney's project is viable from the perspective of both objectives. Glacier Creek Preserve, the research site where the field portion of Courtney's thesis was conducted, provided suitable areas burned with different fire intervals as well as supporting a diverse suite of native bees, although identification has been based solely on appearance. Identification of bees at the Preserve will be facilitated by gene sequencing of four bee species, which will be supplemented by genetic data of other bee species sequenced for a developing bee data base.

Verification of Proposed Budget Needs and Costs: The proposed budget accurately describes Courtney's needs to complete the majority of her research project. I am confident that the $570 shortage shown in her budget will be obtained from other sources should the GRACA proposal be accepted.

Description of Mentor Support: I will oversee Courtney's research with respect to bee diversity and its impact on land management, including data analysis, writing, and presentation of results at an appropriate venue. A PhD candidate in genetics will work with her on gene sequencing.

Description of Student's Background and Preparation for the Project: Courtney is well prepared for both the ecological and the genetics aspects of her thesis research. Her course work provides the background on basic ecological concepts and her knowledge in genetics and gene sequencing as been developed substantially with her work with the genetics PhD candidate as supplemented by her association with Dr. Wu-Smart's Bee Lab at the University of Nebraska - Lincoln.

Courtney's research on fire effects on bee diversity will contribute not only to land management decision making but also, with the gene sequencing component, it will add to the Master Bee data bank being developed for the region. For these reasons, and because there is the potential for this research to lead to a greater understanding of local bee communities, I strongly support Courtney's proposal for consideration in this year's GRACA. Should further information be required, please let me know.

Sincerely,

Thomas B. Bragg
Professor, Plant Community/Fire Ecology