The Garden Club of America Board of Associates Centennial Pollinator Fellowship Application Form

DATE: February 1, 2017

APPLICANT INFORMATION

Full Name	Phillip Alexander Burnham
Home Mailing Address	5 Calarco Ct. Burlington, VT 05401
Telephone Number	(802) 379-0548
Permanent Email Address	pburnham@uvm.edu
University	University of Vermont
Applicant's University Mailing Address	109 Carrigan Drive Burlington, VT 05405 Room 205 Marsh Life Sciences

Biography: In 150 words, provide a biography describing your background, current educational pursuits, future plans, and any personal interests or activities.
After becoming involved in the world of pollinator research with Samantha Alger and Dr. Alison Brody as an undergraduate at the University of Vermont, I decided to make the switch from the pre-medical track to that of a pollinator-focused disease ecologist. I continued my work as an accelerated Master's student at UVM and will be switching to a Ph.D. with Dr. Alison Brody and Dr. Brandon Ogbunu (complex systems and epidemiology) in the Fall of 2017. I wish to continue to study disease from an epidemiological perspective in both native and managed pollinators. My long term goal is to start a bee research lab at a land grant university where I could both continue to acquire knowledge on the many diseases contributing to pollinator decline and disseminate that knowledge to students, beekeepers, the scientific community and the broader concerned public as a whole.

Personal Statement: In 150 words, provide a personal statement describing your interest in pollinators and how it relates to this fellowship.
I grew up around honey bees. My grandfather kept an apiary for many years and decided when I was 11, to delegate the management of the bee yard to me. I was hooked. I had not known very much about bees up until that point but through my grandfather's tutelage and my own research, I eventually figured out how to monitor the bees during the year, extract honey, and most importantly get them through the harsh Vermont winters. This background has always given me a fascination and respect for bees and for pollinators in general. When I started my sophomore year in the biology department at the University of Vermont, I learned that I could spend my life surrounded by these amazing organism, asking questions that are both interesting to science and valuable to pollinator conservation efforts. I decided that this was the only job for me.

Alex Burnham/Plight of the bumble bee: Patterns of temporal variation and coinfection between *Nosema spp.* and two RNA viruses/Centennial Pollinator Fellowship

UNIVERSITY INFORMATION

University Name	University of Vermont
University Mailing Address	Room 120A, Marsh Life Science Building 109, Carrigan Drive Burlington, Vermont 05405
Faculty Advisor's First and Last Name	Alison Brody
Faculty Advisor's Email Address	akbrody@uvm.edu
Enrollment Date	September 2016
Anticipated Date of Graduation	May 2020
Overall GPA	3.74
GPA Within Major	3.74

LETTERS OF RECOMMENDATIONS

Please list the following information for each of the three people who will be sending references for you. Using the letter of recommendation form, all applicants are required to have their three references e-mail (including your name in the subject line) all letters directly to kr@pollinator.org no later than 3PM PST on February 6, 2017. Please make sure that one of your references is from your faculty advisor.

Reference 1: Faculty Advisor	
Full Name	Dr. Alison Brody, Ph.D.
Relationship to Applicant	Primary Faculty Advisor
Telephone Number	(802) 656-0449
Email Address	akbrody@uvm.edu
Reference 2	
Full Name	Dr. Joseph Schall, Ph.D.
Relationship to Applicant	Dissertation Committee Member
Telephone Number	(802) 878-1086
Email Address	Joseph.Schall@uvm.edu
Reference 3	
Full Name	Dr. Jack Rath, DVM
Relationship to Applicant	Beekeeper and colleague
Telephone Number	800-632-3379
Email Address	rath.jack@gmail.com

Alex Burnham/Plight of the bumble bee: Patterns of temporal variation and coinfection between *Nosema spp.* and two RNA viruses/Centennial Pollinator Fellowship

STUDY OBJECTIVES

Study Title	Plight of the bumble bee: Patterns of temporal variation and coinfection between *Nosema spp.* and two RNA viruses
Study Site (State/Region)	Vermont
Proposed Timeline for Research Project	2017 to 2018

Study Description – Provide a Study Objective Summary of 100 words maximum.
The objectives for this study are to analyze bumblebees caught at five different field sites at four time points (~100 bees per time point) for five common bee pathogens (2 species of *Nosema* and 2 RNA viruses) using molecular techniques. I will examine **(1)** patterns of coinfection between the two species of *Nosema*, **(2)** patterns of coinfection between RNA viruses and *Nosema spp.*, **(3)** and study the temporal variation in prevalence of these four pathogens and how it corresponds to bee abundance through time.

Written Proposal (4 page max.) – The proposal must contain a title, priority area focus/foci, an objective, and method. Specify what portion of work has already been completed, if any.

Plight of the bumble bee: Patterns of temporal variation and coinfection between *Nosema spp.* and two RNA viruses

PRIORITY AREA FOCI:

1) Patterns of coinfection between two species of *Nosema* (*N. bombi* and *N. ceranae*)
2) Patterns of coinfection between *Nosema spp.* and two RNA viruses
3) Examining temporal variation in pathogen load between these 4 infectious agents

INTRODUCTION:

The documented decline of important pollinators has garnered much attention and concern in recent years. Bumblebees (*Bombus spp.*) in particular are important native pollinators whose decline has been understudied in light of managed honeybee losses (van Engelsdorp et al., 2008). Certain plants, most notably of the genus *Solanum* (tomatoes, potatoes, eggplant etc.), primarily rely on pollination provided by bumblebees as honeybees are poor pollinators of these plants (Strange, 2015; Thornsbury and Jerardo, 2012). Bumblebee declines in recent years have the potential to drastically disrupt the pollination services they provide and the industries that rely on them. Species such as *B. affinis, B. borealis, B. ashtoni, B. fervidus, B. pensylvanicus, and B. sandersoni* (all species that can be found in Vermont) have decreased in abundance since the 1960s (Colla et al., 2012). In 2015, the state of Vermont listed two species of bumblebees as endangered (*B. affinis and B. ashtoni*) and one as threatened (*B. terricola*) (Vermont Fish and Wildlife Department, 2015). In 2017, *B. affinis* was the first bumble bee to listed as federally endangered.

There are many threats that are thought to be causing bumblebee declines including habitat loss, pesticide use and climate change, bumble bee pathogens and the interactions that occur between them are relatively under studied. Two groups of pathogens that affect bumble bees are the microsporidian parasite *Nosema spp*. as well as a number of RNA viruses. *Nosema* lives in the midgut of its host. It has been shown to cause dysentery and adversely affects forging efficiency (Otterstatter et al., 2005). The two species that affect bumblebees are *N. bombi* (the native species) and *N. ceranae* (an invasive species). *N. ceranae* has become ubiquitous in the European honeybee (*A. mellifera*), and outcompetes *A. mellifera's* unique species of *Nosema*, *N. apis* (A. Bourgeois et al., 2010; M. Natsopoulou et al., 2014). In addition to *Nosema*, RNA viruses originally discovered in honeybees have been found in bumblebee populations (M.A. Fürst et al., 2014). Viruses like Lake Sinai Virus (LSV) and Black Queen Cell Virus (BQCV) cause behavioral abnormalities, inefficient foraging, deformities, abnormal queen cells and death (D. Schroeder and S. Martin, 2012; P. Graystock et al., 2015).

Alex Burnham/Plight of the bumble bee: Patterns of temporal variation and coinfection between *Nosema spp.* and two RNA viruses/Centennial Pollinator Fellowship

Although *Nosema* and RNA viruses have already been documented in bumblebees, interactions between these pathogens and their host have been understudied and not much is known about temporal variation in their pathogen loads. This is important as differential fluctuations in disease load between pathogens might result in certain pathogens peaking in abundance at the same time. This could increase the probability of coinfection. Coinfections (multiple pathogens in one host) might play an important role in colony collapse disorder (CCD) in honeybees (Cox-Foster et al., 2007). Understanding how these patterns work in native bee populations will allow us to make better recommendations for their conservation.

OBJECTIVES:

I propose to conduct assays (viral and fungal) on 400 bumble bees caught at four time points in a Vermont survey I conducted in 2016 to: **(1)** look for patterns of coinfection between *N. ceranae* and *N. bombi*, **(2)** examine if coinfection between *Nosema spp.* and RNA viruses (BQCV and LSV) is common in bumble bees, **(3)** and to examine and model temporal variation in pathogen load between these four infectious agents.

METHODS:

What has already been done:
In 2014 and 2015, I assisted in a survey of RNA viruses in native bumble bees across Vermont. This work was funded by a Centennial Pollinator Fellowship awarded to Samantha Alger. This survey provided the first documentation of deformed wing virus (DWV) and black queen cell virus (BQCV) in Vermont bumble bees. Through this work, we found evidence for disease spillover from managed honey bees into wild bumble bees: bumble bees were more likely to be infected and had higher viral loads when they were caught near a honey bee apiary. We also found differences in viral prevalence between bee species. Most interesting to me, when I reanalyzed these data by grouping sampling events by month, I found seasonal differences prevalence for one virus of interest, Black Queen Cell Virus ($x_3^2 = 70.05$, $p < 0.0001$). In bumble bees, this virus had a high prevalence in June, but dropped below 50% prevalence in August (**Fig. 1**). To my knowledge, this became the first evidence of seasonal variation in RNA viruses among bumble bees. To fully understand this variation and remove the confounding variable of site variation, a survey designed to repeatedly measure pathogen loads for the

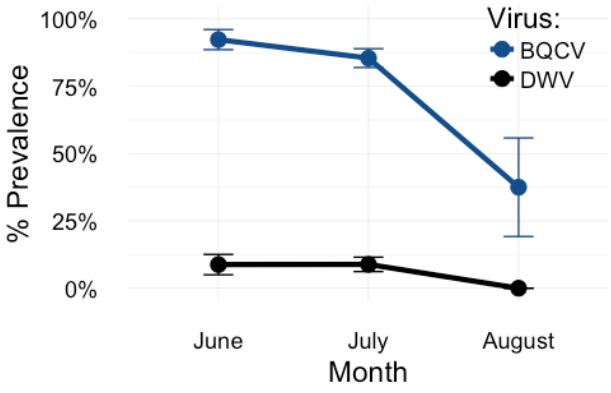

Figure 1 Prevalence for 2 viruses (Black Queen Cell Virus and Deformed Wing Virus) by month. There is evidence for seasonal variation in virus abundance for BQCV ($x_3^2 = 70.05$, $p < 0.0001$) but not for Deformed Wing Virus. In sites sampled in June and July, high prevalences were recorded for BQCV but prevalence dipped below 50% in August.

same sites at discrete time points needed to be conducted. In the summer of 2016, I revisited 5 of the field sites at 4 different time points throughout the summer. For each time point, I collected over 100 bumble bees of two focal species (*B. bimaculatus* and *B. impatiens*) and three castes, as well as conducted species abundance surveys. These specimens are now safely stored in our -80 freezer awaiting analysis.

What will be done during 2017:

In 2017, I will isolate RNA (for RNA viruses) and DNA (for *Nosema*) from 400 bumble bees caught in the 2016 survey. **(1)** I will assay bees for *Nosema* with microscopy. Using primers that I have already designed, I will conduct molecular *Nosema* assays (RT-qPCR) for both species (*N. ceranae* and *N. bombi*) to see how many spores from each species are present. This will give me *Nosema* loads and prevalence data for both species of the parasite allowing me to determine if there are patterns of coinfection between the two. **(2)** I will assay the same bees for two RNA viruses: Lake Sinai Virus (LSV) and Black Queen Cell Virus (BQCV) again using the same molecular methods. By using statistical analysis (repeated measures ANOVA and generalized linear models) I will be able to look for patterns in viral and parasite load between these four pathogens. **(3)** Using these data as well as data from 2015, I will be able to look at how the prevalence and pathogen load fluctuates throughout the course of the growing season. This information will allow me to construct a deterministic model that predicts pathogen load and coinfection probability through time.

IMPLICATIONS:

In general, native bee decline is difficult to document due to the lack of historic records. However, Vermont is unique in that it has one of the best historic collections of bumble bee species dating back to 1915 (Vermont Center for Ecostudies unpub. data). Surveys conducted 2012-2014 by the Vermont Center of Ecostudies confirmed that over half of our state's native bumble bee species are in decline. Among the many threats to our native bees, pathogens are a serious concern. Many have recognized a gap in our knowledge on the interactions of multiple pathogens in a host (Rigaud et al., 2010). The study I propose will examine this understudied area and contribute to our knowledge on the multiple threats affecting our native pollinators.

REFERENCES:

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Budget (1 page max.) As non-profit organizations, The Garden Club of America and the Pollinator Partnership do not pay overhead on funded fellowships. Please indicate whether the proposal is under consideration by other funding organizations.

Qiagen RNA extraction kits (250 count) x 1: $1177.00

Omega DNA extraction kits (50 count) x 2: $220.00

BioRad RT-qPCR kits (RNA) x 2: $1220.00

BioRad RT-qPCR kits (DNA) x 1: $610.00

Primers: $60.00

Lab Consumables (pipet tips, PCR plates, etc.): $713.00

Total: $4000.00

* I currently have partial funding for RNA isolation kits for half of my samples (200 bees) from my lab P.I. This budget is for the remaining RNA and DNA isolation kits and the RT-qPCR kits needed to complete this work.

This proposal is currently not under consideration by any other funding sources.

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SUBMISSION DETAILS

Include "Name of Applicant/Title of Proposal/Fellowship Name" on the top of each page. Please number pages. All applicants are required to submit the following materials to Kelly Rourke via email with subject line "GCA Fellowship Application – Last Name, First Name" at kr@pollinator.org no later than 3PM PST on February 6, 2017. The application is complete when both this application and the three letters of recommendation forms are received.
• **General Submission Form** Complete and sign this form and save in PDF format.
• **Photo** Provide a 300 ppi 4x6 inch color .jpg photograph. It should be a head shot with a plain background in which you are looking directly at the camera. GCA and P2 retain the rights to use any photo submitted on our websites and in materials promoting the fellowship.
• **Résumé** *(2 page max)* Email a PDF of your résumé or curriculum vitae (CV). It should include your education, professional experience, extra-curricular activities, and honors and awards.
• **Three Letters of Recommendations** Using the letter of recommendation form, all applicants are required to have their three references e-mail (including your name in the subject line) all letters directly to kr@pollinator.org no later than 3PM PST on February 6, 2017. Please make sure that one of your references is from your faculty advisor.

DECLARATION

I confirm that the information given on this form is, to the best of my knowledge and belief, true and accurate. I understand that if I have given misleading information on this form, this will be sufficient grounds for terminating my application.

I agree that the information provided on this form may be shared with the Garden Club of America and the Pollinator Partnership.

Signature_____ Date: 2-5-17