Papahānaumokuākea Marine National Monument
Permit Application Cover Sheet

This Permit Application Cover Sheet is intended to provide summary information and status to the public on permit applications for activities proposed to be conducted in the Papahānaumokuākea Marine National Monument. While a permit application has been received, it has not been fully reviewed nor approved by the Monument Management Board to date. The Monument permit process also ensures that all environmental reviews are conducted prior to the issuance of a Monument permit.

Summary Information
Applicant Name: Neil Kirk Hillier
Affiliation: Acadia University, Nova Scotia, Canada

Permit Category: Research
Proposed Activity Dates: December 1, 2011 - February 15, 2012
Proposed Method of Entry (Vessel/Plane): Vessel
Proposed Locations: Nihoa Island, Necker Island, Lisianski Island

Estimated number of individuals (including Applicant) to be covered under this permit:
3
Estimated number of days in the Monument: 10

Description of proposed activities: (complete these sentences):
a.) The proposed activity would…
evaluate populations of Heliothine moths present on individual islands within the monument. Several species of moths, including the Minute Noctuid moth (Helicoverpa minuta) and Pallda moth (Helicoverpa pallida subsp. nihoaensis) have only be recorded from these islands. The overall goal of this research will be to document the pheromone chemical-based communication and mating systems within these moth species. Given the relative isolation of these species, we anticipate that the evolution of both pheromone production and reception may be unique relative to other Heliothine moths with more ubiquitous distributions globally

b.) To accomplish this activity we would ….
Use a series of insect traps (aka. Heliothis traps) baited with female sex attractant pheromone compounds to collect adult male moths for analysis. Selected larvae would be collected and reared to obtain female moths. A small number of animals (i.e. N=20 males and N=20 females for each species) would be required to accomplish this. Moths would be transported to an isolation facility at the Tropical Crop and Commodity Protection Research (Hilo, Hawaii) to complete experimentation. Once in Hilo, moths would be analyzed for pheromone composition produced by females, the sensory sensitivity of males to female odorants, and genetic samples taken from antennal tissues for genotyping of receptors.

c.) This activity would help the Monument by …

This project will provide basic information on insect species distributions within the Monument, and also provides a proof-of-concept for a relatively non-invasive mechanism for assaying potentially disparate insect populations using pheromone attractant traps. If successful, this may provide a potential mechanism for monitoring species which are at very low population densities and/or of conservation concern in the Monument

Other information or background: This research will be integrated into a larger research project investigating olfaction in the Heliothine moths - an economically devastating group of insects worldwide. Collection of insects will be conducted within the monument and other Hawaiian islands including Maui and Big Island. Details below:

Comparative physiology, anatomy and genetics of pheromone processing and production in Hawaiian heliothine moths

In many moth (Lepidopteran) species, odor is used as a primary vehicle for sexual communication, and the olfactory-mediated behavior of the receiving sex is often narrowly-tuned to the signal broadcast by the sending sex. In this group of highly species insects there is abundant evidence that divergence in the olfactory communication channel has occurred frequently. However, our understanding of the neural mechanisms by which the signal produced by one sex becomes matched to signal reception and behavioral preference of the other sex is very limited. Moths of the subfamily heliothinae (aka. Owlet moths) are ubiquitous, with many extant species distributed globally.

Divergence in olfactory communication is evident between these species, based on shifts in the use of two aldehydes in their pheromone blends. Many members of the genera Heliothis and Helicoverpa use Z11-16:Ald as a 'primary' pheromone component, complemented with a smaller amount of either Z9-14:Ald or Z9-16:Ald. The goal of this study is to determine genetic factors which mediate shifts in pheromone production, processing and male preference for these aldehydes as species diverge. Throughout the heliothinae, shifts in male preference for Z9-14:Ald and Z9-16:Ald have occurred, contributing to reproductive isolation and speciation.

We recently found that species isolation between the closely related species Heliothis virescens and Heliothis subflexa was modulated by pheromone preference controlled by a single locus containing 4 receptor genes (which, in turn modulate pheromone sensitivity; Gould et al. 2010). The Gould lab went on to determine the functional role of each receptor, and their differential expression on each species' antennae (Vasquez et al., 2010; Wang et al. 2010).

The heliothine fauna of the Hawaiian Islands is a comparatively large one for the genera Heliothis and Helicoverpa, with at one time at least 8 species present (Hardwick, 1965). This includes immigrants and several 'endemic' species such as Helicoverpa hawaiiensis (found on all major islands), H. pallida which have only been collected on Necker and Nihoa islands, along with H. minuta (Lisianski island), and H. confusa.

Methods

1. Collection: Larvae will be collected from viable host plants during the first 2 weeks, for rearing and emergence later. Adult moths will be collected on 1-2 days at the start of the week using live-trapping Scentry Heliothis traps baited with lure variants of currently known Heliothine pheromones (which all share similar components – Z11-16:Ald, Z9-14:Ald, Z9-16:Ald, Z11-16:OH - with variable ratios). Males and females will be sorted and maintained until experimentation and extraction. Collections will take place on Big Island every week, and excursions will be made to at least 2 other islands – either Nihoa, Lisianski, Necker, Maui, or Kauai (the former 3 pending approval of collection permits for Papahānaumokuākea Marine National Monument).

2. Physiology: The right antenna of each moth will be used for Electroantennograms to determine general sensitivity to sex pheromone components, and single sensillum recording to identify neural odorant affinities. Neurons will be filled with Neurobiotin to identify targets in the antennal lobe (brain). The right antenna and head will be fixed and preserved for confocal microscopic examination at a later date.

3. Extraction: Pheromone glands will be surgically excised from males and females and placed in hexane/dichloromethane for extraction, and identification of sex pheromone components. The remaining left antenna and thorax (N=20) will be placed in ethanol or RNAlater and shipped to Fred Gould's lab at University of North Carolina for extraction of genomic DNA and cDNA. The sequences and expression patterns of receptor genes will be compared with physiological recordings from each Hawaiian species and known pheromone receptor expression in other species.

Anticipated Results:

The results garnered from the investigation of Hawaiian heliothinae will be of benefit in several areas. Tobacco budworm (Heliothis virescens), Corn Earworm (Helicoverpa zea) and Cotton Bollworm (Helicoverpa armigera) are amongst the most devastating species in the world, being cosmopolitan and polyphagous in habit, attacking hundreds of different host plants. Each of these species cost worldwide damage and control costs between $1-5 billion per annum. One of the primary methods for managing these species is the development of pesticide-free pheromone-based monitoring and disruption technologies. This proposal will benefit the development and refinement of pheromone-attractants for these species, which may be ultimately be implemented in pesticide-free monitoring and mating disruption technologies for these devastating pests.

By using a multi-faceted approach, incorporating conservation, ecology, physiology and genetics, we will build significantly upon the currently knowledge of the evolution of pheromone-based species isolation, and basic olfactory processing (and maximize data from samples). The islands of Hawaii provide a unique arena for examining such species, both due to the relative isolation and diversity of species found on each island. By highly effective pheromone live-trapping, we may confirm the presence/absence of presumed extirpated species H. confusa and H. minuta. Results of combined physiology and genetic study will provide valuable information on speciation in this unique archipelago and evaluate if there has been cross-breeding with closely related invasive species. Furthermore, by extracting brains, DNA, and male and female pheromones, the physiological, neuroanatomical and genetic data provided

by this visit will provide for a bank of information to be incorporated with global comparative studies on the heliothinae for years to come.