Full name(s) of VIU faculty involved in the project and their Department(s): Jasmine Janes, Biology; Tim Goater, Biology

Contact person email: Jasmine.Janes@viu.ca

Award: Inquiry Grant

Number of undergraduate student(s) involved: 1 paid

Number of graduate student(s) involved: NA

Number of community partner(s) involved: NA

Abstract for the project:
Pollination ecology provides a means of exploring plant-insect interactions in natural settings. Many plant species rely on insect pollinators to achieve reproduction. Often these relationships are quite specific (i.e. one plant, one insect) while others can be considered generalist (i.e. one plant, many insects). Understanding which strategies and pollinator groups are being used can provide valuable information regarding conservation, population dynamics, ecosystem services and species diversity. Using a combination of diurnal and nocturnal insect trapping methods, this project will investigate the pollination ecology of bog orchids (Platanthera). Members of this orchid group are believed to be pollinated by various insects and to display a variety of pollination syndromes. However, very little work has been conducted on this group in North America and studies for western North America are absent from the literature. This study will provide much needed baseline information on insect diversity, potential pollinators of Platanthera and close relatives, and the extent to which the species rely on nocturnal or diurnal insect species. The project will also provide training for an undergraduate student and facilitate further collections and teaching resources for the VIU Natural History Museum.

Floral visitor assessments of *Platanthera sp.* (Orchidaceae) and relatives

Background

Orchids are one of the most speciose plant families in the world (Cozzolino et al. 2006), yet little is known about the basic biology of certain groups. Many orchid groups comprise taxonomically challenging species as a result of overlap in morphological features (Dressler 1993), recent and rapid radiation of species (Dressler & Dodson 1960), hybridization (Cozzolino et al. 2006) and plasticity to environmental changes (Flanagan 2006). However, these features also provide the opportunity to address long-standing questions relating to ecological and evolutionary biology.

Plantanthera sp. (Orchidaceae, subfamily Orchidoideae) represent a diverse group of terrestrial orchids distributed throughout temperate regions of the Northern Hemisphere (Esposito et al. 2018). The group comprises approximately 40 species, all of which are believed to have undergone recent and rapid radiation with respect to floral morphology and pollination syndrome (Hapeman & Inoue 1997; Esposito et al. 2018). Further confounding their taxonomic status and biology, many of these species are reported to hybridize freely (Wallace 2004) in spite of pollination syndromes that should prevent such crosses. For example, Platanthera dilatata is considered an obligate outcrosser (Boland 1993), while P. aquilonis is believed to be strictly selfing (Wallace 2006). However, molecular evidence suggests that these two purportedly reproductively isolated species are the parents of P. huronensis (Wallace 2004). Thus, insight into the pollinator communities that may be servicing these species may assist in understanding the broader pollination ecology and its influence on speciation within the group.

Some pollination ecology research has been conducted on Platanthera species in Europe (Esposito et al. 2018) and eastern North America (Boland 1993) (Wallace 2006). Such studies suggest that bees may be the primary pollinators during the day and that moths may perform additional pollination at night (Boland 1993; Maad & Nilsson 2004). However, biogeoclimatic conditions are markedly different for the Vancouver Island area thus, pollinator communities are different also. To date, no pollinator surveys have been conducted for this group in the Vancouver Island area, or British Columbia. This project will: 1) assess the diversity of floral visitors associated with Platanthera, and 2) observe the behaviour of floral visitors to make more informed decisions about their role in pollination.

Methods

Site selection

Records from the 2018 Mount Arrowsmith Biosphere Region BioBlitz indicate that *Platanthera* species occur in the Englishman River Falls Provincial Park area. Personal observations of *Platanthera* species have also been made in the Mt Washington area. We will select a minimum of two sites across these areas, but ideally two sites per area will be identified.

Floral visitor observations
Several insect groups are believed to be potential pollinators of *Platanthera*. These groups include beetles (Coleoptera), butterflies and moths (Lepidoptera) and bees (Hymenoptera). To accommodate such insect diversity a variety of trapping and observation methods will be used.

Trapping methods: To assess the diversity of day-time floral visitors we will use pan traps and sweep-net surveys. Pan traps are coloured plates filled with water and a surfactant that attract flying insects with UV-reflective colours (Saunders et al. 2018). For each site, 12 coloured plates (two each of dark blue, orange, yellow, white, light blue and pink) will be laid out, in random order, with 1 m between them. Plates will be available for 1.5 hours on three separate occasions. Timed sweep-net surveys will be conducted nearby at the same time. Platanthera species tend to be visually dull (mostly green) thus, these surveys will facilitate the capture of insects that may not be attracted to UV-reflective colours. Sweep-net surveys will be conducted, at each site, over a 5 m transect for 2x15 minute collection periods on three separate occasions. To assess the diversity of night-time floral visitors, a black light and drop-sheet method (black light method) will be used. This is a common method for surveying nocturnal flying insects (Nielsen et al. 2013). Black light traps will be set up at each site for 1.5 hours on three separate occasions. All insects will be stored in vials until they can be processed.

Observation methods: Insect floral visitor behaviour will be observed through timed surveys to better distinguish visitors from true pollinators. Four 1x1 m quadrats will be laid out randomly at each site on three separate occasions. For each quadrat, insect visitor behaviour will be recorded over 3x15 minute periods. Specifically, we will be interested in distinguishing between behaviours such as: brief fly overs, brief visits (i.e. landing on the labellum), extended visits (i.e. entering the floral tube), feeding and collecting pollen (i.e. pollinia mass observed on the body of the insect). Visiting insects will be collected by sweep-net, where possible, after observing their behaviour. All insects will be stored in vials until they can be processed.

Data analysis
Representative voucher specimens of *Platanthera* and insect species will be lodged with the Royal Victoria Museum and VIU Museum of Natural History. Insect samples will be identified to the lowest taxonomic level possible using local field guides and species keys. Species diversity within sites will be assessed through Shannon's and Simpson's diversity indices. Species diversity among sites and among trapping methods will be assessed using a one-way ANOVA in R. Differences between sites will be explored using a Turkey's honest significant difference test. Paired t-tests will determine whether there were significant differences between observations of insect behaviour among sites or *Platanthera* species.

Outputs/outcomes
This project will provide much needed information regarding pollinator assemblages associated with terrestrial orchid habitats in central Vancouver Island. Considering that very little is known about *Platanthera*, and close relatives, or pollinator communities on Vancouver Island, this project represents an important step toward filling that knowledge gap. Thus, not only will this project address the specific aims of characterizing the floral visitors associated with *Platanthera*

and identifying potential pollinators, it will also enhance our general understanding of plant-insect interactions and insect species diversity on the island. Surveys like this are becoming increasingly important baseline information to assess impacts on insect communities from changing climates and habitat loss (Eisenhauer et al. 2018).

Plans to mobilize/commercialize knowledge/outputs

Findings from this project will be communicated through the VIU Research and Creative Activity Symposium and VIU's Research Week. In addition, the BIOL491 student will be encouraged to present the work at the Pacific Ecology and Evolution Conference (PEEC) in Bamfield. We will also pursue a publication in the *Canadian Journal of Entomology*.

References

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Item	Quantity	Justification	Total cost
Salary	168 hrs x $13.85/hr	As of June 1st 2019, the minimum wage for BC is $13.85/hr. The student will be employed over the summer to begin fieldwork. To ensure that a student is attracted and motivated to perform the work, it is necessary to pay at least minimum wage. The budgeted hours will provide almost five weeks worth of work for the student to conduct field work, sample identification and mounting of specimens. The BIOL491 aspect of the project will commence in Fall 2019, comprising literature reviews, statistical analysis and thesis writing. This component will be unpaid.	$2,326.80
Ethyl acetate	5 x $4.10	Insect euthanasia solution to ensure minimal stress and damage to specimens.	$20.50
Glassine envelopes	5 x $6.00	Glassine envelopes are favoured for Lepidoptera collection as they preserve delicate identifying features on wings.	$30.00
All-weather notebook	3 x $9.50	Detailed field notes are necessary for identification and characterization of behaviour.	$28.50
Plastic kill jars	24 x $3.50	Used to separate insect dispensing agents from actual contact with the insect.	$84.00
Plastic collection vials	30 (12 pk) x $4.00	For collection and storage of insects while in the field and lab	$120.00
Adjustable spreading board & strips	2 x $42.00	Used to pin and spread Lepidoptera wings until set; for long-term storage and display.	$84.00
Insect storage/display box	12 x $50.00	For permanent display and storage of valued insect specimens in the VIU Natural History Museum.	$600.00
DC battery pack with charger & black light & nocturnal collecting sheet	1 x $480.00	UV light attracts numerous nocturnal insects in the area. Previous research suggests that *Platanthera* may be pollinated at night by hawkmoths.	$480.00
Pan traps	4 (6 pk) x $35.00	Collection of day-time floral visitors.	$140.00
Mileage	5 x 50 km @ $0.52 5 x 134 @ $0.52	Travel to field sites (Mt. Washington & Englishman River) from VIU will be conducted in personal vehicles with reimbursement at the current VIU rate.	$478.40
Per diems	2 x 5 @ $60.00	Five days of field work, accounting for myself and the student, at current VIU rates.	$600.00
TOTAL			**$4,992.20**

Project team – roles & responsibilities

Janes – As **principal investigator** I will provide expertise in experimental design, statistical analysis, herbarium sample preparation and plant and insect identification.

BIOL491 student – An exemplary student has been recruited to undertake some of this work for their BIOL491 project. This student (Genevieve van der Voort Maasrchalk) has taken several upper level courses in preparation for this type of research, including: plant ecology, forest entomology, entomology and community ecology.

Dr Tim Goater – Dr Goater (VIU Biology) will collaborate on this project providing expertise in entomology, insect collection and insect mounting.