Competition between Carrion Beetles and Microbes over Carrion: Influence of Hot Temperatures

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1. Project Description

Description of Project

Background. Carrion is a valuable resource (Forbes 2016, Von Hoermann 2018), however, its location and occurrence are unpredictable (Benbow et al 2016). A soon as an animal dies, it becomes a food source for many different organisms, such as microbes and insects (Forbes 2016). The break-down of dead animals occurs in a predictable way (Forbes 2016; Metcalf et al 2013) and is primarily driven by microbes (i.e., bacteria and fungi) and insects (Forbes 2016, Lauber et al 2014). There are five recognized stages of decomposition: fresh stage, bloated stage, active decay stage, advanced decay stage, and remains stage (Matuszewski et al 2008; Metcalf et al 2013).

The fresh stage begins when the heart stops and the microbes that live in and on the animal begin decomposition. Bacteria from the soil soon arrive. Both aerobic and anaerobic processes occur in the fresh stage. The bloated stage is driven by anaerobic bacteria. The bacteria in the intestines produce gas causing bloating. The bloat stage ends when the carcass ruptures and transitions to the active decay stage. Active decay returns to aerobic decay. At the advanced stage little microbe activity occurs due to diminished soft tissues. The remaining stage is characterized by leftover materials that are harder to break down (i.e., dry skin, cartilage, bone). In addition to microbes, insects play an important role in carrion decay as they greatly accelerate the process (Forbes 2016). The timing of insect arrival depends on the insect species and environmental cues, such as the volatiles produced by the microbes (Cammack 2016). The succession of insect communities occurs much like microbes and is predictable.

Dead animals contain many nutrients and their decomposition by insects and microbes releases trapped nutrients into the environment. Releasing nutrients from carrion is part of nutrient cycling in an ecosystem and is extremely important for a healthy ecosystem (Forbes 2016).

Since carrion is a valuable resource, organisms will fight to gain control over it. As insects and microbes are often the main contributors to decomposition, competition between them is intense and both have evolved strategies to deal with their competitors (Janzen 1977). Microbes often utilize toxins which can harm animals or make carrion unsuitable for animal use (Trienens et al 2010). Similarly, insects also use chemicals (e.g., antimicrobial peptides or tannin) when competing over carrion (Bulet et al 1999; Cotter & Kilner 2010, Janzen 1977). In addition, insects can alter the microbiome of carrion by removing parts of the carrion (e.g., the intestine) or transferring symbiotic microbes from their gut onto the carrion (Hwang & Lin 2013, Janzen 1977, Pukowski 1933, Shukla et al 2018).

Temperature is likely to influence competition between insects and microbes over carrion (Catalán et al 2012). Temperature optima and temperature tolerance are species specific. Exposure to heat stress negatively affects growth, longevity, and reproduction in insects and microbes (Hidalgo et al 2019, Nottingham et al 2019). Changes in the outcome of competition between insects and microbes over carrion due to temperature increase may impact the return of energy and nutrient to the ecosystem, especially when microbes outcompete insects and the decomposition of carrion is slowed down (Forbes 2016).

Burying beetles (*Nicrophorus* spp.) are among the first insects to arrive on fresh carrion and exclusively use small vertebrate carrion (i.e., mice) for reproduction (Scott 1998). They use a number of

strategies to clear carrion of microbes; they remove the carrion's gut, secrete antimicrobial peptides, and manipulate the carrion microbiome by transferring symbiotic bacteria onto the carrion (Shukla et al 2017). These behaviors enhance growth and survival of the larvae by preserving the carrion (Shukla et al 2018). Increased temperatures may reduce the ability of burying beetles to produce antimicrobial peptides and preserve the carrion. Higher temperatures increase the metabolic costs for body maintenance and movement (reviewed in Huey & Kingsolver 2019). Consequently, the beetles will need to allocate more resources to metabolisms and potentially reduce their resource allocation to carrion preservation and production of antimicrobial peptides.

The **goal of this project** is to investigate how increased temperatures will affect the competition of burying beetles and microbes over carrion. Specifically, I will address these two **questions**:

1. Do amount and potency of the antimicrobial secretions produced by burying beetles change with increasing temperature?
2. Do increased temperatures change the outcome of the competition over carrion between burying beetles and microbes and thus reduce the ability of burying beetles to preserve the carrion?

Significance. In the wake of global warming, the effect of temperature on ecosystems has become increasingly important to understand, especially in the context of nutrient recycling (Barton et al 2019). If nutrient cycling is slowed in an ecosystem there could be serious consequences. Though microbes are essential for nutrient cycling, the process is slowed without the help of insects.

Methods

As **study objects**, I will use burying beetles from a laboratory population of *Nicrophorus orbicollis* maintained by Dr. Claudia Rauter at the University of Nebraska in Omaha.

Experiment 1. In order to test whether amount and potency of antimicrobial peptides secreted by burying beetles, changes with increasing temperature reproducing females will be exposed to one of 4 different temperatures: 20°C (i.e., standard temperature used in laboratory experiments), 22°C, 24°C, or 26°C (pilot study indicated substantial increase in reproductive failure at 26°C). Using glass capillaries, I will collect oral and anal secretions several times from each female while she is reproducing: i) before the female encounter the carrion (i.e., freshly thawed mouse 18-22g), ii) on the day she laid the first egg, iii) on the day the larvae hatch, and iv) on the day when the larvae leave the brood chamber. The secretions will be stored at -80°C until carrying out standard lytic assays which assesses the ability of the secretions to lyse the bacterium *Micrococcus luteus* (Duarte al 2016). For each sample, the lytic assay will be carried out at two temperatures (20°C and 26°C) which will allow me to investigate whether the antimicrobial potency of the secretions depends on the temperature the female was exposed to during reproduction.

To control for changes in the female's metabolic costs due to increased temperatures, I will determine the resting metabolic rate of each female by measuring the CO_2 production of the female when her larvae are 1 day old and are receiving the most intense parental care (i.e., feeding and preservation of carrion; Berzins, Granville & Rauter unpublished data; Scott 1998). CO_2 production will be measured using a standard flow-through approach (Trumbo and Rauter 2014).

To control for changes in the antimicrobial secretions of larvae in response to temperature increase and thus potentially influencing the female's secretions (Duarte et al 2016), I will also collect antimicrobial secretions from the larvae when they are 1-day and 3-day old. This is the period when the larvae receive the most intense parental care (i.e., are fed by the parents; Scott 1998) and the antimicrobial potency of their secretions is the strongest (Reavey et al 2014).

For this experiment I will randomly assign 40 females from the laboratory colony to each of the 4 temperatures (20°C, 22°C, 24°C, 26°C). 20 females assigned to each temperature will used to assess amount and potency of their antimicrobial production and the other 20 females will used to measure CO_2 production.

Experiment 2. In order to test whether increased temperatures influence a burying beetle's ability to preserve the carrion, I will compare the growth of mold and bacteria on carrion (i.e., freshly thawed mouse, 18-22g) in the presence and absence of female beetles while at the same time exposing each carrion to either 20°C; 22°C, 24°C, or 26°C. To simulate natural soil conditions with its soil microbiome, I will use for his experiment soil from the area the beetles giving rise to the laboratory colony were originally trapped. I will collect the soil immediately before starting this experiment.

To assess the preservation of the carrion, I will take a picture of each carrion every 2nd day until the carrion is completely decomposed. Using ImageJ (https://imagej.nih.gov/ij/index.html), I will determine the amount of carrion covered with bacteria or mold. As second measure of carrion preservation, I will determine the number and size of larvae leaving the brood chamber as the preservation of the carrion is closely associated with growth and survival of the larvae (Shukla et al 2018). For this experiment I will use 20 replicates for each combination of temperature exposure (20°C; 22°C, 24°C, or 26°C) and carrion treatment (beetles present or absence) resulting in a total sample size of 160.

Data Analysis. The data of experiment 1 will analyzed using general linear models with the main factors being the temperature that female beetles were exposed to during breeding and the incubation temperature used in the lytic assay and the covariable larval secretions. The data of experiment 2 will analyzed using general linear models with the main factors, temperature exposure of the carrion and presence/absence burying beetles. For all statistical analyses, I will use R v 3.6.1 (R Core Team 2019).

Project Timeline

Month	Task
Jan – May 2020	Experiment 1: Data collection
May – Sept 2020	Experiment 2: Data collection
Oct 2020	Experiment 1: Carrying out lytic assays and data analysis
Nov 2020 – Feb 2021	Experiment 1: Data analysis and writing corresponding sections for Master's thesis
March 2021 – May 2021	Experiment 2: Data analysis and writing corresponding sections for Master's thesis; presentation of results at RCAF and Biology Seminar

Student/Faculty Role

I will be responsible to carry out the proposed experiment and all data collection, data analysis, and presentation of the results at the UNO Research and Creative Activity in March 2021. My advisor, Dr. Rauter, will advise and support me with the set-up of the experiment, data collection, data analysis, and preparation of the presentation at the UNO Research and Creative Activity in March 2021. Dr. Rauter will also train me in carrying out the lytic assays and determination of the microbe communities.

Previous Funding – None.

IACUC & IBC

IACUC protocols are not required for this project; beetles (*Nicrophorus spp.)* are not vertebrates and the mice used as carrion will be purchased frozen and thus not alive upon arrival at UNO.

IBC protocols are not required for the lytic assays using *Micrococcus luteus*, since *M. luteus* is classified in the risk group 1 (https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/micrococcus.html).

2. Budget Justification

Supplies

Item	Amount ($)	Total ($)
Compact incubator	845	845
Resources for breeding beetles		360
Frozen mice	360	
Assays		745
Glass capillaries	113	
Small Petri-dishes	187	
Square Petri dishes	345	
Freeze-dried *Micrococcus luteus*	100	
Laboratory Supplies		150
Student Summer Stipend		2900
Total		**5000**

Justification

I am requesting funding for a compact incubator to be able to set up all 4 temperature treatments simultaneously as Dr. Rauter's lab has only 3 compact incubators. One compact incubator can hold 4 containers with reproducing females. Thus, I would be able to setup 4 replicates for each temperature at the same time for Experiment 1 and 2 replicates per temperature ×carrion treatment for Experiment 2, respectively. Setting up more replicates at once would be difficult to handle and data collection may be incomplete for some replicates due to time constraints.

As carrion, I will use commercially available, frozen mice which I will purchase from American Rodent Supply, LLC, Indianapolis, IN.

For the lytic assays, I will need glass capillaries to collect antimicrobial secretions from the female beetles. Small Petri-dishes will be used to collect antimicrobial secretions from the larvae. The larvae will be placed into the dishes with a small amount of distilled water for 30 minutes. At the end of the 30 minutes, the larvae will be placed back to their mother and the water-secretion solution will be collected to be used in the lytic assay. Square Petri dishes will be used during the lytic assay as container to pour the agar-*Micrococcus luteus* solution into before adding the antimicrobial secretions. Freeze-dried *Micrococcus luteus* will be purchased from Sigma-Aldrich.

Laboratory supplies will include gloves, pipette tips, centrifuge tubes, and Kim wipes.

The student summer stipend will be used to provide a salary to cover cost of living in the summer semester so that I can work full-time on the project.

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University of Nebraska Omaha

DEPARTMENT OF BIOLOGY

2 October, 2019

TO: GRACA Award Committee

FR: Claudia M. Rauter, Associate Professor of Biology

RE: Letter of Mentor Support, Laura Ney

I enthusiastically endorse Laura Ney's GRACA proposal which she has revised several times based upon my comments and suggestions. Her research project will contribute to my research program which focusses on the impact of environmental factors (e.g., food quantity and quality, population density) on the resource allocation in an insect with elaborate parental care. Laura 's project will concentrate on if and how warmer temperatures affect antimicrobial secretions by burying beetles (*Nicrophorus* spp.). Burying beetles use carrion as a food resource and secrete antimicrobial peptides to preserve the carrion thus improving growth and survival of their larvae. Antimicrobial secretions are therefore an important component of parental care in our study system.

Laura Ney's proposal addresses an essential environmental aspect of ectotherms such as burying beetles, especially in the context of global warming. Higher temperatures can have substantial negative consequences for population growth of ectotherm and in the case of burying beetles also for the recycling of resources within ecosystems. Laura's proposed experiments can be carried out within the proposed time line and allow her to address her questions appropriately. As standard protocols will be used for both experiments, I expect the data collection to be successful.

The proposed budget by Laura Ney is realistic and will allow her to purchase all the supplies needed to carry out her project. The summer stipend will allow her to devote all her time to the project during the summer and make substantial progress.

Laura Ney is a graduate student in my lab and has joined the Biology Graduate program this fall semester. As undergraduate student, Laura conducted research in the area of ecology and has thus research experience. This semester, Laura is receiving training in the practical aspects of using burying beetles as study objects, conducting lytic assays, and measuring metabolic rates of beetles. While Laura Ney works on her research project, I will support her with advice and guidance and, if needed, provide a helping hand. We will have weekly meetings to ensure her project progresses as planned. Based on Laura's enthusiasm for the project and her work ethic, I am convinced that she will achieve the goals of the proposed research project.

Sincerely,

C. Rauter

Claudia Rauter

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