Project Title: Effects of global warming on ectotherm ecosystem engineers: from genes to behavior

Abstract

Global warming affects the entire biosphere. Ectotherms are particularly susceptible to warming as their body temperature depends on environmental temperature. Ants stand out as interesting model organisms. They have large ecological impacts through their huge collective biomass, their numerous interspecific interactions (as preys, predators, aphid farming, seed dispersal, etc.), and because many species are ecosystem engineers (e.g. through soil bioturbation). In addition, owing to their unique biology they may be impacted, and/or show resilience, at both individual and colony levels. In this respect, it is worth noting that in many species, colonies vary dramatically in size throughout their lifecycle, typically starting with a single queen and growing to hundreds of thousands or even a few million workers. Small-sized colonies are most susceptible to warming and will be the focus of the proposal, that aims at investigating how heat stress affects (1) gene expression, (2) underground nest architecture and (3) colony growth and mortality. The current proposal will highlight ant susceptibility to increasing temperatures, which has important cascading ecological effects, and their ability to respond to thermal stress. The proposal fits the third scientific priority (Mondes Durables).

Keywords: global warming, stress-related genes, ant nest architecture, bioturbation, X-ray tomography

I. Background

Global climate changes impact species distribution, adaptation, and survival[1]. Quantifying key species' susceptibilities or resilience to these changes is a necessary step to identify and try to mitigate the incoming cascading changes. Investigating the underlying effects of heat stress is a proxy that can be used to assess the impact of global warming on different species. Global warming may exert some direct effects on ectotherms[2], such as ants, because their body temperature depends on external temperatures. Ants can adjust external temperatures to their needs to some extent, notably through nest architecture, the conductivity of nest material[3] and the use of fermenting materials for nest building. However, this is a costly process that depends on variables such as population and nest size. Founding queens and small-sized colonies are more susceptible to warming than more populous colonies. They have no or a few workers to build their nest, which are thus simpler and shallower than those of more populous colonies, and workers from incipient colonies are typically small-sized and more sensitive to heat and desiccation (less favorable surface/volume ratio).

High temperatures impact gene expression, development pace, size, and metabolism of individual workers[4,5,6,7], and nest architecture at the colony level. Thermophilic ants have evolved heat resistance and have fewer genes affected by heat stress than temperate ant species, where heat affects the regulation of thousands of genes[6]. High gene regulation sensitivity to heat stress can potentially result in aberrant development, size, and metabolism-related traits. Some ants can adjust their circadian rhythms to avoid foraging under heat[8], but such behavioral changes may increase interaction with competing ants' species, exposure to predators, or decrease food availability. At the colony level, modifying above-ground nest architecture adjusts the amount of sunlight hence heat received, while modifying below-ground tunnels adjusts ventilation hence cooling[7,9]. Nest architecture is flexible to some extent and this allows colonies to adjust to ambient temperature to some extent[10]. This process however has the potential of affecting soil bioturbation, a key environmental role displayed by ants in which soil particles are dispersed and organized and that is crucial for soil formation and ecosystem functioning[11,12]. Thus far the studies have addressed independently how heat stress affects ants, notably through the measure of Critical Temperatures, however, it is yet to be determined how modifications in gene regulation are translated into physiological and behavioral traits.

By using an interdisciplinary approach (molecular biology, survivorship and growth analysis, X-ray computed tomography) the current proposal aims at determining how heat stress affects ants, organisms displaying key roles for the environment and that can be directly and negatively affected due to global warming. The project will determine whether species with small colonies are more susceptible than species with populous colonies, and whether species founding new colonies by a solitary queen are more at risk than those founding new colonies by a queen accompanied by hundreds or thousands of workers. This will bring awareness about how ants suffer from heat stress, how ant communities may change in response to it, and what are the potential cascading ecological effects on arthropod communities and ecosystemic services such as bioturbation.

II. Objectives

The main goal is to establish the causal links between heat stress and its underlying consequences in different ant species. The specific objectives include (1) pinpointing stress-related genes that may respond to heat stress, (2) investigating detrimental effects of heat on mortality and colony growth and (3) measuring 3D nest architecture adjustment in response to heat.

III. Justificative

As ectotherms, ants are susceptible to increasing temperature at both the individual and colony levels. Yet, the underlying effects of heat stress over different ant species remain little explored. Heat stress can trigger changes ranging from the activation of stress-related genes to modification of soil bioturbation by ants and differential impacts on the survival of ants with small vs large adult colonies and solitary vs group colony foundation. Addressing these effects fits the third priority theme of the SOUND call (Monde Durables – Solutions Fondées sur la Nature). The project will investigate the underlying effects of both chronic and acute heat stresses on genes expression, survival and nest architecture.

IV. Suitability for the theme

Global warming stands out as one of the most discussed themes of the moment given its direct impact not only over humans but also all the other living species. Humans are responsible for global warming through the emission of greenhouse gases, deforestation, or land clearing activities, for instance. It is essential to study how species are affected at all levels of organization (molecular, behavioral, communities to ecosystem functioning) to anticipate long lasting consequences, and we propose to do this using an interdisciplinary approach combining measures of genes expression, behavior and X-ray computed tomography.

The results will be published in academic journals and communicated to the general public, through participations to the Fête de la Science, conferences for non-professionals, and publication in non-academic scientific journals (e.g. *Frontiers for Young Minds*, *Sciences et Avenir*, *Science & Vie*).

V. Material & Methods

- ***Experiment 1: effects of chronic vs acute heat stresses***. We will test for the effects of chronic vs acute heat stresses by exposing groups of 30 workers to non-lethal temperatures (e.g. 30, 35, 40 and 45°C) and for varied duration (10 min, one hour, one week and one month) using hot plates allowing a precise control of temperature (0.1°C precision, plate model PZ28-1, Gestigkeit, Germany). 10 replicates will be used per temperature and exposure duration. Ants will be checked daily during the duration of exposure to heat to monitor their survival. At the end of the experiment, surviving ants will be stored at -80ºC. A survivorship Kaplan-Meier estimator[13] will be used to measure survival under the varied temperature and duration regime, to document the effects of chronic and acute heat stresses. Genes expression will be measured on a subset of 4 workers per temperature and exposure duration. RNA will be extracted from the heads using a RNeasy Mini Kit (several individuals will be pooled if necessary), and quantified with a NanoDrop 1000 spectrophotometer. Samples will be processed by an Illumina NovaSeq 6000 Sequencing system. The differential expression analysis will be performed using the R package edgeR. The data to be reported will include (1) overall differential expression analysis targeting pathways that may respond to heat stress (e.g. cellular response to heat)[6]; (2) single gene plots to demonstrate the individual expression levels of key genes for the study (e.g. heat shock genes)[6]; (3) Weighted Gene Co-expression Network Analysis (WGCNA) analysis to identify potential modules of genes that may be directly affected by temperature.
- ***Experiment 2: heat avoidance through nest excavation***. We will measure the capacity of ants to avoid heat by excavating deeper nests using earth columns where ants can excavate their nest freely. One trial will use founding queens of the black garden ant, *Lasius niger*. We have shown that it modifies its nest architecture in response to surface temperature[12] (Fig. 1). It founds new colonies (10^4 workers when adult) by one or a few queens without the help of workers, and this likely makes incipient colonies susceptible to heat. Founding queens will be placed on top of the soil columns either solitarily or in groups of four or eight queens, to test for the effect of group size. A second trial will use whole colonies of *M. graminicola*. This species has few workers (~80) and excavates small (acorn-sized) and shallow (ca 5 cm deep) nests which are also likely susceptible to heat. The soil columns will be placed under heat lamps providing varied surface temperatures (20, 30 or 40°C) and tomography will be carried out at regular intervals (7, 14, 28, 88 days) using a medical Computed X-ray Tomograph at the nearby Pitié-Salpêtrière hospital[12]. Images will be processed and quantified with ImageJ software version 1.53 s and visualized with Avizo software version 2021.2 (ThermoScientific)[12]. At the end of the experiment, nests will be excavated to quantify queen survival and colony growth, and the size of the workers produced under the various temperature regimes will be measured using a motorized binocular microscope[12]. For each species, 15 replicates will be used per temperature treatment. All replicates will be used for the demographic and morphometric measurements, but only 5 out of the 15 will also be used for tomography.

Figure 1: CT scan of a *L. niger* nest (from [12])

- ***Study species***. We will start by studying *L. niger* and *M. graminicola*. If time permits, we will add the wood ant *Formica rufa*. It forms very large colonies (10^5-10^6 workers) that have a strong impact on forest ecology, and its founding queens bypasses the solitary stage by entering a colony of *F. fusca* and killing and supplanting its queen.

VI. Research profile of the candidate

The candidate will have a good knowledge in molecular biology and associated statistical analyses. Although not prerequisites, a knowledge of social insects biology would be welcome. The candidate will be autonomous, capable of working independently and will show initiatives. Motivation for learning the different approaches, good English communication and writing skills are also necessary.

VII. References

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