

Molecular underpinnings of adaptive thermal tolerance to climate change in the ant, *Aphaenogaster rudis*

Keywords: Climate change, Molecular Evolution, Adaptation, Ants, Transcriptomics

Background. Global warming is predicted to alter biodiversity, most strikingly across latitude and elevation, especially for arthropod populations whose activity and reproduction are coupled to temperature (1,4). Population persistence in the face of temperature shifts is likely to depend on the extent of temperature-induced phenotypic plasticity (acclimation) and evolutionary adaptation (3,8); however, arthropod populations tend to operate closer to their upper thermal limits at their latitudinal or elevational range margins, so any additional warming is expected to lead to extinction or northward range shifts unless these populations can adapt to rapidly changing conditions (1).

Acclimation and adaptation to thermal stress are ultimately mediated at the molecular level by changes in transcription and protein kinetics; thus, uncovering the molecular basis of temperature response is critical for understanding and predicting how arthropods will respond to climate change (8). The most conserved thermal stress pathway is the heat shock response (HSR), a major transcriptional alteration that up-regulates thermal response genes (heat shock proteins-HSPs) while down-regulating metabolic genes (3,8). Empirical studies of HSR have primarily focused on marine arthropods and model organisms, but are lacking for terrestrial non-model arthropods despite their important role in providing vital ecosystem processes and services (3,6,8).

The numerically abundant terrestrial ant, *Aphaenogaster rudis*, occupies mesic eastern forests from Florida to Maine. This species is an important mediator of nutrient recycling and seed dispersal (5,6), the disruption of which are likely to have cascading effects across the forest ecosystem (6). Recent models predict ants located in warmer and more mesic forests to be the most susceptible to climate warming (2); however, common garden experiments have revealed that although colonies from warmer latitudes decline under severe thermal stress scenarios, they show greater acclimation ability under mild thermal stress relative to colonies from cooler latitudes (7). These findings suggest southern populations may be more resilient than initially predicted and may serve as a reservoir of adaptive thermal tolerance alleles for northern populations.

Questions. I propose to study the molecular underpinnings of adaptive thermal tolerance among *A. rudis* populations across a latitudinal and elevational gradient. I ask two fundamental questions: 1) How does transcriptional regulation of thermal tolerance in *A. rudis* vary with thermal environment, and 2) How has selection shaped molecular mechanisms underlying thermal traits/tolerance?

Hypotheses. 1.) More thermally tolerant populations (southern range) elicit faster or stronger HSR, or have higher basal levels of HSR proteins relative to less thermally tolerant populations (northern range). 2.) Positive selection in nucleotide coding sequences alters protein kinetics to confer thermal tolerance.

Methods. I will use RNA-seq methods to characterize the transcriptome of ants collected from multiple sites along a north-south transect experiencing different temperature regimes, both from natural field variation across their range and from colonies reared in common garden conditions. These will be analyzed with bioinformatics techniques to identify genes and pathways whose expression patterns vary by temperature regime and/or geographic origin. I will measure gene and protein expression via qPCR and

western blots, respectively, to investigate the role of known thermally responsive genes (hsps). I will test for positive selection by sequencing thermally responsive genes and calculating the ratio of non-synonymous substitutions to synonymous substitutions (dN/dS) among populations that differ in their thermal tolerance.

Preliminary and Expected Results. Higher inducibility of thermally responsive gene transcripts in more southern populations would indicate a faster or stronger HSR. Alternatively, adaptive mutations within coding sequences may maintain function under thermal stress and be positively selected in warmer environments. Empirical studies have demonstrated that induction of hsp70 and hsp83 underlie

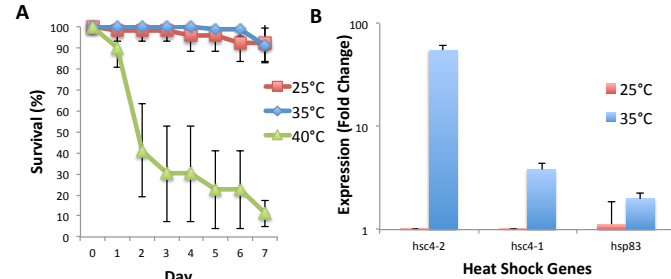


Figure 1 shows the thermal tolerance of *A. rudis* across rearing (25°C), sub-lethal (35°C) and lethal (40°C) temperature regimes (A) accompanied with preliminary inducible gene expression patterns (B).

acclimation ability (3). Thus far, I have discovered two unique inducible HSPs (hsc4-1, hsc4-2) in ants, as well as one (hsp83) shared with *Drosophila melanogaster* (Fig. 1).

Intellectual merit and Broader Impacts. These findings will illuminate the molecular underpinnings of thermal adaptations, which can contribute to building better-informed species distribution models to global warming. The large transcriptome data sets and bioinformatic scripts will be publicly available through the Harvard Forest Data Archive. I plan to focus on public outreach through an ant-oriented website that will distill and communicate scientific discoveries on insects and climate change. I am already mentoring one undergraduate, on the population genetics of *A. rudis*. I plan to mentor additional students for other parts of this work, exposing students to the exciting interface between molecular biology and ecology.

References:

1. Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude Thermal Safety margin. *PNAS*.
2. Diamond, S. E., Sorger, D. M., Hulcr, J., Pelini, S. L., Toro, I. D., Hirsch, C., Oberg, E., et al. (2012). Who likes it hot? A global analysis of the climatic, ecological, and evolutionary determinants of warming tolerance in ants. *Global Change Biology*, 18(2), 448-456. doi:10.1111/j.1365-2486.2011.02542.x
3. Hoffmann, A. a, & Willi, Y. (2008). Detecting genetic responses to environmental change. *Nature reviews. Genetics*, 9(6), 421-32. doi:10.1038/nrg2339
4. Menéndez, R., Megías, A. G., Hill, J. K., Braschler, B., Willis, S. G., Collingham, Y., Fox, R., et al. (2006). Species richness changes lag behind climate change. *Proceedings. Biological sciences / The Royal Society*, 273(1593), 1465-70. doi:10.1098/rspb.2006.3484
5. Ness, J. H., Morin, D. F., & Giladi, I. (2009). Uncommon specialization in a mutualism between a temperate herbaceous plant guild and an ant: are *Aphaenogaster* ants keystone mutualists? *Oikos*, 118(12), 1793-1804. doi:10.1111/j.1600-0706.2009.17430.x
6. Pelini, S. L., Bowles, F. P., Ellison, A. M., Gotelli, N. J., Sanders, N. J., & Dunn, R. R. (2011). Heating up the forest: open-top chamber warming manipulation of arthropod communities at Harvard and Duke Forests. *Methods in Ecology and Evolution*, 2(5), 534-540. doi:10.1111/j.2041-210X.2011.00100.x
7. Pelini, S. L., Diamond, S. E., Maclean, H., Ellison, A. M., Gotelli, N. J., Sanders, N. J., & Dunn, R. R. (2012). Common garden experiments reveal uncommon responses across temperatures, locations, and species of ants. *North*. doi:10.1002/ece3.407
8. Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine “winners” and “losers”. *The Journal of experimental biology*, 213(6), 912-20. doi:10.1242/jeb.037473