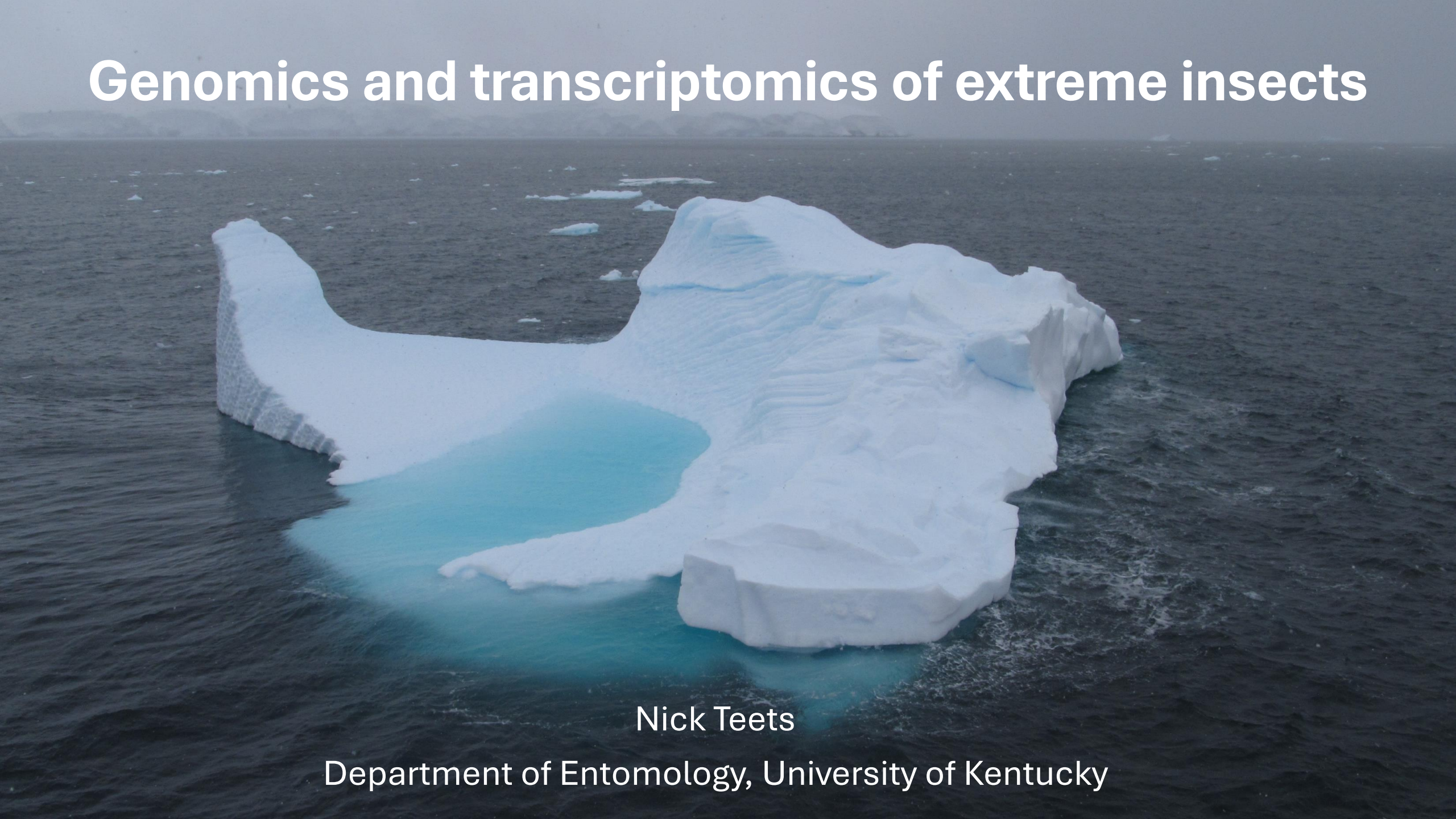
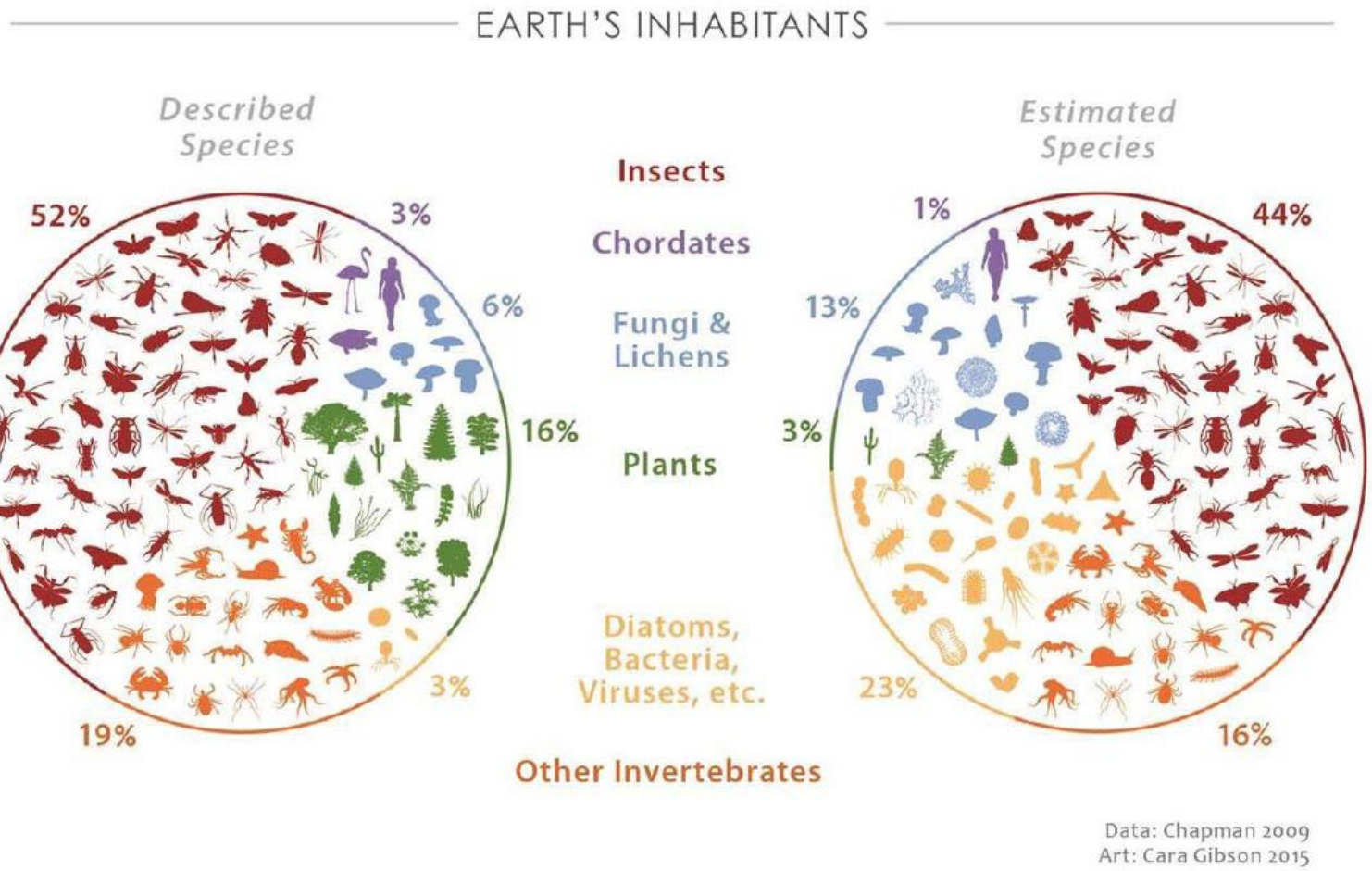
Genomics and transcriptomics of extreme insects



Nick Teets

Department of Entomology, University of Kentucky

Insect Appreciation



- The are 10 quintillion (10,000,000,000,000,000,000) insects alive on earth!
- There are 7.6 billion (7,600,000,000) humans.
- More than 200 million insects for each human on Earth
- 300 pounds of insects for each pound of humans

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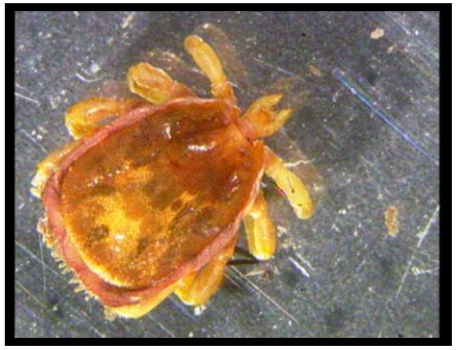
Entomology in Antarctica

Ticks and Mites
~100 species; 50 free-living

Ixodes uriae



Alaskozetes antarcticus

Springtails
~15 species

Cryptopygus antarcticus

Friesea grisea

Insects
2 species

Belgica antarctica

Parochlus steinenii

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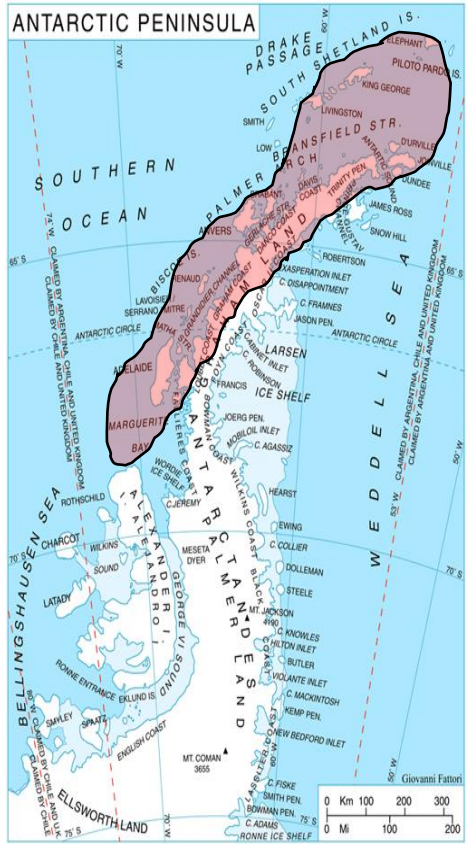
The Antarctic midge, *Belgica antarctica*

- Two-year life cycle
- Adults live <2 weeks in the summer
- Largest terrestrial animal in Antarctica

Male, Left; Female, Right

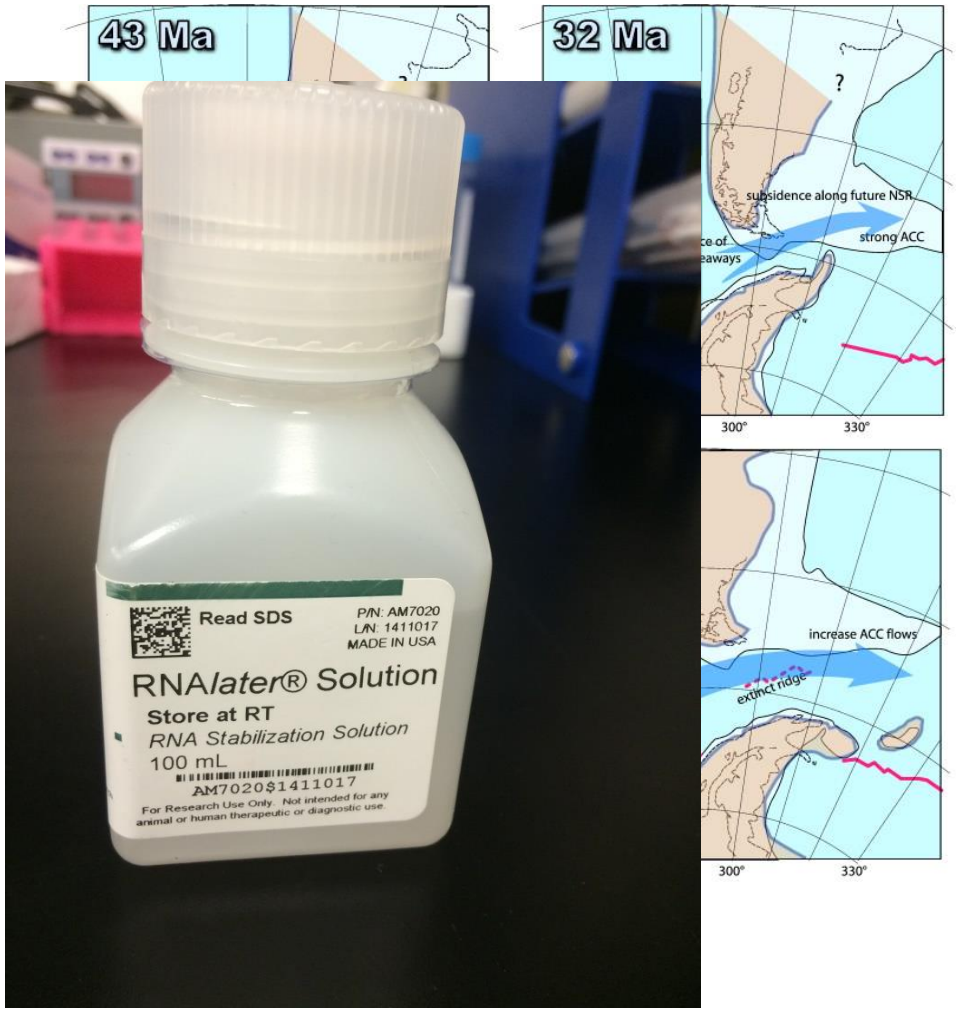


B. antarctica is uniquely adapted to its environment

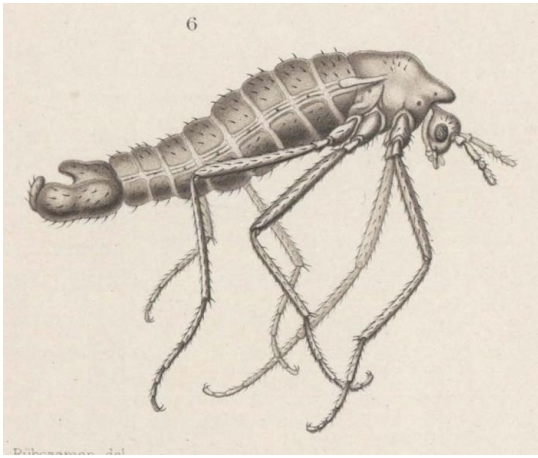
- Apterous (wingless)
- Extended life cycle (2 years)
- Synchronized adult emergence
- Flexible diet
- Stress tolerance

Cold	Freezing tolerant year-round
Desiccation	100% survival at 35% of initial mass
Salinity	95% survival 7 d in 0.5 M NaCl
Freshwater	100% survival for 28 d at 0° C
Anoxia	100% survival for 7 d
Heat	100% survival of +10° C for 7 d
pH	100% survival of pH 3-12 for 14 d

Formation of Drake Passage,
ca. 40 million years ago



Apterous adult



Bergroth, 1906

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Other Antarctic wildlife


Adelie Penguin


Chinstrap Penguin


Gentoo Penguin


Giant Petrel

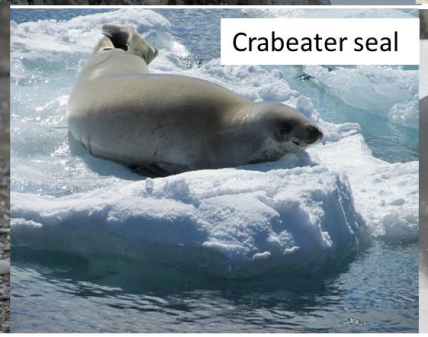

Blue-eyed Shag


Brown Skua


Antarctic Tern


Kelp Gull


Snowy Sheathbill

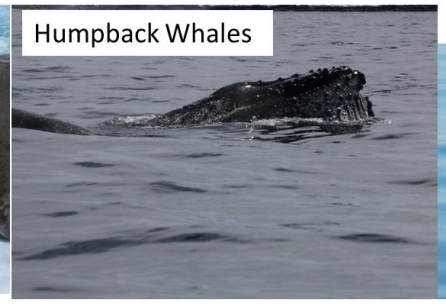

Crabeater seal


Weddell Seal


Elephant Seal


Fur Seal


Leopard Seal


Humpback Whales


Minke Whales


Killer Whales

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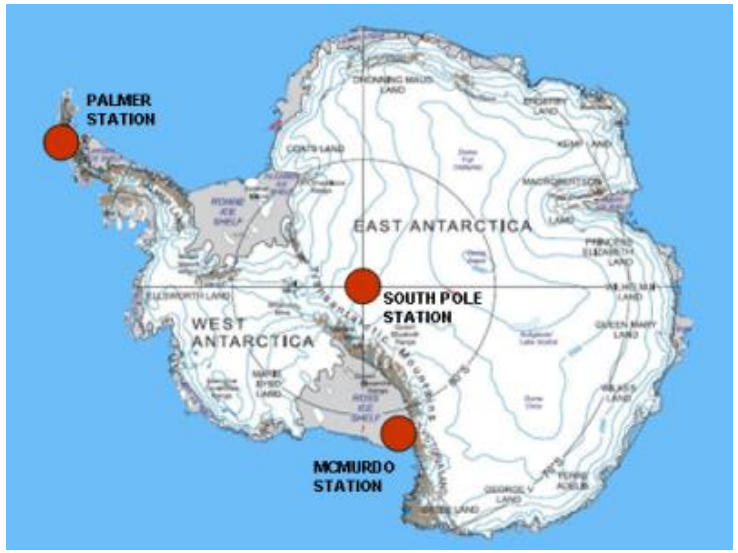
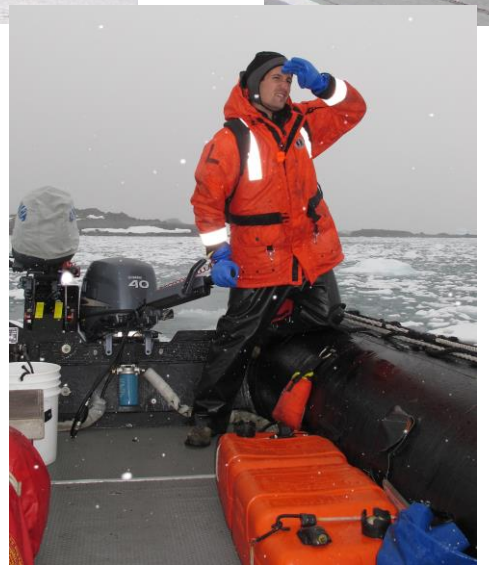
Conducting Research in Antarctica



Palmer Station

Laurence M. Gould

Cleverson Lima
PhD Student in Entomology/Biology



A real Antarctic explorer

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Key questions in our current research

1. What are the physiological and molecular mechanisms that allow *Belgica antarctica* to survive stressful conditions in Antarctica?
2. What are the genomic mechanisms that facilitate adaptation to Antarctica?
   a) Interspecific differences in genome structure and gene content
   b) Population genomics analyses of midges across its geographic range

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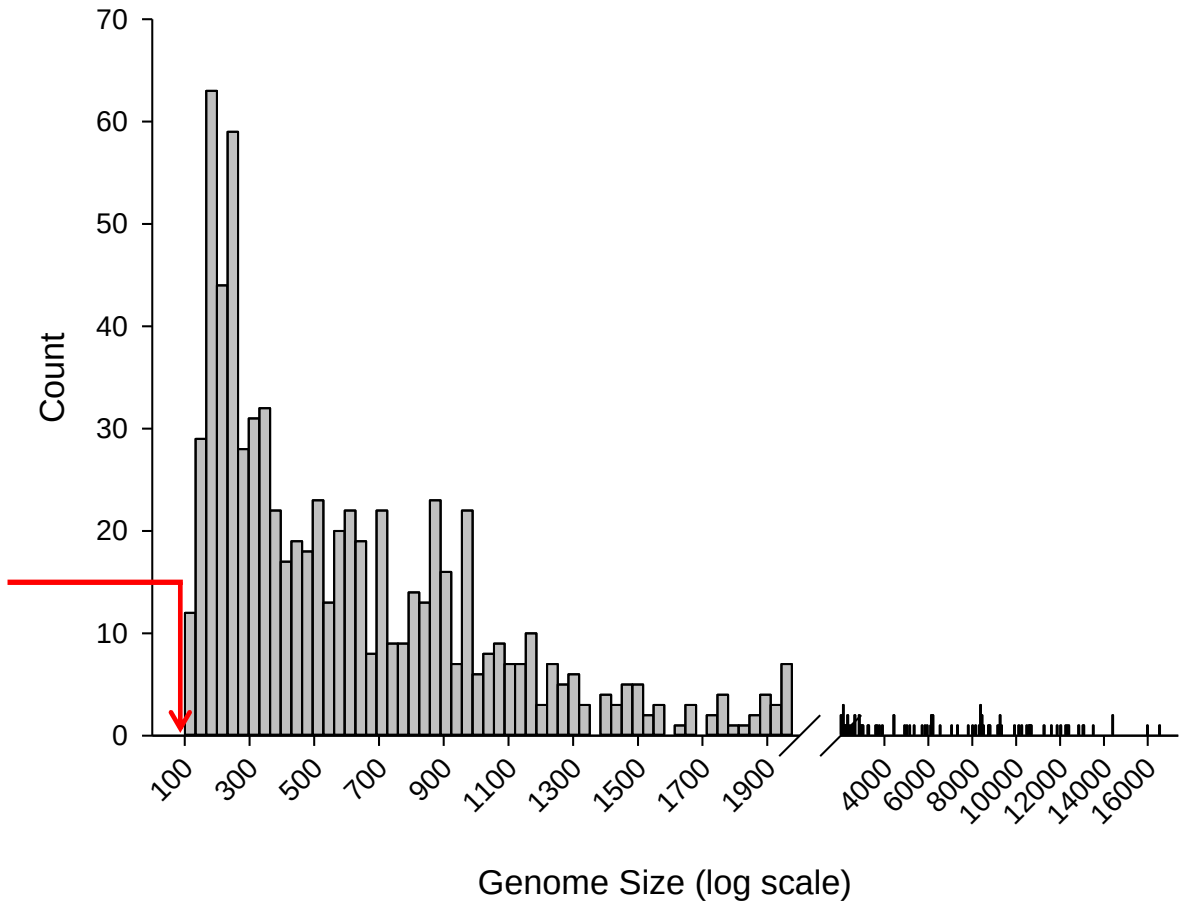
The Antarctic midge genome

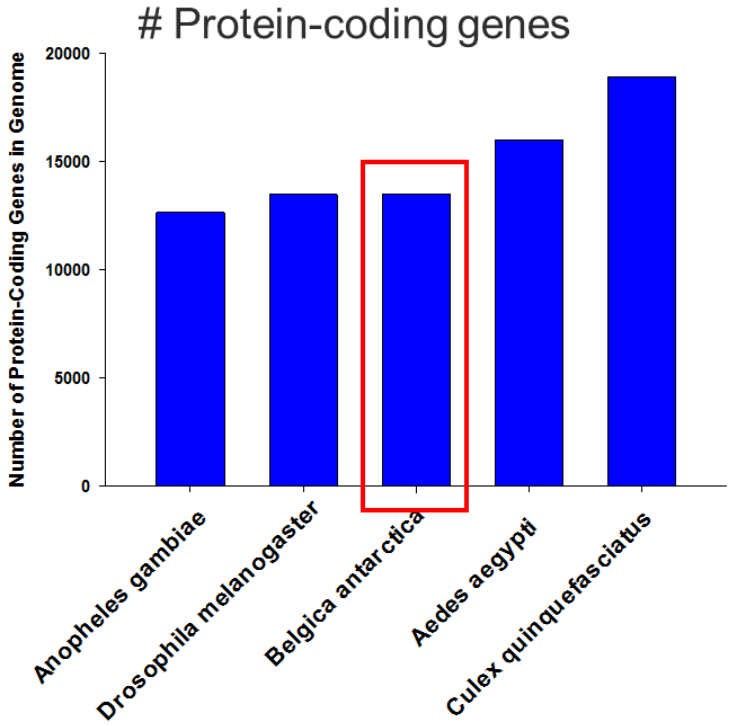
- It’s tiny!

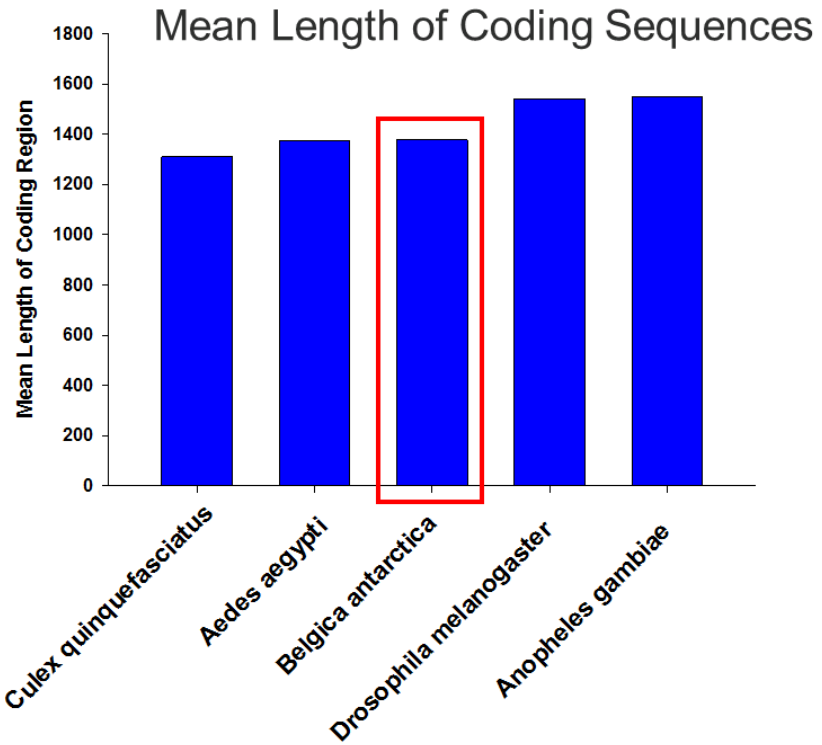
Belgica = 99.3 Mb



Why is it Tiny?

- Gene number of length of coding sequences are comparable to other Diptera





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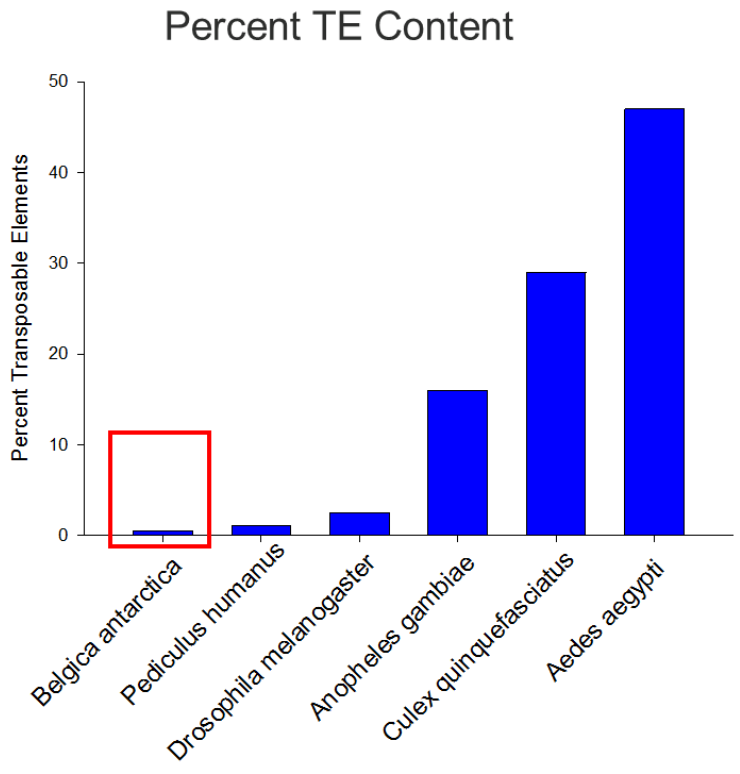
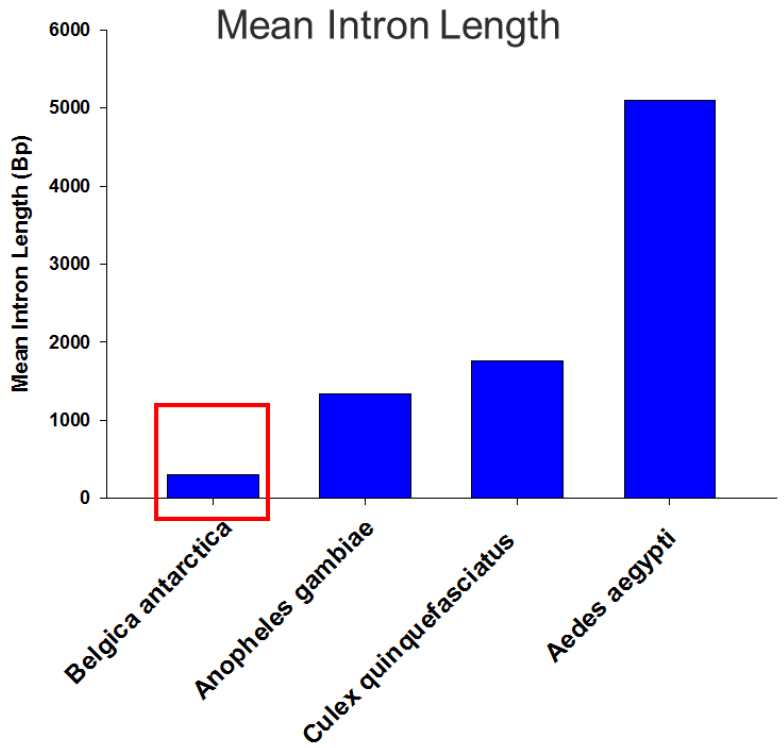
Why is it Tiny?

- However, transposable element (TE) content and intron length are significantly reduced





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Telmatogeton magellanicus

Eretmoptera murphyi

Belgica albipes

Next steps: Comparative genomics

Belgica antarctica

Software to be used on CCS:

- De novo assembly: Hifiasm, Flye, Pilon
- Annotation: MAKER and all of its various dependencies…
- Comparative genomics: BLAST, BLAST2GO, Ka/Ks analysis

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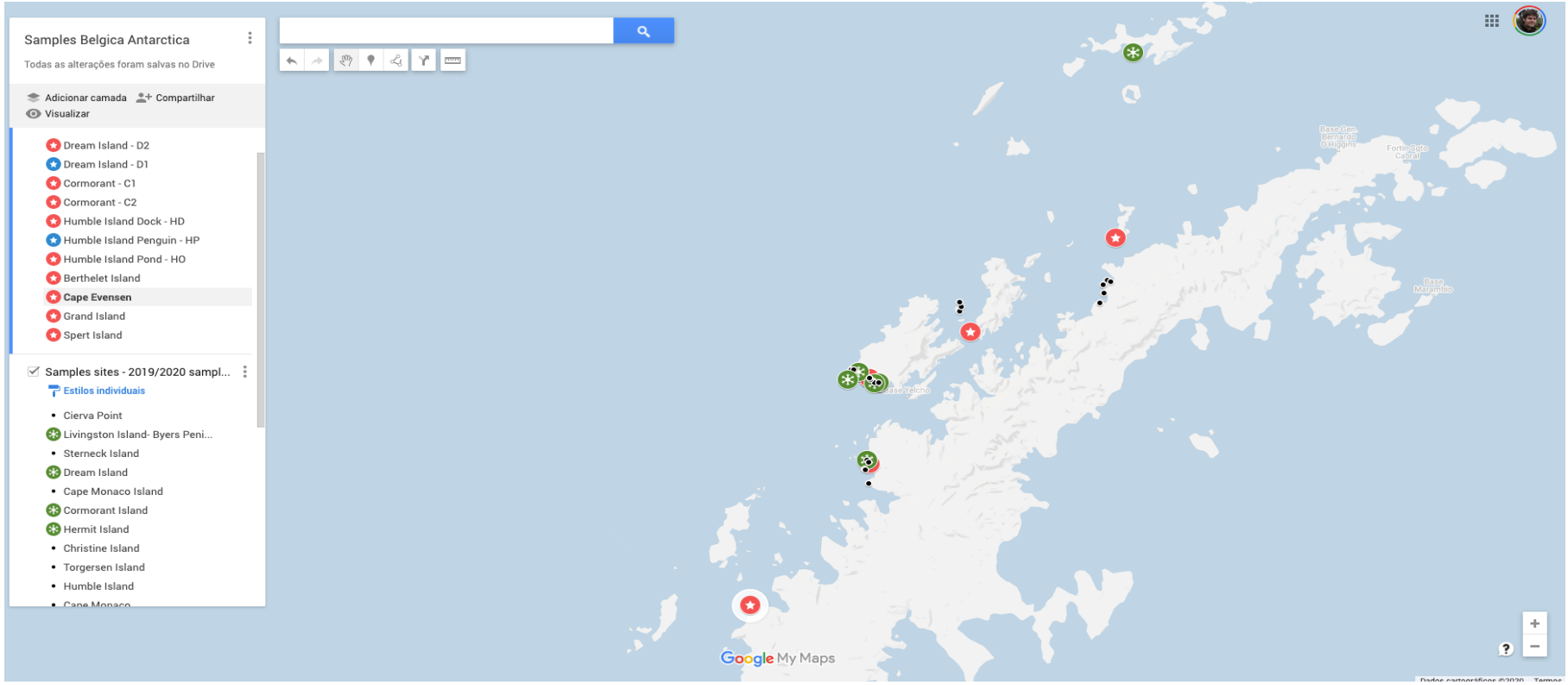
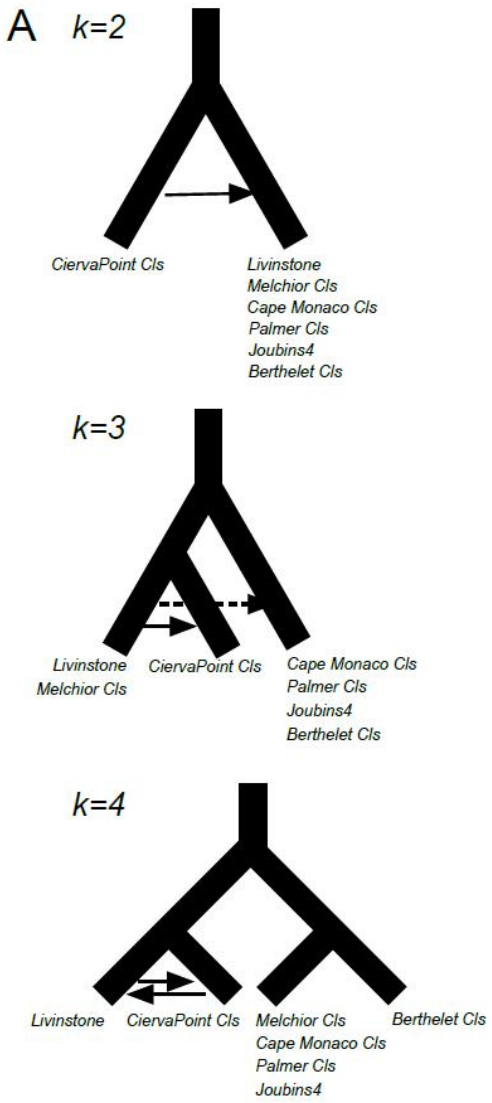
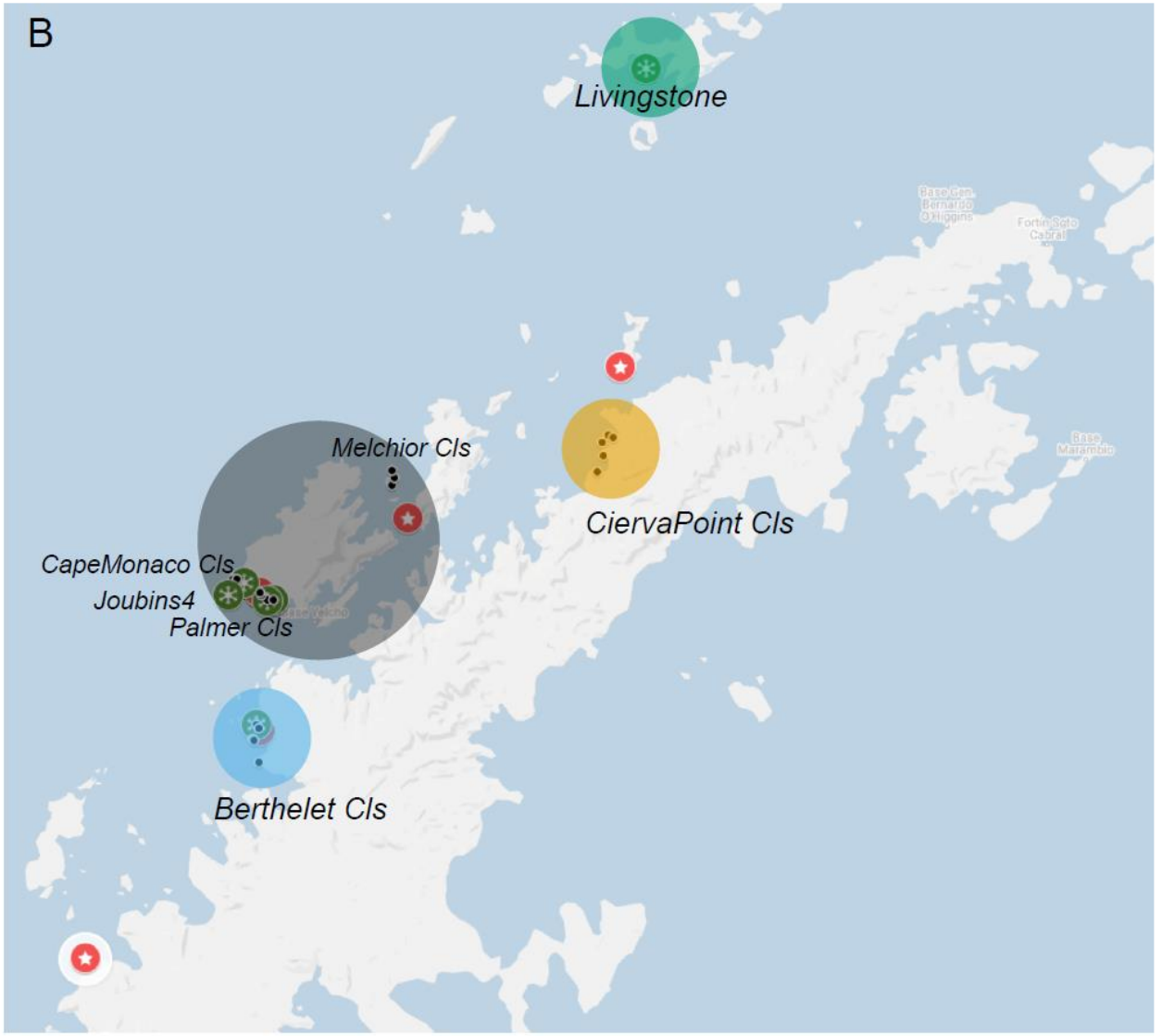
Population genomics

- **Goals:**
  1. Characterize demography of various populations
  2. Recreate biogeographic history using population genetics
  3. Identify signatures of local adaptation across the range



Population genomics – preliminary results





Transcriptomics

- Key questions in recent projects:
  1. **What genes/processes are specifically activated by freezing?**
  2. **Are there long-term physiological consequences following a freezing stress?**
  3. **Do gene expression responses to distinct abiotic stressors reflect cross-talk and cross-tolerance between stressors, thus indicating a common evolutionary origin?**

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Freeze tolerant ectotherms

Freeze Intolerant

Drosophila melanogaster

Tribolium castaneum

Bufo americanus

Anolis carolinensis

Freeze Tolerant

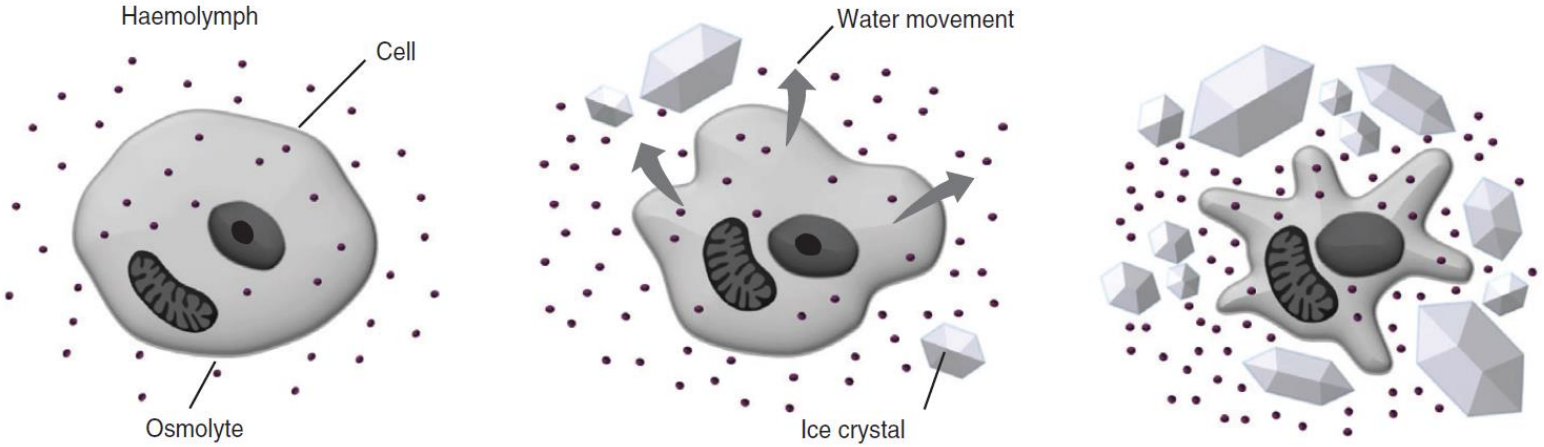
Eurosta solidaginis

Gryllus veletis

Rana sylvatica

Chrysemys picta

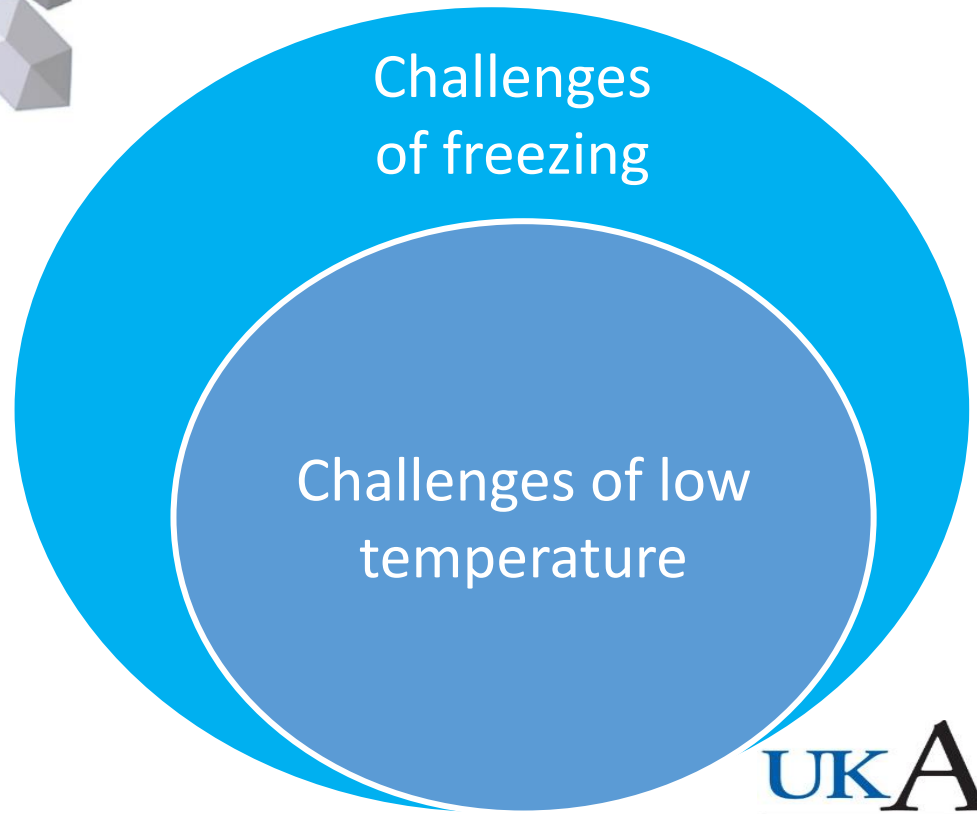
Physiological challenges of freezing



Toxopeus and Sinclair, 2018.

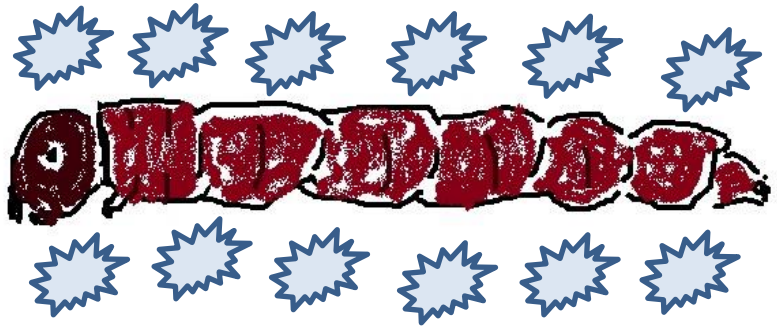
Unique challenges associated with freezing

- Cellular dehydration
- Mechanical damage to cells and tissues
- Anoxia/reperfusion



Experimental Design

Inoculative freezing



Supercooled

- Single control group: kept at 2°C and sampled at onset of experiment
- Frozen and supercooled held at -3°C for 5 d.
- Sampled immediately after treatment and after 2, 12, and 24 h recovery

mRNA-seq.
5 replicates per group

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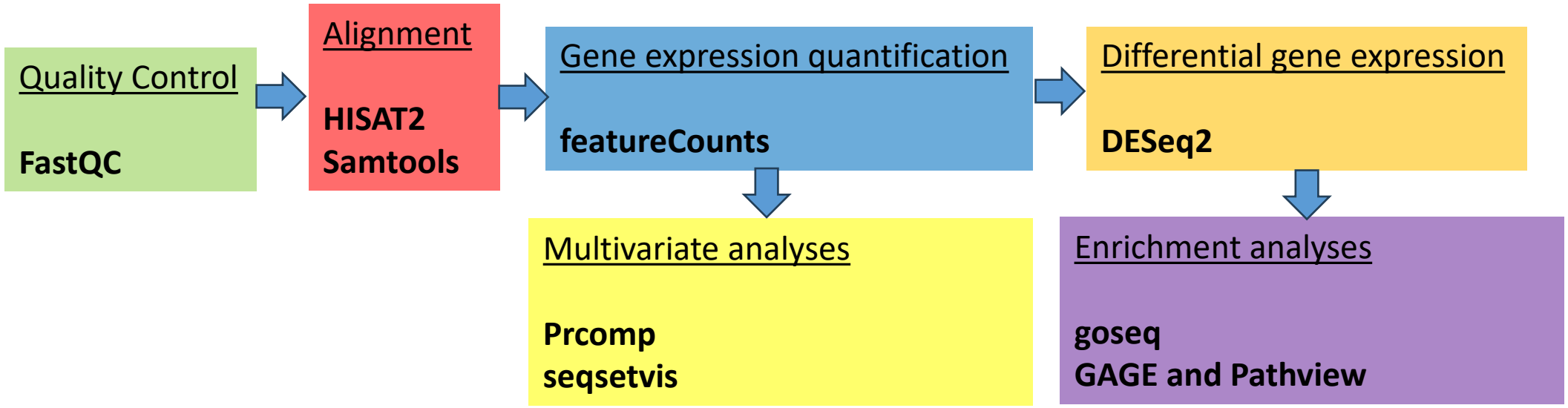
Bioinformatics pipeline



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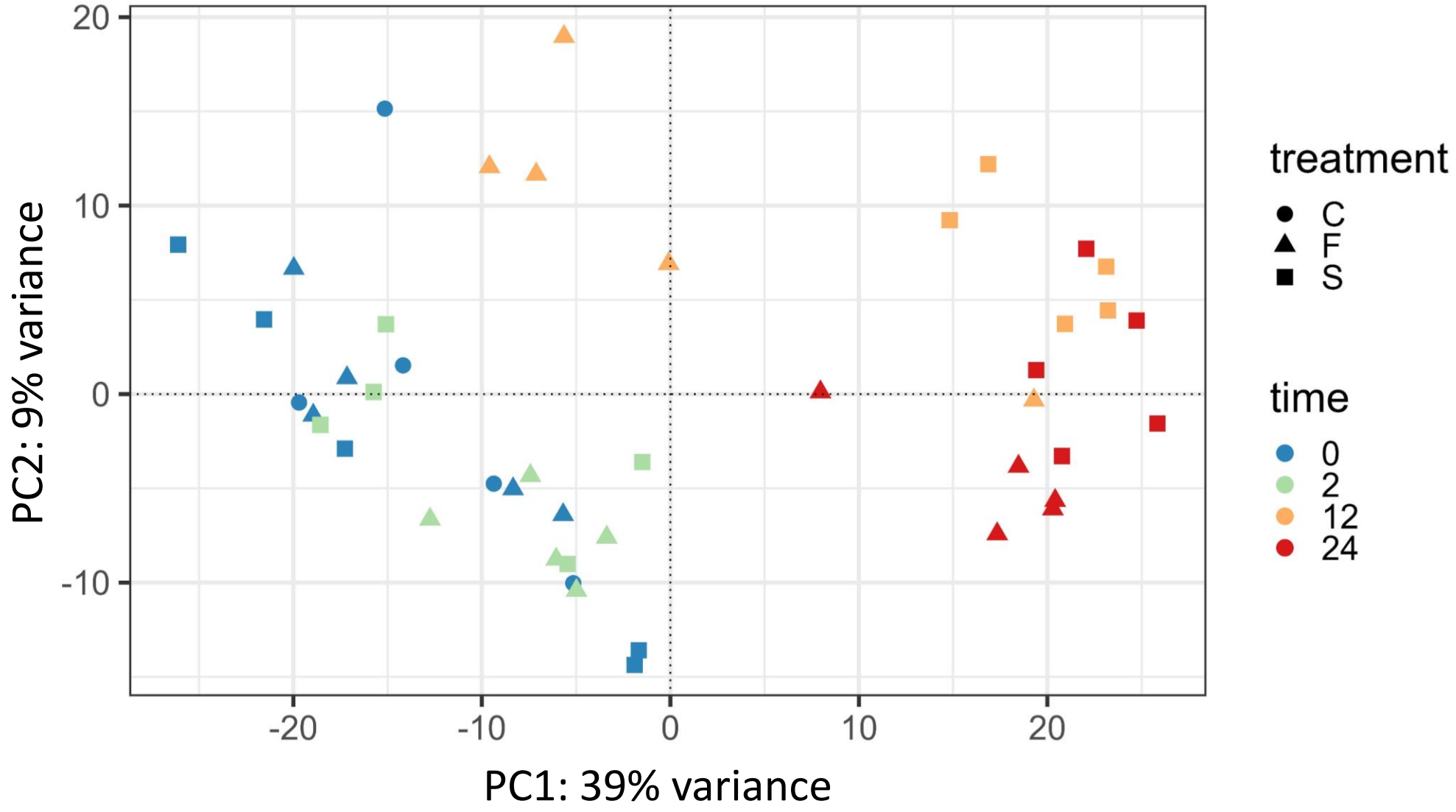
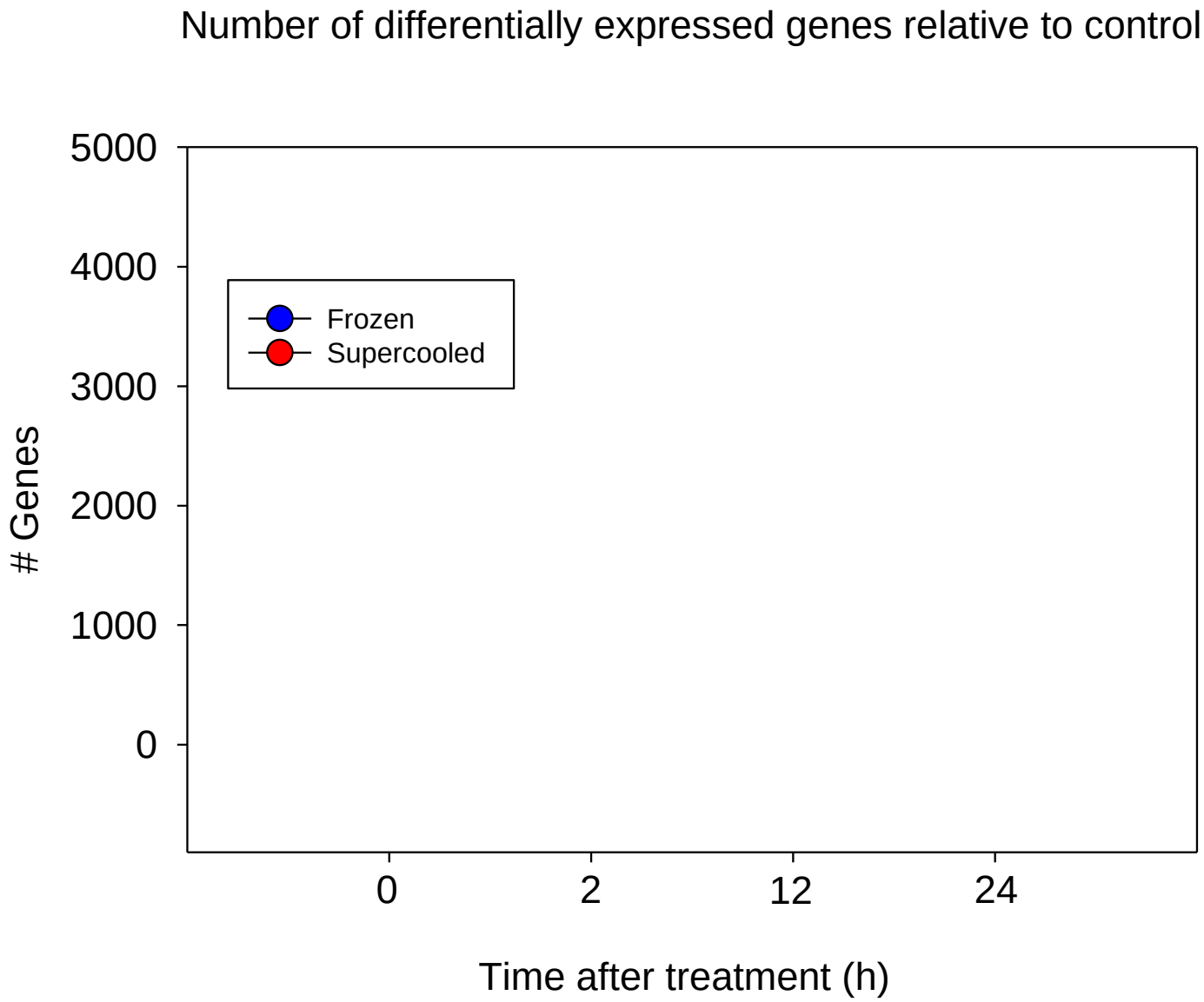
Results: Most variation caused by recovery time

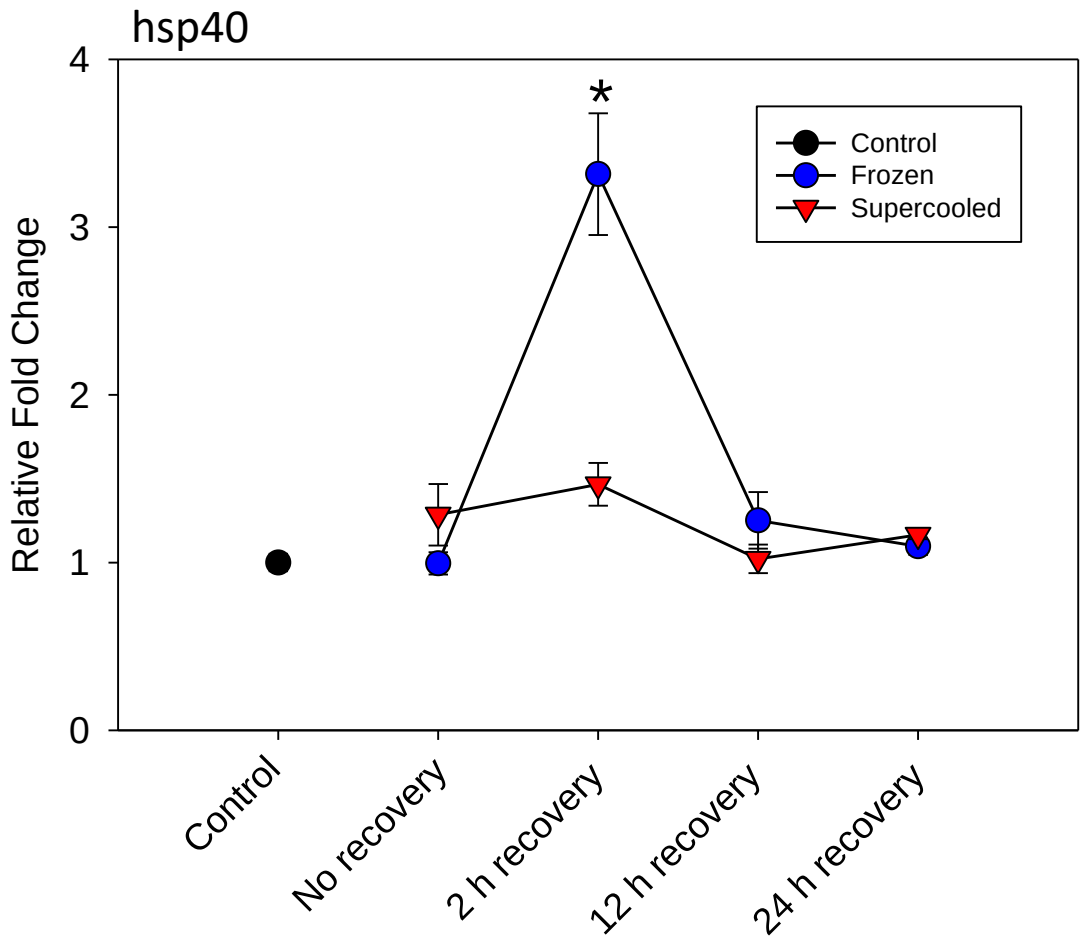


Surprisingly, more DEGs in supercooled larvae



But higher expression of canonical stress genes in frozen larvae

Hsp70 and Hsp40 transcripts tended to be more abundant in frozen larvae



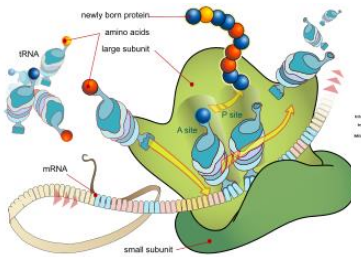
* Indicates significant difference between frozen and supercooled.

Summary of processes involved in recovery from freezing

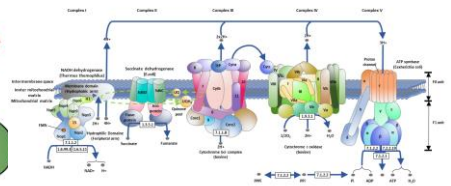
Immediately After

Upregulated:

Translation



Carbohydrate metabolism and OxPhos



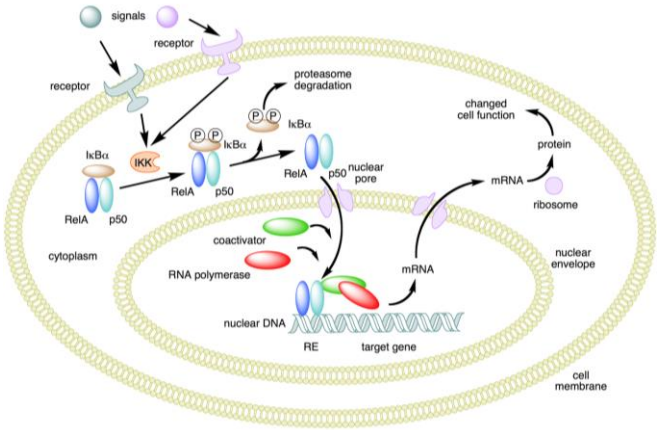
Downregulated:

None

Early Recovery

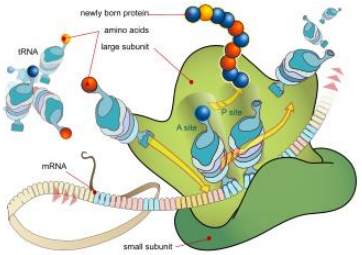
Upregulated:

Heat shock proteins, autophagy, NF-kB signaling

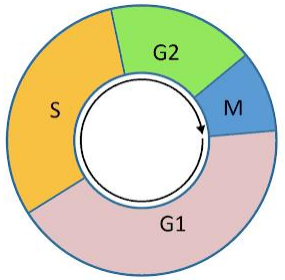


Downregulated:

Translation



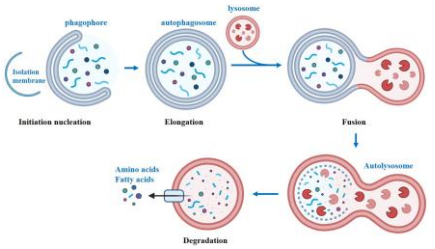
Cell cycle and morphogenesis



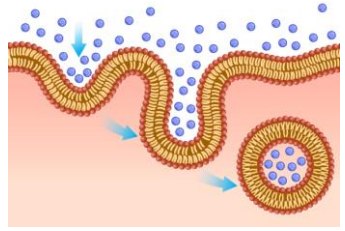
Late Recovery

Upregulated:

Autophagy

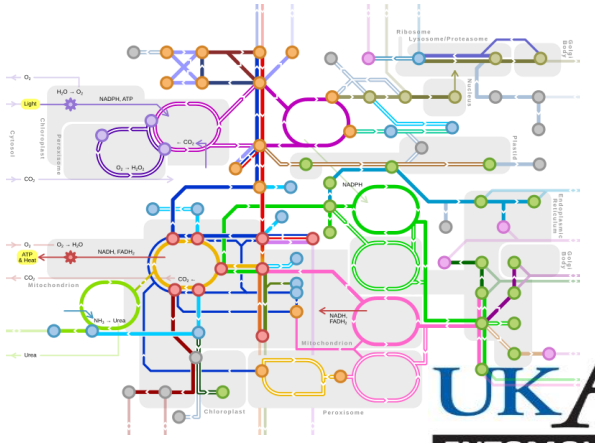


Endocytosis and transport



Downregulated:

Metabolism



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Take-home messages

1. Antarctic insects are an excellent system for studying the mechanisms of extreme adaptation
   a) **Possible applications: aging/senescence, cryopreservation**
2. Genomics provides functional tools for these non-model species
3. Adaptation to extreme environments is complex, and genomic resources are needed from additional species to tease apart the key processes
   a) **Key question: Are Antarctic species better at existing mechanisms, have the evolved new mechanisms, or both?**

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Acknowledgements

Teets lab, October 2023

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Pete Convey, British Antarctic Survey
Paul Frandsen, Brigham Young University

NSF

USDA NIFA

NIH National Institutes of Health

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