Hibernation: insights from comparative genomics of *Zapus* species

Qian Cong[1,2], Alyssa D. McNulty[1], William J. Israelsen[1]

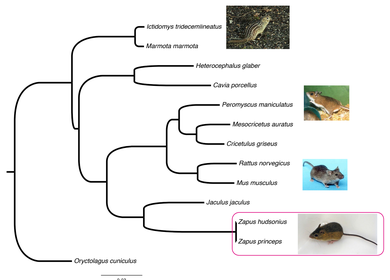
[1]Department of Biochemistry, [2]Department of Biophysics, University of Texas Southwestern Medical Center, Dallas, TX 75390. Correspondence: william.israelsen@utsouthwestern.edu

ABSTRACT

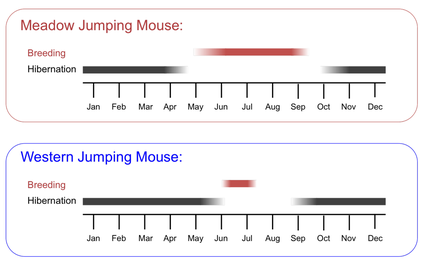
Hibernating mammals employ unique metabolic strategies to survive harsh conditions. They become obese prior to the onset of winter, then conserve their stored energy during the months-long fast of hibernation by slowing metabolism and becoming deeply hypothermic. These extreme hibernation phenotypes may provide insights into control of metabolism and have applications in medicine, but little is known about the molecular mechanisms or genetic underpinnings of hibernation. We have begun using the meadow jumping mouse (*Zapus hudsonius*) as a laboratory hibernation model. We generated whole genome sequences for 7 meadow jumping mice and 7 western jumping mice (*Zapus princeps*), a closely related hibernator. *Z. hudsonius* uses photoperiod as the cue to initiate fall fattening, while *Z. princeps* ignores photoperiod and prepares for hibernation based on food availability. Preliminary comparative genomic analysis reveals a subset of ~1700 proteins that are significantly diverged between the species. These diverged proteins are enriched for genes involved in pheromone sensing and reproduction, immunity, and metabolism. Analysis of individual genes yields insights into speciation and may reveal candidate genes that cause the differences in phenotype between the two species.

1. Jumping Mice: Hibernating Rodents

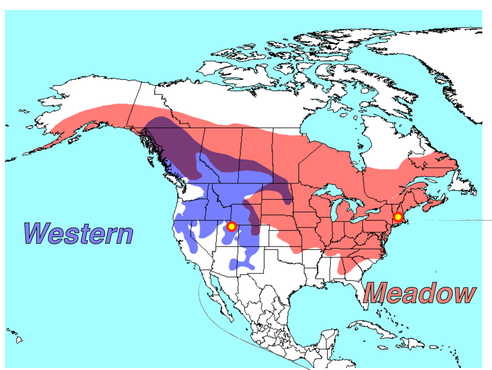
Phylogeny



Life History



Range



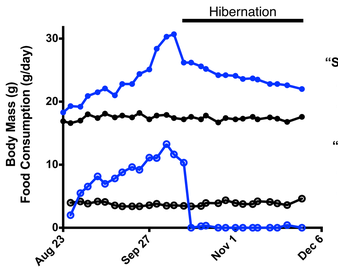
2. Inducing Hibernation in the Lab: Day Length & Temperature

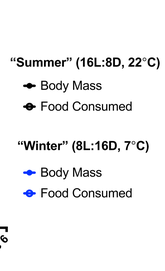
Meadow jumping mice prepare for hibernation based on environmental cues – primarily day length. The mice fatten up and hibernate during simulated fall and winter conditions, but remain reproductively active as long as they are housed in simulated summer conditions.

3. The Hibernation Phenotype of *Zapus hudsonius*

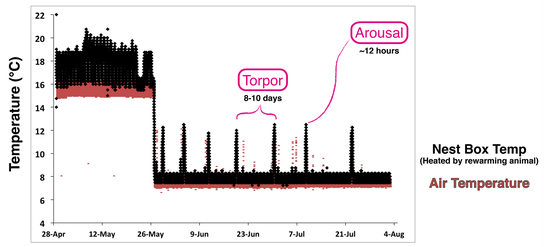
Meadow jumping mice have a hibernation phenotype similar to ground squirrels – once prepared with sufficent fat stores, the animals experience week-long bouts of torpor that are interrupted by short arousals. Body temperature falls to near freezing during torpor. Meadow jumping mice fast during hibernation and do not cache food.

Body Mass & Food Consumption





Torpor and Periodic Arousals



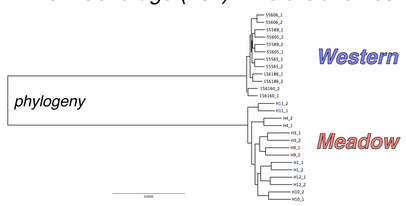
4. Ongoing Genome Sequencing: *Zapus hudsonius & Zapus princeps*

We are in the process of sequencing and annotating the *Zapus hudsonius* genome in collaboration with the Broad Institute. The strategy is to generate a reference genome and perform gene annotation using mRNAseq data and computational methods. We have also generated additional whole genomes of *Z. hudsonius* and *Z. princeps*. These *Zapus* genomes will provide a foundation for comparative genomics and future genetic experiments.

Current de novo Z. hudsonius Assembly

2.7 Gb	**estimated genome size**
290 kb	**contig N50**
90 X	**coverage**
~22,000	**genes**

Low Coverage (~8x) Whole Genomes

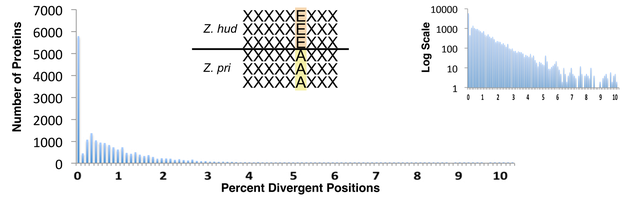


What can we learn about hibernation via comparative genomics?

	Western Jumping Mouse *Zapus princeps*	Meadow Jumping Mouse *Zapus hudsonius*
Long Photoperiod Inhibits Fall Fattening:	**NO**	**YES**
Home Elevation:	**2300-2700 m (7600-8750 feet)**	**70 m (225 feet)**
Individuals Sequenced for Whole Genomes:	**7**	**7**

5. Preliminary Comparative Analysis

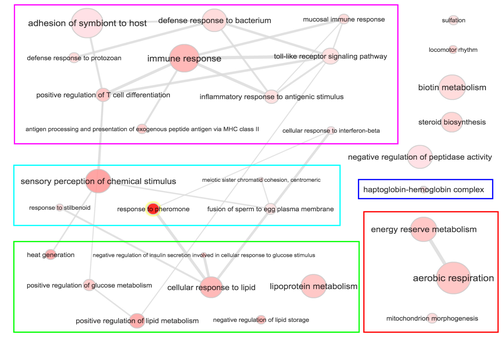
Identify Divergent Genes: Positions that are Fixed in Both Species



Significantly Diverged:
1697 / ~22,000 proteins

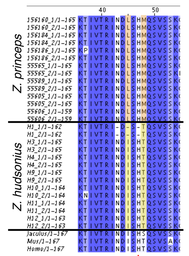
Criteria:
- Fixation Index > 0.8 in both species
- Enriched ($p < 0.05$) in divergent positions

Identify Biological Processes Associated with Diverged Genes

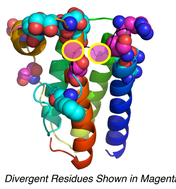


Leptin

Multiple Sequence Alignment

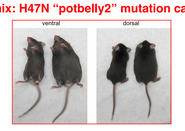


6 Divergent Positions / 167 Amino Acids
dN/dS = 1.44
(>1 suggests postive selection)



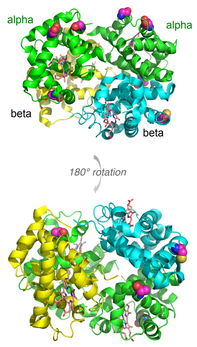
Divergent Residues Shown in Magenta

Beutler Mutagenix: H47N "potbelly2" mutation causes obesity in lab mice



Hemoglobin

3 Fixed Mutations in α-subunit
1 Fixed Mutation in β-subunit
(Shown in Magenta)



Acknowledgements

Jason Malaney, Austin Peay State University
Joe Cook, Museum of Southwestern Biology
Jeremy Johnson, Broad Institute
Alan Muchlinski, Cal State Los Angeles

Massachusetts Division of Fisheries and Wildlife

UT Southwestern Medical Center
Alyssa McNulty
Katie Clark
Robert Wood
Nick Grishin
Rick Bruick
Benjamin Tu
Steven McKnight

MIT
Brian Fiske
Matthew Vander Heiden
Hilda Holcombe
Tyler Caron
Michael Esmail
James Fox
Heather Huckins
Gladys Valeriano
Andrea Vargas

Funding:
NIH Early Independence Award DP5OD021365
Sara and Frank McKnight Fund for Biochemical Research