Hibernation in a novel rodent model: toward the genetic and molecular basis of torpor in mammals

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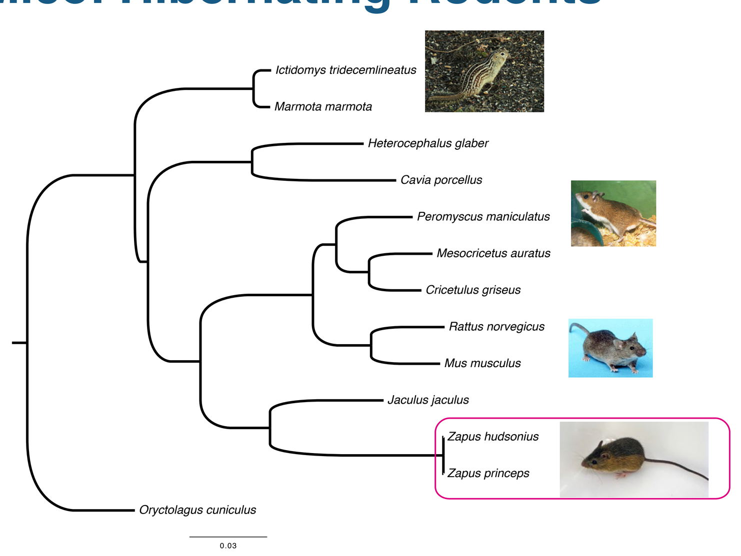
ABSTRACT

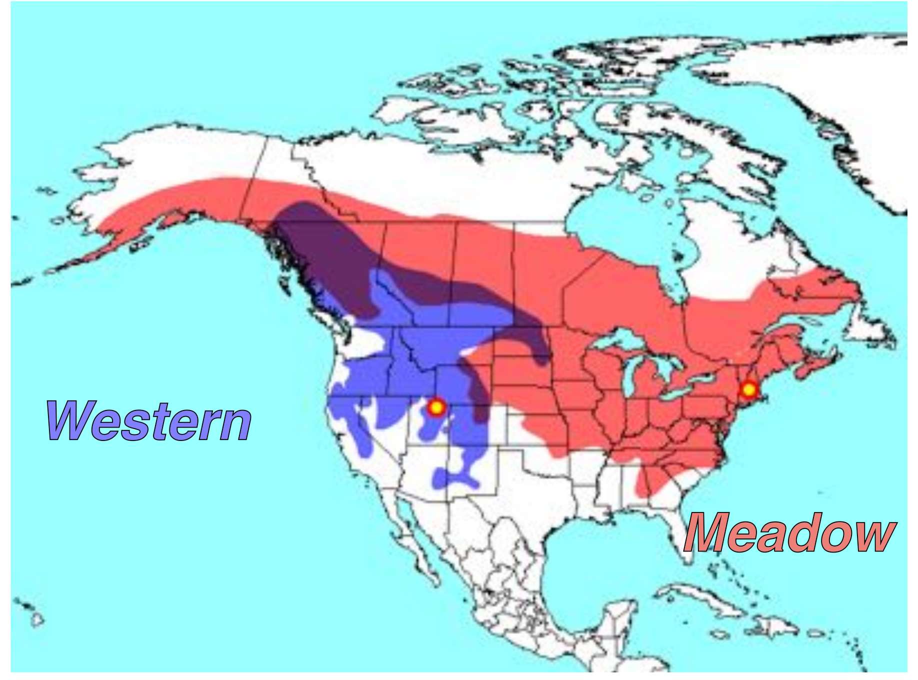
Hibernating mammals provide a natural example of torpor, a state of significantly depressed metabolism with potential applications in medicine. Despite longstanding historical interest in hibernation, a detailed understanding of its genetic and molecular basis is lacking. The meadow jumping mouse (*Zapus hudsonius*) is a small North American rodent that hibernates in response to shortened day length. We have developed these animals as a convenient model of hibernation because they can be maintained in a laboratory setting and induced to hibernate. Our work to de novo assemble and annotate the meadow jumping mouse genome has allowed comparative genomic analysis with other hibernating and non-hibernating species and provided the ability to study gene expression during torpor. To understand the cell-autonomous response to cold, meadow jumping mouse cell lines were exposed to temperatures typical of active and hibernating animals and subjected to RNA sequencing and biochemical analysis. The observed changes in gene expression and other cellular functions in isolated cells serve as a baseline for understanding the changes in cells and tissues seen during hibernation in intact animals. Preliminary results suggest that hibernation in mammals does not require unique genes that are lacking in non-hibernators, but that hibernation is instead based on differential regulation of conserved mechanisms. The meadow jumping mouse model has greatly enabled mechanistic hibernation research and will facilitate future discoveries..

1. Jumping Mice: Hibernating Rodents

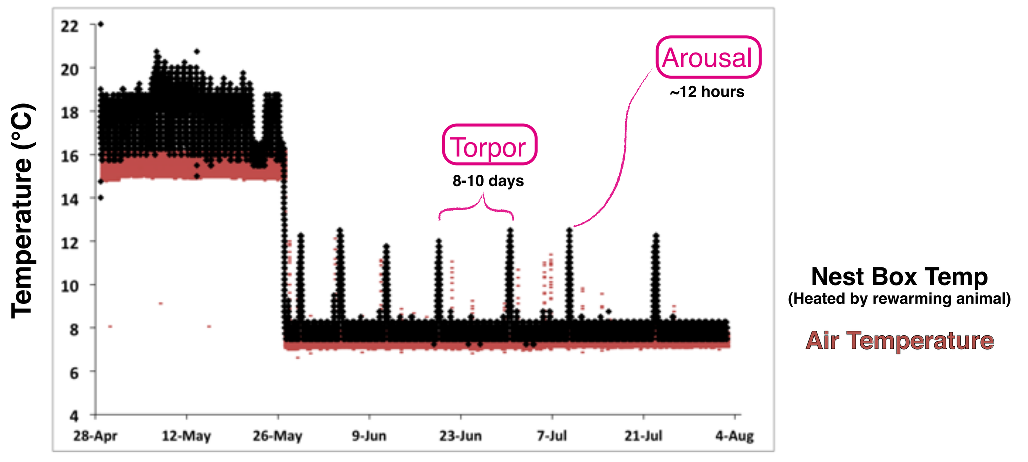
Phylogeny



Range

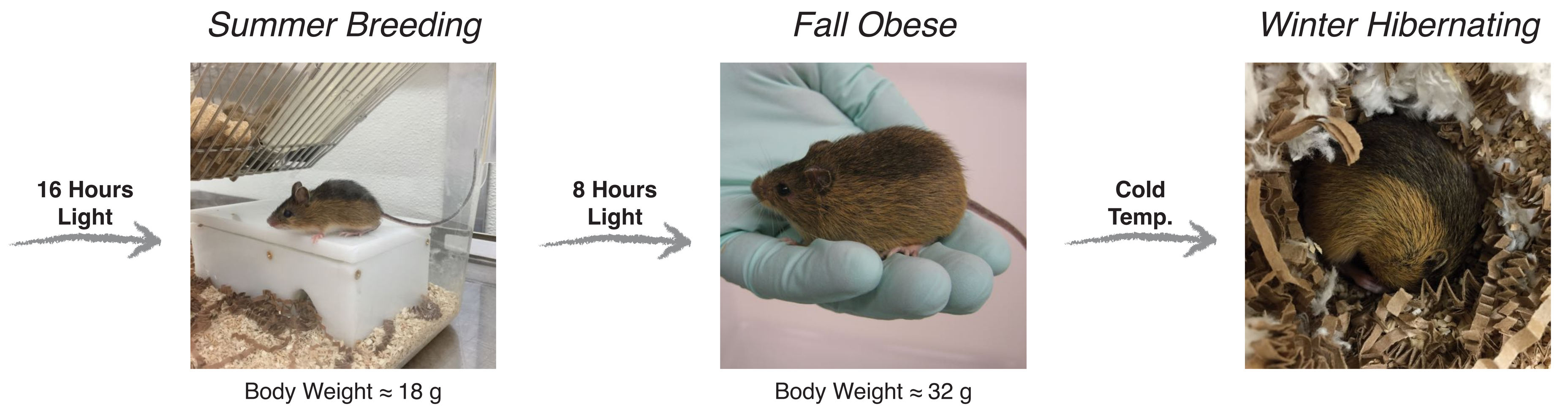


Torpor and Periodic Arousals



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.

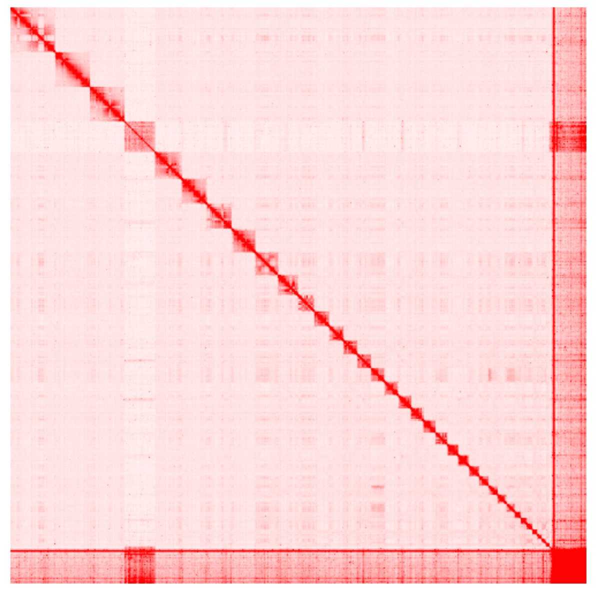


3. Draft Jumping Mouse Genomes

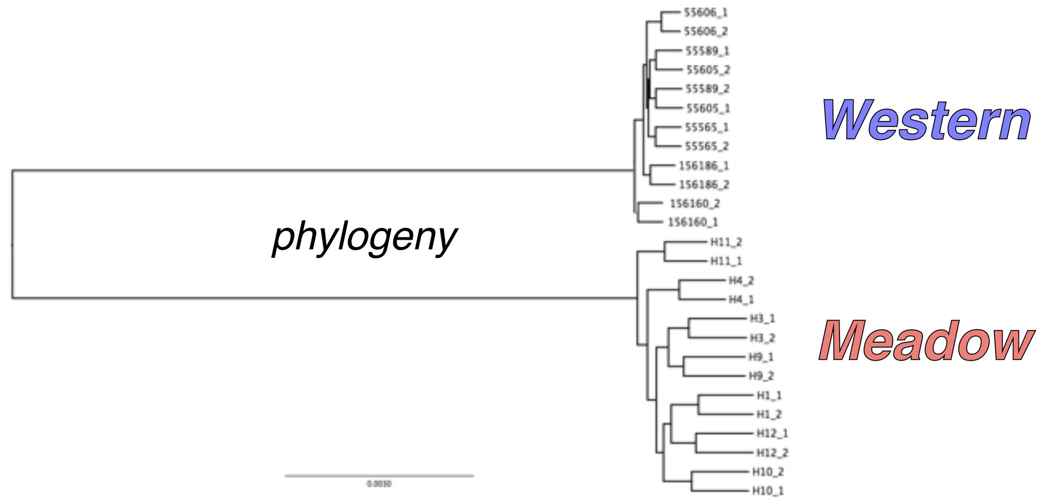
Current Z. hudsonius Draft Assembly

2.7 Gb	estimated genome size
60.4 Mb	scaffold *N50*
~22,000	genes

Hi-C Contact Matrix



Lower Coverage Whole Genomes



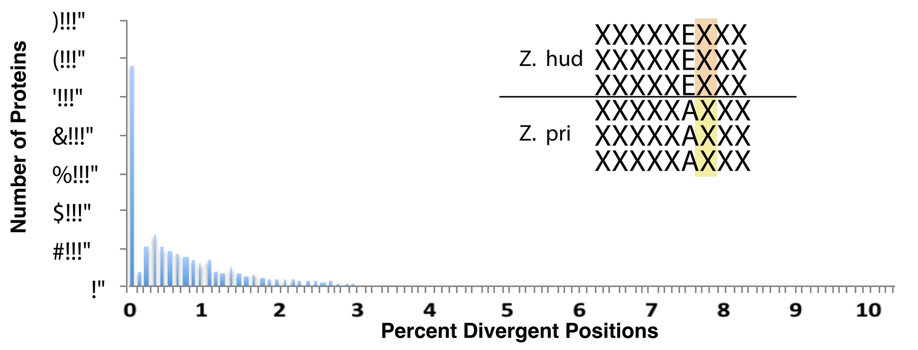
4. Comparative Genomics: *Zapus hudsonius & Zapus princeps*

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

Major Urinary Proteins
Vomeronasal Receptors
Leptin
Hemoglobin

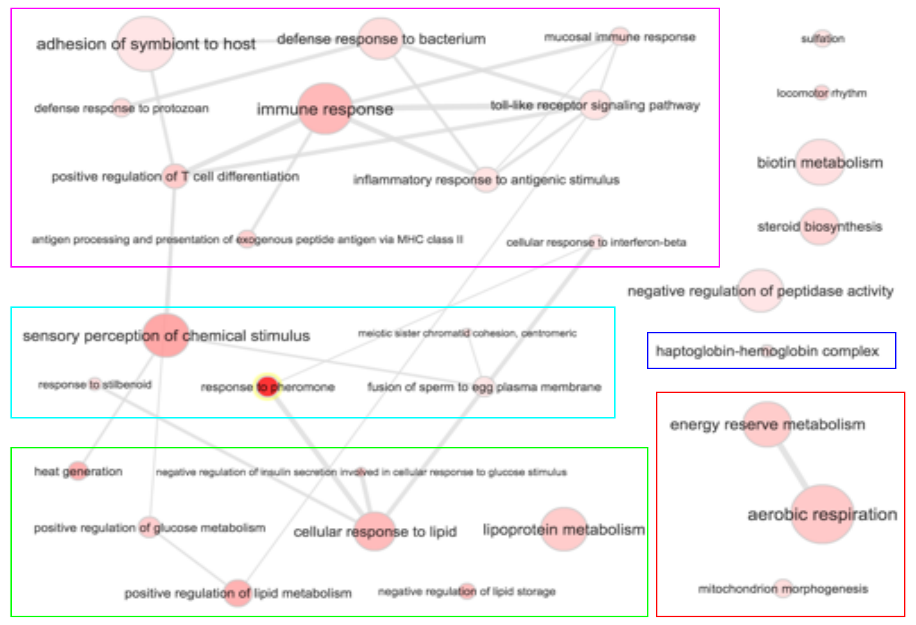
Identify Divergent Genes: Find mutations that became fixed in both species



Significantly Diverged:
1697 / ~22,000 proteins

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

Gene Ontology Analysis



5. Cell Response to Hibernation Temperature

How does cold temperature change gene expression in isolated cells?

Primary Zapus Skin Fibroblasts

37 Degrees
or
6 Degrees for 24 Hours

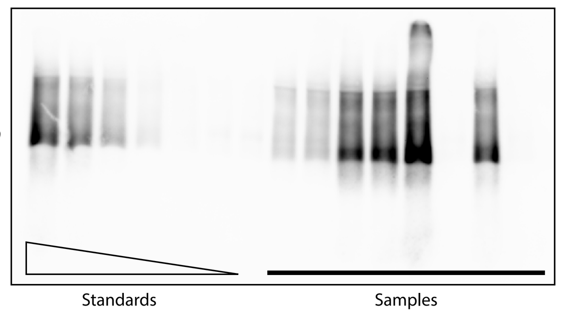
mRNA Sequencing

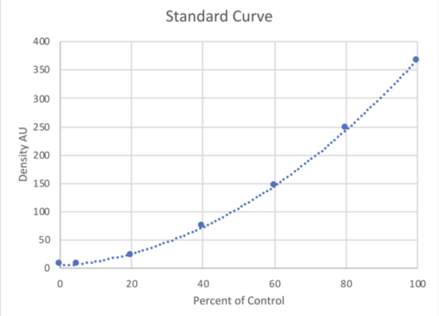
GO Term	Definition	P-value
GO:0007049	cell cycle	3.81E-53
GO:0051301	cell division	6.11E-47
GO:0006260	DNA replication	2.97E-32
GO:0005634	nucleus	1.93E-31
GO:0000775	chromosome, centromeric region	1.65E-27
GO:0005694	chromosome	3.35E-26
GO:0000776	kinetochore	3.07E-25
GO:0006281	DNA repair	2.21E-22
GO:0000278	mitotic cell cycle	7.85E-22
GO:0072686	mitotic spindle	2.13E-19
GO:0007052	mitotic spindle organization	1.95E-18
GO:0005737	cytoplasm	2.21E-18
GO:0005654	nucleoplasm	2.20E-17
GO:0005813	centrosome	3.73E-17
GO:0005856	cytoskeleton	3.70E-16
GO:0006974	cellular response to DNA damage stimulus	8.13E-16
GO:0007059	chromosome segregation	8.55E-16
GO:0060236	regulation of mitotic spindle organization	4.55E-15
GO:0000070	mitotic sister chromatid segregation	3.58E-14

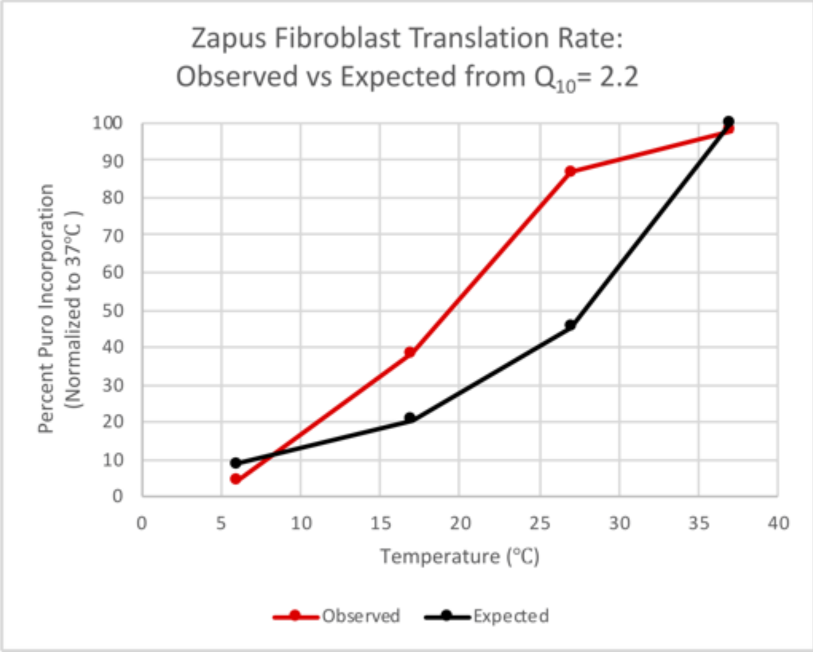
How does translation rate depend on temperature?

Puromycin labeling to determine global transcription level

WB: anti-puro







Preliminary Result: translation rate deviates from expected temperature dependence

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