Mutations in *ceh-17* and its Effect on Sickness Induced Sleep in C. *elegans*

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Background

•Sleeping is a common manifestation that humans and other organisms exhibit when sick. *C. elegans,* a simpler model organism, decreases movement and feeding when exposed to environmental stressors. We characterize this behavior as sickness induced sleep (SIS).
•The Raizen Lab is currently working on a screen using *C. elegans* strains from the million mutation project (MMP) to discover new genes that play a function in SIS. The MMP contains 2007 heavily mutagenized strains of *C. elegans* that are fully sequenced and cataloged (Thomas et al. 2013). As of now, 10-15 worm MMP strains have been shown to have a defect in SIS.
•In this project, I aim to identify the gene responsible for the SIS defective phenotype in the MMP strain VC40258.

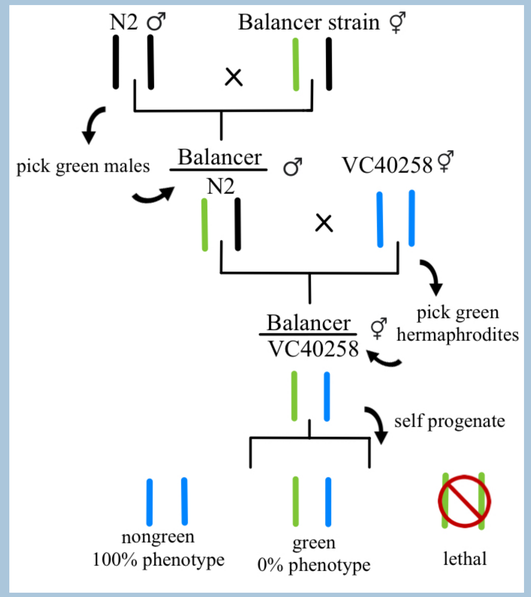
Methods

WorMotels
•A WorMotel is used to quantify the quiescence and activity levels by capturing images every 10 seconds over a period of 4 hours
•Worms are picked the day before at the L4 stage (assay is done on day 1 adults) and are placed into individual wells on the WorMotel (48 total)
•Experimental worms are stressed using a 1500 J/m^2 UVC crosslinker prior to running the assay
•Each WorMotel contains a control strain(s), experimental strain(s), and UVC stressed and unstressed
•The images are analyzed using MATLAB and Prism technology
Pumping Assay
•A pumping assay is used to measure *C. elegans* feeding behavior
•Worms are picked the day before at the L4 stage
•Pumping is measured prior to UVC stress and 2 hours post UVC stress
•Results are quantified as pumping (greater than 10 pumps/min (ppm))) and not pumping (less than 10 ppm)

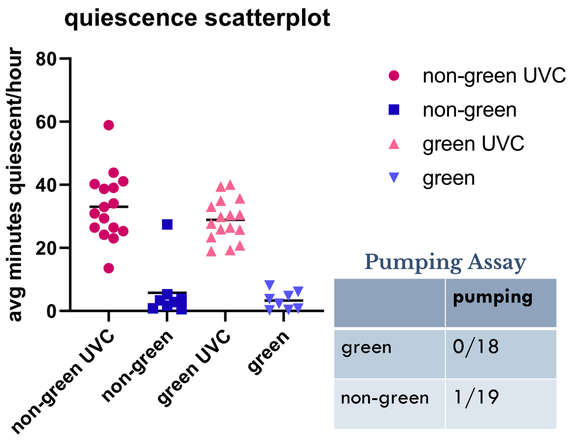
WorMotel Setup

Balancer Mapping

•Balancers for chromosomes I/II, II, and IV/V are used to determine which chromosome the mutation lies on
•Balancers are marked by green fluorescent protein (GFP) and contain a recessive lethal mutation



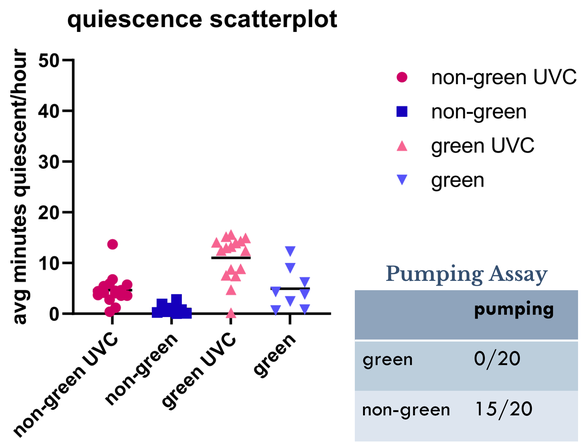
Test for Chromosome II



Pumping Assay

	pumping
green	0/18
non-green	1/19

Test for Chromosome I/III



Pumping Assay

	pumping
green	0/20
non-green	15/20

•If linked to chromosome: expect 100% SIS defect in non-green and 0% SIS defect in green
•If unlinked to chromosome: expect 25% SIS defect in both non-green and green
•Conclude that the gene of interest lies on Chromosomes I or III
•Using the MMP search database for genes on chromosomes I or III mutated in VC40258 led to the gene *ceh-17* which is known to play a role in SIS
•The mutation changes an Arginine at position 153 to a Cysteine, a site located in the HOX DNA-binding domain
•Analysis using MEGA11 software showed that the R153C missense mutation is conserved across *C. elegans, D. melanogaster,* and *H. sapiens*

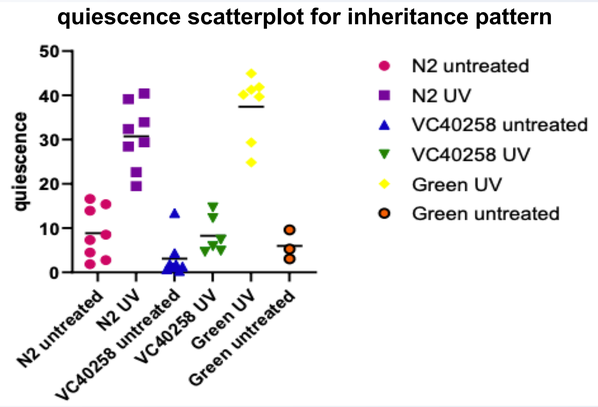
+ → nonpolar amino acid change

Species/Abbrv	*	*	*	*	*	*	*	
1. c. elegans	I	R	T	T	F	T	S	G
2. d. melanogaster	I	R	T	T	F	T	S	N
3. h. sapiens	I	R	T	T	F	T	S	A

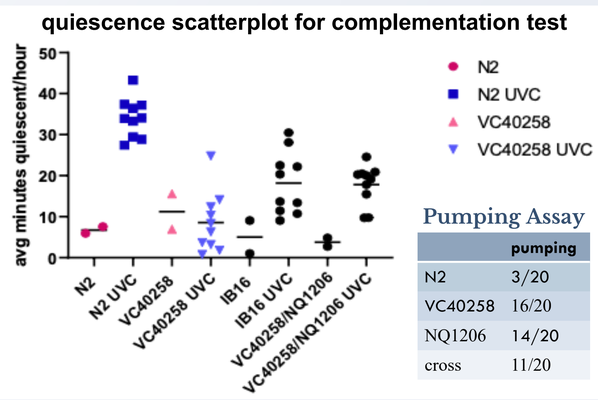
Conserved!

Complementation Testing

•Complementation tests are used to determine if two recessive mutations with the same phenotype are caused by mutations in the same gene



•Mutation is shown to be recessive by showing VC40258 & N2 green cross progeny do not exhibit an SIS defect in quiescence
•A strain with a known SIS defect due to *ceh-17* (NQ1206) was crossed with VC40258
•Results show that the cross progeny exhibit similar levels of quiescence and pumping as the parent strands, indicating that the mutation responsible for the SIS defect phenotype in VC40258 is on *ceh-17*



Pumping Assay

	pumping
N2	3/20
VC40258	16/20
NQ1206	14/20
cross	11/20

Future Steps

•Generating a database of worm sleep genes and their human orthologs to understand the effects in humans
•Categorizing potential SIS defective MMP strains using known SIS genes to make finding new genes more efficient

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