Mapping the allyl alcohol resistant *bet21* in *C. elegans*

SPUR Program
05/25/2010- 08/10/2010

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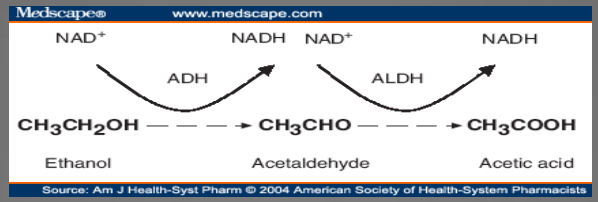
Alcoholism

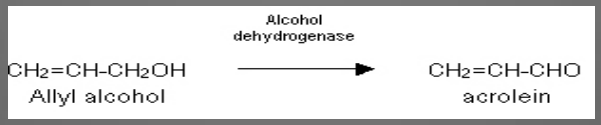
- Alcoholism is a severe disorder that has lasted for centuries and yet we have not found any cure or a clear explanation of how it works.
- Some research has shown that variability in genes encoding ADH enzymes in humans alter the likelihood to become alcoholic.
- Thus, we are interested in determining the exact genes that affect ADH functions.

Alcohol Metabolism

- ADH = alcohol dehydrogenase = an important enzyme to metabolize ethanol into acetaldehyde which will be further metabolized by aldehyde dehydrogenase ALDH into acetic acid.



ADH also metabolize allyl alcohol into acrolein, the volatile substance that can kill the animals.



- Comparing to ethanol, allyl alcohol exposure allows us to identify whether the animals have ADHs or not.

Overview of *C. elegans* Model

- *C. elegans* = *Caenorhabditis elegans*
- Size of the adult ≈ 1.5 mm
- Life cycle ≈ 3 days
- Progeny ≈ 300
- Nervous system ≈ 302 neuron cells for hermaphrodite and 381 neurons cells for male *C. elegans*
- Well mapped wild type circuit diagram (by White)
- Fertilization types:
  1. Inbreeding by self fertilizing hermaphrodite
  2. Out-breeding by crossing with male *C. elegans* which will give 1:1 ratio of male vs. hermaphrodite

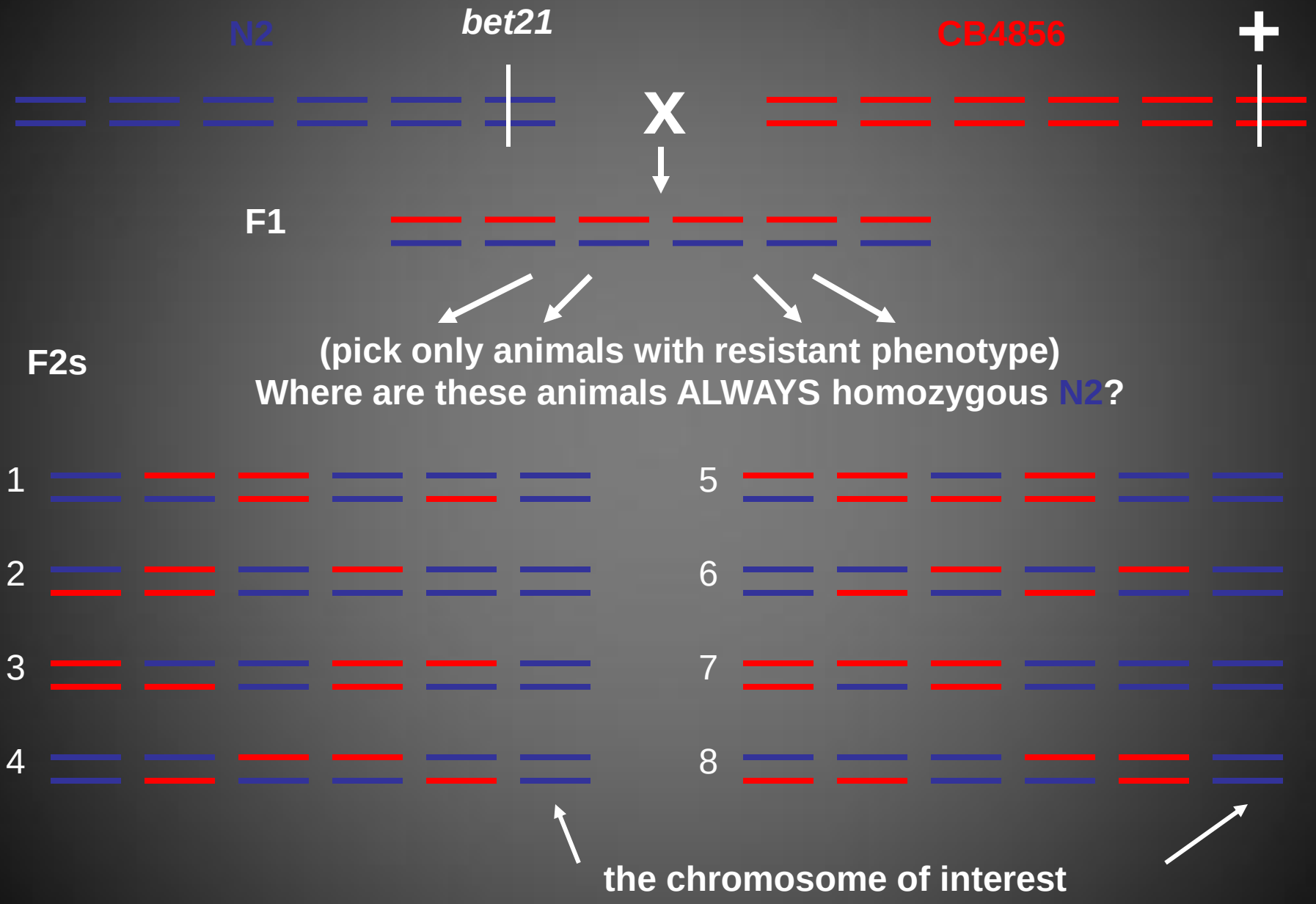
* These stand out points suggest that *C. elegans* is an excellent model to study in Biology especially in Neuronal Biology.

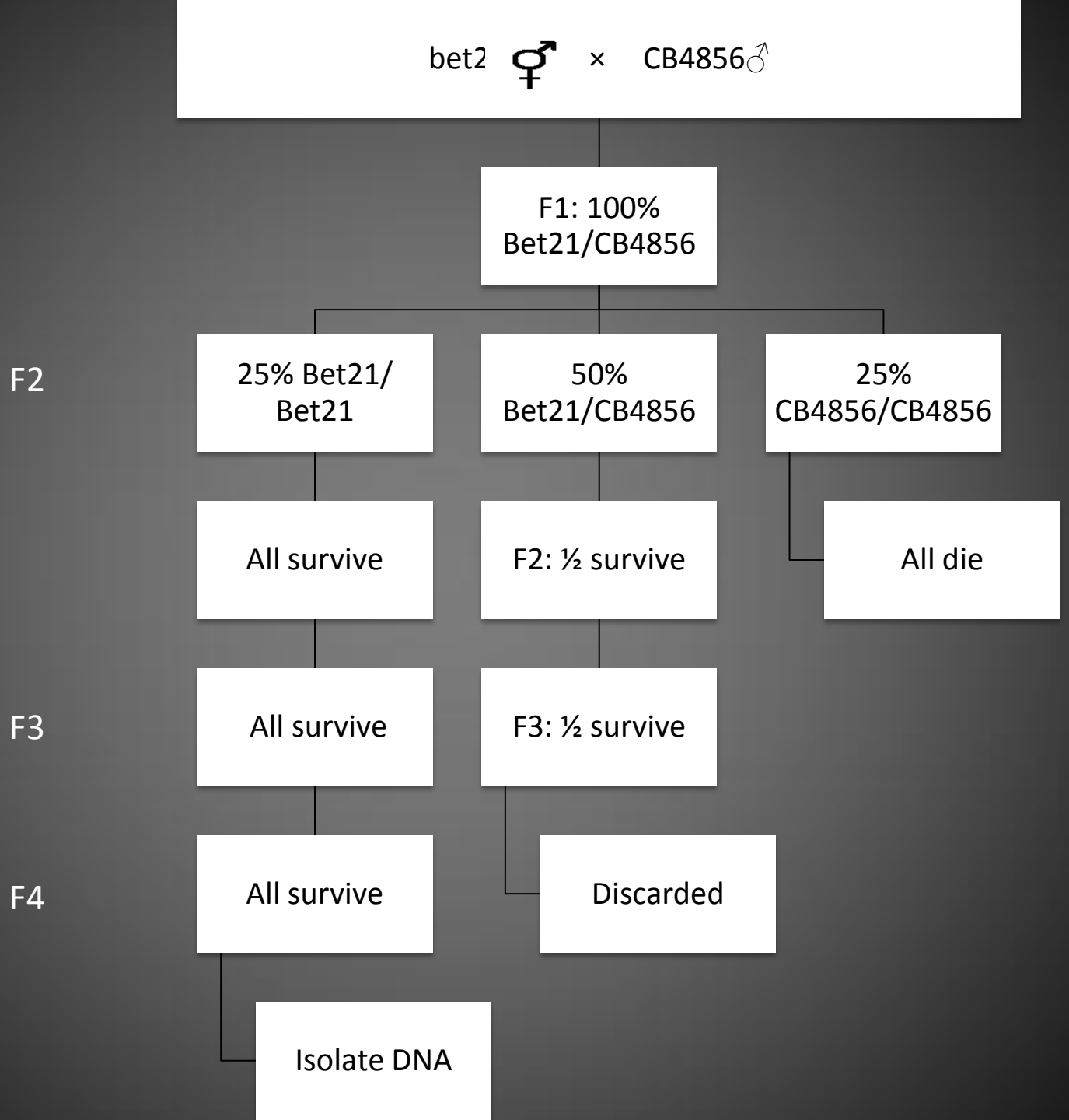
Wild types and *bet21*

- N2 *C. elegans* from London, England.
- CB4856 from Hawaii.
- These two strains' genomic sequence are different in about every 1000 base pairs with a single nucleotide polymorphisms or SNPs. Hence, some of these SNPs are recognition sites for endonuclease restriction enzymes to cut. These are referred as snip SNPs.
- *bet21 C. elegans* were made by mutating *N2 C.elegans* with EMS (Ethyl Methansulfonate), a carcinogenic compound. They exhibit an insensitivity on Allyl Alcohol

  → Hypothetically, *bet21* must carry a mutated gene that leads to a nonfunctional ADH enzyme.
- Where is the gene?

Mapping strategy using SNPs (single nucleotide polymorphisms)




bet2 ♀ × CB4856♂
F1: 100% Bet21/CB4856
F2
25% Bet21/ Bet21
50% Bet21/CB4856
25% CB4856/CB4856
All survive
F2: ½ survive
All die
F3
All survive
F3: ½ survive
F4
All survive
Discarded
Isolate DNA

Materials and Methods

- Cross 5 *bet21* hermaphrodites with 5 *CB4856* male.
- Next day, move each mated *bet21* hermaphrodite to new plates and let them produce F1 progeny which will be all heterozygote of *bet21/CB4856*
- Move 5 L4 F1 hermaphrodites to new plate.
- Let them to produce F2 progeny.
- Pick about 30-40 L4 F2 to prepare for testing on allyl alcohol the next day.
- 50% of all F2 progeny being tested die because *bet21* is not totally recessive to *CB4856* or *CB4856* is semi-dominant to *bet21*.
- Each survivor is moved onto new regular NGM plate and let it reproduce F3 progeny.
- Testing F3 progeny again on allyl alcohol and only pick out plates with full survival rate.
- Retest F4 generation.
- Isolate DNA from the survivors of F4 generation for PCR.

PCR 18 point mapping

- *C. elegans* have 6 chromosome 1 to 5 and X chromosome. There are 3 points on each chromosome (left, right, and middle) to be mapped → 18 point mapping
- The genomic sequence of the recombinants will be easily mapped by using specific restriction enzyme at the different SNPs points.
- Areas on chromosomes that only carry *N2* genes are the interests because the mutation only lies in *N2* genes. From that area we can narrow down the interesting interval and locate the specific location of the gene causing nonfunctional ADH by sequencing within the interval.

PCR results

	Chromosome I			Chromosome II			Chromosome III			Chromosome IV			Chromosome V			Chromosome X		
	-19	-1	+26	-14	+1	+22	-25	-1	+12	-16	+1	+12	-17	+1	+13	-17	+2	+17
	N2: 354, 146	N2: 325, 134, 41	N2: 360, 114, 27	N2: 345	N2: 373, 121	N2: 500	N2: 206, 189	N2: 486	N2: 339, 156	N2: 304, 187	N2:376	N2:313, 77	N2: 307, 87, 79	N2: 435, 70	N2: 282, 205	N2: 540	N2: 409, 133	N2: 409, 34
	CB:500	CB: 495, 41	CB: 474, 27	CB: 236, 109	CB: 494	CB: 368, 132	CB: 395	CB: 354, 132	CB: 495	CB: 491	CB:300, 76	CB: 390	CB: 386, 87	CB: 300, 135, 70	CB: 487	CB: 321, 219	CB: 542	CB: 302, 107, 34
KI 3	N2	N2	CB	N2	CB	N2	CB	N2	HET	HET	HET	HET	CB	CB	CB	CB	N2	N2
KI 4	N2	HET	HET	N2	N2	HET	N2	HET	N2	N2	N2	N2	HET	CB	CB	CB	HET	HET
KI 5	N2	HET	HET	N2	N2	HET	N2	HET	N2	N2	N2	N2	CB	HET	CB	HET	HET	HET
									Controls vary	Controls vary								
KI 6	N2	N2	N2	N2	N2	CB	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2
KI 7	N2	N2	N2	N2	N2	CB	N2	HET	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2
KI 8	N2	N2	CB	N2	CB	HET	CB	N2	HET	HET	N2	HET	CB	HET	HET	N2	HET	HET
KI 9	N2	N2	N2	N2	CB	CB	N2	HET	N2	N2	N2	N2	N2	HET	N2	N2	N2	N2
KI 10	HET	N2	N2	N2	CB	CB	CB	N2	CB	HET	N2	N2	N2	N2	CB	HET	N2	N2
KI 11	CB	N2	CB	N2	N2	CB	CB	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2
KI 12	N2	CB	CB	N2	N2	CB	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2
KI 13	CB	N2	N2	N2	N2	CB	HET	N2	N2	HET	N2	N2	N2	N2	HET	N2	N2	N2
KI 14	N2	CB	N2	N2	N2	CB	N2	N2	N2	N2	N2	N2	CB	N2	N2	N2	N2	N2
KI 15	N2	N2	N2	N2	N2	CB	CB	N2	N2	N2	N2	CB	CB	N2	N2	N2	CB	N2
KI 16	N2	HET	HET						CB			N2	HET	HET	CB	CB	HET	HET
KI 17	HET	HET	HET						CB			HET	CB	CB	CB	N2	N2	HET
KI 18	N2	N2	N2						CB			HET	HET	HET	CB	HET	HET	CB
KI 19	HET	HET	HET						CB			HET	CB	CB	CB	CB	HET	HET
KI 20	HET	N2	N2						CB			N2	CB	CB	CB	HET	N2	N2
KI 21	CB	HET	N2						CB			HET	HET	HET	CB	HET	HET	HET
KI 22	HET	CB	CB						CB			HET	HET	HET	CB	N2	HET	HET
KI 23	HET	N2	HET						CB			HET	HET	CB	CB	N2	N2	N2
KI 24	N2	N2	N2									HET	HET	N2	HET	HET	CB	CB
KI 25	N2	HET	N2						CB			N2	N2	N2	N2	N2	N2	N2
KI 26	N2	HET	N2						CB			N2	N2	N2	N2	N2	N2	N2
KI 27	N2	N2	N2						CB			N2	N2	N2	N2	N2	N2	N2
KI 28	HET	N2	HET						N2			N2	N2	N2	CB	HET	HET	HET
KI 29	HET	N2	N2						N2			HET	HET	HET	CB	N2	HET	HET
KI 30	CB	HET	N2						N2			N2	HET	HET	N2	HET	N2	HET
KI 31	HET	CB	CB						N2			HET	HET	CB	CB	N2	HET	CB
KI 32	N2	N2	N2						N2			N2	HET	HET	CB	N2	HET	N2
KI 33	N2	CB	CB						N2		N2	N2	HET	HET		N2	CB	CB
KI 34	N2	N2	CB						N2		N2	N2		N2	HET	N2	N2	N2
KI 35	N2	N2	CB						N2		N2			N2	HET	N2	N2	N2
KI 36	N2	N2	HET						N2		N2	HET		N2		N2	N2	N2
KI 37	N2	N2	CB						N2		N2	HET		N2	HET	N2	N2	N2
KI 38	N2	N2	HET						N2		N2	HET		N2	N2	N2	N2	N2
KI 39	N2	N2	HET						N2		N2	HET		N2	N2	N2	N2	N2
KI 40	N2	N2	HET						N2		N2	HET			N2		N2	N2
KI 41	N2	N2	HET						N2		N2	HET		N2	N2	N2	N2	N2
KI 42		N2							N2		N2	HET		N2	N2	N2	N2	
KI 43		HET	HET						N2		N2	HET		N2	N2	N2	N2	N2

Discussion

- *N2* only intervals were not clearly found because they were overlapped with either *CB4856* or Het (heterozygote).
- Explanations:

1. Too few recombinants to suggest the correct result
2. The N2 only areas may lie on some other places that were not mapped

Future goals

- Make more recombinants
- Narrow down the interesting interval by PCR
- Sequence the interesting interval.

Acknowledgements

- Dr. Jill Bettinger, Dr. Andrew Davis, and Dr. Pin Lan Li
- Jennifer Gardner
- Joe Alaimo
- Mia, Gina, Ryan, Kapo, Kevin, Chung, Charlotte, and Hersh.

My sincere thanks to everyone!