Caenorhabditis nematodes, population suppression, and gene drives: an emerging story

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1. Rationale

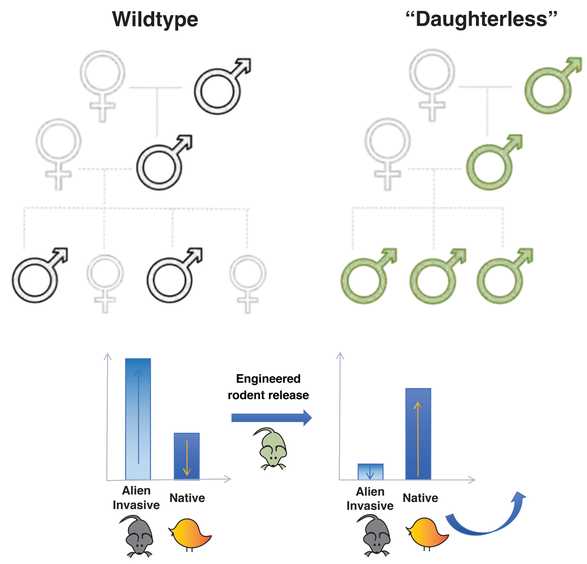
RODENTS

#1 cause of extinctions on islands
>$20b in economic damages in U.S. alone
> 2 billion die in agony from poison yearly

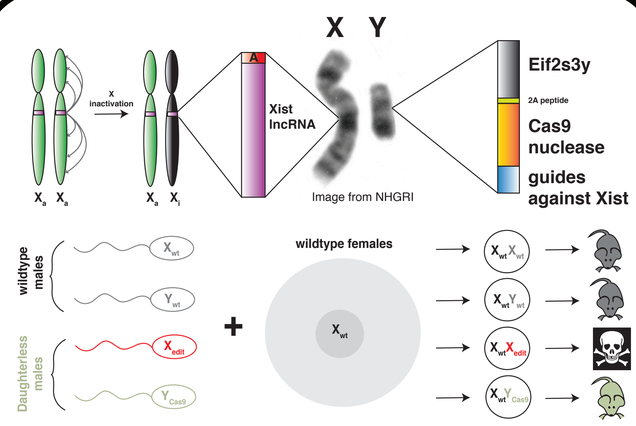
Shown is a rat attacking a birds nest in New Zealand.

New Zealand's native population of fauna is under attack from invasive rodents, and the government has promised eradication of these invasive animals by 2050. This effort will likely require the invention of new technology, as existing tech has allowed us to maintain the status quo rather than reduce populations substantially. Rat poison is the most common tech used, but it induces brain hemorraging, essentially giving rats a migraine for 3 days before death. We consider this method to be inhumane.

2. Sex-biasing population suppression

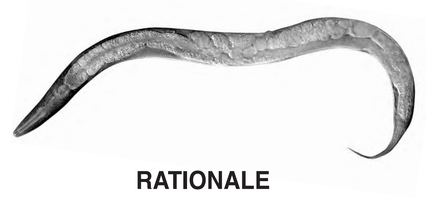


3. "Daughterless" rodent design



Y-Linked Editors, as proposed by Austin Burt in 2003, aim to affect female specific genes involved in fertility or viability as a means to reduce total population size. We will target the conserved "A" region of the Xist long non-coding gene (essential for initiating X-inactivation (Nesterova et al 2001; Hoki et al 2009)) with several guide RNAs. Cas9 will be integrated on the Y as a 2A fusion to the essential male gene Eif2s3y (Li et al 2016). Expression of nuclease and guides in the male germline will induce deletion of the A region of Xist, which will have no effect on male germline cells or Y-carrying embryos. However, inheritance of an edited X chromosome in female embryos will cause failure to inactivate the paternal X in the extraembryonic tissues embryos and induce death (Hoki et al 2009), thereby biasing the sex ratio of a population.

4. Use C. elegans as model to test design



RATIONALE

- Need to test population dynamics of Daughterless design

- Quick reproduction cycle and size of C. elegans make this organism an ideal model for long-term evolutionary stability and population dynamics

OBSTACLES

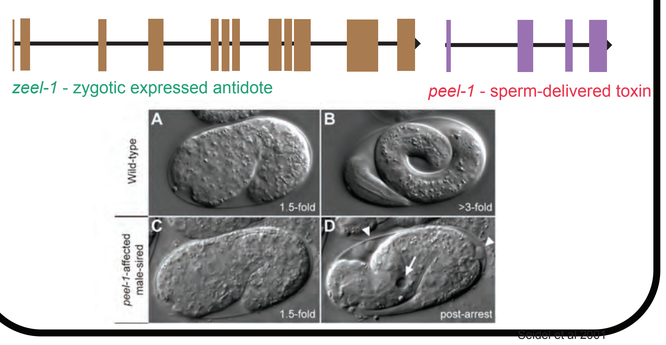
- *C. elegans* are naturally XO, not XY

- *C. elegans* deals with two X chromosomes differently - performs dosage compensation to reduce activity on both X chromosomes by 50%, rather than inactivating a single X. As such, they do not have Xist gene.

5. How to model Daughterless using C. elegans pt 1

- Jonathan Hodgkin created a series of sex-determination mutants that mimic mechanisms from other species - including an XY worm (Hodgkin 2002)
  - whichever chromosome has a male-determining factor becomes the "Y"
    - females: *tra-2(null); fem-1(null); xol-1*
    - males: *tra-2(null); fem-1(null)/fem-1(+); xol-1*

- Will use the adjacent *peel-1/zeel-1* toxin/antitoxin system (adjacent genes) to specifically kill females



Golden et al 2004

6. Is this a useful model?

- **Goal is to determine:**

- evolutionary stability of the suppression system
- do Cas9 and the guides express over multiple generations?
- do repeat regions of guide RNAs recombine over time?
- does resistance to the guide RNAs develop over time?

- population dynamics of suppression system
- does it actually work?
- how many engineered animals need to be released to suppress population?
- how well is our mathematical model fit by the experimental data?

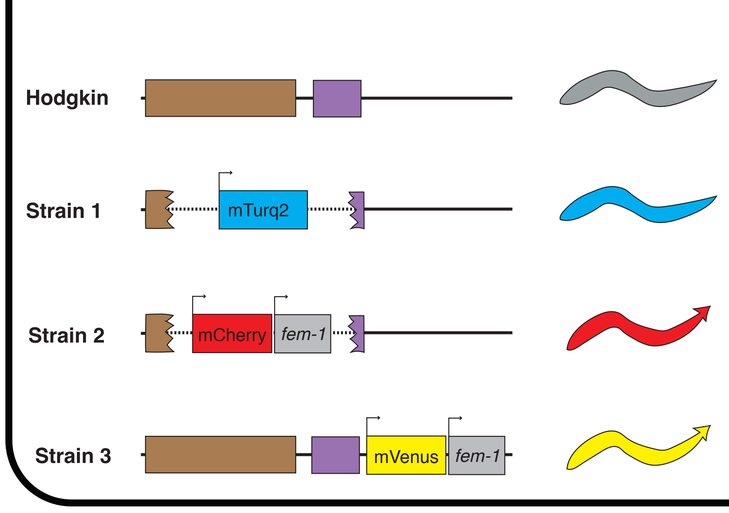
- *C. elegans* will allow us to look at ~100 generations (and over a billion organisms) in the course of a year.

- These data are essential to properly design mammalian strategy

7. How to model Daughterless using C. elegans pt 2

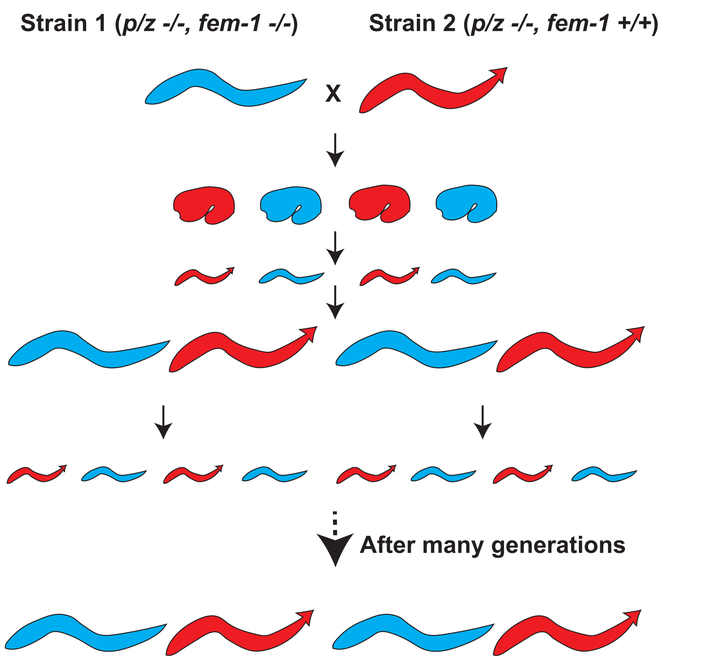
Generate 3 strains marked with different fluorescent colors, with or without *zeel/peel* and *fem-1*

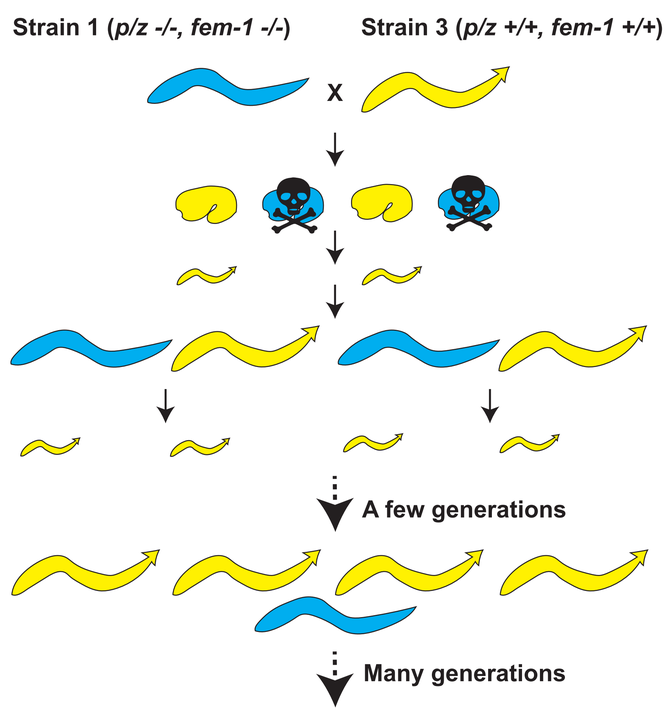
All strains will be made in the Hodgkin "XY" background: *tra-2(null); fem-1(null); xol-1*



Sex	*peel/zeel (p/z)*	*fem-1*
F, XX	*wt/wt*	*null/null*
F, XX	*null/null*	*null/null*
M, XY	*null/null*	*wt/wt*
M, XY	*wt/wt*	*wt/wt*

8. How to model Daughterless using C. elegans pt 3

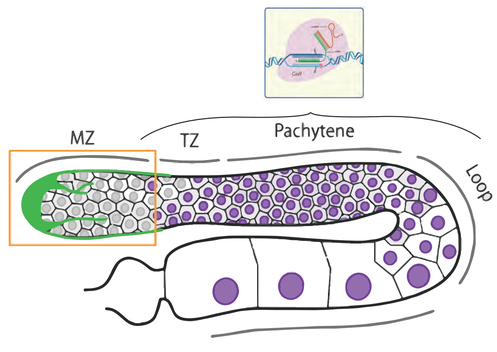




POPULATION CRASH

9. Progress so far

1. Germline-licensed Cas9 nuclease



Narbonne et al 2016; IDT.com

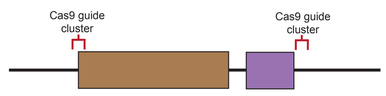
- Default expression in germline is OFF unless overcome by 'licensing' factors

- Jorgensen and Fire groups discovered that many native germline genes have large introns with periodic A/T clusters (PATC)

- Adding PATC introns into a non-germline gene can induce germline expression

- Matt Schwartz generated a licensed Cas9 line using PATC introns (unpublished)

2. Identifying guides to edit the *peel/zeel* locus

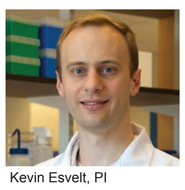


- Bioinformatics using multiple algorithms have identified two clusters of potential guide RNAs to edit the peel/zeel locus.

- Guides will be tested for activity in vitro, and the best will be used to design a knock-in strategy for strain 1, which will be flanked by synthetic, optimal guide sites

- Strains 2 and 3 will be generated by cutting with the optimal guides

10. Acknowledgements



Kevin Esvelt, PI

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Worm Community

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