

Searching for heritable variation in animals' response to radiation

Across the globe background levels of ionizing radiation range by orders of magnitude naturally, with extreme spikes in some areas due to human activity. Many cellular processes are responsible for protecting organisms from DNA damage caused by radiation exposure. Studying heritable variation in animals' ability to tolerate radiation can shed light on human variation in tolerance (i.e. genetic predisposition to cancer), and can suggest biomolecular strategies to disrupt pathologies caused by medical, environmental, or cosmic radiation.

In search of animals with high tolerance to radiation, we collected nematodes from the Chernobyl Exclusion Zone. We chose a set of locations that ranged in ambient radiation levels, and we collected 382 nematode isolates from rotting fruit, soil, and larger invertebrates.

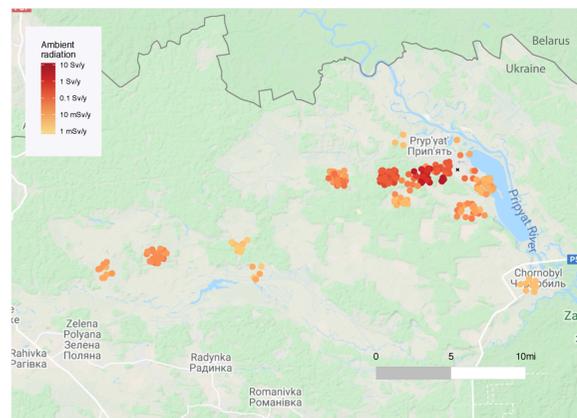


Figure 1 - A total of 250 fruit, soil, and invertebrate samples were collected from an area of ~125 square miles, with background radiation levels spanning four orders of magnitude. 382 nematode isolates were recovered from these samples. Jitter applied to GPS data to make all points visible. Black X marks the Chernobyl Nuclear Power Plant.

Our collection sites spanned the range of biologically relevant background radiation, from levels comparable to New York City, to levels an order of magnitude higher than exposure during space travel.

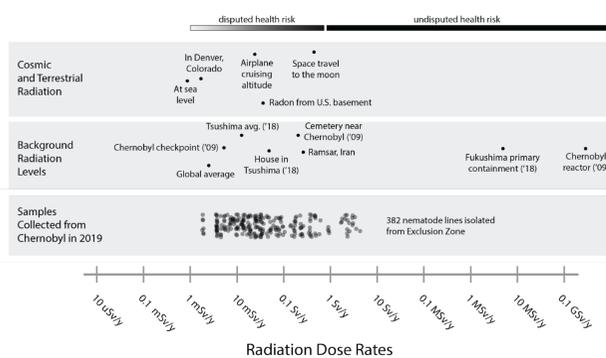
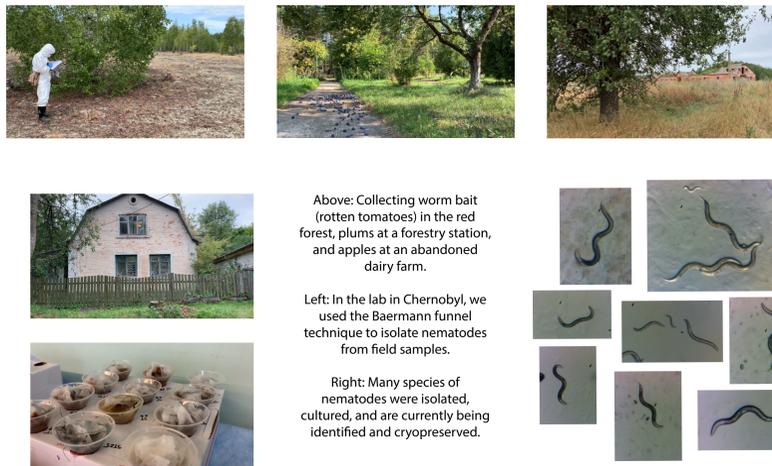


Figure 2 - Radiation dose rates. All values represent continuous exposure for one year. Top and middle: Reference dose rates¹⁻⁴. Data are from a variety of sources using a variety of methods and units, so should be taken as ballpark estimates. Bottom: Ambient radiation at collection site for each nematode isolated in the Chernobyl Exclusion Zone.

Nematodes from Chernobyl: Building an experimental model to study variation in radiation tolerance

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Above: Collecting worm bait (rotten tomatoes) in the red forest, plums at a forestry station, and apples at an abandoned dairy farm.

Left: In the lab in Chernobyl, we used the Baermann funnel technique to isolate nematodes from field samples.

Right: Many species of nematodes were isolated, cultured, and are currently being identified and cryopreserved.

Three clades of nematodes were each isolated from a range of ambient radiation levels

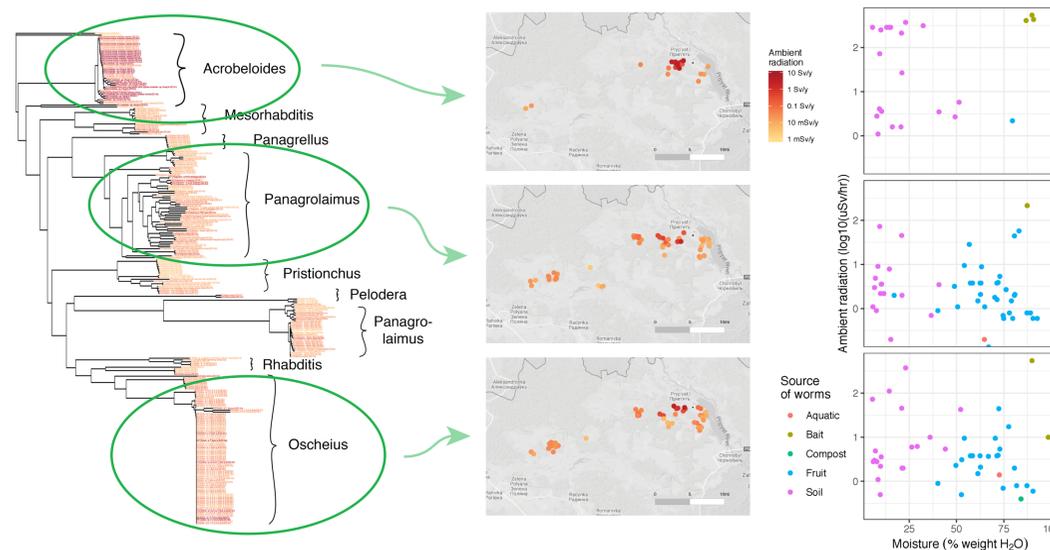


Figure 3 - Collection sites split by clades. Left: Gene tree of 274 wild nematode lines, constructed from a 500 bp region of 18S. Dark blue tips represent isolates from the lowest radiation environments and red tips represent isolates from the highest radiation environments. Acroboloides, Panagrolaimus, and Oscheius clades are composed of isolates from a diversity of environmental backgrounds. Middle: Collection sites for Acroboloides, Panagrolaimus, and Oscheius isolates, color coded by ambient radiation levels at time of collection. Right: Panagrolaimus and Oscheius isolates were collected from sites with comparable ranges of radiation and moisture. Acroboloides were mostly found in soils, which are drier.

What mechanisms contribute to variation in radiation tolerance?

To investigate the genetic and cell biological mechanisms contributing to variation in radiation tolerance, we must first establish our experimental model system: Sets of worms that are genetically similar but divergent in their radiation response.

To identify these sets of worms, we will evaluate relative radiation tolerance for each wild isolate (from Chernobyl as well as nematodes previously collected elsewhere). We are designing a competition assay in which each isolate will be co-cultured with a control strain in normal conditions, and under continuous exposure to ionizing radiation. After several generations we will measure the ratio of experimental : reference population sizes in each condition. The amount that this ratio changes in the presence of radiation will be the tolerance score.

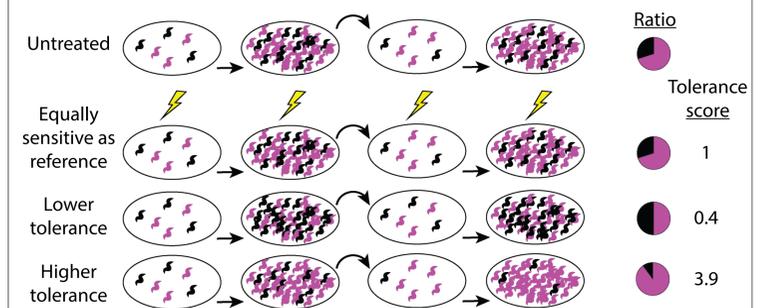


Figure 4 - Schematic of competition assay to evaluate each nematode isolate's relative radiation tolerance.

Pilot tests for co-culturing nematode species for 20 days shows similar population size ratios across replicates.

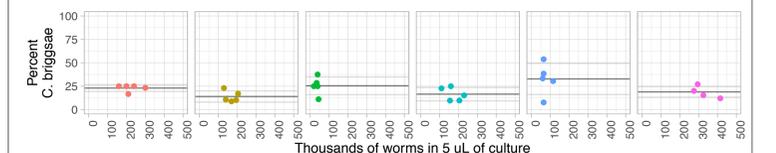


Figure 5 - Co-culturing *Caenorhabditis elegans* and *C. briggsae*. Six small (5 mL) liquid cultures were established with 25 *C. elegans* arrested larvae and 25 *C. briggsae* arrested larvae. On day 20, five 1 μL samples were removed and live worms of each species were counted. *C. briggsae* were identified by a myosin-2::GFP marker. Total mean = 21.76%, standard deviation of six averages = 6.82.

Once we have assigned radiation tolerance scores to each isolate and identified sets of genetically similar worms with diverging radiation responses, we can begin investigating the mechanisms contributing to this divergence.

Does tolerance depend on avoiding, repairing, or otherwise coping with DNA damage?

We will characterize the dynamics of DNA damage and repair in tolerant and sensitive strains to determine the stage(s) where radiation responses differ most.

What are the molecular variants that contribute to radiation tolerance?

We will perform crosses and assay descendants to dissect the genetic variants relevant to radiation tolerance.

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