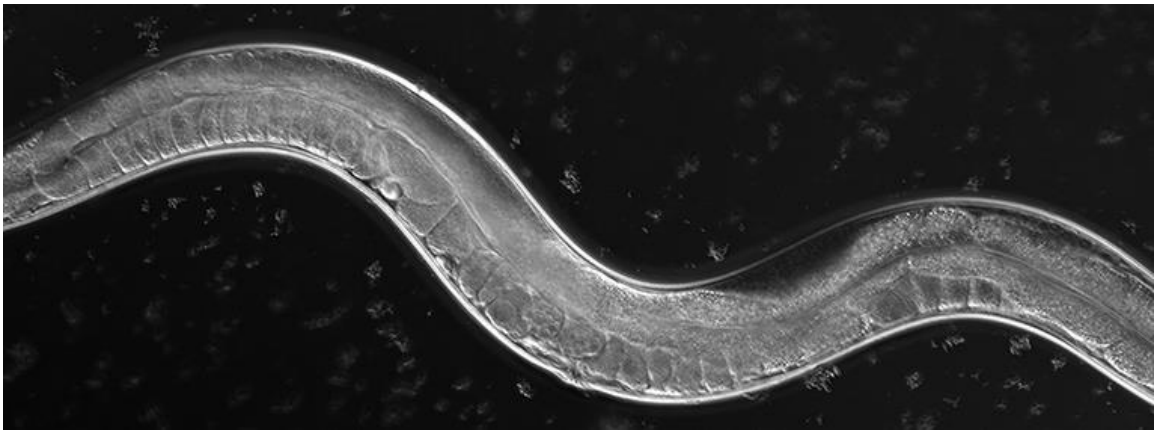

Evolutionary Biology of *Caenorhabditis* and Other Nematodes

20 – 23 June 2022

McMaster University
Hamilton, Canada

Program Book

Schedule overview

Monday 20 June

1600 – 2000	Registration	MDCL 1101
1730 – 1915	Dinner	MDCL 1101
1930	Welcome	MDCL 1102
1930 – 2100	Social	MDCL 1101

Tuesday 21 June

0730 – 0900	Breakfast	MDCL 1101
0900	Opening remarks	MDCL 1102
0915 – 1145	Session 1	MDCL 1102
1145 – 1315	Lunch	MDCL 1101
1330 – 1550	Session 2	MDCL 1102
1600 – 1800	Poster session 1	Student Centre CIBC Hall
1800 – 1930	Dinner	MDCL 1101

Wednesday 22 June

0730 – 0900	Breakfast	MDCL 1101
0900 – 1130	Session 3	MDCL 1102
1145 – 1315	Lunch	MDCL 1101
1330 – 1550	Session 4	MDCL 1102
1600 – 1800	Poster session 2	Student Centre Marketplace
1800 – 1930	Dinner	University Club Alumni Memorial Hall

Thursday 23 June

0730 – 0900	Breakfast	MDCL 1101
0915 – 1115	Session 5	MDCL 1102
1115 – 1145	Closing remarks	MDCL 1102

Detailed Schedule

Monday 20 June

1600 – 2000 **Registration**
Michael DeGroote Centre for Learning and Discovery South-East Corner (MDCL 1101)

1730 – 1915 Dinner
MDCL 1101, South-East Corner

1930 **Welcome**
MDCL 1102
Bhagwati Gupta, McMaster University, Canada
Te-Wen Lo, Ithaca College, USA
Annalise Paaby, Georgia Institute of Technology, USA
Christian Braendle, CNRS, France

1930 – 2100 Social
MDCL 1101, South-East Corner

Tuesday 21 June

0730 – 0900 Breakfast
MDCL 1101, South-East Corner

0900 **Meeting organization & opening remarks**
MDCL 1102
Co-organizers

0915 – 1145 **Session 1** – Chair: Gavin Woodruff, *University of Oklahoma*
MDCL 1102

0915 Deconstructing Male Fertility: Characterizing Fitness and the Functional Role of the NSPF Gene Family during Fertilization
Katja R. Kasimatis
University of Toronto, Toronto, Canada

0927 Programmed DNA elimination in *Caenorhabditis*
Lewis Stevens
Wellcome Sanger Institute, Cambridge, UK

0939 Nematode infection of male fig wasps: potential benefits for nematodes and consequences for fig-fig wasp communities
Justin Van Goor
University of Maryland, College Park, USA

0951 virtual Long-term imaging reveals behavioral plasticity during *C. elegans* dauer exit
Friedrich Preusser
Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

1005 – 1035 Coffee break

1035 Males as agents in controlling brood sex ratio
Solomon Sloat
New York University, New York, USA

1047 Genetic regulation of developmental plasticity in a predatory nematode
Shelley Reich
University of Utah, Salt Lake City, USA

1059 Evolution of Polarity Establishment: The Long and Short Story
Samiksha Kaul
Georgia Institute of Technology, Atlanta, USA

1111 Gene identification and genome annotation in *Caenorhabditis briggsae* by high throughput 5' RNA end determination
Wouter van den Berg
McMaster University, Canada

1123 Evolution of condensin-mediated dosage compensation in nematodes
Avrami Aharonoff
New York University, New York, USA

1135 Virtual poster lightning talks (pre-recorded)

1145 – 1315 Lunch
MDCL 1101, South-East Corner

1330 – 1550 **Session 2** – Chair: Katja R. Kasimatis, *University of Toronto*
MDCL 1102

1330 Updates to the *Caenorhabditis* Natural Diversity Resource
Erik C. Andersen
Northwestern University, Evanston, USA

1342 Understanding adaptive evolution and cryptic speciation in *C. remanei* and *C. latens*
Daniel Fusca
University of Toronto, Toronto, Canada

1354 Sensitivity of *C. elegans* to Orsay virus is suppressed by some bacteria and by a *haao-1* reduction-of-function polymorphism
Rubén González
Institut de Biologie de l'École Normale Supérieure, Paris, France

1406 *Steinernema* nematodes as genetic models of mutualistic and parasitic symbiosis
Erich M. Schwarz
Cornell University, Ithaca, USA

1418 virtual Natural variation in C. elegans genomic defense mechanisms mediated by small RNAs
Gaotian Zhang
Northwestern University, Evanston, USA

1430 – 1500 Coffee break

1500 What is the deal with all these *Medea* elements?
Matt Rockman

New York University, New York, USA

1512 Mutation, selection, and the prevalence of the *C. elegans* heat-sensitive mortal germline phenotype
Sayran Saber
University of Florida, Gainesville, USA

1524 Genetic variation in the *irld* gene family affects starvation resistance
Ryan Baugh
Duke University, Durham, USA

1536 In-person poster lightning talks
Speakers will present in order listed below

1600 – 1800 **Poster session I**
Student Centre CIBC Hall

1800 – 1930 Dinner (Buffet)
MDCL 1101, South-East Corner

Wednesday 22 June

0730 – 0900 Breakfast
MDCL 1101, South-East Corner

0900 – 1130 **Session 3** – Chair: Manuela R. Kieninger, *Wellcome Sanger Institute*
MDCL 1102

0915 virtual The evolution of developmental genetic biases explains the evolution of evolutionary rates
Joao Picao-Osorio
Institut de Biologie de l'École Normale Supérieure, Paris, France

0927 virtual The evolution of an RNA-based memory of self in the face of genomic conflict
Alejandro Burga
Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna, Austria

0939 Single nematode genome assemblies
Erna King
Wellcome Sanger Institute, Cambridge, UK

0951 virtual Sex-determination in the male/female species *C. nigoni*
Jonathan Harbin
Rowan University SOM, Stratford, USA

1005 – 1035 Coffee break

1035 Programmed DNA elimination in *Oscheius* nematodes via precise scission sites is characterized by a shared sequence motif
Pablo Gonzalez de la Rosa
Wellcome Sanger Institute, Cambridge, UK

1047 Extensive natural genetic variation in *Caenorhabditis elegans* egg-laying phenotypes
Laure Mignerot

Institut de Biologie Valrose, Nice, France

1059 Widespread changes in gene expression accompany body size evolution in nematodes
Gavin C. Woodruff
University of Oklahoma, Norman, USA

1111 virtual Evolutionary change in TRA-2 regulation of TRA-1 activator in the sperm/oocyte decision
Yongquan Shen
Rowan University SOM, Stratford, USA

1123 virtual Dissecting intracellular bacterial infection in nematode hosts
Tuan Tran
San Diego State University, San Diego, USA

1145 – 1315 Lunch
MDCL 1101, South-East Corner

1330 – 1550 **Session 4** – Chair: Lewis Stevens, *Wellcome Sanger Institute*
MDCL 1102

1330 Using tetraploids to evaluate Haldane's rule
Ronald E. Ellis
Rowan University SOM, USA

1342 Natural genetic variation in a multigenerational non-genetic phenomena in *C. elegans*
Marie Saglio
Institut de Biologie de l'École Normale Supérieure, Paris, France

1354 Resistance of mitochondrial DNA to cadmium and aflatoxin B_1 damage-induced point mutation accumulation *C. elegans*
Tess C. Leuthner
Duke University, Durham, USA

1406 Conservation of *Nematocida* microsporidia gene expression and host response in *Caenorhabditis* nematodes
Yin Chen Wan
University of Toronto, Toronto, Canada

1418 virtual Allele specific expression suggests that genomic distance amplifies gene regulatory divergence and its compensation
Avery Davis Bell
Georgia Institute of Technology, Atlanta, USA

1430 – 1500 Coffee break

1500 Nigon element evolution and the origin of the XY sex chromosomes of filarial nematodes
Mark Blaxter
Wellcome Sanger Institute, Cambridge, UK

1512 Fine-mapping a novel maternal-effect lethality locus with CRISPR/Cas9-induced meiotic recombination in *C. elegans*
Stefan Zdraljevic

University of California Los Angeles, Los Angeles, USA

1524 Out with the old, in with the new: ion channel evolution
Cody-Jordan Handy-Hart
McGill University, St-Anne-de-Bellevue, Canada

1536 Direct and indirect estimates of the distribution of fitness effects of mutations are not as discordant as they seem at first glance
Charles F. Baer
University of Florida, Gainesville, USA

1600 – 1800 **Poster session II**
Student Centre Market Place

1800 – 1930 Dinner (plated)
University Club Alumni Memorial Hall

Thursday 23 June

0730 – 0900 Breakfast
MDCL 1101, South-East Corner

0915 – 1115 **Session 5** – Chair: Stefan Zdraljevic, *UCLA*
MDCL 1102

0915 Reproductive interference impedes species coexistence in *Caenorhabditis* nematodes with incomplete assortative mating and asymmetric sperm-induced harm
Rebecca Schalkowski
University of Toronto, Toronto, Canada

0927 New Species of Halophile Nematodes Recovered from America's Dead Sea
Michael Werner
University of Utah, Salt Lake City, USA

0939 High-throughput phenotyping of *C. elegans* wild isolates reveals specific resistance and susceptibility traits with distinct microsporidia species
Angcy Xiao
University of Toronto, Toronto, Canada

0951 virtual Quantitative high throughput measurement of selection in an animal system via novel library transgenesis
Zach Stevenson
University of Oregon, Eugene, USA

1005 – 1035 Coffee break

1035 virtual Functional divergence of orthologous temperature-sensitive mutations in *C. elegans* and *C. briggsae*
Satheeja Santhi Velayudhan
Rowan University SOM, Stratford, USA

1047 The Rhabditid Genome Project; creating chromosome-scale reference genomes for all laboratory-cultured Rhabditida
Manuela R. Kieninger
Wellcome Sanger Institute, Cambridge, UK

1059	Whole-genome surveys of variation and linked selection in selfing *Caenorhabditis* species *Ryan McKeown* *Northwestern University, Evanston, USA*
1115 - 1145	**Closing remarks & renewal of organizing committee** MDCL 1102 Co-organizers

Posters

In-person posters

The session on 21 June will take place in the Student Centre CIBC Hall and the session on 22 June will take place in the Student Centre Market Place. All posters may present on both days.

1 Identifying non-coding variants that affect starvation resistance in *C. elegans* using GWAS and data mining
Jameson D. Blount
Duke University, Durham, USA

2 Leveraging the Male Secreted Short (MSS) glycoprotein to characterize the sperm glycocalyx of Caenorhabditis
Asan Turdiev
University of Maryland, College Park, USA

3 Characterizing defects in tail reproductive structures of infertile *C. latens* x *C. remanei* male hybrids
Maia Dall'Acqua
University of Toronto, Toronto, Canada

4 Consequences of X;Autosome Fusions in Filarioidea
Kevin Hackbarth
University of Maryland, College Park, USA

5 Identifying Co-factors that drive TRA-1 activator function
Jibran Imtiaz
Rowan University SOM, Stratford, USA

6 Marvelous Mutants of C. *inopinata*: Forward Screen Reveals Body Size Mutations
Kimberly Moser
University of Oklahoma, Norman, USA

7 Uncovering the effects of reproductive interference on *Caenorhabditis* species coexistence
Jacqueline Jackson
New York University, New York, USA

8 How does the Male Secreted Short (MSS) glycoprotein provide a competitive advantage to *Caenorhabditis* sperm?
Justin Van Goor
University of Maryland, College Park, USA

9 Interaction with TRA-2 Mediates the Sex-specific Function of FOG-2
Eric Haag
University of Maryland, College Park, USA

10 Evaluating possible costs and benefits of variable egg retention in *Caenorhabditis elegans*
Clotilde Gimond
Institut de Biologie Valrose, Nice, France

11 Creation of recombinase-mediated cassette exchange landing pads in genetically diverse wild *C. elegans* strains
Erik C. Andersen
Northwestern University, Evanston, USA

12 Dose-response and quantitative genetic analyses reveals a complex genetic basis underlying susceptibility to diverse toxicants
Erik C. Andersen
Northwestern University, Evanston, USA

Virtual posters

Virtual posters are available for asynchronous viewing at: https://bit.ly/EvoWorm2022_Posters
Password: EBCON2022 *Post your questions on Slack!*

13 Genomic mechanisms of asexual reproduction
George Chung
New York University, New York, USA

14 Conservation and Divergence in the Heterochronic Pathway of *C. elegans* and *C. briggsae*
Maria Ivanova
Rowan University SOM, Stratford, USA

15 Evolution of *fem-1* activity in *Caenorhabditis*
James Kennedy
Rowan University SOM, Stratford, USA

16 Significant differences in the sex determination pathway between *C. elegans* and *C. inopinata*
Ryuhei Hatanaka
Tohoku University, Sendai, Japan

17 Comparative analysis of cellular dynamics of *C. inopinata* and *C. elegans* zygotes
Shun Oomura
Tohoku University, Sendai, Japan

18 Genomic patterns of divergence of *Caenorhabditis brenneri*
Anastasia A. Teterina
University of Oregon, Eugene, USA

19 Genetic regulation of dauer formation in *Pristionchus pacificus* and insights into the dauer hypothesis
Heather Carstensen
California State University, Northridge, Los Angeles, USA

20 The molecular genetics mediating gustatory preferences in *Pristionchus pacificus*
Vivian Vy Le
California State University, Northridge, Los Angeles, USA

21 WormAtlas: New Chapters, New Data, New Worms
Nathan E. Schroeder
University of Illinois at Urbana-Champaign, USA

22 *C. elegans* Male Mobility, Recovery, and Mating After Therapeutic Ultrasound Exposure
Louise M. Steele
Kent State University at Salem, Ohio, USA

23 Compensatory evolution in mitochondrial tRNAs in *Caenorhabditis* nematodes
Ling Wang
Georgia Institute of Technology, Atlanta, USA

24 Generation of genetic resources fo rthe nematode *Caenorhabditis briggsae*
Nikita Jhaveri
McMaster University, Hamilton, Canada

Abstracts

Listed alphabetically by presenting author's last name

In-Person Talk Abstracts

Evolution of condensin-mediated dosage compensation in nematodes

Avrami Aharonoff[1], Jun Kim[1], & Sevinç Ercan[1]
[1]New York University, Department of Biology

In *C. elegans*, X chromosome dosage compensation is accomplished in part by the Dosage Compensation Complex (DCC), which binds to both X chromosomes in hermaphrodites, and represses transcription by a factor of two. The DCC works through a Condensin I paralog belonging to the conserved family of Structural Maintenance of Chromosome (SMC) complexes that regulate 3D genome conformation. Model organisms representing nematodes, flies, and mammals have co-opted different chromatin regulatory complexes to compensate. However, given the limitation of model organisms in accounting for the variation within groups of species, and the high rate of evolution of sex determination mechanisms and sex chromosomes, we cannot say much of their respective groups beyond their models. To address this limitation in nematodes, we performed Hi-C and ChIP-seq in hermaphrodite larvae of the diplogastrid *Pristionchus pacificus*. We observed enrichment of loop-anchored topologically associated domains (TADs) and H4K20me1 on the X chromosome, both of which are features of condensin-mediated dosage compensation. In line with the rapid evolution of dosage compensation proteins, sequence homology and phylogenetic analyses do not produce an ortholog of the DCC in *P. pacificus*. It is therefore possible that the two nematode lineages converged on a similar strategy for compensating. Analysis of published Hi-C data in *C. remanei*, which has an ortholog of the DCC, suggests conservation of condensin-mediated dosage compensation in at least *Caenorhabditis*. We next plan to conduct a wide survey of dosage compensation in rhabditids using our above framework.

Updates to the *Caenorhabditis* Natural Diversity Resource

Robyn E Tanny[1] and Erik C Andersen[1,2]
[1]Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208
[2]Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL 60611

The *Caenorhabditis* Natural Diversity Resource (CaeNDR) has grown significantly over the past two years. During this time, we have created new telomere-to-telomere reference genomes for *C. briggsae* and *C. tropicalis*. These new genomes and long-read RNA-sequencing facilitated the manual curation of over 24 thousand new gene models for the *C. briggsae* reference strain QX1410. Using these gene models and genomes for all three species, the *Caenorhabditis* evolution community has powerful new comparative resource unique among animal (non-human) species. We completed the whole-genome resequencing of 1,860 *C. briggsae*, 1,735 *C. elegans*, and 711 *C. tropicalis* wild strain genomes that comprise nearly all known strains for each of the three species. These new genomes also contain strains

collected from the Hawaiian islands from longitudinal quarterly collections that amplify natural diversity for all three species significantly. We will present new tools and databases added to CaeNDR, including a new genome-wide association mapping package called NemaScan. We hope that these updates facilitate the growth and dissemination of natural diversity studies across the *Caenorhabditis* community.

Direct and Indirect Estimates of the distribution of fitness effects of mutations are not as discordant as they seem at first glance.

Kim Gilbert[1], Tim Crombie[2,3], Moein Rajaei[2], Stefan Zdraljevic[3], Asher D. Cutter[4], Erik C. Andersen[3], José Miguel Ponciano[2], and Charles F. Baer[2]
[1]Institute of Plant Sciences, University of Bern, Bern 3013, Switzerland
[2]Dept. of Biology, University of Florida, Gainesville, FL 32611 USA
[3]Dept. of Molecular Biosciences, Northwestern University, Evanston, IL 60208
[4]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON M5S 3B2, Canada

The distribution of fitness effects (DFE) of new mutations is of fundamental importance in evolutionary biology. However, it is difficult to measure, and estimates are fraught with uncertainty. Methods that attempt to infer the DFE from nucleotide polymorphism have the major advantages of integrating over time, space, and environmental variation. They have the disadvantage of confounding selection with demography. Direct methods, in which fitness effects of known mutant genotypes are measured in the lab, have the advantage of knowing the demography. However, the number of mutations and contexts that can be measured is minute. Here we estimate the DFE indirectly from polymorphism data from hundreds of *C. elegans* wild isolates, and directly from a set of recombinant inbred lines derived from a cross of two laboratory "mutation accumulation" lines, about ~150 mutations total. The DFE estimated from polymorphism data was skewed toward deleterious mutations of "large" effect, with more than half of the distribution having scaled fitness effects $N_e s$ <100. In contrast, the estimated distribution of effects of spontaneous mutations on competitive fitness is nearly symmetrical around 0, with no probability mass associated with mutations with effects (deleterious or beneficial) greater than about 0.002. However, since N_e of *C. elegans* is on the order of 10^5, even mutations with absolute fitness effects on the order of 0.001 would be classified as "large" under this scheme. A model with the mutational effect constrained to 0 fit significantly worse than a model in which fitness effects were allowed to vary. The symmetry around zero is unexpected, and seems unlikely on the face of it. One possibility is that unknown epigenetic mutations explain most of the variation in fitness. Alternatively, it may be that Gillespie's (1995) "House of Cards" reimagining of Fisher's geometric model, in which it is predicted that half of mutations of small (enough) effect should be beneficial is close to the truth, even in experimental systems. The observation that relative fitness almost always decreases with mutation accumulation is inconsistent with the latter hypothesis.

Genetic variation in the *irld* gene family affects starvation resistance

Amy K. Webster[1,5], Rojin Chitrakar[1], Maya Powell[1,6], Jingxian Chen[1], Kinsey Fisher[1], Robyn Tanny[2], Lewis Stevens[2,7], Kathryn Evans[2], Angela Wei[1], Igor Antoshechkin[3], Erik C. Andersen[2], L. Ryan Baugh[1,4]
[1]Department of Biology, Duke University, Durham, NC 27708, USA.
[2]Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208, USA.
[3]Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA.
[4]Center for Genomic and Computational Biology, Duke University, Durham, NC 27708, USA.
[5]Current address: Institute of Ecology and Evolution, University of Oregon, Eugene, OR 97403, USA.
[6]Current address: Environment, Ecology, and Energy Program, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA
[7]Current address: Tree of Life, Wellcome Sanger Institute, Cambridge, CB10 1SA, UK.

The Baugh lab uses L1 arrest and recovery to study nutritional control of development. The lab is interested in developmental quiescence, adult consequences of early-life starvation, multigenerational plasticity, and the genetic basis of starvation resistance, which will be the focus of this presentation. Starvation resistance is important to disease and fitness, but the genetic basis of its natural variation is unknown. We developed a synthetic-population (re)sequencing approach using molecular inversion

probes (MIP-seq) to measure relative fitness during and after larval starvation in *C. elegans*. We applied this competitive assay to 100 genetically diverse, sequenced, wild strains, revealing natural variation in starvation resistance. We confirmed that the most starvation-resistant strains survive and recover from starvation better than the most starvation-sensitive strain, MY2147, using standard assays. We performed genome-wide association with the MIP-seq trait data and identified three quantitative trait loci (QTL) for starvation resistance. These QTL contain several members of the Insulin/EGF Receptor-L Domain (*irld*) family with sequence variation associated with variation in starvation resistance. We used genome editing to show that individual modification of four *irld* genes increases starvation resistance of MY2147. Modification of *irld-39* and *irld-52* together increases starvation resistance of the laboratory-reference strain N2. Increased starvation resistance of the *irld-39; irld-52* double mutant depends on *daf-16/FoxO*, and these worms also show increased nuclear localization of DAF-16 during starvation. DAF-16/FoxO is a widely conserved transcriptional effector of insulin/IGF signaling (IIS), and these results suggest that IRLD proteins modify IIS. This work demonstrates efficacy of using MIP-seq to dissect a complex trait, identifies *irld* genes as natural modifiers of starvation resistance in *C. elegans*, and suggests that an expanded gene family affects a deeply conserved signaling pathway to alter a fitness-proximal trait.

Nigon element evolution and the origin of the XY sex chromosomes of filarial nematodes

Mark Blaxter, Brian Chan*, Pablo Gonzalez de la Rosa, Lewis Stevens
Tree of Life, Wellcome Sanger Institute, Cambridge, UK
* The University of Manchester, Lydia Becker Institute of Immunology and Inflammation, Faculty of Biology, Medicine & Health, Manchester, UK

We have modelled the evolution of rhabditid nematode karyotypes as a dance of rearrangement of seven ancestral chromosomal elements, the Nigon elements. These seven elements, defined by the conserved genes they carry, have been subject to scission, fusion and rearrangement to generate the varied karyotypes (from n=1 to n=>20) observed in extant rhabditid species. Most Rhabditida employ an XX:X0 sex determination system, based on X-to-autosome ratio assessment, as observed in *Caenorhabditis elegans*, with genes allocated to NigonX found on the biological X chromosome in all explored cases. In some filarial nematodes (Oncocercinae within Spiruromorpha) the sex determination karyotype appears to be XX:XY – males are characterised by having heteromorphic sex chromosomes and produce sperm that have either a larger X or a smaller Y chromosome. In analogy to mammalian (and other) XY systems this has been taken to mean that there are male-determining loci on the Y chromosome. Placing reported karyotypes on a phylogenomic tree that incorporates newly sequenced genomes indicates that the filarial XY system has evolved twice independently, through fusion of different "autosomal" Nigons with NigonX, and that the "Y" chromosomes are distinct, autosomal-Nigon-like chromosomes. This in turn suggests that sex determination in these species may still be mediated through X-to-autosome ratio assessment.

Using tetraploids to evaluate Haldane's rule

Ronald E Ellis, Jon Harbin and Abdul Abubaker
Rowan University SOM, Stratford, NJ 08084

In 1922 Haldane noted that when two species form hybrids, if "one sex is absent, rare, or sterile, that sex is the heterozygous sex." Haldane's rule applies to crosses between the closely related species *C. nigoni* and *C. briggsae*, since Woodruff *et al* observed that crosses between *C. nigoni* males and *C. briggsae* hermaphrodites produced no living males, and that the reciprocal crosses between *C. briggsae* males and *C. nigoni* females produced only sterile males. Despite its broad applicability, there is still lively debate about the factors that underlie Haldane's rule.

We have been interested in using polyploid animals to study this phenomenon. To that end, we adapted the Schvarzstein method for producing polyploids through inactivation of *rec-8.* Since *C. briggsae* tetraploid strains already existed, we used RNA interference (carried out by microinjection) to knock down *rec-8* activity in *C. nigoni* females. Among their progeny, we identified rare long animals, the characteristic physical trait of *Caenorhabditis* polyploids. Through a series of crosses, we eventually produced a *C.*

nigoni strain of long males and long females. DAPI staining confirmed that the female animals are polyploid, and most likely *AAAA; XXX*. The males appear to be *AAAA; XX*.

Before we could study hybrid tetraploid animals, we needed to characterize the basic parameters of how the *X:*autosome ratio controls sex in these species. We found that crossing tetraploid males by diploid hermaphrodites produces hermaphrodite progeny in *C. briggsae.* Since these animals are all *AAA; XX,* that means that a ratio of 0.67 produces hermaphrodites in *C. briggsae,* whereas Madl and Hermann found that it yields males in *C. elegans.* Thus, the *X:A* threshold for determining sex has shifted during *Caenorhabditis* evolution. Moreover, we found occasional *AAA; XX* animals that had intersexual tails in an otherwise hermaphrodite body. This observation suggests that 0.67 is close to the threshold, and that fluctuations can be propagated down the pathway, rather than resulting in entirely male or hermaphrodite development.

Using these tetraploid strains, we can now produce interspecies hybrids. These not only include fertile hermaphrodites, but also healthy fertile males. This result strongly supports the model that Haldane's rule in diploid crosses is caused by incompatibility between the genes on the single *X* (which of necessity comes from only a single species), and interacting products made by the pairs of autosomes from both species. As a result, negative interactions should be minimized in tetraploid males, which have one *X* from each parent species. We are now studying backcrosses of these hybrids to each parent species. Although the success rate for individual crosses is low, the resulting progeny tend to be healthy and vigorous.

Understanding adaptive evolution and cryptic speciation in *C. remanei* and *C. latens*

Daniel D. Fusca[1], Asher D. Cutter[1]
[1] Department of Ecology & Evolutionary Biology, University of Toronto, Toronto, Canada

Understanding how adaptive evolution shapes genomes is a major goal of evolutionary biology. Species with very large population sizes and high genetic diversity, such as many *Caenorhabditis* nematode species, make for intriguing study systems to determine the factors that affect adaptive molecular evolution across different parts of genomes. Genome-wide studies of adaptive molecular evolution, however, are rare in *Caenorhabditis* owing to high genetic divergence between species. To address these population genetic questions, we sequenced the genomes of 35 strains of *C. remanei*, including 15 individual worms isolated directly from the wild, and 6 strains of the closely-related species *C. latens*. Demographic analyses indicate only weak differentiation between geographically separated *C. remanei* populations and declines in population size. Surprisingly, we find that the "*C. latens* strain" JU724 is genetically more similar to strains of *C. remanei* than of *C. latens*. Nonetheless, JU724 carries substantial *C. latens* ancestry, especially on chromosome arms, indicative of incomplete lineage sorting of ancestral polymorphism and/or introgression. Based on these findings, and the known partial reproductive isolation observed in crosses of JU724 to both *C. remanei* and *C. latens*, we hypothesize that JU724 may represent a cryptic species separate from both *C. remanei* and *C. latens*. Preliminary analysis of candidate genes under recent positive selection in *C. remanei* suggests that these genes are enriched for chemoreceptors, reminiscent of previous findings in *C. elegans*. This work aims to more comprehensively detect loci in the *C. remanei* genome that have experienced recent adaptive evolution.

Programmed DNA elimination in *Oscheius* nematodes via precise scission sites is characterized by a shared sequence motif

Pablo Gonzalez de la Rosa, Lewis Stevens, Alan Tracey, Manuela Kieninger, Robin Moll, Mark Blaxter
Tree of Life, Wellcome Sanger Institute, Cambridge, UK

Programmed DNA elimination (PDE) is a developmentally regulated process that removes defined parts of the genome from somatic cells. It was first discovered more than 150 years ago by Theodor Boveri in the parasitic nematode *Ascaris megacephala* and it has since been observed in diverse taxa, ranging from ciliates to vertebrates. We previously found that the free living nematode *Oscheius tipulae* performs PDE at all its chromosome ends, eliminating less than 1% of its genome but maintaining the same number of chromosomes. Here we present four new chromosome level assemblies of different *Oscheius* species. In all the species all chromosome ends are remodelled. In addition, two species also eliminate DNA from

regions internal to the chromosome, a process that results in the somatic cells having a greater number of chromosomes than the germline. All scission sites lie within a shared palindromic motif. In one region, nested pairs of these motifs show signs of DNA elimination, indicating regulated usage of the scission sites. Presence of this motif in all sites also suggests it is essential for DNA elimination in *Oscheius*. Some sites of elimination correlate with large scale rearrangements (inversions and a translocation) between species. These results suggest testable hypotheses regarding the mechanisms of PDE and its role in genome evolution. We thank Marie-Anne Felix and colleagues for nematode strains

Sensitivity of *C. elegans* to Orsay virus is suppressed by some bacteria and by a *haao-1* reduction-of-function polymorphism

Rubén González[1], Aurélien Richaud[1], Tony Bélicard, Daria Martynow, Cigdem Alkan, Marie-Anne Félix[1]
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The outcome of viral infections depends on the interaction of three factors: environmental conditions, virus genotype and host genotype. Here we use the pathosystem *C. elegans* – Orsay virus (OrV) to shed light on how the host genotype and the bacterial environment alter viral infection.

First, we describe *haao-1* as a host gene necessary for viral infection. Our team previously identified a *drh-1* indel polymorphism as a major determinant of sensitivity to OrV infection, using a GWAS approach (Ashe, Bélicard et al. 2013). The wild isolate MY10 was found to be an exception in being resistant to OrV infection despite carrying a *drh-1* deletion. In order to determine the cause for this resistance, we built Recombinant Inbred Lines between MY10 and JU1580 – the latter sensitive to OrV infection and also carrying the *drh-1* deletion. Using pooled sequencing of sensitive and resistant lines, we detected a strong QTL on chr. V. This QTL was confirmed by introgressing chr. V in both directions.

In this chr. V region, we found that a non-synonymous polymorphism in the *haao-1* gene was responsible for MY10 resistance to OrV. Indeed, a SNP replacement of the MY10 allele by the JU1580 (and N2) allele makes the nematode sensitive to OrV. Furthermore, a *haao-1* KO mutant in the N2 background is resistant to OrV. The MY10 allele is only found in other strains of the MY10 isotype and is thus a reduction-of-function derived mutation. *haao-1* codes a hydroxyanthranilate oxygenase, an enzyme in the kynurenine pathway that utilizes tryptophan, in particular for de novo NAD synthesis. Our results suggest that a sustained NAD level may be required for OrV infection.

Second, we report that several bacteria from *C. elegans'* natural habitat can reduce its susceptibility to viral infection in comparison to infection levels on *Escherichia coli* OP50. We found bacterial strains on which: (i) the host Intracellular Pathogenic Response to the virus is increased and its susceptibility reduced; (ii) the host barely activates its intracellular pathogenic response and is resistant. If the nematodes initially face the virus in OP50 -where they are susceptible to infection- and then are transferred to the natural bacteria, the reduction in susceptibility is still observed. Dead bacteria fail to induce viral resistance in the host. Finally, these natural bacteria induce resistance even in *drh-1* deletion mutants. This observation suggests that the bacteria may not act through the host antiviral RNA silencing to reduce infection.

Out with the old, in with the new: ion channel evolution

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Muscular movement, controlled by pentameric ligand-gated ion-channels (pLGICs) is a defining feature of animals and a major target for many anthelmintic drugs. New pLGIC receptor classes, especially those unique to parasites, present interesting novel drug targets. The known receptors characterized in *Caenorhabditis elegans*, often have different compositions in related parasites. Details of the mechanisms by which duplicate genes evolve to form new receptors and how subunit composition are regulated are not well understood. We have identified a novel receptor in *C. elegans* that arose by gene duplication of a likely homomeric ancestor, to form a heteromeric receptor responding to a different neurotransmitter. One subunit remains alpha type while the other has lost the alpha YXCC motif and is non-alpha. This provides an ideal system to investigate how the neurotransmitter switch occurred and the mechanisms

regulating receptor subunit organization. Phylogenetic reconstruction identified *Plectus sambesii* possessing subunits with few amino acid differences from that of the nematode ancestor that diverged from Clade I. Characterizing this alongside *C. elegans* by electrophysiology of reconstituted receptors in *Xenopus* oocytes, provides a way to investigate this. Only one subunit from *P. sambesii* can form a homomeric receptor despite both having the alpha motif. Currents when both subunits are present are more robust. Only a heteromeric channel is produced from *C. elegans* subunits. The *P. sambesii* receptor responds fully to the new ligand. This suggests that the switch to a new ligand occurred likely in the homomeric ancestor and that the switch from alpha- to non-alpha was in response to selective pressure independent of the YXCC motif.

Deconstructing Male Fertility: Characterizing Fitness and the Functional Role of the NSPF Gene Family during Fertilization

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Post-insemination molecular interactions involving sperm are an ideal system for linking genotype, phenotype, and fitness. Nematode sperm contain subcellular vesicles known as membranous organelles (MOs) that fuse with the cell membrane upon sperm activation to release their soluble contents into the extracellular space. We hypothesize that one function of MO fusion is to contribute seminal fluid proteins to post-insemination reproductive tract dynamics.

We previously identified Nematode-Specific Peptide family, group F (NSPF) as the second most abundant MO-localized proteins using a novel proteomic survey of the MO protein complement[1]. Molecular evolution analyses for these genes across 12 *Caenorhabditis* species suggested that they play a conserved role in reproduction. To assess NSPF fitness effects, we constructed a CRISPR-based knockout of the three adjacent NSPF genes. Single-generation fertility assays of young and old males under both non-competitive and competitive conditions trended toward a small but non-significant advantage of males containing the wildtype allele over the deletion allele. To overcome the power limitation of single-generation assays, we capitalized on experimental evolution to compound effects over 20 generations. Specifically, we competed the wildtype allele against the deletion allele in 10 replicate *fog-2* feminized *C. elegans* populations. We calculated a significant mean selective disadvantage of 0.1% for the deletion allele, which confirmed that the NSPF genes are an evolutionarily important component of male reproductive success.

We then characterized the functional location of NSPF proteins during fertilization using whole-worm immunostaining of a His-tagged *nspf-1* transgene. Throughout male development, we found that NSPF presence and abundance was correlated with reproductive maturity in males, localizing to the seminal vesicle during L4 and to the spicules throughout adulthood. We confirmed that NSPF proteins are transferred to females during mating and hypothesize that proteins adhere to the spicules during transfer. Transferred proteins localize to the vulva and uterine space of mated females. Interestingly, L4 hermaphrodites showed no NSPF protein signal, whereas adult hermaphrodites showed the same strong signal of NSPF proteins in the uterus regardless of whether they had been mated by a male. These results suggest that the uterine localization of the NSPF proteins is likely a functional property of both self-sperm and male-derived sperm and not simply incidental to the point of transfer during mating.

Our study confirms that NSPF genes are an important component of male reproductive success and potentially act as part of a larger male-female signaling network related to egg laying behavior.

[1]Kasimatis *et al.* (2018) BMC Genomics 19:593.

Evolution of Polarity Establishment: The Long and Short Story

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Polarity establishment, a key element of early embryogenesis, has been studied rigorously in *C. elegans.* This complex developmental process involves partitioning of the maternal effect *par* genes into an anterior and posterior domain within the one cell embryo after fertilization occurs. Establishment and maintenance of these domains that are vital to asymmetric cell division involve interactions between the

par and the actomyosin group of genes. *C. elegans* has been used as a model to study this process and its molecular and cell biological underpinnings are well established within this system. However, the question remains as to how these genes are evolving across long and short evolutionary timescales. How are the two groups of genes involved with polarity establishment evolving across *Caenorhabditis* species? Are the patterns observed interspecifically mirrored at the intraspecific level?

We are using molecular evolution as well as functional experimental assays to evaluate variation in these two groups of genes across *Caenorhabditis* species and within wild strains of *C. elegans*. We demonstrate that actomyosin genes are highly conserved while par genes show more sequence diversity and accumulation changes affecting the protein sequence.

Genomic analysis of *C. elegans* wild strains by Lee et. al. (2021) has demonstrated that there are hyperdivergent regions present within the genome which are being maintained through balancing selection. One region on the left arm of chromosome III consists of five genes, all of which have embryonic phenotypes and two of which (*par-2, chin-1*) are involved in polarity establishment. We focus on this region, specifically in the isolate JU1088, which contains this hyperdiverged haplotype. Using NILs generated with N2 and JU1088 we demonstrate that there is a difference in how the N2 vs JU1088 allele of *par-2* responds to germline RNAi in the opposite genetic background. We also utilize smFISH to tease apart subtle expression differences across early developmental time intervals between N2 and JU1088 in *par-2* and *chin-1*. These results indicate the possibility of the hyperdiverged allele of *par-2* having downstream functional consequences. We hope to explore this region and genes within it further to understand how the hyperdiversity might be contributing to the differences we observe.

The Rhabditid Genome Project; creating chromosome-scale reference genomes for all laboratory-cultured Rhabditida

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Continuous advances in genome sequencing technologies and computational methods mean that it is now relatively straightforward to generate chromosome-level reference genomes for most species. These cutting-edge technologies facilitate closing gaps in the genome assembly, resolve repeat-rich regions, and expose structural changes, even for well-studied genomes, but have thus far only been applied to a handful of nematode species. We are combining long-read HiFi and HiC sequencing to create high quality chromosome-scale reference genomes for all nematode species currently in laboratory culture (>200 species). We achieve substantial increases in contiguity and in assembly span compared to assemblies based on short-read data. For example, we resequenced the entomopathogenic strain M13e of *Heterorhabditis bacteriophora* (supplied by David Clarke, Cork) generating a chromosomal assembly with a scaffold N50 33-fold higher than the published genome (10.3 Mb compared to 312kb).

Using a new very low input method, we are now also able to generate primary assemblies of high contiguity from single nematodes, generating draft genomes from individual wild-caught specimens, including parasites, and to generate assemblies from highly heterozygous cultured strains. These greatly improved assemblies allow us to study patterns of chromosome and genome evolution across Rhabditida and to study processes previously inaccessible, such as programmed DNA elimination.

Single nematode genome assemblies

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Genome sequencing for the phylogenetically diverse nematode species found in marine and estuarine ecosystems has been challenging due to the difficulties in isolating and identifying sufficient numbers of specimens from the field or sustaining them in culture. A novel low input technique overcomes these challenges through optimised long-range library amplification, permitting PacBio HiFi sequencing and genome assembly from single nematode specimens. We have generated draft genome assemblies ranging from 145 to 1062 Mb in size for 26 free-living marine species with high contiguity (N50 range 23 to 700 kb). Transcriptome annotation is also possible as full transcript length cDNA libraries are prepared for these same specimens. These assemblies include the first genomes for species within the orders

Enoplida, Oncholaimida, Chromadorida, Desmodorida and the polyphyletic Monhysterida and Areolaimida. Additional Rhabditida and Diplogasterida genomes have also been sequenced. These new genomes were analysed together with genomes with a selection of published nematode genomes and transcriptomes to construct a phylogeny for the phylum Nematoda based on conserved protein coding genes. The phylogeny supports and expands on previously published Nematoda phylogenies.

Resistance of mitochondrial DNA to cadmium and aflatoxin B_1 damage-induced point mutation accumulation *C. elegans*

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Mitochondrial DNA (mtDNA) is prone to mutation in aging and over evolutionary time, yet the processes that regulate the accumulation of *de novo* mtDNA mutations and modulate mtDNA heteroplasmy are not fully elucidated. Mitochondria lack certain DNA repair processes, which could contribute to polymerase error-induced mutations and increase susceptibility to chemical-induced mtDNA mutagenesis. We conducted error-corrected, ultra-sensitive Duplex Sequencing to investigate the effects of two known nuclear genome mutagens, cadmium chloride and Aflatoxin B_1, on germline mtDNA mutagenesis in *Caenorhabditis elegans*. After 1,750 total generations of mutation accumulation, we detected 2,270 single nucleotide mutations. Heteroplasmy is pervasive in *C. elegans* and mtDNA mutagenesis is dominated by C:G → A:T mutations generally attributed to oxidative damage, yet there was no effect of either exposure on mtDNA mutation frequency, spectrum, or trinucleotide context signature despite a significant increase in nuclear genome mutation rate after Aflatoxin exposure. Mitophagy may play a role in eliminating mtDNA damage or deleterious mutations, and mitophagy-deficient mutants *pink-1* and *dct-1* accumulated significantly higher levels of mtDNA damage compared to wild-type *C. elegans* after exposures. However, in these strains, there were only small differences in overall mutation frequency, spectrum, or trinucleotide context signature compared to wild-type after 3,050 generations, across all treatments. These findings suggest *C. elegans* mitochondria harbor additional previously uncharacterized mechanisms that regulate mtDNA mutational processes across generations.

Whole-genome surveys of variation and linked selection in selfing *Caenorhabditis* species

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Linkage between genomic loci plays a crucial role in understanding how selection shapes a species's evolutionary history and trajectory. Notably, the degree of linkage can vary within a genome and across species and is heavily influenced by factors such as reproductive strategy, making it difficult to characterize the interaction between selection and linkage. In the *Caenorhabditis* genus, a hermaphroditic reproductive strategy (selfing) has independently evolved three times (*C. elegans, C. briggsae*, and *C. tropicalis*). These species have reduced recombination rates and extremely high levels of linkage, particularly in the centers of chromosomes, as compared to outcrossing species. This linkage creates both genetic hitchhiking and background selection, which depress levels of polymorphism in the species. However, non-selective forces, like population size, could generate similar signatures. The relationship between linkage and selection has been best characterized in *C. elegans,* where the observed patterns of polymorphism are driven by an interaction between linkage and both positive and negative selection. However, the relationship between selection and linkage varies between species and likely varies even among the three selfing *Caenorhabditis* species; extensive whole-genome scale characterization of linked selection will likely reveal what factors lead to this.

Here, we compared patterns of polymorphism revealed by whole-genome sequencing of 1,584 strains of *C. elegans,* 1,759 strains of *C. briggsae*, and 705 strains of *C. tropicalis*. We reduced these strains into 540, 610, and 509 genetically unique isotypes, respectively. With new reference genomes

assembled from long-read sequencing data, we analyzed patterns of nucleotide polymorphism; Watterson's theta (Θ), Pi (π), Tajima's D, and the proportion of predicted deleterious variation (a signature of purifying selection) across the three species. Nucleotide diversity and recombination rate are correlated in all three species, suggesting linked selection is an evolutionary driving force in selfing *Caenorhabditis*. Within each species, we explored population structure using principal component analysis, and in *C. elegans* and *C. briggsae*, we demonstrated the influence of demography on signatures of selection. Hyper-divergent regions, which are punctuated regions of high nucleotide diversity enriched for environmental response genes, have been well characterized in *C. elegans* and identified in *C. briggsae* and *C. tropicalis*. We demonstrate that in *C. elegans*, hyper-divergent regions inflate signatures of balancing selection, highlighting the importance of thoroughly characterizing these regions in *C. briggsae* and *C. tropicalis* using long-read sequencing data.

Ultimately, we find that each species displays qualitatively similar patterns of polymorphism, supporting previous studies and indicating that linked selection is an important evolutionary driver in selfing *Caenorhabditis*. Importantly, we see patterns of polymorphism unique to each species, suggesting that comparative studies of selfing *Caenorhabditis* species will be informative in the analysis of linked selection after hyper-divergent regions in the species are characterized.

Extensive natural genetic variation in *Caenorhabditis elegans* egg-laying phenotypes

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Evolutionary transitions from oviparity to viviparity are frequent and many species further display intraspecific variation in egg retention, that is, an intermediate type of parity by laying eggs containing embryos at advanced stages of development. How such natural quantitative variation in egg retention arises through differences in behaviour and physiology – and how this variation ultimately connects to variation in specific fitness components – is not well-understood. Here we focus on intraspecific variation in egg retention of the nematode *Caenorhabditis elegans*. Analysing a panel of ~400 wild strains, we report highly variable intra-uterine retention of fertilized eggs. While the majority of strains differed only subtly in egg retention, a fraction of strains showed either strongly increased or reduced egg retention. We provide evidence for multiple evolutionary origins of such phenotypic extremes and further identify candidate QTL explaining natural variation in egg retention. Focusing on a subset of wild strains, we show that natural variation in egg-laying behaviour contributes to observed divergence in egg retention, likely through variation in underlying neuromodulatory architecture, such as endogenous serotonin availability.

Genetic regulation of developmental plasticity in a predatory nematode

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The nematode *Pristionchus pacificus* exhibits phenotypic plasticity in mouth-form development and has been developed as a model-system for studying developmental plasticity. These worms can develop either a narrow "stenostomatous" (St) mouth with a single tooth or a wide "eurystomatous" (Eu) mouth with two movable teeth. Development of the Eu mouth enables worms to predate on other nematodes in addition to feeding on microbiota in the environment. Mouth-form development is sensitive to environmental conditions and biased by the worm's genetic background. Different strains of *P. pacificus* have been isolated from across the world, and these strains vary in their mouth-form preference from lines that are highly St-biased to highly Eu-biased. A gene regulatory network (GRN) for mouth-form development has been built using a combination of genetic screens and targeted mutagenesis and comprises fewer than 20 genes. To better understand the genetic regulation of mouth-form development, we performed RNA-seq across multiple developmental timepoints and in different culture conditions and

genetic backgrounds. We expect that this dataset will be a useful resource for the *P. pacificus* community. Here, we have specifically examined the expression of known components of the mouth-form GRN and asked (I) which of these genes are environmentally sensitive and (II) if expression of these genes differs between Eu- and St-biased strains. We were surprised to find that only a subset of the mouth-form GRN is environmentally sensitive. Among the genes sensitive to culture conditions are two switch genes, *eud-1* and *sult-1*, enzymes with opposing functions and opposite effects on mouth-form. Contrary to our expectations, only *sult-1* showed significant differences in expression between Eu- and St-biased strains, suggesting different roles for these two switch genes in mouth-form determination. Our data support a model in which *sult-1* expression sets the default state for mouth-form, whereas *eud-1* expression responds to the environment and mediates the developmental response. In summary, our transcriptomic approach indicates that evolutionary forces and environmental conditions modulate the expression of different genes to regulate mouth-form development in *P. pacificus*.

What is the deal with all these *Medea* elements?

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Medea alleles act maternally to kill embryos that don't inherit them. Recent work has shown that polymorphic *Medeas* are particularly abundant in the genomes of selfing *Caenorhabditis* species. In several cases, loci harbor antagonistic *Medea* alleles, such that each allele kills embyros homozygous for the alternate allele. This superficially looks like overdominance, and the elements occur on ancient haplotypes, suggestive of balancing selection. At the same time, these species have genomes that are mosaics of hyper-polymorphic and nearly monomorphic regions, and it's unclear whether and how *Medeas* contribute to this broader pattern of heterogeneous genetic diversity.
I derived analytical results for dynamical models of antagonistic *Medea* elements and their paternal-effect counterparts, *peel* elements. The evolutionary behavior of these alleles is profoundly affected by partial selfing, and by details of the mating system (e.g., monoecy vs androdioecy). Partial selfing generates positive frequency dependence, such that a weakly penetrant allele that is at high frequency in a population can prevent invasion and dis- placement by a much stronger allele. The positive frequency dependence precludes overdominance as an explanation for the ancient haplotypes as there are no stable internal allele frequency equilibria for a single population. Instead, ancient haplotypes in *Caenorhabditis* genomes may result from abundant weak *Medeas* that act as barriers to gene flow among populations. Antagonistic *Medeas* effectively behave as local adaptation loci, locally fixed and resisting homogenization in the face of gene flow, despite the lack of phenotypic benefits for their bearers.

Mutation, selection, and the prevalence of the *C. elegans* heat-sensitive mortal germline phenotype

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C. elegans strains with the heat-sensitive mortal germline (Mrt) phenotype become progressively sterile over the course of a few tens of generations when maintained at temperatures near the upper range of *C. elegans*' tolerance. Mrt is transgenerationally heritable, and proximately under epigenetic control. Previous studies have suggested that Mrt presents a relatively large mutational target, and that Mrt is not uncommon in natural populations of *C. elegans*. The Mrt phenotype is not monolithic. Some strains exhibit a strong Mrt phenotype, in which individuals invariably become sterile over a few generations, whereas other strains show a weaker (less penetrant) phenotype in which the onset of sterility is slower and more stochastic. We present results in which we (1) quantify the rate of mutation to the Mrt phenotype, and (2) quantify the frequency of Mrt in a collection of 95 wild isolates. Over the course of ~16,000 meioses, we detected one mutation to a strong Mrt phenotype, resulting in a point estimate of the mutation rate $UMrt \approx 6 \times 10^{-5}$/genome/generation. We detected no mutations to a weak Mrt phenotype. 6/95 wild isolates have a strong Mrt phenotype, and although quantification of the weak Mrt phenotype is inexact, the weak Mrt phenotype is not rare in nature. We estimate a strength of selection against

mutations conferring the strong Mrt phenotype $\bar{s}$≈0.1%, similar to selection against mutations affecting competitive fitness. The appreciable frequency of weak Mrt variants in nature combined with the low mutation rate suggests that Mrt may be maintained by balancing selection.

Natural genetic variation in a multigenerational non-genetic phenomena in *C. elegans*

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While heredity mostly relies on DNA sequence, additional molecular and cellular features are heritable across generations. This non-DNA based memory could be of importance in the adaptation of organisms to varying environments. Here we test whether and how non-genetic inheritance systems are modulated by natural genetic variation. We use the memory of RNA interference as an experimental paradigm.

As RNAi is heritable in *C.* elegans, we assayed the memory of RNA interference following a *GFP* transgene silencing paradigm. We independently introduced two different germline-expressed *GFP* transgene in genetically divergent wild isolates. We followed the silencing memory of an RNAi trigger provided only in the first generation, targeting the corresponding *GFP* transgene introduced.

We show that *C. elegans* wild isolates differ in the number of generations of silencing. While some isolates consistently show a strong memory for multiple generations in the absence of the RNAi trigger (ie EG4725), others have a very short memory (ie JU775), or no memory at all. Some strains also show an intermediate memory such as the N2 reference strain.

Moreover, genetic variation on chromosome III also underlies the short RNAi memory of the wild isolate JU775 compared to N2.

Overall, we show that multigenerational non-genetic memory is widely modulated by natural genetic variation in *C. elegans*.

Reproductive interference impedes species coexistence in *Caenorhabditis* nematodes with incomplete assortative mating and asymmetric sperm-induced harm

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Species coexistence is shaped by a range of biotic and abiotic factors. Beyond predation, parasitism, and competition, one species may interfere with another's reproductive success in ways that allow one species to sexually exclude the other from a habitat. In this study, we characterize reproductive life-history traits and test for reproductive interference due to adverse effects of inter-species mating between *Caenorhabditis macrosperma* and *Caenorhabditis nouraguensis,* two co-occurring nematode species from French Guiana. We document a higher propensity for intrinsic population growth by *C. nouraguensis* due to greater fecundity of both males and females, indicating its superiority over *C. macrosperma* in resource competition. We also demonstrate that mate discrimination exists between these species, but is incomplete, leading to reduced lifespan and fitness of female *C. nouraguensis* only. We show that these asymmetric costs arise within hours, due to migration of sperm to ectopic somatic locations inside the female. Using this life-history information, we modelled and empirically tested whether reproductive interference to *C. nouraguensis* could offset its intrinsic growth advantage. Multi-generation experiments confirm rapid sexual exclusion of *C. nouraguensis* by *C. macrosperma*. These findings demonstrate the profound ecological implications of reproductive interference for species coexistence through the mechanism of sperm-mediated inter-species harm.

Steinernema nematodes as genetic models of mutualistic and parasitic symbiosis

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Developmentally arrested infective juvenile stage larvae of entomopathogenic nematodes seek and invade insect hosts. Once within the insect's body, the nematode releases symbiotic pathogenic bacteria from within its gut. The nematode and the bacteria rapidly kill the insect, and the bacteria release toxins to protect the insect carcass from colonization by other microorganisms and from other predators. The nematodes consume the bacteria, grow, and reproduce, before eventually sending forth a new generation of infective juveniles, each carrying their bacterial symbionts, to repeat the cycle. An entomopathogenic nematode that is tractable in the laboratory offers the ability to study novel areas of biology, in particular interactions with the nematode's insect prey and the communication and co-evolution underlying the partnership between the nematodes and their bacterial symbionts. Previously available entomopathogenic nematodes have been poorly suited by their reproduction to genetic experimentation, being either male-female (*Steinernema*) or only intermittently hermaphroditic (*Heterorhabditis*). Following the recent rediscovery of the species *Steinernema hermaphroditum*, we have begun developing it as an experimental system. We have demonstrated that *S. hermaphroditum* and its *Xenorhabdus griffiniae* bacterial symbionts grow well in the laboratory and that *S. hermaphroditum* is the first entomopathogenic nematode to consistently develop as self-fertilizing hermaphrodites. We have shown we can isolate and genetically map mutants and can cryopreserve them. We are working to develop a complete molecular and genetic toolset for this species, including forward and reverse genetic approaches and transgenesis. We will report in detail on our progress describing the transcriptome and the genome of *S. hermaphroditum*, including an essentially complete set of protein-coding genes and a third-generation genome assembly.

Males as agents in controlling brood sex ratio.

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In large panmictic populations, sex ratios evolve to be equal. This is due to frequency-dependent selection where alleles that produce more of the rarer sex are selected for until the unbeatable sex ratio of 0.5 is reached. In populations that are highly structured, sex ratios evolve to be female biased. In these metapopulations a small number of founders (<10) migrate every few generations to form a new subpopulation patch. Competition between patches favors a female-biased sex ratio as male offspring do not contribute to population growth as long as mating is assured. Female-biased sex ratios are the ancestral character of the *Caenorhabditis* genus. Historically, models of sex-ratio evolution have neglected the possibility that males control offspring sex. Animal models for female-biased sex ratios have popularly been haplodiploids where females have total control over brood sex. In *Caenorhabditis*, animals have chromosomal sex determination, and males are necessarily complicit in generating brood sex ratios. This is because sperm carrying the sex determining chromosomes compete to fertilize oocytes, generating the observed bias. This sets up the possibility of sexually antagonisitc selection over offspring sex. We tested this using a population genetics simulation in SLiM. We simulated the evolution of sex ratios where four classes of segregating QTL active in either mothers, fathers, or both additively determine the sex ratio of their brood. In our simulations animals exist in a metapopulation where a parameterized number of founders migrate to a new subpopulation every three generations. In simulations where QTL are active in

fathers the sex ratio evolves to be more female biased than in simulations where QTL are active in mothers. This effect is dependent on the number of migrants. When there are fewer migrants and QTL are active in fathers the bias becomes extreme leading to extinction. In simulations where QTL are active in both mothers and fathers the sex ratio evolves to be moderate while rapidly reaching equilibrium faster than when QTL are active in a single parent. We speculate that these results are caused by overlapping generations in our simulations. Males can increase the probability of mating with the next generation by producing an extreme female-biased sex ratio. We outline our strategy to test for sexually antagonistic selection over brood sex ratio in *Caenorhabditis becei*.

Programmed DNA elimination in *Caenorhabditis*

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Programmed DNA elimination (PDE) involves the removal of specific DNA sequences in some cells during development, leading to differences between the germline and somatic genomes. Although originally discovered in the animal parasitic Ascaridid nematode family, PDE is now known to occur in a diverse array of eukaryotic lineages, including ciliates and vertebrates. Recently, new sequencing technologies have revealed that PDE occurs in many taxa where it has previously been missed, including in the free-living nematode *Oscheius tipulae*, which is only distantly related to ascarid nematodes. Here, we unexpectedly discover PDE in the early diverging species of the genus *Caenorhabditis*. Using PacBio HiFi and HiC, we reconstruct the germline and somatic genomes of *C. monodelphis* and show that this species undergoes PDE during embryogenesis, leading to fragmentation of its six germline chromosomes to form 15 somatic chromosomes. Consistent with findings in Ascarididae, we find that the germline-restricted DNA is enriched for satellite repeats and contains genes with roles in germline function, including puf-8, a post-transcriptional regulator that has multiple functions in germline development with homologs in humans and Drosophila. Interestingly, we also find several loci containing ATP-dependent DNA helicase domains in germline-restricted subtelomeric DNA that have homology to those discovered in the eliminated DNA of *Oscheius tipulae*. By generating chromosome-scale genomes for related species, including the outgroup *Diploscapter coronatus*, we reveal that PDE is likely the ancestral state in *Caenorhabditis* and has therefore been lost during the evolution of many *Caenorhabditis* species, including *C. elegans*.

Gene identification and genome annotation in *Caenorhabditis briggsae* by high throughput 5' RNA end determination

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Caenorhabditis briggsae (*C. briggsae*) is a nematode species routinely used for comparative and evolutionary studies. Although the *C. briggsae* genome sequence has been available for quite some time, improvements to the annotation based on experimental evidence are still needed. We have used the Trans-spliced Exon Coupled RNA End Determination (TEC- RED) technique which makes use of Spliced Leader trans-splicing to identify 5' ends of expressed genes. By applying TEC-RED to *C. briggsae* we were able to determine the occurrence of trans-splicing and the genomic location of 5' ends of 4,252 genes, or over one fifth of all known coding genes. TEC-RED tagged sequences that mapped to multiple locations on the genome yielded 362 paralogs, defining 133 sets of two or more genes. Furthermore, a set of 52 tagged 5' ends could not be matched to any annotated coding sequences and likely belong to previously unidentified genes or exons. We also looked for evolutionary changes in trans-splicing and found that of the *C. elegans* orthologs, 14 were uniquely trans-spliced in *C. briggsae*.

The occurrence of Spliced Leader 2 (SL2) splicing to sets of consecutive and closely-spaced genes signals the presence of an operon. This set of criteria allowed us to determine 493 operons in *C. briggsae* with high confidence, of which 334 were fully supported by TEC-RED tags. Functional analysis of operon genes revealed enrichment for germline and growth-associated genes, similar to what has been found in *C. elegans* and other species. We also looked at the conservation of operons and found that in comparison with *C. elegans,* 40% are fully conserved and 22% are novel. By improving the genome annotation, this study serves as a resource for further biological studies by enriching *C. briggsae* as an independent model organism and increasing its reliability as a point of comparison for use in evolutionary studies.

Nematode infection of male fig wasps: potential benefits for nematodes and consequences for fig-fig wasp communities

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All organisms are members of complex biological communities that are characterized by near-constant interactions among species. Such interactions range from obligate mutualism to severe antagonism and shape the evolutionary trajectories of individual species and the community. Interactions can occur between associates in unexpected ways, sometimes leading to profound community-level consequences. An excellent model system to examine these consequences is within the community comprising figs, fig wasps, and wasp-vectored entomopathogenic nematodes. Pollinating fig wasp females are definitive hosts for nematodes because they provide transportation to and nutrition within their reproductive environment (the interior of a new fig). Conversely, wasp males, which emerge as adults 3-5 days before their female counterparts, never leave a natal fig and therefore cannot provide nematodes access to a new fig. Thus, nematode infection of male wasps should be strongly selected against as a reproductive dead-end. Previous observations in Mexico and Panama indicated that such infective events do occur, but the frequency and ecological relevance of this interaction remained uncertain. In a recent survey of the *Ficus petiolaris* community in Baja California, Mexico, we found that nematode infection of male fig wasps is surprisingly common, present in 48% of sampled nematode-infested figs. Nematodes were observed consuming their male hosts and forming enormous mating aggregates on their bodies. This consumption appeared facilitated by a mouthpart polyphenism that differed substantially from nematodes emerging from female wasps. Overlap of nematode generations was also observed, suggesting the development of a second nematode generation within the fig, synchronous with definitive female wasp-host dispersal. This suggests that rates of evolution between nematodes and their hosts may be more dissimilar than previously assumed. Interestingly, infections of non-pollinating fig wasp males were especially common and may result in fewer non-pollinating females exiting figs inseminated, suggesting a novel role for infection on community modulation.

Conservation of Nematocida microsporidia gene expression and host response in Caenorhabditis nematodes

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Microsporidia are obligate intracellular parasites that as a phylum are known to infect most types of animals. Many species of microsporidia can infect multiple related hosts, but it is not known if microsporidia express different genes depending upon which host species is infected or if the host response to infection is microsporidia species specific. To address these questions, we took advantage of two species of *Nematocida* microsporidia, *N. parisii* and *N. ausubeli*, that infect two species of *Caenorhabditis* nematodes, *C. elegans* and *C. briggsae*. We performed RNA-seq at several time points

for each host infected with either microsporidia species. We observed that *Nematocida* transcription was largely independent of its host. We also observed that the host transcriptional response was similar when infected with either microsporidia species. Finally, we analysed if the host response to microsporidia infection was conserved across host species. We observed that although many of the genes upregulated in response to infection are not direct orthologs, the same expanded gene families are upregulated in both *Caenorhabditis* hosts. Together our results show the transcriptional interactions of *Nematocida* infection of *Caenorhabditis* hosts and that these responses are evolutionarily conserved.

New Species of Halophile Nematodes Recovered from America's Dead Sea

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Drought and water diversion are causing saline lakes across the world to decline – threatening benthic communities and the millions of migratory birds that depend on them. In order to protect these critical ecosystems, we must better understand the biological composition and evolutionary limits of benthic microorganisms. Motivated by recent findings of nematodes in Mono Lake, the Siberian permafrost, and deep mines, we ventured into the Great Salt Lake, Utah to search for hypersaline-tolerant nematodes. Several attempts with Baermann funnels were unsuccessful. However, when using a method optimized for sampling in the McMurdo Dry Valleys in Antarctica, we recovered thousands of nematodes from the GSL - challenging the accepted view that only brine flies and brine shrimp can survive such extreme conditions (unpublished). SSU genotyping of over a hundred worms suggests that they represent at least two new species of *Monhysteridae*, a relatively basal nematode family often found in brackish water. However, at 15-20% salinity, to the best of our knowledge, this represents the most saline environment ever recorded for Nematoda. Intriguingly, nematodes were preferentially recovered from microbialites; organo-sedimentary structures built by photosynthetic bacteria that represent the main primary producers in the GSL. Several aspects of microbialite formation are unknown, but they are predicted to require the degradation of cyanobacteria and silica-containing diatoms, both known food sources of nematodes. 16S sequencing of individual worms supports this hypothesis, but further research is required to test it experimentally. If true, nematodes might play a pivotal role in the entire GSL ecosystem. In conclusion, our work extends the limit of saline tolerance by nematodes, upends the current view of biology in the GSL, and provides a new hypothesis for extremophile niche construction.

Widespread changes in gene expression accompany body size evolution in nematodes

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Body size is a fundamental trait that drives multiple evolutionary and ecological patterns. *Caenorhabditis inopinata* is a fig-associated nematode that is exceptionally large relative to other members of the genus, including *C. elegans*. We previously showed that *C. inopinata* is large primarily due to postembryonic cell size expansion that occurs during the larval-to-adult transition. Here, we describe gene expression patterns in *C. elegans* and *C. inopinata* throughout this developmental period to understand the transcriptional basis of body size change. We performed RNAseq in both species across the L3, L4, and adult stages. Most genes are differentially expressed across all developmental stages, consistent with *C. inopinata*'s divergent ecology and morphology. We also used a model comparison approach to identify orthologs with divergent dynamics across this developmental period between the two species. Among such genes were two transcription factors previously shown in *C. elegans* to be important for body size that are regulated by the TGF-β signaling pathway. Multiple hypodermal collagens were also observed to harbor divergent developmental dynamics across this period. *C. elegans*-specific ontology enrichment reveals such genes tend to be expressed in neurons and regulate behavior; they also include genes important for molting and body morphology. A comparison of such genes with previous *C. elegans* experiments reveals overlap with stress response, developmental timing, and small RNA/chromatin regulation. To test if TGF-β signaling has a conserved role in *C. inopinata*, the

CRISPR/Cas9 system was used to generate a 2,647 base pair deletion that removes the entire *dbl-1* coding sequence in *C. inopinata*. Preliminary, anecdotal observations suggest this mutation potentially causes body size reduction and male tail morphological defects, consistent with known roles of the DBL-1 TGF-β signaling ligand in *C. elegans*. Taken together, these transcriptomic results have identified candidate genes that will be further investigated to test their roles in cell size divergence and broaden our understanding of the genetic bases of body size evolution. Ongoing work characterizing its natural microbial communities, patterns of genomic diversity, genomic recombination landscapes, and slow growth rate (among others) is geared toward testing hypotheses regarding the causes and consequences of ecological specialization and morphological divergence with a long-term goal of creating an integrated research program that capitalizes upon the complex ecological and evolutionary relationships among figs, fig wasps, and *C. inopinata*.

High-throughput phenotyping of *C elegans* wild isolates reveals specific resistance and susceptibility traits with distinct microsporidia species.

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Animals are under constant selective pressure from a myriad of diverse pathogens. Microsporidia are a large phylum of eukaryotic obligate intracellular pathogens with wide host ranges including humans and agriculturally important animals such as honeybees, shrimp, and fish. They are ubiquitous animal parasites, but the influence they exert on shaping animal genomes is mostly unknown. Several microsporidian species have been isolated in wild strains of *Caenorhabditis elegans* making these coevolved parasites a powerful tool to study host-pathogen interactions *in vivo*. Using the multiplexed competition assay, PhenoMIP, we measured the impact of four different species of nematode-infecting microsporidia on 22 wild isolates of *C. elegans*. This screen resulted in the identification of 13 strains with altered resistance or sensitivity to infection. Of these identified strains, JU1400, is sensitive to the epidermal-infecting species *Nematocida ferruginous* by lacking tolerance to infection. Conversely, this strain is resistant to the intestinal-infecting species *N. ironsii*, but this infection delays the development of JU1400 animals. Furthermore, coinfection experiments with other microsporidia species demonstrates that JU1400 can specifically recognize and eliminate the invaded *N. ironsii* parasite. Transcriptional analysis of JU1400 response to microsporidia infection revealed few differentially expressed genes during *N. ironsii* infection compared to the sensitive N2 strain. Infection with *N. ferruginous* caused differential regulation of genes that are also induced by toxins. Genetic mapping of JU1400 revealed that several loci contribute to the resistance of *N. ironsii*, including a region on the left part of chromosome II, while sensitivity to *N. ferruginous* is caused by a single locus on the left ends of chromosome I. Construction of near-isogenic lines confirmed that these two opposing phenotypes to different microsporidia species are caused by separate alleles. Overall, our results demonstrate that *C. elegans* can rapidly evolve to recognize and respond to specific microsporidia infections.

Fine-mapping a novel maternal-effect lethality locus with CRISPR/Cas9-induced meiotic recombination in C. elegans

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A recent survey of 609 wild *C. elegans* isolates found that the species-wide *C. elegans* genome is punctuated by divergent regions that contain ~10.3-fold more genetic variation, relative to the reference N2 strain, than the rest of the genome. These divergent regions are enriched for genes involved in environmental sensing and pathogen responses, suggesting that balancing selection may have maintained divergent versions of these genes in different environmental conditions. Therefore, understanding the context in which divergent genes provide an advantage will provide a more comprehensive understanding of *C. elegans* biology. To date however, very few causal genes in divergent regions have been identified, despite many regions being associated with phenotypic variation in the *C.*

elegans population. Identification of causal genes in divergent regions is often hindered by the high levels of genetic variation, unknown genetic content, and low recombination rates.

To facilitate genetic mapping of divergent regions, we constructed multiple large cross populations derived from parents with the largest fractions of divergent genome. During the construction of two of these cross populations, we found that a divergent region on chromosome V quickly reached fixation, suggesting that a genetic incompatibility underlies the large allele frequency distortion at this region. Further characterization of these crosses revealed that the incompatibility element is maternally inherited and causes L1 lethality in F2 offspring that do not contain the element. Though we were able to isolate the incompatibility locus and the surrounding 1 Mb in an incompatibility locus-naive genetic background, the low recombination rate between the divergent haplotypes prevented further fine-mapping. Inspired by work in *Pristionchus pacificus*, we found that targeted meiotic recombination in *C. elegans* can be induced by Cas9. The efficiency of Cas9-induced meiotic recombination (CIMR) ranged from 2-65%, in genomic regions where we observed no recombination in controls without injected Cas9. We suspect that the wide range of CIMR efficiency is caused by a combination of sgRNA efficiency and the level of genetic divergence between haplotypes surrounding the sgRNA target site. Nevertheless, in just three consecutive rounds of CIMR, we fine-mapped the novel incompatibility locus from 1 Mb to 45 kb, reducing the incompatibility locus to 10 candidate genes. We predict that CIMR will greatly facilitate genetic fine-mapping of previously inaccessible genomic regions and therefore broaden our understanding of *C. elegans* biology.

Virtual Talk Abstracts

Allele specific expression suggests that genomic distance amplifies gene regulatory divergence and its compensation

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C. elegans offers unique access into analyses of gene regulatory evolution: their isogenic genomes with genes co-inherited in large, linked groups might make compensatory regulatory changes – *i.e.,* some variants increasing gene expression with others reciprocally decreasing it – likelier to occur and easier to detect. We crossed four wild isolates (JU1088, EG4348, CB4856, and QX1211) to the reference strain N2, then performed RNA sequencing on the F1 heterozygotes and parents. We estimated differential expression across strains and differential expression of alleles within F1s (allele specific expression). The wild strains we chose have widely different levels of divergence from the reference strain, enabling investigation of how gene regulation changes over a range of genomic distances.

We carefully curated methods for quantifying allele specific expression in *C. elegans*, combining best practices at multiple stages to yield an analysis pipeline that avoided reference bias, where reads with reference-genome alleles are better quantified globally, in all crosses. Genes in hyperdivergent haplotypes with detectably different alleles might be expected to exhibit reference bias due to alignment failures of the diverged non-reference allele in these regions. While some genes in these regions were more likely to be reference biased, others had more expression detected from the non-reference allele, and the majority had equal expression detected from both alleles. Therefore, completely excluding genes in hyperdivergent haplotypes from all expression analyses may be an overcorrection.

By comparing allelic expression differences within F1s to the parents' expression differences, we determined if each informative gene had *cis* dominant regulatory changes, *trans* dominant regulatory changes, or compensatory changes. Compensatory changes were inferred at genes where the alleles show a clear, obligately *cis*-driven expression difference but the parents have equivalent expression, implying *trans* compensation of a *cis* regulatory difference. Each of these classes occurred in all four crosses. Of particular interest, *cis* regulatory divergence and *trans* compensation of *cis* regulatory differences appear to increase with genomic divergence, suggesting accelerated regulatory divergence and accelerated compensation of this divergence in *C. elegans*. Further investigations of who, what, and where these genes are – biological function, pathway membership, genomic linkage, population genetic context, and inheritance mode – will shed light on the evolution of robustness, complex traits, and gene expression regulation in this system.

The evolution of an RNA-based memory of self in the face of genomic conflict

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Distinguishing endogenous genes from selfish ones is essential for germline integrity. In animals, small RNAs play a central role in this process; however, the underlying principles are largely unknown. To fill this gap, we studied how selfish toxin-antidote elements (TAs) evade silencing in the nematode *Caenorhabditis tropicalis*. We found that the *slow-1/grow-1* TA is active only when maternally inherited. Surprisingly, this parent-of-origin effect stems from a regulatory role of the toxin's mRNA: maternal *slow-1* mRNA—but not SLOW-1 protein—licenses *slow-1* expression in the zygote by counteracting piRNAs. Our results indicate that epigenetic licensing—known to play a role in *C. elegans* sex-determination—is likely a common mechanism that hinders the spread of selfish genes in wild populations while ensuring a lasting memory of self in the germline.

Sex-determination in the male/female species *C. nigoni*

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The emergence of self-fertility in *Caenorhabditis* is ideal for investigating the origin of new traits, since it evolved recently in three separate lineages. In each case, the self-fertile hermaphrodites are *XX* animals that gained the ability to make sperm during larval development, but still produce oocytes during adulthood. The sex-determination pathway controls spermatogenesis and oogenesis, so it must have been modified to create hermaphrodites. Characterizing the sex-determination pathway in the male/female species *C. nigoni,* which represents the ancestral state of the genus, will help us identify the genetic modifications that cause spermatogenesis in *XX* hermaphrodites of its sister species *C. briggsae*. Characterizing these modifications is essential for defining the changes needed to produce self-fertility.

Using a reverse genetic approach, I generated *C. nigoni* mutations in critical sex-determining genes, along with recessive visible mutations to use as balancers. Genomic edits were made by injecting gravid *C. nigoni* females with Cas9 RNPs. The first gene I targeted was the putative master regulator of the sex-determination pathway, *Cni-tra-1,* which encodes a Gli transcription factor. *C. nigoni tra-1(v481*) masculinizes the somatic tissues of *XX* animals. Moreover, it appears to arrest gonad development and these *Cni-tra-1 XX* animals cannot sire progeny. Although mutations in *C. elegans* or *C. briggsae tra-1* also cause *XX* animals to develop male bodies*,* they become fertile males, producing gonads with sperm and oocytes. Surprisingly, older *Cel-tra-1* or *Cbr-tra-1 XO* males often make sperm and oocytes too. This *XO* switch might be a consequence of self-fertility, as *Cni-tra-1 XO* animals have not been seen producing oocytes.

Next, I targeted *Cni-tra-2,* which encodes the HER-1 receptor. When *C. elegans* or *C. briggsae XX* animals are homozygous null for *tra-2*, they develop imperfect male bodies, but produce only sperm. *Cni-tra-2* also promotes female fates, since *tra-2(v498) XO* animals develop normally but the *XX* animals become imperfect males that produce only sperm. Surprisingly, heterozygosity for *tra-2* induces spermatogenesis in *C. elegans* or *C. briggsae* female mutants, but it does not affect *C. nigoni* females. Thus, low levels of TRA-2 in germ cells might promote self fertility.

My third target was *Cni-fem-3*, which encodes a novel protein that promotes male development in *C. elegans*. The ability of FEM proteins to promote spermatogenesis at multiple points in the sex-

determination pathway might be species-specific, as *C. briggsae fem* genes are not required for spermatogenesis, whereas they are in *C. elegans*. The *Cni-fem-3(v496) XO* animals have a feminized somatic body and germline, producing only oocytes. This phenotype differs from the sister species *C. briggsae,* where the *fem-3(nm63) XO* animals become hermaphrodites.

All of these *C. nigoni* mutants were generated in the inbred strain JU1422, which was used for the first *C. nigoni* genome sequence. However, small broods and low mating efficiency have been a significant struggle. Thus, I'm currently generating more alleles in the distantly related strain CP168, whose draft genome was just shared with us by E. Haag and E. Schwartz. This strain grows more vigorously, which should result in larger broods and increase my ability to detect and score mutants. Final characterization for each gene will use JU1422/CP168 hybrids.

The evolution of developmental genetic biases explains the evolution of evolutionary rates

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Random mutation of the genotype does not generate random phenotypic variation because development biases the mutationally inducible phenotypic spectrum. Therefore, understanding such biases in the introduction of phenotypic variation is essential to reveal which phenotypes can be explored and selected in the evolutionary process. Whether such developmental genetic biases in the construction of phenotypic variation influence evolutionary trends is poorly understood.

Here we address this problem by quantifying the relationship between mutation and wild phenotypic variation within and among nematode species. We use the homologous cellular framework of the six vulval precursor cells (VPC), named P3.p to P8.p, in two clades of nematodes that have divergent evolutionary trajectories of cell fate variation. We generated eight panels of random mutant lines in wild isolates of *Caenorhabditis* and *Oscheius* to quantify the mutability (*i.e.* mutational variance) of VPC fates across micro and macro-evolutionary scales, and compared it with natural genetic variation within and across species of both genera. Our phenotypic analysis of vulva cell fates on over 85,000 nematodes shows, within each species and genus, a strong alignment of the axes of variation upon random mutation with those of wild variation. When represented in a simplified two-dimensional phenotypic space the direction of mutational and natural variation is along the P3.p axis in *Caenorhabditis*, and along the P4.p axis in *Oscheius*. This demonstrates an evolution of the variational properties of VPC fates in *Caenorhabditis* versus *Oscheius*, which can explain the evolution of evolutionary rates.

Long-term imaging reveals behavioral plasticity during *C. elegans* dauer exit.

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During their lifetime, animals must adapt their behavior to survive in changing environments. This ability requires the nervous system to adjust through dynamic expression of neurotransmitters and receptors but also through growth, spatial reorganization and connectivity while integrating external stimuli. For instance, despite having a fixed neuronal cell lineage, the nematode Caenorhabditis elegans' nervous system remains plastic throughout its development. Here, we focus on a specific example of nervous system plasticity, the *C.elegans* dauer exit decision. Under unfavorable conditions, larvae will enter the non-feeding and non-reproductive dauer stage and adapt their behavior to cope with a new environment. Upon improved conditions, this stress resistant developmental stage is actively reversed to resume reproductive development. However, how different environmental stimuli regulate the exit decision mechanism and thereby drive the larva's behavioral change is unknown. To fill this gap, we developed a

new open hardware method for long-term imaging (12h) of *C.elegans* larvae. We identified dauer-specific behavioral motifs and characterized the behavioral trajectory of dauer exit in different environments to identify key decision points. Combining long-term behavioral imaging with transcriptomics, we find that bacterial ingestion triggers a change in neuropeptide gene expression to establish post-dauer behavior. Taken together, we show how a developing nervous system can robustly integrate environmental changes, activate a developmental switch and adapt the organism's behavior to a new environment.

Evolutionary change in TRA-2 regulation of TRA-1 activator in the sperm/oocyte decision

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TRA-1 is a transcription factor that regulates sexual identity in worms. It is subject to complex regulatory interactions that might be involved in the evolution of self-fertile hermaphrodites. We are using gene editing to learn how TRA-1 controls the sperm/oocyte decision. To this end, we generated both *cbr-tra-1(v455)* and *cel-tra-1(v472),* each of which inserts an OLLAS tag near the N-terminus. By comparing them, we showed that *C. briggsae* TRA-1 is cleaved like its *C. elegans* counterpart but forms a slightly larger product. The Zarkower and Spence labs showed that cleaved TRA-1 represses male genes. This cleaved repressor is the predominant isoform in both species.

The Spence and Kimble labs found that TRA-1 can bind the intercellular portion of the TRA-2 receptor. Thus, we made the *cbr-tra-1(v197v383)* allele, which alters 30 residues in the TRA-2-binding domain and showed that it disrupts interactions between TRA-2 and TRA-1 in yeast two-hybrid assays. These *tra-1(v197v383) XX* mutants have many more self-progeny than the wild type, which shows that they make significantly more sperm. The *cbr-tra-2(v403mx)* mutant, which also disrupts the TRA-2/TRA-1 interaction, increases the number of self-progeny too. Furthermore, both mutations restore spermatogenesis to *cbr-she-1(v35) XX* females. Finally, a *cbr-tra-2(v403mx); cbr-tra-1(v197v383)* double mutant shows a complete Mog phenotype — the *XX* animals make only sperm. By contrast, *cbr-fem-3(nm63)* does not alter spermatogenesis and is not needed for these *tra-1* or *tra-2* mutations to have an effect. Thus, *C. briggsae* TRA-2 normally binds TRA-1 to block spermatogenesis and does so independent of FEM activity. This result is surprising, since similar mutations in *C. elegans* have the opposite effect.

To learn how these orthologous *C. elegans tra-2(mx)* alleles work, we are extending gene-dosage studies begun by Tabitha Doniach. Our results imply that *C. elegans* TRA-1 binds TRA-2ic as a negative regulator, preventing it from inactivating the FEM complex. Thus, this section of the pathway has changed dramatically in how information flows during recent evolution.

How might *C. briggsae* TRA-2 affect TRA-1? The *cbr-tra-1(v48)* allele is a missense mutation downstream of the cleavage site. It causes *XX* animals to produce only oocytes and *XO* animals to make oocytes at 25°C but does not affect the sexual development of other tissues. Similarly, both *cbr-tra-1(v405)* and *v406* are frameshifts located downstream of the cleavage site. In a *smg-5* background, these *XX* and *XO* mutants make only oocytes. Since none of these mutations alters the cleaved repressor, there must be a domain at the C-terminus of full-length TRA-1 that promotes spermatogenesis. TRA-2 might block this TRA-1 activator to prevent spermatogenesis.

Finally, our lab found that several chromatin regulators work with TRA-1 to control the sperm/ oocyte decision. Thus, we propose that full-length TRA-1 works with chromatin regulatory factors to activate the expression of genes like *fog-3*, promoting spermatogenesis, and the cleaved form of TRA-1 represses these genes. In *C. briggsae*, TRA-2 blocks the function of full-length TRA-1 by binding to it. By contrast, in *C. elegans* the primary effect of this interaction is to regulate TRA-2.

Quantitative high throughput measurement of selection in an animal system via novel library transgenesis

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Quantifying the selective advantages of mutations is a significant interest in evolutionary biology. In microbial systems, barcoded lineage tracking has dramatically increased the throughput and accuracy of measuring the selection coefficients of individual lineages. However, barcoded lineage tracking has

remained out of reach for animal systems due to technological barriers to generating large, barcoded populations. Here, we present Transgenic Arrays Resulting in Diversity of Integrated Sequences (T.A.R.D.I.S.). TARDIS is the first library-based transgenic system for an animal model, allowing us to rival similar transformation capacity in microbial systems. TARDIS splits the generation of independent transgenics into two distinct components, a large heritable extrachromosomal array and a selectable landing pad. We found that TARDIS arrays can carry several thousand unique barcodes, allowing for the experimental creation of many lineages. To generate a population of barcoded lineages, we applied the TARDIS system in both wildtype (N2) and an ivermectin resistant triple mutant, JD608 (*avr-14*(ad1302) I; *avr-15*(ad1051) *glc-1*(pK54) V) which has been shown to have increased resistance to ivermectin. We carried out large competition experiments in serial liquid culture at various concentrations of ivermectin for several generations. Each generation, genomic samples were isolated, and amplicon sequencing was performed to identify the lineage frequency. The collective lineage selection coefficients were then quantified, and a composite genotypic selection coefficient was measured. We found that the selective advantage or disadvantage can be easily modulated based on the concentration of ivermectin. In the absence of ivermectin, the wildtype background is highly selected, while adjusting the concentration to 1.5nM of ivermectin is enough to flip the selective advantage to the mutant.

Dissecting intracellular bacterial infection in nematode hosts

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The transparency and tractability of *C. elegans* and related nematodes allow for visualization of infection in the context of an intact animal and thus discovery of new interactions between host and pathogen in vivo. From ecological sampling, we discovered a new intracellular bacterial pathogen of the free-living nematode *Oscheius tipulae*. The bacteria, named *Bordetella atropi*, employ a unique mechanism for cell-to-cell spreading. Upon host cell invasion, it undergoes filamentation, a process in which the bacteria continuously divide without septation, resulting in exaggerated elongation. To elucidate the roles of filamentation in vivo, we isolated a filamentation-deficient mutant from in vitro selection that shows reduced anterior-posterior spreading in vivo despite having similar growth rate as the wild-type bacteria. Strikingly, infection by the mutant was constricted to an average of 1 intestinal cell, whereas the wild-type bacterial filaments can spread through an average of 3 adjacent host cells and a maximum of 8 cells, suggesting that filamentation is required for cell-to-cell spreading. Through genome sequencing and complementation, we identified the causative mutation to be a loss-of-function point mutation in the gene *gtaB* of the putative glucose sensing pathway that has been shown to temporarily inhibit cell division to increase bacterial cell size under rich growth conditions. Knockouts of upstream and downstream members in the same glucose sensing pathway phenocopied the mutant either in vitro and in vivo filamentation and spreading capacity, suggesting the involvement of this pathway in regulating filamentation in *B. atropi*.

Interestingly, we observed instances where the bacterial filaments appear to push and disrupt lateral cortical actin filaments of host cells. We hypothesize that host lateral membranes are broken down, but the filaments alone may not generate sufficient physical force to break through host cell lateral membranes, and therefore the bacteria may target some host factors as bacterial filaments cross through neighboring cells. Since genetic manipulations remains scarce in *O. tipulae*, we aim to infect *C. elegans* with *B. atropi* to take advantage of available genetics in the organism to gain insight into how this process occurs in vivo. We first conducted host range assay to gain insight to host species specificity of *B. atropi* and found that *B. atropi* could only infect *O. tipulae* and a related nematode *O. dolichura*. We tested infection against several *C. elegans* mutant strains to potentially identify limiting step(s) that prevent *B. atropi* from infecting *C. elegans*, including *pmk-1*, *tol-1*, and various pharynx mutants, which did not show overt infection. However, we observed a consistent, yet very low infection rate in the *clec-49* mutant that have not been detected in N2 or other mutant tested. Based on this observation, we are conducting forward mutagenesis screens in both N2 and *clec-49* backgrounds. Future work includes verification of putative hits and dissecting the molecular mechanisms of spreading bacterial filaments through cell-cell boundaries and host innate immune response against bacterial intracellular infection.

Altogether, we propose a model in which *B. atropi* evolved a novel mechanism to spread through multiple host cells by coopting a highly conserved glucose sensing pathway that regulate cell sizes to initiate filamentation inside host cells. These bacterial filaments, through some still unknown mechanism(s), break down host lateral membranes for spreading to neighboring cells.

Functional divergence of orthologous temperature-sensitive mutations in *C. elegans* and *C. briggsae*

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Temperature-sensitive mutations manifest a functional wild-type phenotype at the normal (or permissive) temperature and a mutant phenotype at the restrictive temperature, which is usually higher than normal. These *ts* mutations are versatile tools for studying gene function. Fortunately, *C. elegans* has several *ts* alleles that are helpful for genetic, developmental and evolutionary studies. To determine whether the temperature-sensitive behavior of mutations in *C. elegans* and *C. briggsae* is evolutionarily conserved, we constructed orthologs of four *C. elegans* germline *ts* mutations in *C. briggsae* using CRISPR-CAS9 and TALEN mediated gene editing.

FOG-1 is a CPEB-related RNA-binding protein that controls the sperm fate. The *C. elegans fog-1* allele *q253* is a strongly temperature-sensitive allele that causes *XX* animals to become female at 25°C, although most are self-fertile hermaphrodites at the permissive temperature of 15°C. By contrast, the *Cbr-fog-1(v442)*, which is orthologous to *fog-1(q253ts),* is a loss-of-function allele in *C. briggsae*: the *XX* hermaphrodites make only oocytes and the *XO* males produce oocytes at both permissive and non-permissive temperatures.

GLP-1 is a notch receptor protein that regulates the mitotic proliferation of germ cells. The *C. elegans glp-1* alleles *bn18* and *e2141* are strongly temperature-sensitive mutants that block germline proliferation at restrictive temperatures, causing sterility. However, the *C. briggsae* orthologs *Cbr-glp-1(v429)* and *Cbr-glp-1(v438)* are not sterile at restrictive or permissive temperatures, and develop like wild-type worms.

Finally, *C. elegans glp-4(bn2ts)* worms have defective germline development resulting in sterility; they produce very few germ nuclei, all of which arrest at meiotic prophase, when raised at the restrictive temperature. We found that the *C. briggsae* ortholog *cbr-glp-4*(*v473) I* is also a temperature-sensitive allele that results in sterility when grown at 25°C.

Thus, our findings show that one of the four *C.elegans ts* alleles we studied had similar effects in *C. briggsae*. This result shows that mutations orthologous to known *ts* alleles might also be termperature-sensitive in other species. However, the majority of alleles we studied were only *ts* in one species. In these cases, *C. elegans* and *C. briggsae* might differ because some genetic backgrounds are more sensitive to perturbation than others.

Natural variation in *C. elegans* genomic defense mechanisms mediated by small RNAs

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RNA interference is an evolutionarily conserved mechanism for endogenous gene regulation and defense against foreign RNA viruses. Natural variation in exogenous and endogenous RNA interference in *Caenorhabditis elegans* has been suggested to be genetically complex, and the underlying causal variants are largely unknown. Our recent work revealed gene expression variation and possible regulatory mechanisms across 207 genetically distinct wild *C. elegans* strains using genome-wide association mapping. We classified expression quantitative trait loci (eQTL) into local eQTL (located close to the genes that they influence) and distant eQTL (located further away from the genes that they influence). We also identified a diverse collection of genomic hotspots enriched for distant eQTL of multiple genes. Here, we investigated the role of small RNAs in eQTL hotspots. We performed gene set enrichment analysis on genes with eQTL in each hotspot and found hotspots enriched for target genes of small RNAs. Using fine mappings and mediation analysis, we identified candidate variants and genes for each distant eQTL of small RNA targets. Our results showed genetic variants in the gene *eri-6* could underlie the expression

variation of 10 genes. We found the genetic variant in the isoform *eri-6[e]* reduced the expression of *eri-6[e]* but elevated the expression of *eri-6[a-d]*, which encodes the endogenous RNA interference factor ERI-6/7. Elevated levels of ERI-6/7 promote the biogenesis of endogenous ERI-6/7-dependent small interference RNAs (siRNAs) and likely reduce expression of targets, including the 10 genes above. The ERI-6/7-dependent siRNAs primarily target recently acquired, duplicated genes, and pseudogenes with likely viral origins. Strains with the alternative allele allow for strong suppression on the targets to provide extra protection from overexpression of endogenous viral elements and future infections by viruses closely related to endogenous retroviruses. Strains with the reference allele allow for reallocation of resources to other endogenous RNAi pathways to enhance immunity against environmental viruses that are different from the endogenous retroviruses. The distribution of the *eri-6[e]* variant alleles showed geographical differences, indicating adaptive effects of different alleles in wild *C. elegans* to different environments. We also noticed another variant in *eri-7* that might affect the production of siRNAs. Furthermore, the two variants in *eri-6/7* could also influence responses of *C. elegans* to exogenous RNAi. We are currently validating the causality of the two variants using the CRISPR-Cas9 system. We will also test the impacts of these variants on Orsay virus infections and exogenous RNAi response. Our results reveal the diversity in RNA interference pathways among wild *C. elegans* strains and provide evidence of the role of small RNAs in viral defense mechanisms.

In Person Poster Abstracts

Creation of recombinase-mediated cassette exchange landing pads in genetically diverse wild *C. elegans* strains

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The power of genetic techniques and tools using the laboratory-adapted strain N2 is nearly unparalleled, but this one strain is only a single genotype across the genetically diverse wild strain population. We sought to create genome-editing and transgenesis tools to allow the community to rapidly create wild strains with single-copy reporters, overexpression constructs, and additional copies of specific genes. To accomplish this goal, we are adapting the FLP recombinase-mediated cassette exchange method pioneered by the Nonet lab for use in a diverse collection of wild *C. elegans* strains. We have created a single-copy insertion site on chromosome II (nearby the N2 *ttTi5605* site) and a plasmid assembly pipeline (like SapTrap pioneered by the Jorgensen lab). Over the next few months, we hope to create a large number of wild strains that express fluorescent reporters in different tissues and also genes that when overexpressed cause neurodegeneration. To circumvent promoters that might not be visible when driving expression in single-copy, we adapted the split GFP technique with GFP11 driven at high levels in all tissues. Once created, we will make these strains available on the *C. elegans* Natural Diversity Resource. We hope that these new reagents will further enrich the phenotypic diversity of traits that can be studied using the wild strain population.

Dose-response and quantitative genetic analyses reveals a complex genetic basis underlying susceptibility to diverse toxicants

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Toxic exposure is a known risk factor in the onset of many human diseases, but the contributions and abundance of specific genetic variants to xenobiotic-induced disease risk at the population level are unknown. Because of the limited power and scale of toxicological assessments across genetically diverse human subjects, a tractable model system is required to characterize hazard levels of xenobiotic compounds and identify genes linked to susceptibility. Toxicological assessments using *C. elegans* have revealed previously unknown and translational features of xenobiotic metabolism, but investigations of

natural variation in these responses are extremely limited. We measured the susceptibility of eight genetically diverse *C. elegans* wild strains to an array of 28 toxicants, including several heavy metals, mitochondrial poisons, organophosphate insecticides, fungicides, herbicides, and one flame retardant using dose-response assessments. We measured phenotypic responses to each compound by adapting a high-throughput fitness assay using the Molecular Devices ImageXpress Nano automated imaging microscope and developed open-source software to extract and analyze animal morphology measurements from images. Wild strains varied significantly in susceptibility to most toxicants and exhibited variable lowest observed adverse response levels, motivating us to search for quantitative trait loci (QTL) associated with differential responses. To accomplish this search, we measured phenotypic responses to a single dose of each compound across a panel of 200 *C. elegans* strains and performed genome-wide association mapping studies. These analyses revealed dozens of xenobiotic response loci with measurable effects on population-wide toxicant susceptibility and genetically correlated responses to compounds with similar modes of action. We conclude that differential xenobiotic susceptibility among *C. elegans* strains is highly heritable and controlled largely by toxicant-specific genetic architectures. Future work will validate the effects of these QTL in complementary recombinant populations in order to characterize their modes of action, nominate and validate candidate toxicant susceptibility loci, and determine any conserved functions in diverse human populations.

Identifying non-coding variants that affect starvation resistance in *C. elegans* using GWAS and data mining

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Developments in software and genomic resources like more diverse, wild, sequenced strains have the potential to uncover the underlying mechanisms of trait variation involving both protein-coding and non-coding regions by identifying genetic variants associated with the trait. Here we analyze the results of the GWAS software NemaScan (Widmayer et al., 2022), developed by the Anderson Lab and freely available on *Caenorhabditis elegans* Natural Diversity Resource (CeNDR) (Cook et al., 2017), using starvation survival trait data (Webster et al., 2022) from 100 wild *C. elegans* strains. We then compared the resulting list of variants to a map of whole-animal chromatin accessibility dynamics across *C. elegans* development (Jänes et al., 2018). We found a list of genes containing variants associated with variation in starvation resistance, many of them newly identified compared to a previous GWAS using the same trait data, that intersected with these open chromatin regions. We hypothesize that these non-coding variants affect gene regulation to influence the trait. This work highlights the increasing power of new GWAS software to detect variants of interest, reaffirms the members of the *irld* gene family as modifiers of this fitness-proximal trait, and provides a list of genes and non-coding variants for future investigation of their impact on this trait using CRISPR genome-editing studies.

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Characterizing defects in tail reproductive structures of infertile *C. latens* x *C. remanei* male hybrids

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Post-zygotic reproductive barriers that emerge through organismal development separate different species from one another, laying the foundation for the diversification of species that make up life on Earth. *Caenorhabditis* nematodes are an outstanding system for investigating the mechanisms of speciation because of their quick generation times, ease of developmental interrogation, and well-documented genetic makeup. *Caenorhabditis latens* and *Caenorhabditis remanei*, in particular, are two closely-related species that present F1 hybrid dysfunction that contributes to these species' strong but incomplete reproductive isolation. The hybrid defects occur asymmetrically between reciprocal crosses and sexes in a manner consistent with Darwin's corollary to Haldane's rule, with male sterility most severe in *C. remanei*-maternal hybrid males due in part to gonadogenesis defects (Dey et al. 2014). In this study, we use DIC microscopy to characterize reproductive defects in tail reproductive structures of F1 males from reciprocal crosses between *C. latens* and *C. remanei*. Consistent with gonadogenesis defects, F1 hybrid individuals from one direction of the cross had significantly more tail defects than individuals from the reciprocal and pure species crosses. Characterizing the frequency of defects and the distribution of specific dysfunctional phenotypes improves our developmental understanding of hybrid sterility mediating Haldane's rule in this system. In combination with comparisons to known mutant phenotypes in *C. elegans*, these observations suggest mechanistic hypotheses for the genetic causes of post-zygotic reproductive incompatibilities.

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Evaluating possible costs and benefits of variable egg retention in *Caenorhabditis elegans*

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We have quantified natural variation in *C. elegans* egg retention (number of eggs *in utero*) in ~400 wild strains, to identify phylogenetic patterns and genomic regions (QTL) associated with observed phenotypic variation. While the majority of strains differed only subtly in egg retention, a subset of strains showed deviant, either strongly increased or reduced, egg retention. To explore how such phenotypic variation may be maintained, we tested for the presence of potential fitness costs and benefits associated with variable egg retention observed in wild *C. elegans* strains. We show that high egg retention can - but not always - reduce maternal fertility and survival caused by frequent matricidal hatching. In contrast, such genotypes with high egg retention may benefit from improved offspring protection against environmental insults and from a competitive advantage due to reduced extra-uterine egg-to-adult developmental time. Observed natural variation in *C. elegans* egg-laying behaviour may therefore reflect differing, intergenerational trade-offs between fitness components. Altogether, we present a quantitative analysis of natural variation in *C. elegans* egg laying providing first insights into its genetic basis, behavioural links and potential adaptive significance.

Interaction with TRA-2 Mediates the Sex-specific Function of FOG-2

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C. elegans hermaphrodites are essentially XX females that evolved the ability to produce sperm in an ovary [1]. XX spermatogenesis requires a specific protein/mRNA ternary complex composed of the GLD-1 RNA-binding protein, an F-box protein, FOG-2, and the mRNA product of the feminizing gene *tra-2*. GLD-1 and FOG-2 dimerize and bind the 3' UTR of *tra-2* mRNA to repress its activity during spermatogenesis [2,3,4]. Loss of GLD-1, FOG-2, or the GLD-1-binding sites in the *tra-2* 3' UTR eliminates sperm production. GLD-1 is a highly conserved regulator of meiotic progression and oogenesis [5], and has hundreds of RNA targets [7, 8]. In contrast, FOG-2 is a recently evolved *C. elegans*-specific F-box protein [2, 8] only necessary for hermaphrodite spermatogenesis. It has no known role in males, although they express it [9].

The precise mechanism through which FOG-2 in regulating germline sex is unknown, but there are clues. FOG-2 interacts with SKR-1, a component of the E3 ubiquitin ligase complex [8]. This suggests it acts as a canonical F-box protein by targeting another protein for ubiquitin-mediated degradation. However, their similar feminized phenotypes indicated GLD-1 is not that target. Genetic experiments suggest that FOG-2 may have roles independent of its association with the *tra-2* 3' UTR, and potentially independent of GLD-1 as well [10].

As some protein-protein interactions are mediated co-translationally via RNA-binding proteins bound to 3' UTRs [11], we tested the hypothesis that GLD-1 association serves to position FOG-2 to bind and mediate ubiquitination of nascent TRA-2 as it emerges from the ribosome. Consistent with this, we find that the cytoplasmic domain of TRA-2 (TRA-2c) interacts specifically with FOG-2 in yeast two-hybrid assays. This interaction does not require the F-box of FOG-2. An N-terminal portion domain of TRA-2c that overlaps the hypervariable FEM-3-binding domain [12] is sufficient for interaction. Despite this interaction, heat-shock overexpression of FOG-2 cannot masculinize the soma, suggesting GLD-1-binding is essential for substantial FOG-2 activity. Our work suggests FOG-2 specificity for germline sex determination results from an interaction with TRA-2, which would not impact the translation products of other GLD-1 target mRNAs.

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Consequences of X;Autosome Fusions in Filarioidea

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Brugia malayi and *Onchocerca volvulus* are parasitic nematodes in the superfamily Filarioidea that are consequential for public health. Filarioidea are also remarkable for their atypical sex chromosomes and their obligate associations with *Wolbachia* bacterial endosymbionts. In each species, a different

autosome fused with the X chromosome to form a neo-X. As a result, their ancestrally XX/XO systems became XX/XY, where the Y represents a highly degenerated version of the unattached autosomal homolog chromosome (Foster et al., 2020). These independent fusion events offer an opportunity to investigate the evolutionary consequences of X;autosome fusions for the filarial genome in the context of their *Wolbachia* endosymbionts and mammalian hosts (Mattick et al., 2019; Street et al., 2019). We will describe and compare the effects of X;autosome fusions in *Onchocerca* and *Brugia* on dosage compensation, chromatin accessibility, meiotic silencing, gene content, synteny, sex-biased expression, and genetic diversity. Follow-up studies will include further genome sequencing and investigations of the role of *Wolbachia* and host-pathogen interactions in these evolutionary changes.

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Identifying Co-factors that drive TRA-1 activator function

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Gli proteins are involved in cell fate determination, proliferation, and patterning in many species and are major effectors of Hedgehog (Hh) signaling. There are three Gli proteins in humans, and mutations or errors in their regulation can lead to a variety of developmental disorders or cancer. However, the mechanisms by which they interact with co-factors are poorly understood. We are analyzing co-factors of Gli proteins using TRA-1 in *Caenorhabditis* nematodes. The TRA-1 zinc fingers are structurally like those of other Gli proteins, and TRA-1 can be cleaved like other Gli proteins to form a repressor. However, its function has also changed during evolution — in nematodes, TRA-1 controls sexual fates and plays a central role in self-fertility, which makes it easy to assay mutant phenotypes. Furthermore, worms lack classical Hedgehog signaling, so study of nematode TRA-1 should reveal other types of regulation.
Our lab has shown that full-length TRA-1 can work as an activator and promote spermatogenesis, and that the mutation *cbr-tra-1(v48)* disrupts this process and prevents spermatogenesis. We suspect that regulation of TRA-1 activator plays a major role in the evolution of hermaphrodite spermatogenesis in nematodes. Because *v48* was isolated in a classical EMS mutagenesis, we recently made other activator mutations to confirm that all of its phenotypes were due solely to the alteration of TRA-1.

Since TRA-1 activator is likely to interact with a diverse set of co-factors, whose activities help might determine whether specific targets are activated or repressed, we are preparing to purify TRA-1 complexes. We have already identified sites in TRA-1 where an OLLAS tag does not inactivate the protein. We will use gene editing to insert a Tandem Affinity Purification tag into the native *C. briggsae tra-1* gene at one of these sites, purify the complexes and analyze each with mass spectrometry. While doing so, we will look for important modifications to TRA-1 itself, as well as the precise site of cleavage that forms the repressor. In addition, we hope to identify TRA-1 co-factors and learn how they regulate Gli activity. Finally, we will see if any of these co-factors has a novel role in species that produce self-fertile hermaphrodites.

Uncovering the effects of reproductive interference on *Caenorhabditis* species coexistence

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Interspecific sexual interactions play a significant role in driving species coexistence between closely related species. Reproductive interference occurs when individuals of one species engage in reproductive activities with individuals of another species, causing a reduction of fitness of one or both species. Although *Caenorhabditis* elegans has been one of the best studied organisms in biology for decades, researchers have recently established *Caenorhabditis* as a model to study reproductive interference. When collected from nature, multiple *Caenorhabditis* species can be found cohabitating on rotting fruit and flower substrates. How species interact on these ephemeral substrates is unknown. Using *Caenorhabditis* strains collected from Barro Colorado Island, Panama, we ask whether interspecific mating affects the reproductive output and behavior of two androdioecious species, *C. tropicalis* and *C.*

briggsae, when paired with a gonochoristic species, *C. becei*. We paired hermaphrodites with *C. becei* males and then collected data on reproductive output, presence of copulatory plug, and whether animals attempted to leave the mating arena. Preliminary results indicate that *C. becei* is capable of reducing the reproductive output of both *C. tropicalis* and *C. briggsae*. When paired with *C. bece*i males, copulatory plugs are absent on *C. tropicalis* hermaphrodites, but are sometimes present on *C. briggsae* hermaphrodites. Our results are consistent with previous studies of *Caenorhabditis* reproductive interference, which show that gonochoristic species reduce the reproductive output of androdioecious species.

Marvelous Mutants of C. *inopinata*: Forward Screen Reveals Body Size Mutations

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Body size is a fundamental organismal trait varying widely among species. *Caenorhabditis inopinata* grows to be nearly twice as long as its close relative, *C. elegans*. Because of its relationship to this model system, *C. inopinata* is well-positioned to address the causes of body size variation within a comparative molecular genetics context. Here, we report a pilot forward mutagenesis screen to discover genes underlying body size in this species.
We screened 493 mutagenized haploid genomes for recessive body size mutations in the F_3 generation (as *C. inopinata* is a gonochoristic species). We established five mutant homozygous lines after backcrossing for five generations to purge background mutations. Three of these lines harbor a short and fat (dumpy) phenotype, whereas two of these lines have a long mutant phenotype.

Currently we are characterizing mutants to estimate effects on body size dimensions, rates of growth, and the onset of body size differences. Bulk segregant analyses with mutant and wild-type F_2 individuals are also underway to pinpoint the molecular lesions that underlie these mutant phenotypes. Once identified, genes critical for body size regulation in *C. inopinata* can be compared with homologous genes in *C. elegans* using molecular and developmental genetic approaches. This, in tandem with further forward screens, will reveal the extent of functional evolution of body size genes in species with exceptional body sizes.

Leveraging the Male Secreted Short (MSS) glycoprotein to characterize the sperm glycocalyx of *Caenorhabditis*

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Sperm competition is present in all major animal groups, and occurs due to variation in sperm quantity and quality in a competitive environment [1, 2]. While the phenomenological and theoretical aspects of sperm competition have been extensively examined, we still lack a precise knowledge about the underlying molecular mechanisms that influence sperm competitive ability [3]. Sperm are coated in glycoproteins, which form a complex glycocalyx that likely mediates many sperm-female interaction. Using comparative genomics, the Haag lab identified sperm competition protein male secreted short (MSS) that is present in outcrossing *Caenorhabditis* species and was lost in hermaphrodites [4]. MSS is a glycoprotein present on the surface of activated sperm that is both necessary and sufficient for competitive sperm [4]. Its relatively well-conserved N- terminal signal peptide and C-terminal GPI anchor signal sequences are cleaved post-translationally, leaving a poorly conserved glycosylated region. While *mss* is present only in males of outcrossing *Caenorhabditis*, males from both gonochoristic and self-fertile species express several MSS related proteins (MSRP). Like *mss*, expression of most *msrp* paralogs is highly male-biased and associated with spermatocytes [5, 6]. Our work seeks answers to three questions: Is glycosylation essential for the increased competitiveness of MSS+ sperm? Are there glycan-binding receptors on female tissues that can interact with MSS or MSRP? Do MSS and MSRPs perform distinct roles in *Caenorhabditis* worms? I hypothesize: 1) that increased sperm competitiveness is driven by MSS-conjugated glycans, which interact with sugar-binding receptors (e.g. C-type lectins) on female reproductive tissues; and 2) that MSRPs are retained because they are required for baseline fertility.

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How does the Male Secreted Short (MSS) glycoprotein provide a competitive advantage to *Caenorhabditis* sperm?

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By impacting the relative reproductive success of otherwise fertile conspecifics, sperm competition plays a profound role in the evolutionary trajectory of entire species. Sperm competition has been observed in numerous organismal clades, yet our understanding of the molecular mechanisms that mediate it *in vivo* remains extremely limited. MSS (Male Secreted Short) proteins are small sperm surface glycoproteins essential for successful competition in obligately outcrossing *Caenorhabditis*. While MSS proteins provide a competitive advantage to sperm that bear them, the molecular basis for this is currently unknown. MSS-decorated sperm may reach fertile oocytes faster and/or spatially exclude sperm lacking these proteins. Alternatively, MSS-containing sperm may possess an enhanced fertilization ability compared to wild-type sperm without obvious spatial stratification. To test these hypotheses in real time, we mated feminized XX *C. briggsae* with vital dye-stained males that either possessed or lacked a *mss(+)* transgene (from *C. nigoni*). After mating, the distributions of sperm in various sections of the hermaphroditic gonad (spermatheca, uterus, vulva) were quantified. While there were no significant differences between the number of *mss(+/-)* sperm in each gonad area, *mss(+)* sperm were more commonly observed proximal to the spermathecal valve regardless of mating order. More intriguingly, ectopic *mss(+)* sperm were frequently observed beyond the spermathecal valve. This suggests that *mss(+)* sperm reach receptive oocytes more efficiently than *mss(-)* counterparts. Time-lapse imaging revealed that invasivity occurs during ovulation when the valve is briefly open. Ectopic sperm cannot fertilize pre-ovulatory oocytes but may be in position to be the first to do so upon ovulation. To clarify the structural determinants of MSS action, we used microparticle bombardment to overexpress two endogenous MSS-related proteins (MSRPs) in *C. briggsae, msrp-3* and *msrp-8*. Phenotyping experiments and parentage assays indicate that neither confers competitive advantages to the sperm bearing them, suggesting some alternative mechanism underlying MSS success.

Virtual Poster Abstracts

Genetic regulation of dauer formation in *Pristionchus pacificus* and insights into the dauer hypothesis

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Free-living nematodes enter an alternate, stress-resistant larval stage called dauer when faced with harsh environmental conditions. While pathways regulating dauer entry have been extensively characterized in the model nematode *Caenorhabditis elegans*, hormonal and transcriptional analysis have shown only limited conservation in parasitic nematodes. *Pristionchus pacificus*, an entomophilic nematode associated with beetles, also shows limited functional conservation of dauer regulatory genes, making it

an appropriate comparative genetic model to bridging the gaps between *C. elegans* and obligate parasitic nematodes. Despite extensive reverse genetics targeting daf homologs, only mutations in the nuclear hormone receptor *Ppa-daf-12* and the transcription factor *Ppa-daf-16* appear to show the same daf phenotype found in *C. elegans*. Currently only alleles in *Ppa-hsd-2*, a gene necessary for the biosynthesis of dafachronic acids required to suppress dauer formation, have been found to cause a Daf-c phenotype. In contrast to the nuclear hormone pathway, mutations in members of the TGF-β pathway have been found to not affect dauer formation *P. pacificus*. To identify additional genes involved in dauer regulation, we performed a modifier mutagenesis screen on the null *Ppa-hsd-2* mutant and isolated three enhancer alleles that still form constitutive dauers on exogenous Δ7-dafachronic acid, a functionally conserved hormone that suppresses dauer formation in various nematode species. Finally, because mutations of the cGMP-gated ion channel *Ppa-tax-2* result in dauer formation defects, we are testing whether mutant constitutive dauer formation can be ameliorated by exogenous cGMP. Such unbiased genetic approaches may help to discriminate between derived and shared pathways utilized for this fundamental developmental decision in nematodes.

Genomic mechanisms of asexual reproduction

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Diploscapter pachys is a parthenogenetic nematode from a long-lived (est. ~18M years) asexual lineage with an abridged meiosis, a highly heterozygous genome with an unusual karyotype of $2n = 2$ (Fradin, Kiontke, Zegar *et al.* 2017 *Current Biology*). To continue to explore the evolutionary trajectory of *D. pachys* from sexual reproduction to parthenogenesis, we are undertaking a comparative analysis of genome evolution across the clade of parthenogenetic *Diploscapter/Protorhabditis* species against a closely related sexual outgroup. For each species, we are generating phased diploid chromosome-level assemblies using Oxford Nanopore Technologies (ONT) long-read DNA and RNA sequencing complemented with Pore-C chromatin conformation capture. Here, we present evidence for linear *Diploscapter* chromosomes with a long history of fusion and rearrangements of ancestral chromosomes. We are in the process of defining the complete repertoire of telomeric and meiotic genes in this clade. Finally, we plan to determine how the expression patterns of heterozygous alleles have evolved to adapt to non-recombining diploid genomes of the parthenogens in the *Diploscapter/Protorhabditis* clade.

Significant differences in the sex determination pathway between *C. elegans* and *C. inopinata*

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Sex determination is a fundamental process for reproduction that is essential to ensure the continuation of the species. Regulatory mechanisms of sex determination are known to be rapidly evolving and highly diversified. Among nematodes, genetic regulation of sex determination has been studied extensively in *C. elegans*, and comparative analyses were performed in a limited number of closely related species. It remains unclear how sex determination mechanisms are diversified in other nematodes.

In this study, we performed a comparative analysis of genetic pathways of sex determination between *C. elegans* and its sibling species, *C. inopinata*, using comparative genomics, RNAi and transcriptome analysis,

Based on the comparison of genome sequences, most sex determination pathway genes were conserved between these two species. However, *her-1* and *xol-1* in *C. inopinata* had lost their function by transposon insertions. RNAi knockdown in *C. inopinata* targeting *sex-1*, *sdc-2,* and *fem-1* exhibited no detectable phenotypes in both males and females, whereas the knockdown of *tra-1* and *tra-2* showed partial masculinization of the female germline. Also, most of the target genes of *C. elegans* TRA-1 (Berkseth, et al. 2013) showed similar sex- and stage-specific transcription patterns between *C. elegans* and *C. inopinata*.

Taken together, these results suggest that, while the function of downstream genes, including *tra-2* and *tra-1,* are conserved, the upstream part of the pathway has surprisingly diverged between *C. inopinata* and *C. elegans*.

Conservation and Divergence in the Heterochronic Pathway of *C.elegans* and *C.briggsae.*

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The nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae* are close species that have similar morphology and share the same ecological niche. Their post-embryonic development consists of four larval stages separated by molts during which stage-specific proliferation, differentiation, and morphogenesis events occur.
The heterochronic genes of *C. elegans* control developmental timing: mutations in these genes cause the skipping or reiteration of stage-specific post-embryonic events. *C. briggsae* possesses orthologs of the heterochronic genes, but their roles in development are not known. To begin to address the degree of developmental systems drift in the heterochronic pathway, we determined the phenotypes of the *C. briggsae* orthologs of the *C. elegans* heterochronic genes.
lin-14 is a well-studied heterochronic gene that exists only in the *Caenorhabditis* genus. *ce-lin-14* and *cbr-lin-14* mutants have identical heterochronic phenotypes characterized by skipping of L1-stage developmental events and precocious expression of later cell fates.
Several other *C. briggsae* heterochronic genes orthologs, including *cbr-lin-4, cbr-lin-46, cbr-let-7, and cbr-hbl-1*, all display mutant phenotypes that either closely resemble those of *C. elegans,* but differ in severity, or diverge by affecting events of different larval stages.
lin-28 is a heterochronic gene that is conserved in many animals, including arthropods and vertebrates. Among other activities, it acts in stem cells and blocks the maturation of the let-7 miRNA. It is often expressed early in a lineage and is downregulated as cells differentiate.
C. elegans mutants of *lin-28* skip L2-specific events and execute adult cell fates precociously. *cbr-lin-28* mutants have a very different overall phenotype: they arrest their development at the end of L4 stage and develop a disorganized gonad in adulthood.
In summary, the roles of several heterochronic genes differ between *C. elegans* and *C. briggsae*, but their involvement in the developmental timing and certain pairwise genetic relationships are preserved.

Generation of genetic resources for the nematode *Caenorhabditis briggsae*

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Caenorhabditis briggsae is an established model for comparative and evolutionary studies involving its well-known cousin *C. elegans*. With *C. briggsae* being used more frequently as a genetic system of its own, there is a need to develop resources and tools to facilitate rapid identification of genes and study of their function. This project focuses on characterizing differences between *C. briggsae* and *C. elegans* at morphological, physiological, and behavioral levels. As part of this systematic characterization, we performed experiments to quantify the morphology and various other characteristics of the animals. The results show that while *C. briggsae* is similar to *C. elegans* in many ways, there are significant differences between the two species. At the plate level, *C. briggsae* (*AF16*) adults are almost indistinguishable from *C. elegans* (*N2*). However, body measurements revealed that *C. briggsae* is slenderer and longer, and moves in a sinusoidal motion with lower amplitude compared to *C. elegans.* Oil Red O staining of body lipids revealed that *C. briggsae* hermaphrodites are comparatively more transparent. We also observed that *C. briggsae* has a higher incidence of spontaneous male generation.

Investigations of thermal tolerance of *C. briggsae* revealed that while reproductive span, brood size and life span decrease with increasing temperatures, the species shows a much higher resistance to heat stress compared to *C. elegans.*

In addition to the above characterization, we are working to create a genetic linkage map of *C. briggsae* by performing new experiments and consolidating available phenotypic and polymorphic mapping data. An integrated map will accelerate mapping of genes identified in forward genetic screens.

Evolution of *fem-1* activity in *Caenorhabditis*

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Hermaphrodite sex determination is shared by the *Caenorhabditis* species *C. elegans*, *C. briggsae*, and *C. tropicalis*, but the phylogeny shows that it evolved independently in each species. The core pathway that controls sex is largely conserved in this genus which includes the three FEM complex proteins: FEM-1, FEM-2, and FEM-3. These proteins were discovered in *C. elegans,* where the corresponding genes are necessary for male sex determination, since null mutants feminize the soma and germline of *XX* and *XO* animals. However, in *C. briggsae,* the *fem-2* and *fem-3* null mutants feminize the soma of *XX* and *XO* animals but do not affect hermaphrodite spermatogenesis. This difference suggests a divergence in the role of the FEM complex in these two species.

We isolated *C. tropicalis fem-1(v426ts)* by genetic screening and made the null allele *fem-1(v466)* using CRISPR/Cas9. These *fem-1* mutants closely resemble the corresponding *C. elegans* mutants. Both *XX* and *XO* animals develop as females, and there is a strong maternal rescue in null mutants. This result suggests that *C. briggsae* evolved its unique control of sex determination more recently.

To explore this possibility, we used CRISPR/Cas9 to make a *fem-1* null mutant in *C. briggsae,* to see if it behaved like alleles of *fem-2* and *fem-3* in that species. The mutation *fem-1(v508)* is an early frameshift allele. It does indeed show an identical phenotype to *Cbr-fem-2* and *fem-3* null mutants, since *XX* and *XO* animals are both fertile hermaphrodites. The identity of the *XO* hermaphrodites was verified by RT-PCR of *her-1* mRNA, and by using *unc-7 X* in genetic crosses. We conclude that the function of these three proteins in a single FEM complex has been conserved, but that the role of this complex in *C. briggsae* recently diverged from other *Caenorhabditis* species.

Double mutants of *fem-1* with mutations in upstream or downstream genes confirm its place in the *C. briggsae* sex determination pathway. As expected, *tra-1(v181); fem-1(v508)* mutants make *XX* males, whereas *tra-2(nm1); fem-1(v508)* mutants make *XO* hermaphrodites. Finally, we used CRISPR/Cas9 to make a *fem-2(nm27); fem-3(nm63) fem-1(v517)* strain. This triple mutant develops just like the individual *fem* mutants, so each protein regulates sex-determination only through its role in the FEM complex.

The ancestral relationship between the FEM proteins and germline development is complex, as shown by oogenesis in *C. elegans tra-1; fem* double mutants. We extended work begun by the Haag lab to study this relationship in *C. briggsae,* by scoring the germ lines of *tra-1(v181)* mutants with mutations in one or more *fem* genes. Each of the *fem* mutations promotes a switch from spermatogenesis to oogenesis in these *tra*-1 males. Thus, the *fem* genes might retain a residual downstream function in *C. briggsae* too. We are now building double and triple mutants in *C. elegans* to learn what this downstream function might involve.

The molecular genetics mediating gustatory preferences in *Pristionchus pacificus*

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Given that the sensory neuron count is highly constant among nematode species, identifying the genes that mediate species-specific changes in responses toward chemical compounds is an important first step in understanding how sensory preferences evolve. Guanylyl cyclase genes (*gcys*) are known to help detect water-soluble salts and are expressed in the amphid sensory neuron in the model organism *Caenorhabditis elegans*. To determine the role of the *gcy* family in the entomophilic nematode, *Pristionchus pacificus*, we profiled its responses to six salt cues and found major differences to *C. elegans*, such as contrasting responses to iodine and acetate salts.

In *C. elegans*, *gcy-22* is exclusively expressed asymmetrically in the ASER neuron and is known to have a broad effect on salt sensing. However, based on the lack of electrical coupling between the left and right ASE neurons and the absence of the regulator for L/R asymmetry, *lsy-6*, the ASE pair in *P. pacificus* is likely not lateralized in function. Furthermore, there are five *gcy-22* homologs in the *P. pacificus* genome; it is unclear which, if any, of these homologs mediate salt chemotaxis.

Using CRISPR/Cas9 editing system, we investigated the possible gustatory functions of a *gcy-22* homolog, *PPA34960*. We recovered two viable reduction-of-function alleles in *PPA34960* with early stops in the extracellular domain. Interestingly, *PPA34960* (*csu79)* has an enhanced response to all the tested salt cues, different from *Cel-gcy-22*'s broadly weakened ability to detect many salts. We are currently investigating the knockdown effects in other *gcy* genes in *P. pacificus*. In addition to the *gcy-22* homolog, we also examined the roles of genes with 1-1 homologs that are expressed in the amphid neurons known to be involved in salt chemosensation: *Ppa-che-1*, *Ppa-daf-11*, and *Ppa-tax-2*. Whereas loss-of-function mutations in *Ppa che-1* and *Ppa-daf-11* resulted in selective reduction of attraction towards specific salts, we found that *Ppa-tax-2* is necessary for detecting all the salts tested. The characterization of guanylyl cyclase receptors and other genes involved in salt sensing would contribute to understanding the molecular pathway of chemosensation in *P. pacificus* and other host-associated nematodes.

Comparative analysis of cellular dynamics of *C. inopinata* and *C. elegans* zygotes

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C. inopinata is the closest species to *C. elegans*, but these two species have various morphological and ecological differences. In this study, we compared the cellular dynamics of *C. inopinata* and *C. elegans* zygotes using the strains that express GFP::histone and GFP::*β* tubulin. We found differences in 1) the pronuclei dynamics, 2) the mitotic spindle dynamics, and 3) the diffusion pattern of the centrosomes during mitotic telophase. In *C. elegans* zygotes, female and male pronuclei form at the anterior and posterior ends, respectively, and migrate to the center to meet. In *C. inopinata* zygotes, pronuclei formed in various positions, and met at more posterior positions. The mitotic spindles of *C. elegans* form horizontally in the center of zygotes, while that of *C. inopinata* formed at a skewed angle and more anteriorly. While the spindles of most *Caenorhabditis* nematodes oscillate during anaphase, those in *C. inopinata* did not. Despite these differences, the relative position of the cleavage furrow was equivalent to each other. After the cell division, pericentriolar material (PCM) in *C. elegans* diffused rapidly due to the pulling force from the cortex, whereas PCM diffused slowly in *C. inopinata*. The lack of the spindle oscillation and the slow diffusion of PCM suggest that the microtubule-dependent pulling force might be weaker in *C. inopinata* zygotes. To identify the cause of the difference in cellular dynamics, RNAi was performed in *C. inopinata* on the genes known to affect spindle dynamics in *C. elegans*. In *C. elegans* zygotes, RNAi of *klp-7*, a microtubule depolymerization regulator, resulted in a skewed spindles formation at an abnormally anterior position, reminiscent to *C. inopinata* zygotes. In contrast, *klp-7* RNAi had no significant effect on spindle dynamics in *C. inopinata*. Thus, the differences in the microtubule stability may be, at least in part, responsible for the differences in cellular dynamics between *C. inopinata* and *C. elegans* zygotes.

WormAtlas: New Chapters, New Data, New Worms

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The well-characterized anatomy of *C. elegans* has propelled its use as a model organism. Initially characterized through extensive light and electron microscopy (EM) observations, these data continue to provide insight into *C. elegans* biology. The Center for *C. elegans* Anatomy is a hub for the presentation and interpretation of anatomical data through the WormAtlas and WormImage websites. WormAtlas comprises multiple resources designed to assist users in understanding the structure of *C. elegans* and, ultimately, in interpreting their own anatomical data. The WormAtlas handbook chapters include tissue-

specific descriptions of *C. elegans* anatomy. Originally, focused on the adult hermaphrodite, we have expanded our handbooks on dauer anatomy and structural changes that occur during ageing. Our literature section includes pdf and HTML versions of landmark anatomical papers, with a focus on older literature that may not be widely available. This section was recently expanded to include a greater range of literature and reorganized to simplify usage. We have also begun expanding our coverage of anatomy beyond *C. elegans* to other nematode species through the publication of a chapter on *Pristionchus pacificus* anatomy and the addition of a large *P. pacificus* EM data set to our companion WormImage website. In addition to our large collection of physical EM archives of *C. elegans*, mostly now available in digital form on WormImage, we have begun retrieving archival EM data sets of other species. These additional data include approximately 50,000 negatives and prints that are being digitized and annotated for future inclusion on WormImage. During the past year, WormAtlas and WormImage have logged over 263,000 pageviews from more than 48,000 users. Users have been recorded from 173 countries with the U.S. having the largest user base, followed by China and India. Future plans for WormAtlas include new handbook chapters on *C. elegans* organelles and other nematode species, as well as additional sections on tissue specific aging. We will also develop of individual glia webpages to complement our section featuring individual neurons and update WormImage to include 3D models of reference *C. elegans* EM datasets.

C. elegans Male Mobility, Recovery, and Mating After Therapeutic Ultrasound Exposure

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In a previous study, our lab showed that N2 adult hermaphrodites exposed to therapeutic ultrasound had dose-dependent reductions in mobility, fecundity, and survival. Those bioeffects were mainly due to cavitation (formation and violent collapse of gas-filled spaces in the worms' tissues or in the surrounding buffer). In the current study, we have asked how N2 males are affected by therapeutic ultrasound. Experiments were performed at an exposure level that was lethal for half of N2 adult hermaphrodites (1.0 W/cm^2, 1 MHz, 5 minutes at 50% duty). In preliminary mobility assays, N2 males seemed to be less sensitive to ultrasound than were N2 adult hermaphrodites. Writhing N2 males also seemed to be more likely to recover their mobility on agar and survive than were N2 adult hermaphrodites. However, males that recovered their mobility appeared to have a reduced mating index compared to unexposed controls. Further work will confirm these results and explore males' responses at other ultrasound intensities.

Genomic patterns of divergence of _Caenorhabditis brenneri_

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Caenorhabditis brenneri is an outcrossing species of nematodes in the 'Elegans' subgroup (*Rhabditida*; Sudhaus & Kiontke 2007), which it known to be one of the most genetically diverse eukaryotes, with nearly one in ten nucleotides being polymorphic, making its population diversity level comparable to that of bacteria (Dey et al. 2013). Prior to studying patterns of such enormous population diversity of *C. brenneri* on the genome level, we aimed to generate a high-quality chromosome-scale reference genome and estimate rates of evolution along the genome. We assembled a chromosome-scale reference genome for an inbred strain of *C. brenneri* (VX0223, that being created by 300 generations of inbreeding) using short and long reads and chromosome conformation capture data. We employed genomes of *C. doughertyi*, *C. wallacei*, *C. tropicalis*, and *C. elegans* for phylogenomic analysis and assessed the lengths of tree branches for *C. brenneri*, additionally we used pairwise genome alignments for some of these species. To explore the divergence pattern along *C. brenneri* genome, we performed the wavelet transformation, a signal processing technique used for the detection of spatial patterns by analyzing frequencies of the signal at multiple scales simultaneously (as in Spencer et al. 2006). In

particular, we were interested in differences among spatial patterns in regions of lower and higher recombination. This data may be utilized in the future to correlate with diversity levels at multiple scales and test hypotheses that involve both micro- and macroevolutionary processes.

Compensatory evolution in mitochondrial tRNAs in *Caenorhabditis* nematodes

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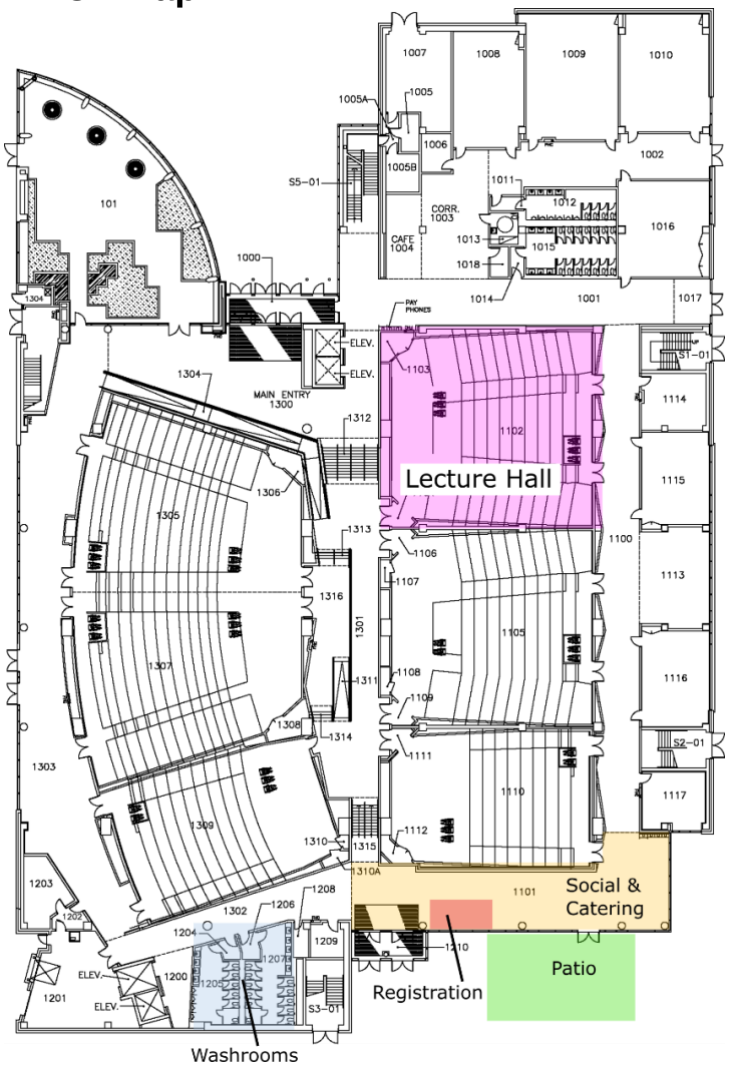
Deleterious mutations can accumulate in natural populations, as reversal mutations are likely to be rare and selection cannot always purge all low-fitness alleles. However, mutations at other sites can potentially compensate for genetic changes that would otherwise be deleterious, either by occurring first and "pre-compensating," or by occurring second and reversing fitness. Thus, interdependency among sites may facilitate evolutionary divergence, even in critical and highly constrained sequences. However, identifying such compensatory or conditional mutations is difficult without an *a priori* expectation of sequence function. In this project, we use the canonical structure-function relationship of the cloverleaf-like tRNA secondary structure, as well as the high mutation rate of tRNAs, to study compensatory evolution. We explore mutations in mitochondrial tRNAs (mt-tRNAs) in *Caenorhabditis* nematodes to identify patterns of compensatory evolution over short and intermediate evolutionary timescales, including interactions between mt-tRNAs and associated factors encoded in the nuclear genome.

Maps

Campus Map

McMaster campus map link : https://discover.mcmaster.ca/map/

MDCL Map



Travel Info

Closest bus stops to McMaster University
Sterling at University
Sterling at Life sciences
University at Forsyth
Sterling at Forsyth
Go bus Transit platform number 1 on Cootes drive
Main opposite Emerson-2 mins walk to McMaster by Hwy 8
Longwood at King take bus number 5/10 from this bus stop and get down at Sterling at University

How to reach McMaster by air
John C. Munro Hamilton International Airport (6.8 miles / 11.0 kilometers)

In Car/cab 16-22 minutes via ON-6 N
https://goo.gl/maps/tLCkn393jHttJC9Z8

Bus route
Bus number-20 from Airport passenger terminal-get down at John at King William
walk 2 mins to King at John
Take bus number 10 from King at John and get down at Main opposite Emerson, walk 2 mins to reach Mcmaster university
https://goo.gl/maps/dgoBUXzxWnXsT2mr6
Toronto Pearson International Airport (31.5 miles / 50.7 kilometers)

In Car/Cab 40 minutes
https://goo.gl/maps/Ca1gR7VP4NdywnYv6

Bus Route
Bus number 40 from Pearson Airport Terminal 1, get down at Main at Longwood, take bus number 10 from Main at Longwood and get down at Main opposite Emerson, walk 2 mins to McMaster University.
https://goo.gl/maps/akMpKYe4eDm7T81SA

How to reach Mcmaster from Hamilton Go Bus centre
https://goo.gl/maps/CdSFK66vQ9TuWziv6
Bus numbers- 1A get down at university at Sterling
5C get down at university at Sterling
5 Get down at Main opposite Emerson

Cab services other than Lyft and Uber
Hamiton cab service-(905)-777-7777
Blue line transportation-(905) 525-0000
Hamilton airport taxi service-(289) 925-2629

Links for places to visit in Hamilton
https://www.planetware.com/canada/top-rated-things-to-do-in-hamilton-ontario-cdn-1-265.htm

Recommended food & drink venues

Food

Near Campus

1. Saigon
   Location: 1024 King Street West. Westdale neighbourhood. A 12 minute walk.
   Opens: 11 AM – 9 PM
   Prices: $
   East-Asian cuisine.

2. Nannaa
   Location: 1010 King Street West. Westdale neighbourhood. A 13 minute walk.
   Opens: 11:30 AM – 8:30 PM
   Prices: $$
   Persian cuisine.

3. Valentino's
   Location: 824 King Street West. Westdale neighbourhood. Not walking distance.
   Opens: 11 AM – 9 PM
   Prices: $$
   Italian cuisine.

4. Royal Spice
   Location: 1685 Main Street West. Towards Dundas. Not walking distance.
   Opens: 11:30 AM – 11 PM
   Prices: $
   Indian and Hakka Chinese cuisine.

Dundas

1. Quatrefoil
   Location: 16 Sydenham Street
   Opens: 4 – 11 PM, Wed – Sat
   Prices: $$$$
   French cuisine.

2. Betula
   Location: 225 King Street West
   Opens: 12 – 9 PM
   Prices: $$
   Seasonal cuisine.

3. India Village
   Location: 100 King Street West
   Opens: 3 – 9 PM
   Prices: $$
   Indian cuisine.

Downtown

1. Brux House
   Location: 137 Locke Street South
   Opens: 4 – 10 PM
   Prices: $$$
   Gastropub with European-inspired menu. Fun fact: Justin Trudeau went here recently.

2. Earth to Table: Bread Bar
   Location: 258 Locke Street South
   Opens: 9 AM – 9 PM
   Prices: $$
   Restaurant with locally sourced ingredients.

3. Bon Temps
   Location: 61 Young Street
   Opens: 4 – 10 PM
   Prices: $$$
   French cuisine.

4. Shakespeare's Steak and Seafood
   Location: 181 Main Street West
   Opens: 5 – 9:30 PM, Wed-Sat
   Prices: $$$
   Steak and Seafood.

5. Aberdeen Tavern
   Location: 432 Aberdeen Avenue
   Opens: 4 – 9 PM
   Prices: $$$
   Canadian cuisine.

Coffee

Near campus

1. Williams Café
   Location: 1309 Main Street West. Walking distance.
   Opens: 8 AM – 10 PM
   Prices: $
   Coffee, breakfast and sandwiches.

2. Starbucks
   Location: 1341 Main Street West, at large intersection. Walking distance.
   Opens: 6 AM – 9 PM
   Prices: $$
   Coffee. No surprise there.

Dundas

1. Detour Café
   Location: 41 King Street West
   Opens: 8 AM – 5 PM
   Prices: $$
   Artisan Coffee.

Downtown

1. Tim Hortons
   Location: everywhere
   Opens: 5 AM – 11 PM
   Prices: $
   Coffee.

2. Democracy*
   Location: 202 Locke Street South
   Opens: 7 AM – 9 PM
   Prices: $$
   Coffee and vegan food.

3. Café Oranje
   Location: 312 King Street East
   Opens: 8 AM – 4 PM
   Prices: $$
   Dutch-inspired coffee shop.

Drinks of the intoxicating kind

Near campus

1. Phoenix Bar & Grill
   Location: On campus. Walking distance.
   Opens: 11:30 AM – 10 PM
   Prices: $
   The university pub! Seating inside and patio.

2. Grain & Grit Brewery
   Location: 11 Ewen Road. West of campus, towards Dundas.
   Opens: 12 – 9 PM, Tue – Sun.
   Prices: $$
   Local beer brewery. Seating inside and patio.

3. Fairweather Brewing Company
   Location: 5 Ofield Road. West of campus, towards Dundas.
   Opens: 12 – 9 PM, Tue – Sun.
   Prices: $$
   Local beer brewery. Seating inside and patio.

4. Snooty Fox
   Location: 1011 King Street West. Westdale neighbourhood. A 13 minute walk.
   Opens: 12 PM – 12 AM. Mon – Sun.
   Prices: $$
   Bar. Seating inside.

Dundas

1. Winchester Arms
   Location: 120 King Street West
   Opens: 12 – 9 PM. Tue – Sun.
   Prices: $$
   Pub.

Downtown

1. The Brain Bar
   Location: 199 James Street North
   Opens: 12 PM – 2 AM. Mon – Sun.
   Prices: $$
   Cocktail bar. Seating inside and patio.

2. Bar Sazerac
   Location: 278 James Street North
   Opens: 4 PM – 2 AM. Mon – Sun.
   Prices: $$
   Cocktail bar. Seating inside and patio.

3. The Ship
   Location: 23 Augusta Street
   Opens: 12 – 11 PM. Mon – Sun.
   Prices: $$
   Bar. Seating inside and patio.

Conference Code of Conduct

The organizers value your attendance and want to make your experience productive and inspiring by fostering an open exchange of ideas in a professional setting. Our Code of Conduct was established to communicate a transparent set of standards and guidelines for acceptable behavior at the conference and to provide a positive, safe, and welcoming environment for all attendees.

All conference participants are expected to follow the Code of Conduct while attending any portion of the conference, including but not limited to talks and poster sessions, both in-person and virtual, and all conference Slack channels. These types of communications include Zoom chat, Zoom Q&A window, poster Q&A, Slack, email, social media, and texts.

Unacceptable Behaviors
Unacceptable behaviors include, but are not limited to:

- Intimidating, harassing, abusive, discriminatory, derogatory, or demeaning speech or actions by any participant and at all related events
- Harmful or prejudicial verbal or written comments or visual images related to gender, gender expression, gender identity, marital status, sexual orientation, race, religion, political orientation, socioeconomic, disability or ability status, or other personal characteristics, including those protected by law
- Inappropriate use of nudity and/or sexual images (including presentation slides, posters, Slack channels, or Zoom chat)
- Deliberate intimidation or stalking
- Violating the rules and regulations of the online provider, Zoom
- Sustained disruption of scientific sessions or other events
- Unwelcome and uninvited attention or contact
- Real or implied threat of physical harm
- Real or implied threat of professional or financial damage or harm
- Photographing or reproducing slides of oral presentations and posters without permission
- Recording of scientific and other sessions without permission

Taking action or making a report
Need to file a complaint? Please email one of the organizers (Annalise Paaby, Bhagwati Gupta, Christian Braendle, Te-Wen Lo).

Consequences of non-compliance
Anyone asked by staff, organizers, session chairs or presenters to stop unacceptable behavior is expected to comply immediately. Retaliation toward the aforementioned or toward someone reporting an incident or after experiencing any of the following consequences will not be tolerated and may result in additional sanctions.
The consequences of non-compliance with the Code of Conduct may include:

- Immediate removal from in-person meeting
- Immediate removal from accessing the online meeting
- Immediate removal from Slack channels and meeting app without warning
- Restrictions from future meeting attendance
- Incidents may be reported to the proper authorities

Diversity and Inclusion
We are committed to promoting equality, diversity, and inclusion to create greater opportunity for any individual to fulfill their scientific potential, irrespective of their background, gender, or circumstances. This diversity leads to innovation by attracting the widest possible talent to the community and fostering a greater diversity of ideas, approaches, and perspectives. The Organizing Committee aims

to select speakers and session chairs that represent the breadth and diversity of the discipline and conference participants.

Social Media/Photo/Video Policy
Attendees are permitted to live tweet during presentations, unless the speaker explicitly opts out by stating so at the start of their talk. Taking or sharing photos, videos, or reproductions of posters is not permitted unless you have the presenter's consent.

Posters
When you view poster materials at the conference, whether in person or via Slack, remember that posters are typically works in progress. Please do not cite or reproduce any part of posters without the presenter's permission.

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