The Caenorhabditis Genetics Center (CGC) & *Caenorhabditis elegans* Natural Diversity Resource (CeNDR)

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Introduction to *C. elegans* as a model organism

Caenorhabditis elegans is a small, free-living nematode commonly found feeding on bacteria in decaying organic matter, such as compost piles or rotting fruits (Félix, 2010). Caenorhabditis species, including *C. elegans*, were studied well before 1963 when Sydney Brenner turned to nematodes in his quest to "tame a small metazoan organism to study development directly" (Brenner, 1988), but it was the efforts of his laboratory and descendents who shaped modern biology with numerous discoveries that truly set the stage for the role of *C. elegans* as a premier model organism.

Concerned that the "classical problems of molecular biology have either been solved or will be solved in the next decade," Brenner shrewdly predicted that the future of biology lie in the molecular biology of development, and in particular, the nervous system. Coming from a background in microbiology, he believed the nematode offered advantages similar to microbial systems due to its small size, short life cycle, and ease of cultivation in the lab (Figure 1A&B; Brenner 1988). Although Brenner initially proposed *Caenorhabditis briggsae* as the ideal model system in which to examine developmental processes, he later settled on the closely related species *Caenorhabditis elegans* because it was easier to cultivate in his laboratory (Félix 2008).

By the mid-1970s, Brenner's focus expanded beyond neuronal development into the developmental biology of the worm, setting up *C. elegans* to become a premier model organism. In his seminal paper (Genetics, 1974), he laid the foundation of *C. elegans* as a model system for elucidating molecular regulation of development and emphasized the power of genetic analysis that was possible. In the years since that initial paper, researchers throughout the world have exploited the worm's essentially invariant cell lineage, simple anatomy, and transparent body, cementing the role of *C. elegans* as a model system ideally suited for dissecting genetic pathways controlling developmental processes from egg to adulthood.

C. elegans offers many advantages as an animal model system. The worms are small enough (0.25 mm long at hatching to ~1 mm long as adults) to be grown on agar-filled petri dishes, yet large enough to be easily observed under a dissecting microscope. Perhaps most critical from the standpoint of a stock center, *C. elegans* is amenable to cryogenic storage, which allows the collection of strains to be preserved indefinitely, reducing genetic drift that occurs while maintaining actively growing stocks over generations. The worms develop externally and are transparent, allowing observation of developmental events throughout an animal's

entire life history (Figure 1A). Dr. Jonathan Sulston and his collaborators did exactly that, mapping out every cell division and revealing that the cell lineage is essentially invariant (Sulston and Horvitz, 1977; Kimble and Hirsch, 1979; Sulston et al., 1983). This cellular blueprint further enhanced the genetic power of the worm, as researchers can identify developmental defects in mutants by following cell lineages to identify precisely when and where cell divisions go awry. Advances in microscopy, microfluidics, and computing technology can now allow 4D (3D time-lapse) imaging of cells during development, automating the process of tracking cell divisions, even during larval stages (Dutta, 2105; Zacharias, 2016; Keil, 2017).

C. elegans is an androdioecious (hermaphrodite-male) species with a short generation time, maturing from embryos to adults in about three days (Figure 1A). A single hermaphrodite typically produces ~300 progeny through self-fertilization and can quickly give rise to a hearty population (Wood, 1988). Under typical laboratory culture conditions, a healthy hermaphrodite with ample food will have a lifespan of several weeks. However, stressful conditions such as crowding, starvation, or high temperature, can induce *C. elegans* larvae to enter an alternate developmental state known as dauer diapause (Cassada and Russell, 1975; Golden and Riddle, 1984; Antebi et al., 1998). Dauer larvae form a specialized cuticle that protects them from environmental stresses, including desiccation, and allows them to survive for several months. When conditions become more favorable, dauer larvae will resume development and mature much as they would if conditions had always been tolerable.

Working with *C. elegans* in a lab often requires little more than a basic dissection microscope with which to observe worms and a worm “pick” (an implement somewhat akin to a bacterial loop) used to manipulate worms (Figure 2A-B). Worms can be grown at a wide range of temperatures from roughly 15°C to 25°C, meaning room temperature is often sufficient for maintaining stocks. The rate of development is influenced by temperature (it is faster at warmer temperatures), providing researchers some control over the timing of development by altering ambient temperature. Worms are usually grown on a standardized agar nutrient medium (nematode growth medium, or NGM) supplemented with a lawn of OP50, an auxotrophic strain of *E. coli* selected because it forms a thin, translucent lawn in which worms are still visible (Figure 2B). Alternatively, large populations of worms can be grown in liquid culture to facilitate biochemical work. Synchronized populations can be easily established through several means, including selection of embryos using hypochlorite (the eggshell is resistant to bleach), selection of dauer larvae with SDS (dauer cuticles are

resistant to the detergent), or even automated sorting by size or fluorescent protein expression in flow-based large particle sorters (Stiernagle 2006; Pulak, 2006).

C. elegans was the first animal to have its DNA completely sequenced, revealing a relatively compact genome of ~100 Mb containing ~20,500 protein-coding genes (The *C. elegans* Sequencing Consortium, 1998; Hillier, 2005), paving the way for molecular analysis, bioinformatics, and reverse genetics. The use of RNA interference (RNAi) – a molecular phenomenon first identified in *C. elegans* (Fire, 1998) – to knock down gene function in animals is trivial in *C. elegans*, facilitating high-throughput screening for genes regulating developmental processes of interest to a user. RNAi can be administered in worms through injection of double-stranded RNA (dsRNA), feeding the worms bacteria expressing the dsRNA, or even soaking the worms in a solution of dsRNA (Tabara, 1998; Timmons, 1998, Timmons, 2001). More recently, genome engineering using CRISPR/Cas9 to edit endogenous gene function has taken reverse genetics to the next level, allow researchers to not only knock-out gene function, but to make specific modifications to endogenous genes, mimicking known disease models or dissecting loci to identify key regulatory elements or functional domains, for example (reviewed in Dickinson and Goldstein, 2016).

In 2000, the establishment of WormBase (wormbase.org), a founding member of the Alliance of Genome Resources (alliancegenome.org), fueled the revolution in worm research that had begun with the collection and dissemination of systematic genetic, bibliographic and nomenclature data by the CGC. Composed of an international consortium of biologists and genomic specialists, WormBase provides the research community with an easy to use centralized online compendium of freely available, up-to-date information covering all things worm: the genetics, genomics, and general biology of *C. elegans* and related nematodes, the pedigree of Caenorhabditis labs tracking the academic history of past and present lab members, as well as maintaining a comprehensive bibliography of *C. elegans* publications and meeting abstracts (Lee, 2018). WormBase is nicely complemented by WormAtlas (http://www.wormatlas.org/), an online database featuring behavioral and structural anatomy of *C. elegans,* which includes numerous detailed drawings and high-resolution micrographs.

Thousands of research laboratories use *C. elegans* as the primary system in their basic and applied research, and educators world-wide use the nematode to demonstrate genetic and molecular biology techniques in

collegiate teaching labs as many mutant phenotypes are obvious and easy for even novices to score. Additionally, *C. elegans* is not parasitic or harmful to humans, making it safe for students with basic knowledge of lab safety. These traits make *C. elegans* ideal for hands-on science education in high school classes and student research projects.

Research on this premier model organism has led to fundamental insights into basic biological mechanisms, including the genetic basis of programmed cell death and cell signaling, the discovery of microRNAs, and the identification and subsequent elucidation of the mechanism of RNA interference in animals (Horvitz, 1999; Lee, 1993; Fire, 1998). The nematode has also proved important for understanding mechanisms of cancer progression and other diseases including Alzheimer's and Parkinson's, as well as for revealing basic mechanisms underlying human development (Ewald, 2010; Johnson, 2010). In addition, *C. elegans* serves as a key model for broadening our understanding of parasitic nematodes, and was used to identify the mode of action of the first new livestock anthelmintic brought to market in twenty-five years (Kaminsky, 2008; Waterman, 2010). Researchers in all of these areas, and myriad others, depend upon the availability of strains critical for their work.

By the mid-1970s, leaders in the field had already recognized that worm strains were important reagents, and the sharing of stocks among labs would greatly benefit the entire community (R. Herman, personal communication). They created a central repository for key strains, thereby protecting them from loss and making them freely available to all members of the *C. elegans* community: the Caenorhabditis Genetics Center (CGC) was born.

A Brief History of the CGC

The *C. elegans* community was founded with an attitude of cooperation and sharing of research tools among researchers, and the CGC was built on these principles. The CGC was established to promote research on the nematode *C. elegans* by acquiring, maintaining, and distributing genetically characterized nematode stocks upon request, providing the community of researchers and educators around the globe with easy access to *C. elegans* and related strains. CGC services enhance research progress by making key strains carrying mutations and transgenic lines readily available, allowing researchers to dedicate time and reagents to novel research efforts. Distribution of strains from a centralized location removes the burden on individual research labs to

distribute popular published strains requested by other researchers, and in some instances, relieving the tension and potential conflict of requesting strains from, or providing strains to, competing labs.

CGC operations began in 1979 at the University of Missouri under the direction of Dr. Donald Riddle. Strains were sent out in response to written requests from researchers, and thanks to the financial backing from the National Institutes of Health (NIH), strains were distributed free of charge to academic researchers. A mere fifteen strains were distributed in that first year, but demand for strains grew slowly and steadily over the next decade as the field began to mature (Figure 3), reaching nearly 1,400 strains in its final year at Missouri before Dr. Riddle transferred Directorship of the CGC to Dr. Robert Herman on June 1, 1992. Mark Edgley, the highly efficient curator of the CGC under Dr. Riddle, loaded liquid nitrogen tanks containing frozen stocks of more than 1,600 strains into a rented moving van, and heroically drove them 500 miles to personally deliver the strain collection to Dr. Herman and its new home at the University of Minnesota's St. Paul Campus. In 2003, the collection made a much shorter trip, moving to its current home on the U of M's Minneapolis (East Bank) Campus. Dr. Herman oversaw CGC activities for the next fifteen years, a period of rapid growth in the CGC and worm community overall (Figure 4 & 5), until his retirement in 2007. Demand for strains continued to climb steadily, each year setting another new record high number of strains distributed and orders fulfilled by the CGC.

Dr. Ann Rougvie assumed directorship of the CGC on June 1, 2007, at which time the NIH mandated implementation of a cost recovery scheme as part of the CGC funding mechanism. This change in funding strategy meant that for the first time, academic users were required to pay an annual lab fee (essentially a one-year subscription for CGC services) in addition to a nominal fee per strain ordered, ending twenty-eight years of free distribution to researchers. An audible gasp from the crowd in attendance was heard when the change in policy was announced at the International Worm Meeting that year, followed by the first ever drop in strain distribution (Figure 4). The community quickly adjusted to the new policy, and the demand for strains resumed its steady climb, hitting an all-time high of 31,242 strains shipped by the CGC in 2013. Demand has remained steady since then, leveling off around 30,000 strains distributed annually, with approximately 115 strains shipped each business day. The single day record was set on January 20, 2009, when a staggering 619 strains were shipped.

The number of orders processed annually has followed a similar trend, averaging roughly 7,500 requests in recent years (Figure 4). The historic average is around four strains requested per order, a figure that has remained fairly constant from year to year. The largest single order processed was for 3,894 strains in 2009. Over 90% of the strains in the collection have been distributed at least once; a remarkable figure considering several large collections have been added to the catalog in recent years. More than three quarters of the strains in the collection have been requested within the last four years, showing continued demand for older strains as well.

After nearly forty years, the CGC continues to be the sole general stock center collecting strains from research labs and distributing them within the worldwide community of Caenorhabditis researchers. The CGC continuously works to meet the rising demand of the community while maintaining its high standards with a small but efficient staff. For many years only the director and a curator, who single-handedly performed all tasks necessary for daily operation, staffed the CGC. Additional technicians and undergraduate support staff were added as the collection was expanded and demand for strains grew. Presently, the CGC staff is composed of the director, the head curator Dr. Aric Daul, four scientists, and undergraduate support staff, with administrative support provided by the University of Minnesota Accounts Receivable Office and the Department of Genetics, Cell Biology & Development.

Diversity of species in the collection

Though *C. elegans* is by far the most widely studied Caenorhabditis species (more than 98% of the >20,700 genetically distinct strains available in the CGC catalog are *C. elegans*), more than 50 species comprise the genus, and nearly forty of these are represented in the CGC. *C. briggsae,* the second-most popular by strain count, is represented by only ~100 strains in the CGC collection, and most other species are represented by a few isolates. All told, the CGC collection is composed of around 50 different species of Caenorhabditis and related genera. Most species in the clade are gonochristic (female-male), but the two most commonly used species – *C. elegans* and *C. briggsae* – are androdioecious (Haag, 2005). From the standpoint of the stock center, this provides a significant advantage as most *C. elegans* and *C. briggsae* stocks can be maintained as hermaphrodites, eliminating the need for intersex matings to propagate lines. Though primarily a

hermaphroditic species, males can be found in most populations of *C. elegans* and can arise spontaneously from chromosomal nondisjunction of the X chromosome during meiosis. Sex in *C. elegans* is determined by the ratio of autosomes to X chromosomes: hermaphrodites carry two copies of the X chromosome (XX), whereas males carry a single copy (XO) (Hodgkin, 1987). Males are significantly different from hermaphrodites in morphology and behavior; some anatomical differences can be observed in early development, though most become more apparent in later larval stages when the asymmetric gonad and distinctive tail fan (a specialized copulatory structure) are formed (Figure 1C-E).

Technological breakthroughs in whole-genome sequencing have led to newfound appreciation for understanding how genetic variation can affect biological processes. The identification, preservation, and distribution of wild isolates (isolated from nature) are critical for research in comparative genomics, evolutionary genetics, statistical genetics, and other fields utilizing whole-genome analyses. Although a few wild isolates are maintained in the CGC collection, detailed curation of the stocks and their genomic information is beyond the scope of CGC operations. To meet the needs of a growing number of investigators interested in incorporating studies of natural variation across wild populations and time into their work, Dr. Erik Andersen (Northwestern University) spearheaded the establishment of the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR). CeNDR is a resource center that complements the role of the CGC by specializing in the curation, maintenance, and distribution of wild isolates of *C. elegans* and their whole-genome sequences. By focusing on natural isolates, CeNDR is able to maintain these stocks in ways that avoid genetic drift or behavioral changes in response to laboratory conditions, thereby easing the burden on the CGC and ensuring that these key resources are available to evolutionary genetics researchers well into the future.

CeNDR: Managing wild isolates and their genomes

The overwhelming success of *C. elegans* as a model system results from the use of N2, a single, laboratory-adapted strain from Bristol, England (Sterken, 2015) as the standard wild-type strain throughout the community. This standardized genetic background has enabled fine-scale analyses of experimental perturbations without the worry of divergent genetic backgrounds between labs, as is often found in other model organisms. The flipside of this, however, is that because differences between populations are caused by allelic variation, the *C. elegans* community has many more discoveries yet to be made by studying the natural

diversity present within this species (Braendle, 2009; Felix, 2010; Frezal, 2015; Nigon, 2017; Sterken, 2015). To address this gap in the community's experimental toolkit, a large population of wild strains has been collected from nature by *C. elegans* researchers and citizen scientists worldwide (Figure 6) (Andersen, 2012; Cook, 2016). These strains provide a reservoir of natural genetic variation that can be used to understand genetic drivers of evolutionary processes and identify the underlying molecular mechanisms for traits relevant to genetic differences among individuals in a population. CeNDR was created to maintain a comprehensive database on this collection of strains and provide a set of tools for examining natural variation in *C. elegans* wild strains to perform genome-wide association (GWA) mappings (Cook, 2017; Zdraljevic, 2017). Since its release in fall of 2016, CeNDR has had over 45,000 unique visitors investigating one of the three main areas of focus. This overwhelmingly positive response and growth of CeNDR has moved studies of *C. elegans* natural variation to many laboratories across the community.

CeNDR provides a specialized platform for collecting, distributing, and maintaining strains isolated from nature (www.elegansvariation.org). Currently, CeNDR contains 766 wild strains that make up 330 distinct isotypes (isotypes are genome-wide haplotypes that are shared across strains from the same isolation location, as explained more below). *C. elegans* researchers or citizen scientists can donate wild strains to CeNDR through a web form. Following the receipt of new strains, a single hermaphrodite animal is propagated to ensure that the genotype is genetically distinct from a potentially heterogeneous wild population.

CeNDR collects information on each strain, including its isolation location, date of collection, substrate where nematodes were found, elevation, etc. These data are integrated into the CeNDR database and can be browsed via a geographic interface on the website. This dataset is also available for download or accessible through an application programming interface (API). After isolation and propagation of the strains, the population is split to cryopreserve animals for long-term storage and to isolate DNA for whole-genome sequencing. This step ensures that the genotype information obtained from whole-genome sequencing can be connected directly back to a specific strain. Sample mix-ups and strain contamination are possible when managing many strains and samples, especially because most wild strains look phenotypically identical. However, CeNDR's ability to retain frozen stocks allows verification of the genetic identity of strains should the need arise. CeNDR checks all frozen samples by low-coverage whole-genome sequencing to ensure that genotypes are as reported. If strain fidelity issues are discovered, as has been observed with other wild strains

in the past (Andersen, 2012; McGrath, 2009), stains are recovered from long-term cryopreserved stocks in liquid nitrogen.

Additionally, this data check and cryopreservation improve the data fidelity for downstream genome-wide association (GWA) mappings. Passaging of strains drives evolution and adaptation to laboratory conditions, so CeNDR strains are never passaged more than three times in a given year to reduce these effects.

CeNDR provides strains to the community in three different ways. First, individual strains are sent on agar NGM plates when individual strains are requested. Second, a collection of 12 divergent wild strains are sent for users to optimize quantitative trait assays. These strains are sent cryopreserved in strips of 12-well tubes. Third, six unique collections of 48 wild strains are sent for users to perform species-wide quantitative trait assays. Again, these strains are sent cryopreserved but in 48-well formats. Over 2,000 wild strains have been sent to the community since CeNDR was announced in the fall of 2016.

CeNDR offers whole-genome sequence and variant data for all isotype reference strains, along with metadata on gene conservation and functional studies. Because most reproduction in *C. elegans* occurs through self-fertilization by hermaphrodites, most natural substrates are colonized by genetically clonal individuals. Sometimes, genetically distinct strains are found in the same isolation location. Therefore, the concordance of genetic variation among strains is examined and whole-genome sequence data for identical or nearly identical strains is combined into isotypes, which represent genetically distinct genome-wide haplotypes from the same isolation location. The strain set for future experiments comprises a single representative strain (called the isotype reference strain) from each isotype set. By combining sequence coverage of all strains within an isotype, CeNDR obtains high-coverage sequence data that are aligned and used to perform variant calling. CeNDR sequences all wild strains to a minimum of 30-fold depth of coverage, a level that facilitates reliable calling of single-nucleotide variants (SNVs) along with other classes of genomic variants. All variant data are available through an API or can be downloaded in tab-delimited format or Variant Call Format (VCF) files, and aligned sequence data is available in binary alignment map (BAM) format. For users familiar with genomic applications, these file types and data enable individual studies beyond just looking at single-nucleotide variation. Additionally, CeNDR has developed a genome browser for querying and visualizing genetic variation across the *C. elegans* species, allowing users to toggle different tracks that detail genomic information

including genes, SNVs identified in individual strains, variant effects predicted with SnpEff (Cingolani, 2012), raw sequence reads from the BAM files, and conservation scores across nematode species (*e.g.*- phyloP and phastCons). This functionality gives users the ability to query their favorite gene and discover whether natural variation might affect its function, suggesting future phenotypic studies if these alleles have interesting mutational profiles (gain-of-function or reduction-of-function). Importantly, these studies set the context for what *C. elegans* does in nature. The laboratory environment is drastically different than the nature niche, and the roles of genes and pathways might be very different than the standard laboratory-adapted N2 genetic background.

CeNDR's GWA mapping process is optimized for *C. elegans* and combines whole-genome genotype data with measurements of quantitative traits to perform association mappings, an approach that has been used successfully in many studies (Cook, 2016; Zdraljevic, 2017). So far, approximately 3,100 unique GWA mappings have been performed on CeNDR. The GWA mapping portal is designed for non-experts and has several user-defined options along with drag-and-drop capabilities. Multiple traits can be submitted simultaneously and organized within an easy-to-read report. Within these reports, users are presented with figures, tables, interactive elements, and data in a tab-delimited format. Once a quantitative trait locus from GWA mapping is identified, CeNDR provides tools to browse the genes and potential functional connections underlying that genomic region, including integrated functional studies based on RNA interference (RNAi) screens and biochemical pathway predictions. Lastly, system features enable CeNDR to interact with other services (Wormbase and the CGC) and allow access to the underlying databases through an API, which can be used to query, among other things, genetic variants, strain information, mapping report data, and *C. elegans* genes and homologs.

Over the next few years, CeNDR expects to incorporate over 1,000 wild *C. elegans* strains and associated whole-genome sequence data. These strains come from many different locations across the globe, but collections from the Pacific Rim are encouraged, as this region harbors the highest genetic diversity in the species (Andersen, 2012; Cook, 2016; Zdraljevic, 2017). GWA mappings will be enhanced by the addition of two new features. CeNDR will update the genetic markers used for GWA mappings to better define genomic regions and make better predictions of haplotype structures. Most importantly, CeNDR hopes to expand to

wild strains from two related androdioecious Caenorhabditis species, *C. briggsae* and *C. tropicalis*. Analyses of these two species will enable powerful comparative approaches of natural variation across the genus.

Nomenclature in *C. elegans*

C. elegans has had a strict set of rules regulating system nomenclature in place since its introduction as a model system (Horvitz,1979; Tuli, 2018). Drawing from annotations used in the bacterial, yeast, and fly communities, the founders of the worm community established a system of standardized nomenclature ensuring that worm strains and alleles are named in a manner that makes the unique identity and source of each strain unambiguous. Each laboratory dedicated to long-term use of *C. elegans* is assigned a set of unique alphabetic laboratory codes by WormBase (genenames@wormbase.org) and registered with the CGC for naming new strains and alleles generated in that lab. A lab code is assigned to the principal investigator (PI) of each lab because they are ultimately responsible for maintaining records of named strains and alleles in their lab and ensuring their strains are accurately described in publications, on WormBase, and when distributed to the scientific community, CGC, or CeNDR.

A PI's lab code also serves as a unique identifier for the CGC to use for tracking the lab's account, including the lab's location and billing information, their history of strains received from the stock center, strains they have contributed to the CGC collection, and so forth. Once assigned, a code is permanently affiliated with that researcher. If a lab relocates to different institution, the lab code moves with the PI to preserve the historical records of strains and alleles generated, as well as their order history at the CGC. Codes are also not reassigned to other groups if a lab ceases to work on *C. elegans*, for example, due to retirement or a change of direction in their research. A post-doc should not plan to take over their mentor's code; they will receive their own code when establishing their lab or research group (for many new PIs, being assigned their lab code is a rite of passage).

A strain is defined as a set of individuals of a particular genotype with the capacity to produce more individuals of the same genotype. Each strain should be assigned a unique name consisting of the originating lab's code (two or three non-italicized uppercase letters) followed by a number without a hyphen. Lists of lab codes and corresponding PIs are available on both the CGC website (cgc.umn.edu) and WormBase, allowing users to quickly determine that the strain RG559 originated in the Rougvie Lab (University of Minnesota), and CL2006 in

the lab of Dr. Chris Link (University of Colorado), for example. The numerical portion of a strain name is assigned by each laboratory, typically sequentially, and numbers should never be reused or reassigned to a different strain. Any modification of an existing strain (such as out-crossing) that makes a stock distinct from the parental strain requires the newly-derived strain be assigned its own strain name. Labs maintaining bacterial strains used in conjunction with the worm work may also assign names to these strains using their strain designation followed by "b" to distinguish bacterial stocks from nematode strains. A small collection of such bacterial strains are maintained and distributed by the CGC.

In *C. elegans*, genes are given names consisting of three or four italicized letters, a hyphen, and an Arabic number, as in the example *hbl-1*. Assignment of new gene names is tightly regulated to prevent different groups from using the same name to describe more than one gene or using different names to describe the same gene, and requires approval from a senior member of the worm community who acts as the registrar of gene names. Requests for new gene names or species prefixes should be directed to genenames@wormbase.org. Dr. Robert Herman regulated genetic nomenclature for a brief period in the late 1970s until the founding of the CGC in 1979. For the next thirteen years, Don Riddle and Mark Edgley oversaw genetic nomenclature as well as the strain curation while the CGC was housed at the University of Missouri. When CGC operations moved to the University of Minnesota in 1992, administration of gene names and nomenclature was transferred to Dr. Jonathan Hodgkin (Oxford University), who retained the role through 2013, an era spanning multiple landmark events that reshaped the worm community, notably the complete sequencing of the *C. elegans* genome in 1999, and the establishment of WormBase in 2000. In 2013, Dr. Hodgkin handed responsibilities to Dr. Tim Schedl (Washington University), who currently gives final approval of new gene names.

Gene names may be followed by an italicized Roman numeral indicating the linkage group (chromosome) on which it resides (I, II, III, IV, V, or X). The genotype of an animal is specified by listing relevant known differences between its genotype and wild-type, which to date has been defined by convention as the Bristol N2 strain. When necessary, a standardized three-letter prefix and hyphen can be added preceding the gene name to specify the nematode species of origin (for example, the orthologs of *C. elegans tra-1* in *C. briggsae* and *Pristionchus pacificus* can be annotated as *Cbr-tra-1* and *Ppa-tra-1*, respectively).

When a principal investigator is assigned their alphabetic lab code for naming strains, they typically also receive a second alphabetic designation, or allele code, for naming mutations and other genetic variations generated in their laboratory. Each allele is assigned a unique name consisting of the originating lab's allele code (one to three italicized lowercase letters) followed by an italicized Arabic number without spaces or hyphens, for example *ve18* or *ma298*. In these examples, the "*ve*" indicates the allele *ve18* was generated in the Rougvie Lab (University of Minnesota), and the "*ma*" indicates that *ma298* originated in the lab of Victor Ambros (University of Massachusetts Medical School). When gene and mutant allele names are used together, the mutation name is included in parentheses after the gene name, e.g., *hbl-1(ve18*), or *mir-795(ma298)*. These designations allow the community to easily identify the source of alleles, including those in strains carrying combinations of mutations that were each originally generated in different labs, making it straightforward for researchers and curators to contact the appropriate lab should questions arise.

Other genetic modifications are annotated in a similar manner, but include an additional 1-2 letter designation following the lab's allele code to identify the type of change. Currently recognized modifications include: partial duplication of chromosomes (*Dp*), deficiencies removing multiple genes (*Df*), translocations (*T)*, extrachromosomal, integrated, and single-copy insertion of transgenes (*Ex*, *Is*, and *Si*, respectively), transposon insertions (*Ti*), and introgressed regions (*IR*). Targeted modifications, such as those made using CRISPR/Cas9 genome editing, should be assigned allele names, but can optionally include information about the specific changes in brackets (*bus-50(e5000[T110E])* is an engineered missense mutation. Complete current nomenclature guidelines can be found on WormBase (https://wormbase.org/about/userguide/nomenclature) and WormBook (Tuli 2018). Nomenclature guidelines are updated as needed to accommodate advances in technology, addressing issues such as alleles generated through genomic engineering – tagged endogenous loci or gene replacements – in ways that adhere to the established doctrine while providing users with specific information about each new strain. Presently, genetic nomenclature for *C. elegans* and related species is regulated by WormBase, working in close collaboration with the CGC. Input from the community of worm researchers is welcomed and carries significant weight when changes to the guidelines are considered.

The CGC cooperates extensively with WormBase on matters of genetic nomenclature and information about strains in our collection. The CGC website interfaces with WormBase regularly to keep databases up to date, track changes in gene names, curate molecular data and descriptions of phenotypes associated with alleles

and transgenes, and keep strain lists synchronized. Changes in reported genotypes are tracked and updated to ensure strain information is accurate and consistent between the two sites. This cross-talk between sites also provides users with alternate means of locating strains needed for their work, allowing WormBase users to quickly see which strains of interest are available to order from the CGC (Figure 7A), and CGC users to more efficiently search our catalog by having our search engine use synonymous gene names (such as the sequence ID or alternative gene names). Furthermore, each strain entry in the CGC catalog (Figure 7B) provides a link to that strain and the alleles it carries on WormBase, allowing users to quickly access whatever additional information might be available beyond what is provided in the CGC description, including additional publication references.

Distribution of Strains

In early years when strains were distributed free of charge, the CGC sent out strains in response to written or emailed requests from researchers. Once user fees were imposed in 2007, the CGC developed an online ordering system to accommodate credit card payment and simplify invoicing against purchase orders. In 2017, the CGC moved to a custom database software package that merged key large databases (strains information, freezer locations, shipping history, and lab information) and integrated them with the ordering system, greatly increasing the efficiency of daily operations in the CGC. The online searchable catalog of the CGC strain collection is available through the CGC website and ordering system (cgc.umn.edu), which have been redesigned to add features that enhance the user's experience while simplifying the ordering process. Changes to the strain collection are now updated in real time, including modification to strain descriptions as new information is made available (such as identification of molecular breakpoints or linked background mutations, additional aspects of phenotypes, reported problems currently being examined, etc.), and new strains are automatically added to special pages such as the list of recently acquired strains. The new site provides users with advanced search functions in the on-line catalog and the ability for lab members to set up their own individual sub-accounts to view their personal order history including order status and ship dates. PIs or lab managers have administrative regulatory privileges over any user sub-accounts linked to their lab.

All strain orders must be submitted through the CGC online ordering system, and credit card payments are strongly encouraged. In cases of financial hardship, users who do not have funds available may email the

director or curator to request a waiver of the fees. Waivers are considered for specific strains requested on a case-by-case basis. If the request is approved and a waiver granted, the user is provided with a one-time code to remove the fees when submitting their order through our website. Waivers are capped at a maximum of 25 strains per lab within a year.

New orders are processed daily. Requested strains are thawed from frozen stocks or prepared from stocks already out in the lab for other orders. At any given time, roughly 15-20% of the strains in the collection are actively growing in the lab as they are prepared for other orders or stored on starved plates at 11°C (Figure 2C). A strain that is actively growing in the lab might take as little as 1-2 business days to prepare for shipping, but requested strains not already out in the lab take longer because they must be thawed from frozen stocks. Aliquots stored in -80°C freezers contain a small amount of agar in the freezing medium, allowing a portion of the frozen sample to be removed with a sterile microspatula and placed on an NGM plate. Thawing survival rates vary somewhat in mutant strains and non-*C. elegans* species, but the small size of the worms makes it possible to have hundreds, if not thousands, of animals preserved in each frozen aliquot. Thawed strains are allowed to recover 1-3 days before animals are transferred to new plates for verification and further processing. All strains prepared for orders are transferred to new plates of food (bacteria), decontaminated (if necessary), and assessed for proper phenotypes before being sent to users.

Homozygous strains usually require little maintenance beyond examining the plate to confirm phenotypes. Heterozygous strains and strains carrying chromosomal duplications or extrachromosomal transgenic arrays are checked for correct segregation of progeny. Mutants with temperature-sensitive alleles are periodically shifted to restrictive temperatures to confirm phenotypes. Strains carrying fluorescent reporters are checked for expression when practical, using dissecting microscopes equipped for epifluorescence. Animals are not normally mounted for examination at high magnification unless a strain is being verified in response to suspected problems such as silencing of transgene expression or inappropriate expression patterns. Strains are normally shipped via first class US post at no additional cost to user; shipping costs are built into the annual operating budget. The CGC will ship strains with a private courier such as FedEx or DHL when requested by the user, but the user must provide an account number or prepaid label to use when setting up the shipment. The agar-filled petri dishes on which the strains are raised and shipped are sealed with paraffin film to protect against desiccation and contamination, and the small petri dishes (35 mm diameter) are packed in padded

envelopes, allowing strains to be shipped at relatively low cost (most packages weigh only a few ounces). Strains are normally sent in a starved condition because starved animals are much more tolerant of environmental stress than fed animals. Starvation makes the worms more likely to survive swings in temperature and prolonged shipping times, requiring no special shipping conditions are necessary for most orders – a key advantage for a stock center sending samples around the globe. Still, if local weather conditions are forecast to be warmer than 85^{o}F or colder than 0^{o}F, the CGC will hold shipments until weather is more favorable to prevent death of strains during shipping (though our comfortably cool winter temperatures are embraced by most Minnesotans, our small nematode friends typically do not survive prolonged exposure below zero).

Upon receiving their requested worm strains, users are encouraged to recover strains by transferring individual animals or a chunk of agar to a new plate with a flame-sterilized scalpel or spatula (Stiernagle, 2006). Even if no motile animals are seen on the surface of the agar plate, there are often starved animals that have burrowed into the agar that will crawl out when moved to a plate with bacterial food. Sadly, conditions encountered during shipping are sometimes too extreme for the worms to survive. In the rare event that the user is unable to recover a strain within a few days after transferring to a fresh plate, we will typically send a replacement at no cost if notified in a timely manner. Similarly, if a user finds a problem with a strain received from the CGC, we ask that they notify us as soon as possible to help ensure the validity of strains for all users. When a potential problem with a strain is reported, attempts are made in-house to verify if the stock is correct. When needed, the originating lab is consulted for further information or asked to validate the stock. If deemed necessary, a replacement is requested from the originating lab. CGC staff will always work with users to troubleshoot potential problems with strains obtained from the CGC.

Serving the World Community

Approximately 6,000 labs are registered users of the CGC. The overwhelming majority of these groups are academic research labs, though *C. elegans* is growing in popularity for instructional purposes in teaching colleges and high schools. Only a small percentage of registered users are commercial (for-profit) research groups. More than half of all the labs registered with the CGC are located within the US, with steady growth in the number of new labs ordering from the CGC both within the US and abroad. Historically, most of the strains shipped to labs outside of the US were sent to members of the worm community in the United Kingdom and

Europe, but the CGC has seen a pronounced increase in the number of requests from academic research labs across Asia, especially within China, in recent years.

The CGC has sent strains to researchers in 74 countries, some of which present special challenges due to extreme climate conditions or import regulations. Recipients residing in extremely hot or cold climates will sometimes make special shipping accommodations to ensure the requested strains arrive in good condition. The CGC will accommodate such requests when feasible, for example, arranging temperature-controlled shipping (at recipient's expense) with the recipient's preferred courier, or having the order shipped to collaborator located in a more temperate region of the destination country. Additionally, some users who have experienced difficulties in clearing their shipments through customs prefer to arrange shipment through particular freight-forwarding agencies or customs brokers contracted with their university to aid in the process, but the user is responsible for making any such arrangements and dealing with any problems that might arise during transit.

International delivery is generally reliable, but can be slow, with customs clearance often being the largest hurdle. Despite the non-hazardous and non-infectious nature of *C. elegans*, many countries have specific regulations for the import of live specimens. Carrier rules and import regulations are prone to changes and can vary between destination countries (and sometimes even at different ports of entry within the same country), so in some situations private courier will typically be more successful than US post when shipping to some countries and *vice versa*. Through experience, CGC staff members have learned to avoid pitfalls in the shipping process that are likely to trigger red flags at certain destinations and give each shipment its best chance of arriving in a timely manner. Regardless of which shipping method is used, the CGC will declare the full dollar amount of order and contents of the shipment are accurately described as required by each courier. If additional permits, special shipping forms, or declaration letters are required for importing strains into the destination country, the recipient is responsible for providing any such documentation.

Acquisition of new resources

The primary aim of the CGC is to obtain a reference allele of every identified gene and all useful chromosome rearrangements generated in the laboratory strain N2. The CGC is actively seeking strains carrying mutations in genes not yet represented in the collection: null alleles of genes absent from the collection or represented only

by hypomorphic alleles, unusual alleles of a gene (e.g. - gain-of-function or conditional alleles, tagged-endogenous loci), difficult-to-make mutant combinations, and useful genetic or molecular tools (balancer chromosomes, deficiencies, inducible or tissue-specific transgenes, etc.). However, the CGC will consider strains not fitting these criteria if the user provides adequate justification of why a strain should be maintained by the CGC. Many new strains are obtained through donation requests from labs looking to deposit popular strains in the CGC to relieve the burden of distributing the strains themselves. These are often key reagents from recent papers that are in high demand from other research labs. A small number of new strains are acquired in response to requests from members of the community contacting the CGC looking for particular alleles or transgenes significantly different from those already available in the CGC catalog.

Though the CGC aims to preserve unique strains and make them available to the community far into the future, it is not feasible to accept every strain that a lab has generated or published. With the rapid expansion of the collection in recent years (Figure 5), the CGC has been forced to become more selective about additions to the collection. This selectivity has become a greater challenge in recent years as the first wave of PIs dedicated to worm research reach retirement age and seek a long-term home for their lab's collections of strains, many of which are not preserved anywhere else outside their own laboratories. The CGC has also seen an increased number of requests from labs closing or changing research direction with no former post-docs or other lab members interested in or able to inherit the lab's frozen stocks. In such cases, the CGC will work with the PI or lab manager to narrow down the list of potential donations to strains that fill the needs of the CGC, focusing on the acquisition of strains that enhance the current catalog while avoiding redundancy in the collection, primarily by excluding multiple lines of similar transgenes and collections of genotypically or phenotypically similar alleles.

All donors must complete an online strain donation form available on the CGC homepage. The CGC curator vets all strain donation requests before a strain is accepted as part of the collection. Each donation request must include enough information for the curator to determine the value of adding that strain to the CGC catalog, including the complete genotype and a brief description of the phenotype, and provide enough information in the strain description that it will be meaningful to other users in the community. The description of a transgenic line must include all components of the transgenic array, including any transformation or co-injection markers used, as well as instruction on how to maintain the array in the

population (picking individuals with appropriate markers or how to select against animals that have lost the transgene). For balanced strains, the description should include information about how to identify heterozygous animals to pick to maintain the stock and the expected classes segregating among their progeny. When considering donations of characterized wild isolates, required information includes where a strain was isolated, from what habitat it was isolated, when it was isolated, how it was isolated, and by whom it was isolated. The CGC advisory board is often consulted when vetting donations of non-*C. elegans* species and deciding whether strain submissions should be redirected to CeNDR.

The CGC is dedicated to the long-term preservation and maintenance of strains and discourages contributions of stocks not suitable for long-term stability, such as transgenes with especially poor transmission rates or that are prone to silencing, mutants with unstable genomes or mortal germ lines, unbalanced heterozygous mutants, etc. Although important strains may be excluded by these criteria, it is not practical for the CGC staff to dedicate time and resources to maintain such difficult stocks. Additionally, it is unfair to users to advertise availability of strains unlikely to be recovered from frozen stocks.

Large collections or sets of strains (genetics toolkits) may be donated to the CGC, but require special consideration before acceptance. This practice was initially reserved for collaboration with large community resource projects, such as the *C. elegans* Knock-out consortium (The *C. elegans* Deletion Mutant Consortium 2012) and the *C. elegans* Gene Expression Project (Hunt-Newbury 2007), each of which submitted more than 2,000 strains to the CGC. However, individual research labs may also now submit collections of strains in this manner as they develop tool kits, such as genetic mapping strains, transposon-insertion strains, strains for tissue-specific expression (or knock-down of expression), etc. In these cases, strain information for large collections may be submitted as a spreadsheet or CSV file using a template provided by the CGC, allowing the data to be uploaded by CGC curators *en masse*. Each strain being donated must meet the same requirements for inclusion as if it were being donated individually. When submitting large collections of strains, we request the donors send them as frozen aliquots rather than on NGM petri plates, and can provide a cryoshipper to streamline the process. Once the frozen stocks are received, the strains will appear in our online catalog and be available to order. Frozen stocks can be simply stored in a freezer until the strain is ordered, allowing CGC staff to process the new stocks as needed, rather than processing dozens (or hundreds) of strains simultaneously upon receipt. The first time a strain from a large collection is ordered, the original aliquot is

thawed, the population is expanded, and the strain is cleaned and processed as any other newly acquired strain would be before the stock is sent to the requesting lab and refrozen in aliquots for CGC freezer stocks (described in the following section).

Implicit in any strain donation to the CGC is the agreement that the strain may be distributed to academic labs without constraints. The CGC does not use material transfer agreements (MTAs) for any strains it receives or distributes but will respect requests from labs specifying that their strains not be provided to commercial groups or used in human research as required by their funding sources or institutions. Any such restrictions are clearly noted in a strain's description in our online catalog. The CGC does not retain any intellectual property (IP) rights or otherwise regulate how strains are used by recipients after distribution. Any potential problems or conflicts over IP rights should be resolved between the user and lab in which the strain originated.

Preservation of New Strains

New strains are normally sent to the CGC on small agar petri plates (Figure 2B). Upon arrival, plates are inspected for any sign the stock might be compromised, such as crushed or broken plates that might have allowed different populations to mingle. A visual inspection is performed, examining each plate for mites, which can prey on nematodes and carry worms or embryos between plates, before unsealing the plate. Worms are transferred to new plates to establish a stable population before attempting to decontaminate the stock, and gross phenotypes are observed. Nearly all incoming stocks are treated with hypochlorite to eliminate unwanted bacteria or other micro-organisms present on the original plate received (Stiernagle, 2006). Once the stock is clean, the strain is evaluated to determine if the observed phenotypes match the description of strain provided by the donor. In some cases, published references are checked for additional information about phenotypes and expression patterns. If inconsistencies are noted between observed phenotypes and submitted strain descriptions or published descriptions, the lab submitting the strain is contacted and asked for clarification.

Frozen stocks of each strain are made as soon as possible after receipt. At least seven frozen stocks of each strain are made: one is thawed to check the quality of the frozen stocks, and other frozen aliquots are distributed across four locations for long-term storage in both -80C and liquid nitrogen freezers on site in the CGC lab space, in liquid nitrogen freezers in a neighboring building, and in liquid nitrogen freezers at the USDA-

National Animal Germplasm Program (NAGP) in Fort Collins, CO. Samples tested at the CGC have remained viable when thawed after more than 30 years of frozen storage. Utilizing multiple storage facilities ensures the long-term viability of the collection to make it a permanent community resource. Multiple locations at the University of Minnesota protect the collection against localized loss due to fire or equipment failure, while maintaining a complete mirror copy of the collection at the NAGP provides insurance in event of catastrophic loss at the primary location in Minnesota.

Enhancing CGC operations

Though the primary focus of the CGC is the collection and distribution of strains, the CGC funding was switched from a government contract to a grant in 2012, adding a small research component and aligning the CGC with other NIH-supported animal resource centers. As part of our research component, CGC staff members are actively developing new strains and molecular tools that will benefit researchers throughout the community.

C. elegans has proven itself as a top-tier genetic system, but until recently, the collection of available balancer chromosomes lacked complete coverage. The National BioResource Project (NBRP; headed by Dr. Shohei Mitani, Tokyo Women's Medical University School of Medicine) generously provided to the CGC for distribution an exceptional collection of chromosomal inversions that, in combination with preexisting balancers, covers nearly 90% of the genome (Dejima, 2018). Many of these new balancer chromosomes are enhanced by fluorescent markers so that users do not have to rely on phenotypic markers, which are sometimes not apparent until later stages of development or that can be difficult to score in some mutant backgrounds. CGC staff have similarly used CRISPR/Cas9 engineering to insert fluorescent markers into some of the unlabeled inversions from the NBRP, as well as many of the preexisting classical balancers, providing users with new genetic tools to quickly and easily distinguish homozygous mutants from heterozygous animals in a population, even in relatively early stages of development.

As other new molecular techniques are developed or adapted for use in *C. elegans,* they drive demand for genetic toolkits or certain sets of strains. For example, the CGC catalog offers series of strains for driving targeted expression of transgenes through the Gal4-UAS system (Wang, 2017) and targeted knock-down of gene expression in specific tissues or cell types mediated by auxin-inducible degradation (AID) (Zhang, 2015). Other recent acquisitions by the CGC include sets of *C. elegans* and *C. briggsae* recombinant inbred lines (RILs),

strains carrying fluorescent markers for cell lineage and expression studies, genetic mapping kits, and a series of strains with transposon insertion sites at defined positions throughout the genome (Murray, 2008; Sarov, 2006; Zhong, 2010; Bi, 2015; Frøkjær-Jensen, 2014). It is not feasible, of course, for the CGC collection to include all promoter constructs that might be created for use in these systems, but as additional technologies are adapted for use in *C. elegans*, the CGC will strive to make basic toolkits available by providing critical component strains of such systems with the aim of acquiring additional strains as demand might warrant.

The CGC has long maintained a close relationship with the consortia of labs generating gene knock-out mutations in *C. elegans*. Strains generated by the Vancouver (and previously, Oklahoma) group as part of the *Caenorhabditis elegans* Gene Knockout Consortium (2012) are sent to the CGC for distribution. An additional sizable collection of deletion mutants isolated by NBRP are available directly from the Mitani Lab upon request, and once characterized, may be sent to the CGC with permission from the NBRP. In addition, a small consortium of labs is also working to develop Crispr-Cas9 pipelines to delete genes of specific interest to the human disease and parasitic nematode communities, and those deletions have begun to enter the CGC collection (Au, 2018; Wang, 2018).

The CGC catalog also includes a set of ~2,000 strains comprising the Million Mutation Project (MMP), a joint project by the Moerman and Waterston Labs to identify multiple mutations in virtually every *C. elegans* gene (Thompson, 2013). Because many of these mutations are single nucleotide changes, it is important that researchers analyzing these strains have an appropriate control strain (and genome sequence) to use in their experimentation. While a single strain from Bristol, England known as N2 (Sterken, 2015) has served as the standard wild-type reference strain among the community for many years, the *C. elegans* genome was originally assembled piecemeal from sequence obtained from multiple different N2 isolates, resulting in some haplotypes reported in the reference genome that are not found in the stock of N2 maintained by the CGC. Researchers using high-throughput, short-read DNA or RNA sequencing have identified genetic differences between the reference genome and stocks of N2 maintained in their individual labs, suggestive of genetic drift in some labs' stocks. With the introduction of the MMP strains, the need for a true reference strain and corresponding genome became apparent. The CGC is collaborating with a consortium led by Dr. Shinichi Morishita and Dr. Andy Fire to establish a new fully-sequenced wild-type reference strain derived from N2 (Yoshimura J, submitted). The new strain was strategically chosen as the parental strain used in the Million

Mutation Project. The CGC has many aliquots of the new reference strain, with protocols in place for maximizing the number of archived samples while minimizing the number of generations grown each an original aliquot is thawed. These steps will ensure a large supply of worms frozen at essentially the same time to provide researchers with a long-term source of a standardized reference genome control strain that is common to all labs in the community. Distribution will begin once the annotated sequence is available on WormBase.

A look ahead

The future of the worm community is bright. *C. elegans* continues to grow in popularity as a model organism, and with users registering an average of six new CGC lab accounts in each week over the last year, shows no signs of slowing down. Many new users are entering the field without coming from experience in an established worm lab, and are instead using *C. elegans* as a complement to the other organisms that are the primary system in their laboratory. The use of *C. elegans* in high school and college biology labs is introducing new generations of scientists to the worm. The worm's versatility will only continue to grow as new technologies are adapted for use in this efficient, easy to use, and cost-effective model system. The simplicity, speed, and relative ease of working with worms continue to provide advantages as new technologies are applied to worm models. The compact genome and worldwide distribution of worms in the wild makes Caenorhabditis ideally suited for comparative genomics studies, which will only gain traction as community resources such as CeNDR grow.

The collaborative spirit that has invariably been a hallmark of the worm community endures in new generations of researchers openly sharing exciting new reagents with their colleagues around the globe, and it is just as important today, as it was 40 years ago, that these shared reagents continue to be made available to researchers worldwide. Although technology is making the world a smaller place by making it easy for scientists in different countries to communicate and share information, customs and international shipping regulations are continually becoming more restrictive, threating the exchange of reagents between these same researchers. Resource centers such as the CGC and CeNDR provide vital services for the community, not only by maintaining stocks and strain information, but also by using their experience and knowledge to ensure key strains will be successfully delivered to the researchers who rely upon them for their research.

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Figure 1. *C. elegans* life cycle, nervous system and sexually dimorphic tail. (A) Hermaphrodite life cycle. Under ideal growth conditions, *C. elegans* will develop from a fertilized egg to an adult in just over two days. The first few hours of development occur *in utero*, the egg is then laid and embryogenesis continues until the basic body plan is complete and the first stage (L1) larva hatches. Development continues, punctuated by molts, through three additional larval stages (L2 to L4) until the sexually mature adult stage is reached. When conditions are harsh, for example due to high temperature, starvation, and/or crowding, *C. elegans* L1 larvae can enter an alternate developmental stage known as a dauer larva, which confers resistance to environmental stress and promotes survival. If conditions become favorable, dauer larvae can resume development. Reprinted with permission from WormAtlas. **(B)** The simple *C. elegans* nervous system is visualized by pan-neuronal expression of the GFP transgene *evIs111* [F25B3.3::GFP + dpy-20(+)]. **(C,D & E)** Micrographs showing tail morphology. Scale bars are 50 μm. A hermaphrodite's tail tapers gently to a point **(C)**, whereas an adult male tail has a fan-like copulatory structure shown in lateral **(D)** and ventral **(E)** views.

Figure 2. Tools for basic *C. elegans* manipulation and storage. (A) A typical stereomicroscope station. *C. elegans* are typically observed using a dissecting microscope with variable magnification and equipped with visible light transillumination. **(B)** Nematodes grown on petri dishes are manipulated using wire picks while viewing them through a dissecting microscope with ample working distance. Similar to an inoculation loop, a worm pick (arrow) is typically fashioned from a piece of platinum wire embedded into a handle of sorts, for example, with the wire melted into the tip of a glass Pasteur pipet. **(C)** A partial view of the active CGC collection. Stocks of *C. elegans* can remain viable for several months if kept in a starved condition on sealed petri dishes stored at cool temperatures.

Figure 3. CGC strain distribution. The number of strains shipped per year is graphed. Demand grew annually until 2007, when it dropped briefly in response to implementation of user fees and strain charges mandated by the National Institutes of Health (NIH). Recent demand has remained steady around 30,000 strains shipped per year. Several key events within the worm community are indicated, including the sequencing of the *C. elegans* genome, the launch of WormBase, and the awarding of three Nobel prizes for research on *C. elegans* (Sydney Brenner, Robert Horvitz, and John Sulston, Physiology or Medicine, 2002; Andrew Fire and Craig Mello, Physiology or Medicine, 2006; and Martin Chalfie, together with Roger Tsien and Osamu Shimomura, Chemistry, 2008).

Figure 4. Number of orders. The number of orders processed by the CGC per year is graphed. The number of orders grew steadily each year until the implementation of user fees in 2007. Recently, demand has remained steady at about 7,500 orders annually, and for the last 25 years, the average number of strains requested per order has remained remarkably consistent at about four.

Figure 5. Expansion of the CGC collection. Growth of the CGC collection is graphed. The CGC collection continues to grow steadily, with an average of about 700 new strains added each year. Large collections such as the Million Mutation Project (Thompson, 2013) strains have recently boosted the collection.

Figure 6. Global origins of wild *C. elegans* isolates available in CeNDR. Partial world map indicating the origins of some of the 766 wild isolates of *C. elegans* available through CeNDR. The clickable map (available through the CeNDR homepage or at https://www.elegansvariation.org/strain/global-strain-map/) allows users to quickly access more information about specific strains isolated from regions of geographical interest.

Figure 7. Crosstalk between the CGC website and WormBase. The CGC works closely with WormBase to ensure that strain information is kept current and synchronized between the databases. **(A)** Selecting the Genetics tab from the *hbl-1* gene page reveals, in part, this typical WormBase page view listing curated strains containing mutant alleles of *hbl-1.* At a glance, users learn which strains are available through the CGC (yellow box) and can identify which strains are *hbl-1* single mutants (blue box). The table below links directly to CGC strain pages, as shown in **(B)** for *hbl-1(ve18)*, and the user can quickly add the strain to their CGC order. The CGC strain information pages provide essential information about strains and also link to WormBase, allowing users to access additional information.

References

Andersen, E.C., J.P. Gerke, J.A. Shapiro, J.R. Crissman, R. Ghosh, J.S Bloom, M.A. Félix, and L. Kruglyak. 2012. Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. Nat Genet. 44(3):285-90. (PMID: 22286215)

Antebi, A., J.G. Culotti, and E.M. Hedgecock. 1998. daf-12 regulates developmental age and the dauer alternative in *Caenorhabditis elegans*. Development. 125(7):1191-205. (PMID: 9477318)

Au V., E. Li-Leger, G. Raymant, S. Flibotte, G. Chen, K. Martin, L. Fernando, et al. 2018. Optimizing guide RNA selection and CRISPR/Cas9 methodology for efficient generation of deletions in *C. elegans*. bioRxiv. doi: https://doi.org/10.1101/359588

Bi Y., X. Ren, C. Yan, J. Shao, D. Xie, and Z. Zhao. 2015. A Genome-wide hybrid incompatibility landscape between *Caenorhabditis briggsae* and *C. nigoni*. PLoS Genet. 11(2):e1004993. (PMID: 25692300)

Braendle C., and M.A. Felix. 2009. The other side of phenotypic plasticity: a developmental system that generates an invariant phenotype despite environmental variation. J Biosci. 34(4):543-51. (PMID: 19920340)

Brenner S. 1974. The genetics of *Caenorhabditis elegans.* Genetics. 77(1): 71–94. (PMID: 4366476)

Brenner S. 1988. The nematode *Caenorhabditis elegans*. ed. B.W. Wood, Foreword. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Cassada R.C., and R.L. Russell. 1975. The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev Biol. 46(2):326-42. (PMID: 1183723)

Cingolani P., A. Platts, L. Wang le, M. Coon, T. Nguyen, L. Wang, S.J. Land, X. Lu, and D.M. Ruden. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin). 6(2):80-92. (PMID: 22728672)

Cook D.E., S. Zdraljevic, R.E. Tanny, B. Seo, D.D. Riccardi, L.M. Noble, M.V. Rockman, et al. 2016. The genetic basis of natural variation in *Caenorhabditis elegans* telomere length. Genetics. 204(1): 371–383. (PMID: 27449056).

Cook D.E., S. Zdraljevic, J.P. Roberts, and E.C. Andersen. 2017. CeNDR, the *Caenorhabditis elegans* natural diversity resource. Nucleic Acids Res. 45(D1):D650-D657. (PMID: 27701074)

Dejima K., S. Hori, S. Iwata, Y. Suehiro, S. Yoshina, T. Motohashi, and S. Mitani. 2018. An Aneuploidy-Free and Structurally Defined Balancer Chromosome Toolkit for *Caenorhabditis elegans*. Cell Rep. 22(1):232-241. (PMID: 29298424)

Dickinson D.J., and B. Goldstein 2016. CRISPR-Based Methods for *Caenorhabditis elegans* Genome Engineering. Genetics. 202(3):885-901. (PMID: 26953268)

Dutta P., C. Lehmann, D. Odedra, D. Singh, and C. Pohll. 2015. Tracking and Quantifying Developmental Processes in *C. elegans* Using Open-source Tools. J Vis Exp. (106):53469. (PMID: 26709526)

Ewald, C.Y., and C. Li. 2010. Understanding the molecular basis of Alzheimer's disease using a *Caenorhabditis elegans* model system. Brain Struct Funct 214(2-3):263-83. (PMID: 20012092)

Félix MA. 2008. RNA interference in nematodes and the chance that favored Sydney Brenner. J Biol. 7(9):34. (PMID: 19014674)

Félix M.A., and C. Braendle. 2010. The natural history of *Caenorhabditis elegans*. Current Biology. 20(22):R965–9. (PMID: 21093785).

Fire, A., S. Xu, M.K. Montgomery, S.A. Kostas, S.E. Driver, and C.C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature *391:*806–811. (PMID: 9486653)

Frézal L., and M.A. Félix. 2015. Elife. Mar 30;4. doi: 10.7554/eLife.05849. (PMID: 25822066)

Frøkjær-Jensen C., M.W. Davis, M. Sarov, J. Taylor, S. Flibotte, M. LaBella, A. Pozniakovsky,D.G. Moerman, and E. M. Jorgensen. 2014. Random and targeted transgene insertion in *Caenorhabditis elegans* using a modified Mos1 transposon. Nature Methods 11(5):529–534. (PMID: 24820376)

Golden J.W., and D.L. Riddle. 1984. A pheromone-induced developmental switch in *Caenorhabditis elegans*: Temperature-sensitive mutants reveal a wild-type temperature-dependent process. Proc Natl Acad Sci USA 81(3):819-23.

Haag, E. S. 2005. The evolution of nematode sex determination: *C. elegans* as a reference point for comparative biology. *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.120.1, http://www.wormbook.org. (PMID: 18050417)

Hillier L.W., A. Coulson, J.I. Murray, Z. Bao, J.E. Sulston, and R.H. Waterston. 2005. Genomics in *C. elegans*: so many genes, such a little worm. Genome Res. 2005 Dec;15(12):1651-60. (PMID: 16339362)

Hodgkin J. 1987. Primary sex determination in the nematode *C. elegans*. Development. 1987;101 Suppl:5-16. (PMID: 3503722)

Horvitz H.R., S. Brenner, J. Hodgkin, and R.K. Herman. 1979. A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol Gen Genet. 175(2):129-33. (PMID: 292825)

Horvitz H.R. 1999. Genetic control of programmed cell death in the nematode *Caenorhabditis elegans*. Cancer Res. 59(7 Suppl):1701s-1706s. (PMID: 10197583)

Hunt-Newbury R., R. Viveiros, R. Johnsen, A. Mah, D. Anastas, L. Fang, E. Halfnight, et al. 2007. High-throughput in vivo analysis of gene expression in *Caenorhabditis elegans*. PLoS Biol. Sep;5(9):e237. (PMID: 17850180)

Johnson J.R., R.C. Jenn, J.W. Barclay, R.D. Burgoyne, and A. Morgan.. (2010). *Caenorhabditis elegans*: a useful tool to decipher neurodegenerative pathways. Biochem Soc Trans 38(2):559-63. (PMID: 20298221)

Kaminsky R., P. Ducray, M. Jung, R. Clover, L. Rufener, J. Bouvier, et al. 2008. A new class of anthelmintics effective against drug-resistant nematodes. Nature 452(7184):176-80.

Keil W, L.M. Kutscher, S. Shaham, and E.D. Siggia.. 2017. Long-Term High-Resolution Imaging of Developing *C. elegans* Larvae with Microfluidics. Dev Cell. 40(2):202-214. (PMID: 28041904)

Kimble J. and D Hirsh. 1979. The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev Biol. 70(2):396-417. (PMID: 478167)

Kiontki K, M.A. Félix, M. Ailion, M.V. Rockman, C. Braendle, J.B. Pénigault, and D.H. Fitch. *2011.* A phylogeny and molecular barcodes for Caenorhabditis, with numerous new species from rotting fruits. *BMC Evolutionary Biology. 11: 339.* doi:10.1186/1471-2148-11-339*. (*PMID: 22103856*)*

Lee R.Y.N., K.L. Howe, T.W. Harris, V. Arnaboldi, S. Cain, J. Chan, W.J. Chen, et al. 2018. WormBase 2017: molting into a new stage. Nucleic Acids Res. 46(Database issue):D869–D874. (PMID: 29069413)

Lee R.C., R.L. Feinbaum, and V. Ambros. 1993. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75, 843-854. (PMID: 8252621)

McGrath P.T., M.V. Rockman, M. Zimmer, H. Jang, E.Z. Macosko, L. Kruglyak, and C.I. Bargmann. 2009. Quantitative mapping of a digenic behavioral trait implicates globin variation in C. elegans sensory behaviors. Neuron. 61(5):692-9. (PMID: 19285466)

Murray JI, Z. Bao, T.J. Boyle, M.E. Boeck, B.L. Mericle, T.J. Nicholas, Z. Zhao, M.J. Sandel, and R.H. Waterston. 2008. Automated analysis of embryonic gene expression with cellular resolution in *C. elegans*. *Nat Methods* 5(8):703-9. (PMID: 18587405)

Nigon V.M., and M.A. Félix. 2017. History of research on *C. elegans* and other free-living nematodes as model organisms. *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.181.1, http://www.wormbook.org. (PMID: 28326696)

Pulak R. 2006. Techniques for analysis, sorting, and dispensing of *C. elegans* on the COPAS flow-sorting system. Methods Mol Biol. 351:275-86. (PMID: 16988441)

Sarov M. S. Schneider, A. Pozniakovski, A. Roguev, S. Ernst, Y. Zhang, A.A. Hyman, and A.F. Stewart. 2006. A recombineering pipeline for functional genomics applied to *Caenorhabditis elegans*. Nat Methods 10:839-44. (PMID: 16990816)

Sterken M.G., L.B. Snoek, J.E. Kammenga, and E.C. Andersen. 2015. The laboratory domestication of *Caenorhabditis elegans*. Trends Genet 31(5):224-31. (PMID: 25804345)

Stiernagle, T. 2006. Maintenance of *C. elegans*. *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.101.1, http://www.wormbook.org. (PMID: 18050451)

Sulston, J. E. and H.R. Horvitz, 1977. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. 56:110-156. (PMID: 838129)

Sulston J.E., E. Schierenberg, J.G. White, and J.N. Thomson. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. 100: 64-119. (PMID: 6684600)

Tabara H., A. Grishok, and C.C. Mello. 1998. RNAi in *C. elegans*: soaking in the genome sequence. Science *282*, 430–431. (PMID: 9841401)

The *C. elegans* Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. Science. 282:2012-2018. 10.1126/science.282.5396.2012. (PMID: 9851916)

Timmons L., and A. Fire, A. 1998. Specific interference by ingested dsRNA. Nature *395*, 854. (PMID: 9804418)

Timmons L., D.L. Court, and A. Fire. 2001. Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in *Caenorhabditis elegans*. Gene *263*, 103–112. (PMID: 11223248)

The *C. elegans* Deletion Mutant Consortium. 2012. Large-scale screening for targeted knockouts in the *Caenorhabditis elegans* genome. G3 (Bethesda). 2012 Nov; 2(11): 1415–1425. (PMID: 23173093)

Thompson O., M. Edgley, P. Strasbourger, S. Flibotte, B. Ewing, R. Adair, V. Au V, et al. 2013. The million mutation project: a new approach to genetics in *Caenorhabditis elegans*. Genome Res. 23(10):1749-62. (PMID: 23800452)

Tuli M.A., A.L. Daul, and T. Schedl. 2018. Caenorhabditis nomenclature. *WormBook*, ed. The *C. elegans* Research Community. Published as WormBook Early Online doi:10.1895/wormbook.1.183.1. http://www.wormbook.org.

Wang H., J. Liu, S. Gharib, C.M. Chai, E.M. Schwarz, N. Pokala,and P.W. Sternberg. 2017. cGAL, a temperature-robust GAL4-UAS system for *Caenorhabditis elegans*. Nat Methods. 14(2):145-148. (PMID: 27992408)

Wang H., H. Park, J. Liu, and P.W. Sternberg. 2017. An efficient genome editing strategy to generate putative null mutants in *Caenorhabditis elegans* using CRISPR/Cas9. G3 (Bethesda). 2018 Sep 17. pii: g3.200662.2018. (PMID: 30224336)

Waterman C., R.A. Smith, L. Pontiggia, and A. DerMarderosian. 2010. Anthelmintic screening of Sub-Saharan African plants used in traditional medicine. J Ethnopharmacol *127*, 755-759. (PMID:19962435)

Wood W. 1988. The nematode *Caenorhabditis elegans*. ed. B.W. Wood, 1-16. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory. (Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory).

Yoshimura J., K. Ichikawa, M. Shoura, K.L. Artiles, I. Gabdank, L.Wahba, C.L. Smith, et al. "Recompleting the *C. elegans* genome." In preparation.

Zacharias A.L., and J.I. Murray. 2016. Combinatorial decoding of the invariant *C. elegans* embryonic lineage in space and time. Genesis. 54(4):182-97. (PMID:26915329)

Zdraljevic S., C. Strand, H.S. Seidel, D.E. Cook, J.G. Doench, and E.C. Andersen. 2017. Natural variation in a single amino acid substitution underlies physiological responses to topoisomerase II poisons. PLoS Genet. 13(7):e1006891. (PMID: 28700616)

Zhang L., J.D. Ward, Z. Cheng, and A.F. Dernburg. 2015. The auxin-inducible degradation (AID) system enables versatile conditional protein depletion in *C. elegans*. Development. 142(24):4374-84. (PMID: 26552885)

Zhong M., W. Niu, Z.J. Lu, M. Sarov, J.I. Murray, J. Janette, D. Raha, et al. 2010. Genome-wide identification of binding sites defines distinct functions for *Caenorhabditis elegans* PHA-4/FOXA in development and environmental response. PLoS Genet 6(2):e1000848. (PMID: 20174564)

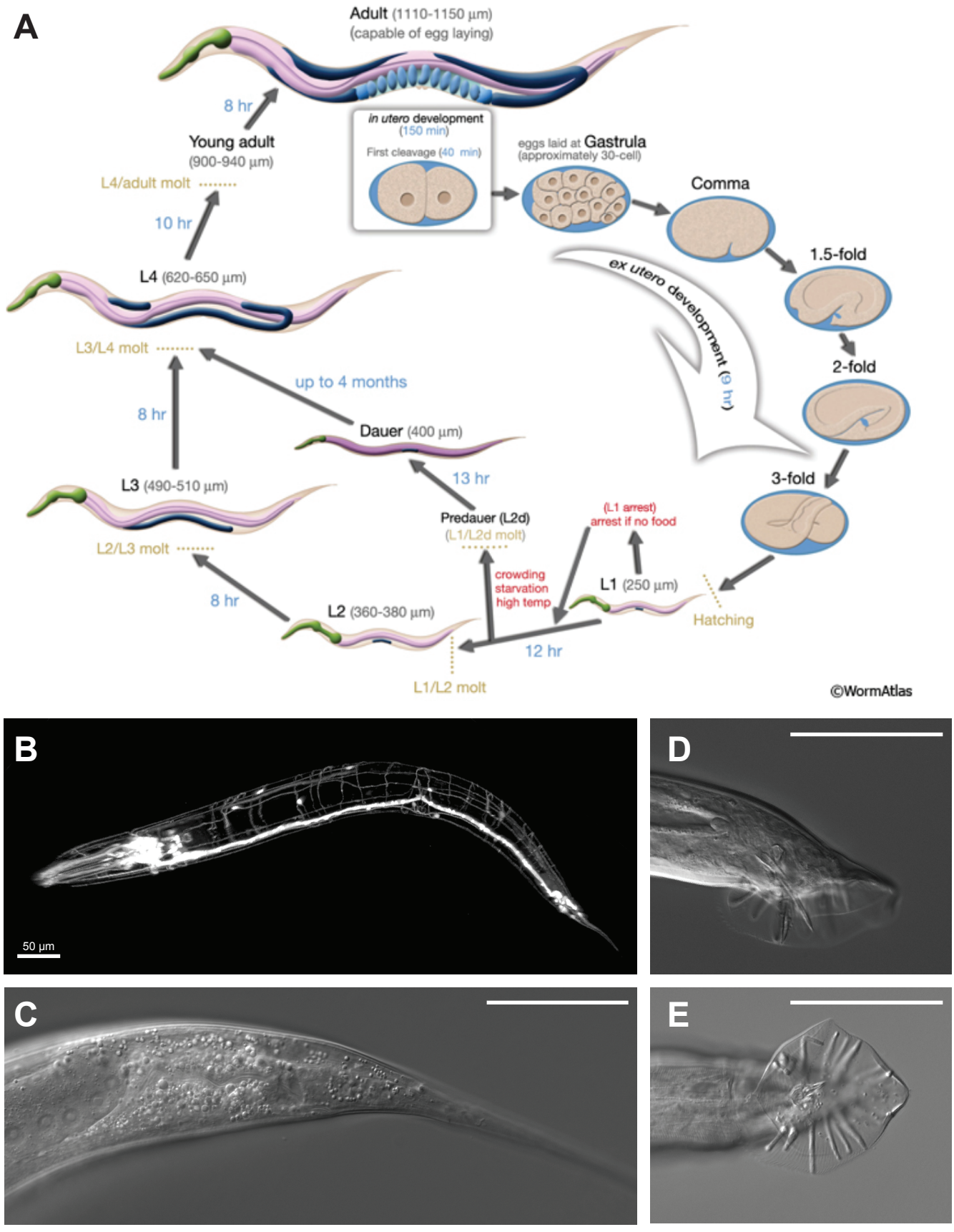
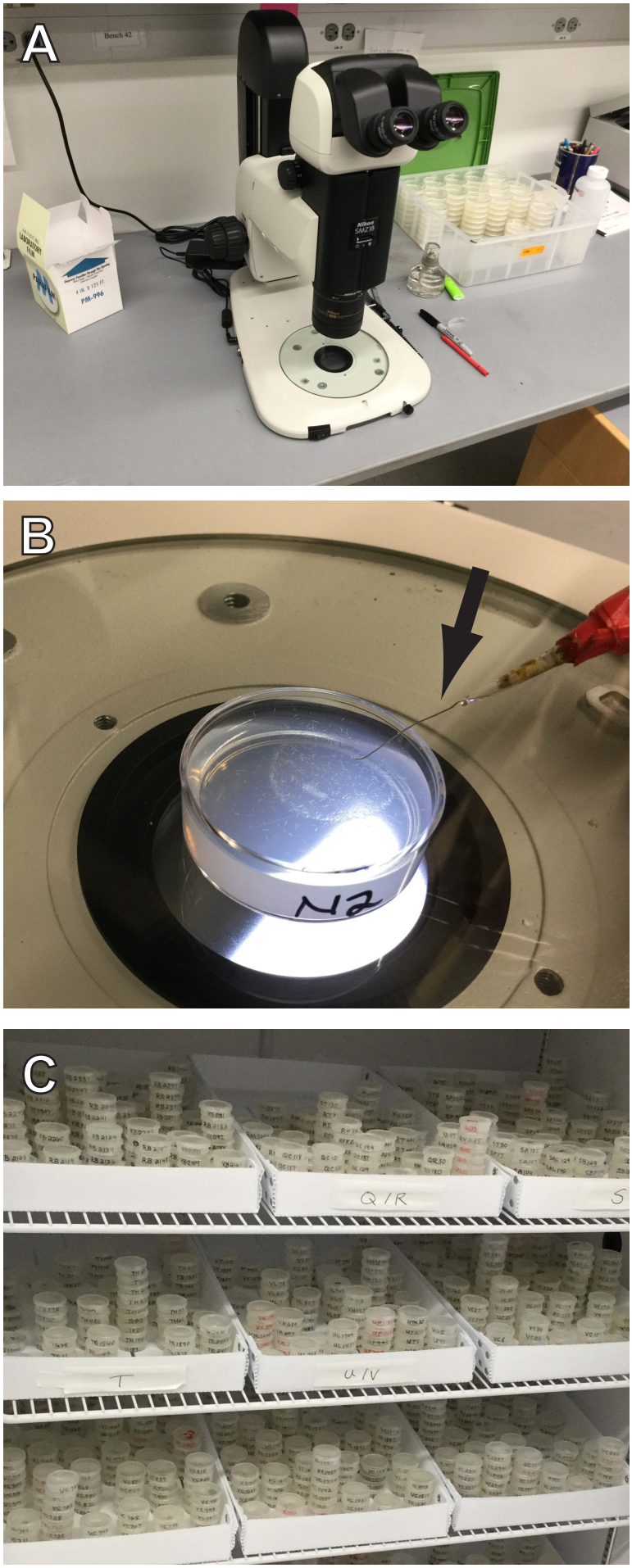


Figure 1. Daul et al. CGC & CeNDR



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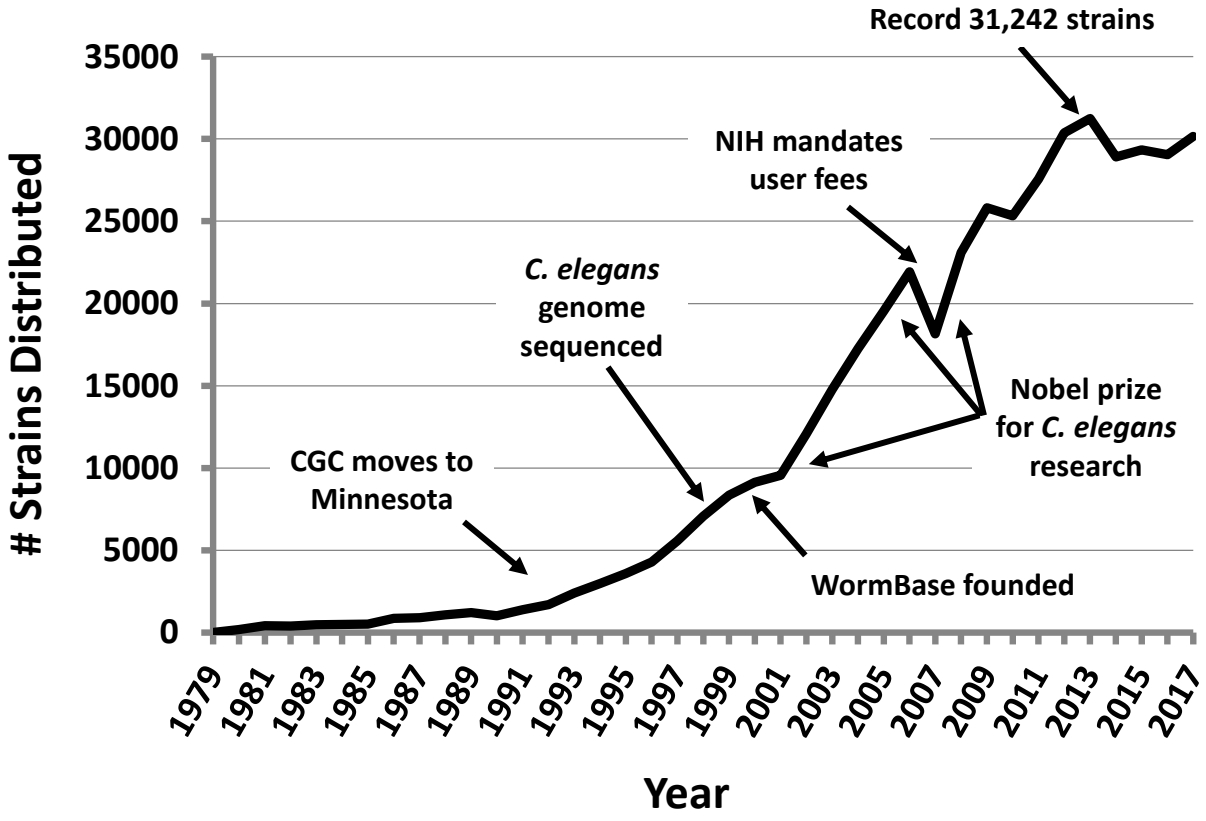
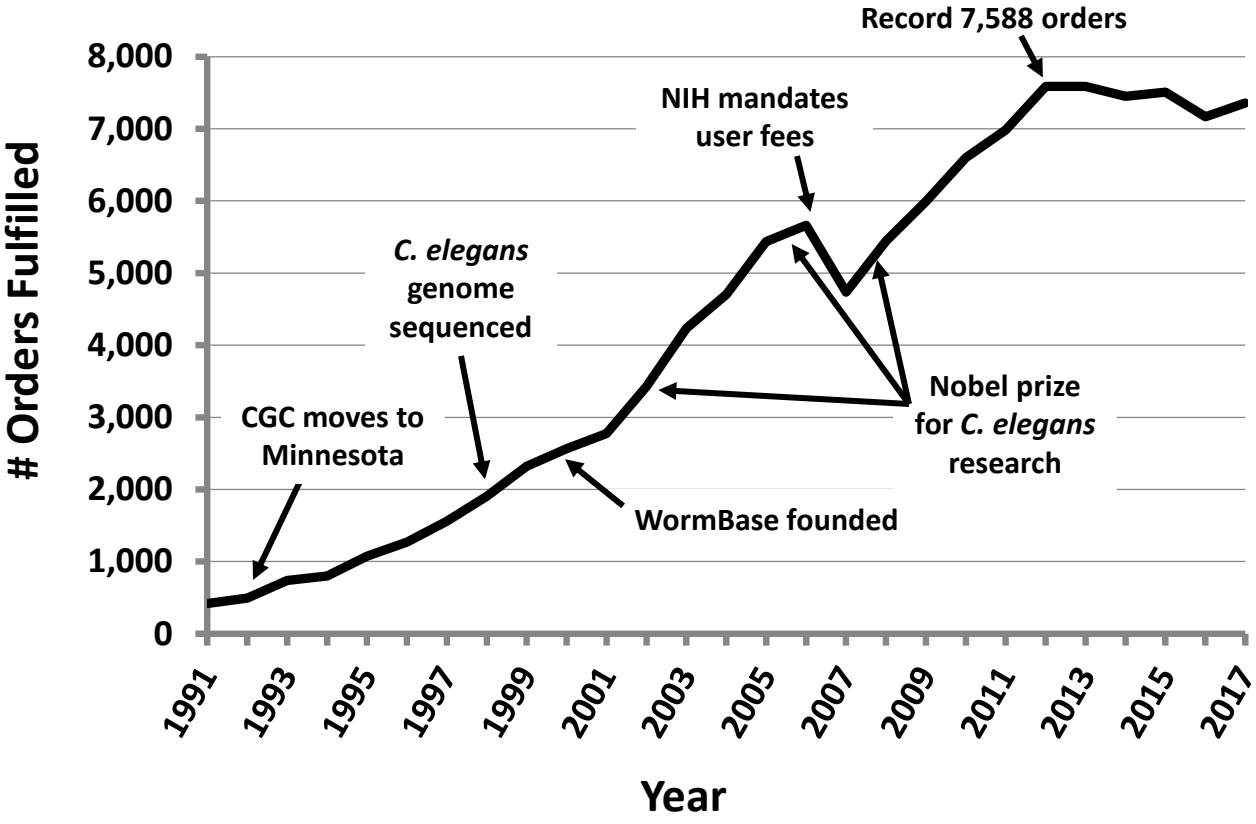
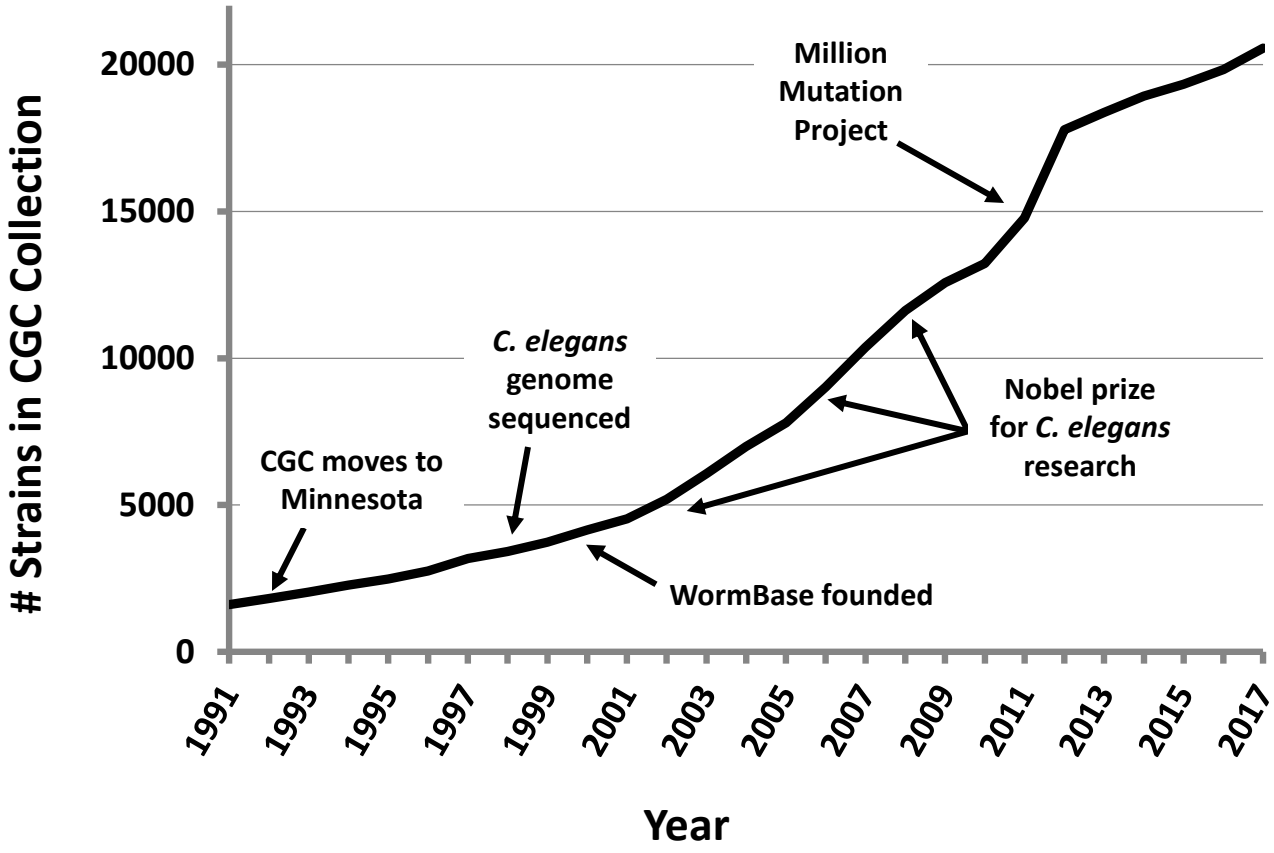


Daul et al. CGC & CeNDR Figure 3



Daul et al. CGC & CeNDR Figure 4



Daul et al. CGC & CeNDR Figure 5

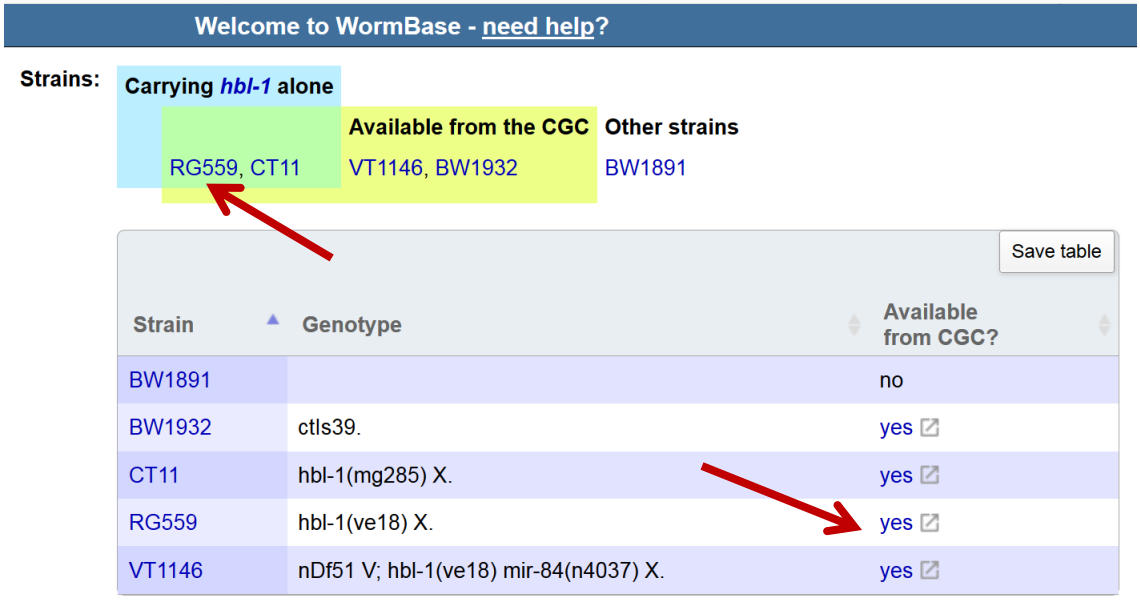
Global Strain Map

Home / Strain / **Global Strain Map**

Hover over or click a pin to see information about a *C. elegans* wild isolate

Daul et al. CGC & CeNDR Figure 6

A.



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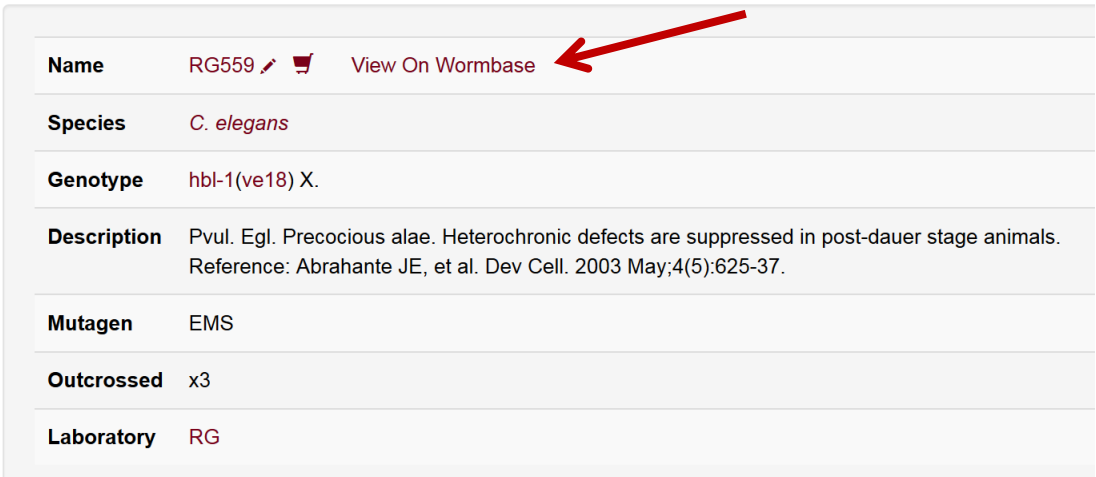

Taken from https://cgc.umn.edu/strain/RG559

Daul et al. CGC & CeNDR Figure 7