

Sampling and Isolation of *C. elegans* from the Natural Habitat

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Abstract

Wild populations of the model organism *C. elegans* allow characterization of natural genetic variation underlying diverse phenotypic traits. Here we provide a simple protocol on how to sample and rapidly identify *C. elegans* wild isolates. We outline how to find suitable habitats and organic substrates, followed by describing isolation and identification of *C. elegans* live cultures based on easily recognizable morphological characteristics, molecular barcodes and/or mating tests. This protocol uses standard laboratory equipment and requires no prior knowledge of *C. elegans* biology.

Key words *Caenorhabditis elegans*, Natural genetic variation, Natural populations, Wild isolates, Natural habitat, Ecology

1 Introduction

Insights into *C. elegans* biology are almost exclusively based on the analysis of a single reference strain (N2) but recent research has started to investigate genetic and phenotypic variation among *C. elegans* wild isolates [1]. Key findings are that the reference strain N2 shows extensive laboratory adaptation [2, 3] and that *C. elegans* natural populations show overall very little genetic differentiation across the globe [4]. Nevertheless, *C. elegans* wild isolates may exhibit marked phenotypic differences, some of which have been genetically and molecularly characterized using quantitative genetic mapping approaches [1]. Such characterization of *C. elegans* natural variation generates novel insights into *C. elegans* biology and further places the wealth of mechanistic knowledge of this model organism into a much-needed ecological and evolutionary context [5].

Hundreds of *C. elegans* isolates have been collected worldwide, and the species is clearly more abundant in temperate than in tropical regions [4–8] (Fig. 1). The few *C. elegans* isolates found in the tropics occurred mostly at high altitude [8, 9] and indicate, consistent with experimental determination of thermal optima in



Fig. 1 Geographic distribution of *C. elegans* wild isolates (adapted from refs. [4, 7, 13, 15] and C. Braendle, unpublished data). A list of available *C. elegans* wild isolates can be consulted at http://www.wormbase.org/species/c_elegans/strain#2-10-5. For information on other *Caenorhabditis* species, see [8] and for updates on species discovery, see http://evolution.wormbase.org/index.php/Main_Page

the laboratory [10], that *C. elegans* does not tolerate prolonged exposure to temperatures above 25 °C.

Current insights into the global distribution of *C. elegans* are limited due to strong sampling bias, with some regions (e.g., France) having been frequently sampled [5, 6, 11–13] whereas others (e.g., Eastern Europe) have never or only rarely been sampled. Although it is likely to colonize highly diverse habitats, *C. elegans* has primarily been isolated from anthropogenic habitats, such as gardens or agricultural lands [11–17], and only very rarely from more unperturbed sites (e.g., forests) [13], which still await in-depth sampling for *C. elegans*.

C. elegans and other *Caenorhabditis* species have been isolated from a wide variety of organic substrates, mainly decomposing vegetal matter, such as compost, fruits or plant stems [8, 18]. Despite commonly being referred to as a soil nematode, *C. elegans* has virtually never been isolated from soil samples but mostly from decaying vegetal matter on or above the soil layer. *C. elegans* has also been isolated from a range of live or dead invertebrates (e.g., beetles, isopods, millipedes, snails, and slugs) [4–6, 11, 13]. Note that in contrast to other nematode species there is no strong evidence for taxon-specific phoretic or necromenic invertebrate associations of *C. elegans* [5, 19].

In this chapter we provide a simple protocol for sampling and isolation of *C. elegans* wild isolates, chiefly aimed at researchers having minimal working knowledge using *C. elegans* as a study organism. Nevertheless, acquiring basic knowledge of *C. elegans* handling and maintenance as presented in [20] is highly recommended. Note that several alternative, yet usually more time-consuming nematode isolation protocols are available [6].

2 Materials

2.1 Collection and Storage of Substrate Samples

1. Sampling containers, e.g., resistant plastic zip-lock bags.
2. Disposable plastic gloves.

2.2 Nematode Isolation in the Laboratory

1. Dissecting stereomicroscope with transmitted light source (5–50× magnification).
2. Nematode growth medium (NGM) [20]: 1 L : 3 g NaCl, 25 g agar, 2.5 g Bacto Peptone, 975 mL H₂O. After autoclaving, add 1 mL 1 M CaCl₂, 1 mL 5 mg/mL cholesterol in ethanol, 1 mL 1 M MgSO₄, and 25 mL 1 M KPO₄. Mix well and dispense solution in Petri dishes (e.g., 90 and 55 mm diameter). (The increased agar concentration (2.5 % instead of the usual 1.7 % helps preventing burrowing of nematodes into the culture plates). Store at room temperature for 2–4 days.
3. Seed NGM plates with a central spot of *E. coli* OP50 (100–200 µL) and store at room temperature for 2–4 days. OP50 is available from the *Caenorhabditis* Genetics Center (CGC): <http://www.cbs.umn.edu/research/resources/cgc>.
4. Disposable plastic gloves.
5. Clean water or M9 buffer (1 L): 3 g KH₂PO₄, 6 g Na₂HPO₄, 5 g NaCl; autoclave; add 1 mL 1 M MgSO₄ before use [20].
6. Platinum worm pick [20].
7. Parafilm.

2.3 Morphological and Molecular Identification of *C. elegans*

1. Dissecting stereomicroscope with transmitted light source (50×–100× magnification).
2. Standard PCR reagents and equipment and access to sequencing service.

2.4 Genetic Identification Through Crosses with Established *C. elegans* Strains

1. Dissecting stereomicroscope with transmitted light source (5–50× magnification).
2. Identified *C. elegans* strain, e.g., N2 (available from the CGC: <http://www.cbs.umn.edu/research/resources/cgc>).
3. NGM plates (55 mm diameter) (*see* Subheading 2.2, item 2).

2.5 Establishment and Cryopreservation of *C. elegans* Wild Isolate Stocks

1. NGM plates (55 mm diameter) (*see* Subheading 2.2, item 2).
2. Freezing solution [20]: 300 mL: Start with 100 mL of dH₂O and add 1.76 g NaCl, 2.04 g KH₂PO₄, 1.7 mL of 1 M NaOH, 1.2 g agar, 71.6 mL glycerol. Heat to boiling until the agar is dissolved. Bring to 300 mL with dH₂O. Distribute 50 mL per 100 mL bottles; autoclave. After autoclaving or when first using, add 1.5 mL 0.1 M MgSO₄ and 100 µL 1 M CaCl₂ per 50 mL bottle [6, 20].
3. Cryovials (2 mL).
4. -80 °C freezer and liquid nitrogen storage facilities.

3 Methods

3.1 Finding Suitable Habitats and Substrate Samples

Potentially, *C. elegans* may be found in diverse, anthropogenic as well as unperturbed natural sites; however, the latter have rarely been sampled. In temperate regions of France, temporal sampling of natural populations uncovered *C. elegans* from September to November, but rarely during the summer months [13]. Such population fluctuations further depend on the specific habitat and substrate sampled, given that certain habitats, e.g., composts heaps in gardens [12], may represent more stable habitats than others, such as, seasonally occurring fruits. As a general rule, hot, dry or very cold habitats are very unlikely to yield *C. elegans* although individuals in the dauer stage may be uncovered at low densities.

Substrates potentially containing *C. elegans* encompass any organic sample that contains or contained microbial organisms serving as possible *C. elegans* food source, including fruits, plant stems and leaves, compost, animal carcasses, animal feces. Fruits in advanced stages of decomposition on the ground represent ideal target samples due to their high microbial content and likely also because they attract diverse invertebrates that might carry *Caenorhabditis* nematodes [5]. Direct sampling of such invertebrate carriers (arthropods, including insects, isopods as well as mollusks, such as snails or slugs) has yielded *C. elegans* and other *Caenorhabditis* species [5, 6, 12]. Sampling success is highest for rotting fruit/plant material that is not dry and not directly exposed to sun light.

3.2 Collection and Storage of Substrate Samples

1. Using disposable plastic gloves, collect samples from suitable habitat (*see* above) and place into container, ideally transparent plastic zip-lock bags for plant substrates (for invertebrates, use hard plastic containers, e.g., Falcon tubes). Leave ample air in the bag and add some paper towel if the sample is very liquid, to avoid fermentation.
2. Note date of collection, locality (e.g., GPS coordinates), habitat, and substrate type (e.g., take photographs).

3. Store samples in the dark, and keep at temperatures of 15–25 °C (above 25 °C may lead to sterility in *C. elegans*). Before processing in the laboratory, substrate samples may be stored for several weeks in plastic bags but samples should be regularly aerated. For high success in nematode recovery, analyze substrates within 1 week after sampling.

3.3 Nematode Isolation in the Laboratory

1. Place substrate samples on large (e.g., 100 mm diameter) NGM plates seeded with a spot of *E. coli* OP50 in the center of the plate. Tear up or cut large samples into smaller pieces and place them around the *E. coli* spot (sacrifice live invertebrates). Add 1–3 mL of clean water or M9 buffer onto the substrate. Avoid damaging the agar plates as this will facilitate burrowing of nematodes into the culture plates, making subsequent visual observation difficult. Cover the plate with lid. Samples, such as rotting fruit, usually contain diverse invertebrates (e.g., mites, insect larvae, flies): to avoid their spread and cross-contamination among plates, wrap plates with Parafilm. Keep samples at 15–20 °C.
2. Bactivorous nematodes, such as *C. elegans*, will crawl onto the *E. coli* lawn and can be easily observed using a standard dissecting stereomicroscope equipped with a transmitted light source. Nematodes may colonize the *E. coli* lawn within minutes to hours after placing the sample on the culture plate, but for samples with low densities (or individual dauer individuals) it may take several days to detect the nematodes. Keep sampling plates for 5–7 days and, after nematode isolation, autoclave before disposal.
3. Check for the presence of nematodes on the plate using a dissecting scope. Diverse bacteria and fungi stemming from the sample will also grow on the NGM plate, making observation difficult over time (*see Note 1*). If this is the case, cut out a piece of agar containing nematodes and transfer to a new plate to facilitate visual observation.
4. Pick individual nematodes to fresh NGM plates (55 mm diameter) using a platinum worm pick and amplify population (*see Note 2*).

3.4 Morphological and Molecular Identification of *C. elegans*

The following protocol for rapid and easy identification of *C. elegans* uses minimal morphological knowledge of *C. elegans* allowing an efficient initial elimination of nematodes that do not represent members of the genus *Caenorhabditis*. In a second step, only nematodes with a hermaphroditic mode of reproduction are selected. After these two initial steps of selection, analysis using species-specific DNA sequence-tag is performed to distinguish between different hermaphroditic *Caenorhabditis* species [8], which are morphologically very similar. Currently, only three androdioecious

(hermaphrodite-male) *Caenorhabditis* species have been described: *C. elegans*, *C. briggsae*, and *C. tropicalis* (*C. sp.* 11); all other species (>23) show a gonochoristic (female-male) mode of reproduction [8, 21]. For a detailed overview of *Caenorhabditis* identification methods, see [6].

1. Diverse nematode taxa may be uncovered in substrate samples, but a given sample often contains only one or two different nematode species. To check whether picked nematodes may represent *C. elegans*, use the following two criteria:
 - (a) The pharyngeal head region contains two distinct bulbs: the middle and the basal pharyngeal bulbs, the latter bulb exhibits a grinder (Fig. 2). This morphology is typical for all *Caenorhabditis* species [6] and can be examined using a dissecting scope at 50× or higher magnification (or using light microscopy).
 - (b) Test whether reproduction occurs through self-fertilizing hermaphrodites: isolate juvenile stages onto individual NGM plates and determine whether fertile progeny is produced after a 2–5 days of growth at 20°. (For isolation of gonochoristic *Caenorhabditis* species, see Note 3).

Note that several additional simple morphological criteria (e.g., long and pointy tail, central vulva, oval embryos) are characteristic for *Caenorhabditis* and may also be used for identification [6]; for detailed morphological characterization of *Caenorhabditis* species, see [8, 22, 23].

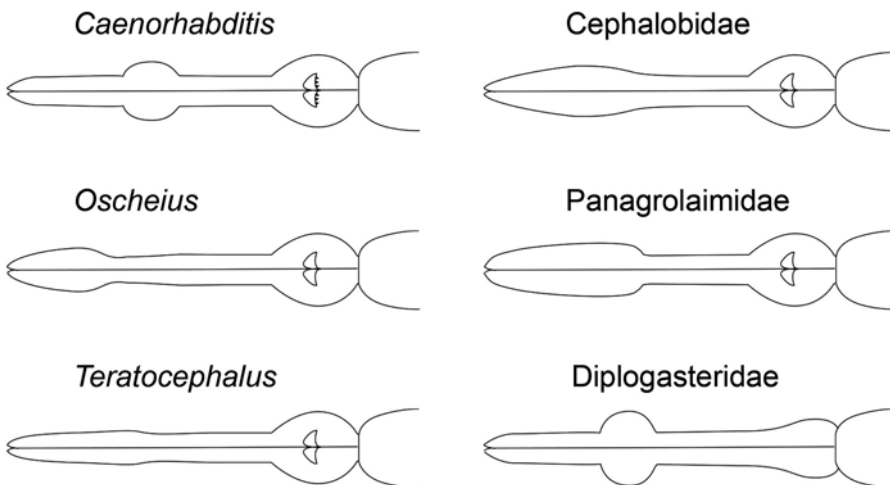


Fig. 2 Morphological characteristics of the *C. elegans* pharynx compared to a selection of other nematode taxa (adapted from [6, 24]). The presence of two circular pharyngeal bulbs is a distinctive feature of *Caenorhabditis* species and can be observed using a dissecting stereomicroscope at >50× magnification and good illumination. Commonly found in the same habitat/substrate are nematodes of the genus *Oscheius*, which superficially show an overall morphology and size similar to *C. elegans*, yet present a clearly different pharyngeal structure

2. Molecular identification of *C. elegans*: Kiontke et al. [8] have established a *Caenorhabditis* species barcoding method using sequencing of the ITS2 (Internally Transcribed Spacer) region, localized between the 5.8S and 28S rDNA genes. To amplify the region of approximately 2 kb, use the following primers:

5.8S-1: 5'-CTGCGTTACTTACCACGAATTGCARAC.

and

KK28S-4:5'-GCGGTATTTGCTACTACCAYYAMGATCTGC.

Sequence the amplified fragment with the sequencing primer KK-28S-22 (5'-CACTTTCAAGCAACCCGAC) [8].

Use NCBI Blast to check for species identity of sequence. *Caenorhabditis* ITS2 sequences are highly species-specific and isolates of a given species differ by a maximum of two nucleotide polymorphisms [8].

3.5 Genetic Identification Through Crosses with Established *C. elegans* Strains

In combination with ITS2 sequence analysis (Subheading 3.4), or as an alternative, presumptive *C. elegans* species identity of a new isolate can be verified through crosses with known *C. elegans* strains, e.g., the reference strain, N2 (available from the CGC). Place three to four L4 hermaphrodites of the new isolate together with five to ten males of the identified *C. elegans* strains, and vice versa. The presence of a high proportion of males (>30 %) in the F1 progeny indicate successful crossing, thus confirming *C. elegans* species identity.

(Note that *C. elegans* does not produce any cross progeny with any other known *Caenorhabditis* species.)

3.6 Establishment and Cryopreservation of *C. elegans* Wild Isolate Stocks

1. Strain establishment: derive any new *C. elegans* isolate (i.e., strain) from an isolated single L4 stage hermaphrodite and amplify resulting populations over 4–5 generations, so that isolates can be considered nearly isogenic [6]. Unless individuals are isolated within hours after sampling, it is advisable to derive only a single isolate from a given sampling bag/unit. Given that *C. elegans* proliferates very rapidly, also within the sample after collection, this procedure limits strain establishment of isolates with identical genotypes. Each new *C. elegans* isolate should have a unique name, ideally following *C. elegans* strain naming procedures, i.e., Lab name abbreviation followed by number ID [20].
2. Cryopreservation: All *Caenorhabditis* species can be cryopreserved [6] using standard *C. elegans* protocols, and stored at –80 °C and liquid nitrogen [20]. For a given isolate to be frozen, grow 5–10 NGM plates (55 mm diameter) until starvation when cultures contain a maximum of freshly starved L1 animals. Collect individuals from plates using M9 buffer and place solution into sterile test tube (e.g., 15 mL Falcon tube) and place on ice for 15 min. Centrifuge tube and keep 3 mL of solution containing worms, then add an equal volume

of Freezing Solution to tube and mix well. Aliquot mixture to three cryovials, labeled with strain name. Place cryovials in a small Styrofoam box with slots for holding microtubes (to ensure slow freezing of the worms) and place box in a -80°C freezer overnight. Transfer tubes to stock collections at -80°C or liquid nitrogen.

3. Database entry: Add strain information of newly discovered *C. elegans* wild isolates to Wormbase using the following form: http://tazendra.caltech.edu/~azurebrd/cgi-bin/forms/wild_isolate.cgi.

4 Notes

1. To limit fungal contaminations on NGM plates, add 0.01 % thimerosal to water or M9 solution used to moisten the substrate samples.
2. Substrates may harbor diverse microbes that are pathogenic to *C. elegans* [5]. *C. elegans* and other nematode species therefore may show pathologies preventing development or growth, and may also complicate morphological analysis. Moreover, many bacterial contaminants generate thick films on NGM plates rendering observations difficult. To generate clean plates and *C. elegans* cultures, use NaOH–sodium hypochlorite mixture to decontaminate [20].
3. To isolate gonochoristic *Caenorhabditis* species, place either a single mated female (often visible by the presence of a mating plug) or a female plus male to a fresh plate. For detailed isolation methods of gonochoristic *Caenorhabditis* species, see [6].

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References

1. Gaertner BE, Phillips PC (2010) as a platform for molecular quantitative genetics and the systems biology of natural variation. *Genet Res (Camb)* 92:331–348
2. Andersen EC, Bloom JS, Gerke JP et al (2014) A variant in the neuropeptide receptor npr-1 is a major determinant of *Caenorhabditis elegans* growth and physiology. *PLoS Genet* 10, e1004156

3. McGrath PT, Xu Y, Ailion M et al (2011) Parallel evolution of domesticated *Caenorhabditis* species targets pheromone receptor genes. *Nature* 477:321–325
4. Andersen EC, Gerke JP, Shapiro JA et al (2012) Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. *Nat Genet* 44:285–290
5. Félix M-A, Braendle C (2010) The natural history of *Caenorhabditis elegans*. *Curr Biol* 20:R965–R969
6. Barrière A, Félix M-A (2014) Isolation of *C. elegans* and related nematodes. In: *The C. elegans Research Community* (ed) *Wormbook*. <http://dx.doi.org/10.1895/wormbook.1.115.2>
7. Gimond C, Jovelin R, Han S et al (2013) Outbreeding depression with low genetic variation in selfing *Caenorhabditis* nematodes. *Evolution* 67:3087–3101
8. Kiontke K, Félix M-A, Ailion M et al (2011) A phylogeny and molecular barcodes for *Caenorhabditis*, with numerous new species from rotting fruits. *BMC Evol Biol* 11:339
9. Dolgin ES, Felix MA, Cutter AD (2008) Hakuna Nematoda: genetic and phenotypic diversity in African isolates of *Caenorhabditis elegans* and *C. briggsae*. *Heredity* 100:304–315
10. Anderson JL, Albergotti L, Ellebracht B et al (2011) Does thermoregulatory behavior maximize reproductive fitness of natural isolates of *Caenorhabditis elegans*? *BMC Evol Biol* 11:157
11. Barrière A, Félix M-A (2005) High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. *Curr Biol* 15:1176–1184
12. Barrière A, Félix M-A (2007) Temporal dynamics and linkage disequilibrium in natural *C. elegans* populations. *Genetics* 176:999–1011
13. Felix MA, Duvéau F (2012) Population dynamics and habitat sharing of natural populations of *Caenorhabditis elegans* and *C. briggsae*. *BMC Biol* 10:59
14. Haber M, Schüngel M, Putz A et al (2005) Evolutionary history of *Caenorhabditis elegans* inferred from microsatellites: evidence for spatial and temporal genetic differentiation and the occurrence of outbreeding. *Mol Biol Evol* 22:160–173
15. Petersen C, Dirksen P, Prah S et al (2014) The prevalence of *Caenorhabditis elegans* across 1.5 years in selected North German locations: the importance of substrate type, abiotic parameters, and *Caenorhabditis* competitors. *BMC Ecol* 14:4
16. Sivasundar A, Hey J (2005) Sampling from natural populations with RNAi reveals high outcrossing and population structure in *Caenorhabditis elegans*. *Curr Biol* 15:1598–1602
17. Caswell-Chen EP, Chen J, Lewis EE et al (2005) Revising the standard wisdom of *C. elegans* natural history: ecology of longevity. *Sci Aging Knowl Environ* 40:pe30
18. Felix MA, Jovelin R, Ferrari C et al (2013) Species richness, distribution and genetic diversity of *Caenorhabditis* nematodes in a remote tropical rainforest. *BMC Evol Biol* 13:10
19. Kiontke K, Sudhaus W (2006) Ecology of *Caenorhabditis* species. *The C. elegans Research Community* (ed) *Wormbook*. <http://dx.doi.org/10.1895/wormbook.1.37.1>
20. Stiernagle T (2006) Maintenance of *C. elegans*. *The C. elegans Research Community* (ed) *Wormbook*. <http://dx.doi.org/10.1895/wormbook.1.101.1>
21. Felix MA, Braendle C, Cutter AD (2014) A streamlined system for species diagnosis in *Caenorhabditis* (Nematoda: Rhabditidae) with name designations for 15 distinct biological species. *PLoS One* 9, e94723
22. Sudhaus W, Fitch D (2001) Comparative studies on the phylogeny and systematics of the Rhabditidae (Nematoda). *J Nematol* 33:1–69
23. Sudhaus W, Kiontke K (1996) Phylogeny of Rhabditis subgenus *Caenorhabditis* (Rhabditidae Nematoda). *J Zoo Syst Evol Res* 34:217–233
24. Chiang JT, Steciuk M, Shtonda B et al (2006) Evolution of pharyngeal behaviors and neuronal functions in free-living soil nematodes. *J Exp Biol* 209:1859–1873