INTRODUCTION TO RESUBMISSION APPLICATION

This proposal is a resubmission for application R21AG053638, "High-throughput multi-modal analysis of natural variation in *C. elegans* healthspan," which was reviewed in February 2016 at the CMAD scientific review group. The initial application was not discussed. We are grateful for the opportunity to address the reviewers' concerns in this revised application. Our interpretation of the summary statement for the initial submission is that the reviewers found the proposed project to be of great interest. They recognized the innovative technology in the project ("*the concept and technologies…are innovative*", *"The experiments utilize creative and cutting–edge technologies*") and the talents of the investigators ("*pioneers and experts in [their] fields*"). Reviewers praised the potential of the proposed work *("If successful, this project will develop a broadly useful automated system for multi-modal assays of C. elegans healthspan and provide the first mechanistic understanding of natural variation in aging.")*

The primary major concern expressed the reviewers was that the aging phenotypes of the N2 *C. elegans* strain may not be applicable to wild isolate strains. We address this concern in several ways. First, we tested the WorMotel method with four representative wild isolate strains and found no technical issues (for example, animals escaping from the wells) that would prevent wild isolates from being studied with our technique. Second, these preliminary studies yielded results for healthspan metrics that varied with age in a manner qualitatively similar to that of the laboratory strain. This result shows that *C. elegans* wild isolates are representative of how the species ages and not very different from the laboratory strain N2 in this regard. Finally, while our data from wild isolate strains were qualitatively similar to N2, they were *quantitatively* different both from N2 and from one another, demonstrating that wild isolates have heritable trait differences that can be mapped in our project.

Reviewer #3 stated that "the successful completion of the aims will not yield biological insight but rather will establish technology and preliminary results that might be useful in future studies." In our view, biological insight into the relevance of wild *C. elegans* strains into the mechanisms of healthspan and aging is itself a crucial discovery. To determine the broad relevance and translational potential of discoveries using *C. elegans*, we need to study more than just one strain in the species and we need to use quantitative assays and approaches. The future studies enabled by our project will be directly relevant to the use of model organisms to understand healthspan. Reviewer #3 also expressed concern about the lack of testable hypotheses in our proposal. Our long-term central hypothesis is that our analysis of natural variation in *C. elegans* will reveal novel conserved regulators of aging. As intermediate goals of the proposed work, we will test the hypothesis that declines in a battery of non-locomotory healthspan measures will predict lifespan to a greater degree than for locomotory metrics in Aim 1. Furthermore, we will test in Aim 2 the hypothesis that heritable variation in quantitative healthspan measures from Aim 1 are present in the *C. elegans* species.

One reviewer was concerned that the development of new assay capabilities requires significant innovations that are of uncertain feasibility. We note that only one assay (automated measurement pharyngeal pumping) will require significant technical development, and under Pitfalls and Alternative Approaches we describe a simple alternative strategy of conducting pumping assays manually. Therefore, we believe the overall level of risk in the proposal is low. Reviewer #3 suggested that stress resistance might not yield any more information than aging itself. Considering that that oxidative stress is only one potential mechanism of aging, and a rather controversial one at that, we do not think it can be safely assumed that oxidative stress resistance will vary in a manner identical to longevity in *C. elegans*.

Although we found no evidence that healthspan assays for wild isolates differ in a fundamental way from that of the laboratory strain, it is still conceivable that the biology of aging in some wild isolates will be different in some respects from that of N2. We believe that knowledge of such differences would be critical for the use of *C. elegans* to study aging. There is growing recognition that to fully realize the potential of *C. elegans* to understand the mechanisms of aging, we must go beyond the laboratory strain. Indeed, a core objective of the NIA's *Caenorhabditis* Intervention Testing Program (CITP) is to identify interventions that robustly affect longevity in multiple *C. elegans* strains and across diverse *Caenorhabditis* species. This goal reflects an acceptance that the laboratory strain should not be the sole basis for conclusions about this species or clade. Our proposal to study natural variation in *C. elegans* aging also supports this idea.

We have made several other improvements to the proposal as well. In the Approach, we added preliminary data from four diverged wild strains and expanded the Innovation section to make our approach more understandable. In summary, we believe this research plan will be an important complement to the CITP and a critical step in expanding the *C. elegans* aging research beyond the laboratory strain.

RESEARCH STRATEGY

A. SIGNIFICANCE

Natural variation in human aging

Aging varies greatly across the human population. The association between chronological age and health status is much more variable than is often recognized [1]. Although environmental factors play an important part in this variability, twin studies have shown that 23% (for males) and 26% (for females) of the population variance in lifespan is caused by genetic factors [2]. Understanding this genetic component will be important for developing strategies to slow or mitigate aging and its associated illnesses, but progress toward this goal has been limited. Genome-wide association (GWA) studies, which test for correlations of genotype and phenotype for a particular trait [3], have identified only a small number of candidate genes for human longevity [4]. To date, the only reproducible finding across different human populations is that genetic variants in the apolipoprotein E gene *APOE* are correlated with differences in lifespan [5]. Less powerful gene-specific tests have also revealed a link between lifespan and the gene encoding the forkhead family transcription factor FOXO3A [6–9], which is homologous to *C. elegans* DAF-16. Many expensive GWA studies have been contentious [10] and nearly all of them were statistically underpowered [11].

Human geneticists next turned to whole-genome sequencing (WGS) to identify variant genes common to long-lived individuals [12]. However, the expense of WGS has limited this approach to a handful of individuals and no common protein-coding variants have been discovered [13]. The NIH Precision Medicine Initiative along with commercial efforts aim to complete millions of human sequences. These efforts are promising but their results remain years away. Moreover, such studies may encounter the same difficulties as statistically underpowered GWA studies because the addition of more genomes, each with more rare variants, encumbers more statistical tests and a more strict significance threshold. Identifying the genetic basis of aging variation ideally requires (1) uncorrelated measures of aging that can be used as traits to track healthy aging across the population and (2) scaling of quantitative assays to measure healthspan for a large number of individuals.

C. elegans as a model for aging variation

The roundworm *C. elegans* has been enormously useful for the discovery of genes that regulate aging [14]. A number of aging-associated pathways studied in *C. elegans*, including insulin/insulin-like signaling, DAF-16/FOXO, TOR, and dietary restriction, have proven to be conserved in other animal models and possibly in humans [15]. One limitation of longevity studies in model systems, which typically rely on a single laboratory-adapted strain, has been the lack of a connection to population variation. In the biology of aging and other areas, investigations of natural variation between and within species have served as an important complement to other genetic approaches. For example, studies of naked mole rats, which have lifespans more than ten times that of typical laboratory rats, have revealed processes and compounds that modulate aging [16,17]. Within *C. elegans*, natural variation has been exploited to analyze genetic incompatibilities, pathogen avoidance, oxygen sensation, offspring production, growth rate, drug resistance, and a variety of other traits [18–22]. We propose to develop quantitative assays for healthspan to make *C. elegans* a powerful model for natural variation in aging. As in human genetics, we require scalable and quantitative assays to measure *C. elegans* healthspan for a large number of independent strains. We hypothesize that we can use the variation in aging present in the *C. elegans* population to identify novel factors that regulate healthy aging.

Our work will complement that of the NIH *Caenorhabditis* Intervention Testing Program (CITP), which seeks to identify pharmacological interventions that increase lifespan and/or healthspan in multiple *Caenorhabditis* species and multiple *C. elegans* natural strains. While the CITP explores the effects of compounds on aging, we will focus on understanding the genetic basis of natural variation in aging. At present, CITP does not perform any characterization of healthspan or lifespan beyond the measure of fraction surviving each day. Our proposed work will define additional and untested lifespan/healthspan traits that can be evaluated as a part of CITP. Additionally, once preliminary data on healthspan are collected and analyzed for heritability and correlations, we will use the entire collection of *C. elegans* wild strains to identify molecular mechanisms for how aging varies across this species. This information will be invaluable towards understanding how drug treatments affect multiple *Caenorhabditis* species and translate these results to the divergent human population. We are therefore enthusiastic about potential collaborations with the CITP.

An integrated platform for the measurement of healthspan traits

We propose to create and optimize an experimental platform to measure *C. elegans* healthspan for a large number of independent strains by adapting a previously developed device for automated analysis of

lifespan in a scalable microfabricated multi-well format [23]. Using this system, we will assay a collection of quantitative traits for 16 of the most divergent *C. elegans* wild isolates and determine the genetic contribution for each trait. This platform and statistical analyses will provide the necessary preliminary data to perform association mapping studies, facilitating the first genome-wide and species-wide approach to identify functional healthspan variation in a metazoan. We will fine map using whole-genome sequence data from a large panel of over 500 wild *C. elegans* strains to identify the variant genes. These results will give mechanistic insights into healthspan that can make causal connections between the thousands of correlated variants discovered by human genome sequencing and lifespan variation. In addition to its application in quantitative genetics, our platform will be a broadly useful tool for analysis of *C. elegans* aging. We will actively work to disseminate our plans and software with the research community, including CITP.

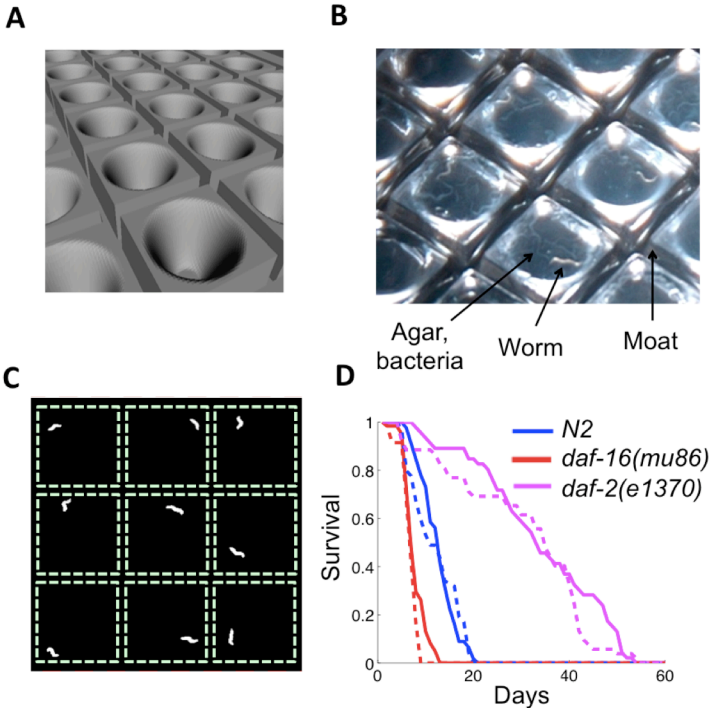


Fig. 1. WorMotel multi-well array. (a) 3D rendering showing well and moat geometry. (b) Filled WorMotel array. (c) Thresholded image of nine wells. (d) Survival curves from WorMotel (solid lines) and manual methods (dashed lines).

B. INNOVATION

Automated tracking of healthspan phenotypes

As described above, the quantitative analysis of natural variation in aging has been stymied by the lack of comprehensive and scalable phenotypic assays. To address these limitations, we have developed the WorMotel, a system for long-term cultivation and imaging of *C. elegans* in microfabricated multi-well arrays (Fig. 1). By using an automated plate-handling robot, this system is capable of longitudinal tracking of locomotion and lifespan in a large number of animals.

Previous automated or semi-automated techniques for analysis of aging in *C. elegans* have been reported. Genetic and compound screens for nematode aging have generally employed liquid culture conditions in microtiter plates for moderately high throughput but have been dependent on manual observation and were not capable of longitudinal analysis [24]. Another method employs microfluidic chambers, which have limited scalability and are usually restricted to specific developmental stages [25]. Another system employs flatbed scanners to image tens of thousands of animals on agar plates [26]. However, because of a low frame rate (~1 / hour), it cannot track young animals or easily quantify behavior. As a result, none of these technologies are suitable for quantitative genetic analysis of natural variation in aging.

In the proposed work, we will expand the phenotypic capabilities of the WorMotel system by integrating assays for different aspects of health: avoidance behavior, feeding rates, presence of aging pigments, and stress resistance. Each of these characteristics has been assayed in the context of previous studies, but they have been studied largely in isolation using manual methods. Our work will be the first to consider them together as an ensemble description of health, integrate them with a high-throughput automated assay, and probe their contributions to lifespan using statistical genetics. This approach necessitates a quantitative ascertainment of the traits at high accuracy and replication, facilitating a study of correlations among the traits to better describe healthspan as an organismal trait.

Genes underlying natural variation in aging

Longevity was one of the first traits investigated by quantitative geneticists using *C. elegans*. More than thirty years ago, researchers noticed that the laboratory strain and a wild strain had differences in lifespan [27]. Initial studies found that 34-52% of the differences in lifespan across the population were controlled by genetic factors [27,28]. With these encouraging calculations of lifespan heritability, investigators initiated mapping experiments to identify the genes that vary to cause differences in lifespan. Several different *C. elegans* strains and recombinant progeny were measured for lifespan, including the laboratory strain and four wild strains [29–32]. A variety of simulations [33] and empirical results [19] show that these mapping experiments were statistically underpowered because few recombinant strains were created and analyzed, reflecting the difficulty of measuring lifespan for hundreds of independent strains.

Notably, no genes have been identified in these mapping experiments, but some major conclusions can be drawn. First the visual inspection of living or dead animals is noisy and subjective, leading to statistical block effects in the best cases and large unknown confounders in the worst cases. Second, only a small number of mapped genomic regions were replicated in independent studies. This lack of replication could be caused by the choice of wild strains, in addition to the limited statistical power and lifespan measurement noise. Many of these strains have active transposition of mobile genetic elements, leading to unstable genotypes as strains are propagated over time. Although nearly every study had these major problems, a common locus on chromosome IV was identified using only these four strains in the species. Along with high heritability of the lifespan trait, this result indicates that an understanding of natural variation in lifespan is experimentally tractable with a better strain set, more quantitative measures of aging, and larger populations. These initial published results (and our preliminary results discussed later) indicate the basic biology of aging in wild *C. elegans* strains is shared with the laboratory-adapted N2 strain.

Using our automated assays of health phenotypes, we will define the traits that are most reproducible and have the largest genetic contributions. These results will position us to perform subsequent mappings to identify the genes responsible for variation in healthy aging. Our work will greatly improve on previous studies, which were statistically underpowered because of a small number of sampled genotypes (five) and small population of recombinant individuals analyzed. Our 16 chosen strains represent 74.4% of the total known variation in the *C. elegans* species. This significant sampling of genetic diversity will allow us to characterize phenotypic variation and perform subsequent statistical analyses. Once quantitative healthspan assays are optimized and heritability established using the 16 divergent wild strains, we will be able to measure aging traits for our entire collection of more than 500 fully sequenced wild isolates. The phenotypes from this panel will be correlated with existing genotype information in genome-wide association mappings and subsequent fine mappings using whole-genome sequence data.

C. APPROACH

Aim 1. To develop a system for multi-modal automated healthspan assays based on a microfabricated multi-well imaging device.

Automated assays of lifespan using the WorMotel

Each WorMotel consists of a transparent polymer substrate containing a rectangular array of 240 wells each 3 mm in diameter (Fig. 1). Each well is filled with 15 µL of NGM agar and seeded with bacteria, which the animal eats. A single animal is added to each well, either manually or using a COPAS Biosort (Union Biometrica) nematode sorter. The rounded, aspheric geometry of each well is designed to minimize interference from optical scattering at the edge of each well and to prevent animals from burrowing under the agar surface (Fig. 1b) [23]. A narrow liquid-filled moat surrounding each well prevents animals from escaping from their wells. Our original devices were fabricated from polydimethylsiloxane (PDMS) molded from a 3D-printed master. We are also developing an injection-molded polystyrene version.

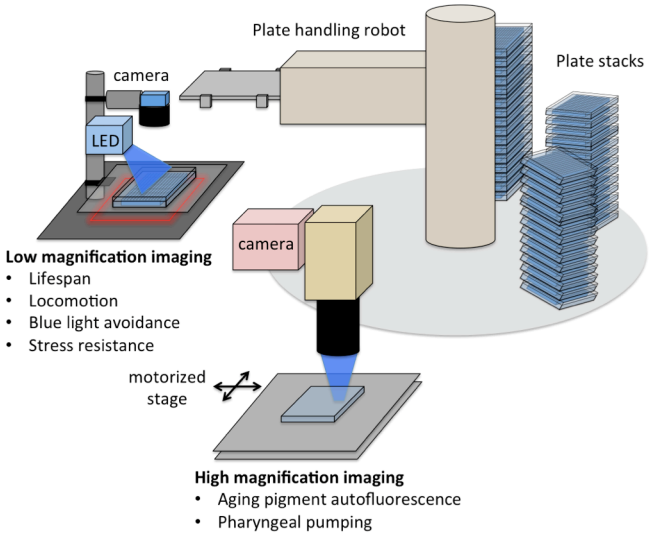


Fig. 2. Proposed automated system for screening health phenotypes. Upper left: low magnification imaging system. Upper right: Plate handling robot. Below: High magnification imaging system.

After being loaded with nematodes, the arrays are imaged at 1-10 frames per min. under LED dark field illumination with a 5-10 megapixel camera [34]. A machine vision algorithm quantifies the amount of movement in each well and records body shape properties such as centroid, area, and posture. Survival curves for long-lived, normal, and short-lived strains in the WorMotel were in excellent agreement with results from standard manual methods carried out in parallel (Fig. 1d). These results show that the WorMotel environment is similar to the standard laboratory conditions with regard to lifespan. To scale up lifespan measurements, we have integrated the WorMotel imaging system with a plate handler robot and a microplate stacker that can serially image ~90,000 individual worms (Fig. 2).

Automated healthspan assays

In addition to measuring lifespan, the WorMotel system also measures the activity levels of each animal over time. Locomotor activity has been used as a measure of health in many studies of aging in model systems

and humans [35,36]. Although locomotor activity is an important indicator of the animal's status, it is only one of several indicators of organismal health. Moreover, evidence suggests that locomotor activity alone provides an incomplete picture of healthspan. For example, we found that mutants in the *daf-2*/insulin/insulin-like signaling pathway exhibit quiescence during aging. However, upon removal from food, they are as active as wild-type animals of the same age. Therefore, we need additional measures of health span. Toward this end, we will develop a collection of health assays, based on previously validated studies [37] and adapted for high-throughput, automated or semi-automated analysis of animals in the WorMotel. We will expand the current mobility-based healthspan assay in part by incorporating a high-magnification imaging system in parallel to the low-magnification system (Fig. 2). We will develop assays to measure:

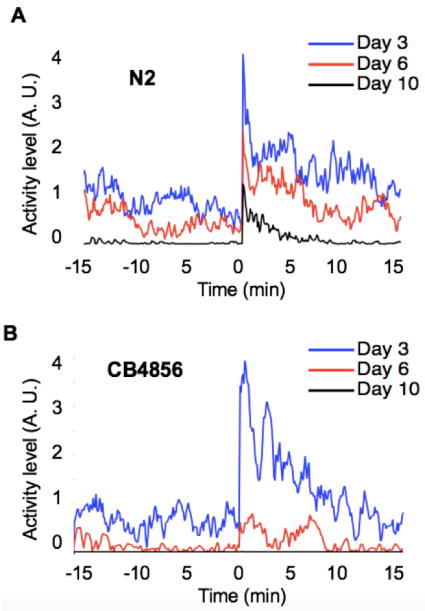


Fig. 3. A. Average activity level of (a) laboratory N2 and (b) wild isolate CB4856 worms in a WorMotel before and after a 10 s blue light pulse at time 0. B. Data from other wild isolate strains are similar (not shown)

A. *Avoidance behavior*. A number of stimuli generate aversive behaviors in *C. elegans*. These include increased temperature, mechanical touch, osmotic shock, and blue or UV light. We will use a high power LED array (Luminus) to illuminate the 240-well WorMotel with blue light at 480 nm, with irradiance 1 mW/mm^2 for ten seconds. This dose is sufficiently small to avoid phototoxicity. We will measure the activity (amount of movement) of each animal for 15-minute periods before and after illumination. Preliminary results show that blue light robustly induces an increase in activity, and that this response declines with age (Fig. 3) in the laboratory (N2) strain and for four wild isolates tested.

B. *Stress resistance*. Aging has been described in terms of its resistance to cellular stress. Some long-lived mutants are more resistant than wild-type animals to external stressors such as temperature or reactive oxygen species (ROS) generating compounds. To assay stress resistance, we will expose some animals to the oxidative stressor paraquat (20 mM) and record their subsequent activity and lifespan within the automated imaging system. Unlike the other assays described here, stress resistance assays can only be performed once in a given animal's lifetime and these individuals will be removed from the study after the assay is complete. This assay will be done only on a subset of plates with the remainder left to age normally. We will conduct a stress resistance assay at two time points: days 1 and 6 of adulthood. Preliminary results show that resistance to oxidative stress varies significantly between different wild isolate strains [33].

***C. Pharyngeal pumping*.** *C. elegans* feeds by rhythmic contractions (pumps) of the pharynx, a neuromuscular tube connecting the mouth and intestine. In wild-type animals, pharyngeal pumping rate declines rapidly with age from about 3 Hz during early adulthood to about 1 Hz at one week [38]. Pumping is most readily visible as an anterior-posterior movement and change of shape of the grinder, a tooth-like structure in the second (terminal) bulb of the pharynx. Imaging pharyngeal pumping requires high spatial and temporal resolution (2-3 micron resolution at 30 fps). We will use a scanning-stage microscope setup to view one well at a time. Then, we will use machine vision algorithms to segment the animal and track its terminal bulb over time. Pharyngeal pumping will be measured by estimating the velocity of the grinder via particle image velocimetry algorithms. Similar tracking algorithms have been used to quantify pumping rate in previous studies by our group [39]. We have confirmed that pumping rates decline with age in a similar manner in the laboratory strain and a diverged strain from Hawaii (CB4856).

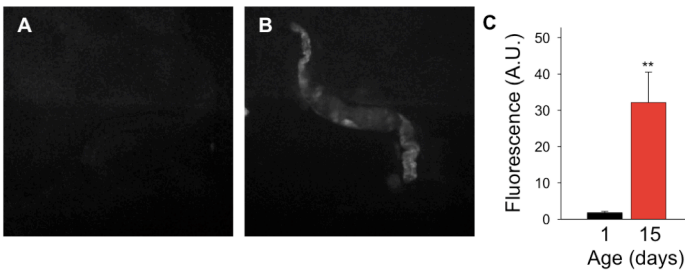


*Fig. 4. (a) Weak autofluorescence of a first-day N2 adult animal in a WorMotel well. (b) Autofluorescence of a day 15 animal. (c) Comparison of young and old animals (n=10-15). **p<0.01*

***D. Aging pigment autofluorescence*.** The *C. elegans* intestine has granules containing autofluorescent aging pigments that increase in brightness with age (Fig. 4). These pigments, which are visible under a GFP fluorescence filter, are a measure for assaying the overall health of the animal. Using the scanning microscope, we will quantify autofluorescence of each animal once per day using GFP filters.

We will perform blue light avoidance and stress

resistance assays in the low magnification system (Fig. 2). Pharyngeal pumping and autofluorescence assays will be performed in the high magnification, which will be equipped with motorized XY and focus stages to scan through individual wells through the 2X, 0.50 numerical aperture objective lens of a fluorescence stereomicroscope (Leica). Coordination of the stages, cameras, and robot will be performed using MATLAB.

These measures will define a battery of metrics providing a more complete picture of health. The rate of locomotory decline during the first few days of adulthood can predict some degree of variation in the lifespan. We will test the hypothesis that declines in a battery of nonlocomotory healthspan measures will also predict lifespan to a similar or greater degree.

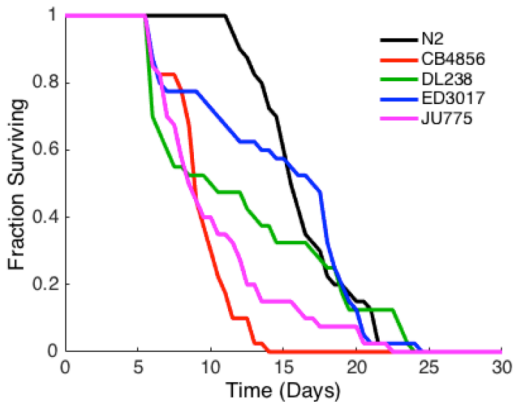


Fig. 5. Survival curves of the laboratory strain (N2) and four wild isolates, assayed on a single WorMotel array (n=40 for each strain)

Potential pitfalls and alternative strategies for Aim 1

Relevance of aging assays: It is conceivable that some healthspan assays developed using the reference N2 strain will not be appropriate for some or all of the wild isolates, due to physiological differences among the strains. First, we have tested four diverged wild isolate *C. elegans* strains with our WorMotel aging assays. We found that all are compatible with the methods and exhibit qualitatively similar (although quantitatively different) aging profiles as the reference N2 strain (Fig. 5). These results suggest it is unlikely that these variants of a single species will have fundamentally different aging characteristics. Second, even if one or more aging assays proves to be inappropriate for wild isolates, finding and describing any such differences will be very valuable for the field, as it would call for the reinterpretation of specific aging assays and/or the use of the laboratory strain for translation of aging studies beyond nematodes. Third, we note that our major goals do not depend on any specific healthspan parameter.

***Pharyngeal pumping*:** It may be difficult to conduct automated assays of pharyngeal pumping with high reliability. If this proves to be a limitation, we will use manual or semi-manual analysis of pumping rate based on reviewing videos in a smaller cohort (20 animals per strain, each assayed for one minute every four days).

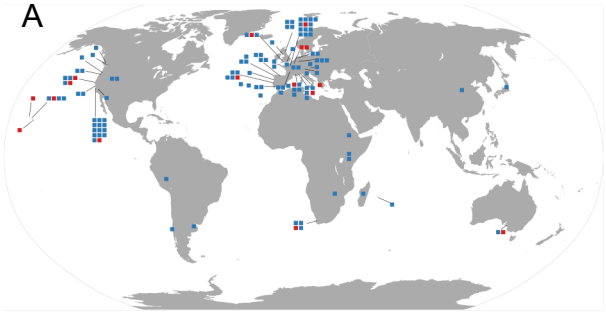
Aim 2. Carry out longitudinal assays for 16 genotypically diverged *C. elegans* strains to determine traits correlated with healthspan decline

Choice of 16 genotypically diverged strains

The Andersen group has amassed a collection of over 500 wild *C. elegans* strains from colleagues and collaborators throughout the world. This collection encompasses all of the known isolation locations and samples with at least two independent strains derived from each natural substrate (rotting fruit, compost, isopods, etc.). We used both short- and long-read whole-genome sequencing to identify and characterize the variation of these strains. Thus far, we identified over 1.5 million single-nucleotide variants (SNVs), 600,000 short insertion/deletion variants, and 1,200 mobile element transposition events. We determined the 16 most genotypically diverged strains in the *C. elegans* species by calculating the level of variation and the relatedness of strains (Fig. 6). These 16 strains are diverged from one another and from the laboratory reference strain, together capturing 74.4% of the total variation in the species. We chose to characterize the variation in healthspan of these 16 genotypically diverged strains because we expect that genotype will be correlated with phenotype and the strains will differ in diverse healthspan traits.

Determination of heritability and the level of replication required for genetic mapping experiments

In order to use GWA mapping to identify the genes that vary to cause differences in healthspan across the *C. elegans* species, we need to determine which traits are genetically tractable and the level of replication required to observe statistically significant differences. Heritability describes the fraction of variance across the population that is influenced by genetic factors. Because we ultimately want to map genetic variants, this parameter is an important determinant for each trait. We will calculate broad-sense heritability as the amount of trait variance that can be assigned to strain divided by the total trait variance. Broad-sense heritability for lifespan is up to 26% in humans and up to 52% in *C. elegans*. We have already established an estimate of this parameter using a small number of strains and will define this parameter for each of the traits described in Aim 1 using the diverse set of 16 strains. Our subsequent studies will focus on traits with heritabilities greater than 20% because statistical power calculations show that these traits are most addressable by GWA mapping in *C. elegans* [33]. To determine the level of replication necessary to obtain statistically significant results, we will



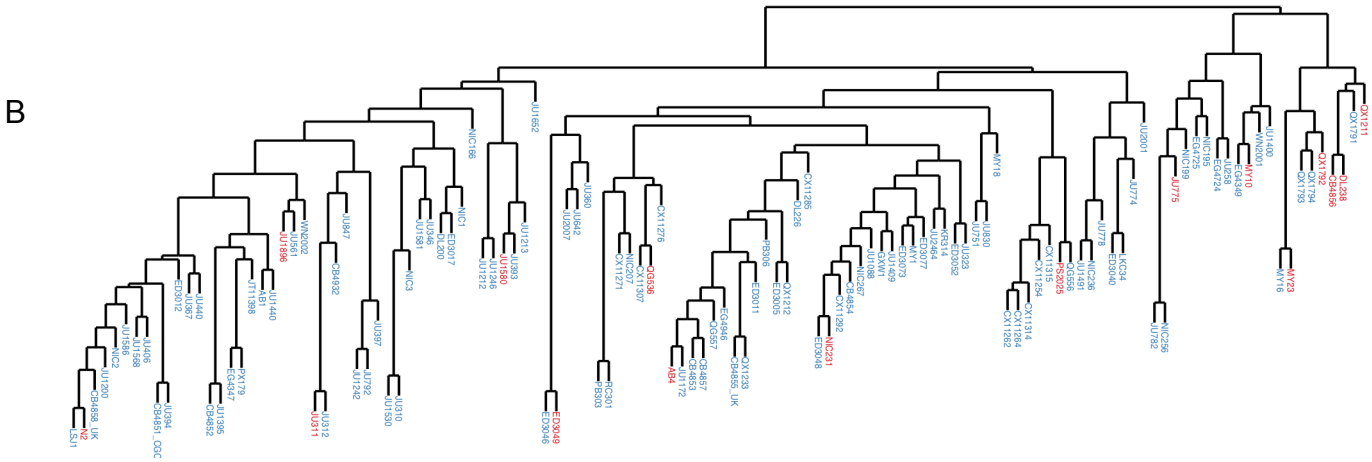


Fig. 6. *(A) Isolation locations of a set of 124 fully genotyped C. elegans wild isotypes are shown as filled squares. Red squares denote the 16 strains used for our analyses. (B) The relatedness of the C. elegans wild strains shown as a phylogenetic tree with the 16 strains in red.*

assay at least eight replicates of these 16 divergent strains in at least three separate trials for the traits optimized in Aim 1. We can subsample these replicate measurements and calculate heritability at each level of sampling. Oftentimes, heritability values will plateau when assaying a large number of replicates because increasing the number of measurements only marginally increases the reproducibility. To maximize our experimental efforts, we will determine the number of replicates that reach that plateau level and design an experimental block structure. These two calculations will provide the parameters that allow us to phenotype our collection of 500 wild strains in a variety of measures of healthspan.

Once we have collected the replicate data for the large suite of traits across the set of 16 diverged *C. elegans* strains, we will investigate the correlation structure of these traits at each time point and in aggregate over the course of the healthspan assays. This analysis of the large data set will give us measures for how different traits relate to one another and which traits provide independent information about healthspan. For example, locomotion and pharyngeal pumping might be correlated but evoked responses to blue light might be uncorrelated from both. Therefore we can optimize and measure a smaller subset of uncorrelated, independent traits for the large collection of wild strains and perform GWA mapping to identify healthspan regulators.

Potential pitfalls and alternative strategies for Aim 2

Some of our traits might have heritability values less than 20%. In which case, we will determine whether the trait measurement is variable or whether the strains have little phenotypic variation using ANOVA. For traits in which the measurement is noisy, we can optimize the trait further using a few more wild strains. For traits in which the 16 strains do not differ phenotypically, we will not pursue them further. Some of our healthspan traits might require large levels of replication to get accurate measurements of phenotype, making GWA mapping experiments with more than 500 strains difficult or impossible. In this case, we will work to optimize the traits further or pursue other traits from our diverse suite of healthspan measurements.

Project timeline and future directions:

We expect that the development and optimization of healthspan trait assays using the multi-well imaging device (Aim 1) will be completed within the first year. These iterative design and experiment optimizations will be performed on the laboratory strain and a divergent wild strain from Hawaii (CB4856). Once a trait is optimized, we will measure the phenotypes of the 16 divergent strains using high replication (Aim 2). This effort will begin in the first year and continue through the second year of support. After assays are complete and the traits with heritability about 20% are determined, we will seek additional funding (*e.g.* an R01) to measure the healthspan phenotypes of our *C. elegans* wild isolate panel of nearly 500 strains and perform GWA mappings to identify the genes that vary to cause differences in these important traits.

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