A ROBOTIC PLATFORM FOR FULLY AUTOMATED DEVELOPMENTAL AND AGEING STUDY IN C.ELEGANS: APPLICATION TO THE GENETIC MAPPING OF LIFESPAN AND MITOCHONDRIAL STRESS RESPONSE

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ABSTRACT

C. elegans is a powerful model organism for biomedical studies. However, the traditional protocols, which continue to be broadly used, rely on manual handling, making them labor-intensive and time-consuming. Automation of these processes would greatly benefit long-term studies of *C. elegans*. Significant progress has been achieved over the past decade in the techniques to study worm's biology: the introduction of microfluidic approaches for different assay types and the use of machine learning-based algorithms for data processing offer an increase in experimental throughput and a better control of experimental conditions.

We propose here a novel solution for automated developmental and ageing studies in *C. elegans* aggregating these new mentioned methodologies. Our microfluidic-based robotic platform is capable to fully automate all the key aspects of *C. elegans* experimentation, including worm culture, treatment, imaging, as well as data recording and analysis. The unique characteristics of the platform allow high content phenotypic studies on multiple worm populations in parallel that go beyond a simple tracing of growth or survival curves. We present here a panel of standardized bioassays allowing automated: (1) monitoring of *C. elegans* lifespan, (2) assessment of worm fitness, (3) testing of different stress responses activation and (4) identification of developmental and reproductive phenotypes that can serve as potential predictors of ageing.

To validate the performance of the assays, we mapped the genetic determinants of lifespan in a worm genetic reference population – the recombinant intercross advanced inbred lines (RIAILs). From 85 worm lines, we assessed the life-history traits on-chip, including the development time, growth dynamics, and reproduction. RIAIL lifespans, previously generated with the traditional on-plate method, exhibited large variations, and were positively correlated with developmental time on-chip. Among the top candidates obtained from QTL mapping, novel longevity modulators were identified and validated.

MICROFLUIDIC PLATFORM OVERVIEW

Our microfluidic technology allows **large-scale** studies for the parallel characterization of drugs and chemicals in *C. elegans*. The SydLab™ One platform provides **fully-automated culture, treatment, imaging and analysis** of the worms over long-term experiments. The high-content information extracted using our image processing and data interpretation algorithms enables detailed multi-phenotypic screening at the whole-organism level.

Features

- Patented microfluidic design, relying on passive hydrodynamics
- 16 fluidic lines, enabling tests of 16 independent conditions
- Plug & play chip-to-device connectivity and fluidic operations

Features

- Active culture, treatment and study of 64+ independent conditions
- Programmable acquisitions of BF and fluo images and videos
- Active temperature control in the 10-40°C range

Features

- User-friendly software to design, run and monitor experiments
- Time-resolved/high-content data extraction based on AI
- Integrated statistical analysis /data interpretation algorithms

Worms are **automatically injected into the microfluidic platform** and confined within dedicated chambers of the microfluidic chips. They are then continuously fed with *E. coli* solution and can be exposed to the test compounds according to the defined treatment plan. The pictures of each microfluidic chamber **are acquired via time-lapse microscopy** at desired frequency.

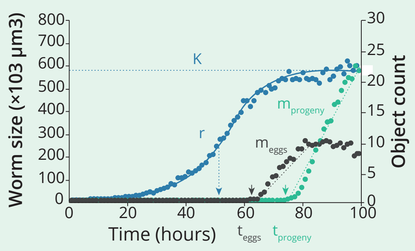
DATA PROCESSING PIPELINE

The time-lapse brightfield pictures obtained during the experimental procedure are post-processed by a **machine learning (ML) software (A)**. Through this ML software we monitor the **growth rate** of the worm population within the microfluidic chip over several days, as well as the **fertility** (eggs appearance and number) and **progeny production (B)**, with a high degree of reproducibility accross replicates **(C)**.

A. ML-based objects' detection

The object recognition allows the ML algortihms to extract the following parameters: maximal length ($\mathbf{K}_{length}$), time required to reach ½ max length($\mathbf{r}_{length}$), the time point when the first egg is detected ($\mathbf{t}_{eggs}$), the time point when the first larvae is detected ($\mathbf{t}_{progeny}$), the speed of egg ($\mathbf{m}_{eggs}$) and progeny accumulation ($\mathbf{m}_{progeny}$).

B. Automated data analysis



C. Objects detected

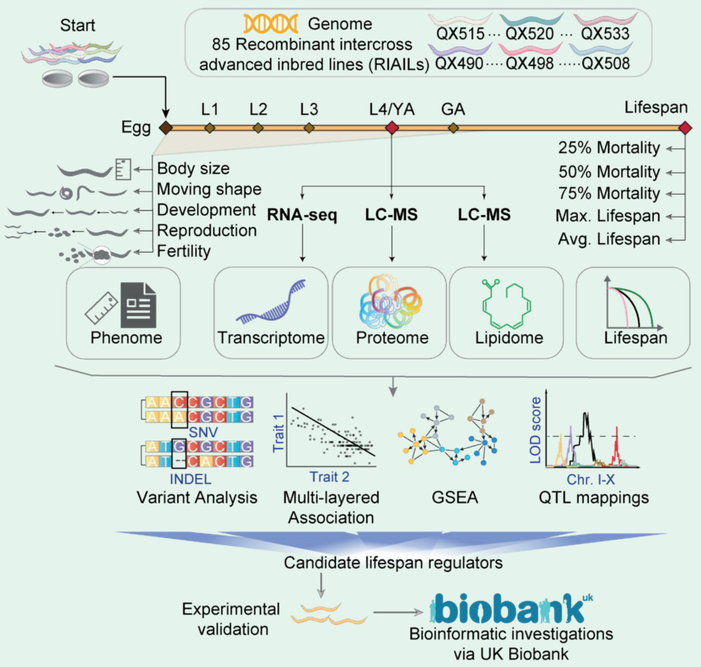
● Worm (size) ● Eggs (number) ● Progeny (number)

Reproducibility

Parameter	Value	Variation	Experiments
Maximum size (K)	570'388 (µm³)	±6.5%	15 repeats
Growth dynamic (r)	2.24 (days)	±5.6%	15 repeats
Sexual maturity (t_{eggs})	56.44 (hours)	±5.4%	15 repeats

OVERVIEW OF THE STUDY DESIGN

From Phenome analysis to QTL mappings

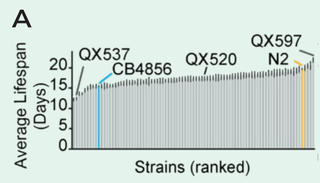


85 RIAILs derived from the crossing of QX1430 (N2 Bristol) and CB4856 strain (Hawaii) were used. Lifespan, early life-history traits, transcriptome, proteome, and lipidome were collected for each strain. We applied a systems genetics approach to study relations between different phenotypes and molecular traits to identify candidate lifespan genes. After prioritization of the candidate genes, we validated them through wet lab experiments.

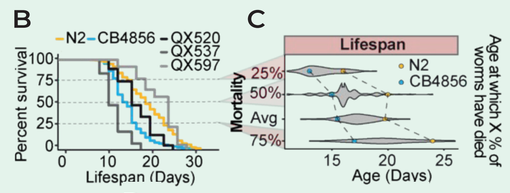
We collected data from all RIAILs using three pipelines: (1) worms were cultured and scored for their lifespans; (2) worms were cultured in the SydLab™ One platform for ~100 h to collect early life-history traits, including body size, moving shapes, developmental parameters, reproduction, and fertility; (3) worms were cultured to reach L4/young adulthood, and collected for multi-omics

RESULTS

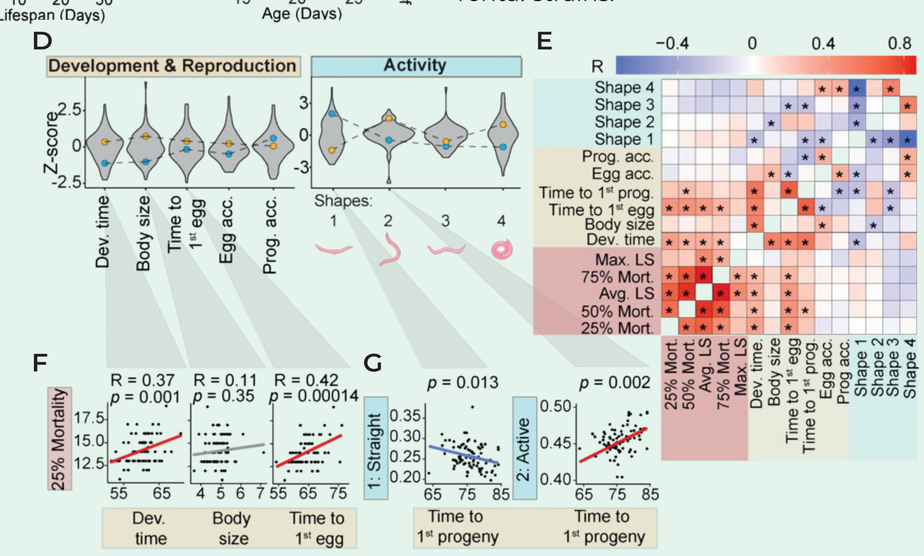
RIAILs exhibit extensive variation in lifespan and life-history traits



A. Bar plot showing the average lifespan of 85 RIAIL strains and two wild-type parental strains. Grey bars: RIAILs; Orange bar: N2 (Bristol); Blue bar: CB4856 (Hawaii, HW). Examples of strains with different average lifespans are labeled.



B. Examples of differences in the average lifespan of RIAILs (QX537, QX520, and QX597; grey) and parental N2 (orange) and CB4856 (blue) strains.
C. Violin plots of the RIAIL lifespan traits. Dots represent the average value of the trait for the two parental strains.

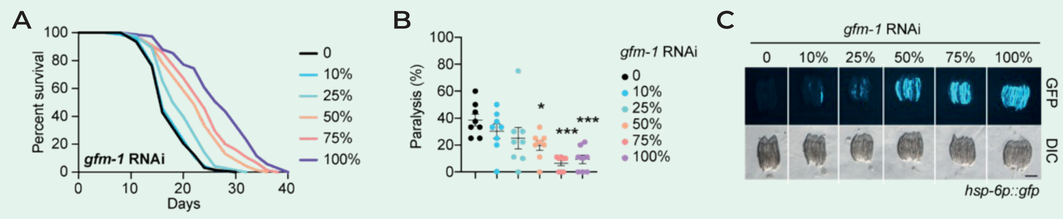


D. Violin plots of early (Developmental & Reproduction) and activity (shapes) life-history phenotypic traits. Dev. time: developmental time; Egg acc.: egg accumulation; Prog. acc.: progeny accumulation; Shape 1: straight; Shape 2: active; Shape 3: swimming; Shape 4: supercoiled
E. Pearson correlation between lifespan and physiological traits. Stars represent non-adjusted *p*-values (*: adjusted BH *p*-value < 0.05). LS: lifespan. Mort: mortality.
F. Correlation of 25% mortality with developmental time, body size, and the time to the 1st egg, respectively. R: Pearson correlation coefficients *p*: *p*-values.
G. Scatter plots of time spent in 2 moving shapes and time to the 1st progeny of each RIAIL strain. The *p*-value indicates the coefficient in the linear model.

Identification of a lifespan-modulating locus on Chromosome II and prioritization of candidate genes

QTL mapping of lifespan and life-history traits identifies two significant QTL on Chr. II, and V for average lifespan, and time to 1st progeny, respectively. Eight genes were located in the region of the lifespan QTL on Chr. II. We employed an integrative systems approach to prioritize potential candidate lifespan regulatory genes. Among the candidate genes found under this locus, *gfm-1* met at least half of the selection criteria.

RNAi of *gfm-1* induced UPR^{mt} activation and prolonged lifespan in *C. elegans*



A. RNAi of *gfm-1* extends worm lifespan in an RNAi dose-dependent manner. Worms fed with ev (control RNAi) or 10%-100% *gfm-1* RNAi; control RNAi was used to supply to a final 100% of RNAi.
B. Age-related paralysis of worms fed with ev or 10%-100% *gfm-1* RNAi. Error bars denote SEM. Statistical analysis was performed by one-way ANOVA followed by Tukey post-hoc test (*$p < 0.05$; ***$p < 0.001$).
C. RNAi of *gfm-1* induced the UPR^{mt} (*hsp-6p*::gfp reporter) in a dose-dependent manner. Worms fed with ev or 10%-100% *gfm-1* RNAi. Scale bar: 0.5 µm.

CONCLUSION AND OUTLOOK

We presented a novel microfluidic platform designed for fully automated analyses of *C. elegans* nematodes. This study highlighted the strong advantages of our innovative approach, including a good reproducibility and accurate results thanks to standardized protocols and multi-phenotypic readouts in real time. Our study unveiled a specific genetic locus that plays a role in determining lifespan variations within the RIAIL population. Furthermore, we identified known and novel longevity modulators, including *gfm-1* RNAi, which we validated experimentally. The comprehensive multi-layered characterization of the RIAIL population is now also made accessible through an open-access web resource (https://lisp-lms.shinyapps.io/RIAILs/), which provides a valuable tool for investigating the intricate relationships between biochemical and whole-body phenotypes and for hypothesis generation for the scientific community.

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