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RÉSUMÉ

Les différences d'espérances de vie entre espèces sont principalement d'origines génétiques. En revanche, à l'échelle d'une espèce, les conditions environnementales peuvent influencer significativement la longévité des individus. Lorsque des animaux sont élevés dans des environnements contrôlés et protégés ils meurent principalement de causes liées au vieillissement. Il est alors frappant, que la variabilité des espérances de vie est la même au sein de populations isogéniques qu'au sein de populations non isogéniques. Ces résultats démontrent que les facteurs génétiques seuls ne suffisent à expliquer la mort et le taux de vieillissement des individus. Afin de mieux comprendre quels sont les phénomènes à l'échelle cellulaire pouvant expliquer le vieillissement prématuré d'individus génétiquement identiques, nous avons choisi de travailler sur des populations clonales de *Caenorhabditis elegans* élevées dans un environnement contrôlé et protégé.

Comme il a été montré chez C. elegans que les performances locomotrices pouvaient être utilisées comme un biomarqueur du vieillissement, nous avons développé une méthode permettant de quantifier différentes variables associées à la locomotion. En tirant parti de la capacité des nématodes à s'orienter puis à se déplacer lorsqu'ils sont placés dans un champ électrique, nous avons pu isoler des populations de nématodes ayant des vitesses de déplacement différentes.

Nous avons ensuite montré l'efficacité de cette méthode pour séparer à l'intérieur d'une population isogénique dans un même environnement des nématodes ayant une courte espérance de vie de ceux ayant une longue espérance de vie, et ce dès le début de la phase post-reproductive. Nous avons alors pu mettre en évidence que le niveau de carbonylation du protéome était significativement plus élevé chez les individus ayant une espérance de vie courte.

SUMMARY

Genetic differences explain most of the lifespan variability within animal kingdom. However, at the level of the species, environmental factors can significantly affect lifespan. When animals are raised in controlled and protected environment such as laboratory conditions they mainly die from age related causes. However it is striking to observe that in these conditions lifespan variability is similar in isogenic and non isogenic populations. These results show that genetic determinants are not sufficient to explain death occurrence and aging rate of individuals. Therefore we decided to investigate mechanisms that can influence biological aging. To address this issue we used *C. elegans*, a nematode. Indeed, this model gives the opportunity to raise clonal populations.

Because locomotion has been shown to be one of the most reliable biomarker of aging we decided to employ electrotaxis to sort groups of nematodes with similar crawling velocity performance and therefore life expectancy. We first showed the relevance of electrotaxis as a general method to realize quantitative measurement of *C. elegans* locomotory performance either between different strains or between individuals at different age. Then we investigated effect of age on locomotory performance of *C. elegans* N2 in electric fields. We found that crawling speed is the major factor affecting self-sorting of *C. elegans* N2 hermaphrodites just after the reproductive period. We demonstrated that we were able to sort groups of nematodes having different life expectancy and finally showed that protein carbonyl levels, the proxy we used to measure proteome quality, was correlated with lifespan in *C. elegans* N2 hermaphrodites. These results are consistent with an accumulation of damage model of aging.

INTRODUCTION

Genetic differences explain most of the lifespan variability within animal kingdom. However at the level of the species environmental factors can affect significantly lifespan. When animals are raised in controlled and protected environment such as laboratory conditions they mainly die from age related causes. However it is striking to observe that in these conditions lifespan variability is similar in isogenic and non isogenic populations (Finch and Tanzi 1997) **(Tab. 1)**. These results show that genetic determinants are not sufficient to explain death occurrence and aging rate of individuals. Therefore we decided to investigate mechanisms that can influence biological aging.

To address this issue, we used the nematode *C. elegans*, because this model gives the opportunity to raise clonal populations (Brenner 1974). We first developed a method, that allows us to separate isogenic animals that age differently, then we analysed proteome maintenance in separated groups of *C. elegans*.

In the first part of the introduction, I will introduce the concept of aging and review the main causes of aging in living organisms. I will then focus on *C. elegans* and what we know about its aging.

Species	Heritability of life-span* (%)	Coefficient of variation of life-span† (%)	Mean life-span (temp.)
Nematode			15 days (25°C)
Within line	0 (self-fertilizing)	34	
Between lines	34	19 (16–24)	
Flies			
Fruit fly, inbred lines			40 days (25°C)
Within line	<1		
Between lines	6–9	11	
Medfly, outbred	ND	45	21 days (25°C)
Mouse, inbred lines			27 months
Within line	<1	24 (19–71)	
Between lines	29	16	
Human twins	23–35	MZ, 19; DZ, 25	72 years

Table 1: Lifespan variation in selected vertebrates and invertebrates. Coefficient of variation of lifespan represents the standard deviation of the distribution of the lifespan divided by the mean lifespan of the population. From (Finch and Tanzi 1997)

I- Overview of the aging phenomenon

A- What is aging?

1- Definition of aging

In 1956 Harman proposed to characterize aging as "*the progressive accumulation of changes with time associated with or responsible for the ever increasing susceptibility to disease and death which accompanies advancing age*" (Harman 1956). The father of the free radical theory of aging used a loose description of the processes linked with aging by employing the word "changes". These modifications are defined by a teleological criterion; only future events—death or disease—will determine if this change was deleterious or not. Moreover, Harman relates aging with chronological time. Chronological age, as opposed to physiological age, is the simplest way to measure aging, but we will see later that this criterion can be confounding to the study of aging. In this work we will narrow the definition of aging to a progressive and intrinsic accumulation of damage that on the one hand decreases the ability to adapt to the environment and on the other hand increases the occurrence of disease and the probability of dying. In some contexts it is necessary to distinguish aging from senescence, however in this work these two words will be used synonymously.

2- Is aging an inherent property of a living system?

Since the work of Weismann at the end of the 19th century, there has been a differentiation of the germline, which produces the gametes, from the soma, which gives rise to the other tissues of the body. The germline is considered to be immortal as it is transferred between individuals of the same species vertically (Jones 2007, Medvedev 1981). On the contrary, the soma dies with the individual.

A fundamental question raised from this description of soma and germline is: How come aged parents do not beget "old" offspring? In other words, does the germline accumulate damage, but is then able to rejuvenate—after or just before—the zygote is created, or does this cell lineage remain free from injury over time? Recent works on *C.*

elegans and *Mus musculus* show that rejuvenation phenomena occur either in the oocyte just before fertilization or during the early phase of development (Hernebring et al. 2006) (Goudeau and Aguilaniu 2010). Goudeau and Aguilaniu showed that the germline of nematodes, corresponding to their gonads, shows a high level of protein carbonylation until a certain stage of oocyte formation when this level drops. This rejuvenation of the proteome seems to be coupled with proteasome activity (Goudeau and Aguilaniu 2010). In the mouse, the proteasome also appears to be implicated in the elimination of damaged proteins (Hernebring et al. 2006). Additionally, these results show the importance of removal of damage proteins in the rejuvenation process.

These examples demonstrate that some tissues are able to reverse the process of damage accumulation. So, this accumulation is temporary and not progressive. It is however important to note that individual gametes are not immortal since accumulation of damage can lead to their death. The "immortality" of the germline is, in fact, associated with specific events like such as fertilization and a phenomenon of natural selection between the gametes.

3- Measurement of aging

a- Measurement of aging at the level of the population

SURVIVAL KINETICS

Disease and Aging. Dorland's illustrated medical dictionary defines a disease as "*any deviation from or interruption of the normal structure or function of any part, organ, or system of the body (or their combination) that is manifested by a characteristic set of symptoms and signs and whose etiology, pathology, and prognosis may be known or unknown"* (Dorland 2000). Thus, disease occurrence is a discrete event compared to the intrinsic and progressive nature of the aging process. Although it was stated previously that chronological time is an imperfect proxy to represent aging, it is interesting to see that the major causes of death in industrialized country are diseases that correlate well with time in a logarithmic scale (Pellay, personnal communication) **(Fig. 1)**. In this context, aging can be seen as a predisposing factor for the development of the diseases.

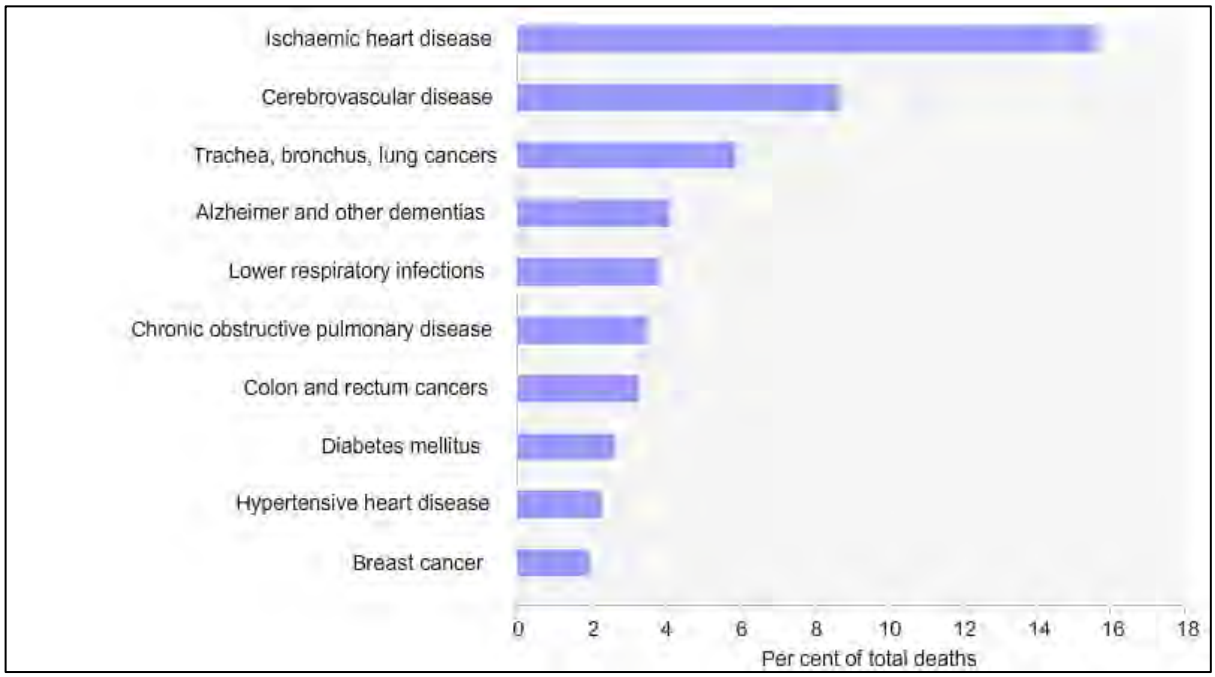

Figure 1: Ten leading causes cause of deaths in 2008 in High-income countries. Source World Health Organization (World Health Organization 2008)

Aging and death. Alterations in aging, provoked by genome modifications or environmental conditions, are commonly detected and quantified by survival kinetics (Ghirardi et al. 1995). In survival kinetics a binary event is counted: death or non-death of an individual within a population. Counting of a binary event in survival kinetics allows for the estimation of a continuous phenomenon: aging. Survival kinetics yields information on the aging process at the population level but it does not provide information on aging at the individual level, just as the death of an individual does not necessarily inform us of its age. Indeed, an organism can be at risk of a disease and die even if it has not reached its last stage of senescence. Therefore, it is possible, by working with large populations, to measure the mean or the median lifespan of a population for a given condition. In this case, the median lifespan of a population is a proxy for aging measurement.

SPECIAL TRANSFORMATION OF SURVIVAL DATA: MORTALITY CURVES

Benjamin Gompertz, while working for an insurance company, popularized the representation of survival data by a mortality curve. Gompertz found it interesting to transform survival data in this way because it made it possible to directly perceive the age specific probability of dying for a given individual in the population. He observed that the probability of dying was increasing exponentially with time except at very old ages (see also below). We now refer to this phenomenon as the Gompertz law (Wu et al. 2009). On a semi-logarithmic scale this law can be represented by a linear curve that reaches a plateau at older ages. Two parameters can be extrapolated from the linear part of the curve: the y-intercept and the slope. The y-intercept estimates the initial probability of dying (which can be seen as initial vulnerability). The slope constitutes the mortality rate of individuals in the population.
Compared to survival curves, mortality curves provide additional information but require more individuals. As each point of a mortality curve represents the probability of dying for a randomly chosen individual, it is necessary to have a population big enough to reduce noise in the curve.

Since the pioneering work of Gompertz, mortality curves of many organisms have been established (Vaupel et al. 1998). Many data sets show an exponential increase in dying probability followed by a deceleration of the probability of dying for old ages. If we assume that populations are homogeneous, deceleration of dying probability for old ages would mean that living organisms age slower at old ages. Even if such an explanation cannot be automatically rejected, it does not seem to be likely. In the following paragraph we will briefly describe two alternative models that try to explain this phenomenon.

MODEL EXPLAINING THE SHAPES OF MORTALITY CURVES

Frailty Model. Vaupel, et al. invoked the concept of heterogeneity to explain the mortality plateau at old ages (Vaupel 1979). Instead of representing the probability of dying for all the individuals in the population, the mortality curve represents the mean of the probability of dying of all the individuals. Frail individuals will have a high initial probability of mortality whereas robust animals will have a low initial probability of mortality. This scenario

preserves the idea that there is an exponential increase of probability of dying with time for all individuals **(Fig. 2)**.

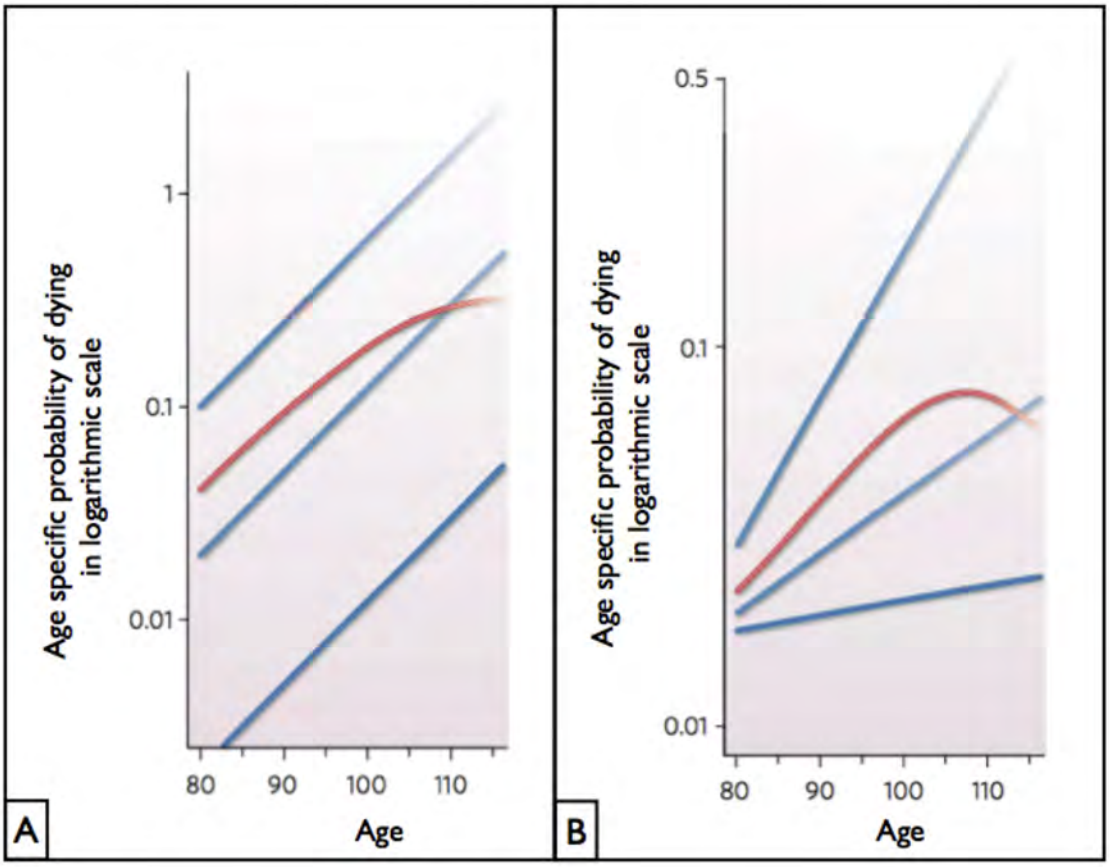

Figure 2: Alternative explanations of the shape of a mortality curve according to the frailty model. The blue curves represent individuals probability to die depending on their age. The red curves represent the mortality rate measured at the level of the population. Source (J. Vaupel 2010)

Reliability theory of aging and longevity. The reliability theory of aging and longevity found its inspiration in the engineering field. To increase the "survival" of a mechanical or electronic device, designers will double or triple the components fulfilling essential functions. If one piece fails, the others still ensure the "vital" function. These designs for mechanical and electronic devices lead to a failure curve that looks similar to the mortality curve of biological systems with an exponential increase of the failure rate with time and a deceleration of the failure rate for old devices.

Based on these ideas Gavrilov and Gavrilova developed the redundancy model to explain the shape of the mortality curve for biological systems (Gavrilov and Gavrilova 2004). In this model essential functions are performed by redundant components. These components have a

constant probability to fail with time but as a consequence the probability for the function to fail increases with time. The particularity of this model is that it does not imply aging of the building component **(Fig. 3)**. They are either functional or not. However, the whole organism ages because its probability to die increases with time.

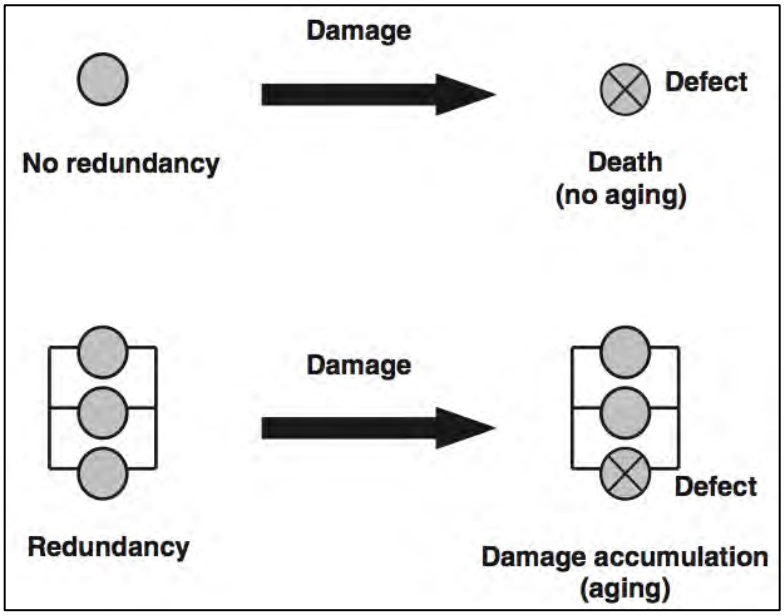

Figure 3: Damage accumulation in redundant and non-redundant systems (Gavrilov and Gavrilova 2004)

In conclusion, survival kinetic analysis is popular for short-lived animals with large population sizes because of (i) the simplicity of the measurement, an animal is either dead or alive, and (ii) the short duration of the experiment. Moreover, it is possible to extract from survival data, parameters that characterize aging of the studied population. As already mentioned, these parameters provide information about the population dynamic and not about the individuals. Different models interpret the values of the parameters in different ways. A complementary approach to the survival analysis is the use of age-related changes. In fact, for long-lived species such as humans, they are the exclusive way to study aging.

b- Measurements of aging at the level of individuals

Age-related changes can occur at different levels of organization such as cells, organs or the whole organism. Depending on the variable measured and the sensitivity of the techniques used, it can be necessary to pool many individuals. In cross-sectional studies, at a given time point, measurements are taken on all the individuals of one cohort. For the next

time point another cohort is used. Therefore, in this method a single measurement is taken per individual. Differential mortality between the cohorts may affect the quality of the data. Possible erroneous interpretations of the results are illustrated in figure 4 **(Fig. 4)**. However, this methodology is the only solution when the measurement can alter the aging processes or require the death of the animal. In contrast to cross-sectional studies, in longitudinal studies, measurements of the biomarkers are taken repetitively on the same individual. These studies are more straightforward because it is possible to follow age-related changes over time on the same individual. It is therefore possible to compare individual trajectories for a given age-related change. The major drawback of this method is the "practice effect". For example, for a functional test the subject tested may respond "better" if he already knows the test. These studies are also much more difficult to organize.

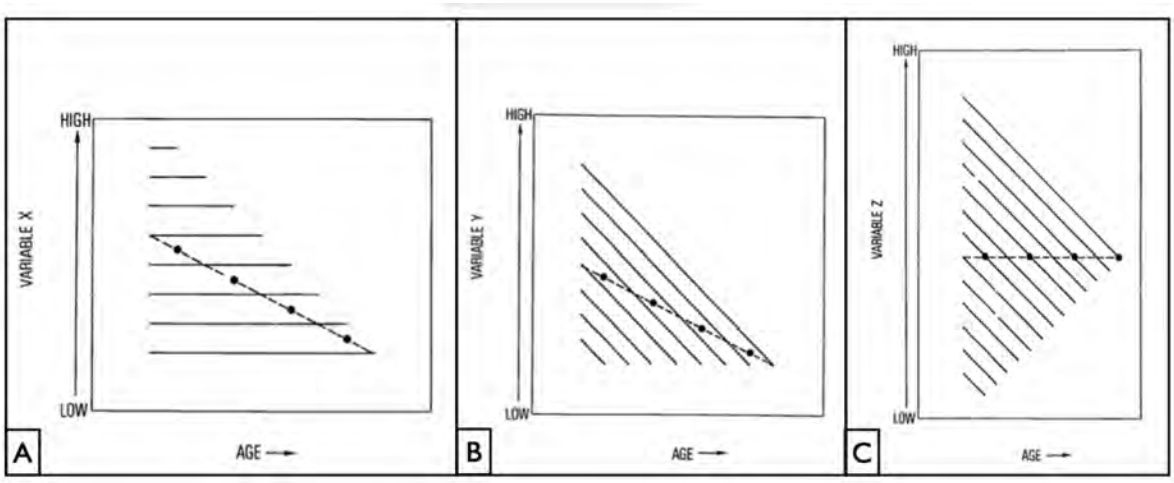

Figure 4: Possible erroneous interpretation of the trend of a variable with age, when measurements of different individuals are pooled. Differential mortality can affect the mean value of the variable measured. (A) The individual values do not vary with age but the mean value decrease with age. (B) Individual values decays with a higher rate than the mean value. (C) Individual values decrease but not the mean value.

A biomarker is usually defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic response to a therapeutic intervention”. Some age-related changes are poorly informative of individuals’ aging status (i.e., greying hair in humans or defecation rate in *C. elegans*). Aging biomarkers should thus be restricted to a category of age-related changes that have a relatively good predictive power to evaluate individuals’ remaining lifespan (Butler et al. 2004). Some aging biomarkers are segmental, meaning that they only have a predictive

value during a part of the lifespan. For instance, grip strength is only a good biomarker of premature mortality for men after 60 years of age (Metter et al. 2002). Conversely, high cholesterol level is a predictor of high risk of mortality only before 40 years of age (Karasik et al. 2005). Ideally, the function evaluated by the biomarker should represent the aging status of the whole organism. In the example of grip strength, one can argue that the measurement coincides with hormonal status change, which modulates muscle metabolism, joint health and the psychological status of the person. Finally, a potential pitfall is that a biomarker may indicate only a predisposition to a certain disease rather than an accurate measure of the overall aging process. For instance, high cholesterol level may mainly be a good predictor for cardiovascular diseases (Karasik et al. 2005).

Although very "desirable" the quest for an "ideal" aging biomarker may be impossible as the work of Borkan and Norris illustrates. Indeed, they measured 24 biomarkers in a group of 1086 males (Borkan and Norris 1980). To compare results of participants to a virtual biological age, they transformed the physiological values to an age-specific score. When the authors plotted the results for each participant, they observed inconsistency between the physiological score of the different biomarkers of the panel **(Fig. 5, Tab. 2)**.

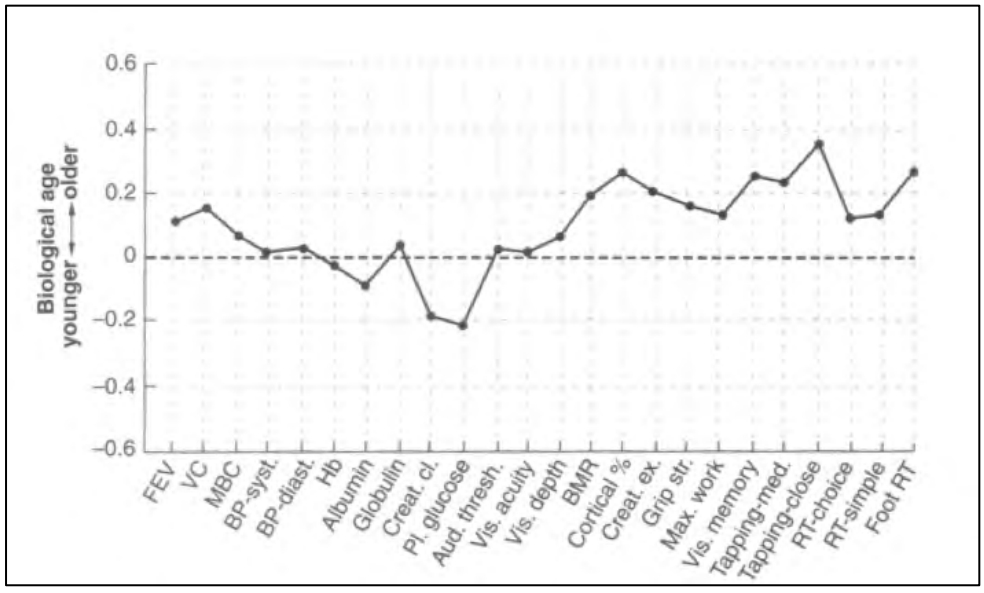

Figure 5: A biological age profile of a single individual, using the parameters given in table 2. Depending on the parameter the biological age of the individual is different (Borkan and Norris 1980).

Biomarker	Correlation coefficient (*r*)
Forced expiratory volume (1 second)	−0.698*
Vital capacity	−0.606*
Maximum breathing capacity	−0.547*
Systolic blood pressure	0.538*
Diastolic blood pressure	0.368*
Hemoglobin levels	−0.223*
Serum albumin levels	−0.356*
Serum globulin levels	0.092*
Creatinine clearance	−0.602†
Plasma glucose levels	0.279
Auditory threshold (4000 cycles/sec)	0.549
Visual acuity	−0.306†
Visual depth perception	−0.232*
Basal metabolic rate	−0.337*
Cortical bone percent	−0.435*
Creatinine excretion	−0.538*
Hand grip strength	−0.501*
Maximum work rate	−0.511*
Benton visual memory test (errors)	0.502*
Tapping time (medium targets)	0.468*
Tapping time (close targets)	0.366*
Reaction time (simple)	0.287*
Reaction time (choice)	0.220*
Foot reaction time	0.222*

Table 2: Mean Correlation with age for selected biomarkers in 1086 Males studied in the Baltimore Longitudinal Study on Aging. From (Borkan and Norris 1980)

B- Why aging?

In his paper “Cause and Effect in Biology” E. Mayr elaborated on the distinction between evolutionary biology and functional biology (Mayr 1961). Most of the time, these two fields of biology presented complementary explanations of the aging phenomenon. Indeed, while evolutionary theories focused on the constrains that gave rise to the aging phenotype through the large time-scale of evolution, mechanistic theories have focused on the mechanistic basis of aging processes at the level of the organism.

1- Evolutionary explanations of aging

a- Evolutionary genetics of aging

Evolutionary theories distinguish death caused by aging, here referred to as intrinsic mortality, and death caused by environmental threats and accidents, here referred to as extrinsic mortality. In fact they emphasized the role of extrinsic mortality as a driving force to select or counter select gene mutations involved in the aging process (Medawar 1952) (Williams 1957). Indeed, in a context where extrinsic mortality is the main cause of death, the age-specific proportion of animals decays exponentially and only a few aged animals contribute to the renewal of the population **(Fig. 6)**. Thus, mutations affecting the reproductive input of aged animals will be weakly counter-selected because the deleterious consequences of these mutations can only be observed in a protected environment. In fact, the strength of the selective pressure against a mutation favoring accelerated aging will be proportional to the negative effect of this mutation against the reproductive input of its carrier. According to this principal Medawar explained how various mutations related to aging processes accumulate over generations when extrinsic mortality is predominant over intrinsic mortality. It is important to note that only mutations occurring in the germline have participated in the evolution of aging within different species.

Different groups have shown experimental evidence of the importance of the age of the parents that contribute to the renewal of the population to modulate a population’s lifespan in an evolutionary time scale. R. Arking, for instance, showed that it was possible to

increase the lifespan of Drosophila over generations by allowing only old individuals to reproduce (Arking 1987).

In addition to the work of Medawar, Williams discussed the concept of antagonistic pleiotropy, which assumes that genes can have opposite effects on the reproductive success of the individual depending on their age (Williams 1957). He postulated that mutations increasing the reproductive capacity of young individuals would be partly protected against counter-selection even if they have deleterious effects on old individuals. Ruvkun's team illustrated the idea of antagonistic pleiotropy by investigating the effect on lifespan of genes known to be essential for normal development. Interestingly, they identified 67 genes out of 2700 screened that increased lifespan when inhibited only during adulthood. The main classes of these genes are related to protein synthesis, mitochondria and signaling (Curran and Ruvkun 2007).

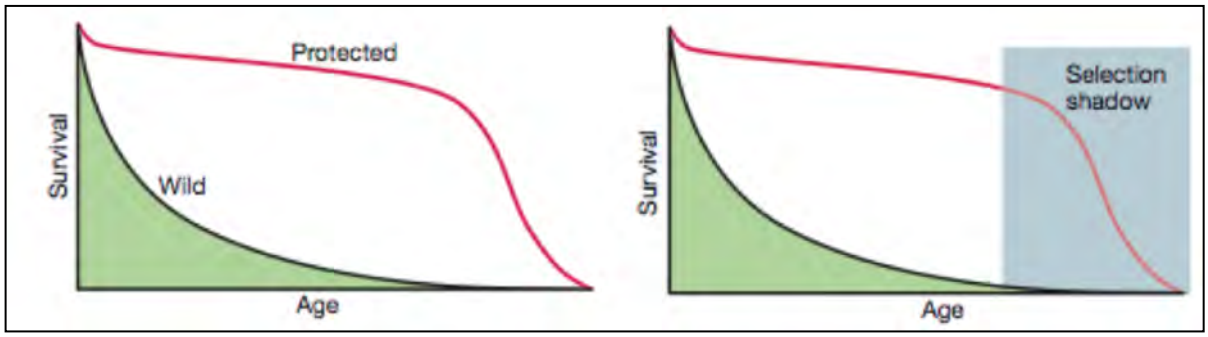

Figure 6: Age specific proportion of animals that participate to the renewal of the population. (Black curve) In the wild, extrinsic mortality is high, the age specific population decay is exponential because the probability to dye is constant with time. (Red curve) In protected environment, most of the animals die from age related cause. The probability of dying increases exponentially with time. Genes involved in aging processes affecting reproductive success of aged individuals are not counter selected because of the selection shadow. From (Kirkwood and Austad 2000)

b- Evolutionary physiology of aging

The disposable soma theory of aging, developed by Kirkwood and Holliday, relies on two main concepts: extrinsic mortality and trade off in energy allocation (Kirkwood 2005). Indeed, as energy supply is limited for an organism, the distinct fate of the soma and the germline introduce a trade off between investment in reproductive success or in somatic

maintenance. Disposable soma theory postulates that the investment in somatic maintenance is adjusted according to the level of extrinsic mortality. The higher the extrinsic mortality, the better it is to allocate energy to reproductive success at the expense of somatic maintenance.

2- Mechanistic causes of aging.

Events linked with aging are multiple, and they occur at different levels of organization within organisms. Moreover, maintenance processes may be regulated differently, even in phylogenetically close species, if they have encountered different selective pressures in their history. Thus, within the scope of this paper, it would take too long to make an exhaustive list of the mechanisms that can lead to aging. In the following paragraphs we will focus on the molecular basis of aging. In addition, we will restrict our description to the mechanisms that lead to molecular damage or to production of toxic molecules such as aggregates. We will not review the deleterious consequences of errors of the cell machinery.

a- Reactive molecules

SOURCE OF REACTIVE SPECIES IN THE CELL

Since Harman discussed the possibility that damage generated by free radicals could be the leading cause of aging processes, endogenous sources of highly reactive species (RS) have been investigated. RS are no longer narrowly limited to free radicals and are subdivided into two groups: reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Dröge 2002).

In Metazoa, the electron transfer chain (ETC) of the inner membrane of the mitochondria is the main producer of ROS. In humans, it has been estimated that 0,12 to 2 % of O_2 consumed during normal respiration is converted into superoxide (O_2^-) (Nathan and Ding 2010). This phenomenon is due to a passive leakage of electrons from the ETC to molecules of O_2. Indeed, electrons, normally, transit into the inner membrane of the mitochondria through five molecular complexes and finally react with a molecule of O_2 and H^+ to form water:

$$2H^+ + 2e^- + ½\ O_2 \rightarrow H_2O.$$

The aim of this series of oxido-reduction reactions is to produce ATP. Interestingly, the mitochondrial genome encodes only thirteen proteins, twelve in nematodes, which are all involved in the molecular complexes of the ETC. The channeling of the electrons in the ETC is, however, imperfect and at some stage of their transit, electrons can be captured by O_2 in the matrix and generate a molecule of $O_2^{\cdot -}$. This molecule cannot cross the phospholipid membrane, is highly unstable and can dismute naturally in hydrogen peroxide (H_2O_2). If superoxide encounters nitric oxide ($NO^{\cdot}$) before its dismutation, it leads to the formation of peroxynitrite (ONO_2^-) and finally $OH^{\cdot}$, both of which are highly reactive (Dröge 2002). These reactions are not enzymatically controlled and can be deleterious for the cell. The enzyme superoxide dismutase (SOD) can greatly increase the rate of transformation of $O_2^{\cdot -}$ into the less reactive H_2O_2. This molecule can diffuse through the phospholipid membrane and be decomposed into water and O_2 by catalases. Leakage of electrons increases when the ETC is highly reduced and when the membrane potential of the inner mitochondrial membrane is high (Dröge 2002). Recently it has been discovered that an uncoupling agent initially thought to work only in brown adipose tissue can decrease both the potential of this membrane and the production of ROS in other tissues (Echtay et al. 2002).

If we set aside detoxification reactions (to be described later), generation of ROS is not always a passive phenomenon in the cell. For example, superoxide is produced both, in phagocytes when NADPH oxidase produces superoxide during the immune response (Nathan and Ding 2010) and by xanthine oxidase. H_2O_2 is notably created by the flavoenzyme Ero1 in the Endoplasmic Reticulum (ER) during oxidative folding (Sevier and Kaiser 2008). Indeed, correct folding of proteins often requires the formation of disulfide bonds through oxidation of two-neighboring cysteines. Moreover, redox homeostasis of the cell is usually maintained by oxidative stress sensors and redundant antioxidant defenses (Dröge 2002).

In addition of endogenous production of ROS, exogenous production can occur. Xenobiotics and irradiation are the main sources (Hyun 2008) **(Fig. 7)**.

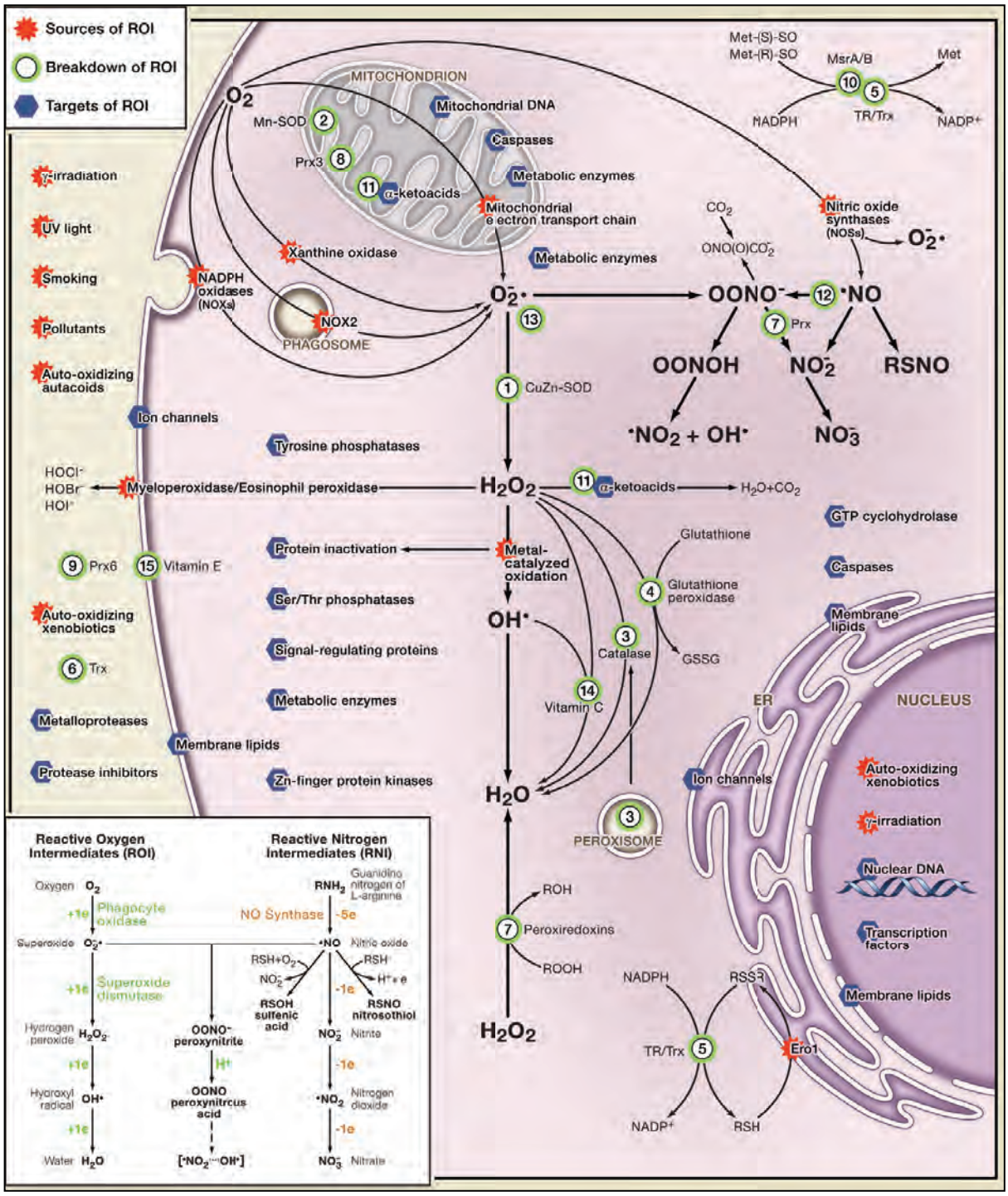

Figure 7: Reactive oxygen intermediates in human cells. From (Nathan and Ding 2010).

ANTIOXIDANT DEFENSES

Both passive and active generation of ROS can have deleterious effects on the homeostasis of cells if they are not controlled. Antioxidant defenses rely on two classes of molecules:

- detoxifying enzymes like SOD, catalases, and glutathione peroxidase/ glutathione reductase. Concentrations of these enzymes are low but they have a high turnover.
- intermediate compounds (antioxidant metabolites), like glutathione, methionine, and vitamins. These molecules react stoichiometrically with ROS; their role is to scavenge highly reactive molecules before they impair vital functions (Jocelyn 1967). Some of these molecules can be recycled by $NADPH,H^+$ or other antioxidant metabolites to recover their scavenging property.

The most abundant ROS produced in the cell is the anion superoxide (Nathan and Ding 2010). Cells usually possess SOD in every compartment where this molecule is generated. For example, the *C. elegans* genome encodes five different SODs localized either to the cytoplasm, the mitochondria's matrix or the extracellular matrix. SODs are metalloenzymes, they use copper and zinc as intermediates to transform superoxide and protons into molecular oxygen and hydrogen peroxide (H_2O_2):

$$2\ O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

H_2O_2 is less reactive than superoxide, but it diffuses easily in the cell and can react with some transition metals such as iron through the Fenton reaction to form the hydroxyl radical ($OH^\cdot$) a highly reactive radical (Jenkins 2003):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^\cdot + OH^-$$

To limit production of hydroxyl radical cells possess different systems that degrade H_2O_2 two of which are described here. Catalases are enzymes that use iron to transform hydrogen peroxide into water and O_2. Catalases have one of the highest turnover known.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

The second system, uses an enzyme couple, glutathione peroxidase (GPx) and glutathione reductase. GPx needs glutathione - a tripeptide made of L-cysteine, L-glutamic acid, and glycine - to transform hydrogen peroxide into water. At the end of the reaction glutathione is oxidized into glutathione disulfide. Then glutathione reductase uses $NADPH,H^+$ to regenerate

glutathione (Fig. 8). The function of this enzyme couple is not limited to hydrogen peroxide detoxification, it can detoxify a wide variety of peroxides (e.g., lipid peroxides).

In fact, many detoxification systems in cells rely on the ability of methionine and cysteine to be oxidized reversibly. Stadtman even postulated that methionine at the surface of proteins could be used like a shield to protect essential sites (Levine and Stadtman 2001). Additionally, vitamins such as alpha-tocopherol – vitamin E – or ascorbic acid -vitamin C- can be used as intermediates like glutathione to scavenge highly reactive ROS.

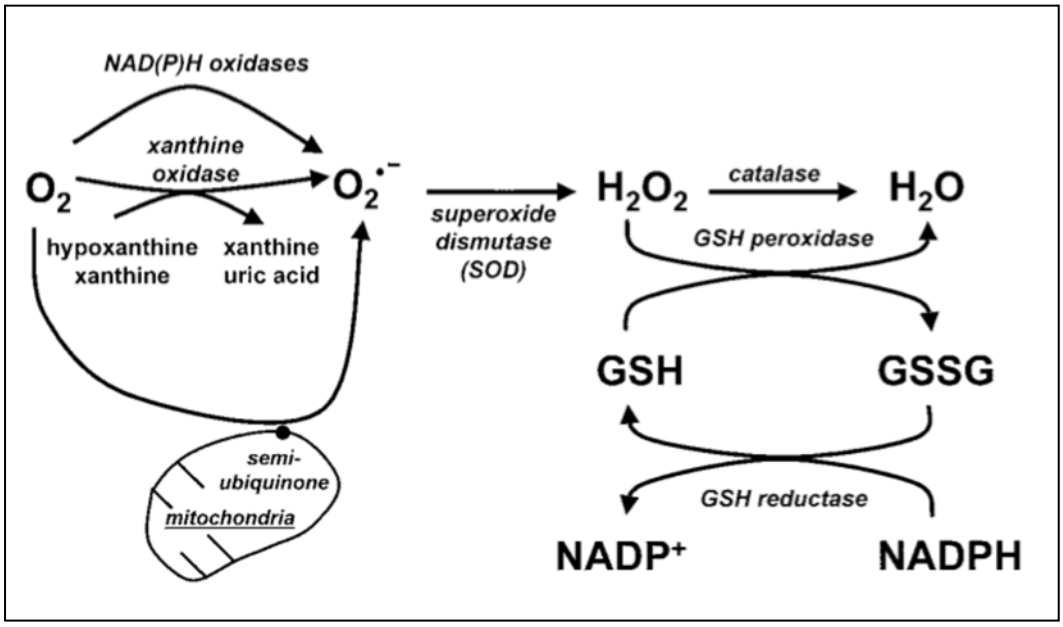

Figure 8: Generation and detoxification of superoxide anion. Superoxide anion is first transformed in hydrogen peroxide by the superoxide dismutase. Then either catalase or the GSH peroxidase/GSH reductase system can eliminate H_2O_2. From (Dröge 2002)

Artificial selection for long-lived fruit flies increases the content and the activity of antioxidant defense systems (ADS) and decrease the level of oxidative damage (Arking et al. 2000). Conversely, reverse selection for short-lived fruit flies decreases the content and activity of ADS and increases the level of oxidative damage. In parallel to these observations, different teams tried to increase the lifespan of fruit flies by overexpressing ADS (Sohal 1995, Orr and Sohal 2003, Parkes 1998). However results were inconsistent. Orr et al. drew the conclusion that overexpression of ADS was able to increase the lifespan of *Drosophila melanogaster* only in short-lived strains. Indeed, long-lived strains of fruit flies usually exhibit higher levels of SOD activity than short-lived strains. In mice, results of ADS gene knockouts (KO) on lifespan are not consistent (Muller et al. 2007).

2- Molecular damage

LIPID OXIDATIVE DAMAGES

Molecules of O_2 as well as free radicals can concentrate in the phospholipid bilayers of cells. If they react with polyunsaturated fatty acids (PUFAs) they can generate potentially toxic compounds such as lipid peroxides or aldehydes. Aldehydes result from the breakdown of lipid hydroperoxides (Pamplona 2008).

Lipid peroxidation is a chain reaction with three primary steps: (i) initiation, free radicals react with PUFAs to create lipid radicals, (ii) propagation, with formation of lipid peroxides and free radicals such as lipid peroxyl radicals and lipid radicals, (iii) and finally, termination by formation of non-radical products (NRP) through reaction between the free radicals (Kelly et al. 1998) **(Fig. 9)**. Accumulation of lipid peroxides in the membrane increases its permeability. Moreover, they can breakdown into aldehydes such as malonaldehyde (MDA) and 4-hydroxyal-kenals, or in particular 4-hydroxynonenal (HNE) and 4-hydroxyhexenal (Mark et al. 1997). These molecules are able to diffuse within the cell and react with proteins, DNA and other macromolecules. Susceptibility to peroxidation of PUFAs increases proportionally with the amount of unsaturation (Pamplona and Barja 2006). Alpha-tocopherol – vitamin E – can arrest the propagation step. Indeed, this molecule is lipid soluble and can scavenge lipid peroxyl radicals. Alpha-tocopherol is oxidized in reaction with radicals, however ascorbic acid – vitamine C – and ubiquinol can recycle it (Winterbourn 2008).

Removal of lipid peroxides can be directly accomplished via the glutathione peroxidase/glutathione reductase enzyme couple through the previously described mechanism. Nevertheless, Phospholipase A2, can first catalzyse the hydrolysis of phospholipids hydroperoxides into fatty acid hydroperoxides (Hulbert et al. 2007).

In *C. elegans*, Shmookler Reis et al. showed that the increase in longevity of mutants was correlated with modification in the fatty acid composition (Shmookler Reis et al. 2011). Indeed, compared to short-lived strains, long-lived strains had an increase from 34% to 48% in monounsaturates and a decrease from 37% to 26% in polyunsaturates. In fact, the peroxidation index, which represents the sensitivity of the membrane to lipid peroxidation, goes from 141 to 81 between the short-lived and the long-lived strains. These results are consistent with observations that maximum lifespan (MLSP) and peroxidation index are also correlated in mammals and birds (Hulbert et al. 2007).

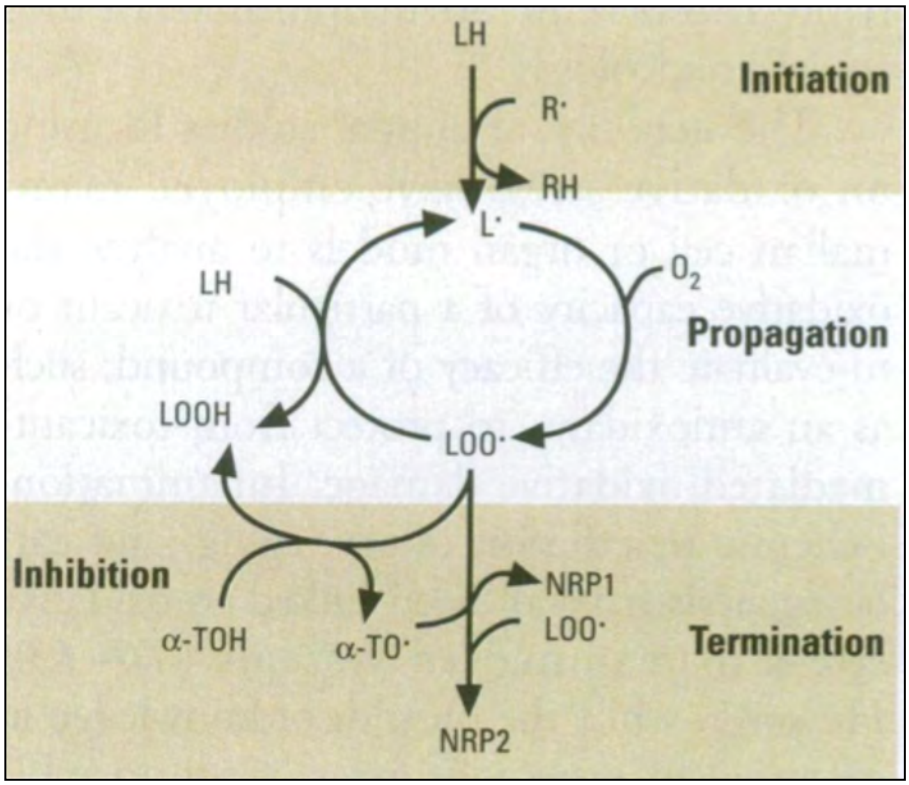

Figure 9 : Lipid peroxidation is a chain reaction: Initiation, Propagation, Termination. LH: Lipid Substrate. $LOO^{\cdot}$: Lipid peroxyl radical. LOOH: Lipid Hydroperoxide. α-TOH: α-tocopherol – Vitamin E. NRP: Non Radical Product. From (Kelly et al. 1998).

DNA OXIDATIVE DAMAGES

In eukaryotes, DNA is located in a specific cellular compartment, the nucleus, moreover histone proteins facilitate the formation of a compact superstructure, the heterochromatin, where DNA is relatively protected against harmful attack of reactive molecules. Nevertheless, ROS or derivatives of lipid peroxidation can cause DNA lesions, including double strand breaks, single strand breaks or DNA oxidation in less compact zones. In spite of a large battery of enzymes that can repair these damages (Cooke et al. 2003), some may persist and give rise to deleterious genome rearrangements or mutations.

8-Oxoguanine is the most common form of oxidative damage to DNA. Although, this marker is commonly used to measure aging, results taking into account the evolution of DNA oxidation are not always consistent and are subject to experimental artifacts (Jean Boiteux, personal communication). DNA base lesions can be removed by a base excision repair mechanism, involving removal of single lesions by a glycosylase action or by a more

complex mechanism leading to the removal of lesions containing oligonucleotides by the nucleotide excision repair system (Marnett 2000). If lesions are not repaired they can give rise to mutations during the next replication cycle or during transcription. Initially, mutations were thought to be the culprit due to DNA damage during aging, however, genome rearrangements appear to have potentially more dramatic consequences (Hastings et al. 2009, Vijg and Dollé 2002).

Another common form of damage sustained by DNA in dividing cells is telomere shortening. The precise mechanisms linking telomere shortening and aging processes are still undergoing highly intensive research activity, (Sahin and Depinho 2010, Passos et al. 2010) but interestingly even the lifespan of *C. elegans,* an animal which soma is exclusively composed of post-mitotic cell, can be extended through overexpression of a protein that lengthens telomeres (Joeng et al. 2004). As ROS are particularly produced in the mitochondria, mitochondrial DNA is more prone to oxidation. Indeed, several groups have reported a higher level of 8-oxoguanine and mutations in mitochondrial DNA as compared to nuclear DNA (Denver et al. 2000, Wallace 2010).

PROTEIN OXIDATIVE DAMAGES

ROS can react directly on proteins to cause oxidative damage or they can react with other molecules such as lipids and sugars that eventually react downstream with proteins. ROS reactions with proteins can lead to the breakage of an amino acid and/or a modification of the three-dimensional structure of the protein through the loss of a bond between two amino acids (Stadtman 2006). Regulatory sites, such as phosphorylation sites or methylation sites can also be targeted by ROS.

All amino acid residues of proteins are susceptible to oxidation by ROS (Stadtman 2006). Most of these modifications are irreversible, except for the creation of disulphide bonds between two cysteines or the conversion of methionine into methylsulfoxide (Levine and Stadtman 2001). Interestingly, several enzymes regulate their activity depending on the oxidative status of two neighboring cysteines within their active site (Dröge 2002, Sevier and Kaiser 2008). The most frequent and stable oxidative damage, aside from methionine and cyteine oxidation, is the formation of protein carbonyl derivatives (PCD). They can originate from direct oxidation of specific amino acids - lysine, arginine, proline and threonine – by $OH^{\cdot}$ via a metal-catalyzed oxidation (MCO), or they can result from the addition of aldehydes

to proteins produced during lipid peroxydation or reactive carbonyl derivatives (Nyström 2005). Measurement of PCD is classically done by derivatization of the carbonyl function by 2,4-Dinitrophenolhydrazine **(Fig. 10)**. The level of carbonyl functions can then be determined by immunofluorescence (Aguilaniu et al. 2003).

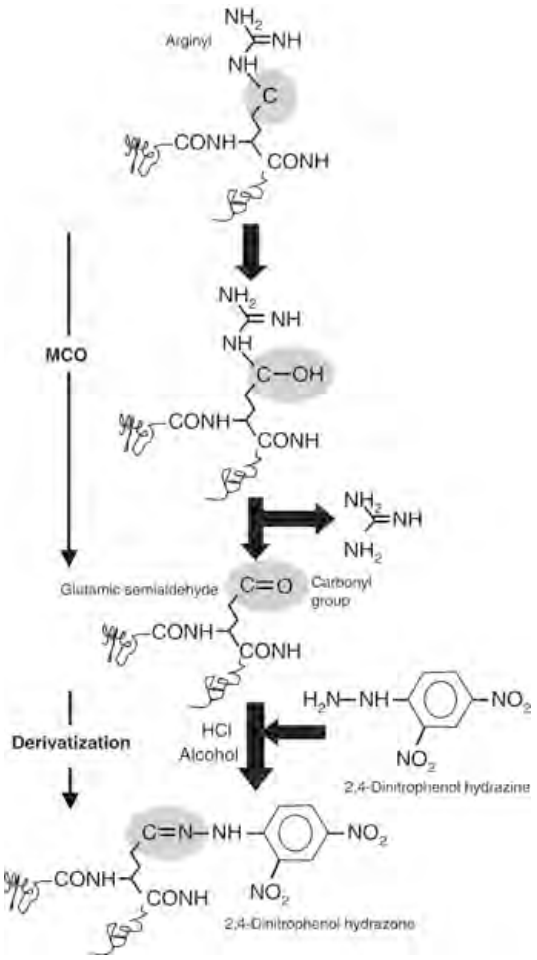

Figure 10: Carbonylation and derivatization of a protein amino-acid side chain. Metal Catalized Oxidation (MCO) of amino-acids can lead to protein carbonyl formation. 2,4-Dinitrophenol hydrazine addition to the protein carbonyl result in a protein 2,4-dinitrophenol hydrazone. This product can be detected by specific monoclonal or polyclonal antibodies (Nyström 2005).

PCD content increases over the lifespan of several model organisms and in senescent cell cultures (Levine and Stadtman 2001) **(Fig. 11)**. Accumulation of PCD has also been shown to correlate with life expectancy in *Drosophila* experiencing different life conditions (Sohal et

al. 1993). Moreover, Das et al. showed that the age associated decrease of aconitase activity due to increased carbonylation may be directly linked with the aging process (Das et al. 2001). Indeed, inhibition of aconitase by fluoroacetate can decrease life expectancy of fruit flies in a dose-dependent manner.

It is important to note that sensitivity of proteins to oxidation depends not only on ROS quantity but also on the folding state of the proteins (Dukan et al. 2000). Indeed, Dukan et al. showed that misfolded proteins are especially sensitive to protein carbonylation. Thus, chaperone proteins, involved in protein folding or refolding play a central role in the protection of proteins against oxidative damage. However if proteins are oxidized they are preferentially degraded by the proteasome and an imbalance between damaged protein production and proteasome activity can lead to the formation of aggregates of crosslinked proteins (Carrard et al. 2002).

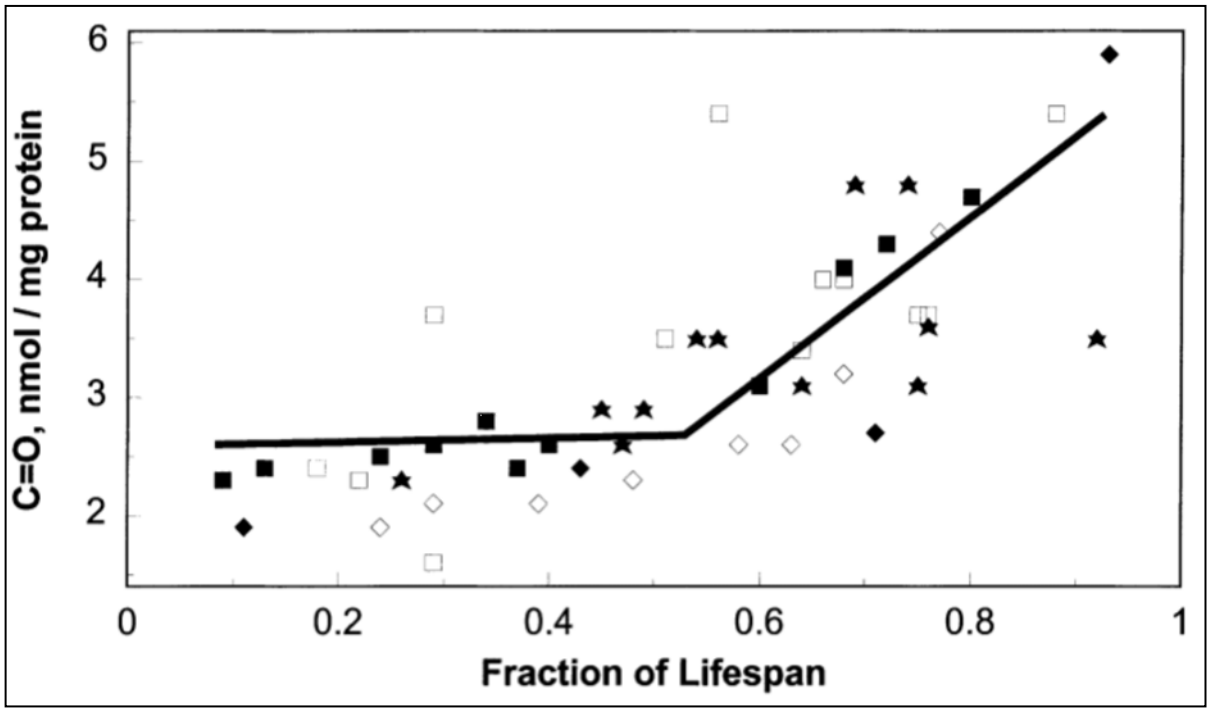

Figure 11: Protein carbonyl level increase with age. However increase is more pronounced during the last third of the lifespan. The line is the semi-logarithmic fit to all the data points. The data points were taken from published reports: ■, human dermal fibroblasts in tissue culture; ★, human lens; □, human brain obtained at autopsy; ◆, rat liver; and ♢, whole fly (Levine and Stadtman 2001).

AGE-PRODUCTS, LIPOFUSCIN AND OTHER AGGREGATES

The size and chemical nature of cell aggregates can vary. Most of the time they result from non-enzymatic reactions. Although they can be the signatures of specific diseases (e.g., beta-amyloid aggregates in Alzheimer's disease), they usually occur in non-pathogenic conditions (Lindner and Demarez 2009). Indeed, lipofuscin accumulates with age in postmitotic cells (Brunk and Terman 2002). This is thought to originate from oxidized unsaturated lipids, but reactions in the lysosome with autophagocytosed materials give rise to the final structure of lipofuscin-like products. These molecules are autofluorescent (340 nm/430 nm) so their accumulation can be easily monitored (Gerstbrein et al. 2005). Due to their complex structure they may be difficult to break down, which favors their accumulation. High levels of lipofuscin in cells may interfere with processes including autophagy and degradation of oxidized proteins by the proteasome (Keller et al. 2004).

Advanced Glycation End products (AGE) are another well-studied class of molecules that accumulate with age. They are the end products of the Maillard reaction, a series of non-enzymatic reactions between reducing sugars like glucose and proteins, lipids or nucleic acids. These reactions mainly affect structural components of the connective tissue matrix or basement membrane such as collagen (Singh et al. 2001).

II- Aging in *C. elegans*

A- Biology of *C. elegans*

C. elegans is a 1 mm long roundworm of the phylum Nematoda **(Fig. 12)**. Nematodes are pseudocoelomates that possess neither vascular nor respiratory systems but despite their apparent anatomical simplicity, members of this phylum are very diverse with many life styles represented: plant parasites species (*Trichodorus*), mammals parasites species (*Ascaris*, *Trichostrongylus*), free living species (*Caenorhabditis*). The lifespan ratio between the shortest lived, *Rhabdias bufonis* (3 days), and the longest lived nematode, *Loa loa* (15 years) is around 2000. *C. Elegans* is not the only model organism of this phylum, *C. briggsae* and *Turbatrix aceti* are also studied (Gems 2000).

C. elegans is a free-living species that can be found in rotten fruits and organic compost (Félix and Braendle 2010). They eat bacteria and yeasts. As opposed to other nematodes species such as *Heterorhabditis* and *Steinernema*, they do not seem to have specific commensal bacteria in their digestive tract (Münch et al. 2008, Marie-Anne Félix personal communication). In fact, the ecology of *C. elegans* is still poorly known. For example, it is not known how they survive during winter. In laboratory conditions nematodes are bred between 15°C and 25°C and their standard food is *E. coli* OP50.

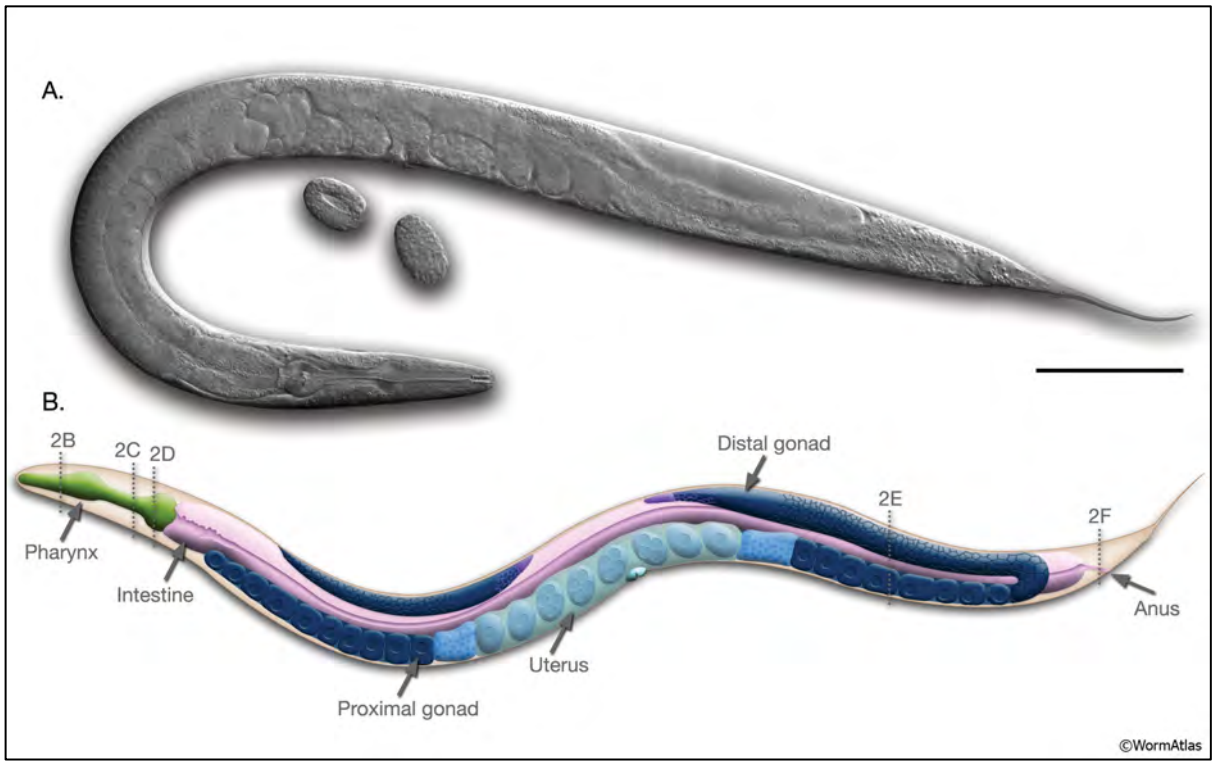

Figure 12: Anatomy of an adult hermaphrodite. **A.** DIC image of an adult hermaphrodite, left lateral side. Scale bar 0.1 mm. **B.** Schematic drawing of anatomical structures, left lateral side. Dotted lines and numbers mark the level of each section (accessible on the website). From (Altun and Hall 2010)

1- Reproduction function of *C. elegans*

C. elegans is usually considered as a self-fertilizing hermaphrodite. However, males do arise in laboratory, at a proportion of 0,1% due to genetic rearrangement during meiosis (Félix and Braendle 2010). Stressful conditions during oogenesis (e.g., high temperature) can increase their proportion. Whether males play a role in nature is not known. They have a distinct number of somatic cells, they produce only sperms but in greater numbers than hermaphrodites and the fertilizing ability of their spermatozoids is higher. Males can be recognized by the typical shape of their tail.

After hatching *C. elegans* larvae have more or less the morphology of an adult. Individuals progress through four larval developmental stages (L1, L2, L3, and L4) **(Fig. 13)**. Their adulthood can generally be divided into a reproductive period and a post-reproductive period. An alternative stage, the dauer state, may occur under specific stressful conditions (e.g., high temperature, high population density and low quantity of food) at the end of the L1 stage (Hu 2007). Dauers have a specific metabolism and are especially resistant to stress as

compared to adults or other larval stages. Moreover, they can survive for months and if environmental conditions become favourable, they can finally reach L4.

Germ cells begin to proliferate at the L1 stage. The fate of the germ cell, i.e., whether they will become sperms or oocytes, seems to be decided in the distal tip cell (DTC). Sex determination depends on time differential expression of specific genes like *fog-3* or *fem-3*. From late L4 to young adult, germ cells differentiate into sperms. Thereafter, oocytes are produced. For hermaphrodites, number of progeny depends on the quantity of sperms, which is the limiting factor (Kimble and Crittenden 2007).

In lab conditions, that is to say, at a constant temperature and with food ad libitum *C. elegans* display reproductive aging (Luo and Murphy 2011). After 4 days of adulthood, *C. elegans* stops producing fertilized eggs although their remaining lifespan is about 15 days. If hermaphrodites mate with males during their reproductive period they will produce more progeny. This is due to the fact that sperm is the limiting factor. Johnson's group showed that even old hermaphrodites are still able to produce eggs (Mendenhall et al. 2011). So, the end of the reproductive period does not seem to trigger the degradation of remaining oocytes. In fact, germline usually swell in old animals; Golden et al. observed polyploidy of the oocytes due to a new round of meiosis after sperm depletion (Golden et al. 2007).

Reproductive lifespan in *C. elegans* does not correlate with the total adult lifespan when different mutant strains are compared (Huanget al. 2004). It is noteworthy that the method of survival analyses as performed in laboratory may lead to artifacts. Indeed, adult *C. elegans* in the wild experience cycles of hotter and cooler temperature with the alternation of day and night. We know that at temperatures higher than 28°C wild type (WT) nematodes stop laying eggs. Thus, we cannot extrapolate that nematodes display reproductive aging in nature and to our knowledge this phenomenon has never been investigated.

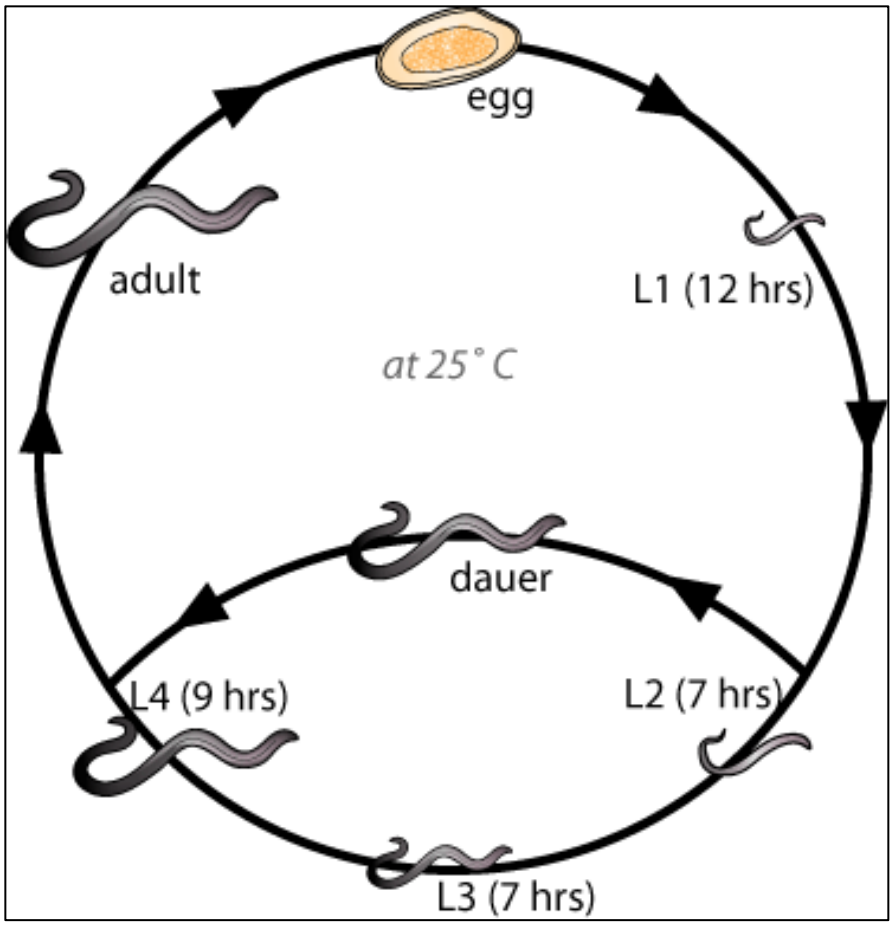

Figure 13: Life cycle of *C. elegans*. After L1 stage, *C. elegans* larvae can enter in the dauer state in harsh environmental conditions including high temperature, high density, and low food supply. When environmental conditions become more favorable dauers they can finally reach L4 (Yuan 2004).

2- Nutrition function of *C. elegans*

Even if the ecology of *C. elegans* is poorly known, this nematode seems to feed, at least partially, on live microorganisms in its natural habitat (Félix and Braendle 2010). Thus, digestion and immune response are complex to disentangle. In the paragraphs below, we will see how the structure of the digestive tract of *C. elegans* can provide nutriments from its microbial food and protect itself from infection and finally, we will describe what is known about aging of the digestive tract.

The pharynx composes the first part of the digestive tract of *C. elegans*. It is dedicated to pumping and mechanical digestion of the food. This organ is made up of 9 epithelial cells, 20 muscle cells, 9 marginal cells, 4 gland cells and 20 neurons (Franks et al. 2006). The pharynx has intrinsic myogenic activity that is regulated by its nervous system. The nervous system, in turn, integrates internal signals such as the animal's nutritional status and external

signals such as the presence or absence of food (Avery and Horvitz 1989). The mechanical action of the pharyngeal grinder is essential for the protection of the worm against infection because *phm-2* mutant worms with grinder malformation are more susceptible to death from infection (Gravato-Nobre and Hodgkin 2005). However these worms grow normally on *E. coli* OP50, which therefore means that this strain is not too pathogenic for the worm.

The intestine is composed of 20 large epithelial cells. The pH in the lumen has never been measured directly, however proteases secreted in the lumen have an optimal activity ranging from pH 4 to pH 5 (McGhee 2007). Thus, it is assumed that the pH of *C. elegans'* gut is slightly acid. The nematode possesses different kinds of molecules to chemically digest microorganisms. It is assumed that lysozymes, encoded by 10 genes in nematodes, and saposin-like proteins act synergistically to disrupt the cell wall of microorganisms. Overexpression of lys-1 leads to an increased resistance of the worms against *Serratia marcescens* infection (Mallo et al. 2002). Transit is passive but the gut is voided 45-50 seconds due to autonomous defecation cycles (McGhee 2007).

During aging, defecation cycle frequency decreases, but Bolanowski et al. showed that there was no correlation between the rate of decrease and the life expectancy of the worms (Bolanowski et al. 1981). Conversely, pumping rate of *C. elegans* is a good biomarker of aging (Huang et al. 2004). McGee et al., have recently investigated the aging of the intestine and they observed that the digestive tract is compressed in some places because of the swelling of the reproductive system (McGee et al. 2011). This phenomenon may prevent the lumen from being voided correctly and lead to the formation of bacterial pockets. By day 12, at 20°C, aging of the enterocytes is associated with a decrease in the size of the nuclei and, in more advanced ages, with a loss of these nuclei. This phenomenon occurs especially in the anterior half of the digestive tract. The loss of nuclei is not due to the physical pressure of the germline because *glp-4* mutants, that do not display mitosis of their germ cells, have a similar phenotype compared to wild type. But interestingly, *daf-2* mutants exhibit an increased resistance to the loss of enterocyte nuclei until old ages.

Some authors argue that *E. coli* OP50 could promote aging of the nematode. Indeed, Garrigan et al. showed that after addition of carbenicillin to the bacterial lawn of *E. coli* OP50, the proliferation of the bacteria is stopped and the nematodes live longer (Garigan et al. 2002). Moreover, if we replace the standard food OP50 by the laboratory strain *Bacillus subtilis* PY79, *C. elegans* live also longer (Garsin et al. 2003). However so far, it is impossible to conclude that *E. coli* OP50 directly damages the nematode or if this deleterious effect would be mediated by the sensory system of the worm.

3- Relation function of *C. elegans*

Five percent of the genome of the worm is devoted to sensory perception (Bargmann 2006) and *C. elegans* is able to adapt its behavior according to signals from the environment. So far, a number of taxes have been described. They include reactions to chemicals –(e.g., butanedione, NaCl) or to physical phenomena (e.g., temperature, light) (Ryu and Samuel 2002), and electric fields-. In addition, nematodes adapt their foraging strategy to the "quality" of their environment (Shtonda and Avery 2006) or to pathogen threat. Pathogen avoidance behaviour is well described in the literature (Zhang et al. 2005). Neuron circuits regulating these behaviors have, for the most part, been elucidated.

In the following section we will describe the basic components of the locomotive function of *C. elegans*. Then we will describe electrotaxis.

a- Locomotion in C. elegans

Locomotion requires the coordinated action of neurons and muscles. Information about locomotion output is integrated at the level of interneurons. These cells receive orders from sensory neurons and transmit their commands to motor neurons. The muscle system of *C. elegans* is composed of 95 rhomboid-shaped body wall muscle cells (Altu and Hall 2010) (Sulston and Horvitz 1977). These cells are arranged in four longitudinal bundles located in four quadrants. Nematodes can only make dorsoventral bends, so to crawl they have to be either on their right or left side. Innervations of the different muscle quadrants are specific. Muscles from the head are only innervated by motor neurons of the nerve ring, muscles from the neck are innervated from motor neurons of the nerve ring and the ventral cord, muscles from the body and the tail are only innervated by the ventral cord. Nerve information is transmitted from the motor neuron to the muscle cell at the level of the neuromuscular junction. *C. elegans* exhibit 4 types of motor neurons (A, B, C, and D) (Altun and Hall, Nervous system, general description 2011). The C-type is not linked to locomotion; it innervates the vulva muscle. The A- and B-type motor neurons are stimulatory; they use acetylcholine to mediate their signal. The D-type is inhibitory; it uses gamma-aminobutyric acid (GABA). The D-type of motor neuron is strictly post-synaptic of other motor neurons. This means that it is the same input from the ventral cord that triggers the contraction of the

ventral muscle and the release of the dorsal muscle via the inhibitory neuron. The post-synaptic muscle junction contains two kinds of receptors for acetylcholine, a nicotinic receptor and a levamisole receptor, and one kind of receptor for GABA (Gottschalk et al. 2005).

The locomotive behavior of *C. elegans* can be dissected into different specific movements: (i) forward movement, (ii) reversal, which can be short or long, (iii) the omega turn, if animals turn from an angle superior to 120°. This movement is called the omega turn because from the top, the nematode seems to draw the Greek letter with its body (Gravato-Nobre and Hodgkin 2005).

b- Sensing the environment

The initial work reporting electrotaxis of *C.elegans* was published in 1978 by Sukul and Croll (Sukul and Croll 1978). However, this phenomenon had been observed earlier with other nematode species. In 2007, Samuel's team at Harvard investigated the determinants of this behavior and showed that it was mediated by a specific neural circuit (Gabel et al. 2007).

When nematodes are subjected to electric fields (EF) they crawl toward the negative pole. This behavior is observed for EF between 4 V/cm and 14 V/cm. When the EF is higher than 14 V/cm, worms are temporarily paralyzed until the current is switched off. At intermediate values of potential difference, nematodes crawl toward the negative pole with a deviation from the direction of the EF proportional to its strength. The cause of this deviation is not known. EF strength has no influence on the speed of the nematodes.

Samuel's team showed that electrosensory behavior was mainly mediated by the ASJ and ASH neurons **(Fig. 14)**. ASJ neurons are known to play a role in formation and recovery from dauer stage by detecting dauer pheromone and food. They also have a minor role in positive chemotaxis for water-soluble chemoattractant. ASH neurons are known to be involved in mechanosensation. They mediate a reversal movement after a head-on collision or a nose touch. Laser ablation of these neurons prevents worms from moving toward the negative pole without impairing their normal locomotion. Ablation of ASK, AWC and AWB neurons has only a slight effect on the electrosensory behavior whereas ablation of ASI neurons has no effect. During experimental monitoring of intracellular calcium dynamics, Gabel et al. observed that the strongest signal was always observed when the immobilized

nematode was pointing its head toward the positive pole. Thus, it seems that worm decision to crawl toward the negative pole is associated with a decrease of the amphid sensory neurons activity (Gabel et al. 2007).

Compared to chemotaxis, trajectories in electrotaxis are straighter and the experiments are shorter.

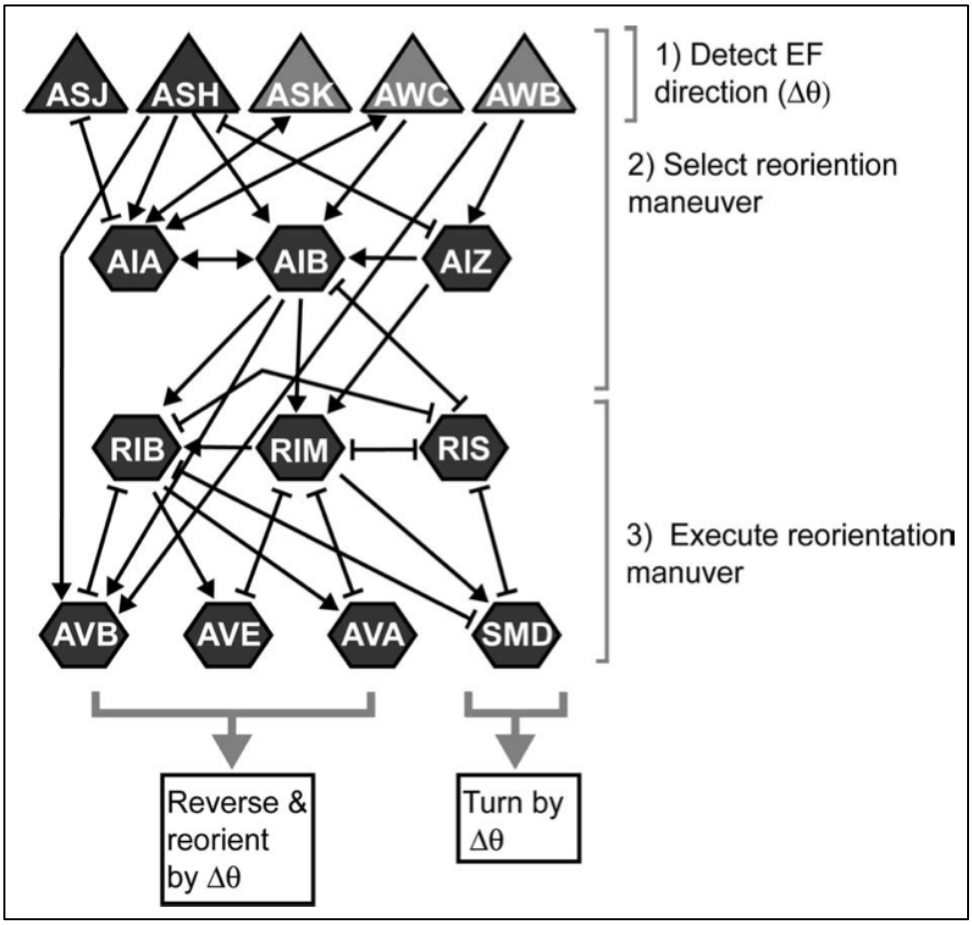

Figure 14: Neural circuits for electrosensory behavior. ASJ and ASH are the neurons that sense electric field. Sensory neurons are indicated by triangles. Interneurons and command neurons by hexagons. Arrows indicate Chemical synaptic connections between neurons. (Gabel et al. 2007)

c- Aging of locomotion ability

In 1980, Hosono set a classification describing different stage in *C. elegans* locomotory decline:

Type I: worms progress with rhythmic sinusoidal movement.

Type II: worms are able to progress but their movement is irregular and not active.

Type III: worms are unable to progress but move their head after a gentle touch with a tooth-pick.

He demonstrated that according to this classification it was possible to sort worm having different life expectancy (Hosono et al. 1980). Herndon et al. employing the same classification showed that worms locomotory decline was correlated with sarcomeres disorganization in muscle cell (Herndon et al. 2002). At the same time Glenn et al. reported that treatment with arecoline, an analogue of acetyl choline could improve locomotory performance of 10 days old nematodes (Glenn et al. 2004). More recently, Hsu et al. reported that rate of motor activity decay from day 3 to day 9 was even better than punctual measurement to predict life expectancy of worms (Hsu et al. 2009).

B- What we know about aging in *C. elegans*

1- Modulation of aging in *C. elegans*

Modulation of lifespan, and more particularly, increase of lifespan can be done by different ways: (i) modulation of the environment (Lucanic et al. 2011), (ii) genetic manipulation (Kenyon 2010), (iii) chemicals (Onken and Driscoll 2010, Evason et al. 2008). Around the beginning of the 80's, different teams discovered single mutations that were able to extend significantly lifespan of nematodes. These finding were in apparent opposition to evolutionary theories. Indeed, aging is supposed to be caused by the sum of multiple mechanisms that escape natural selection. Since then, hundreds of mutations have been showed to extend significantly lifespan of nematode (Mendenhall et al. 2011). For now, Ayyadevara et al., found the most dramatic one: null mutants of *age-1* gene, involved in the IIS, after prolonged developmental times at 15-20 degrees C, exhibit a 7 fold increase of lifespan compared to a control worms raised on a bacterial lawn (Ayyadevara et al. 2008). Most of our tools to investigate modulation of longevity have been based on generation of mutants or RNAi screen, so the mechanisms found to increase lifespan are for now mainly loss of function one. Only few experiments, using transgenic lines that over express specific gene, have been shown to be relevant to increase lifespan (Back et al. 2010).

Manipulations with a beneficial effect on lifespan are usually linked with pathways dealing with nutrient sensing, energy homeostasis or reproduction (Kenyon 2010). In this

chapter we will see how different input coming from nutrient sensing systems, reproductive system or mitochondria can modulate the lifespan of the nematodes. Issues related to protein homeostasis will be more described in the next paragraph.

a- Alteration of aging by nutrient sensing systems inputs

INSULIN/INSULIN LIKE SIGNALING (IIS) PATHWAY

As mentioned previously, *C. elegans* can enter in a specific state, the dauer larvae state, during its development when environmental conditions are not favorable. Dauers can survive for months. This duration is huge compared to the lifespan of adult worms which is around two weeks at 20 °C. Several pathways control the entry in this stage including the Insulin Like growth Signaling (ILS) pathway (Honda et al. 2008). Mutations in this pathway can have major consequences on *C. elegans* life history traits. For example they increase the probability of worms to form dauers and larvae that reach adulthood are long-lived but most of the time less fertile (Tissenbaum and Ruvkun 1998). When DAF-2 (abnormal DAuer Formation), the only receptor of the Insulin-Like Signaling pathway (ILS), is activated, a cascade of phosphorylations leads to sequestration of DAF-16 in the cytoplasm. DAF-16 is a transcription factor that controls the expression of genes involved in stress resistance. So, mutations that counteract the cascade of phosphorylation, favor the translocation of DAF-16 in the nucleus and lead to a stress resistant and long-lived phenotype whereas mutations preventing the action of daf-16 can give rise to short-lived animals (Mukhopadhyay et al. 2006).

Upon insulin like peptide stimulation, the DAF-2 tyrosine kinase receptor activates the phosphatidyl Inositol Kinase AGE-1. AGE-1 can in turn convert the PhosphatidylInositol-4,5-biphosphate (PIP2) in PhosphatidylInositol-3,4,5-triphosphate (PIP3). A mutation in each of these genes can lead to an increase of two to three folds of the lifespan of nematodes (Kenyon 2005). Null mutation in *age-1* can even lead to an increase of seven times of the nematode lifespan (Ayyadevara et al. 2008). The *daf-18* gene, an orthologue of the tumor suppressor gene PTEN, downregulates ILS pathway by PIP3 dephosphorylaton. This phosphatase is required for dauer entry and *daf-18* mutants are short lived (Masse et al. 2005). Activation of various kinase: AKT-1, AKT-2, SGK-1, and PDK-1, by PIP3 ultimately lead to phosphorylations of daf-16 (Yen et al. 2011). These phosphorylations will favor the

binding of DAF-16 with 14-3-3 proteins and the sequestration of the transcription in the cytoplasm (Berdichevsky et al. 2006) **(Fig. 15)**. In conditions favoring DAF-16 translocation into the nucleus, a set of various genes involved in stress resistance is activated (Murphy et al. 2003, Murphy 2006). These genes are involved in Anti-oxidant Defense Systems (ADS) process, such as *sod-3* and *mtl-2*, in proteome maintenance, for example *hsp-16.2*, or anti-microbial defense, *lys-7* and *lys-8*. As a consequence, *daf-16* mutants are short-lived. Effects of ILS signaling are not only mediated by DAF-16. Indeed, the transcription factor SKN-1, a cap'n'collar related transcription factor, is also under the control of DAF-2 (Tullet et al. 2008). As for DAF-16 SKN-1 effect is mediated by the three kinases AKT-1, AKT-2 and SGK-1. SKN-1 activates the expression of genes necessary for the phase II detoxification systems and enzymes involved in anti-oxidant defense such as glutathione S-transferase. SKN-1 is necessary to observe the long-lived phenotype of *daf-2* mutants. The *skn-1* mutants are short-lived (An and Blackwell 2003).

ILS can modulate autophagy in a mechanism that remains to be discovered but independent of DAF-16. When autophagy is compromised *daf-2* mutants do not exhibit anymore a long-lived phenotype. Though *daf-2;daf-16* double mutants exhibit autophagy, they are not long-lived (Hansen et al. 2008).

Different pathways modulate effects of ILS by promoting DAF-16 and SKN-1 translocation into the nucleus. JNK pathway involved in stress response in drosophila and mammalian can promote daf-16 translocation into the nucleus independently of ILS (Oh et al. 2005). In fact, Oh et al. have shown that JNK-1, a mitogen activated protein kinase, can phosphorylate DAF-16. Mutations in *jnk-1* shorten lifespan of nematodes whereas a transgenic line overexpressing this gene exhibit a long-lived phenotype with increased resistance to heat-shock treatment and oxidative stress. Similarly, overexpression of SIR-2.1 mediates an increase in lifespan in a 14-3-3 and DAF-16 dependent way. An association between these three proteins seems to specifically promote the expression of genes involves in anti-oxidant defense (Berdichevsky et al. 2006). During oxidative stress the p38MAPK pathway can promote translocation of SKN-1 in the enterocyte nuclei. In normal conditions this effect is counterbalanced by the glycogen synthase-3 (GSK-3).

Cofactors can affect the transcriptional outputs of DAF-16 and SKN-1. DAF-16 normally triggers the synthesis of HSP-16.2 in response to heat-shock, however expression of HSP-16.2 is higher when DAF-16 acts in synergy with heat-shock factor 1 (HSF-1) (Hsu et al. 2003). Indeed, Heat Shock Proteins 90 (HSP 90) normally sequestrate HSF-1 in the

cytoplasm, but when *C. elegans* experience conditions like heat-shock, that increase the number of misfolded proteins, HSF-1 is released and can stimulate expression of Heat-Shock proteins in synergy with DAF-16.

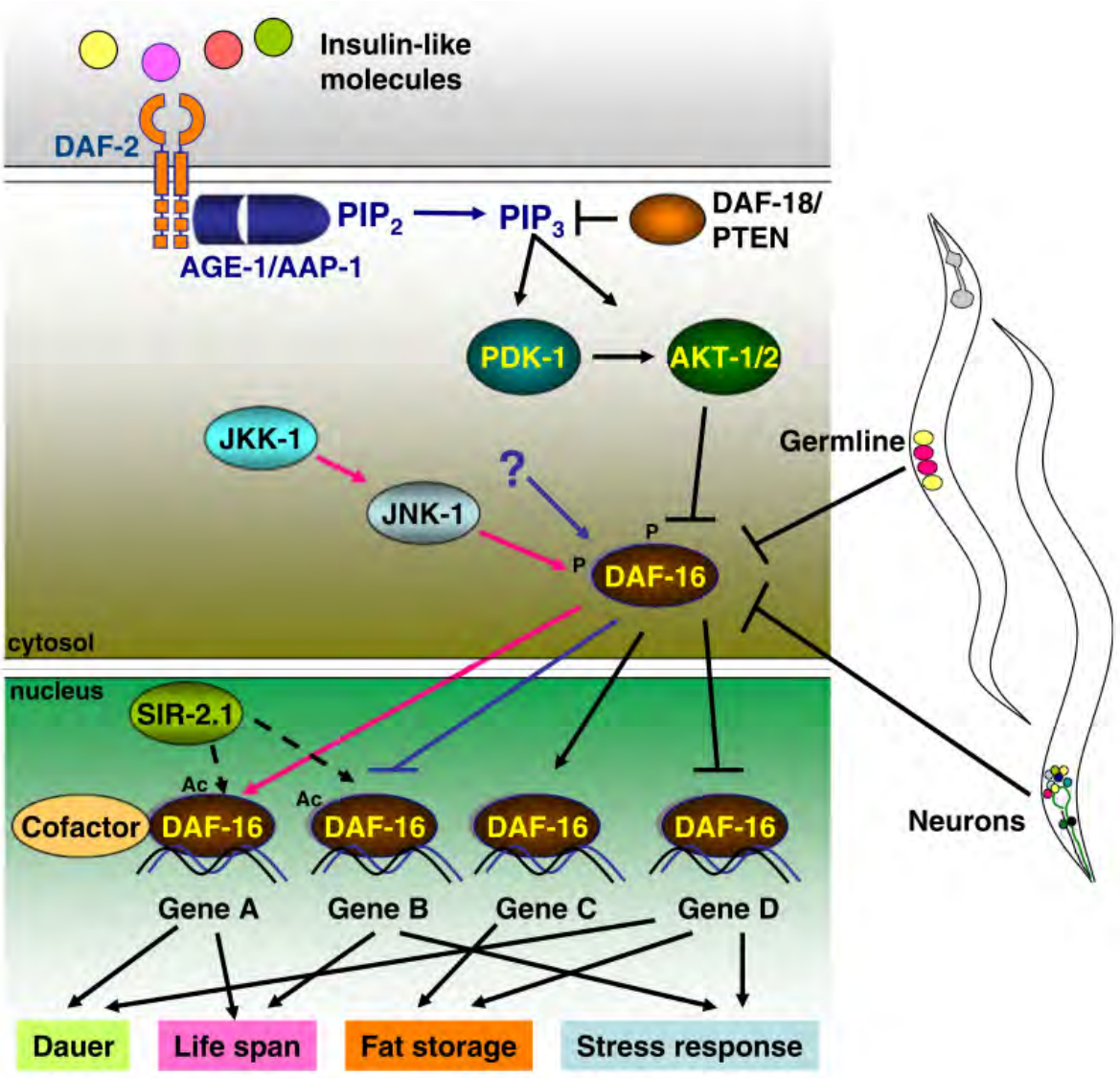

Figure 15: Regulation of Daf-16 activity in the ILS pathway context. Low insulin signaling will promote translocation of DAF-16 in the nucleus. Independently or in synergy with different cofactors DAF-16 will activate transcription of genes implicated in the long-lived phenotype. From (Mukhopadhyay 2006)

Taken together, these results show that to the long-lived phenotype *daf-2 C. elegans* mutants is dependent on the synergistic action of heat-shock proteins, autophagy and antioxidants enzymes. To understand how this pathway was regulated at the level of the organism the team of Ruvkun studied the cell autonomous/non autonomous action of *daf-2*

mutations. Presence of this mutation only in neurons or to a lesser extends in enterocytes is sufficient to observe the long-lived phenotype (Wolkow et al. 2000). Libina et al. investigated whether *daf-16* was required in all tissues to observe the long-lived phenotype of *daf-2* mutants (Libina et al. 2003). They used the background genotype *daf-16*, *daf-2* double mutants, and they specifically expressed in enterocytes a transgenic *daf-16* construction. They observed a 60% increase of lifespan in the transgenic double mutant. The increase is only 3-15% when daf-16 is only expressed in neurons and there is no effect on lifespan when *daf-16* is only expressed in muscle.

Dillin et al observed whether low ILS was required during the whole life of *C. elegans* to observe a long-lived phenotype (Dillin et al. 2002). They exposed WT to daf-2 RNAi at different moment of their life. They found that exposure of nematodes to *daf-2* RNAi during development is not required to have the long-lived phenotype. Conversely, exposure of *C. elegans* to *daf-2* RNAi after reproductive period has only minor effect on lifespan.

DIET RESTRICTION

Lifespan of organisms from yeast to rodents are increased by diet restriction. The long-lived phenotype is usually associated with a decrease in reproductive capacity (Shanley and Kirkwood 2000). In animals including fruit flies and rats the diet-restricted phenotype is induced by a low level in protein intake. Grandison et al., demonstrated in fruit flies, that the long-lived phenotype and the low fertility can be decoupled by adjusting specific proportion of each amino acid in the diet (Grandison et al. 2009). In *C. elegans*, diet restriction can be observed in different contexts such as mutants with a low pumping rate -eat-2 mutants-, and modulation of the concentration of food available (Houthoofd et al. 2003, Panowski et al. 2007). In addition to lifespan extension, a low accumulation of lipofuscin and a low level of fat mass characterize diet restriction induced phenotype in *C. elegans* (Gerstbrein et al. 2005). Diet restriction is mediated by several transcription factors such as PHA-4, which is antagonized by the TOR pathway, and SKN-1 (Panowski et al. 2007) (Bishop and Guarente 2007). The *daf-16* gene is not required to observe the diet restricted phenotype (Houthoofd et al. 2003).

TOR pathway. In *C. elegans*, the TOR pathway modulates protein synthesis and antagonizes PHA-4 (Sheaffer et al. 2008). ILS and TOR pathway are interlinked at the level of DAF-15 the regulatory subunit of *let-363*/TOR, because DAF-16 can repress transcription of *daf-15* (Jia et al. 2004). TOR activation lead to an increase in the cell translation capacity. Phosphorylation of the eIf4-BP binding proteins by TOR releases eIf4E, a translation initiation factor (Hansen et al. 2007). TOR also favors translation by phosphorylating the S6K, which in turn phosphorylate the S6 ribosomal protein. Pha-4 is necessary to observe the DR phenotype in target tissues. Pha-4 controls autophagy and genes involved in anti-oxidant defense (Panowski et al. 2007). TOR mutations increase lifespan of nematodes but this effect is abolished when Pha-4 expression is inhibited (Vellai et al. 2003) (Sheaffer et al. 2008).

Regulation of Diet restriction by ASI neurons. Bishop and Guarente have shown in nematodes that laser ablation of ASI neurons can abolish the diet restriction effect (Bishop and Guarente 2007) **(Fig. 16)**. The *skn-1* gene expression is necessary in ASI neurons to observe the diet restriction induced phenotype. SKN-1 exists in different isoforms and these isoforms are tissue specific. Skn-1b is especially active in neurons whereas skn-1c is especially active in enterocytes. Translocation of SKN-1b is constitutive in neurons but diet restriction increase the level of SKN-1b in the nucleus. In a *skn-1(zu135)* background mutant, expression of SKN-1b in neurons rescues diet restriction induced phenotype whereas expression of SKN-1c in enterocyte did not. The increased respiration observed in dietary restricted nematodes is not observed anymore in SKN-1 mutants. The expression of *skn-1b* in neurons is sufficient to rescue the increased respiration whereas the expression of *skn-1c* in the gut is not (Houthoofd et al. 2003, Bishop and Guarente 2007)

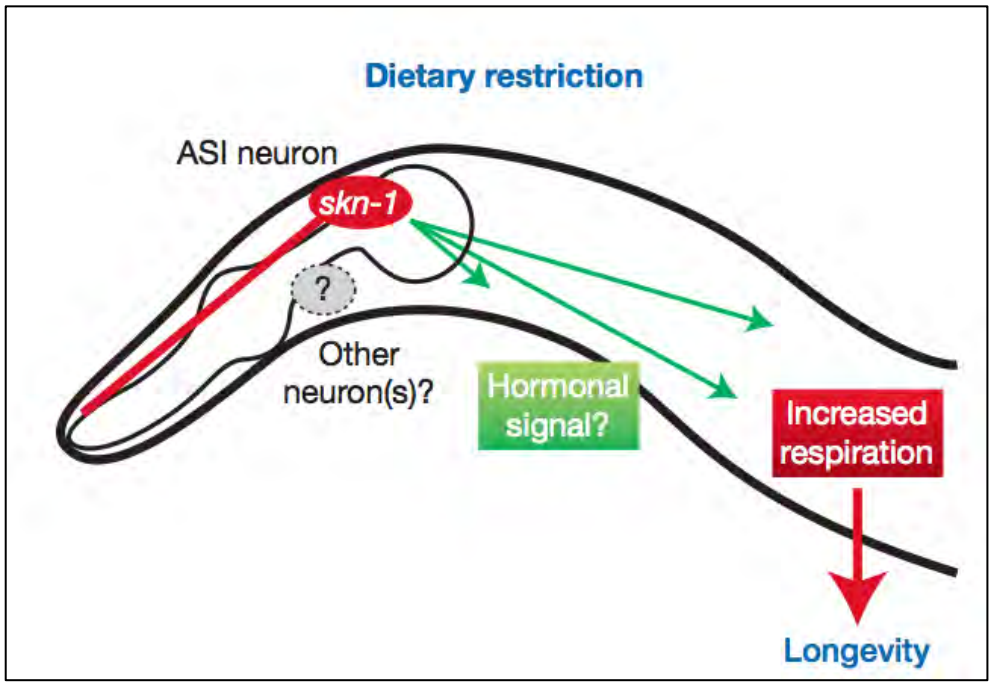

Figure 16: Regulation of diet restriction induced phenotype at the level of the organism. ASI neurons trigger increase respiration at the level of the target tissues. From (Bishop and Guarente 2007)

Results presented above show that in *C. elegans* the diet restriction induced phenotype is coordinated at level of the neurons by the isoform SKN-1b and in the target tissues by PHA-4. The main processes implicated in lifespan extension seem to be autophagy, with a reduced level of protein synthesis, and a high respiration rate, associated with a high level of antioxidant proteins production.

b- Modulation of aging by mitochondria

In the free radical theory of aging, overproduction of ROS is a leading cause of the aging process. Indeed, these molecules can react with different components of the cell and then disrupt key functions. Therefore, accumulation of oxidative damage is thought to be deleterious for the cells of an organism. However, as mentioned previously, a high respiratory rate is necessary for lifespan extension by dietary restriction and partial loss of superoxide dismutase function does not always prevent life extension in long-lived mutants.

It seems obvious that reducing the functioning of the ETC could be beneficial. Lowering the quantity of ROS produced and therefore the amount of oxidative damages seems to be a straightforward mechanism to slow down aging. Nonetheless, functional impairment of mitochondria in humans can lead to disease, for instance in Frederich ataxia. Rea et al. with an elegant set up showed that the long-lived phenotype of ETC "mutants" is dependent on the level of inhibition of the different components of the ETC (Rea et al. 2007). The beneficial effect can only be seen in a slight window. Some beneficial effect on lifespan has been observed with inhibition of protein of any complex of the ETC.
Interestingly, the effect on lifespan is concomitant with effects on development and egg production and is associated with a slow development and low number of progeny. The effect on lifespan extension can only be seen when *C. elegans* are in contact with RNAi during the development. When adult worms experience RNAi during adulthood they produce less ATP but there is no effect on lifespan. This effect seems counter-intuitive because ROS is supposed to have harmful effect also during adulthood. In addition, Rea et al. did not observe correlation between ROS production and oxidative damage on the one hand and lifespan on the other hand. However it is noteworthy that there is a dramatic increase in the number of mitochondria and mitochondrial DNA during L3 and beginning of L4 stage in *C. elegans*. It may be possible that crucial events happen at this time. The team of Dillin recently showed that the long-lived phenotypes of mit mutants can be regulated locally. Indeed, manipulations of the ETC in neurons were able to trigger a response in the whole organism (Durieux 2011).

Results of experiments manipulating the physiology of mitochondria are not easy to interpret. It is always hard to know if the ultimate phenotype is due to a beneficial mutation or if it is a hormetic response following a stress induced by the initial mutation. Yang et al. have shown that, although lifespan extension by alteration of given gene activity was a robust result, depending on the technique used to alter the gene activity, RNAi or mutation, the mechanisms involved could be divergent (Yang and Hekimi 2010).

c- Alteration of aging processes by Reproductive system inputs

Disposable soma theory postulates a trade off in resource allocation between investment in reproductive success and investment in somatic maintenance. Investigation of the mechanisms ruling the interaction between reproduction and survival can be achieved by

different strategies in *C. elegans* hermaphrodites. Laser ablation of gonads or mutations inhibiting germline proliferation can abolish progeny production while mating with male can increase it.

Complete removal of the gonad by laser ablation does not increase *C. elegans* lifespan. In fact somatic gonad and germline affect differently *C. elegans* longevity (Hsin and Kenyon 1999). When germline precursors are laser ablated or germline proliferation inhibited in mutants such as glp-1, only somatic gonad develops and the lifespan is increased. Ablation of the precursors of the somatic gonad lead to a normal lifespan compared to control. Increased lifespan in germline-less nematodes is dependent on different pathways involved in stress resistance and fat metabolism. The transcription factor DAF-16, the steroid receptor DAF-12 and the Nuclear Hormone Receptor NHR-80 are necessary to observe the long-lived phenotype (Mukhopadhyay et al. 2007, Goudeau et al. 2011). Though DAF-16 is both implicated in insulin like signaling and germline-less nematodes signaling, mechanisms controlling its action are different. Indeed, extended longevity mediated by DAF-16 is dependent on KRI-1/TCER-1 in germline-less nematodes whereas they are not necessary in daf-2 mutants (Ghazi et al. 2009).

Gems and Riddle observed that when *C. elegans* N2 hermaphrodites mate with male they live shorter (Gems and Riddle 1996). However, they found no correlation between lifespan of hermaphrodites and total oocyte production. Unfortunately, they did not examine viability of the progeny in order to evaluate quality of oocyte. These results demonstrate that investment in oocyte production is not directly linked with lifespan in *C. elegans*.

2- Proteostasis and aging

Adults *C. elegans* are post-mitotic animals, their somatic cells no longer divide after development. Therefore accumulation of non-functional proteins is critical because they cannot be diluted in daughter cells. We will describe here maintenance processes specifically involved in proteostasis.

a- Unfolded Protein Response and Protein Synthesis

Folding is a critical step during protein synthesis. If the supply of chaperones, which normally aids this process, is not sufficient there is a risk to produce misfolded proteins. Endoplasmic reticulum possesses a system to detect unfolded proteins. If the Endoplasmic reticulum contains too many misfolded proteins, activation of the transmembrane ribonuclease IRE-1 leads to XBP-1splicing (Ali et al. 2011, Calfon et al. 2002). This transcription factor will then trigger the synthesis of chaperone proteins acting specifically in the endoplasmic reticulum. This response is called the unfolded proteins response. An extreme case is observed when pathogenic bacteria infect *C. elegans* during their development. Indeed, nematodes have to secrete a high amount of proteins to counteract pathogens infection. Richardson et al. have shown that xbp-1 mutants are unable to withstand pathogens infection during their development (Richardson et al. 2010).

b- Proteome maintenance

HSF-1 is a transcription factor regulating genes encoding for heat shock proteins. Either overexpression of HSF-1 or the small heat shock protein HSP-16.2 can increase *C. elegans* lifespan (Fonte et al. 2008, Hsu et al. 2003). Conversely HSF-1 mutations decrease lifespan of wild type *C. elegans* N2 and abolish the long-lived phenotype of daf-2 mutants. To test when the heat shock response become limiting with age Ben-Zvi et al. first submitted nematodes to heat-shock at day 1, 4 and 7. They observed at day 4 and 7 that the heat-shock response was dampened 3-8 fold compared to day 1. Then, they examined whether in normal condition heat-shock proteins activity was also decreasing early. They used thermosensitive mutants of the proteins dynamin and paramyosin. At permissive temperature these mutants exhibited defects in folding and localization of these proteins from day 6. However, when

HSF-1 was overproduced these ts mutants had a wild type phenotype. These results show that collapse of proteostasis is an early phenomenon in *C. elegans* aging (Ben-Zvi et al. 2009).

Cohen et al investigated in *C. elegans* the toxicity of protein aggregates. They express in transgenic lines of *C. elegans* the human $A\beta_{1-42}$ peptide and found that the molecular weight of $A\beta_{1-42}$ peptide was not correlated with aggregates toxicity. In fact they observed small $A\beta_{1-42}$ peptide oligomers were the more toxic aggregates. To protect the cell HSF-1 and DAF-16 had two opposing effect on $A\beta_{1-42}$ peptide aggregates size. HSF-1 was acting on $A\beta_{1-42}$ peptide disaggregation while DAF-16 was favoring their polymerization (Cohen et al. 2006).

c- Oxidative stress response induced by proteasome dysfunction

In *C. elegans* six subunits of the proteasomal machinery contain Antioxidants Response Elements (AREs), where SKN-1 can bind. Kahn et al., investigated whether proteasomal disruption could lead to SKN-1 activation. They observed that knock-out of almost all subunits of the proteasome lead to nucleolar relocalization and increased cytosolic fluorescence of an SKN-1-GFP. Moreover GST-4 (Glutathione transferase-4) an enzyme involved in phase II of the oxidative stress response was specifically expressed when proteasome core subunit α and ß were knock down but not in regulatory subunit knock-down (Kahn et al. 2008).

Results

AIMs of this study:

General aim:

In this work we wanted to investigate the link between biological aging and proteome maintenance

Specific aims:

To achieve our global aim we proceeded this way:

- Develop a simple and general method to quantify motility of *C. elegans* (first paper).
- Validate our method to study biological aging in *C. elegans*
- Establish the links between proteome maintenance and biological aging in *C. elegans*

I- *C. elegans* self-sorting by electrotaxis

A- Running worms: *C. elegans* self-sorting by eletrotaxis

Here, we report an inexpensive method to measure worms crawling velocities and sort them within a few minutes by taking advantage of their electrotactic skills. This method allows to quantitatively measure the effect of mutations and aging on worm's crawling velocity. We also show that worms with different locomotory phenotypes can be spatially sorted, fast worms traveling away from slow ones. Groups of nematodes with comparable locomotory fitness could then be isolated for further analysis.

Running worms: *C. elegans* self-sorting by electrotaxis

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ABSTRACT

The nematode *C. elegans* displays complex dynamical behaviors that are commonly used to identify relevant phenotypes. Although its maintenance is straightforward, sorting large populations of worms when looking for a behavioral phenotype is difficult, time consuming and hardly quantitative when done manually. Interestingly, when submitted to a moderate electric field, worms move steadily along straight trajectories. Here, we report an inexpensive method to measure worms crawling velocities and sort them within a few minutes by taking advantage of their electrotactic skills. This method allows to quantitatively measure the effect of mutations and aging on worms crawling velocity. We also show that worms with different locomotory phenotypes can be spatially sorted, fast worms traveling away from slow ones. Groups of nematodes with comparable locomotory fitness could then be isolated for further analysis. *C. elegans* is a growing model for neurodegenerative diseases and using electrotaxis for self-sorting can improve high-throughput search of therapeutic bio-molecules.

Introduction

The nematode *Caenorhabditis elegans* [1] is routinely used as a model organism to investigate key biological processes including aging [2-4], functioning of the neural system [5], and muscle degeneration [6] to cite but a few. Its genetic and phenotypic traits are extremely well documented [1]. Moreover, a comprehensive library of mutants is available [7] and powerful tools, such as RNAi, allow manipulation of gene expression. The locomotion abilities and the dynamical behaviors of worms provide important displays of their phenotype/genotype and can thus be used as powerful proxies for quantitative analysis. For instance, multiple drugs – *e.g.* those affecting synaptic transporters such as serotonin [8] – and chemicals – *e.g.* those involved in chemotaxis [9] – are known to affect the behavior of worms. Morphological abnormalities – *e.g.* long, dumpy or roller mutants – and neural deficiency – *e.g.* uncoordinated mutants – also correlate with a more or less severely impaired locomotion [1,5]. In practice, screening for a phenotype of interest, such as abnormal locomotion, is done by visual scoring followed by manual selection. For example, behavioral classes of motility are still the standard way to evaluate the locomotor abilities of *C. elegans*. This is time consuming and hardly quantitative.

Several image-based tracking softwares have been developed to automatically extract locomotion properties of freely crawling worms [10-13]. However, freely moving worms have highly unsteady kinematics –worms typically switch between active foraging and resting periods – and their trajectories are complex, rendering a quantitative description difficult. Moreover, the number of analyzed worms cannot be too large to allow for unambiguous worm identification. Other devices, such as worm sorters, are dedicated to high-throughput screening. They are expensive and sort worms only according to a static phenotype (*e.g.* their shape or the expression level of a reporter gene). Recently, an *in vivo* high-throughput microfluidic worm sorter was designed by Rohde *et al.* [14]. Worms were sequentially immobilized one at a time thanks to a pressure controlled valve, analyzed by fluorescence microscopy, released and dispatched to the appropriate exit. Although such a worm sorter is an excellent strategy for high-throughput screening, it requires a high degree of expertise and is, unfortunately, not applicable to analyze locomotion patterns since it deals with mechanically immobilized worms. In this article, we describe an elementary method that

combines a direct measurement of the velocity of single worms and the ability to sort multiple worms according to their locomotory skills.

Results

Our method is based on the electrotactic ability of *C. elegans* [15,16]. As first evidenced by Sukul *et al.* [15], *C. elegans* can detect the presence of an electric field. If this field is larger than typically 3 V/cm [16] worms move steadily in the direction of decreasing potentials (Fig.1 and Fig. 2). Gabel *et al.* evidenced that mutation such as *che-13* and *che-2* and laser ablation that disrupt the functions of amphid sensory neurons also disrupt electrotaxis. Yet, *C. elegans* electro-sensory navigation is still not well understood. Nevertheless, such a robust behavior opens the possibility to sort population of worms. Here, we combined a classic DNA-electrophoresis box (see Fig. 1 and Methods) with a LED ring, for proper illumination, and a video camera to create an inexpensive worm-sorter platform. In a typical experiment, one or several worms are transferred on an agar gel placed in the electrophoresis chamber which is filled with an electrophoresis buffer. The agar pad is typically ten centimetres long, flat and has walls to prevent buffer inflow. As we will discuss next, this elementary setup was sufficient to get reproducible electrotactic runs.

Quantitative electrotaxis. Figure 2 shows how a group of wild type worms (N2 strain) spread over the gel surface in function of time with or without an electric stimulation. Without an electric field, worms displayed complex locomotion patterns with reorientations, “omega” reversals, backward motions and pauses. As shown on Figure 2, the resulting trajectories were not oriented (Fig. 2a). Worms only slowly invaded the surface of the agar gel (Fig. 2b), with no preferred movement orientations (Fig. 2c). This can also be seen on the histograms of the components of the velocity perpendicular, $v_{\perp}$, and parallel, $v_{//}$, to the electric field, which were found to be centred on 0 (Fig. 2d). In contrast, during an electrotactic run, a wild type worm moved steadily in a well defined direction (Fig. 1b, Fig. 1c and Fig. 2; Supplementary movie 1). This is the trace of directed locomotion: there were very few events of slow, hesitating forward or backward motion. Repeating this experiment with several worms (> 100) showed that all young adult worms were responsive to a difference of potential of 120V applied to the electrophoresis box. They displayed straight trajectories oriented in average along the electric field direction (Fig.1, Fig. 2a, and Fig. 2c). For a given worm, the trajectory orientation remained surprisingly constant on the entire length of the gel (5 cm ~ 50 times a young adult worm length) (Fig. 1 and Fig. 2c).

Accordingly, the histogram of $v_{\perp}$ was centered on 0, while the histogram of the velocity component parallel to the electric field direction, $v_{//}$, was shifted towards positive velocity, with an average value of 140 µm/s in good agreement with previously reported measurements [10] (Fig. 2d). Only on rare occasions, worms got confused and operated an omega loop before resuming their motion (Supplementary movie 2). Increasing the difference of potential from 100V to 250V did not affect the worms speed. This means that worms are forced to move by the presence of an electric field but not moved by it, as DNA is by electrophoresis. When suddenly reversing the electric field intensity, worms display a typical omega loop (see supplementary movie 3) before they resume their trajectory, evidencing that worms are indeed sensing the existence of an electric field and adjusting its locomotion to it. However, as observed by Gabel *et al.* [16], the trajectories were inclined with respect to the electric field orientation. They reported that the larger the electric field, the larger the angle between the trajectory and the electric field orientation. In our setup, working with 120V ensured almost parallel trajectories (Fig. 2c). Therefore, electrotaxis appears as an efficient way to quantitatively measure a worm (forced) velocity within a few minutes and *a priori* in a much more reproducible way than what can be achieved by observation of freely moving worms. Performing electrotactic runs with 2 or more worms should allow discriminating between slow and fast worms. Therefore, using such a simple electrotaxis apparatus gives an efficient way to serial sort worms based on their locomotor fitness.

In the following, we explore and validate this approach on three biologically relevant examples: (1) the quantitative comparison between wild type and mutants displaying altered locomotion (Fig. 3, Fig. 4), (2) the effects of aging on the locomotory rate of worms (Fig. 3) and (3) the actual separation of a mix of two worm strains (Fig. 4, Fig. 5). We then discuss the potential of this method.

Wild type *vs. slow* worms. *C. elegans* body wall muscles have two functional acetylcholine receptors activated by levamisole and nicotine respectively. UNC-29 is a subunit of the levamisole sensitive receptor [17,18] and ACR-16 is a subunit of the nicotine sensitive receptor [19]. Both *unc-29* and *unc-29;acr-16* mutants have been shown to move at a slower rate with uncoordinated phenotype, the double mutant being less active [19]. However, it is difficult to quantitatively measure the velocity of such phenotypes because they only move occasionally. We conducted several electrotaxis runs on wild type *C. elegans*, the single mutants *unc-29* and *acr-16, and* the double mutant *unc-29*, *acr-16*. All mutant strains were reactive to the presence of an electric field. They showed a directed locomotion allowing us

to measure their velocity precisely. Wild type worms were the fastest worms ($v_{//}$ = 110 μm/s), followed by *acr-16* ($v_{//}$ = 80 μm/s), *unc-29* ($v_{//}$ = 35 μm/s) and finally *unc-29, acr-16* mutants ($v_{//}$ = 15 μm/s). This simple experiment confirms that the double mutant has a much more pronounced phenotype, in good agreement with the fact that *unc-29* and *acr-16* mutations impair both acetylcholine receptors. Moreover, we were able to discriminate between *acr-16*, *unc-29* and wild type worms on the sole base of their velocities difference.

Quantitative influence of aging on locomotion. Locomotion has been proposed as a qualitative way to score for aging [20,21]. The worm electro-tactic abilities relationship with age has not been studied in details yet, but a recent experiment suggested that all larvae stages and young adult worms were responsive to an electric field [22]. Here, we subjected worms of increasing ages, from young adult at day 1 (*D1*) to old worms at day 8 (*D8*), to electrotaxis trials. Worms of all ages were responsive and run directionally, although older worms tend to follow less straight trajectories. The average worm velocity decreased with age by 70% between day 1 and day 7 (Fig. 3). Hence, directed locomotion by electrotaxis gives a quantitative, user-independent indicator of physiological aging. An interesting follow-up, would be to test whether this physiological aging is related to lifespan and how does it correlate with aging-related muscle degeneration.

The two previous examples evidence how electrotaxis can be used to perform quantitative measurements of single worm velocity. Such measurements can then be used to identify a given phenotype or to get a quantitative estimate of a worm physiological state. Although it is a quantitative method, it remains time consuming to perform such experiments using one worm at a time. An alternative approach is to force many worms to race against each other.

Worms self-sorting. If all worms do not move at the same speed exactly, the population will spread on the gel, creating a phenotypical gradient from slow to fast worms (Fig. 4a). In other words, electrotaxis could be used to spatially sort worms according to their velocity, very much like DNA is sorted by molecular weight during electrophoresis. Sorted population of worms can be recovered after the trial by selecting worms at a given distance from the original starting point. As a proof of concept, we tested this method on a population mix of wild type worms and *dbl-1* mutants (Supplementary movie 4). DBL-1 is the TGF-β-related ligand for the Sma/Mab pathway [22]. Loss of *dbl-1* activity results in smaller animals which makes them easy to distinguish from wild type animals. Interestingly those worms turned out to be slower than wild type worms. Figure 4b shows the number of wild type and *dbl-1*

worms at different time and position along the gel. All worms that have traveled at least 4 cm after 8 min were wild type worms, thus demonstrating in practice the efficiency of self sorting by electrotaxis. We therefore achieve to sort the initially mixed population. Since population separation is the result of differential locomotory rate between the worms, the variability of velocities within a population can affect the sorting process. We checked numerically that indeed the sorting efficiency is decreased by increasing the velocity variability between worms of the same population as shown in figures 5a and 5b. Finally, to try realistic velocities distribution, we used our experimental data on mutants (reported on Fig. 3) and analyzed how a 50 / 50 mix of two populations of such worms would self-sort. We computed the relative enrichment in fast worms (wild type) as a function of the distance at which worms would be captured after a given fixed time (Fig. 5c-f). In every case, populations were quickly enriched into the fastest worm (WT).

Discussion

Taken together, our experiments show that electrotaxis can be used to quantitatively measure the speed of single worms and to sort a population based on its worms' velocities within only a few minutes. Physical sorting only depends on the distance worms are able to crawl in a given amount of time. Whereas, it is possible to design complex electrotaxis setup [16,23], we shall insist that a simple, commercial electrophoresis system is sufficient to physically sort the faster worms from a population. A vision system placed above the electrophoresis box is only needed to get quantitative measurements.

A recent study proposed a microfluidic approach to sort worms by electrotaxis according to their swimming speed differences [23]. Although this approach was interesting it suffered from two intrinsic limitations: (i) the difficulty to use it with a very large number of worms – high throughput micro-fluidics are highly demanding – and (ii) its small dimensions, since it is always more efficient – and easier – to use a large device for sorting. Indeed, the spatial resolution increases with the length on which worms are allowed to run. Finally, it is important to note that catching the worms back from the micro-fluidic channel was an apparently unsolved challenge. Our elementary method overpasses those limitations. In particular, using a macroscopic electrophoresis setup increases the resolution of the sorting process. The sorting resolution is limited by the size of the electrotaxis gel and by the number of worms. As a matter of fact, two (populations of) worms with well defined velocity (Fig. 5a) are separable if the length of the gel is longer than $\delta\, v_{max} / \Delta v$, where Δv is the relative

difference of their average speed, v_{max} the velocity of the fastest worm and δ the typical size on which worms are captured afterwards. With δ = 1 cm, v_{max} = 150 μm/s and a gel of 10 cm, the speed resolution is Δv = 15 μm/s, which is smaller than the velocity standard deviation within a population. However, the effective resolution is lowered by the variability of velocities within a population (Fig. 5a,b). If the two populations are not well separated after one run, isolating a sub-population and performing another electrotaxis race will allow further separation of this sub-population. Iterating this process will increase the degree of sorting of the sub-population and remove almost all the slow worms (mutants) after a few trials. This method allows increasing the resolution but at the expense of decreasing the number of worms that can be sorted and collected. Since even isogenic population exhibits a rather large variability of their navigation velocity, this method could be used to prepare population samples with well defined locomotion abilities and presumably similar physiological state. Uncoordinated worms or worms defective in sensing the field should also be separable from wild type worms since they will remain near the starting area.

We demonstrated here the practical potential of our method in separating a large number of worms to select for the desired phenotype as long as it is related to locomotion, which is very quite often the case for nematodes [1]. It may be efficiently combined with a worm sorter, transcriptomics, RNAi, or biochemical analysis, to correlate the physiological state of the worms with the expression level of specific genes. We also showed how gel-electrotaxis assay can be used to get quantitative data of the dynamical behavior of worms within a few minutes only. *C. elegans* is a growing model for neurodegenerative diseases and diseases linked with muscle degeneration. These dysfunctions affect locomotory behavior. We anticipate that gel-electrotaxis serial sorting combined with high-throughput screening of bioactive molecules could help to find innovative therapeutic strategies to these diseases.

Methods

Strains. We used wild type strain (N2), *dbl-1* mutants and the mutants *acr-16(ok789), unc-29(x29), unc-29(x29); acr-16(ok789)* obtained from the laboratory of J.-L. Bessereau (ENS, INSERM U 789). *C. elegans* worms were developed at 15°C and then at 25°C during adult-hood. They grew on NGM plates seeded with *E. coli* OP50.

Electrotaxis assay. In each experiment approximately 10-15 worms were selected from a cultivation plate of a synchronized population of adults and rinsed with M9 buffer solution. They were then transferred on an agar gel in a drop of M9. The agar gel was composed of:

de-ionized water, 2% of Bacto-Agar, glycerol (6,2 mL of glycerol 60% for 1L), NaCl (0,250 mmol/L) as previously described in [16]. The gel was cast by pouring a first layer of agar and adding a PDMS (Polydimethylsiloxane) block onto it so that it will shape the future cavity where nematodes will crawl (6x8 cm). A second layer of gel was then poured around the PDMS block. Once solidified the PDMS block was removed. The resulting agar pad was then placed in an electrophoresis box filled with a buffer. It was composed of de-ionized water, glycerol (3,7 mL of glycerol 60% for 1L) and NaCl (0,250 mmol/L) as previously described in [16]. The electrophoresis power supply was an APELEX PS305 and the electrophoresis box from Biorad, Wide Mini-Sub^{tm} cell.

Image analysis. Experiments were imaged with a 6.6 Mpixel CMOS monochrome Camera (Pixelink) with a close focus zoom lens 10x (13x130 mm FL), Edmund Optics Ltd). We used a white, bright field / dark field ring light (Edmund Optics Ltd), to enhance the contrast . Since the worm trajectories are ideally straight, image analysis was straightforward. Trajectories of worms were computed from images by using successively ImageJ http://rsbweb.nih.gov/ij/[24] and its *analyze particles* plug-in to detect worms position at every time step (1 frame per second), the GNU software Octave (http://www.gnu.org/software/octave/) and finally Igor Pro (Wavemetrics) to construct trajectories, perform data manipulation and compute statistical tests. Velocities were computed by averaging the displacement of the center of mass of nematodes over 10 frames (10 s).

Numerical analysis. The histograms and enrichment proportion displayed in Figure 5a, b were numerically computed. We computed the evolution with time of the position of 1000 worms assuming that every worm has a speed set by a Gaussian distribution with a standard deviation of 50 μm/s (Fig. 4c,d). Positions were updated every second during 240 s. To increase the variability of the total population, we allowed the average velocity of single worms, v_s, to vary from one worm to another by adding a Gaussian noise: $v_s = v_0(1+g(\mu))$, where g is a function that returns a random value from a Gaussian distribution of standard deviation μ The resulting population have 1000 worms with normally distributed averaged velocity and display larger variability for larger μ.

Acknowledgments

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Author Contributions. X.M performed the experiments. F.L contributed numerical codes for image analysis and F.L and P. H analyzed the data. X.M, F.L., I.M, B.L, J-M. DM and P.H wrote the manuscript.

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Supplementary Information. This section contains supplementary movies illustrating worms sorting by electrotaxis.

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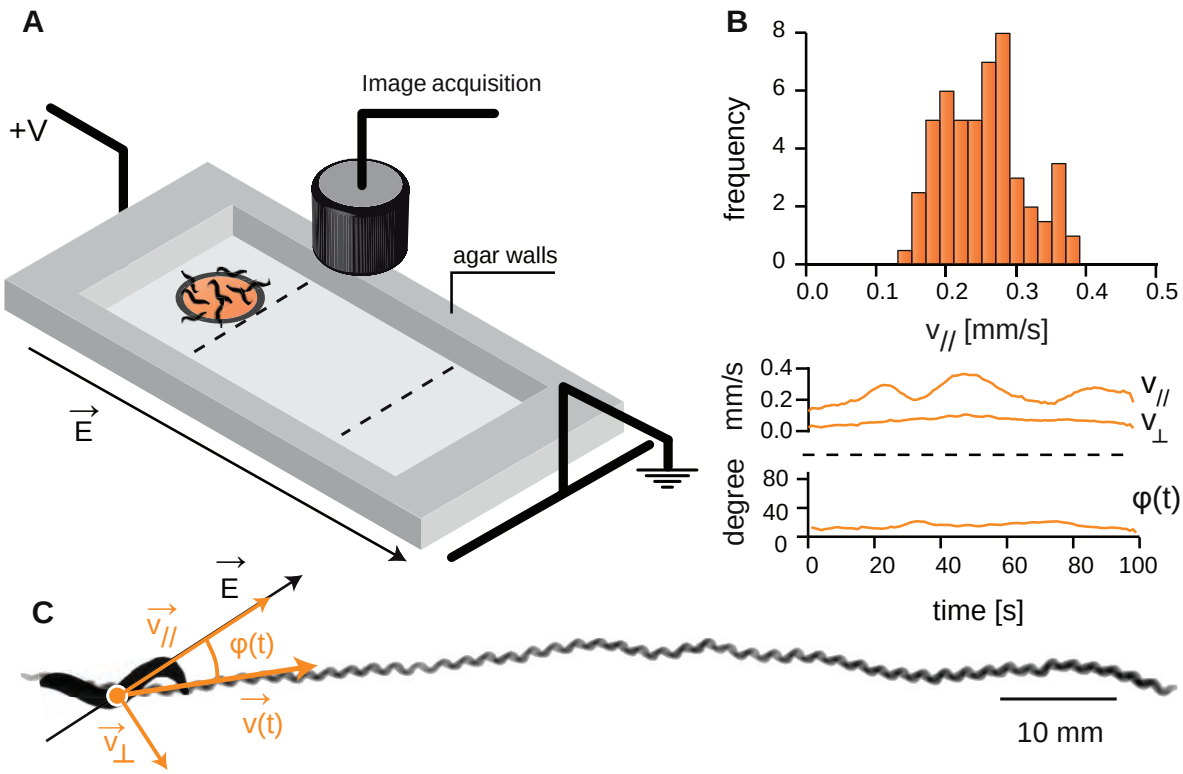

FIG. 1: Experimental setup. (a) The setup combines a classic electrophoresis box (~ 20 cm long) with a video camera and a LED ring to record images of nematodes moving at the surface of an agar gel. The gel is flat and has walls (in grey) to prevent buffer inflow in the electrotaxis area. (b) Velocity distribution during an electrotactic event and evolution with time of the velocity and the orientation of the trajectory of a single worm performing electrotaxis. This shows that during electrotaxis, a single worm moves steadily in a relatively constant direction. (c) The corresponding trajectory is relatively straight and has an angle θ of 15° with the electric field orientation. The characteristic sinusoidal shape of the nematode crawling gait can be observed, indicating that the worm is moving by generating a rearward flexural wave on its body.

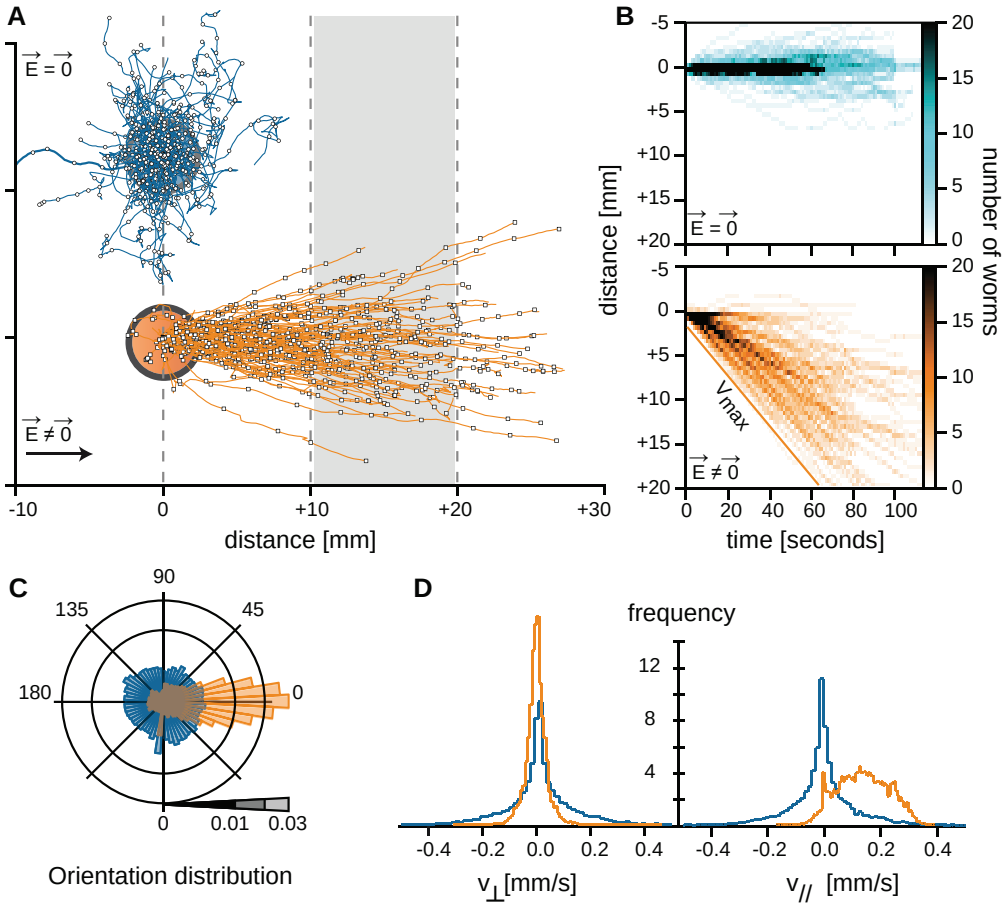

FIG. 2: Electrotaxis and directed locomotion. (a) Trajectories obtained from several distinct experiments done with 10-15 worms are displayed on the same graph. Directed locomotion by electrotaxis (orange, N = 130) is observed for a difference of potential of 120 V, while without any electric field, trajectories are randomly oriented (blue, N = 146). (b) From those trajectories, one can extract a spatio-temporal diagram of the density of nematode (graded in orange or blue intensity) at the surface of the gel. Electrotaxis leads to a directed spreading at the surface of the gel. Note that even in a synchronized population of worms there is a large variability in velocity. (c) Orientations of the trajectory are mostly parallel to the electric field (orange), though they vary from one worm to another. It is not known yet how the electrotaxis orientation is set by worms without electrotaxis, trajectories do not exhibit any preferred orientation (blue). (d) The histograms of parallel and perpendicular velocity with (orange, $v_{//}$ = 140 ± 90 µm/s) or without (blue, $v_{//}$ = 1 ± 70 µm/s) an electric field. The measured velocities may depend on environmental conditions such as the presence or absence of nutriments. This is why worms were systematically rinsed in M9 buffer before their transfer. Similarly, the poro-elastic properties and humidity of the agar gel can affect the worms velocity. It is therefore recommended to run a control with wild type worms to set a reference speed.

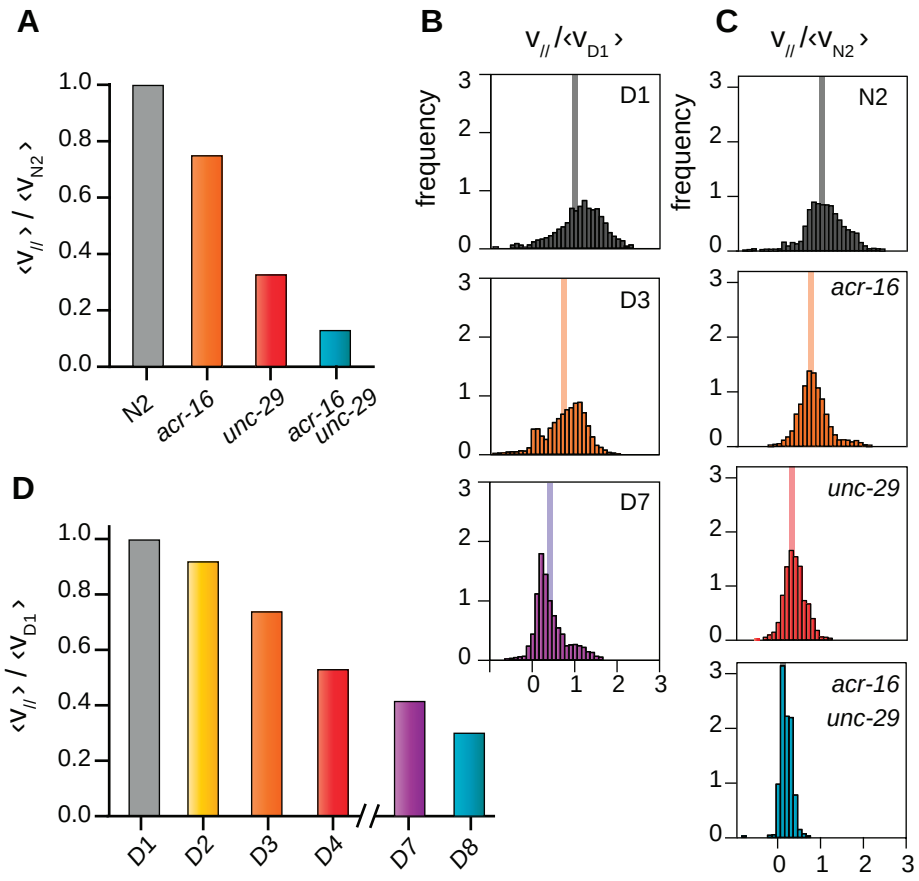

FIG. 3: Comparative analysis of mutant worms (a, c) and chronological aging effects on forced locomotory abilities (b, d). (a) *acr-16* ($v_{//}$ = 80 µm/s ± 40 µm/s, N = 28), *unc-29* ($v_{//}$ = 35 µm/s ± 34 µm/s, N=26) and *unc-29*, *acr-16* mutants ($v_{//}$ = 15 µm/s ± 19 µm/s, N = 22) exhibit a decrease of their velocity when compared to the control population (N2, $v_{//}$ = 110 µm/s ± 50 µm/s, N = 28) in successive electrotactic runs. Errors are computed as standard deviations. (c) The histograms of $v_{//}$ for mutant worms are significantly different ($p < 0.05$). Here, the wild type average velocity along the direction of the electric field, $\langle v_{N2} \rangle$ = 110 µm/s, is taken as a reference of velocity. (d) Populations of worms also show a decrease of the average velocity as they get older, from the first day (D1) to the 8th day (D8). Here, the average parallel velocity at Day 1, $\langle v_{D1} \rangle$ = 120 µm/s is taken as a .reference Number of worms: D1 / N=17; D3/ N=15; D7/N = 6. The normalized average velocity is indicated by a vertical line on the histograms (b, c).

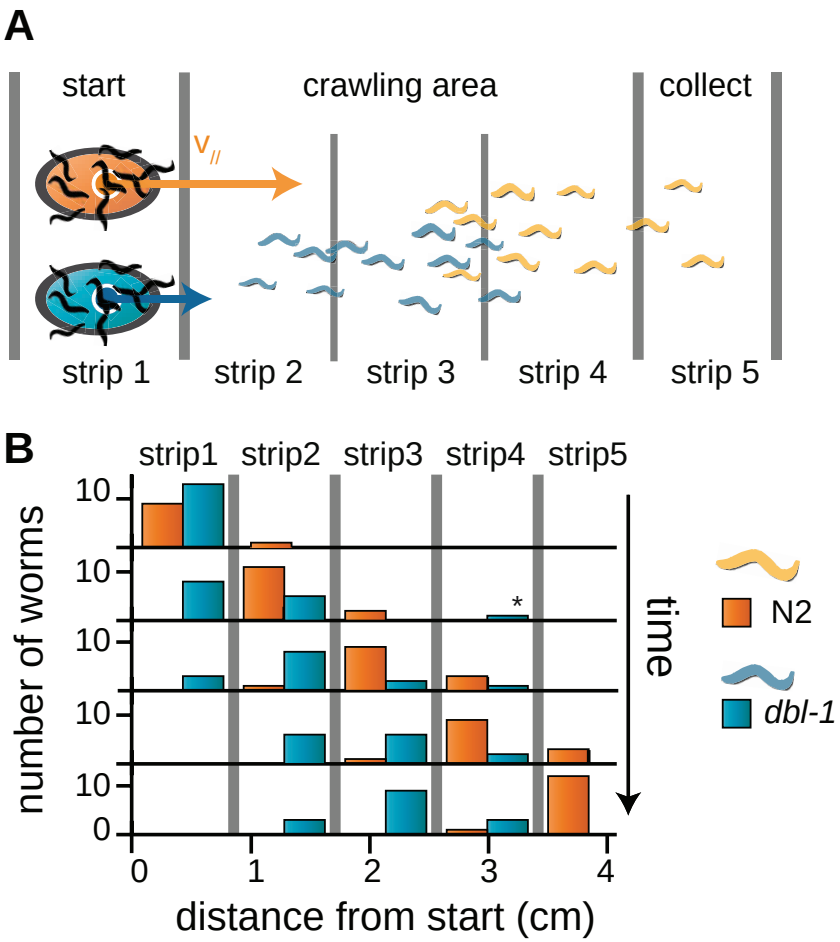

FIG. 4: Population sorting I. (a) Principle of population sorting. (b) Sorting in practice. We conducted a sorting experiment with a mix of 15 wild type and 15 *dbl-1* worms. The number of wild type worms (orange) and *dbl-1* mutant worms (blue) are shown as a function of time and space. We divided the observed area into 5 slices of equal size and computed the number of worms of each strain at different time points (every 2 minutes). Progressively, the wild type worms separate from the initial mix. The final stripe contains only wild type worms, while, the 2nd and 3rd stripes contain only *dbl-1* mutant. The experiment was repeated three times. See also supplementary movie 4.

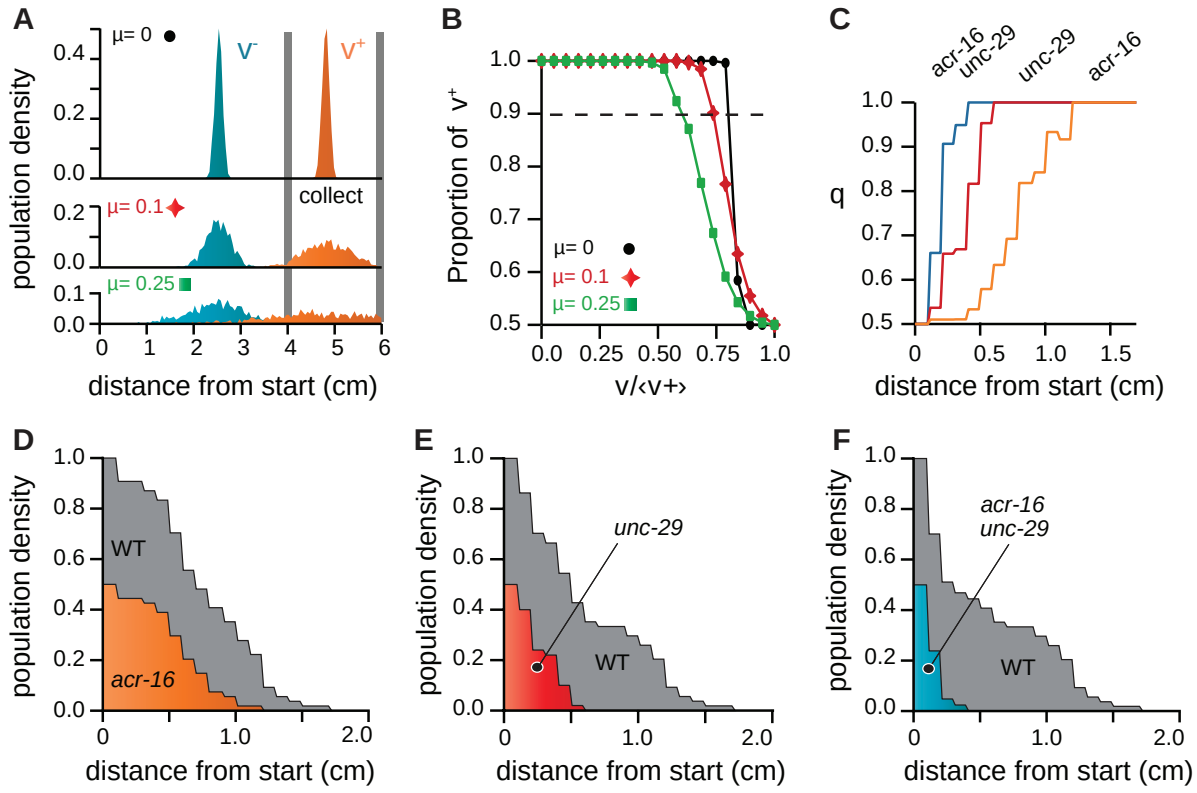

FIG 5: Population sorting II. (a) We numerically computed the histograms of the distance traveled by a fictitious population of 1000 worms assuming a Gaussian velocity distribution for each worm with an average parallel velocity given by $v_0(1+\text{gnoise}(\mu))$ and a standard deviation of 50 μm/s. The resulting population has 1000 worms with normally distributed averaged velocity and displays larger intra-population variability for larger μ. We then compared two populations with different v_0. Wild type worms (orange) are moving at $\langle v^+ \rangle$ = 200 μm/s and slow worms (blue) are moving at $\langle v^- \rangle$ = 100 μm/s. (b) The same principle allows to compute the proportion of wild type worms (v0 = 200 μm/s) in the collect area (> 4 cm from start) as a function of the ratio of the average velocity of the two populations. As expected, population with similar dynamics and intra population variability of velocities (larger μ) decrease the sorting efficiency. (c) Using the experimental data displayed on Fig. 3 we computed the distribution of worms position at time τ = 100s. Calling $f_1(x)$ and $f_2(x)$ the two position distributions, the fraction of population 1 over population 2 as a function of the distance traveled is given by $q(d) = \left(\int_d^{+\infty} f_1(x)dx\right) \Big/ \left(\int_d^{+\infty} f_1(x)dx + \int_d^{+\infty} f_2(x)dx\right)$. When starting from a fictitious 50% mix of wild type and any of the mutant strains *acr-16*, *unc-29* or *unc-29;acr-16* (see text), the sub-population is quickly enriched in wild type worms (faster worms) as the distance of capture increases. Ideally, capturing worms as far as possible from the starting

point ensure a perfect sorting. (d,e,f) However, since not all worms move at their maximum velocity during electrotaxis, there is a trade off between the degree of separation and the total number of worms that can be captured. As shown on sub-figures 5d,5e and 5f, the population density decreases with the distance to start.

B- Effect of age on C. elegans locomotion in electric fields.

The aim of our project was to investigate whether quality of proteome maintenance was determining lifespan in *C. elegans* N2 nematodes. At 25°C, *C. elegans* N2 nematodes begin to die at day 5. We thus decided to sort nematodes at day 5, before first animal died, in order to study the proteome of the whole population. As crawling velocity has been shown to be one of the most reliable biomarker for aging (Huang, Xiong and Kornfeld 2004) (Hsu, Feng, et al. 2009), we decided to employ electrotaxis to sort groups of nematodes with similar crawling velocity and therefore life expectancy. Two days old adult worms move with straight trajectory in electric field, so distance crawled during a given period of time correlates with crawling velocity. However, it was not known how electro sensory behavior was evolving with time. We studied locomotion of wild type *C. elegans* N2 worms in electric fields during the first five days of adulthood to know what were the main phenotype affecting worms sorting at day 5.

1- Effect of electrotaxis on *C. elegans* lifespan.

To use electrotaxis in longevity studies we had to test the effect of this method on worms' lifespan (Figure 6). During an experiment of electrotaxis, in addition to the run that usually last from 5 to 10 min, worms are rinsed before and after the run and they are transferred via a pipette. We thus compared the lifespan of worms having experienced all the steps of the experiment to worms that did not move from their bacterial lawn, because we wanted to be sure that steps associated to the run did not affect the lifespan of the worms. We found no significant difference between the longevity of 5 days old worms submitted once to an Electric Field (EF) compared to the controls (P value =0,67).

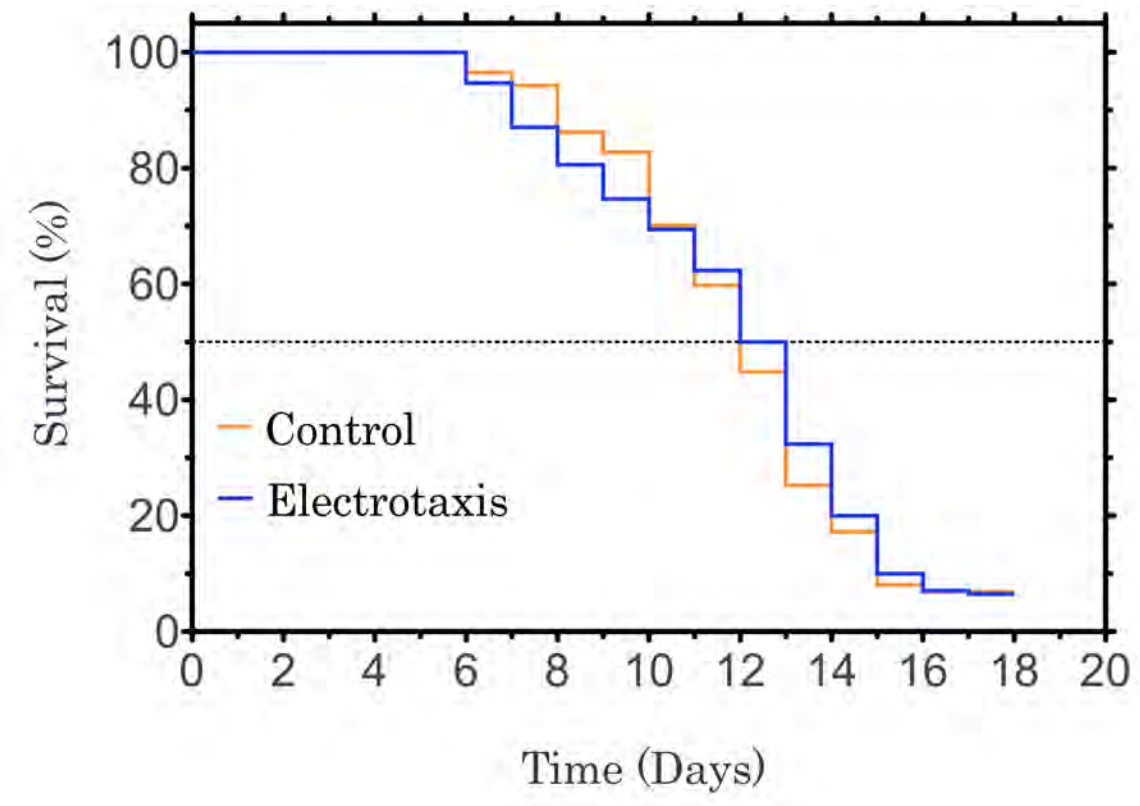

Figure 6: Effect of electric field and steps associated with electrotaxis assay on *C. elegans* survival. The blue curve represents worms submitted to the electric field at day 5 (n=159), the orange curve represents the control (n=81). Experiments were done using wild type *C. elegans* N2 for both conditions. We did not found significant difference between these two curves Log-rank (Mantel-Cox) Test P Value = 0,67. Experiments were repeated at least once

2- Description of *C. elegans* behavior in electric field

During a run nematodes may interrupt their forward movement by a backward movement here referred as reversal or an omega turn, an omega turn occur when head of the nematode touch its tail (Gravato-Nobre et Hodgkin 2005). We did not differentiate these two behaviors and just called pause, these interruptions in the forward movement (Fig. 7). Directional fluctuations were calculated as the standard deviation of the approach angle to the EF (Gabel, et al. 2007). It is important to note that we only calculated speed, here referred as crawling velocity, approach angle, and directional fluctuation of the nematodes during the forward movement. Moreover, we defined distance as the length between initial and final coordinates of the worms.

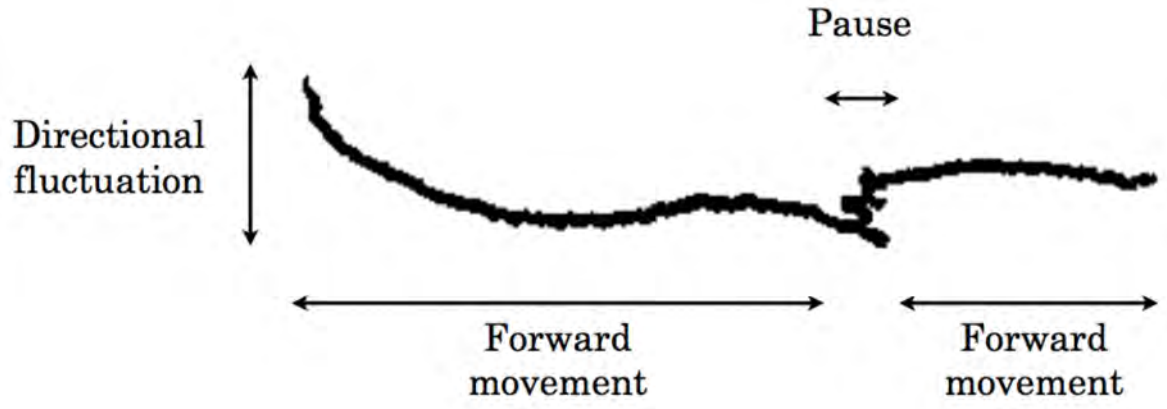

Figure 7: Trajectory of a nematode during electrotaxis. Forward movement may be interrupted by reversals or omega turns here referred as pause. Directional fluctuation represents the standard deviation of the approach angle to the electric field.

3- Effect of age on the relative standard deviation of crawling velocity

As shown in our published work (Manière, et al. 2011), *C. elegans* crawling velocity decreases with time. We thus decided to examine whether the crawling velocity was decreasing with constant variability or if there was an increasing heterogeneity with time. We observed a decrease in the mean crawling velocity of nematodes cohort of 57% from day 1 to day 5 whereas maximum speed only decrease of 31%. To quantify the evolution of crawling velocity heterogeneity, we calculated the Relative Standard Deviation (RSD), the standard deviation divided by the mean, of the crawling velocity of different cohorts for each day **(Fig. 8)**. RSD is higher in day 1 than in day 2 (but the difference is not significant: P value = 0.52), it increases of 125% from day 2 to day 5. During day 1 and day 2, a few worms did not respond to the EF, however we always noticed presence of eggs next to them. As egg laying act on locomotive behavior we hypothesized that it could be the cause of the absence of reaction to the EF but, as we were not sure that the eggs came from these worms we did not censure them.

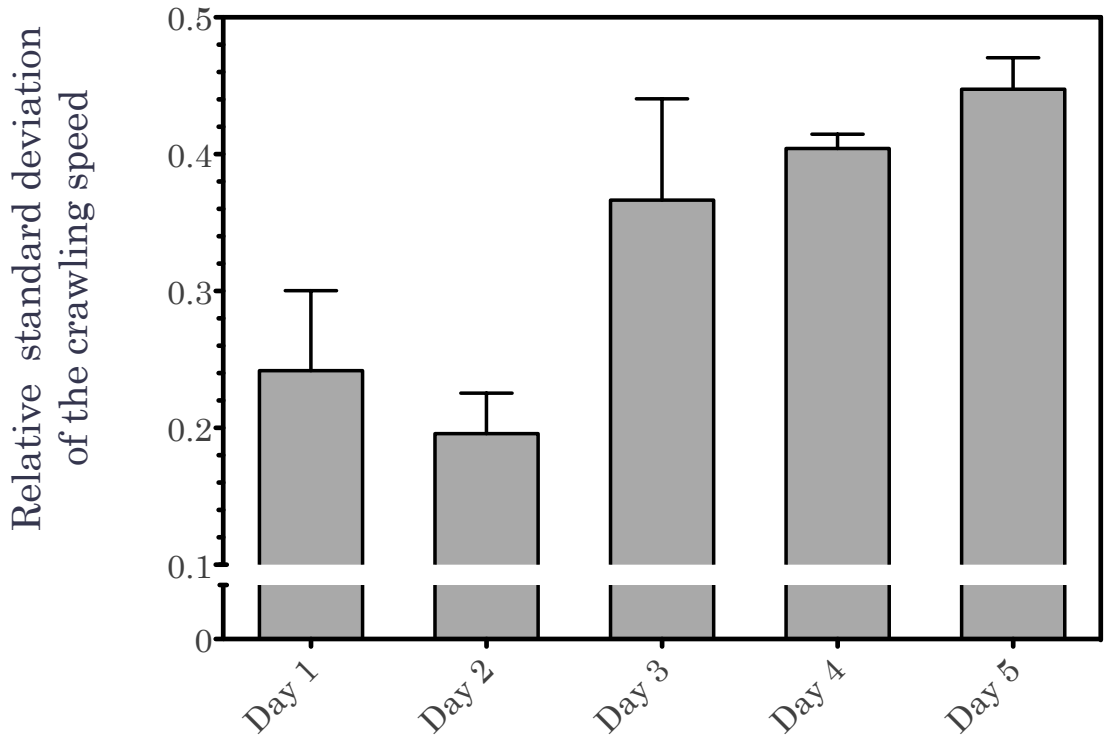

Figure 8: Effect of age on the relative standard deviation of crawling velocity in *C. elegans* N2 cohorts (n=63-98). Difference between day 1 and 2 is not significant (P value = 0.52, unpaired t test). Relative standard deviation increases of 125% from day 2 to day 5. Error bars are computed as the SEM from three replicates.

In order to evaluate reproducibility of worm crawling velocity we compared the speed of individuals between two different runs (3 min) separated by a break (1 min). We found that r correlation between speeds of the two runs were always above 0.66 for the first four days of adulthood **(Fig. 9)**.

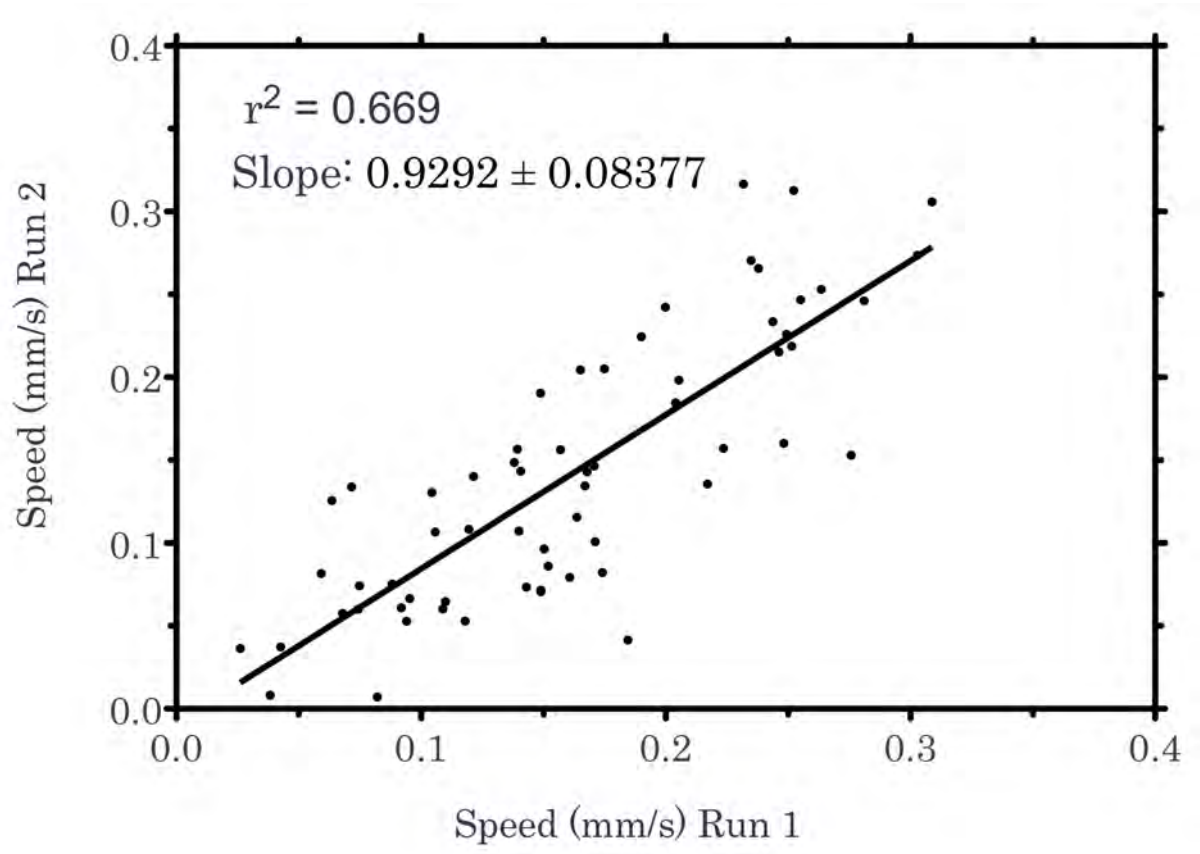

Figure 9: Reproducibility of *C. elegans* crawling velocity measurement. The graphic shows the correlation between two runs of 3 min separated by a break of 1 min of 4 days old *C. elegans* N2. r^2 was evaluated by a linear regression (P value < 0.0001)

4- Effect of age on *C. elegans* approach angle to the electric field

Gabel et al. noted that, for EF ranging from 4 V/cm to 14 V/cm, the angle to the electric field is proportional to the EF strength while the crawling velocity remains constant. Our observation confirmed these results. To determine whether the approach angle was an age dependent variable, we measured the means approach angle of different cohorts of worms during the first five days of adulthood. We observed that mean approach angle is significantly smaller for day one than for the other days (Day 1=10.8, Day 2=18.0, Day 3=14.9, Day 4=19.8, Day 5=19.8), however there was no clear trend with time **(Fig. 10)**. Moreover, for a given individual the reproducibility of the approach angle was low (data not shown).

As for a population of *C. elegans* submitted to an EF the distribution of the angles to the EF was relatively wide, we tested the correlation between approach angle and crawling velocity but we found no correlation **(Fig. 11)**.

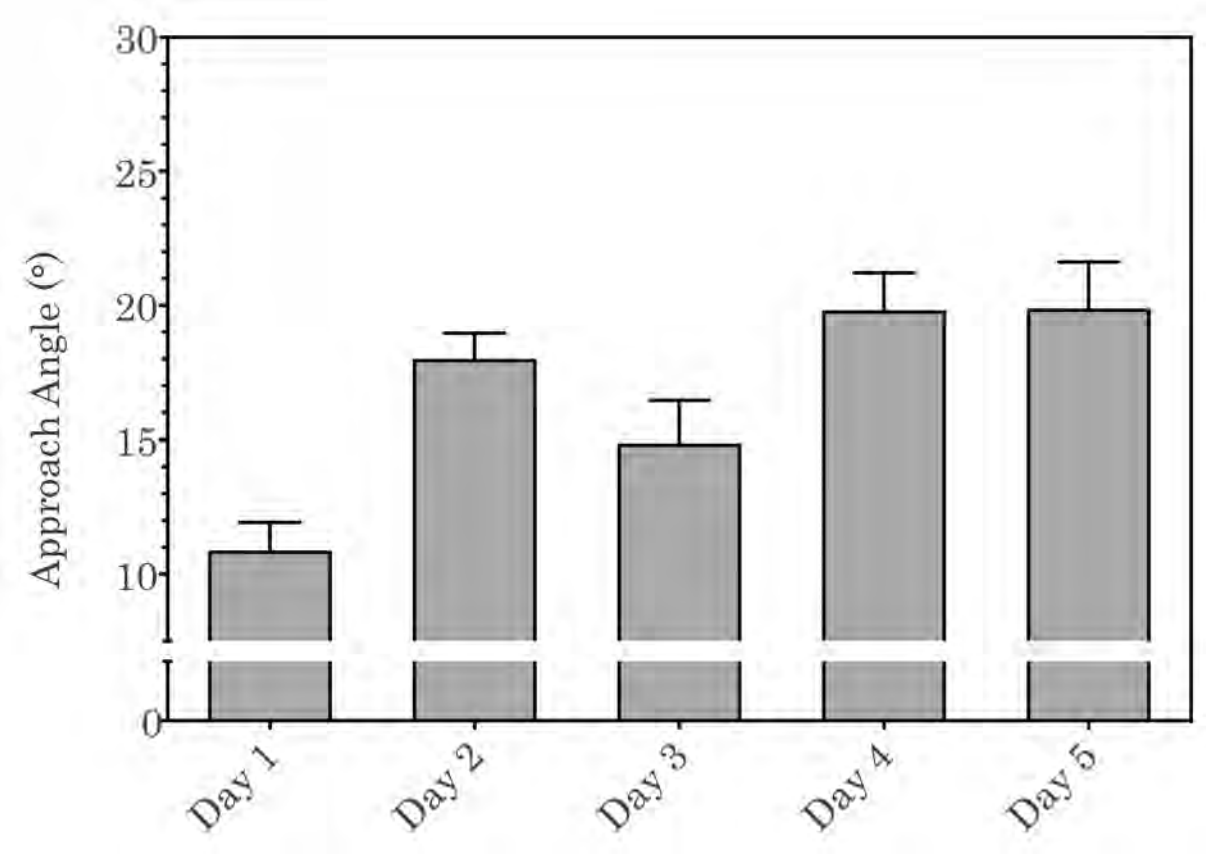

Figure 10: Effect of age on *C. elegans* approach angle to an electric field of 4V/cm. We measured the approach angle of *C. elegans* for each of the first five days of adulthood. We took these measurements on different cohort (n=63-98 for each cohort). Approach angle is significantly lower for day 1 than for the other day, however there is no clear trend with time. Error bars are computed as the SEM from three replicates.

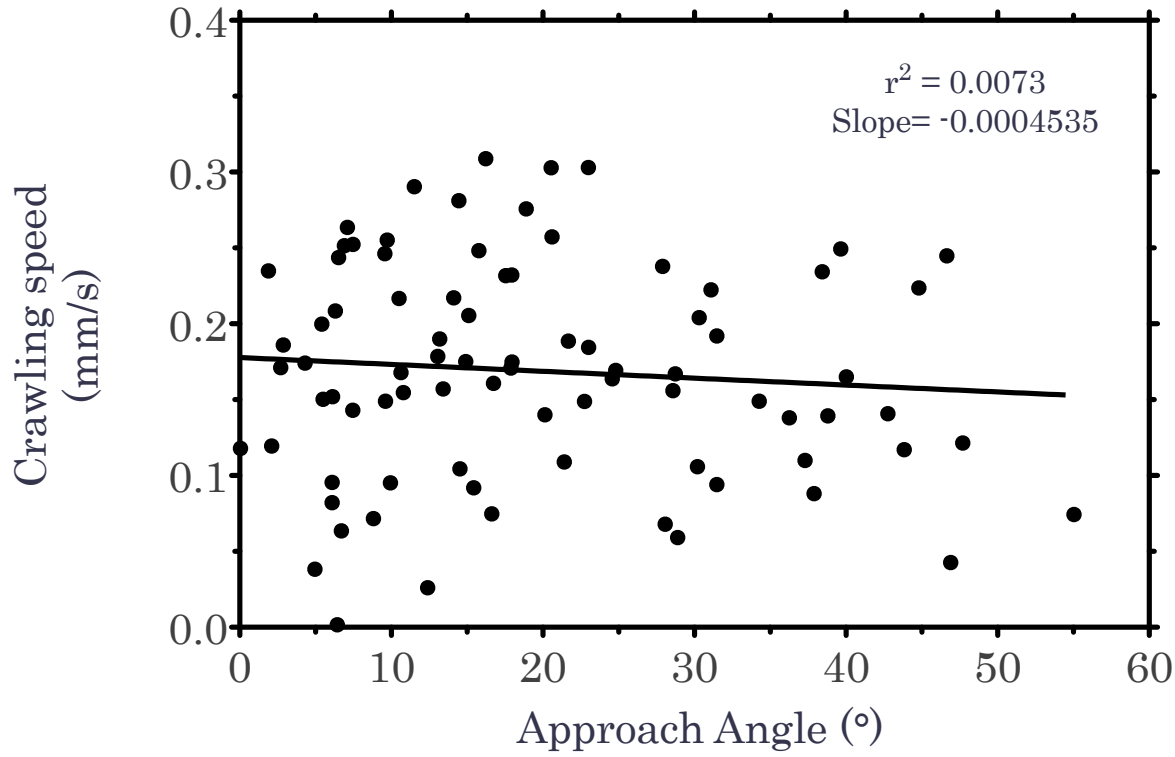

Figure 11: Correlation between the angle to the electric field and the speed of nematodes. Wild type *C. elegans* N2 (n=84) were submitted to electric field at day 4. We represented on this graph the speed (mm/s) of the worms in function of their angle to the electric field. We observed no correlation (r^2=0.0073) between these two variables.

Approach angle does not vary with time, however we observed that trajectories of nematodes in EF are less straight with time **(Fig. 12)**. We thus quantified directional fluctuations of worms depending on their age. We observed again that the results for day 1 were reproducible but not consistent with the global trend with age. From day 2 to day 5, mean directional fluctuation have a 110% increase **(Fig. 13)**. We hypothesized that directional fluctuations may be caused by a decline (detection, transmission, integration) of the efficiency of the navigation circuit. However we did not investigate further this question because it was out of the scope of our project.

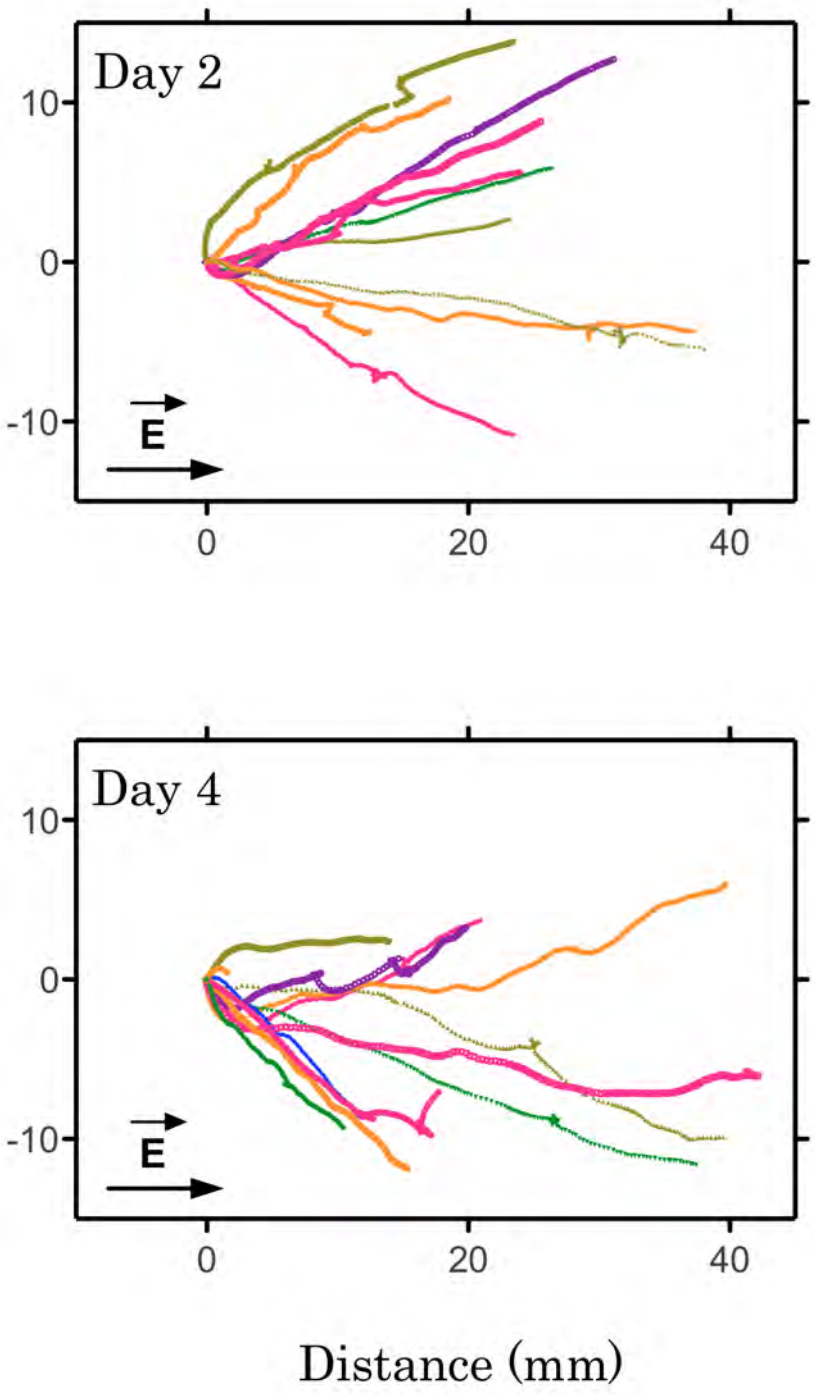

Figure 12: Trajectories of *C. elegans* N2 (N=15) at day 2 and day 4 in an EF of 4 V/cm. *C. elegans* ability to sustain a straight trajectory decreases with age.

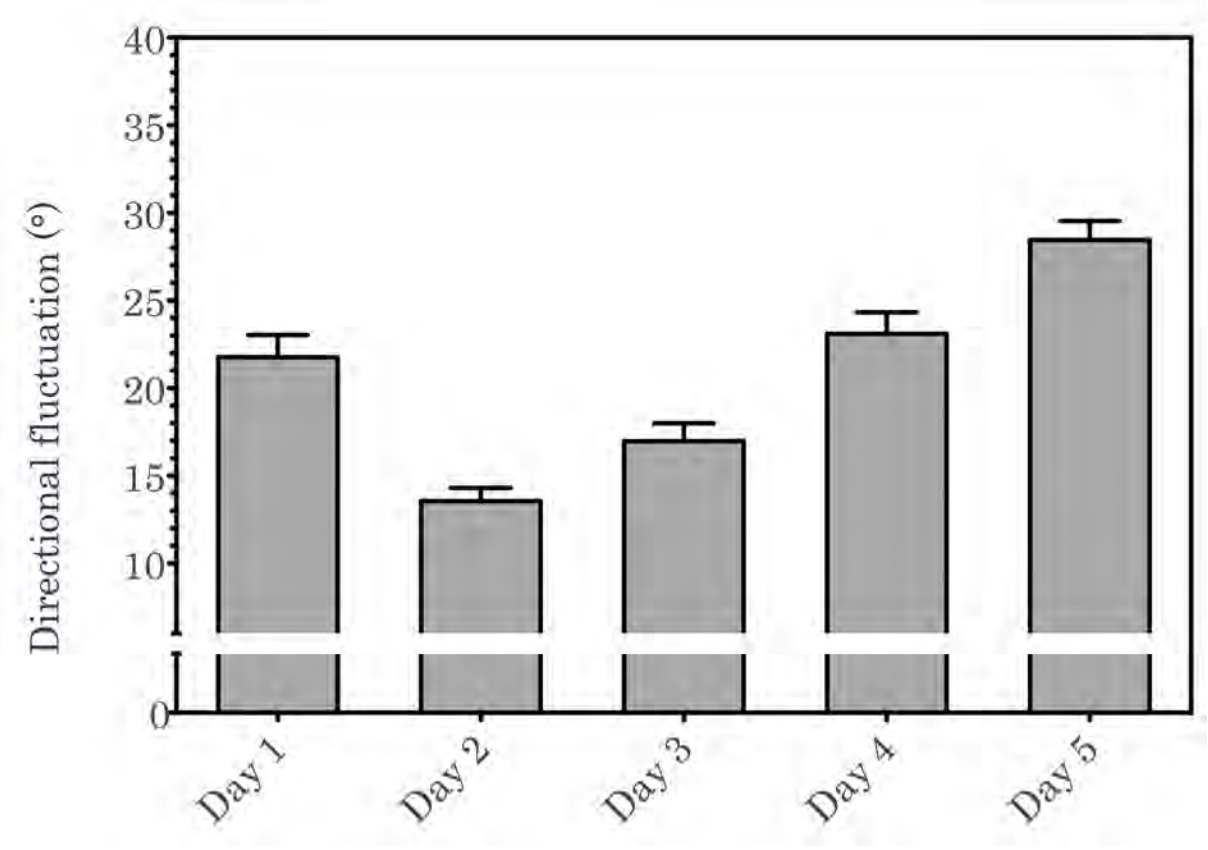

Figure 13: Effect of age on *C. elegans* directional fluctuation in an Electric field of 4V/cm. We measured on different cohorts (n=63-98) the standard deviation of *C. elegans* approach angle to an electric field. Results represent the mean of the standard deviation for each cohort. Directional fluctuation have a 110% increase from day 2 to day 5. Errors computed are SEM of three replicates.

5- Effect of age on *C. elegans* mean number of pauses.

Electrotaxis runs can be interrupted by maneuvers such as reversals and omega turns, we referred to these interruptions as pauses. The total number of pauses does not increase between day 1 and day 3 but increases significantly between day 3 and day 5 (81% increase) **(Fig. 14)**. Nonetheless, the median duration of the pauses does not increase from day 1 to day 5 (day 1 = 4s, day 2 = 2s, day 3 = 3s, day 4 = 3s and day 5=4s).

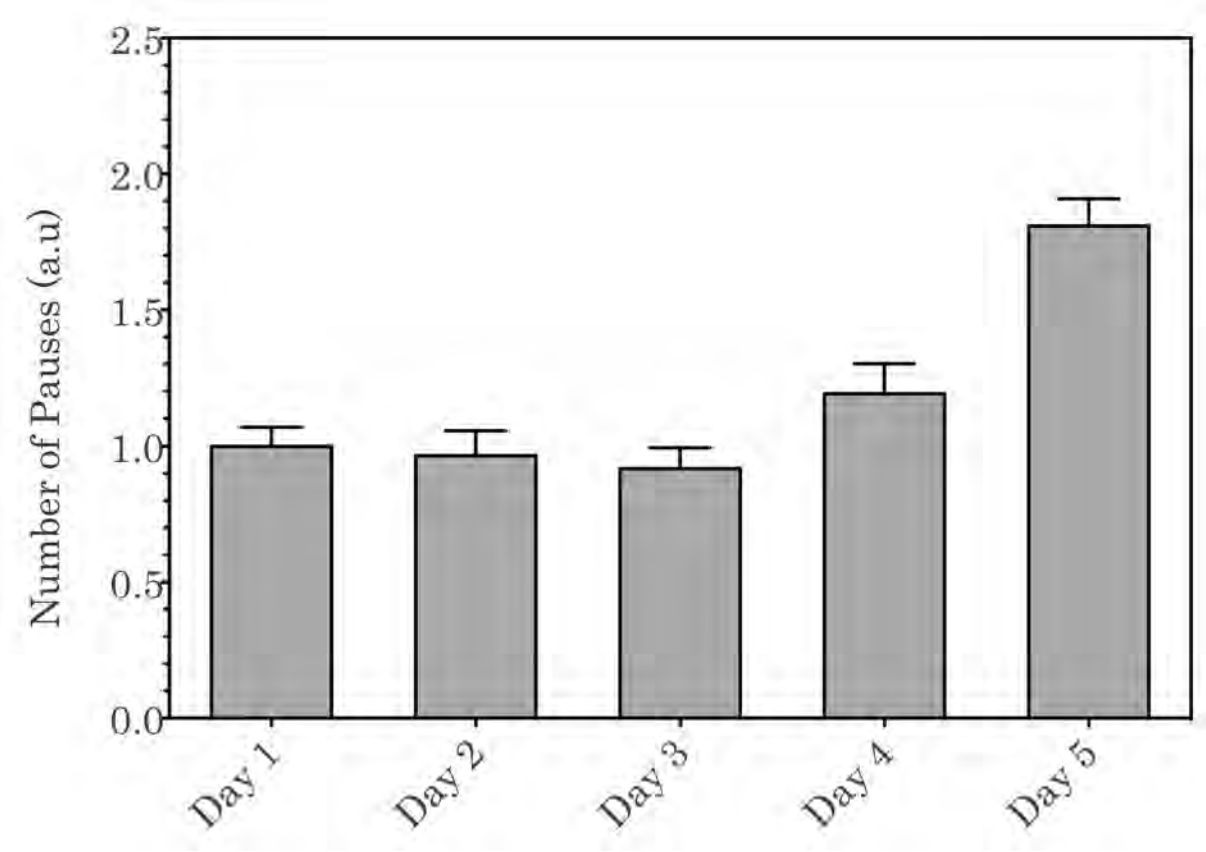

Figure 14: Effect of age on mean number of pauses of *C. elegans* during a run of 3 min. We represent here the mean number of pauses for different cohort of *C. elegans* N2 (n=63-98) at different age. Results are normalized to the mean of day 1. Error bars are computed as the SEM from three replicates.

6- Major factor affecting distance crawled by *C. elegans* at day 5

As we sorted nematodes at day 5 by selecting them depending on the distance they crawled we examined factors that most affect the distance worms crawled during a run of electrotaxis. We thus compared at day 5 correlations between the different variables that we monitored during the 5 first five days of adulthood. The best correlation with distance at day 5 is crawling velocity (crawling velocity > directional fluctuation > number of pauses). As expected, approach angle does not correlate with distance crawled by nematodes **(Fig. 15)**.

	Distance (mm)	Speed (mm/s)	Direct. Fluctuation (°)	Number of pauses	Approach Angle (°)
Distance (mm)					
Speed (mm/s)	0.912				
Direct. Fluctuation (°)	-0.595	-0.406			
Number of Pauses	-0.546	-0.472	0.521		
Approach Angle (°)	-0.001	-0.159	-0.164	-0.025	

Figure 15: Correlation between factors potentially affecting self-sorting of nematodes at day 5 electrotaxis. We measured correlation between the different factors that we monitored during the first five days (this experiment represent pooled results of 3 replicates, n=63).

7- Conclusion

We explore in this study evolution of locomotion in electric fields during the first five days of *C. elegans* adulthood. The aim of this work was to gain insight into the specific behavioral components affecting *C. elegans* self-sorting. We found that crawling velocity was the major factor affecting distance crawled by nematodes at day 5. As *C. elegans* crawling velocity has been shown to be a good biomarker for aging, electrotaxis seems to be well adapted to sort nematodes depending on their life expectancy at day 5 (Hsu, Feng, et al. 2009).

Directional fluctuation and pauses frequency evolve with age in *C. elegans*. They may also be good biomarkers for aging. However, they are not the major factors affecting self-sorting by electrotaxis, so it is difficult to sort big populations according to these variables. However in studies at the level of the individual they may be used to improve the predictivity of crawling velocity or even to replace it. In chemotaxis, Pierce-Shimura et al. interpreted pauses – they refer to this behavior as pirouettes – as a strategy that worms employ after a run so as to reorient themselves in order to be sure that they crawl in the right direction (Pierce-Shimomura, Morse and Lockery 1999). During electrotaxis runs nematodes stop instantaneously to move forward if current is switched off. So, it seems that they constantly

evaluate presence of electric field. Moreover Gabel et al demonstrated that *C. elegans* were able to reorient themselves without pause in time varying EF rotating at a frequency of 7.2°/s (Gabel, et al. 2007). Therefore, pauses and pirouettes should have distinct roles in chemotaxis and electrotaxis. However, at the level of the navigation circuit we do not know whether mechanisms that control it are the same.

Locomotory decline in *C. elegans* is usually interpreted as a consequence of muscle frailty (Herndon, et al. 2002) (Glenn, et al. 2004). However, recent findings report sensory function loss in aging *C. elegans*. In this context, it could be interesting to investigate the molecular basis of behavioral decline measured by electrotaxis.

8- Materials and methods

Nematode and bacterial strains. *C. elegans* N2 (ancestral) strain kindly provided by J.J. Ewbank (Marseille, France) was used for all experiments, unless otherwise indicated.
E. coli strains used in this study was the uracil deficient strain OP50

Nematode maintenance and synchronization. Nematodes were maintained at 25ºC on nematode growth medium (NGM) agar plates seeded with stationary phase bacterial culture incubated at 37°C. Age-synchronized populations of nematodes were initiated from eggs recovered following sodium hydroxide (0.5M final) and sodium hypochlorite (0.96 % final) treatment of gravid adults maintained at 25° and fed E. coli OP50. All assays were carried out with nematodes synchronized twice: once before development by sodium hydroxide/hypochlorite treatment as described above, and a second time at the end of development by selecting exclusively nematodes at the end of the 4th larval (L4) stage based on vulva morphology.

Survival assays. At L4 stage 75 nematodes were transferred in Petri dishes of 9 cm of diameter where almost all the surface was covered by OP50 bacterial lawn. All the Petri dishes necessary for the six first days of experiments were prepared one day before day 0. Nematodes were transferred onto new plates every day during the first 5 days to avoid progeny contamination, and every 2-3 days thereafter. Dead nematodes were scored every 24h. A nematode was considered dead when it failed to respond to plate tapping or a gentle touch with a platinum wire. Only nematodes that died as a result of getting stuck to the wall of the plate were censored. Lifespan was measured as the time from the end of L4 larval stage (beginning of adulthood) until death.

Electrotaxis assays. In each experiment approximately 10-15 worms were selected from a cultivation plate of a synchronized population of adults and rinsed with electrotaxis buffer (ref Samuel). They were then transferred on an agar gel in a drop of electrotaxis buffer. After the run, in experiments were we controlled for the innocuousness of the electrotaxis assay, the piece of agar was then rinsed and nematodes transferred via a pipette to new bacterial lawn of OP50 (of the innocuousness of electrotaxis assay)

The agar gel was composed of: deionized water, 2% of Bacto-Agar, glycerol (6.2 mL of glycerol 60% for 1 L), NaCl (0.250 mmol/L) as previously described in [16]. The gel was cast by pouring a first layer of agar and adding a PDMS (polydimethylsiloxane) block onto it so that it will shape the future cavity where nematodes will crawl (668 cm). A second layer of gel was then poured around the PDMS block. Once solidified the PDMS block was removed. The resulting agar pad was then placed in an electrophoresis box filled with a buffer. It was composed of de-ionized water, glycerol (6 mL of glycerol 60% for 1 L) and NaCl (0.250 mmol/L) as previously described in [16]. We used a PS305 electrophoresis power supply (APELEX, France) and the Wide Mini-Subtm Cell electrophoresis box (Biorad, USA). Temperature of the room was keep constant and was controlled by air-conditioned.

Image Analysis. Experiments were imaged with a 6.6 Mpixels CMOS monochrome camera (Pixelink) with a close focus zoom lens 10X (136130 mm FL, Edmund Optics Ltd, UK). We used a white, bright field/dark field ring light (Edmund Optics Ltd), to enhance the contrast. Since the worm trajectories are ideally straight, image analysis was straightforward. Trajectories of worms were computed from images by using successively FIJI including the plugin M2Track. I then computed a program in excel to analyse trajectories.

Statistical analyses. Statistical analyses and graphic displays were made using Prism 5.0c from GraphPad Software, Inc.

Work contribution:

Xavier Manière performed the experiments and analysed the results.

Xavier Manière and Ivan Matic designed the experiments

II- Relationship between proteome maintenance and biological age

Phenotypic variability is usually similar in homogenous population and heterogeneous populations. For example, the coefficient of variation of lifespan is even higher in isogenic population than in non isogenic population of *C. elegans* (Finch and Tanzi 1997) (Kirkwood et al. 2005). We hypothesized that these relatively wide distributions could come from different aging rates of individuals.

Ben-zvi et al. showed that the collapse of proteostasis was an early event in *C. elegans* (Ben-Zvi et al. 2009). Therefore, we decided to investigate whether differences in proteome maintenance success could be involved in the variability of lifespan in isogenic population of *C. elegans* raised in the same environment

To compare proteome maintenance success between short-lived and long-lived population of *C. elegans*, we first show that we were able to sort groups of nematodes having different life expectancy by employing electrotaxis. Second, we used protein carbonylation assay to measure worms proteome quality. Third, we tested for the ability of proteome maintenance processes to withstand stress in the different population.

A- Level of protein carbonyls in *C. elegans* with age

We measured protein carbonyl content in *C. elegans* adults from day 0 to day 10. Measurements were taken on protein extracts of different cohorts of wild-type N2 worms raised on bacterial lawn of *E. coli* OP50 at 25°C. As already observed, by Adachi et al. the amount of protein carbonyls increases with age in *C. elegans* (Adachi et al.1998). From day 1 to day 4, also known to be the nematode's reproductive period, the amount of protein carbonyls derivatives increases slowly (15%). By contrast, during the post-reproductive period, between day 4 and day 10, the increase is higher (132%). This increase can be fitted by an exponential curve. However, due to the lack of time points, we cannot conclude whether this is a two steps increase or an exponential increase. First deaths in *C. elegans* population appear around day 5 five **(Fig. 16)**. Thus after this age the mean level of protein carbonylation between the different cohorts is affected by differential mortality. Moreover heterogeneity in protein carbonyls within the population has never been quantified, so we do not know the dynamic of protein carbonyls level at the individual level. To measure level of protein carbonyls on the totality of the population we decided to test if we were able to sort population of nematodes having different life expectancy by employing electrotaxis at day 5.

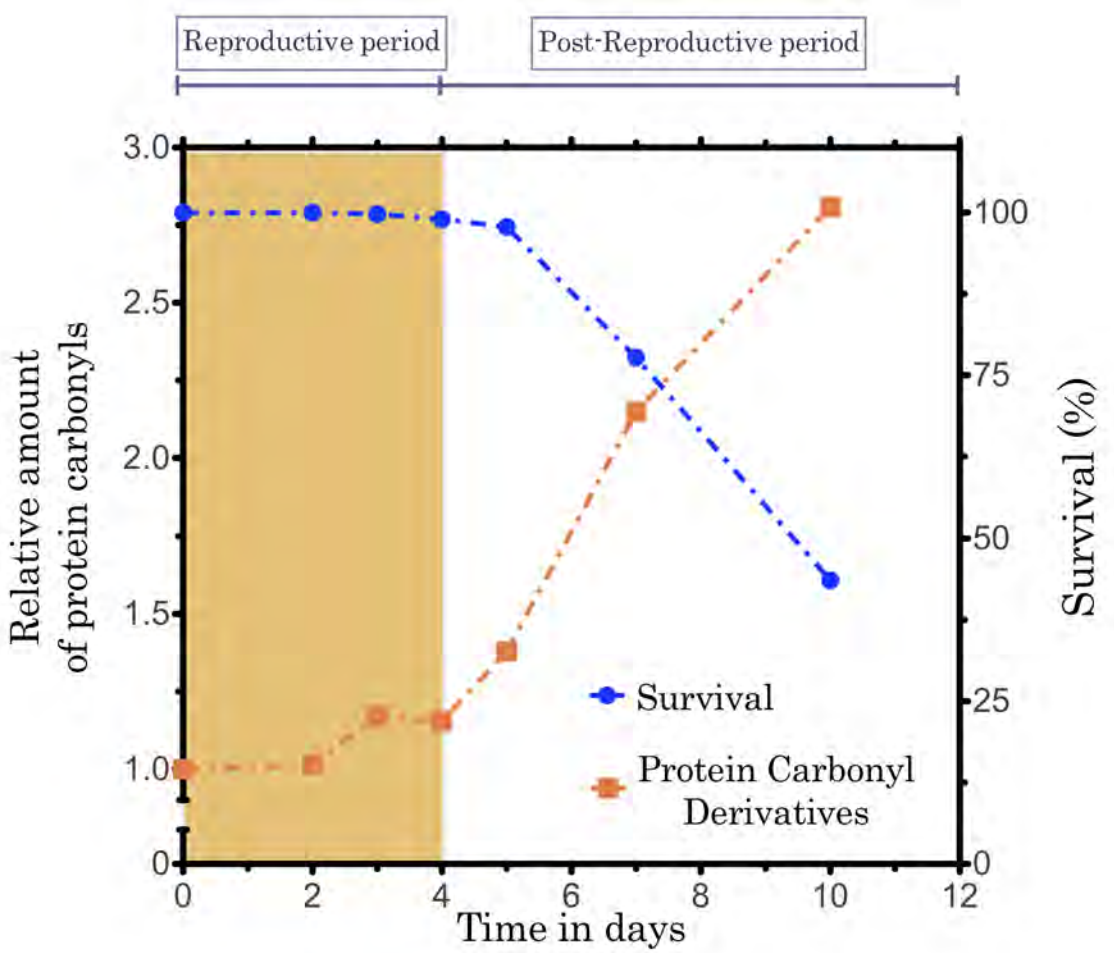

Figure 16: Relative amount of protein carbonyls at different chronological age measured on *C. elegans* N2 nematodes. Orange curve represents measurement taken on different cohorts of *C. elegans*. Amount of protein carbonyls are expressed in relative amount compared to day 0. Blue curve represents survival (%) of control worms used in this set of experiments. Experiments were repeated once with similar results.

B- Life expectancy of nematodes from different crawling velocity categories.

We decided to make up three groups according to protein carbonyls measurement constrains and worms locomotory performance distribution. Our assay to measure protein carbonyls require at least 60 individuals and we wanted to have big enough differences in the mean crawling velocity of each group of worms. The first group is referred as the fast category, it contains the 25% fastest worms, the second group is referred as the intermediate category, it contains 50% percent of the worms, the third group is referred at the slow category, it contains the 25% slowest worms **(Fig. 17).** We found that worms from the fast

category lives significantly longer than worms from the intermediate category (15%, P value < 0.0001) and worms from the intermediate category lives 44% longer than worms from the slowest group (44%, P value < 0.0001) **(Fig. 18)**. We then compared the mortality rate of these three populations. We observed that the fast category had the higher mortality rate but the lower initial mortality. Conversely, the slow category group had the higher initial mortality but the lower mortality rate **(Fig. 19)**.

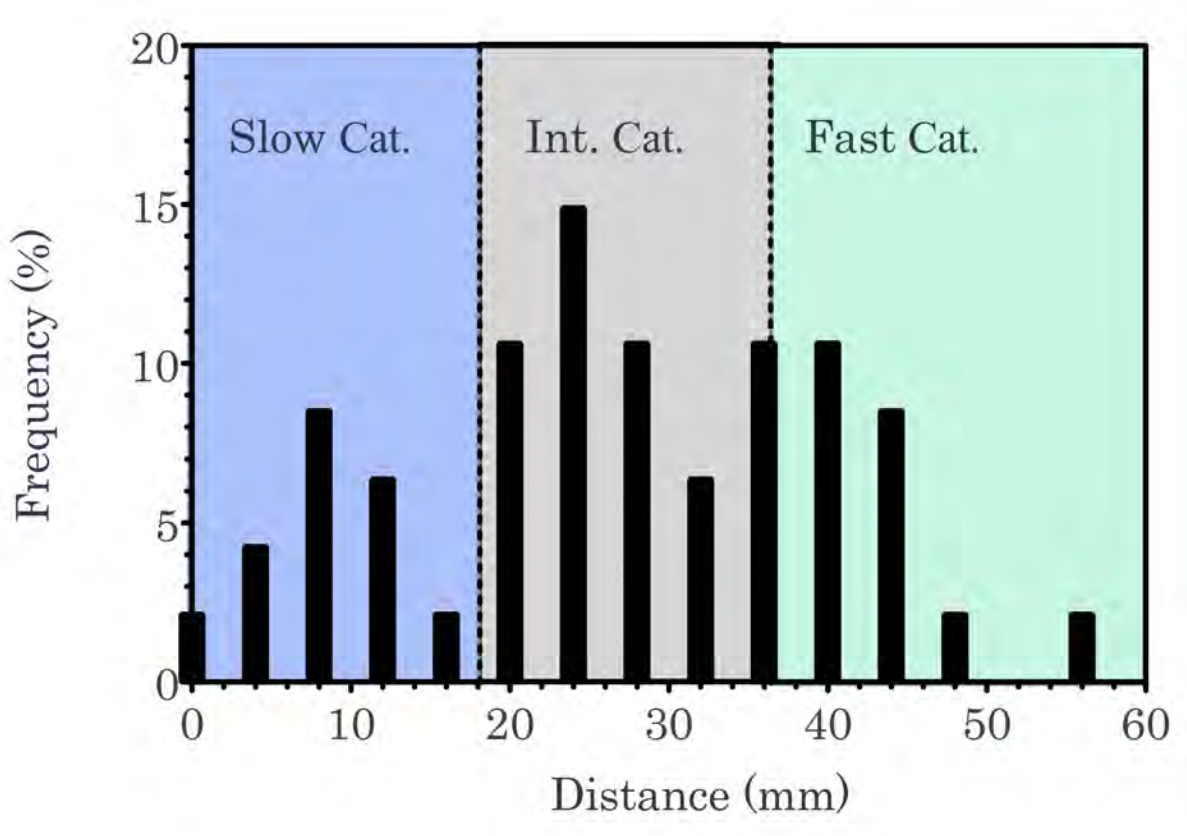

Figure 17: Nematode positions on the gel after an electrotaxis run of 8 minutes at day 5 of adulthood. Worms (N=47). Slow category gather the 25% slowest nematodes, the fast category the 25% fastest nematodes and the intermediate category the rest of the nematodes.

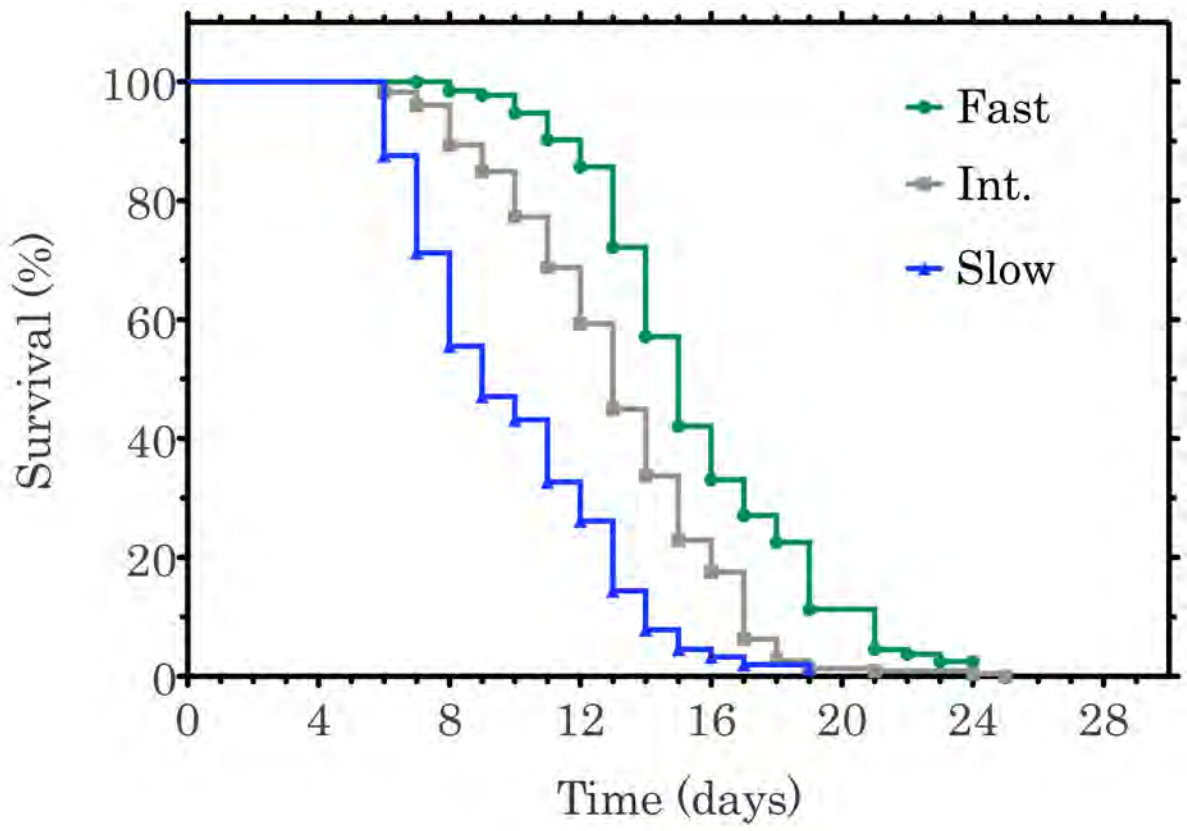

Figure 18: Comparison of *C. elegans* N2 survival depending on their category. Green curve represents survival of worms from the fast category. Grey curve represents survival of worms from the category Intermediate. Blue curve represents survival of worms from the category slow. Difference between the life expectancies was significant between the three groups. Experiments have been repeated at least two times and with similar results. Log-rank (Mantel-Cox) Test, P value < 0.0001)

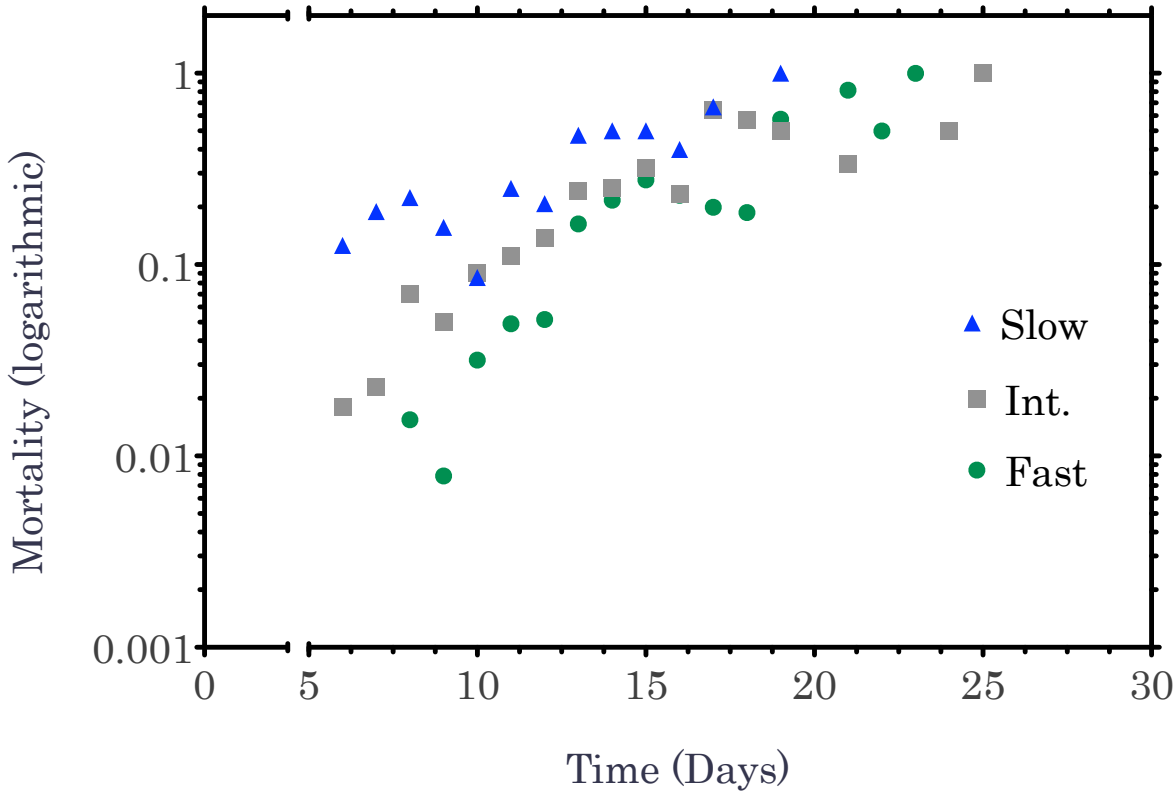

Figure 19: Mortality rate of nematodes from different categories. Worms from the category fast (green dots) exhibit a low level of initial mortality but a high mortality rate while worms from category slow (blue dots) exhibits a high level of initial mortality but a low mortality rate, worms from intermediate category (grey dots) exhibits an intermediate initial mortality and an intermediate mortality rate. Experiments were repeated twice with similar results.

C- Protein carbonyls content of nematodes from different categories

Collapse of proteostasis is an early event in *C. elegans* aging (Ben-Zvi et al. 2009). We thus wanted to test whether worms with shorter life expectancy had a more damaged proteome. We used measurement of protein carbonyls as a proxy to evaluate quality of the proteome. We measured the amount of protein carbonyls in protein extracts from nematodes of the three categories that we previously described. We found that nematodes of the slow category have significantly more protein carbonyls than worms from the fast category. There was only a slight but not significant difference between animals from the intermediate category and worms from the fast category **(Fig. 20)**. These results show that worms having the shorter life expectancy have more protein carbonyls in their proteome.

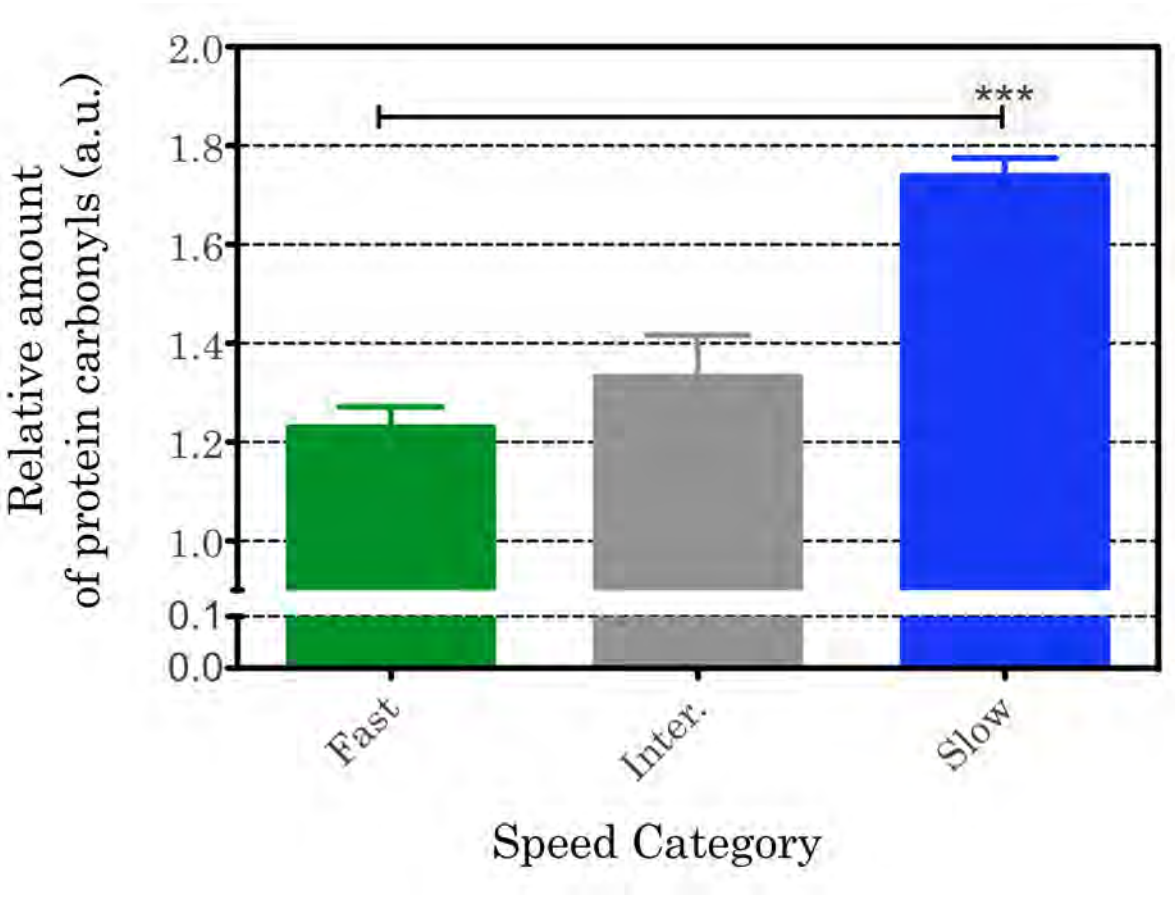

Figure 20: Protein carbonyls level of nematodes from different category at day 5. Fast bar, represent the relative amount of protein carbonyl of worms from the fast category, Inter. bar, represent results of worms from the intermediate category, and Slow bar, represent results of worms from the slow group category. The Values are expressed as the relative fraction of protein carbonyl content of day 0. The amount of protein carbonyl is 41% higher in category Slow than in category Fast (unpaired two-tailed t-test P-Value=0,0007). These data represent results of three independent experiments.

D- Quality of proteome maintenance after heat shock depending on speed category

In order to gain insight into proteome maintenance after heat-shock characterizing categories of worms with different life expectancy we measured protein carbonyl levels subsequently to heat shock stress. We reasoned that worms with a proteome containing large amount of misfolded proteins would exhibit a high level of protein carbonyls after heat-shock and that worms with defect in proteasome or other degradation system would not be able to degrade protein carbonyls after the heat shock. After two hours at 35°C, we observed that

level of protein carbonyls was correlated with crawling velocity categories. Indeed, level of protein carbonyls increase 85% after the heat shock for category fast, 98% for category intermediate, and 156% for category slow. After 4 hours of recovery at 25°C, level of protein carbonyls decreased more for category intermediate, 28%, than for category fast, 19%, though their level after recovery was the same. For category slow, decrease of the level of protein carbonyls was only of 9% after the recovery **(Fig. 21)**.

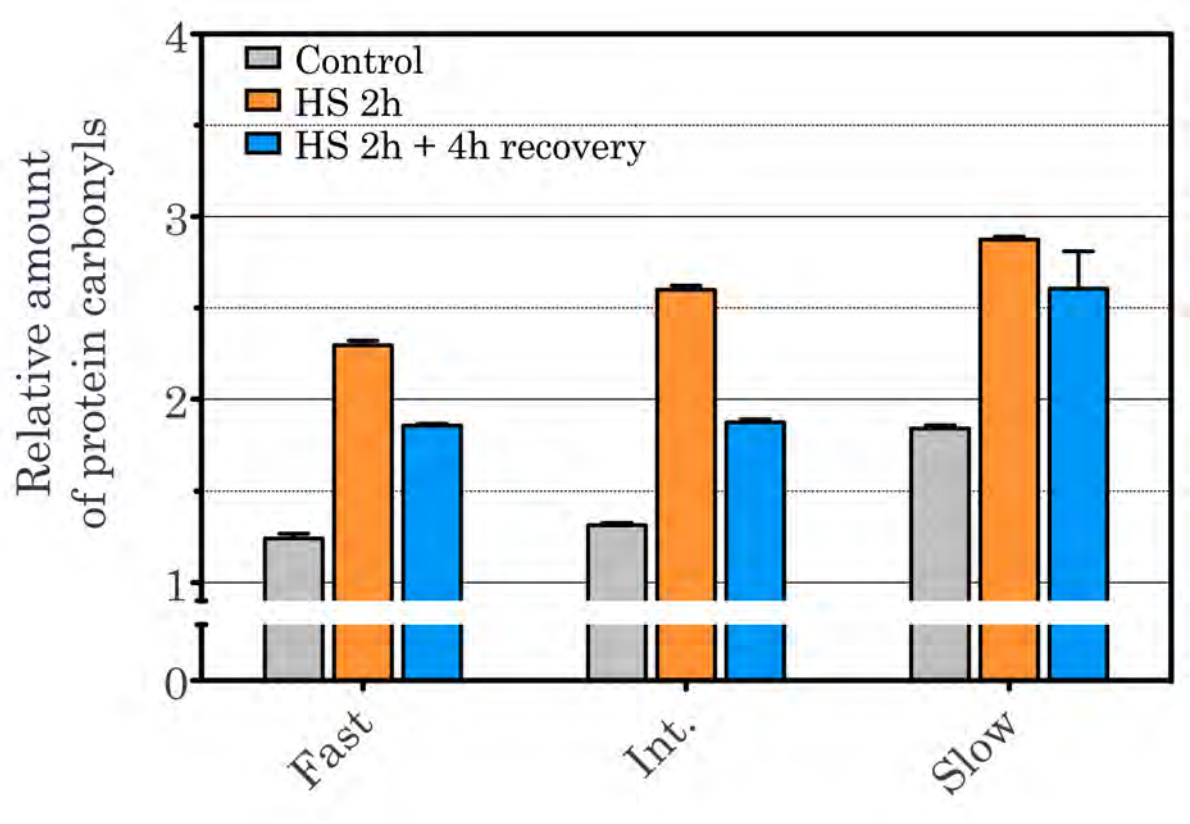

Figure 21: quality of proteome maintenance after heat shock depending on speed category. To evaluate proteome maintenance quality of proteome maintenance we measured protein carbonyl content after a heat shock (35°C). We worked with three cohorts of worms. We measured protein carbonyl content before heat shock (grey bars), after two hours of heat-shock (orange bars) and after 2 hours of heat shock followed by four hours of recovery at 25°C (blue bars). The values are expressed as the relative fraction of protein carbonyl content of day 0. We observed that quality of proteome maintenance correlates after heat shock correlates with speed category. These graphics represent results from two independent experiments.

Finally, we compare survival at 35°C of worms from the different categories (fast, intermediate, slow). We stopped kinetic after 50% of the worms of category “slow” died, at

this point 22 % of the worms of category intermediate were dead and no worms from the category fast were dead **(Fig. 22)**.

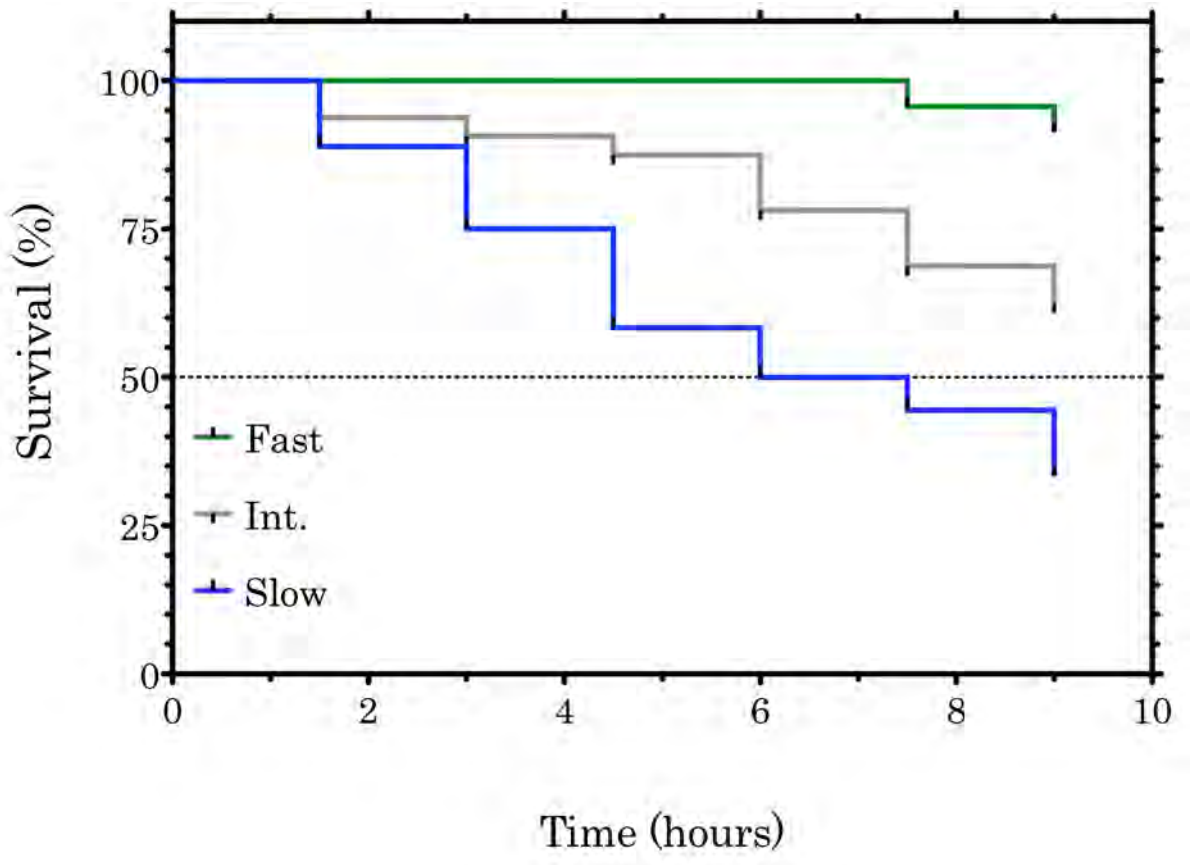

Figure 22: Survival of *C. elegans* depending on their speed category and therefore their life expectancy. Time is expressed in hours. The green curve represents survival of the fastest worms, the blue curve represents survival of slowest worms, and the grey represent the survival of worms from the intermediate group. P values were calculated using the Log-rank (Mantel-Cox) Test. The three curves were significantly different between fast and Intermediate (P Value= 0.0141) and between Intermediate and slow (P-Value = 0.0113). Experiments were repeated twice with similar results.

E- Conclusion

We measured protein carbonyls levels in nematodes at different ages. We found that during the reproductive period there is only a slight increase in protein carbonyls compared to the post reproductive period. To compare the level of proteome damage of nematodes having different life expectancies we set up different categories gathering worms with similar crawling velocity, a biomarker that have been demonstrated to have predictive power on life expectancy (Hsu, Feng, et al. 2009). We employed electrotaxis to sort worms. We found that worms from the slow category have the shortest life expectancy and the higher level of protein carbonyls moreover their protein carbonyl levels were higher after a heat shock than for the intermediate and fast category. Worms from the fast category had the longest life expectancy and the lowest initial mortality but their mortality rate was also higher.

According to the disposable soma theory, aging is caused by accumulation of damage in somatic tissues. We measured protein carbonyls on protein extract. Therefore, protein carbonyls levels represent the sum of the protein carbonyls from the soma and the germline. We have planned to complete this study by monitoring mRNA production of proteome maintenance systems. In addition we want to measure ROS production in the different categories and in the different conditions. Indeed, Dukan et al. showed that protein carbonyls formation is not only affected by ROS level but also by the presence of aberrant proteins. Protein carbonyl level is a proxy to measure proteome damage (Dukan et al. 2000).

F- Materials and methods

Nematode and bacterial strains. *C. elegans* N2 (ancestral) strain kindly provided by J.J. Ewbank (Marseille, France) was used for all experiments, unless otherwise indicated.
E. coli strains used in this study was the uracil deficient strain OP50

Nematode maintenance and synchronization. Nematodes were maintained at 25ºC on nematode growth medium (NGM) agar plates seeded with stationary phase bacterial culture incubated at 37°C. Age-synchronized populations of nematodes were initiated from eggs recovered following sodium hydroxide (0.5M final) and sodium hypochlorite (0.96 % final) treatment of gravid adults maintained at 25° and fed E. coli OP50. All assays were carried out with nematodes synchronized twice: once before development by sodium hydroxide/hypochlorite treatment as described above, and a second time at the end of development by selecting exclusively nematodes at the end of the 4th larval (L4) stage based on vulva morphology.

Survival assays. At L4 stage 75 nematodes were transferred in Petri dishes of 9 cm of diameter where almost all the surface was covered by OP50 bacterial lawn. All the Petri dishes necessary for the six first days of experiments were prepared one day before day 0. Nematodes were transferred onto new plates every day during the first 5 days to avoid progeny contamination, and every 2-3 days thereafter. Dead nematodes were scored every 24h. A nematode was considered dead when it failed to respond to plate tapping or a gentle touch with a platinum wire. Only nematodes that died as a result of getting stuck to the wall of the plate were censored. Lifespan was measured as the time from the end of L4 larval stage (beginning of adulthood) until death.

Electrotaxis assays. In each experiment approximately 60-75 worms were selected from a cultivation plate of five days old adults and rinsed with electrotaxis buffer (ref Samuel). They were then transferred on an agar gel in a drop of electrotaxis buffer. After the run, the agar gel was cut in three in order to isolate the 25% slowest nematodes, from the intermediate group and the 25% fastest nematodes. The different piece of agar were then rinced and nematodes transferred via a pipette to new bacterial lawn of OP50.

The agar gel was composed of: deionized water, 2% of Bacto-Agar, glycerol (6.2 mL of glycerol 60% for 1 L), NaCl (0.250 mmol/L) as previously described in [16]. The gel was cast by pouring a first layer of agar and adding a PDMS (polydimethylsiloxane) block onto it so that it will shape the future cavity where nematodes will crawl (668 cm). A second layer of gel was then poured around the PDMS block. Once solidified the PDMS block was removed. The resulting agar pad was then placed in an electrophoresis box filled with a buffer. It was composed of de-ionized water, glycerol (6 mL of glycerol 60% for 1 L) and NaCl (0.250 mmol/L) as previously described in [16]. We used a PS305 electrophoresis power supply (APELEX, France) and the Wide Mini-Subtm Cell electrophoresis box (Biorad, USA).

Measurements of nematode heat shock resistance. After the electrotaxis assay *C. elegans* of the different categories were transferred onto OP50 lawn (≈25 nematodes per bacterial lawn). After allowing nematodes to recover during 12h at 25°C, Petri dishes were transferred at 35°C. Dead nematodes were scored every hour.

Measurements of nematode heat shock stress and recovery. After the electrotaxis assay *C. elegans* of the different categories were transferred onto OP50 lawn (≈60 nematodes per bacterial lawn). After allowing nematodes to recover during 12h at 25°C, Petri dishes were transferred at 35°C during two hours. If necessary worms were then transferred at 25°C to let them recover during 4 hours.

Image Analysis. Experiments were imaged with a 6.6 Mpixels CMOS monochrome camera (Pixelink) with a close focus zoom lens 10X (136130 mm FL, Edmund Optics Ltd, UK). We used a white, bright field/dark field ring light (Edmund Optics Ltd), to enhance the contrast. Since the worm trajectories are ideally straight, image analysis was straightforward. Trajectories of worms were computed from images by using successively FIJI including the plugin M2Track. I then computed a program in excel to analyse trajectories.

Preparation of Protein Extracts and Protein Carbonylation Measurement. Samples of C.elegans were pelleted by centrifugation and resuspended in the lysis buffer of the OxyElisa protein carbonylation detection kit (Millipore) supplemented with amixture of protease inhibitors (Roche). Resuspended worms were broken by using a mechanical homogenizer in a combination with glass beads 150-202 μm (Sigma), and then centrifuged 20 min at 12,000 × g. Samples were supplemented with 10 mg/100 ul lipid removal agent

(Sigma 13360-U), kept 1 hr at RT and centrifuged 15 min 10,000 g. Genomic DNA fragment were removed by using carboxylated nano beads (SHANGHAI EHOO BIOTECNOLOGY co., LTD, average particle size: 80 nm, surface carboxyl volume ≥0.25 mmol/g) at the final concentration of 2 mg/mL, kept at RT for 15 min followed by centrifugation of 15 min at 10000 g. The amount of protein in the supernatant was measured by the BCA kit (Pierce) and total protein carbonylation using the OxyElisa kit. Protein extracts diluted to 10 µg/mL were loaded into wells (provided in the kit) and incubated over night at 4 °C to allow proteins to adsorb to the surface, followed by DHR derivatization of adsorbed proteins and detection of derivatized dinitrophenol (DNP)-carbonyl by a mouse DNP specific monoclonal antibody conjugated to HRP. Subsequent incubation with enzyme substrate 3,3′,5,5′ tetramethylbenzidine resulted in a colored product that was quantified using a microplate reader with maximum absorbance at 450 nm.

Statistical analyses. Statistical analyses and graphic displays were made using Prism 5.0c from GraphPad Software, Inc.

Work contribution:

Anita Krisko measured protein carbonylation.

Xavier Manière performed all the other experiments.

Xavier Manière analysed the results.

Xavier Manière and Ivan Matic designed the experiments.

Discussion

Obtained results

The question we wanted to address in our project was: do differences in proteome maintenance success could be involved in lifespan variability in isogenic population of *C. elegans* raised in the same environment? Because locomotion has been shown to be one of the most reliable biomarker of aging we decided to employ electrotaxis to sort groups of nematodes with similar crawling velocity performance and therefore life expectancy. We first show the relevance of electrotaxis as a general method to realize quantitative measurement of *C. elegans* locomotory performance, either between different strains or between individuals at different age. Then we investigated the effect of age on locomotory performance of *C. elegans* N2 in electric fields. We found that crawling speed is the major factor affecting self-sorting of *C. elegans* N2 hermaphrodites just after the reproductive period. We demonstrated that we were able to sort groups of nematodes having different life expectancy and finally showed that protein carbonyl levels, the proxy we used to measure proteome quality, was correlated with lifespan in *C. elegans* N2 hermaphrodites.

Predictive categories: rate of decay or punctual measurement?

To be able to compare proteome quality within *C. elegans* populations having different life expectancy, we needed to sort a relatively high number of individuals. Therefore electrotaxis was well suited because it is possible within a single experiment of less than ten minutes to sort 60 nematodes in a 6 x 8 cm gel. Hsu et al showed that rate of motor activity decay measurement from day 3 to day 9 - at 20°C - may be more accurate than punctual measurement to predict remaining lifespan (Hsu et al. 2009). Unfortunately, this strategy requires daily measurements during the first part of *C. elegans* lifespan, we thus considered that it was difficult for big populations.

To improve the predictive power on life-expectancy at the individual level, it would be interesting either to do repetitive measurement during the first part of *C. elegans* life as Hsu et al did or/and to build a score integrating results of the different variables measured during

electrotaxis run (crawling speed, directional fluctuation, mean run duration, etc.). As manipulation of worms can affect their lifespan, the challenge is to find the best balance between multiple measurements that will increase the predictive power of the assay and reduction of worms manipulation in order to limit artifacts.

Locomotory decline: what are we measuring?

Herndon et al observed that tissues in *C. elegans* age at different rates. For example, when they looked for aging patterns in *C. elegans*, they did not observe significant alterations of neuron physiology with time, whereas sarcomeres disorganization in muscles cells was well correlated with motility categories and life expectancy. Consequently, muscle degeneration is usually interpreted as the major cause of locomotory decline. However, recent observations gave evidences of neuronal aging in *C. elegans*. Cai and Sesti examined the consequences of oxidation of the KSV-1 potassium channels, key regulators of neurons excitability (Cai and Sesti 2009). They observed that oxidation of these channels was associated with a loss of excitability of the neurons and that nematodes chemotaxis was impaired. Moreover Pan et al. reported age-dependent defect of touch neurons (Pan et al. 2011) and Glenn et al. demonstrated that treatment with arecoline, an analogue of acteylcholine can improve locomotory performances of 10 days old nematodes.

Therefore, some of the variables we measured during electrotaxis including directional fluctuation, run duration may be linked with neuronal aging. For example, turn rate is governed by head motor neurons (Schafer 2005). Ryu et al showed that run duration in thermotaxis have neuronal basis (Ryu and Samuel 2002). *C. elegans* is a growing model for neurodegenerative diseases. Development of behavioral assays targeted on neurons performances would allow to gain insight on neuronal aging.

Measurement of Oxidative damage in C. elegans

We measured protein carbonyls on worms extract. During our protocol we removed lipid derivatives and nucleic acids in order to measure only protein carbonyl. Moreover, non-soluble aggregates were also eliminated during the protein extraction. Using this protocol we observed significant differences in protein carbonyls levels between fast and slow categories of nematodes at day 5 of adulthood. Our technique had two limitations, first, we pooled many individuals (n≈ 40-60) so it was not possible to study neither inter-individual variability in protein carbonyl of each category nor to observe specific tissue localization of protein carbonyl derivatives in individual worms. Second, as we measured only soluble proteins, we measured the equilibrium between formation of protein carbonyl and aggregation in insoluble aggregates. Goudeau et al., developed an in situ protocol to measure protein carbonyl in *C. elegans* (Goudeau et al., 2010). It would be interesting to implement this technique in aging studies at the level of individuals. This would enable us to compare the relative distribution of protein damage within *C. elegans* tissues and correlate it with locomotory decline and/or lifespan. One limit of in situ measurements would be auto fluorescence of worms' tissues in aged nematodes.

Aging and protein oxidative damage

The free radical theory developed by Harman postulates that damage originated from ROS production was the leading cause of aging in biological organisms. Studies in the 1990's, using *C. elegans* mutants over-producing free radicals species were consistent with Hartman theory (Adachi, Fujiwara and Ishii 1998). However, recent results are challenging this theory (Gems and Doonan 2009). For example, high ROS production is observed in different physiological conditions like in long-lived dietary restricted animals. Moreover, high level of protein carbonyls in *C. elegans* double mutants *sod-3;daf-2* does not shorten their lifespan compared to the *daf-2* single mutants (Yang and Hekimi 2010). Our results show that the level of protein carbonyls correlates with life expectancy of isogenic nematodes at day 5. Our conclusion is that within an isogenic cohort the individuals that are the less capable of maintaining a low level of protein oxidative damage are the one that are the most likely to die first. It is noteworthy that it was out of the scope of our experiments to determine if an absolute level of carbonylation was a good biomarker of biological age. To our knowledge it

is the first time that the amount of carbonyls showed a predictive value of life expectancy for isogenic organisms that experienced the same environmental conditions. Usually, the level of protein oxidative damage is compared between different mutants strains or between isogenic animals experiencing different conditions. These set-ups did not allow to evaluate the predictive power on life expectancy of protein carbonyls.

Hekimi reported that *sod-2* and even *sod-2;sod-3* deletions increase protein oxidative damage without altering lifespan of *daf-2* and *clk-1* long-lived mutants (Van Raamsdonk and Hekimi 2009). They concluded that ROS mediated damage of the proteome was not a leading cause of aging in *C. elegans*. According to the work of Nakamura et al., it seems that oxidative damage does not harm all the proteins the same way, or at least, that some proteins are more likely to be oxidized than others (Nakamura et al. 1999). Nakamura et al. showed that vitellogenin was a major carbonylated protein in aged nematodes. This protein is expressed in the enterocytes during the egg laying stage and is part of the yolk composition. During the post-reproductive period these proteins are exported in the body cavity. As vitellogenin possesses metal binding capacity, it makes it more prone to oxidation (Nakamura et al. 1999). So, it would be interesting to know, in the long-lived mutants lacking *sod-2* and *sod-3*, the nature of the carbonylated proteins. Indeed, vittelogenin may have played the role of ROS scavenger. In this situation, measurement of carbonylated protein would not have reflected damage of functional proteins.

Phenotypic variability and aging rates in homogeneous population.

Lifespan variability is usually similar in isogenic and non isogenic populations (Kirkwood and Finch 2002). This phenomenon demonstrates the importance of stochastic events in lifespan determination. A possible explanation for this phenomenon is that phenotypic variability in cells might lead to different perceptions of the same environment. Thus, one can speculate that phenotypic variability could lead to differential allocation of energy in somatic maintenance and reproduction within individual of a theoretically homogeneous population. To investigate whether there is such a trade off in homogeneous population of *C. elegans* N2, Klass investigated biological factors influencing lifespan of *C. elegans* N2 (Klass 1977). He

found neither correlation between lifespan and number of eggs produced, nor between offspring and parents lifespan. In addition, *C. elegans* reproductive span does not correlate with lifespan neither at the individual level nor at the strain level. Interestingly, in early adulthood *C. elegans* crawling speed shows a weak negative correlation with life expectancy (data not shown) (Hsu et al. 2009). However this phenomenon has never been investigated specifically. In our work we show that groups of nematodes with shorter life expectancy have more protein carbonyl. This phenotype may be caused by a lack of investment in somatic maintenance but we still do not know whether this is mainly due to excessive ROS production or protein folding issues. Detoxification of oxidizing compounds is partly mediated by intermediate species including gluthatione, thioredoxin or vitamins that need to be recycled by NADPH (Stadtman et al. 2005). The pentose phosphate pathway, an alternative pathway to glycolysis, generates NADPH which is also involved in anabolism (Geer et al.1979) (Arking et al. 2002). It is thus tempting to speculate that investment in anti-oxidant defense can interfere with anabolism and vice versa.

Design in biological systems

Stressful conditions may occur at any moment in biological systems. These situations may challenge normal functioning and need rapid response. Thus it is common to find fail-safe designs in biological systems (Hartwell et al. 1999) (Kitano 2004). The fail-safe concept comes from the engineering field. These designs eliminate harmful consequences when a failure or a malfunction occurs. This usually involves a mechanic response that needs no energy or action by an integrating system. Such design can be found in biological systems including *C. elegans*. Indeed, transcription factors involved in stress response such as HSF-1 are persistently present in the cell. HSF-1 is sequestrated by DAF-21, a heat shock protein, in the cytoplasm (Singh and Aballay 2006). When denaturated protein content increases in the cytoplasm, DAF-21 bind to them and HSF-1 is mechanically released and can translocate into the nucleus to promote expression of more heat-shock proteins. Another example deals with adaption to hypoxic stress. The Hypoxia Inducible Factor (HIF) is constitutively targeted to proteasome degradation. This transcription factor activates genes involved in resistance to proteotoxic stress. When the proteasome does not function properly, HIF is not degraded and

can trigger expression of genes involved in proteostasis (Mehta et al. 2009). Interestingly, the latter example shows that cell waste continuously energy in order to be ready to cope with potential challenge to their homeostasis. Fail-safe design has two main consequences on cell biology. First, it explains why some mutations increase lifespan. Loss of function mutations that prevent stress response during normal functioning lead to permanent fail-safe state in which stress response genes are always activated. Low Insulin like signaling is an example in *C. elegans*. Indeed, DAF-16 is usually sequestrated in the cytoplasm due to insulin like signaling. If insulin like signaling is compromised DAF-16 is constitutively targeted to the nucleus and activate stress response genes (Yen et al. 2011). Second, action of maintenance processes can be classified into three categories: prevention of damage, detection of damage, and degradation of damaged products. In the case of proteome maintenance, prevention can be fulfilled at the level of protein structure, for example, poor folding or proteins will increase their probability to be oxidized and/or to become non-functional. Detection involves presence of damage sensors. For example in the unfolding proteins response, IRE-1 can detect high load of unfolded proteins in the endoplasmic reticulum and trigger the synthesis of new chaperone proteins (Ali et al. 2011). Each maintenance process has a cost and failure of this process has short or long term consequences. In the case of the HIF transcription factor, continuous degradation of the transcription has a cost but small compared to the cost of degrading the whole proteome subsequently to a too slow reaction to a hypoxic stress. In the context of our study we would like to know whether there is a pattern in the progressive failure of proteome maintenance processes and if this could be related to the process cost or the consequences of the alterations. An alternative approach of the fail safe concept to proteome maintenance system would be to test whether proteins involved in oxidative stress detection and/or oxidative stress response are more robust against oxidation than protein repressing oxidative stress response. Indeed, this mechanism would allow an automatic response of cell to ROS challenge.

Conclusion

Behavioral decline in an isogenic population of *C. elegans* is asynchronous. This phenomenon may be due the fact that individuals do not age at the same rate. As somatic tissues of *C. elegans* are composed of post-somatic cells, we reasoned that capacity to maintain proteostasis was a key determinant in *C. elegans* biological aging. We report in this work that behavioral decline correlates both with life expectancy and protein carbonyl level. This result does not allow us to conclude that collapse of proteostasis is the cause of premature aging in *C. elegans*. Future work will help us to gain insight into this hypothesis. For example, it would be interesting to investigate whether some interventions aimed at restoring proteostasis in worms predicted to have a short life expectancy could improve their life expectancy.

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Appendix

I- Developmental modulation of C. elegans longevity

A- *C. elegans* resistance to heat shock following development on pathogen

DAF-2/DAF-16 insulin-like pathway controls the expression of the Heat Shock Factor 1 (HSF-1), a transcription factor whose induction limits the accumulation of damaged proteins, increases stress resistance, resistance to pathogens, and lifespan. First, we tested whether nematodes developed on 536 have higher resistance to heat shock than nematodes developed on OP50 and found that former nematodes indeed resist significantly (Mann Whitney test, p-value=0.001) better to a 10h heat shock at 35°C (Fig. 2A). Second, because it was reported that capacity to induce the heat shock response early in life correlates positively with the life expectancy, we measured induction of the heat shock response in these two groups of nematodes. For this, we used a *C. elegans* strain with a Green Fluorescent Protein (GFP) fusion to the hsp-16.2 promoter to monitor the induction of this chaperone protein and its upstream signaling pathway. The expression of this gene is regulated by HSF-1. We observed that development on the 536 strain, compared to development on OP50, induced a significant (unpaired t-test $p<0.0001$) upregulation of hsp-16.2 expression in the young adult nematodes (Fig. 2B).

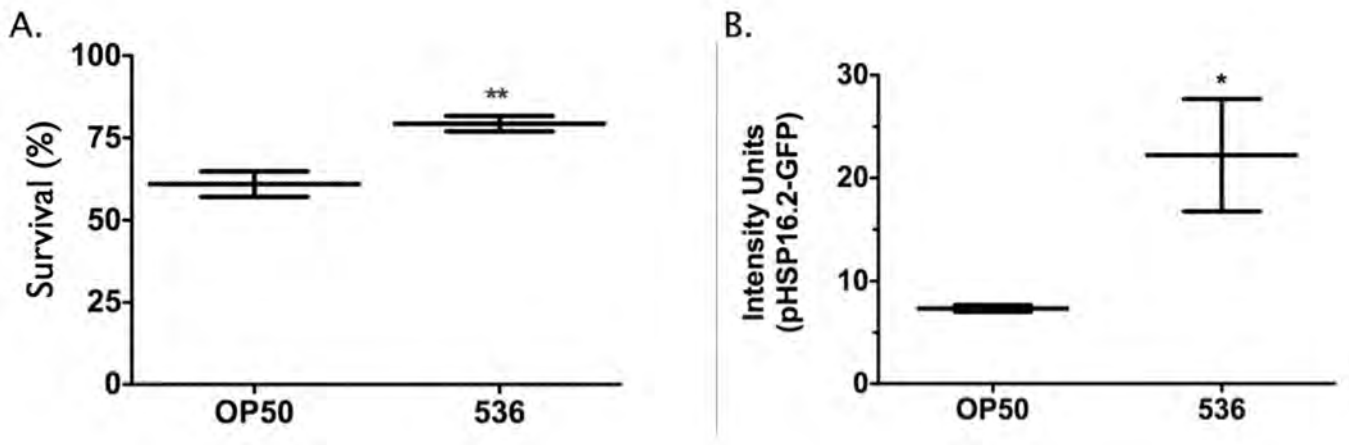

Figure 4. Heat shock resistance and hsp-16.2 induction in *C. elegans* developed on different *E. coli* strains. (A) Survival of nematodes developed on OP50 or 536 bacterial strains after 10h at à 35°C. (**B**) The level of fluorescence of the pHSP-16.2::GFP reporter was greater in young adult nematodes (day 0) developed on the pathogen 536 (n=75) than in nematodes developed on OP50 (n=75). Graph - mean ± s.e.m. Statistics: unpaired t-test and. Data presented in A, and B are the average of at least three independent experiments compiled together after statistical analysis showed no significant difference between individual experiments.

B- *C. elegans* N2 survival following development on pathogens

Overexpression of HSP-16.2 can increase lifespan in *C. elegans*. We reasoned that if development on the pathogenic E. coli 536 increase HSP-16.2 production nematodes developed on this strain should have a longer lifespan. However, we found no difference between survivals of *C. elegans* N2 between these two conditions. These results may be probably due to a transient overexpression of HSP-16.2 in worms developed on 536.

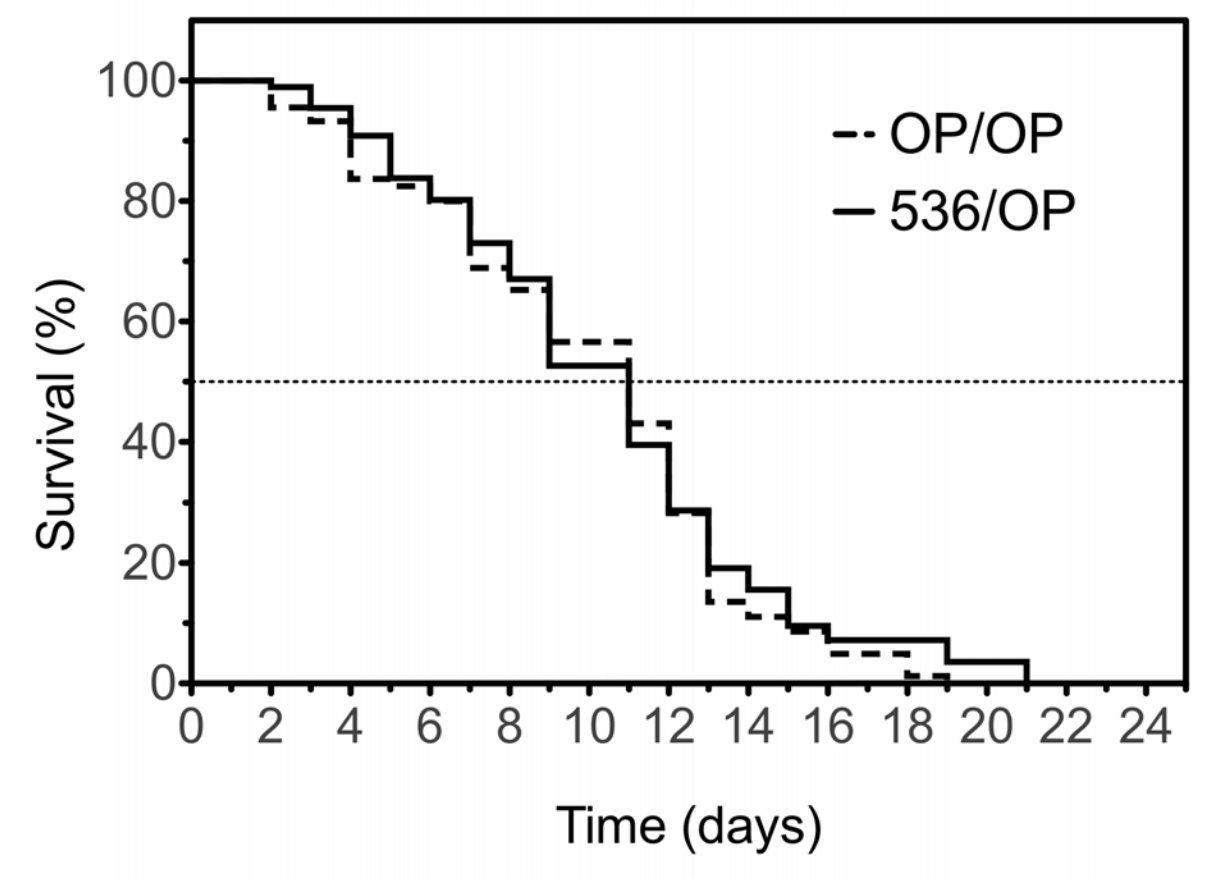

Figure 2. Survival of *C. elegans* developed on different *E. coli* strains. (A) Survival of the N2 nematodes is similar for worms developed on E. coli OP50 or E. coli 536 and maintained on OP50 during adulthood (536/OP50, n=81) (OP50/OP50, n=82). These results come from one experiment representative of three independent experiments.

Survival assays. For survival assays nematodes were transferred onto new plates every day during the first 5 days to avoid progeny contamination, and every 2-3 days thereafter. Dead nematodes were scored every 24h. A nematode was considered dead when it failed to respond to plate tapping or a gentle touch with a platinum wire. Only nematodes that died as a result of getting stuck to the wall of the plate were censored. Lifespan was measured as the time from the end of L4 larval stage (beginning of adulthood) until death.

Elimination of bacteria from nematode intestine. Nematodes were washed in M9 minimal medium and then incubated in NGM medium for 1h. Then, nematodes were incubated in M9 medium complemented with 20 mg/ml Polymyxin B (Fluka) for 1h. Finally, nematodes were

washed in M9 medium, and transferred on plates containing the appropriate bacterial strain. We verified that antibiotic treatment did not affect nematode survival by comparing survival curves of nematodes that were treated with antibiotic with those that were not treated, and we did not find any difference. Nematodes from the survival experiments were regularly checked for presence of the bacterial strain that was supposed to be eliminated by antibiotic treatment.

Measurements of nematode heat shock resistance. L4 nematodes developed on OP50 or 536 were washed in antibiotic Polymyxin B in order to eliminate bacteria from the nematode guts. Then, nematodes were transferred onto OP50 lawn in order to measure heat shock resistance using nematodes that have the same bacterial strain in the intestine. After allowing nematodes to recover during 12h at 25°C, they were transferred at 35°C. Dead nematodes were scored after 10h of incubation.

Measurements of nematode heat shock protein (HSP) expression. HSP expression measurement assays were carried out with *C. elegans* strain TJ375 carrying a fluorescent reporter under the control of the HSP-16.2 promoter (gpIs1[hsp-16-2::GFP]). Gene reporter levels were quantified with the COPAS Biosort (Union Biometrica

Work Contribution:

Xavier Manière developed the protocol for eliminating pathogenic bacteria.
Xavier Manière and Diana Fernandez did the survival kinetic and the measurement of the heat-shock resistance
Magali Leroy did the measurement of GFP fluorescence in Collaboration with the Ewbank group.
Magali Leroy and Ivan Matic designed the experiment.

II- Modulation of aging profiles in isogenic population of Caenorhabditis elegans by bacteria causing different extrinsic mortality rate

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RESEARCH ARTICLE

Modulation of aging profiles in isogenic populations of *Caenorhabditis elegans* by bacteria causing different extrinsic mortality rates

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Abstract It has been postulated that the presence of parasites causing high extrinsic mortality may trigger an inducible acceleration of the host aging. We tested this hypothesis using isogenic populations of *Caenorhabditis elegans* nematodes and different *Escherichia coli* strains. When exposed to pathogenic bacteria, nematodes showed up to fourfold higher mortality rates, reproduced earlier, produced more H_2O_2, and accumulated more autofluorescence, than when exposed to an innocuous strain. We also observed that mortality increased at a slower rate in old animals, a phenomenon known as mortality deceleration. Mortality deceleration started earlier in populations dying faster, likely as a consequence of lifelong heterogeneity between individual tendencies to die. Taken together, our results strongly suggest that the high extrinsic mortality imposed by the pathogens results in the modulation of nematodes' life-history traits, including aging and reproduction. This could be an adaptive response aiming at the maximization of Darwinian fitness.

Keywords *Caenorhabditis elegans* · Aging rates · Pathogen · *Escherichia coli* · Mortality deceleration

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Introduction

Aging is a gradual deterioration of structure and function resulting in a higher susceptibility to the environmental challenge, a decline in reproduction, and an increasing susceptibility to disease and death (Kirkwood 2005). The evolutionary theory postulates that natural selection can modulate aging rates by tuning the allocation of metabolic resources among essential life processes like growth, reproduction, and maintenance. Williams (1957) proposed that the high rates of extrinsic mortality imposed by the environment should result in the evolution of faster aging. This theory has been largely supported by comparative studies on aging both in nature and experimental settings (Finch and Ruvkun 2001). For example, when genetically heterogeneous populations of *Drosophila melanogaster* were subjected to different levels of adult mortality, populations with elevated extrinsic adult mortality evolved shorter life spans than those with low-mortality (Stearns et al. 1998, 2000).

However, when environment changes frequently, evolution may be too slow because it acts over several generations. Under such conditions, phenotypic plasticity of aging may evolve, i.e., the capacity to change phenotype in response to changes in the environment without alteration of the genotype. Such adaptive phenotypic plasticity was indeed observed for *Strongyloides ratti* nematode species (Gardner et al. 2006). Depending on the environment, aging rates of these nematodes can differ by 30-fold. Similarly, aging trajectories of genetically identical *D. melanogaster* and *Caenorhabditis elegans* lineages were shown to be modulated by food availability (Lenaerts et al. 2007; Mair et al. 2003). It has been postulated that the presence of parasites causing high extrinsic mortality may also trigger accelerated aging of the host (Pletcher et al. 2007). Our goal was to test this hypothesis in a detailed and systematic investigation of the alteration of aging patterns by pathogens.

For this study, we used isogenic populations of *C. elegans* and different *Escherichia coli* strains. Due to several convenient features, *C. elegans* is an established model for studies of aging and host-pathogen interactions (Ewbank 2002; Olsen et al. 2006; Riddle et al. 1997; Sifri et al. 2005). The self-fertilizing hermaphrodites can be held in homozygous, isogenic populations (Hope and Hames 1999), eliminating genetic heterogeneity as a confounding factor in aging studies. *C. elegans* can be fed on a lawn of bacteria to ensure the same bacterial strain is present both in the intestine and the environment of the nematode, in the absence of any other microorganisms. Its translucent tissues greatly facilitate in vivo fluorescence measurements of both nematode and bacterial markers. The nematode is standardly maintained and fed on a non-virulent *E. coli* OP50 strain. *E. coli* is a facultative anaerobic bacterial species present in the intestine of warm-blooded animals, including humans (Nataro and Kaper 1998). Although most *E. coli* strains are harmless and confined to the intestinal lumen as commensals, other strains can cause moderate to severe diseases, including urinary tract infection, diarrhea, sepsis, or meningitis. Different pathogenic *E. coli* kill *C. elegans* with different efficiencies, depending on virulence factors and other determinants (Diard et al. 2007). Therefore, *E. coli* presents the possibility to choose closely related bacteria from the same species that cause various levels of host mortality.

Combining those two well-studied model organisms, we investigated how different characteristics of *C. elegans* mortality curves were affected by various *E. coli* pathogens. Particular consideration was given to the rate of exponential mortality increase (Gompertz 1825) and the onset of mortality deceleration at late ages (Vaupel et al. 1998). In addition, we monitored how pathogens affect fertility, because an ultimate measure of aging should include both survival and reproduction (Partridge and Barton 1996). As age-dependent accumulation of autofluorescent compounds is a universal biomarker of aging (Gerstbrein et al. 2005), we also measured this in *C. elegans* fed different bacterial strains.

We found that nematodes have up to fourfold higher mortality rates in presence of pathogenic bacteria, and that mortality increased at a slower rate in old nematodes. This mortality deceleration started earlier in populations with higher mortality rates, which is very likely a consequence of lifelong heterogeneity between individual nematodes in their tendency to die. When fed bacteria causing high mortality, nematodes show increased accumulation of autofluorescence. *C. elegans* exposed to pathogenic bacteria shift towards earlier reproductive schedule. Therefore, our results indicate that nematodes response to bacteria causing high mortality modulates their life-histories, i.e., they reproduce earlier and age faster. Such phenotypic plasticity may be part of an adaptive response to pathogens aiming at the maximization of Darwinian fitness.

Results

C. elegans mortality curves

We performed comparative longevity studies of genetically identical populations of *C. elegans* growing on different bacterial food sources under otherwise identical experimental conditions. According to standard practice, nematodes were kept at 25°C on *E. coli* OP50, an uracil biosynthesis deficient mutant, during development, i.e., the first 48 h between egg-stage and adulthood. Afterwards, nematodes were fed either on *E. coli* OP50, on the laboratory strain MG1655, or on one of three natural isolates: IAI1, ED1A, and F11. IAI1 and ED1A were isolated from the feces of healthy humans, and F11 was isolated from a patient with

urinary tract infection. For further information on these strains see (Diard et al. 2007; Johnson et al. 2006; Picard et al. 1999). Moreover, nematodes were also fed on OP50 and IAI1 bacteria killed by UV irradiation.

Different survival models including two to five parameters were fitted by maximum likelihood estimation to the collected survival data from all the experiments (see Figs. S1, S2, and Supporting text S2 for a detailed description and discussion of the models). The maximum likelihood estimation framework provides straightforward significance tests for determining the appropriate number of parameters to explain the data (Pletcher 1999). In all cases, the piecewise exponential four-parameters model (also called Gompertz 2-stage model, (Fukui et al. 1993)) fitted data best, i.e., mortality first increased exponentially and then significantly decelerated on every food source, leading to a kink-like shape in the graph (Figs. 1, S2). In this model, the fitted parameters were: the initial mortality (intercept a_1), the rate of mortality increase before deceleration (exponential slope b_1), the onset of mortality deceleration (time-point of kink t_{kink}), and the rate of mortality increase after the kink (exponential slope b_2). Two examples of fitted mortality curves and a scheme explaining the different parameters are shown in Fig. 1.

Our results show that the rate of increase before the kink differed considerably between different experimental conditions; the fitted exponential slope on F11 bacteria was $\sim$4 times the exponential slope on OP50 bacteria (log(mortality)/day 0.59 and 2.03, respectively). The rate of mortality increase before deceleration was highly correlated to the mean survival ($R^2 = 0.97$; $P < 10^{-2}$). Moreover, we observed that it is possible to modulate the timing of the onset of *C. elegans* mortality deceleration by changing the bacterial food source (Figs. 1a, S2; Table 1). On OP50 bacteria, the kink was observed after 8.6 days, which is nearly identical to the previously published observation (Vaupel et al. 1994). Under the different experimental conditions we tested, mortality deceleration set on as early as day 4 or as late as day 11. However, unlike the data obtained for nematodes fed on live bacteria, the mortality curves for nematodes grown on UV-killed OP50 or IAI1 were very similar (Table 1; Fig. S2), as is their overall survival (Fig. S1, Kaplan–Meier test: $P = 0.41$, $N = 945$ and 481, respectively).

The variations in the rates of mortality increase before deceleration were inversely related to the time-point of the kink (Table 1). This was not true for the intercept (Table 1), which should, however, be affected by any time-independent multiplicative effect as postulated in the often-used proportional hazard models. Therefore, a more detailed analysis is appropriate. The fitted values for the exponential slope after the kink were significantly greater than zero in all cases, meaning that we did not find a flat plateau under any condition. The mortality rates after deceleration differed between different food sources, although not in a pattern linked to the onset of mortality deceleration as for the first slope (Table 1). Remarkably, the survival at the moment of the kink (S_{kink}) remained quite unaltered around the value 0.8 for all conditions,

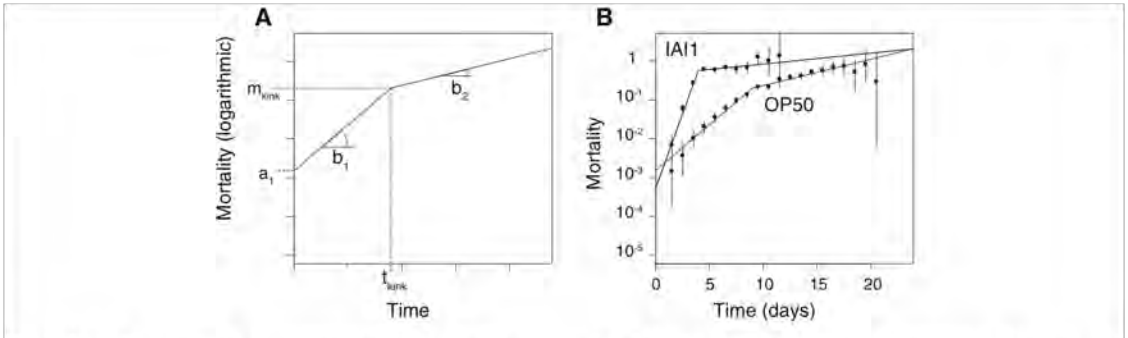

Fig. 1 Mortality rates of a 2-stage-Gompertz model. **a** Schematic representation of the fitted parameters: initial mortality (intercept a), aging rate before deceleration (exponential slope b_1), onset of mortality deceleration (time-point of kink t_{kink}), and mortality rate after the kink (exponential slope b_2). **b** Fitted mortality curves for nematodes fed on *E. coli* strains OP50 and IAI1. *Circles* represent daily estimates of mortality; *lines* represent fitted models. *Error bars* represent 95% confidence intervals based on binomial distribution estimates

Table 1 Analysis of different features of mortality curves

Condition	Mean survival (days)	Slope b_1	t_{kink} (days)	Intercept a_1	Slope b_2	m_{kink}	S_{kink}	N
F11	5.56 (0.05)	2.03 (0.15)	3.79 (0.09)	2.58×10^{-4} (1.14×10^{-4})	0.09 (0.03)	0.52 (0.04)	0.78 (0.02)	1,257
IAI1	5.46 (0.04)	1.78 (0.09)	3.96 (0.07)	5.31×10^{-4} (1.70×10^{-4})	0.06 (0.03)	0.58 (0.04)	0.72 (0.02)	1,446
ED1A	7.12 (0.07)	1.62 (0.32)	4.46 (0.28)	2.92×10^{-4} (2.18×10^{-4})	0.19 (0.03)	0.25 (0.03)	0.85 (0.04)	1,142
MG1655	8.04 (0.10)	1.35 (0.39)	5.09 (0.32)	3.82×10^{-4} (2.41×10^{-4})	0.11 (0.02)	0.24 (0.03)	0.83 (0.04)	7,93
OP50	11.14 (0.09)	0.59 (0.07)	8.62 (0.78)	1.30×10^{-3} (4.29×10^{-4})	0.17 (0.03)	0.19 (0.04)	0.73 (0.07)	1,411
IAI1[†]	11.42 (0.14)	0.69 (0.10)	8.99 (0.55)	5.17×10^{-4} (2.48×10^{-4})	0.16 (0.03)	0.21 (0.04)	0.74 (0.06)	481
OP50[†]	11.46 (0.09)	0.66 (0.04)	9.74 (0.19)	4.08×10^{-4} (1.26×10^{-4})	0.21 (0.02)	0.25 (0.02)	0.69 (0.03)	945

Mean survival, the fitted parameters, and derived values are each shown in a separate column. Rows contain the different experimental conditions (bacterial food strains). Standard errors are given in parentheses, as calculated directly by the statistical software for mean survival, or estimated from the standard deviation of the bootstrap distribution for the model-specific values

b_1, the rate of mortality increase before deceleration (exponential slope before the kink); t_{kink}, the onset of mortality deceleration (time-point of kink); a_1, the initial mortality (intercept); b_2, the rate of mortality increase after deceleration (exponential slope after the kink); m_{kink}, the mortality at the kink as estimated from the fitted model; S_{kink}, the surviving part of the population at the kink as estimated from the fitted model; N, number of nematodes in each experiment

[†] UV-killed bacterial food was used throughout life of the nematode

while the mortality rate at the kink (m_{kink}) changed more than threefold between OP50 (0.18 day^{-1} on day 9) and IAI1 (0.57 day^{-1} on day 4) (Fig. 1a; Table 1).

Available quantity of bacteria and *C. elegans* survival

To control for potential effects of food quantity on *C. elegans* survival, bacteria were enumerated in lawns of F11, OP50, which is uracil auxotroph ($Uracil^-$), and an OP50 $Uracil^+$ mutant. Under identical growth conditions where uracil is limiting, OP50 $Uracil^+$ strain provided the same amount of bacteria as the F11 strain and produced twofold more biomass than OP50 $Uracil^-$ (Table 2). Bacterial densities were then compared to the survival of nematodes on these three strains. F11 shortens life span by more than 50% compared to OP50 $Uracil^+$ despite an identical biomass. There was no significant difference between life span of nematodes fed on OP50 $Uracil^+$ and on OP50 $Uracil^-$ (Table 2). Therefore, the available amount of bacterial food cannot account for the differences in nematode survival.

Modeling of mortality deceleration as a consequence of frailty heterogeneity

The timing of the kink in mortality curves varied between different food sources (Figs. 1a, S2; Table 1).

Table 2 Colony forming unit (CFU) counts in bacterial lawns and corresponding life spans of the host

Food strain	Mean life span (SEM) (Days)	CFU count (SEM)
F11	5.56 (0.05)	17.48×10^9 (0.41×10^9)
OP50 wt ($Uracil^-$)	11.14 (0.09)	8.12×10^9 (0.74×10^9)
OP50 $Uracil^+$	10.10 (0.18)	17.60×10^9 (1.07×10^9)

The mean life span was calculated as the average time until death for all nematodes fed on the respective bacterial strain (censored observations were excluded). Standard error of the mean (SEM) is given in parentheses. The amount of live bacteria in the food lawn was determined as follows: bacterial lawns were prepared as in (Hope and Hames 1999), by dripping 25 µl of bacterial culture on NGM agar plates and incubating overnight at 37°C. The entire bacterial lawn was then re-suspended in 10^{-2} M $MgSO_4$ and the solution plated in appropriate dilutions

A possible explanation of mortality deceleration is frailty heterogeneity (Beard et al. 1959; Vaupel et al. 1979), i.e., variability of the lifelong individual tendency to die resulting in a reduced mortality rate increase at older ages (Fig. 2). In order to understand the observed variations of the mortality deceleration patterns, we investigated by modeling whether the observed variations of the kink could be explained in the framework of frailty theory. Mathematically, frailty is a lifelong multiplicative effect on mortality

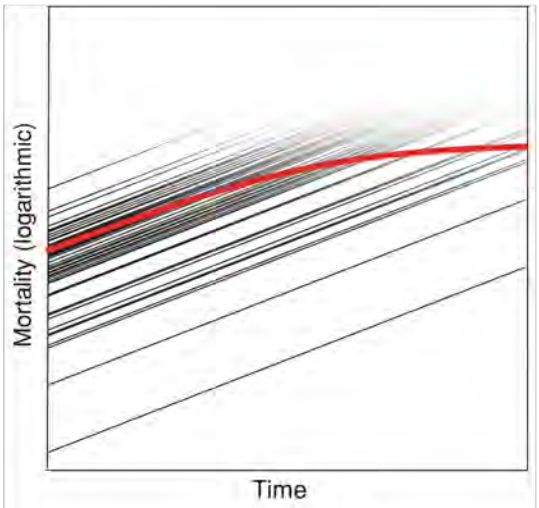

Fig. 2 Illustration of frailty heterogeneity. Heterogeneous frailty can lead to apparent mortality deceleration at the population level whilst individual mortalities do not level off at late ages. Individual frailties are stochastically distributed. In the case of a gamma distribution of frailties, as shown here, this effect exactly offsets the effect of individual aging, leading to a flat plateau. *Black/gray lines* individual mortality trajectories, *grey-level* the probability to still be alive. *Red line* apparent population mortality

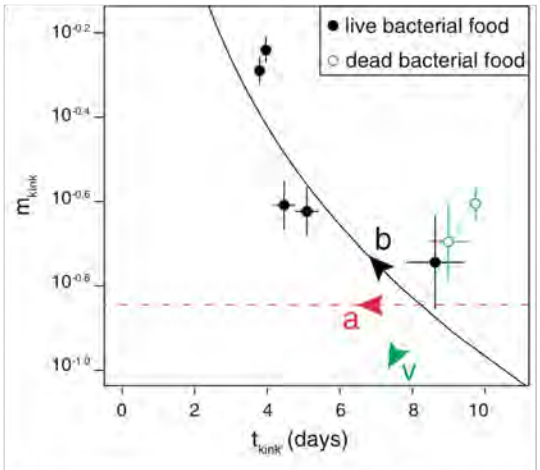

Fig. 3 Relationship between time point and mortality at the kink in survival curves. *Filled black circles* nematodes fed on different strains of live bacteria, *open green circles* nematodes fed on dead bacteria. *Values* and *error bars* are from Table 1. *Solid black line* trajectory of simulated kinks when varying only parameter *b* in a frailty heterogeneity model with Gompertz baseline mortality and power variance function frailty distribution. *Dotted green line* trajectory of simulated kinks when varying only parameter *v*, the variance of frailty distribution, in the same model. *Dashed red line* trajectory of simulated kinks when varying only parameter a in the same model. See Supporting text S3 for a derivation of the underlying equations

that differs between individuals (Beard et al. 1959; Vaupel et al. 1979). The observed deceleration of mortality at population level is caused by a process often referred to as "selective culling" (Steinsaltz and Wachter 2006). Essentially, more frail individuals die earlier than less frail ones, leaving the most robust part of the population to live longer (Fig. 2). In our multiplicative frailty model, individual mortality can be expressed as Zae^{bt}. Here, the relevant parameters are the average initial mortality a, the aging rate b, and the parameters describing the statistical distribution of frailties Z, of which the variance v is most important.

For various frailty distributions, variations of each of those parameters have specific consequences on the shape of the mortality curves. The goal of our analysis was to explain the observed pattern (Fig. 3) by the influence of the environment on the different parameters, after we had determined that the observed deceleration phenomena could not be accounted for by heterogeneity between experimental replicates (Supporting text S1). For the distribution of frailties, we modeled the case of Power Variance Functions (Supporting text S3). These include the gamma distribution, which is often used as a frailty distribution for mathematical convenience. We concentrated our analysis on the relationship between the time point of the onset of mortality deceleration (t_{kink}) and the mortality at that time point (m_{kink}, Supporting text S3). In our model, the change of the aging rate b explains to a large degree the position of the kink in the $m-t$ coordinate system for nematodes fed different strains of live bacteria (Fig. 3, Supporting text S3). Moreover, a decrease in inter-individual variability is predicted to shift the kink towards the upper right (increase in both t_{kink} and m_{kink}), as measured for nematodes fed on killed bacteria (Fig. 3).

Fertility of *C. elegans* fed on different bacteria

Another major life-history related trait, reproduction, may also be affected by the bacterial food strain. We therefore compared reproductive performance during

the 4-day long reproductive period by daily counting of hatched larvae for individual nematodes. *C. elegans* N2 were fed on *E. coli* OP50, IAI1, or F11 throughout life, including development. We found that the total number of hatched larvae did not differ significantly between different experimental conditions. Total brood size per nematode was (mean ± standard deviation) 146 ± 50 for OP50; 136 ± 46 for IAI1; 133 ± 58 for F11. However, the timing was significantly different (Fig. 4). During the first 24 h of adulthood, nematodes produced 32 and 27% more larvae in the presence of bacteria causing high mortality, respectively IAI1 and F11, than on the innocuous OP50 strain ($P < 10^{-4}$, two-tailed Students *t*-test with unequal variances). From day 1 to day 2, nematodes produced 46 and 38% fewer larvae when fed on IAI1 and F11, respectively, than on the innocuous OP50 ($P < 10^{-4}$). No nematodes died during reproductive period when fed on OP50, while 17 and 25% of nematodes were dead before the end of the reproductive period when fed on IAI1 and F11, respectively. Similar results were obtained in all experiments we performed using N2 nematodes fed the studied bacterial strains throughout life, including development. Therefore, nematode reproduction was shifted to earlier performance in presence of pathogenic bacteria, probably in order to maximize Darwinian fitness.

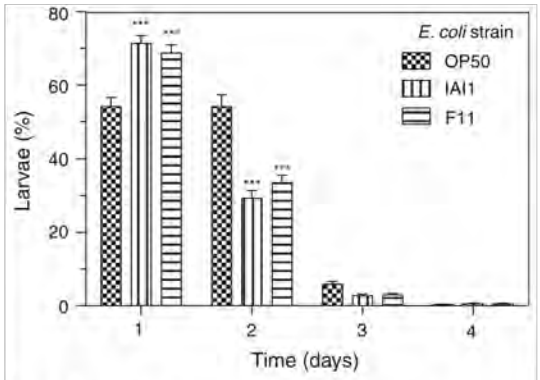

Fig. 4 Comparison of the reproductive performance between nematodes fed on different *E. coli* strains. Nematodes were fed on *E. coli* OP50, IAI1, or F11 throughout life, including development. Hatched larvae were counted daily for individual nematodes. Number of days corresponds to the time from the beginning of adulthood (end of L4 development phase). Data were collected for 69, 91, and 113 nematodes fed on OP50, IAI1, or F11, respectively. The total number of hatched larvae did not differ significantly between different experimental conditions. For every individual nematode, the percentage of hatched larvae per day out of the total number of the hatched larvae during reproductive period was calculated. The mean values for all nematodes/day/bacterial food strain ± standard errors, are presented. Data were obtained from two independent experiments, each with four replicates. The difference between the reproductive performance of the nematodes fed on OP50, and of the nematodes fed on IAI1 or F11 strains was significant on day 1 and on day 2 (***; two-tailed Students *t*-test with unequal variances: $P < 10^{-4}$)

Induction of H_2O_2 production in nematodes

It was reported that *C. elegans* challenged with *Enterococcus faecalis* produce H_2O_2, probably as a part of the antimicrobial response (Chavez et al. 2007). H_2O_2 production results in oxidative stress and in the accumulation of autofluorescence in the nematode intestinal tissues. As accumulation of the autofluorescent compounds is considered an universal biomarker of aging (Gerstbrein et al. 2005), a tempting hypothesis is that reactive oxygen species produced by *C. elegans* as part of the antimicrobial response may cause faster aging. This would be in accord with the free radical theory of aging, which postulates that aging results from the accumulation of damages caused by reactive oxygen species. We tested this possibility by measuring H_2O_2 production by the nematodes after exposure to OP50, IAI1, or F11 bacteria. H_2O_2 production was significantly higher in nematodes exposed to bacteria causing higher mortality rates, IAI1 and F11, than in those exposed to OP50 (Fig. 5, Mann−Whitney test: $P < 10^{-4}$). We also quantified accumulation of autofluorescence in nematodes exposed to OP50 or IAI1. The autofluorescence was 17% stronger in nematodes fed on IAI1 than on OP50 ($N = 40$ and $N = 41$, respectively, Mann–Whitney *U* test: $P < 0.05$). Therefore, faster accumulation of the autofluorescence in nematodes exposed to bacteria causing higher mortality may result from self-injury inflicted by the innate immune response. However, H_2O_2 acts also as a signaling molecule in many cellular processes like growth, development, and proliferation (de Magalhaes and Church 2006; Giorgio et al. 2007). Therefore, it cannot be excluded that H_2O_2 production signals for the modulation of nematode life-histories as a response to pathogenic bacteria.

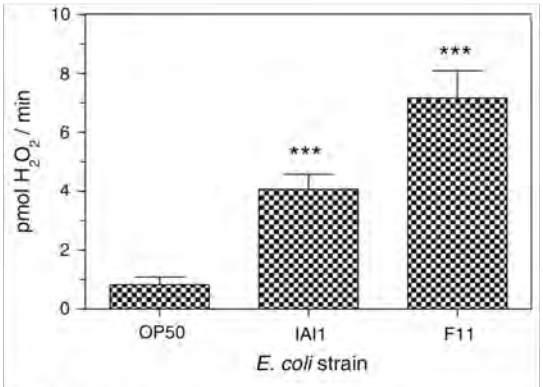

Fig. 5 Hydrogen peroxide production by *C. elegans* exposed to different *E. coli* strains. Averaged absorbance measurements from three replicates of each condition, with the no-nematode controls subtracted out. The amount of hydrogen peroxide produced per minute was calculated by comparison to a standard curve (data not shown). The mean values ± standard deviation, are presented. The difference between the amount of hydrogen peroxide produced by nematodes fed on IAI1 or F11 strains, and nematodes fed on *E. coli* OP50 was significant (***; Mann–Whitney two-tailed test: $P < 10^{-4}$). Repetitions of this experiment showed similar results

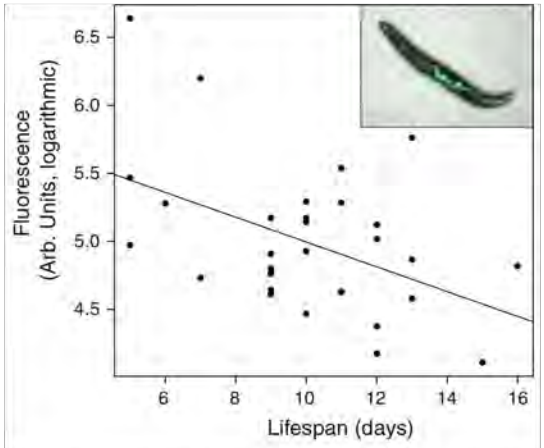

Fig. 6 Quantification of intestinal bacterial load. Correlation of individual life span of nematodes with the quantity of live intestinal bacteria measured early in life (day 3), as estimated by bacterial fluorescence intensity ($R^2 = 0.21$, $P < 10^{-2}$). *Inset* photography showing fluorescent bacteria in the nematode intestine. Bacterial fluorescence correlates very well with the number of colony forming units in nematode intestine (see Fig. S3)

Variability of intestinal bacterial loads and life expectancy

In order to elucidate the effect of bacteria on their host, it is essential to determine their fate after ingestion. The quantity of live bacteria in the nematode intestine is influenced by nematode pumping rate, grinder integrity, and efficiency of the nematode digestive system, and may therefore vary with the overall physiologic state and individual life span of the nematodes. Seeking to gather data amenable to a quantitative analysis, we constructed an OP50 mutant carrying the yellow fluorescent protein under the control of a strong and constitutive promoter on the chromosome (P_L-*lacO* in a Δ*lacI* background, see Table S1 (Elowitz et al. 2002; Lutz and Bujard 1997)). This tool enabled us to determine the quantity of living bacteria within the nematode intestine in vivo by fluorescence microscopy as the fluorescent signal corresponds well to the amount of living bacteria ($R^2 = 0.78$, $P < 10^{-8}$; Fig. S3). We measured fluorescence on day 3 (Fig. 6) because we had determined previously that variability was largest on that day (data not shown), and subsequently measured individual life span. The amount of living bacteria in nematodes intestine indeed negatively correlated with subsequent survival ($R^2 = 0.21$, $P < 10^{-2}$).

Discussion

Many studies comparing natural populations or artificial evolution experiments have demonstrated how genetic differences translate into differing aging schedules and therefore yielded deep insights into the evolutionary mechanisms of aging (Finch and Ruvkun 2001; Hughes and Reynolds 2005). Yet, adaptive phenotypic plasticity, i.e., the capacity to change the phenotype in response to changes in the environment without alteration of the genotype (Pigliucci 2001), also plays a role in tuning aging rates (Pletcher et al. 2007). Modulation of the aging trajectories by food availability and temperature is a well-documented phenomenon (Partridge et al. 2005). For example, dietary restriction increases life span of *C. elegans* by lowering the slope of the mortality increase with age (Lenaerts et al. 2007). The question is whether another very common environmental factor, parasites, can also modulate host aging rates. Alteration of longevity associated with the presence of parasites has been observed in several studies, e.g., for

Biomphalaria glabrata snails (Blair and Webster 2006). However, the modulation of hosts' aging rates was not investigated.

Modulation of *C. elegans* life-history traits

We tested whether parasites causing high extrinsic mortality trigger an inducible acceleration of the host aging using genetically identical *C. elegans* feeding on different *E. coli* strains. We found that genetically identical *C. elegans* show up to fourfold higher rates of mortality increase (slope b_1) in presence of *E. coli* bacteria causing high mortality relative to the innocuous OP50 strain (Table 1). The most pronounced acceleration was caused by a human opportunistic pathogen, followed by two human commensal isolates, and a common laboratory strain, while the standard food strain, OP50, or killed bacteria led to the slowest rate of increase.

In our study, the observed modulations of mortality rates could be due to the food quantity or quality. However, three pieces of evidence strongly suggest that a dietary-related mechanism is a very unlikely cause for the observed variation of mortality rates. First, overall number of larvae laid by N2 nematodes fed on OP50, IAI1, or F11 did not differ significantly, whereas the estimated aging rate on IAI1 and F11 is several-fold faster than on OP50 (Figs. 4, S2). This is not expected for dietary restricted animals as it has been shown that slower aging resulting from the dietary restriction in nematodes is associated with low brood size (Bishop and Guarente 2007; Klass 1977). Second, we showed that there is no effect of available quantity of live bacterial food on nematode survival (Table 2). The third piece of evidence stems from nematodes grown on killed bacteria. Food quantity-related difference and most food quality-related differences between bacterial strains are not altered by killing the bacteria. We therefore compared survival of nematodes grown on UV-killed bacteria of the strains OP50 and IAI1, and found that survival is not significantly different between nematodes grown on dead OP50 and dead IAI1. Survival studies on killed bacteria bear the implication that bacterial viability is an essential factor of the effect on the mortality rate.

Observation that only live bacteria modulate nematode mortality rates raises another question: whether the increased mortality is caused by toxicity/damage due to the pathogenic bacteria and/or as a consequence of a nematode response to extrinsic stress? These two hypotheses cannot be distinguished by analyzing survival data alone. Therefore, we studied another major life-history trait, reproductive output, by comparing fertility between N2 nematodes fed on OP50, IAI1, and F11. Although total number of produced larvae did not differ, we found that the schedule was significantly altered. Nematodes fed on bacteria causing higher mortality, IAI1 and F11, produced significantly more larvae during early reproductive period relative to nematodes fed on OP50 (Fig. 4). Identical patterns were obtained by counting oocytes laid by *fer-15* mutant nematodes fed on different bacterial strains (data not shown). No nematodes died during reproductive period when fed on OP50, while they started dying before the end of the reproductive period when fed on IAI1 and F11. Therefore, our data indicate that nematodes actively respond to pathogens by modifying reproductive schedule in order to maximize Darwinian fitness. Similar inducible host life-history changes have been reported in other animal systems, for instance *B. glabrata* snails that were exposed to a pathogen, but not infected by it, showed accelerated reproduction (Minchella 1985).

A second argument in favor of the theory that different mortality rates result from a response of the host, is based on the investigation of antimicrobial defense with potentially detrimental effects on the nematode. The generation of H_2O_2 has been proposed as a mechanism of immunological antimicrobial defense, analogous to the action of mammalian phagocytes (Chavez et al. 2007). Our measurements indicate that the production of H_2O_2 is greater in *C. elegans* feeding on IAI1 and F11, than on OP50 (Fig. 5). Apart from their role in immunity, reactive oxygen species are a well known cause of molecular damage central to many mechanistic theories of aging (Harman 1956). It is therefore plausible that nematodes show a higher mortality rate, at least partially, because they respond to pathogenic bacteria by producing H_2O_2, which results in faster accumulation of damage. This conjecture is corroborated by a report showing that bacterial pathogen infection of the nematode intestine causes protein damage, as a consequence of the H_2O_2 production (Mohri-Shiomi and Garsin 2008). Further confirmation comes from the related fact that accumulation of autofluorescent

pigments, an established biomarker of aging (Gerstbrein et al. 2005), is accelerated as a result of oxidative stress in nematodes (Chavez et al. 2007). We also observed faster accumulation of autofluorescence in nematodes fed pathogenic bacteria compared to innocuous ones. Therefore, faster accumulation of the autofluorescence we observed in nematodes exposed to bacteria causing higher mortality may result from self-injury inflicted by the innate immune response. Such trade-off between longevity and pathogen resistance was previously described for *D. melanogaster* (Libert et al. 2006). In this study, up-regulation of the immune response resulted in the reduction of *D. melanogaster* life span even in the absence of the pathogens. However, H_2O_2 has also been reported to act as a signaling molecule in many cellular processes like growth, development, and proliferation (de Magalhaes and Church 2006; Giorgio et al. 2007). For example, H_2O_2 was shown to enhance insulin receptor signaling, which is involved in modulation of longevity and stress resistance in many species. Hence, H_2O_2 production could be innate immune response to pathogens, but it could also be involved in triggering alternative life-history, e.g., the observed modulation of nematode reproductive schedule and aging rates. Both cases are a consequence of a nematode adaptive response to extrinsic stress.

Late-life mortality deceleration

We also observed that mortality increased at a slower rate in old nematodes, a phenomenon known as mortality deceleration. A consistent feature of the mortality data for nematodes fed different bacteria was that under conditions leading to a faster aging rate, mortality deceleration occurred earlier but at a higher level of mortality (Table 1; Fig. 3). Since its first description as downward deviation from the Gompertz law of exponential mortality increase in human populations (Gompertz 1825, 1872), the phenomenon of late-life mortality deceleration has been observed in many different species (Vaupel et al. 1998). Different underlying causes have been proposed, but their relative contribution are still subject to discussion (Carey and Tuljapurkar 2003; Steinsaltz and Wachter 2006). While data collected from various wild and artificially evolved populations have been analyzed thoroughly, (e.g., Charlesworth 2001; Drapeau et al. 2000; Mueller et al. 2003; Steinsaltz 2005; Vaupel et al. 1998, 1994), experimental approaches to modulate the shape of mortality profiles are scarce. Our experimental system provides a new opportunity to shed light on this subject. It allowed us for the first time to investigate late-life mortality deceleration in nematode populations aging at different rates without affecting other factors like temperature or genotype, that have pleiotropic effects (Johnson et al. 2001; Riddle et al. 1997).

Two main hypotheses explaining late life mortality deceleration have both received some evidential support. The first one explains mortality deceleration as an evolutionary result of the plateau in the force of natural selection after the end of reproduction, and therefore a feature of every individual mortality curve (Charlesworth 2001; Mueller and Rose 1996). The second explains mortality deceleration as a strictly demographic result of heterogeneity in life-long tendency to die (frailty) between individuals, whereas individual mortality does not level off (Fig. 2; Steinsaltz and Wachter 2006; Vaupel et al. 1979; Vaupel and Yashin 1985). Frailty theory is centered around lifelong heterogeneity that can be based on genetic as well as non-genetic differences between individuals (Vaupel et al. 1994). It is therefore pertinent to analyze variations between several different aging profiles shown by genetically identical animals.

The results of our mathematical modeling (see Supporting text S3) imply that if individual frailty is heterogeneous, then the variations in the aging rate cause the pattern of mortality deceleration as proposed Beard et al. (1959) and Vaupel et al. (1979). We adjusted only one feature of the model in order to incorporate the fact that, experimentally, mortality keeps increasing after the onset of late life deceleration instead of plateauing as in (Beard et al. 1959). Thus, we used a wider family of frailty distributions, the power variance functions, that encompass the traditionally used gamma distribution as a special case (Hougaard 1984, 2000). Of course, such consistence between a theoretical model and experimental data cannot be sufficient to reject all alternative explanations. It is nevertheless compelling that a model as basic as frailty theory still manages to catch the main features of our experimental data.

As heterogeneity between individuals is the centerpiece of frailty theory, we examined possible

variability between single nematodes. In a similar experiment, it had recently been reported that in vivo measurements of individual heat-shock response in an isogenic population of *C. elegans* could reveal hidden heterogeneity and thus predict life span (Rea et al. 2005; Wu et al. 2006). We sought a means to observe such heterogeneity under native conditions, without subjecting the animals to heat-shock or similar treatments. We considered the bacterial load in the intestine as a possibly meaningful marker, as it depends on pharyngeal pumping, expression of anti-bacterial effectors, defecation, and bacterial growth. Of these factors, the first three are known to vary with age (Huang et al. 2004; Kenyon et al. 1993) and thus probably also change with overall state of the nematode. We therefore tested whether the bacterial load in the nematode intestine was linked to subsequent survival by feeding the nematodes on the standard food strain OP50 carrying a strongly and constitutively expressed fluorescent construct (Fig. 6). The negative correlation we found indicates that the amount of live bacteria in the intestine of young nematodes could reflect robustness/frailty of the host and thus presents a reliable mean to demonstrate heterogeneity between individual *C. elegans*. It is possible that frailer individuals are infected by bacteria to a higher degree. This might amplify existing heterogeneity between nematodes and thus provoke more pronounced mortality deceleration. However, the observed mortality deceleration cannot be ascribed to heterogeneity caused by bacteria alone, since substantial mortality deceleration was also observed on dead bacteria (Table 1). As suggested by our model, variability between nematodes was similar on harmless and pathogenic bacteria, but slightly lower on dead bacteria (Supporting text S3; Fig. 3). This is plausible in view of the reported fact that variance of the life span distribution was smaller on dead bacteria than on living ones (Gems and Riddle 2000). We concluded that our results are consistent with frailty theory, and that frailty theory provides a useful framework to analyze the variations of the shapes of mortality curves in a systematic way.

Concluding remarks

Our study shows that in the presence of bacteria causing high mortality rates, nematodes reproduced earlier and produced more H_2O_2, which is probably a form of the innate immunity defense. Pathogens also cause faster nematodes' body deterioration with age exemplified by faster accumulation of autofluorescence and by the faster decline of nematode motility (unpublished data). As aging process is characterized by an increase in the likelihood of death and a decline in fertility with advancing age, our results taken together strongly suggest that the high rates of extrinsic mortality imposed by the pathogens could result in faster aging of nematodes. Such phenotypic plasticity in aging is probably an adaptive response to pathogens in order to maximize Darwinian fitness. Such scenario would fit into the dispensable soma theory, which explains aging as a result of the optimization of resources between maintenance and repair of its soma and the functions that maximize Darwinian fitness (Kirkwood 2005).

Experimental procedures

Bacteria and nematode strains

The *Caenorhabditis elegans fer-15* conditional sterile mutant, fertile at 15°C and sterile at 25°C, (kindly provided by J. J. Ewbank, CIML, Marseille, France) was used for all experiments, except for the measurements of fertility and H_2O_2 production for which wild-type N2 and *fer-15*/*spe-9* nematodes were used, respectively. The *Escherichia coli* bacterial isolates used in this study have been previously described in (Diard et al. 2007). The *uvrA*::Kan^R derivatives of OP50 and IAI1 as well as OP50 Δ*lacZ*::YFP-Cm^R were constructed as previously described (Datsenko and Wanner 2000; Table S1). The spontaneous OP50 Uracil$^+$ mutant was obtained by selection on minimal medium.

Nematode maintenance and survival assay

Nematodes were maintained at 25°C on nematode growth medium (NGM) agar plates for survival assays as previously described (Hope and Hames 1999). *E. coli* OP50, an uracil biosynthesis deficient mutant, was used as a standard food strain for *C. elegans* (Hope and Hames 1999). Nematodes were kept on OP50 bacteria during development (first 48 h between egg-stage and adulthood at 25°C), unless

otherwise indicated. For experiments on dead bacteria, nematodes were kept on dead OP50 bacteria during development. Dead bacteria were obtained by UV-irradiating (Gems and Riddle 2000) lawns of Δ*uvrA*::Kan^R strains (UV sensitive DNA repair mutants) grown on plates supplemented with Kanamycin (100 μg/ml). Dead nematodes were scored usually every 24 h, otherwise interval-censoring was used. Life span was measured as the time from the beginning of adulthood until death. A nematode was considered dead when it failed to respond to plate tapping or a gentle touch with a platinum wire. Nematodes that died as a result of getting stuck to the wall of the plate or handling were taken into account as right-censored observations.

Measurement of bacterial fluorescence/nematode autofluoresence

Fluorescence microscopy photographs were taken at 2.5× magnification on a Zeiss Axioplan 2 or Axiovert 200 M microscope equipped with a digital camera (Photometrics CoolSnap HQ, Evry, France). For measurement of endogenous autofluorescence, a CFP filter (Chroma Technology Corp., Rockingham, USA) was used. Image analysis was performed using ImageJ (version 1.38e) software. For in vivo measurements of fluorescence, the nematodes were transferred onto bacteria-free NGM agar (Hope and Hames 1999). Autofluorescence was measured on the second day of nematode adulthood. The quantity of live intestinal bacteria was measured on day 3 of the nematode adulthood because we previously established that the variability was largest on that day (data not shown). On day 3, nematode size was not found to be significantly different between nematodes grown on OP50 or IAI1 ($P = 0.7$, two-tailed Students *t*-test with unequal variances, $N = 43$ and 44, respectively).

Comparison of the reproductive performance

For measurement of reproductive performance, *C. elegans* N2 were fed on *E. coli* OP50, IAI1, or F11 throughout life, including development. Nematodes were maintained individually in 12-well plates and examined every 24 h using a dissecting microscope (Huang et al. 2004). Self-fertile reproduction was assessed by counting the number of hatched larvae in each well after an additional 24 h incubation of laid eggs. Nematodes that formed bag or were dead before the end of reproductive span were excluded from this analysis.

H_2O_2 measurements assay

Caenorhabditis elegans fer-15/spe-9 mutants were used for this assay in order to avoid hatching of any progeny during measurements. Nematodes were exposed for 72 h to bacterial strain, from hatching to first day of adulthood, at 25°C. H_2O_2 measurements were made using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Molecular Probes, Invitrogen) as previously described (Chavez et al. 2007) with initial washes performed in M9 and correction for nematode density based on measurements at 600 nm. OP50, IAI1, or F11 bacteria do not produce H_2O_2 in exponential nor in stationary phase cultures. The H_2O_2 production by bacteria was tested in vitro using c.a. 10^8 CFUs of OP50, IAI1, or F11.

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RÉSUMÉ

Les différences d'espérances de vie entre espèces sont principalement d'origines génétiques. En revanche, à l'échelle d'une espèce, les conditions environnementales peuvent influencer significativement la longévité des individus. Lorsque des animaux sont élevés dans des environnements contrôlés et protégés ils meurent principalement de causes liées au vieillissement. Il est alors frappant, que la variabilité des espérances de vie est la même au sein de populations isogéniques qu'au sein de populations non isogéniques. Ces résultats démontrent que les facteurs génétiques seuls ne suffisent à expliquer la mort et le taux de vieillissement des individus. Afin de mieux comprendre quels sont les phénomènes à l'échelle cellulaire pouvant expliquer le vieillissement prématuré d'individus génétiquement identiques, nous avons choisi de travailler sur des populations clonales de *Caenorhabditis elegans* élevées dans un environnement contrôlé et protégé.

Comme il a été montré chez C. elegans que les performances locomotrices pouvaient être utilisées comme un biomarqueur du vieillissement, nous avons développé une méthode permettant de quantifier différentes variables associées à la locomotion. En tirant parti de la capacité des nématodes à s'orienter puis à se déplacer lorsqu'ils sont placés dans un champ électrique, nous avons pu isoler des populations de nématodes ayant des vitesses de déplacement différentes.

Nous avons ensuite montré l'efficacité de cette méthode pour séparer à l'intérieur d'une population isogénique dans un même environnement des nématodes ayant une courte espérance de vie de ceux ayant une longue espérance de vie, et ce dès le début de la phase post-reproductive. Nous avons alors pu mettre en évidence que le niveau de carbonylation du protéome était significativement plus élevé chez les individus ayant une espérance de vie courte.