Experimental evolution of Caenorhabditis elegans

Mravak, Antonija

Master's thesis / Diplomski rad

2016

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Split, University of Split, Faculty of science / Sveučilište u Splitu, Prirodoslovno-matematički fakultet**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:166:617128

Sveučilište u Splitu
Prirodoslovno-matematički fakultet
Odjel za fiziku

Antonija Mravak

EXPERIMENTAL EVOLUTION OF *CAENORHABDITIS ELEGANS*

Diplomski rad

Mentorica: prof. dr. sc. Jasna Puizina

Split, 2016.

Ovaj rad, izrađen u Splitu na Mediteranskom institutu za istraživanje života (MedILSu), pod mentorstvom prof. dr. sc. Jasne Puizine te vodstvom dr. sc. François-Xaviera Pellaya te dr. sc. Juraja Simunića, predan je na ocjenu Odjelu za fiziku Prirodoslovno-matematičkog fakulteta Sveučilišta u Splitu radi stjecanja zvanja magistre (mag.) fizike, smjera biofizike.

TEMELJNA DOKUMENTACIJSKA KARTICA

Sveučilište u Splitu
Prirodoslovno-matematički fakultet
Odjel za fiziku

Diplomski rad

EKSPERIMENTALNA EVOLUCIJA *CAENORHABDITIS ELEGANS*

Antonija Mravak
Teslina 12, 21 000 Split

Sažetak

Više eksperimentalnih istraživanja na različitim modelnim organizmima dokazuje da se dugovječnost može povezati s otpornošću na stres. To upućuje da bolje nošenje s okolišnim izazovima igra ulogu u regulaciji životnog vijeka. Postizanje otpornosti na stres kroz evoluciju moglo bi pomoći u rasvjetljavanju teorije starenja. U svrhu ispitivanja te hipoteze, proveli smo eksperimentalnu evoluciju na vrsti *Caenorhabditis elegans*. Dvije populacije crva bile su tijekom njihova razvoja i života podvrgnute uvjetima povišene temperature i UV zračenja kroz više generacija. Među njima pojavila se jedna populacija otporna na visoku temperaturu i jedna na UV zračenje. Te su populacije zatim bile podvrgnute testovima podnošenja visoke temperature ('heat stress assay') i duljine životnog vijeka ('lifespan assay'). Crvi koji su dobro podnosili višu temperaturu, pokazali su značajno bolji odgovor na toplinski stres u usporedbi s divljim tipom. Ta otpornost pojavila se i nekoliko generacija nakon izlaganja nižoj neletalnoj temperaturi, stoga vjerojatno nije posljedica hormeze. S obzirom da je eksperimentalna evolucija provedena na relativno malom broju generacija, životni vijek tih crva nije se znatnije razlikovao od divljeg tipa. Crvi izloženi UV zračenju nisu pokazali značajnu razliku na oba testa, što može značiti da je otpornost na UV zračenje teško postići. Omjer smrtnog rizika kao i opažanja njihovog stanja upućuju na to da je izloženost UV radijaciji možda uzrokovala oštećenja, a ne razvoj otpornosti. Obe eksperimentalne populacije možda tek trebaju proći kroz značajniju evolucijsku promjenu koja će dovesti do povećane otpornosti na stres i produženja njihovog životnog vijeka.

(28 stranica, 7 slika, 3 grafa, 4 tablice, 42 literaturna navoda, jezik izvornika: engleski)

Ključne riječi: dugovječnost, otpornost na stres, UV zračenje, toplinski stres.

Voditelji: prof. dr. sc. Jasna Puizina, dr. sc. François-Xavier Pellay, dr. sc. Juraj Simunić.

Ocjenitelji: prof. dr. sc. Jasna Puizina, prof. dr. sc. Paško Županović, dr. sc. Nada Ilić.

Rad prihvaćen: 22.1.2016.

BASIC DOCUMENTATION CARD

University of Split
Faculty of Science
Department of Physics

Graduation Thesis

EXPERIMENTAL EVOLUTION OF *CAENORHABDITIS ELEGANS*

Antonija Mravak
Teslina 12, 21 000 Split

Summary

Several experimental studies on different model organisms have shown that longevity can be associated with stress resistance. That fact suggests that better coping with environmental challenges plays a role in lifespan regulation. Obtaining stress resistance through an evolutionary process might help to enlighten aging theory. To examine this hypothesis, we performed experimental evolution experiment on *Caenorhabditis elegans*. During their development and life, two populations of worms were exposed to high temperature and separately to UV radiation for few generations. One population among them became resistant to high temperature and one to UV irradiation. Those populations were then analyzed with heat stress assay and lifespan assay. Worms that had better response to high temperature as an evolution constraint have shown a significantly better response to heat stress comparing to wild-type. This resistance appeared even several generations after pre-exposure to lower sublethal temperature, so is most unlikely to be the consequence of hormesis. Considering that experimental evolution was done on a relatively small number of generations, lifespan assay of those worms showed no significant difference from the wild type. For UV evolved worms there was no significant change in both assays, that might mean that resistance to UV is harder to acquire. Observation of their condition and hazard ratio indicate that irradiation might have caused damage instead of resistance. Both experimental populations probably have yet to reach the significant evolution change that would lead to increase of stress resistance and increase of their lifespan.

(28 pages, 7 figures, 3 graphs, 4 tables, 42 references, original in: english)

Key words: longevity, stress resistance, UV radiation, heat stress.

Supervisors: Prof. Dr. Jasna Puizina, Dr. François-Xavier Pellay, Dr. Juraj Simunić.

Reviewers: Prof. Dr. Jasna Puizina, Prof. Dr. Paško Županović, Dr. Nada Ilić.

Thesis accepted: 22.1.2016.

TABLE OF CONTENTS

1. INTRODUCTION

Coping with unfavorable environmental conditions is an important task for living beings. Through evolutionary processes, different organisms have found ways to deal with those challenges. Research has shown that in long-lived mutants, longevity is associated with stress resistance. To examine the impact of stress resistance acquired through experimental evolution on lifespan, *Caenorhabditis elegans* (*C. elegans*) was used as a model organism. Specifically, strains evolved under high temperature and separately UV light have been analyzed.

Firstly, a brief introduction on evolution will be given. The main characteristics of *C. elegans* together with experimental evolution research will be followed by the overview of the main aging theories. In materials and methods, details of the experimental design will be described. Finally, a conclusion will follow results and discussion.

1.1. Biological evolution

Evolution can be regarded as heritable successive changes in natural populations that lead to the diversity of biological systems. In the 19th century, Charles Darwin[1] and Alfred Russel Wallace proposed a theory of evolution by natural selection which was a breaking point in the scientific theory of evolution. It happens on the small and large scale. The first one is known as a microevolution and it describes heritable change on the genetic level within the population. Macroevolution, on the other hand, explains the evolution of the different species that arise from the same ancestor (Hall and Hallgrímsson, 2008).

Natural populations tend to evolve under different environmental conditions due to processes of selection, mutation and genetic drift. Genetic and phenotypic changes that are created in that way are of interest to evolutionary biology (Alcántara-Díaz *et al.*, 2004). Natural selection states that some individuals who are more adapted to environment have more offspring. In that way, desirable traits are inherited and lead to genetic change of the population. Selection is the result of both environment and genetic variation. Genetic drift happens by chance whereas natural selection does not (Hall and Hallgrímsson, 2008).

More recently, the role of epigenetics in evolution has been shown. Apparently, some traits arise from factors above DNA which can be passed through generations and selected for or

[1]In 1858 he wrote a book on that subject, "On the Origin of Species" (Hall and Hallgrímsson, 2008).

against, contributing in that way to evolution (Dickins and Rahman, 2012). Two main mechanisms behind epigenetics are DNA methylation and histone modifications, which affect expression of genes (Novak, 2004). The concept of acquiring certain characteristics during lifetime to deal with environmental challenges and passing those new characteristics on the progeny is not new. Jean-Baptiste Lamarck proposed it even before Darwinian theory (Hall and Hallgrímsson, 2008).

1.2. Experimental evolution

Experimental evolution is an important method for research of evolutionary mechanisms that occur under imposed laboratory conditions. Research consists of quantitative and qualitative descriptions of changes. So far, most of the experiments have been done on *Escherichia coli* (*E. coli*) (Lenski, 2011). But, the need for understanding more complex organisms has led to the use of new models. Although *C. elegans* has been used frequently as a model in molecular biology, its benefits in evolutionary biology are also being explored (Gray and Clutter, 2013).

C. elegans experimental evolution has contributed to the topics of coevolution, mating systems, population structure, effects of accumulated mutations and life history. Some of the particularly interesting themes are the pleiotropic cost of increased lifespan and the link between individual vigor and lifespan. Research has shown that there is a positive correlation between the latter two (Chen and Maklakov, 2012).

Generally, two types of manipulations are used in evolution experiments, genetic and environmental ones. The first one implies mutagenesis, genomically integrated transgenes and similar manipulations. Experimental evolution through environmental pressure, which is investigated in this work, include different conditions imposed upon population like change of the temperature, irradiation, nutrients or reproduction timing (Gray and Clutter, 2013).

1.2.1. *C. elegans* as a model of experimental evolution

Some of the advantages of *C. elegans* as a model organism are its relatively short life cycle and the fact that it is isogenic, meaning that all individuals in the population are genetically identical. It is also very well studied and can be maintained at low costs (Gray and Clutter, 2013). Due to those traits, that organism is also suitable for aging research[2]. Because of the

[2]More details on the traits of *C. elegans* will be given in Materials and methods.

conditions that are predominantly unfavorable, in its natural habitat *C. elegans* is mostly found in special so-called dauer stage. Interestingly, it has evolved so it can survive unfavorable conditions for months and during favorable ones it develops and reproduces fast before next difficult period (Félix and Braendle, 2010).

Considering that *C. elegans* body temperature depends on the temperature of the environment, its lifespan varies considerably with temperature. Thus, it is adapted to live and function in the temperature range of 15-25°C that is close to the one of their habitat. But, it is still not entirely clear how do they cope with different temperatures.

1.2.2. Heat stress

Although worms can survive short periods of exposure to heat stress, everything above 25°C is considered to be a stressful condition. As reported by several research groups, second generation of *C. elegans* at 27°C becomes sterile (Begasse *et al.*, 2015). Thus, there is a local extinction at such condition. In addition, lifespan differs at different temperatures. It is shorter at higher temperature in their optimal range (Begasse et al., 2015).

Longevity of *C. elegans* is associated with increased thermotolerance. Experiments have been performed by examining *C. elegans* mutants with longer lifespan. Heat stress assay of those mutants has shown significantly better heat resistance comparing to the wild-type (Lithgow *et al.*, 1995).

Paradoxically, even if *C. elegans* lifespan decreases with temperature, worms that were shortly pre-exposed to sublethal heat stress show significantly longer lifespan than without pre-exposure (Lithgow *et al.*, 1995). That phenomena is known as hormesis. Heat stress is associated with overexpression of genes that encode molecular chaperones which extend lifespan and enhance stress resistance (Tatar *et al.*, 1997; Walker and Lithgow, 2003).

Interestingly, worms that were exposed to high temperatures like 28°C tend to recover and produce progeny after they have been returned to 20°C. Moreover, from the test on *C. elegans* at 28, 29 and 31°C and recovery at 20°C, worms that were at 31°C recovered better than the ones at 29°C (Aprison and Ruvinsky, 2014).

1.2.3. UV radiation

Ultraviolet radiation is a small part of electromagnetic spectrum of the solar radiation. Wavelengths of UV are shorter than wavelengths of the visible light and larger than the X-rays. Three subtypes of UV have been introduced with respect to the wavelength; UVA with range 315-400 nm, UVB with 280-315 nm and UVC with 100-280 nm (Diffey, 2002). Only 5% of the total UV radiation that reaches Earth is UVB and the rest of it is UVA. UVC is absorbed in the stratosphere and does not reach the biosphere. The UV light that reaches the Earth's surface with biggest energy is UVB but is mostly screened out by ozone which is changing due to increasing depletion of the ozone layer. Consequences of UV irradiance on humans, plants, animals and the whole ecosystem are numerous. UV light plays role in production of vitamin D, vision of vertebrates and invertebrates, formation of pigments in plants. On the other hand, UV light energy is also cause of the production of ROS and DNA damage leading to skin cancer, eyes and tissues damages (Yagura *et al.*, 2011).

UV radiation leads to mutagenesis and cellular death primarily through DNA lesions and ROS production. Induction of DNA lesions depends on wavelength of UV radiation but also on the composition of DNA. It has been shown that the relative absorption which was obtained from the data from different species depends on the guanine-cytosine content (Sutherland and Griffin, 1981). Since the maximum of absorption of DNA is around 260 nm, UVC most effectively induces DNA photoproducts. Those represent DNA damages that are formed by dimerization of the neighboring pyrimidines. The main products are cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproduct. In addition, there is an indirect DNA damage from UVA and UVB that arises from reactive oxygen species (ROS). DNA lesions formed because of ROS can be recognized as a formation of dihydro-8-oxoguanine and they are considered to be a premutagenic lesion (Figure 1).

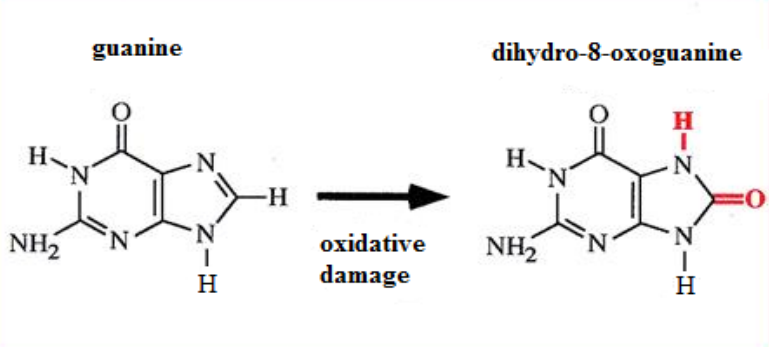


Figure 1. Illustration of formation of dihydro-8-oxoguanine (Nakabeppu, 2014).

To deal with DNA damages, during evolution cells have developed DNA repair mechanism. Processes of mutation and selection have led to increase resistance of bacteria and other microorganisms to UV light (Yagura *et al.*, 2011).

Considering that UVA light has the smallest energy in the UV range, its deleterious effects on DNA are not as damaging as of UVB and UVC. Nonetheless, UVA has the most penetrating power of all UVs and as such has great influence on organisms. In particular, it was shown that UVA mostly induces oxidative damage to lipids and proteins which is believed to lead to both photoaging and cancer in humans skin (Vile and Tyrrell, 1995).

When it comes to researches of UV influence on *C. elegans*, most of them focused on the effect of UVC for practical reasons. Furthermore, experiments of directed evolution with irradiation have been performed mostly on bacterias. The key model for research of radiation resistance is *Deinococcus radiodurans* (*D. radiodurans*). It has been demonstrated that their resistance to ionizing radiation is linked to desiccation resistance as a part of evolutionary adjustment to environment. Desiccation resistance requires DNA repair ability and protein protection against oxidative stress and in turn leads to radiation resistance (Mattimore and Battista, 1996).

The predominance of UVA in ecosystems and their major role in light induced oxidative stress are the reasons why it is taken into account in our experiments. *C. elegans* lives in soil and is not constantly exposed to UV irradiation and other sources of genotoxic chemicals might be in their environment or food. Thus, it is not known whether the main source of DNA damage for them is UV irradiation (Kiontke and Sudhaus, 2006). Research of early exposure of *C. elegans* to UV has shown that it leads to mitochondrial dysfunction. Younger developmental stages are less resistant to UV radiation comparing to adults. That might be connected with the fact that an adult *C. elegans* is protected by a thicker cuticle. Also, there is a shielding effect which explains why do multicellular organisms stand higher doses of irradiation comparing to mammalian organisms samples in monolayer culture (Lans and Vermeulen, 2011).

1.3. Theories of aging

Senescence (aging) is a deterioration of fitness and function of an organism with age. Arguably, it is a common feature of almost all biological kingdoms, but its cause and even definition are still largely debated (Hughes and Reynolds, 2005). Considering that we are

tackling in this project the mechanistic cause of aging through experimental evolution, aging will be introduced from perspective of both evolutionary and mechanistic theories.

The core idea of evolutionary theories is that there is a decrease of natural selection that comes with age due to extrinsic mortality which represents non-age related deaths. So, genes that act on reproduction or survival early in life are of bigger importance for Darwinian fitness than the ones that act similarly but are expressed later (Hughes and Reynolds, 2005).

Mutation accumulation as a part of evolutionary theory describes the contribution of genes that have dominantly damaging effect. When accumulated, those damages are lethal and cause aging. Also, deleterious genes can be dispersed through genome and individually might not particularly influence late life. Natural selection is supposed to be more effective when it comes to genes that are strongly detrimental (Hughes and Reynolds, 2005).

Antagonistic pleiotropy states that some genes give rise to two or more life traits between which there is a trade-off. In that way, some mutations might benefit an organism in early age and be detrimental late in life. Those mutations will be selected over mutations that work conversely (Hughes and Reynolds, 2005; Zhou *et al.*, 2011). An example is an experiment that revealed antagonistic pleiotropy in *C. elegans* in timing of reproduction but not the lifespan. Worms have been selected for early fecundity over more than 40 generations. Thus, it was expected to get a decrease of late fecundity and longevity. The hypothesis was confirmed when it comes to fecundity. Clearly, there was a trade-off between early and late reproduction. Contrary to expectation, longevity was not confirmed as a trait involved in antagonistic pleiotropy. That has led to conclusion that longevity and early fecundity can be uncoupled (Anderson *et al.*, 2011).

In addition to other evolutionary theories of aging, there is the disposable soma theory, often considered to be a special case of the antagonistic pleiotropy theory. It is based on the assumption that somatic maintenance requires energy expenditure and consequently metabolic cost that is necessary for reproduction. Therefore, there is selective benefit for genes that enhance resources used for development or reproduction. Substantial somatic damage that piles up over the time causes death. Also, the disposable soma theory explains longevity induced by dietary restriction. Stressful conditions shift allocation of resources to somatic maintenance so that organisms survive until the favorable conditions prevail and organisms can reproduce normally. Complete removal of food in experiment with *C. elegans* has given rise to an increase

in lifespan even higher than when the food was partly reduced (Hughes and Reynolds, 2005; Zhou *et al.*, 2011; Kaeberlein *et al.*, 2006). This experiment also illustrates to which extent *C. elegans* is a very special model, able to not only survive but even live longer without food, due to the fact that in adult stage, it is a post-mitotic organism, meaning that its somatic cells do not divide.

When it comes to mechanistic theories, there is also many different explanations for senescence. Oxidative stress resistance will be mentioned in particular.

Reactive oxygen species (ROS) contribute to aging by causing damage of proteins, lipids, DNA and consequently cellular damage. It is noteworthy that ROS, though dangerous in large extent, has a role in normal functioning of an organism (Figure 2). In the case of absence or too few of ROS, some physiological functions like host defense might become impaired (Finkel and Holbrook, 2000).

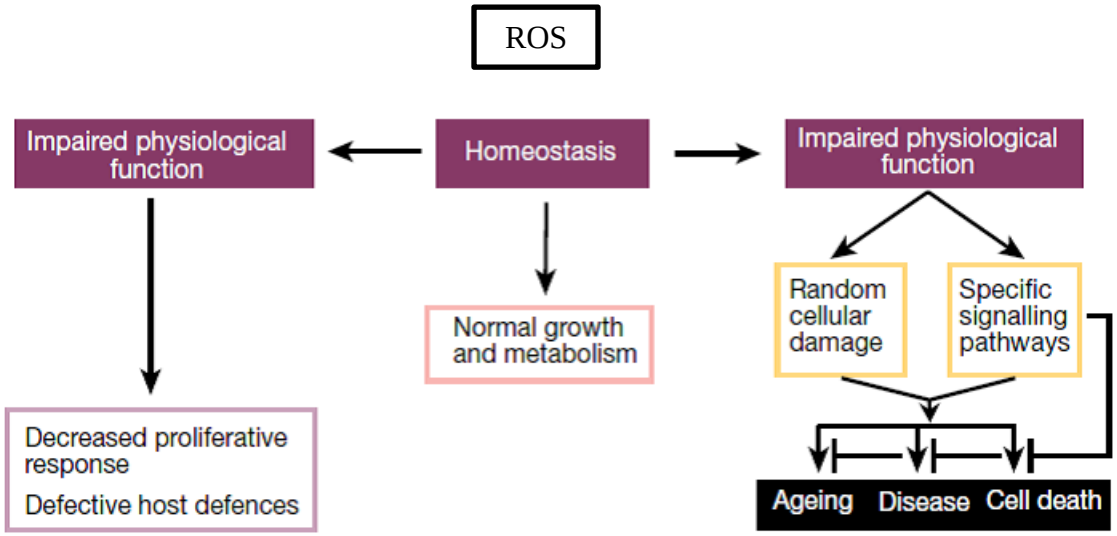


Figure 2. Three possible states of an organisms depending on the ROS concentration. If there is not enough or too many ROS, the state is not in homeostasis and impaired physiological function occurs (Finkel and Holbrook, 2000).

Endogenous ROS are mostly produced in mitochondria because most of the oxygen is used there for respiration and production of ATP. Superoxide radicals are byproducts of electron transport chain in mitochondria. Therefore, the rate of production of ROS is connected to the rate of oxidative metabolism which contributes to senscence. Enzymes such as superoxide dismutase

(SOD), catalase, glutathione peroxidase are used as a protection against ROS and are known as antioxidants. Among exogenous sources of ROS is UV light.

All oxidative damage cannot be completely eliminated, so it might accumulate over time causing even bigger damage (Zhou *et al.*, 2011; Gems and Doonan, 2008). In *C. elegans*, aging is also followed by accumulated molecular damage but it is still not clear why that accumulation occurs. Also, it is unknown to what extent ROS causes that damage (Gems and Doonan, 2008). Oxidative stress resistance has shown to be connected to increased lifespan (Lithgow *et al*. 1995).

Obviously, there is no solely one explanation for senescence and which factor is the most influential is yet to be explored.

1.3.1. Genetic regulation of lifespan

It has been shown that resistance to oxidative and heat stress increases the lifespan (Lithgow *et al.*, 1995). So, there is a positive correlation between UV and heat resistance and longevity of *C. elegans*. That leads to the conclusion that there might be a mechanism that regulates both lifespan and stress resistance.

Lifespan of *C. elegans* varies under different conditions. Mutations in several genes has been shown to strongly influence longevity. As an example, *age*-1 and *daf*-2 mutants have significantly increased lifespan. Additionaly, mechanisms that regulate the dauer stage that worms enter in unfavorable conditions, also influence longevity. That stage is regulated with insulin/insulin-like growth factor signaling (IIS) pathway. It includes DAF-2 (transmembrane protein), DAF-16 (transcription factor) and few intracellular kinases. Inactivation of IIS causes extension of lifespan but also resistance to biotic and abiotic stress. By inactivation of IIS, worms exit dauer stage and continue developing normally (Lithgow *et al.*, 1995; Kaletsky and Murphy, 2010; Amrit *et al.*, 2010). The regulation of that pathway is included into mechanistic theories of aging.

2. AIM OF THE THESIS

Research has shown that there is a coupling between stress resistance and aging mechanism. In particular, heat stress and UV stress resistance were shown to be associated with longevity. In this work we want to prove the causality within this association by showing that the acquisition of stress resistance through evolutionary process leads to longer lifespan. Based on this hypothesis, two outcomes of the experiment are expected.

- UVA and heat-evolved worms will be more resistant to stress comparing to wild-type worms.
- UVA and heat-evolved worms will have longer lifespan comparing to wild-type worms.

If both results are valid, this will confirm that longevity is partly shaped through acquisition of stress resistance.

3. MATERIALS AND METHODS

3.1. *Caenorhabditis elegans*

Caenorhabditis elegans (lat. *caeno*-recent, *rhabditis*-rod, *elegans*-elegant) is a model organism that was introduced as such by Sydney Brenner in 1974. It is a non-parasitic nematode that can be found in soil (Riddle *et al.*, 1997).

The worms body consists of a tube covered with a cuticle. It is divided into two tubes inside of which are the pharynx, the reproductive system and the gut (Figure 3). Their body is transparent so the cell lineage can easily be seen (Sulston and Horvitz, 1977).

An adult worm has 959 somatic cells that do not divide in an adult, 302 of them are neurons. The main part of its nervous system is circumpharyngeal nerve ring[3]. Sens organs are in head, so *C. elegans* can taste, smell, touch and respond to heat. Considering that they do not have eyes, their reaction to light is poor. They have four muscle bands whose flexing and relaxing create dorso-ventral stimulus and consequently movement (Kenyon, 1988; Riddle *et al.*, 1997).

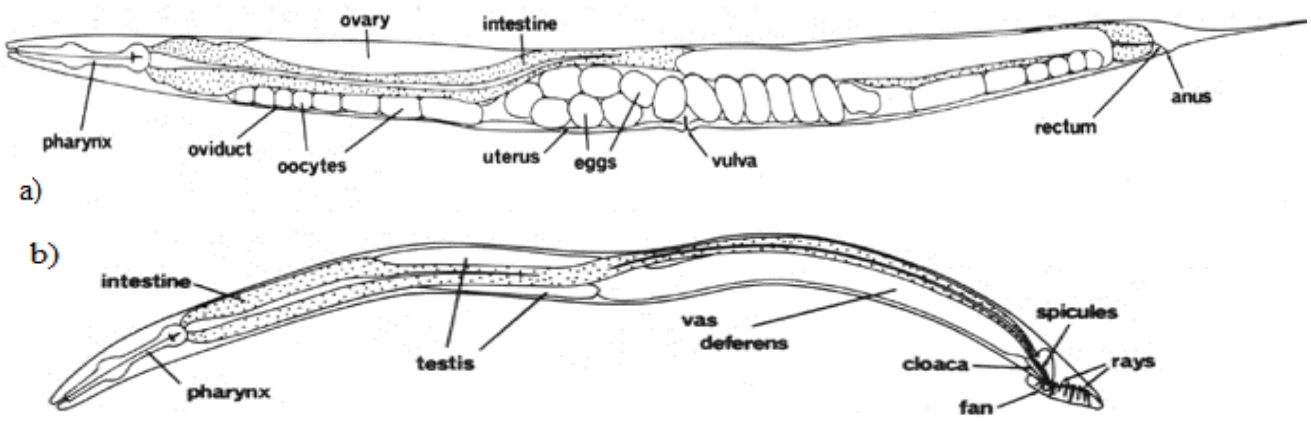


Figure 3. Main parts of the a) hermaphrodite and b) male worm (Sulston and Horvitz, 1977).

Some of the advantages of *C. elegans* as a model organism is a short life cycle (3 days), a size which is around 1 mm for an adult and easy obtaining big populations. They have five pairs of autosomes and one pair of sex chromosomes. Their genome (100 million base pairs) was entirely sequenced in 1998. There are two different sexes, males and hermaphrodites. The latter can be fertilized by males or self-fertilized and they produce around 300 eggs. Their life cycle

[3]That is a structure of nerves and ganglia that surrounds the pharynx (Sulston and Horvitz, 1977).

consists of egg, four larval stages (L1-L4) and adulthood. Also, there is one special state that worms enter from L2 stage if they are in starvation (Figure 4). That stage is called the dauer stage in which worms can survive up to 6 months without eating (Riddle *et al*., 1997).

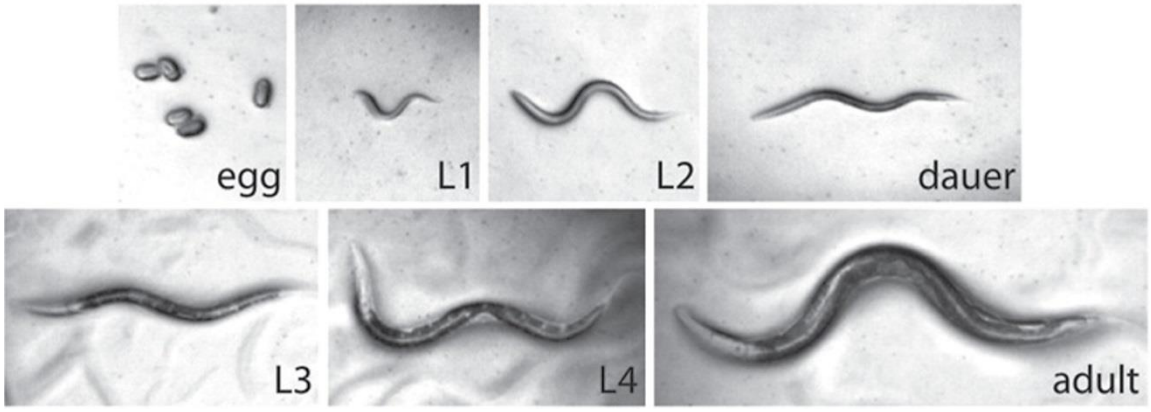


Figure 4. *C.elegans* phenotype according to developmental stages; from eggs to adults (Fielenbach and Antebi, 2008).

When the conditions become favorable again, they go straight to L4 stage and continue developing normally (Figure 5). Also, *C. elegans* can survive freezing over years in L1 diapause stage in which they can also survive starvation. An average lifespan of *C. elegans* is 2-3 weeks, it varies depending on the temperature of the environment. The temperature range at which *C. elegans* is viable is 15-25°C (Kenyon, 1988; Riddle *et al.*, 1997; Begasse *et al.*, 2015).

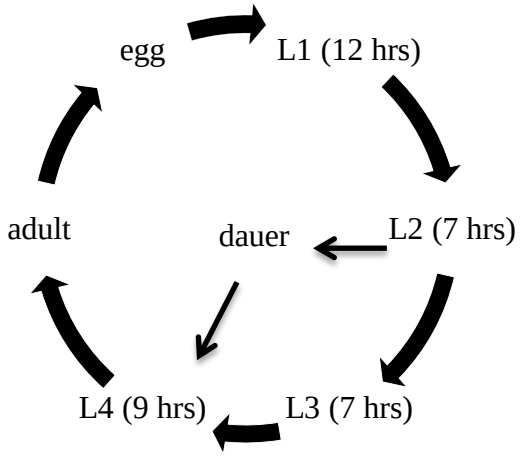


Figure 5. Life cycle of *C. elegans*. There are four stages, L1-L4 that precede the adult stage. Also, there is a dauer stage that worms enter when they are in unfavorable conditions. Time intervals that are written apply for the interchange of the stages at 25°C (made according to Riddle *et al*., 1997).

Worms grow, reproduce and age. Considering the complexity of this animal, it is an excellent model, among other, for studying embriology, morphology, aging and evolution. The so-called N2[4] strain has been adopted as a reference wild-type strain. Other wild-types of *C. elegans* around the world might have different traits but they are still interfertile with the N2 (Riddle *et al.*, 1997). It is important to stress out that different *Caenorhabditis* species vary in length of the lifespan, temperature range to which they are adapted as well as other traits. For example, another *Caenorhabditis* species, *Caenorhabditis briggsae* (*C. briggsae*) is fertile at higher temperature comparing to *C. elegans* (it goes up to 30°C) and has a longer average lifespan (Begasse *et al.*, 2015).

3.2. Strain maintenance

N2 was used for experiments as a reference wild-type. Worms were grown at 20°C on nematode growth medium (NGM) and they were fed with *E. coli* OP-50 strain. Evolved strains were maintained on NGM or/and in liquid. The key steps in the maintenance of all three strains will be described.

3.2.1. Preparation of bacteria

The *E. coli* OP-50 was used as a food for worms. That strain is auxotroph so the growth of the bacteria on the plate is constrained. L-Broth (LB)[5] is a rich media used for bacterial growth (Stiernagle, 2006). Inoculation was performed aseptically by using the colony from the streaked plate and immersing it into LB. Inoculated cultures were left overnight on the shaker at 37°C. Prepared bacterias were stored on 4°C.

3.2.2. NGM plates

NGM medium[6] was prepared according to protocol and cooled to 55°C. Then, it was dispensed into petri plates under sterile conditions using a peristaltic pump. Around 20 ml, 10 ml and 5 ml of NGM was used for the preparation of 100 mm, 60 mm and 35 mm petri plates,

[4]Needed strain can be obtained from Caenorhabditis Genetics Center (CGC) that started in 1978 and it is supported by the National Institutes of Health-Office of Research Infrastructure Programs. It is placed at the University of Minnesota, Twin Cities. Our strain was also obtained from CGC.
[5]See appendix for preparation protocol.
[6]See appendix.

respectively. After cooling, NGM was solidified. Plates were left to completely dry for two-three days before use (Stiernagle, 2006).

3.2.3. Seeding plates

Using a pipette, needed amount of bacteria was applied in the middle of the plate (usually 50-100 μl depending on the size of the plate). A gentle circular movement of the plate allows spreading of bacteria that should not reach the edges. Worms mostly stay in the bacterial area and if they crawl to the edges, they dry out and die (Stiernagle, 2006). Plates were dried before use and afterward stored at room temperature.

3.2.4. Liquid medium

Worms can also be grown in a liquid medium which is used for bigger populations. In the experiment, liquid medium was used for worms that were exposed to higher temperature. It was consisted of S medium and bacteria. Also, antifungal drug[7] was added to prevent fungal contamination. When there was a need for antibiotic[8], different strain of *E. coli* was used (OP-51 which is resistant to it). It was important to ensure that there is enough oxygen when worms were maintained in described way.

3.2.5. Synchronization

Synchronization was performed via bleaching when assays were prepared or when contamination was noticed. Worms are ready for the bleaching when there are a lot of gravid adults and eggs. Washing of the plate was done with M9[9], worms were then transferred to the tube and put in the centrifuge. After removal of the supernatant, bleaching solution[10] was added. It is used for killing and dissolving worms, but it does not damage eggs unless it is left to work longer than few minutes (typically 5-7 minutes). Centrifugation lets the eggs to sediment and the following step is washing the eggs in M9 so that the rest of the bleaching solution is completely removed. The final step is to leave the eggs with few milliliters of M9 in the tube overnight at

[7]Amphotericin B was used (400 μl on 1l of solution).
[8]Streptomycin was used (1 ml on 1l of solution).
[9]See appendix.
[10]See appendix.

20°C. Eggs hatch and the next day worms in the L1 stage can be put on plates with bacteria to grow (Sulston *et* Hodgkin, 1988).

3.3. Evolution of strains

Two different experimental designs were prepared, one for the worms that were exposed to heat and the other for the worms exposed to UV light. In both cases, N2 was a strain from which the evolved ones were obtained.

3.3.1. Evolution by heat

N2 worms were grown in a liquid medium in the incubator. 26.6°C was shown to be an egg-laying limit temperature (Figure 6). Every two days food was added. Also, cleaning protocol which consisted of a change of the medium and addition of antifungal drug and antibiotic was done. The number of young/adult worms was counted and the temperature was increased when progeny increased considerably. The maximum temperature reached in the experiment was 27.6°C. The progeny of the worms that laid eggs on that temperature was transferred on the petri plate on 20°C and used for lifespan and heat stress assay.

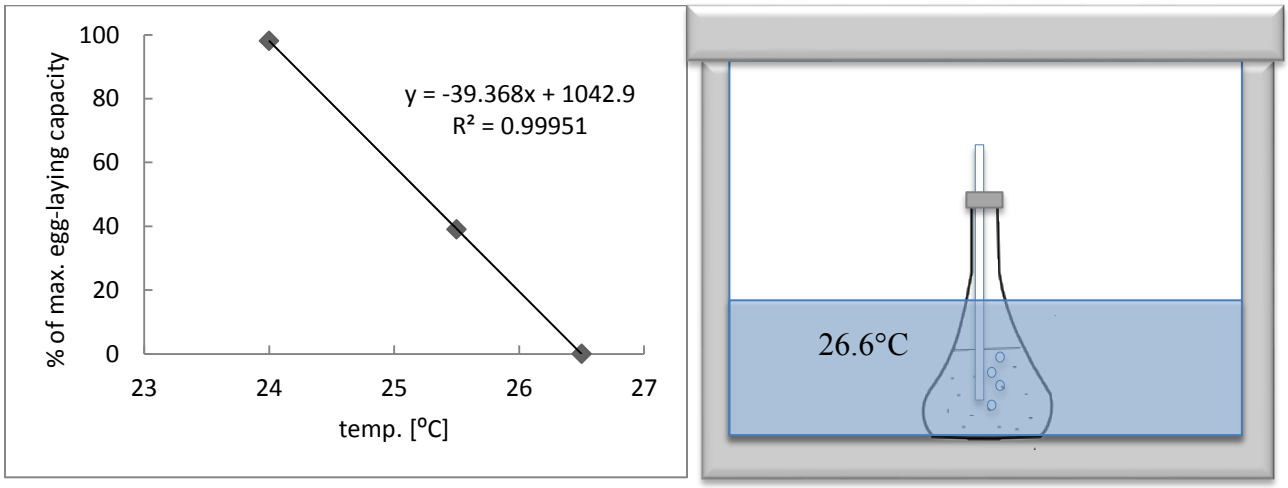


Figure 6. Heat protocol. Right side: It was shown that there is a linear decrease in fecundity between 24°C and 26.6°C. Left side: We designed an experiment in which thousand of worms were grown in liquid culture in a glass vial with an oxygenation system, with a controlled temperature in a bath incubator starting just below the limit of egg-laying capacity. This temperature was raised regularly for 0.2°C when the population of worms increased to a define amount.

3.3.2. Evolution by UVA irradiation

The N2 worms in the L1 stage were irradiated with UVA. The fast recovering worms that reached adult stage and started to hatch were taken out and bleached. Worms in the L1 stage were left to grow until they were ready for the next bleaching. Their progeny (in L1 stage) was exposed to UVA again (Figure 7). The irradiation dose[11] was raised after noticing faster recovery of the irradiated worms and if the number of the worms that recovered was considerably bigger.

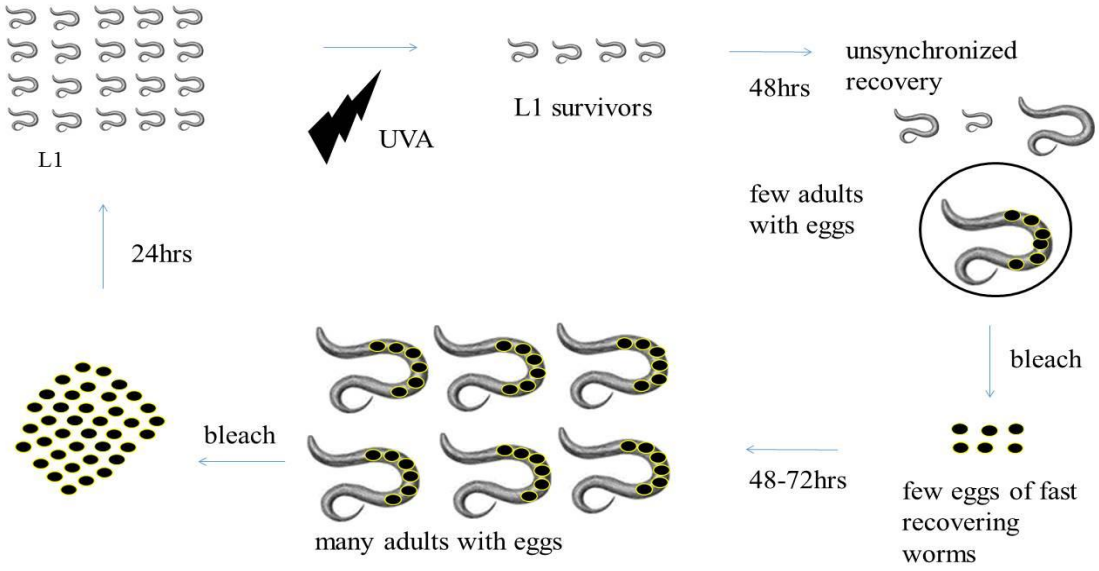


Figure 7. Radiation protocol. Worms in L1 stage were exposed to UVA. In few days some of them recovered and the gravid adults with eggs were taken out and bleached. After they had grown to adults, they were bleached again and L1 worms were exposed to UVA.

We noticed that worms did not survive above 24 J/cm^2. Worms were put on petri plates and after every irradiation, they were stored in the incubator on 20°C. A device that was used in the experiment was UV LED chamber BSL-02 with the controller for dose-controlled operations.

3.4. Heat stress assay

In this assay worms were heat shocked on 37°C, which is more than 15°C higher than their optimal temperature (Gill *et al*., 2003). Worms were washed off the petri plates using M9 and around 50 of each strain (N2, evolved under heat and UVA) were put in 96-well plate. Three plates were prepared in the same way and protected with cover so the liquid did not evaporate too quickly. Measurements were done after three, four and five hours of exposure to heat, each

[11]Exposure dose or radiant exposure is a time integral of the irradiance (Diffey, 2002).

time taking one 96-well plate out of the incubator. In that way, the time needed for counting did not influence the following measurement. Number of alive and dead worms was counted and used for analysis.

3.5. Lifespan assay

Plates for the lifespan were prepared by adding fluorodeoxyuridine[12] (FUDR) on standard NGM plates. This drug prevents reproduction by blocking the cell division process. After they were dried, OP-50 was applied on the surface of the plates. Worms were synchronized by bleaching and let to grow to L4 stage (Sutphin and Kaeberlein, 2009). Around 50 worms per strain were transferred with sterile platinum pick on few petri plates. Every day worms were counted. The ones that crawled to the edge or died due to the handling, were censored. Gentle tapping of the worms head helped to distinguish dead and alive worms that did not move. When contamination was noticed, worms were transferred to the new plate. Usually those plates would be thrown away, but in our experiment contamination appeared in all of the plates even when the lifespan assay was repeated so the plates were kept and the experiment was continued. Lifespan finished when the last worm died. Collected data consisted of numbers of the alive, dead and censored worms at each day (Sutphin and Kaeberlein, 2009). Inverted optical microscope was used for visualization of worms on the plate.

3.6. Data analysis

We used the software GraphPad Prism 6 (GraphPad Software Inc., USA) for the statistical analysis and the data plotting. It is used for scientific graphing but it is particularly useful for survival analysis. Statistics that was performed for determining if there is a significant difference between different species was log-rank method (Mantel-Cox test). Also, for both lifespan and heat stress assay Kaplan-Meier curve was plotted. It includes survival percentage plotted against time (days in lifespan and hours in heat stress assay), but also censored data. Graph of evolution of worms exposed to high temperature was plotted in Microsoft Excel (Microsoft Corporation 2010, USA).

[12]Drug that inhibits DNA synthesis. It is used because it prevents egg production and growth of contamination on plate but it does not influence development of the adult worms or their aging (Mitchell *et al.*, 1979).

3.6.1. Statistics

GraphPad Prism 6 uses an algorithm for log-rank. That method computes the number of deaths (observed and expected) in each group at all time events and it adds that to get a summary at all time points. Specifically, P-value is used for determining whether the hypothesis is true. The hypothesis is rejected if the P-value is bellow certain threshold (usually 0.05 which was taken as a limit in our analysis). In other words, if the P-value is bigger than the decided limit, it means that experimental variables did not have a significant effect on the result. To determine P-value by the conservative method, a chi-square value has to be calculated as well as degrees of freedom (Machin *et al.*, 2006). Chi-square is defined as $chi - square = \sum_{all\ curves} \frac{(O_i - E_i)^2}{E_i}$, where O_i represents observed number of deaths in curve i and E_i expected number of deaths. Expected data is usually based on literature and observed data are actual results. Degrees of freedom is $df = n - 1$ where n is the number of categories that are examined (Machin *et al.*, 2006). One can determine P-value from the reference table by knowing just the chi-square value.

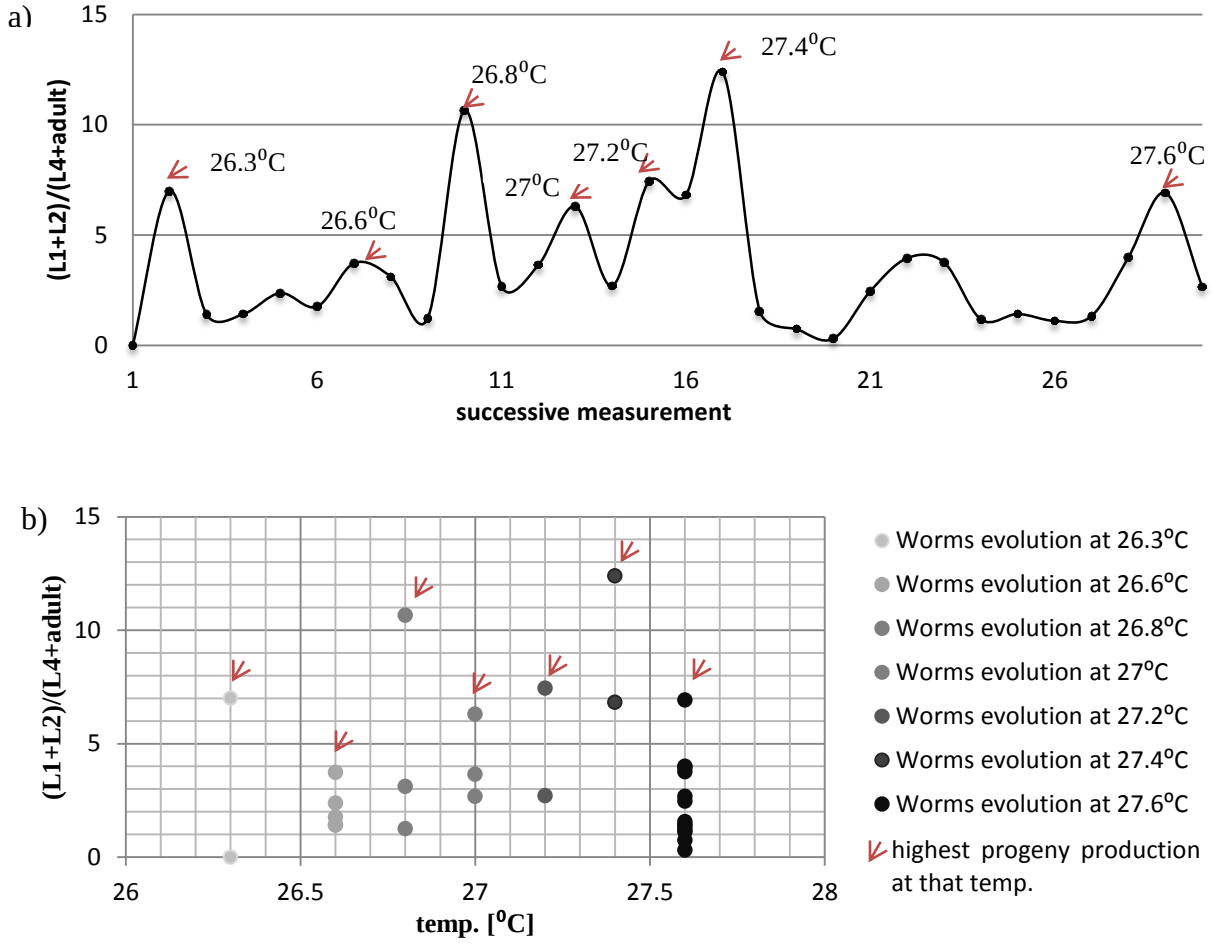
Hazard defines the slope of the survival curve and hazard ratio compares two groups. It takes all the data for calculations and it is important that hazard ratio of population is consistent at all time points (Machin *et al.*, 2006).

3. RESULTS

The main results of the experiment are lifespan and heat stress assay, but there are some qualitative results that follow from the observations of worms condition.

4.1. Evolution by heat

Flask in which worms were maintained was mixed well and 1 ml was taken out daily. Few drops of 5 µl were put on the microscope slide and worms of each stage including the dead ones were counted. Progeny was considered to be the main indicator of a heat adaptation due to the fact that it is described that egg-laying is limited on high temperature. Graphs 1a and 1b represent evolution of worms at high temperature.



Graph 1. Evolution of N2 exposed to high temperatures. a) Progeny production is represented gradually with each measurement during evolution process. The peaks with appointed temperatures represent high progeny production after which the temperature was increased. b) Same data as in first graph is represented in dependence on temperature.

The ratio of L1 and L2 over L4 and adults was taken into account as a measure of progeny production. Every time when the temperature was increased, that ratio was very low. But, with longer exposure, worms started to adjust and produce offspring. Peaks that can be noticed on the Graph 1a demonstrate a point after which the temperature was increased. 27.6°C seemed to be a limiting temperature for the worms evolution and it took a longer time to achieve an egg production. The experiment was stopped when they produced progeny at 27.6°C and those were used for further analysis. We also noticed that this evolution process was not linear and the time between 2 increase in temperature varied from few days to few weeks.

4.2. Evolution by UVA

Ten generations of worms were irradiated at L1 stage. It was noticed that UVA dose at which all of the worms die is 25 J/cm^2. So the maximum dose of irradiation to which they were exposed was 24 J/cm^2.

To compare the population of N2 worms to those frequently exposed to UVA, around 20 worms of each were taken at L1 stage. They were put on the 35 mm petri plates with OP-50 and then they were separately exposed to 10, 20 and 24 J/cm^2. Results are summed up in Table 1. Just after irradiation, most of the worms did not move but might have still been alive. Considering that they were in the L1 stage, it was hard to see them and they were too small to be tapped to check their response. To avoid that, data were collected one and two days after exposure to UVA.

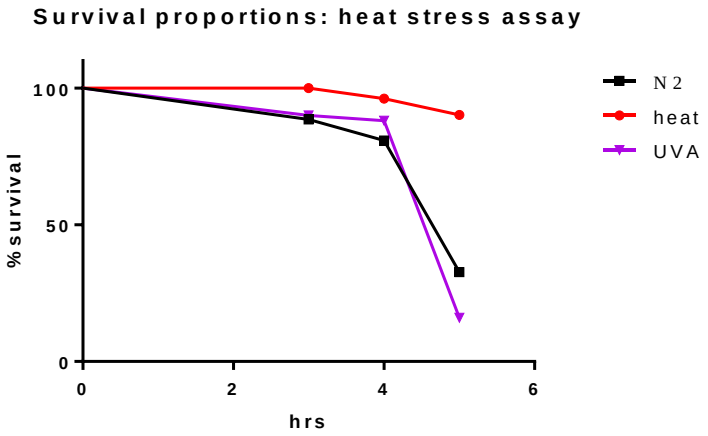
Table 1. Worms first and second day after UVA irradiation.

First day after irradiation			
[J/cm^2]	10	20	24
N2	50% alive; L2/L3 stage	26% alive; L2 stage	none of them moves
UVA	92% alive; L2/L3 stage	60% alive; L2 stage	none of them moves
Second day after irradiation			
N2	50% still alive ; young adults	26% still alive ; some are in L3, some in L4 stage	5% alive, L3 stage
UVA	92% still alive ; young adults stage	60% still alive ; L2 and L3 stage	5% alive L2 stage

Apparently, worms developed slower than when they were under optimal conditions (see Figure 5). Also, more of the UVA-evolved worms survived the irradiation comparing to the wild-type. Nevertheless, some of N2 worms that survived 10 and 24 J/cm^2 developed a little faster than UVA ones.

4.3. Heat stress assay

Heat stress analysis was performed in liquid medium (M9) in the 96-well plate at 37°C. Three plates were prepared with approximately 50 worms per strain. After 3, 4 and 5 hours of exposure to heat, worms were observed under the microscope and the alive ones were counted. As can be seen from Kaplan-Meier curve in Graph 2 and from the data analysis in Table 2, there is a significant difference between N2 and heat-evolved worms.



Graph 2. Heat stress assay at 37°C. Kaplan Meier curve is plotted for N2, UVA and heat-evolved worms. In all three cases there were alive worms after 5hrs of exposure to 37°C. N2 and UVA worms have dropped to less than 50% of survived ones whereas the heat strain showed the best resistance to high temperatures. More than 80% of them were alive after 5 hours of exposure to heat.

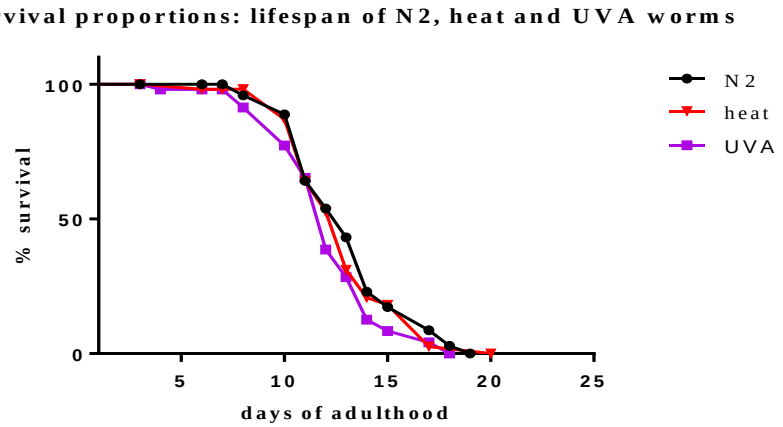
Table 2. Analysis summary of heat stress assay.

Log-rank (Mantel-Cox) test: N2 and heat		Log-rank (Mantel-Cox) test: N2 and UVA	
Chi square	34.14	Chi square	1.05
P-value	< 0.0001	P-value	0.31
Are the survival curves significantly different?	Yes	Are the survival curves significantly different?	No
Hazard ratio N2/heat	7.92	Hazard ratio N2/UVA	0.69

Heat-evolved worms stand higher temperatures better comparing to N2. The P-value for comparison of N2 and heat-evolved worms is < 0.0001 and >0.05 for N2 and UVA-evolved worms. In support of that goes the observation of their condition after exposure to 37°C. Heat-evolved worms who were observed after 5 hours of exposure moved as usual while N2 and UVA worms reacted poorly on heat. Their movements was slow and some of them twitched and huddled up. UVA worms looked the worst comparing to the other two strains. Also, hazard ratio shows that there is almost 8 times faster death occuring in wild-type comparing to heat-evolved worms. Difference between wild-type and UVA-evolved worms is considerably smaller and goes in favor of N2 that were dying slower.

4.4. Lifespan

Lifespan was performed on 35 mm petri plates with FUDR. Approximately 50 worms of each strain were monitored from young adulthood to the day when the last worm died (Graph 3). Statistical analysis showed that P-value is >0.05 for both strains, so there is no significant difference between curves of N2 and UVA-evolved worms, and N2 and heat-evolved worms (Table 3). Therefore, there was no increase of lifespan of the evolved strains.



Graph 3. Lifespan assay. Kaplan-Meier curve for N2, worms evolved under higher temperature and UVA irradiation was plotted. Curves practically do not differ.

Table 3. Analysis summary of lifespan assay.

Log-rank (Mantel-Cox) test: N2 and heat		Log-rank (Mantel-Cox) test: N2 and UVA	
Chi square	0.12	Chi square	2.19
P-value	0.73	P-value	0.14
Are the survival curves significantly different?	No	Are the survival curves significantly different?	No
Hazard ratio N2/heat	0.94	Hazard ratio N2/UVA	0.75

A median[13] of survival was also computed. Usually, it is 2-3 weeks long for wild-type. In our experiment, it is the same for N2 and heat-evolved worms and one day shorter for UVA-evolved ones (Table 4).

Table 4. Median of survival for each strain.

Median of survival	**N2**	**heat**	**UVA**
days	13	13	12

Also, the contamination on all of the plates (of all three strains) was noticed, so it might have influenced the lifespan. Importantly, all strains were under the same conditions. Maximum lifespan is 18, 19 and 20 days for UVA-evolved, N2 and heat-evolved worms, respectively.

[13]It coresponds to time at which 50% of worms are alive.

5. DISCUSSION

Lifespan is connected to numerous factors among which is oxidative stress resistance (Lithgow *et al*. 1995). Our hypothesis stated the same, heat and UVA presented sources of stress for worms, so we expected that strains that acquired stress resistance through experimental evolution will live longer. The hypothesis that both UVA and heat-evolved worms will be stress resistant was partly confirmed whereas lifespan assay showed no significant difference between strains.

Median lifespan was almost the same in all strains and shorter than the one from the literature. A reason for relatively short lifespan might be due to the improper instruments and also bacterial contamination. Still, it is hard to say whether the contamination affected equally all of the strains. Because of the fact that all worms have been under the same conditions, we took into account observations and measurements of the difference between them.

Heat stress assay has shown that worms that were exposed to higher temperature deal with heat significantly better comparing to wild-type strain whereas UVA worms have not shown notably different heat stress resistance. Nevertheless, UVA-evolved worms have shown better stress resistance comparing to wild-type strain when they were both irradiated with UVA (at 10 J/cm^2). It should be examined whether worms will show the same response to biotic stress (like frequently used pathogen bacteria *Pseudomonas aeruginosa*[14]) and other abiotic stress (like paraquat[15]). If they show considerably better resistance to those stress assays, that would imply that they are resistant to stress in general.

Heat stress experiment was done in liquid after washing the NGM plates with OP-50 on which worms have grown until they reached the adult stage. Most of the previous research has been done on worms that were in the 5-7th day of adulthood. So, it is hard to compare survival data from those assays with ours. There was a limitation in the way of determining whether the worms are alive, they could not have been tapped to check their reaction. As a workaround, the same experiment might be performed on NGM plates that are previously heated at 37°C. Also, it might be useful to use coloring (like SYNTOX green[16]) to distinguish alive and dead worms. Additionally, a healthspan analysis can be performed to examine the coping of each strain with

[14]It usually causes death or hypoxic response (Kirienko *et al*., 2013).
[15] It interferes with electron transfer and it is toxic for humans and animals (Gems and Doonan, 2008).
[16]That is green fluorescent dye that is used as a marker of worms death (Gill *et al.*, 2003).

heat stress (Bansal *et al.*, 2015). Observations of the movement of worms go in favor of heat-evolved strain that looked healthy and active even after 5 hours of exposure to heat. UVA worms looked the worst after every check. Their movement was slow, they twitched and reacted poorly on shaking.

Results from the heat stress assay, lead to a conclusion that heat-evolved worms are adapted the best to heat stress, which was expected because those worms were exposed to high temperature during evolution experiment. Considering that we took worms for lifespan and heat stress assay 5 generations after the last exposure to heat and their acquired stress resistance was stable through generations, it is most likely not due to hormesis and epigenetics.

Still, it is expected for worms that are resistant to heat stress to have a longer lifespan. Some other pathway can be triggered during evolution process, like in the case of *D. radiodurans*. Resistance to ionizing irradiation was an unexpected and indirect consequence of resistance to desiccation (Mattimore and Battista, 1996). However, the evolution experiment was performed for only small number of generations so that is not probable. Maybe the resistance to UVA and heat has activated the resistance to other stress sources, and that should also be examined. Furthermore, there might have been a damage of enzyme or some other proteins that influenced the lifespan. Change that occur might have disappeared before we performed lifespan and heat stress assay.

For UVA-evolved strain, significant change comparing to wild-type in both lifespan and heat stress assay was not achieved. Further analysis should be performed to examine them, to be able to differentiate between actual evolution, epigenetic modification and hormesis. UVA worms might not have been irradiated enough to become stress resistant. Although a significant difference between UVA and wild-type was not shown, if we look at the hazard ratio in both lifespan and heat-stress experiments, it can be noticed that UVA-evolved worms were dying with higher speed rate. Thus, UVA has maybe caused damage rather than stress resistance.

6. CONCLUSION

Evolution experiment was performed using two environmental factors, UVA and heat to develop two different strains from a wild-type strain of *C. elegans*. The main hypothesis that was proposed was based on previous research on coupling of the stress resistance and longevity and would be useful for determining factors that influence aging. Also, it would be interesting for evolutionary biologist to answer the question of the impact of the stress factors on vigor and their influence on species during evolution. Temperature changes are common in natural habitat of *C. elegans*, but when it comes to UVA irradiation, its impact is unclear.

Result of the heat stress assay has shown significantly better heat stress response of heat-evolved strain comparing to wild-type whereas UVA-evolved worms have shown better response only to UVA irradiation. However, lifespan assay of both evolved strains was not different from the wild-type one.

Although we could not confirm the main hypothesis in the timeframe of the internship, we have mostly been able to show that we did obtain some degree of resistance in both our evolved strains. For the heat-evolved strain, we have demonstrated that this resistance is stable through generations. Worms should further be exposed to UVA/heat because evolution process takes time and many generations in which at some point a significant genetic change might occur. Maybe our populations have yet to achieve more evolution changes. Worms that were used for assays have previously been stored at 20°C and few generations after the last one exposed to heat/UVA was used. So, possible changes that have happened could have been epigenetic ones and have already dissapeared within few generations.

Lastly, *C. elegans* has shown to be suitable model organism for this kind of research. Examining evolutionary impact of different ecological factors can help in understanding how organisms coped with them during evolution and how did that influence their lifespan.

LITERATURE

Alcántara-Díaz, D., Breña-Valle, M., Serment-Guerrero, J., Divergent adaptation of *Escherichia coli* to cyclic ultraviolet light exposures, Mutagenesis **19** (5): 349-354, 2004.

Amrit, F.R.G., Boehnisch, C.M.L., May, R.C., Phenotypic covariance of longevity, immunity and stress resistance in the *Caenorhabditis* nematodes, PLOS ONE **5** (4): 1-13, 2010.

Anderson, J.L., Reynolds, R.M., Morran, L.T., Tolman-Thompson, J., Phillips, P.C., Experimental evolution reveals antagonistic pleiotropy in reproductive timing but not life span in *Caenorhabditis elegans*, J Gerontol A Biol Sci Med Sci: 1-9, 2011.

Aprison, E.Z., Ruvinsky, I., Balanced trade-offs between alternative strategies shape the response of *C. elegans* reproduction to chronic heat stress, PLOS ONE **9** (8): 1-13, 2014.

Bansal, A., Zhu, L.J., Yen, K., Tissenbaum, H.A., Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants, PNAS: E277-E286, 2015.

Begasse, M.L., Leaver, M., Vazquez, F., Grill, S.W., Hyman, A.A., Temperature dependence of cell division timing accounts for a shift in the thermal limits of *C. elegans* and *C. briggsae*, Cell Rep **10** (5): 647–653, 2015.

Chen, H., Maklakov, A.A., Longer life span evolves under high rates of condition-dependent mortality, Curr Biol **22**: 2140–2143, 2012.

Dickins, T.E., Rahman, Q., The extended evolutionary synthesis and the role of soft inheritance in evolution, Proc R Soc B **279**: 2913–2921, 2012.

Diffey, B.L., Sources and measurement of ultraviolet radiation, Methods **28**: 4–13, 2002.

Félix, M.A., Braendle, C., The natural history of *Caenorhabditis elegans*, Curr Biol **20** (22): R965-R969, 2010.

Fielenbach, N., Antebi, A., *C. elegans* dauer formation and the molecular basis of plasticity, Genes Dev **22** (16): 2149–2165, 2008.

Finkel, T., Holbrook, N.J., Oxidants, oxidative stress and the biology of ageing, Nature **408**: 239-247, 2000.

Gems, D., Doonan, R., Aging medicine: oxidative stress in aging: from model systems to human diseases; Chapter 6: The nematode *Caenorhabditis elegans*: oxidative stress and aging in the nematode *Caenorhabditis elegans*, Humana Press, 81-108, 2008.

Gill, M.S., Olsen, A., Sampayo, J.N., Lithgow, G.J., An automated high-throughput assay for survival of the nematode *Caenorhabditis elegans*, Free Radic Biol Med **35** (6): 558–565, 2003.

Gray, J.C., Cutter, A.D., Mainstreaming *Caenorhabditis elegans* in experimental evolution, Proc R Soc B **281**: 20133055, 2014.

Hall, B.K., Hallgrímsson, B., Strickberger's evolution-5th ed.; Part 1: Evolution and Species, Jones & Bartlett Learning, 3-19, 2008.

Hughes, K.A., Reynolds, R.M., Evolutionary and mechanistic theories of aging, Annu Rev Entomol **50**: 421–445, 2005.

Kaeberlein, T.L., Smith, E.D., Tsuchiya, M., Welton, K.L., Thomas, J.H., Fields, S., Kennedy, B.K., Kaeberlein, M., Lifespan extension in *Caenorhabditis elegans* by complete removal of food, Aging Cell **5**: 487-494, 2006.

Kaletsky, R., Murphy, C., The role of insulin/IGF-like signaling in *C. elegans* longevity and aging, Dis Model Mech **3**: 415-419, 2010.

Kenyon, C., The nematode *Caenorhabditis elegans*, Science **240**: 1448-1453, 1988.

Kiontke, K., Sudhaus, W., Ecology of *Caenorhabditis* species, WormBook, 1–14, 2006.

Kirienko, N.V., Kirienko, D.R., Larkins-Ford, J., Wählby, C., Ruvkun, G., Ausubel, F.M., *Pseudomonas aeruginosa* disrupts *Caenorhabditis elegans* iron homeostasis, causing a hypoxic response and death, Cell Host Microbe **13** (4): 406-416, 2013.

Lans, H., Vermeulen, W., Nucleotide excision repair in *Caenorhabditis elegans*, Mol Biol Int: 1-12, 2011.

Lenski, R.E., Evolution in Action: a 50,000-generation salute to Charles Darwin, Microbe **6** (1): 30-33, 2011.

Lithgow, G.J., White, T.M., Melov, S., Johnson, T.E., Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress, P Natl Acad Sci USA **92**: 7540-7544, 1995.

Machin, D., Cheung, Y.B., Parmar, M., Survival Analysis: A Practical Approach, 2nd edition, Wiley, 2006.

Mattimore, V., Battista, J.R., Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation, J Bacteriol **178**: 633–637, 1996.

Mitchell, D.H., Stiles, J.W., Santelli, J., Sanadi, D.R., Synchronous growth and aging of *Caenorhabditis elegans* in the presence of fluorodeoxyuridine, J Gerontol **34** (1): 28-36, 1979.

Murakami, S., Johnson, T.E., A genetic pathway conferring life extension and resistance to UV stress in *Caenorhabditis elegans*, Genetics **143**: 1207-1218, 1996.

Nakabeppu, Y., Cellular Levels of 8-Oxoguanine in either DNA or the nucleotide pool play pivotal roles in carcinogenesis and survival of cancer cells, Int J Mol Sci **15** (7): 12543-12557, 2014.

Novak K., Epigenetics changes in cancer cells, Med Gen Med **6** (4): 17, 2004.

Riddle, D.L., Blumenthal, T., Meyer, B.J., *et al*. editors, *C. elegans* II.-2nd edition: Section I: The Biological Model, Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press, 1997.

Stiernagle, T., Maintenance of *C. elegans*, WormBook, 1-11, 2006.

Sulston, J.E., Horvitz, H.R., Post-embryonic cell lineage of the nematode *Caenorhabditis elegans*, Devel biol **56**: 110-156, 1977.

Sulston, J., Hodgkin, J., The Nematode *Caenorhabditis elegans*, Cold Spring Harbor, 587-606, 1988.

Sutherland, J.C., Griffin, K.P., Absorption spectrum of DNA for wavelengths greater than 300 nm, Radiat Res **86**: 399–410, 1981.

Sutphin, G.L., Kaeberlein, M., Measuring *Caenorhabditis elegans* life span on solid media, J Vis Exp **27**, 2009.

Tatar, M., Khazaeli, A.A., Curtsinger, J.W., Chaperoning extended life, Nature **390**: 30, 1997.

Vile, G.F., Tyrrell, R.M., UVA radiation-induced oxidative damage to lipids and proteins in vitro and in human skin fibroblasts is dependent on iron and singlet oxygen, Free Radic Biol Med **18** (4): 721–730, 1995.

Walker, G.A., Lithgow, G.J., Life span extension in *C. elegans* by a molecular chaperone dependent upon insulin-like signals, Aging Cell **2**: 131–139, 2003.

Yagura, T., Makita, K., Yamamoto, H., Menck, C.F.M., Schuch, A.P., Biological sensors for solar ultraviolet radiation, Sensors **11**: 4277-4294, 2011.

Zhou, K.I., Pincus, Z., Slack, F.J., Longevity and stress in *Caenorhabditis elegans*, Aging **3** (8): 1-21, 2011.

APPENDIX

Solutions were prepared according to protocol (Stiernagle, 2006).

LB

Mix:

- 10 g NaCl,
- 10 g bacto-tryptone,
- 5 g bacto-yeast,
- add H_20 to 1 litre.

Mix and autoclave.

NGM

Mix:

- 3 g NaCl,
- 17 g agar,
- 2.5 g peptone,
- 975 ml H_2O.

After autoclaving for 50 min and cooling the mixture to 55°C, add:

- 1 ml 1 M $CaCl_2$,
- 1 ml 5 mg/ml cholesterol in ethanol,
- 1 ml 1 M $MgSO_4$,
- 25 ml 1 M KPO_4 buffer (108.3 g KH_2PO_4, 35.6 g K_2HPO_4, H_2O to 1 litre).

Swirl to mix well.

S basal

Mix:

- 5.85 g NaCl,
- 1 g K_2HPO_4,
- 6 g KH_2PO_4,
- 1 ml cholesterol (5 mg/ml in ethanol),
- H_2O to 1 litre.

After autoclaving add:

- 1 M Potassium citrate pH 6.0 (20 g citric acid monohydrate, 293.5 g tri-potassium citrate monohydrate, H_2O to 1 litre and autoclaved),
- Trace metals solution (1.86 g disodium ethylenediaminetetraacetic acid, 0.69 g $FeSO_4 \cdot 7 H_2O$, 0.2 g $MnCl_2 \cdot 4 H_2O$, 0.29 g ZnSO4 $\cdot$ 7 H_2O, 0.025 g $CuSO_4 \cdot 5 H_2O$, H_2O to 1 litre, autoclaved and stored in the dark),
- 1 M $CaCl_2$ (55.5 g $CaCl_2$ in 1 litre H_2O, autoclaved).

S Medium

Using sterile technique, add:

- 1 litre S Basal,
- 10 ml 1 M potassium citrate pH 6,
- 10 ml trace metals solution,
- 3 ml 1 M $CaCl_2$,
- 3 ml 1 M $MgSO_4$.

Mix and storage.

M9 Buffer

- 3 g KH_2PO_4,
- 6 g Na_2HPO_4,
- 5 g NaCl,
- 1 ml 1 M $MgSO_4$,
- H_2O to 1 litre.

Sterilize by autoclaving.

Bleaching solution

- 9 ml H_20,
- 1 1 M NaOH,
- 3 ml bleach[17] and mix.)

[17]Solution (4-6%) of sodium hypochlorite NaOCl.