

## The Study on Life Span of the Nematode, *Teratorhabditis palmarum*

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**ABSTRACT:** Life span is the length of time for which a person or an animal lives during which various biological processes regulated by several genetic pathways. A lot of genes have been identified in nematodes whose mutations cause several folds increase in lifespan. It was predominantly found that life span of males of nematode, *Teratorhabditis palmarum* was shorter than that of females when compared by taking different parameters. It was also observed that isolated females always lived longer than the solitary males. The average life span of male and female in isolated condition of *T. palmarum* was found to be greater than that of the mated and single-sex groups of males and females. The isolated male and female life span was found to be  $11.4 \pm 1.34$  and  $13.2 \pm 2.18$  days, respectively and when kept together, it was found to be  $9.2 \pm 1.91$  and  $10.65 \pm 2.23$  days for males and females, respectively. Mating affected the life span of both sexes. Lastly in single-sex groups, there was also reduction in life-span of males.

**Keywords:** *Caenorhabditis elegans*, Lifespan, Mating, Sexes, *Teratorhabditis palmarum*

Aging is a universal process that leads to progressive deterioration of muscular, metabolic, reproductive and cognitive functions which finally affects lifespan. Generally it is a biological process that cannot be easily measured or determined by the organism. However, life span is defined as the number of days an animal remains responsive to external stimuli. It is only a single estimate parameter that define period of time an organism is alive but does not give any actual explanation to the cause of aging. Over the last decade, several different types of genes have been identified that modulate life span. Klass (1983) was the first who reported a method for isolating longevity mutants in *Caenorhabditis elegans* and it was used to isolate two most important mutants, Age-1 and daf-2, for which there is an increased lifespan, are associated with reduced calorie intake. Nematodes exhibit a significant diversity of life histories, which includes considerable variation in rate of ageing and adult life span. Genetically, DAF-16 expression and/or activation are an important mechanism by which species undergoes longer life. Amrit *et al.* (2010) observed that hermaphroditic species are generally shorter lived than the gonochoristic species and show higher levels of daf-16 expression. The overall fitness is primarily contributed by the interactions within and between the two sexes

(Andersson, 1994; Arnqvist and Rowe, 2005). If the females capacity is condition dependent in order to permit the damaging effects of males, than selection will delay senescence, favouring robust individuals with longer life spans (Williams and Day, 2003; Maklakov *et al.*, 2015).

Life span in nematodes is affected by several factors such as temperature, food availability, stochastic factors (such as lifestyle) and reproductive activity. In *C. elegans*, there is reduction in life span by several parameters, including *E. coli* food availability (Gems and Riddle, 2000; Garigan *et al.*, 2002; Garsinet *et al.*, 2003), higher temperature (Klass, 1977), mating between the sexes (Gems and Riddle, 1996) and attempted mating between males (Gems and Riddle, 2000). Furthermore, lowering of temperature, food availability and reproductive activity can all increase life span. Klass (1977) observed maximum life span of *C. elegans* at 20° C to 19 days, at 25° C to 11 days. Beside *C. elegans*, a lot of work on life span has been done in other nematodes. In the free-living nematode, *Panagrellus redivivus*, maximum life span of males and females at 25° C was 6 days and 20 days, respectively (Duggal, 1978 a&b). In contrast to this, lifespan is also affected by the gender specific differences in nematode

and depend upon whether or not nematode mate. Weadick *et al.* (2016) observed that hermaphrodites were the shortest lived, females the longest with males intermediate life span. Culture conditions also have an important impact in estimation of lifespan. In *C. elegans*, there is 40% increase in life span under axenic liquid culture as compare to culture on *E. coli* agar plates (Croll *et al.*, 1977; Mitchell *et al.*, 1979). It varies with different culture conditions and methodical problems. In *C. elegans*, *P. Pacificus* and *P. superbus* the survival ratio also affected by anaerobic conditions (Kitazume *et al.*, 2018). Lifespan which was measured in NGM medium showed variations in N2 strain of *C. elegans*. Chen *et al.* (2007), Train *et al.* (2008), Klass (1977) and Shook and Johnson (1999) observed lifespan, 14.8, 15, 19 days and 13.3 days in NGM medium, respectively.

In this study, we estimated the average life span of *Teratorhabditis palmarum* by certain parameters. For that, we calculated life span by taking both sexes either separately, combined each other's or single-sex groups of males and females. We also compared it with others nematodes.

## MATERIAL AND METHODS

The free living nematode *Teratorhabditis palmarum* was isolated near the fort (27.8° N, 78° E) in Aligarh. Nematodes were extracted from manure by modified (Cobb's, 1918) sieving and decantation and modified Bermann's funnel techniques. *T. palmarum* was cultured on nematode growth medium (NGM) supplemented with *E. coli* OP50 strain as a food source at  $23 \pm 1^\circ\text{C}$  in 5 mm petridishes. The bacterial strain OP50, maintained in laboratory, was tested. The pure culture of the strain was maintained and stored as slants, stabs and frozen permanents as glycerol cultures at  $-20^\circ\text{C}$ . The strain was sub-cultured on regular basis on Nutrient broth medium. For preparing the broth culture of the strain, single OP50 colony was inoculated in 100 ml nutrient broth and kept at  $37^\circ\text{C}$  for overnight at bacterial rotary shaker. After obtaining the population, nematode were regularly propagated by transferring 5–10 gravid females to fresh *E. coli* plates in order to obtain age-synchronized population and continued the progeny of nematodes. In

order to collect J4 stage, handpicked the J4 stage of males and females from the culture by picking needle and placed onto fresh OP50 containing NGM agar plates.

## Life Span Analysis

First, the life span of male and female was studied separately. So, followed the age-synchronized population, particularly L4. Most L4 nematodes could be clearly distinguished as male or female according to their genital morphology. Both sexes were segregated at L4 stage and transferred it into 12-well sterile tissue culture agar plates seeded with *E. coli* OP50 as a food source. 20 L4 larvae of each sex were taken and estimated the lifespan separately. Secondly, the life span of male and female was also estimated when placed together. For this, 20 L4 larvae of each sex were taken and placed in same well plates with five replicates for both experiments. Thirdly, measured the life span of single-sex groups of males and females. For this, we took 5, 10 & 15 animals per plates of both sexes and had three replicates for this experiment. The wells were observed daily under a dissecting microscope and the numbers of live and dead worms were counted in each well. Worms that were not moving, did not respond to gentle probing with a needle, or were missing were counted as dead. Transferred the worms daily in new fresh plates. Recorded the date and the number of worms that were alive and dead.

## Statistics

All results were expressed as mean  $\pm$  standard deviation. Graphs were plotted using Origin 6 software. Analysis of variance was performed to identify the significant differences from the control group.

## RESULTS

### Unmated Female and Male Life Span

Considerable variation was observed in estimated life span of male and female. The life span of solitary unmated females was  $13.2 \pm 2.18$  days. While the solitary males was shortest lived than females i.e.,  $11.4 \pm 1.34$  days. Male and female survival curves are drawn in Fig. 1 A & B.

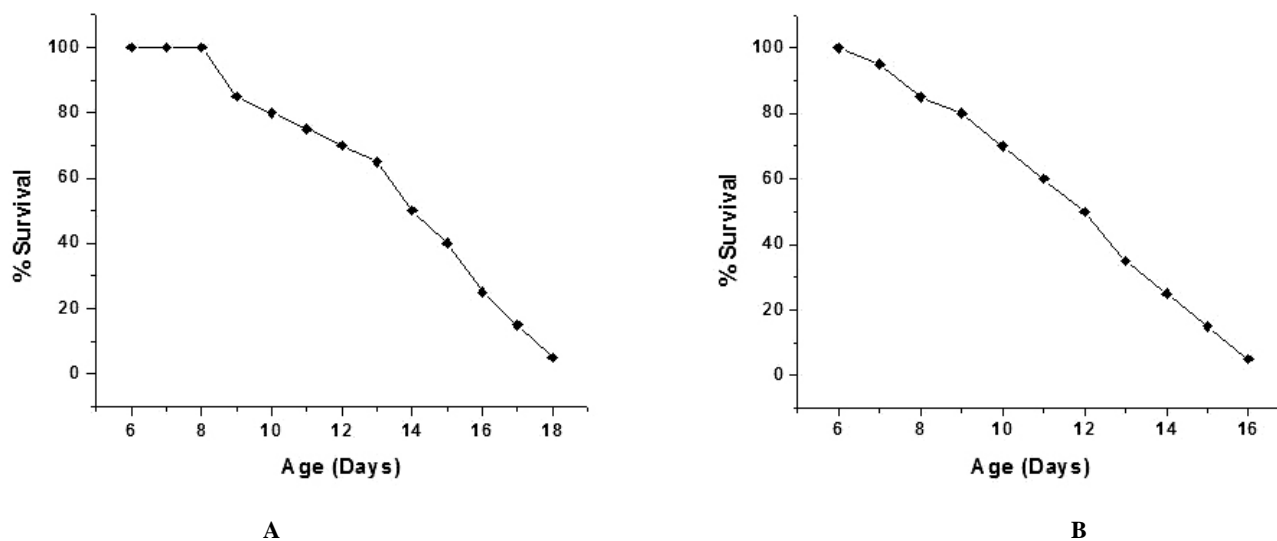


Fig. 1. (A) Survival curve for females and (B) Survival curve for males

### Mated female and male life span

Generally mating reduced overall lifespan for both mated male and female. Mating with male for their lifetime reduced female lifespan compared with its isolate. The observed life span of mated males and females were  $9.2 \pm 1.91$  and  $10.65 \pm 2.23$  days respectively. Mated survival curves are shown in Fig. 2.

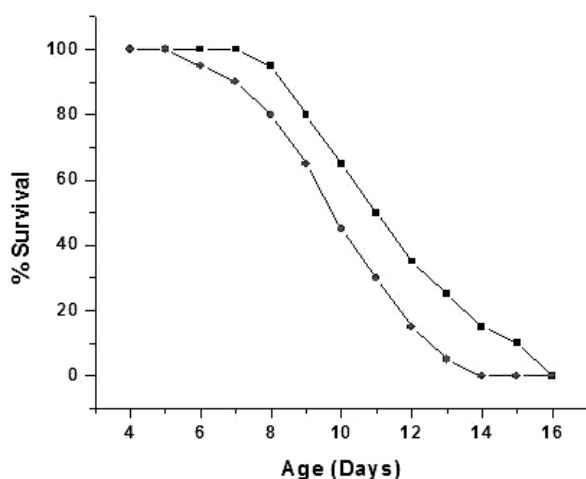


Fig. 2. Combined survival curves for males & females

### Effect of single-sex groups on life span

It is common that male attempted mating resulted in reduction of life-span in single-sex groups of males. As

the males congregated into clumps, caused decrease in longevity compared with solitary and mated individuals. Since male life span was greatly affected by population density as it nearly was unaffected in female. In three groups of males, animals acquired such a mating plug after 2-3 days. While no mating plugs were observed on solitary males. Finally, observed life span of 5, 10 & 15 animals per plates of male were  $9.3 \pm 2.7$ ,  $8.4 \pm 1.3$  and  $7.6 \pm 0.8$  days. In contrast to this, slight variations were recorded against the population density in female life span. It was found to be  $12.8 \pm 2.7$ ,  $12.4 \pm 1.4$  and  $11.4 \pm 0.7$  days of 5, 10 & 15 animals per plates. Survival curves are drawn in Fig. 3 A&B.

## DISCUSSION

Generally it has been observed that there is sex differences in life span of animal species, in which male typically shorter lived than female (Comfort, 1979; Smith, 1989). It was also found that virgin male & female live longer than non-virgin. For *Turbatrix aceti*, both virgin male and female had greater life span as compared to their mated counterparts (Kisiel and Zuckerman, 1974). Similarly these findings were reported for *P. redivivus* (Abdulrahman and Samoiloff, 1975) and *Rhabditis tokai* (Suzuki *et al.*, 1978). In our nematode, both virgin male and female were lived 17% & 19% longer than mated male and female. This reduced longevity was due to sex-related behaviour or reproductive activity in mated

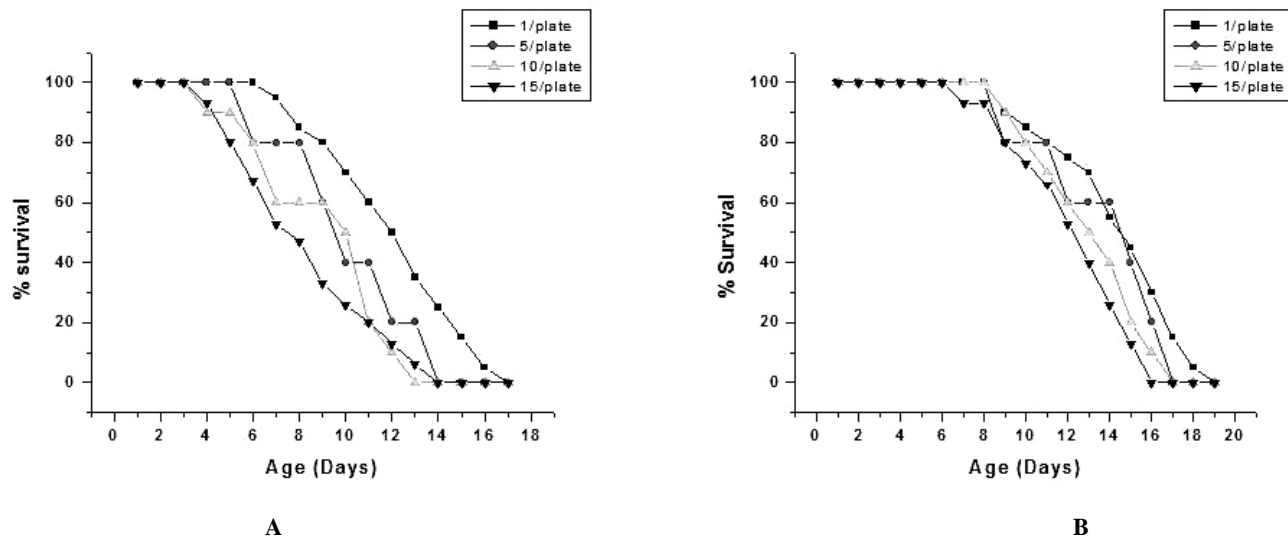


Fig. 3. Effect of population density on male survival (A) and female survival (B)

individuals. The continuous presence of mating competent males significantly shortens the lifespan of the opposite sex (Maures *et al.*, 2014).

However, Gems and Riddle (1996) found that mated males have a greater life span than that of unmated and grouped males. They also concluded that beside reduction in the longevity of mated hermaphrodite by 43%, male remain unaffected by mating. Honda (1925) observed that mating was also found to reduce the mean lifespan of females of the dioecious species *Diplogastera aerivora* from 52 days (range, 33–68 days) to 25 days (range, 11–54 days) and in males also reduced mean life span from 43 days (range, 15–71 days) to 33 days (range, 10–54 days). Possibly one of the reasons in the reduction of male longevity is the capacity to produce mating plugs. If so, this would be an example of a trade-off between fitness traits, in which the deposition of mating plug enhances reproductive success (Barker, 1995) but limits longevity. Similarly in this study, males which attempted to mate and formed the copulation plug caused reduced lifespan in both male and female. While in female it was due to the effect of copulation rather than an increase in egg production (Gems and Riddle, 1996).

However, male have reduce life span either they maintained separately or combined with female & single sex groups. It showed 14% reduction with combined

female, when cultured separately reduced by 16% and 27–33% reduced by single sex groups compared with the female. Male life span showed inverse relation with increase in population density, whereas life span of female was not density dependent. This was due to either male clumping or mating that deposited mating plug on each other. Solitary males were more variable than those of grouped males. Its life span was increased by 24%, suggesting that male-male interaction greatly reduced the life span. Male longevity was reduced by the presence of even one other male (Gems and Riddle, 2000b). In *C. elegans*, males showed the homosexual mating clumps formation when they were present in grouping which resulted in halves male lifespan compared with solitary males (Gems and Riddle, 2000b). Recent work had shown in *Caenorhabditis*, the copulation and male harassment can be quite damaging for females and hermaphrodites (Maures *et al.*, 2014; Ting *et al.*, 2014). In this study, it was found that there is significant variations in estimation of life span under the different forms.

## REFERENCES

- Abdulrahman, M. & Samoiloff, M.R. (1975). Sex-specific aging in the nematode *Panagrellus redivivus*. *Canadian Journal of Zoology* **53**: 651–656.

- Amrit, F.R.G., Boehnisch, C.M.L. & May, R.C.** (2010). Phenotypic covariance of longevity, immunity and stress resistance in the *Caenorhabditis* nematodes. *PLoSone*, **5**: e9978.
- Andersson, M.B.** (1994). Sexual Selection. Princeton University Press, Princeton, N.J. 624 pp.
- Arnqvist, G. & Rowe, L.** (2005). Sexual conflict. Princeton University Press, Princeton, N.J. 352 pp.
- Barker, D.M.** (1995). Copulatory plugs and paternity assurance in the nematode *Caenorhabditis elegans*. *Animal Behaviour* **48**: 147-156.
- Chen, J.J., Senturk, D., Wang, J.L., Müller, H.G., Carey, J.R., Caswell, H. & Caswell-Chen, E.P.** (2007). A demographic analysis of the fitness cost of extended longevity in *Caenorhabditis elegans*. *The Journal of Gerontology* **62**: 126-135.
- Cobb, N.A.** (1918). Estimating the nema population of the soil. U.S. Department of agriculture. *Agricultural Technical Circular of US* **1**: 48p.
- Comfort, A.** (1979). The Biology of Senescence. Elsevier, New York. 414 pp.
- Croll, N.A., Smith, J.M. & Zuckerman, B.M.** (1977). The aging process of the nematode *Caenorhabditis elegans* in bacterial and axenic culture. *Experimental Aging Research* **3**: 175-189.
- Duggal, C.** (1978a). Copulatory behaviour of male *Panagrellus redivivus*. *Nematologica* **24**: 257-268.
- Duggal, C.** (1978b). Initiation of copulation and its effect on oocyte production and life span of adult female *Panagrellus redivivus*. *Nematologica* **24**: 269-276.
- Garigan, D., Hsu, A.L., Fraser, A.G., Kamath, R.S., Ahringer, J. & Kenyon, C.** (2002). Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. *Genetics* **161**: 1101-1112.
- Garsin, D., Villanueva, J., Begun, J., Kim, D., Sifri, C., Calderwood, S.B., Ruvkun, G. & Ausubel, F.M.** (2003). Long-lived *C. elegans* daf-2 mutants are resistant to bacterial pathogens. *Science* **300**: 1921.
- Gems, D. & Riddle, D.L.** (2000a). Defining wild-type life span in *Caenorhabditis elegans*. *The Journals of Gerontology* **55**: 215-219.
- Gems, D. & Riddle, D.L.** (2000b). Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. *Genetics* **154**: 1597-1610.
- Gems, D. & Riddle, D.L.** (1996). Longevity in *Caenorhabditis elegans* reduced by mating but not gamete production. *Nature* **379**: 723-725.
- Honda, H.** (1925). Experimental and cytological studies on bisexual and hermaphroditic free-living nematodes. *Journal of Morphology* **40**: 191-233.
- Kisiel, M. & Zuckerman, B.** (1974). Studies of aging *Turbatrix aceti*. *Nematologica* **20**: 277-282.
- Kitazume, H., Dayi, M., Tanaka, R. & Kikuchi, T.** (2018). Assessment of the behaviour and survival of nematodes under low oxygen concentrations. *PLoS One* **13**: e0197122.
- Klass, M.R.** (1977). Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mechanisms of Ageing and Development* **6**: 413-429.
- Klass, M.R.** (1983). A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. *Mechanisms of Ageing and Development* **22**: 279-286.
- Maklakov, A.A., Rowe, L. & Friberg, U.** (2015). Why organisms age: evolution of senescence under positive pleiotropy? *Bioessays* **37**: 802-807.
- Maures, T.J., Booth, L.N., Benayoun, B.A., Izrayelit, Y., Schroeder, F.C. & Brunet, A.** (2014). Males shorten the life span of *C. elegans* hermaphrodites via secreted compounds. *Science* **343**: 541-544.
- Mitchell, D.H., Stiles, J.W., Santelli, J. & Sandini, D.R.** (1979). Synchronous growth and aging of *Caenorhabditis elegans* in the presence of fluorodeoxyuridine. *The Journals of Gerontology* **34**: 28-36.
- Shook, D.R. & Johnson, T.E.** (1999). Quantitative trait loci affecting survival and fertility-related traits in *Caenorhabditis elegans* show genotype-environment interactions, pleiotropy and epistasis. *Genetics* **153**: 1233-1243.
- Smith, D.W.E.** (1989). Is greater female longevity a general finding among animals? *Biological Reviews* **64**: 1-12.

- Suzuki, K., Hyodo, M., Ishii, N. & Moriya, Y.** (1978). Properties of a strain of free-living nematode, *Rhabditis* sp.: life cycle and age-related mortality. *Experimental Gerontology* **13**: 323-333.
- Tain, L.S., Lozano, E., Sáez, A.G. & Leroi, A.M.** (2008). Dietary regulation of hypodermal polyploidization in *C. elegans*. *BMC Developmental Biology* **8**: 28.
- Ting, J.J., Woodruff, G.C., Leung, G., Shin, N.R., Cutter, A.D. & Haag, E.S.** (2014). Intense sperm-mediated sexual conflict promotes reproductive isolation in *Caenorhabditis* nematodes. *PloS Biology* **12**: e1001915.
- Weadick, C.J. & Sommer, R.J.** (2016). Mating system transitions drive life span evolution in *Pristionchus* Nematodes. *The American Naturalist* **187**(4): 517-531.
- Williams, P.D. & Day, T.** (2003). Antagonistic pleiotropy, mortality source interactions and the evolutionary theory of senescence. *Evolution* **57**: 1478-1488.