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Isolation of a long lifespan strain of *Drosophila melanogaster*.

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There is a substantial interest in the relationship between longevity and oxidative stress. It is well-known that longevity of an organism is a quantitative trait and is determined by the action of both genetic and environmental components. The longevity of an organism is thought to be evolved in relation to demands by the environment for fitness. The fruitfly *Drosophila melanogaster* has been used as a model in biological research on aging for exploring the longevity phenotypes, artificial and natural selection responses (Paaby and Schmidt, 2009). In the past decade or so, various groups have tried employing artificial selection to generate extended longevity strains and to use them as a tool to examine the mechanisms underlying aging. In *D. melanogaster*, much of the longevity research has been based on the artificial selection in the laboratory. The factors shown to be involved in longevity include coordinated and specific upregulation of different antioxidant genes, expression of heat shock proteins, mitochondrial differences, decreased ROS production or increased ROS scavenging activity, and calorie restriction (Arking, 2005). In *Drosophila*, energy metabolism and SOD account for less than 40% of the average difference in lifespan between long-lived and normal flies (Rose *et al*., 1992; Tyler *et al*., 1993). We have isolated a long lifespan (LLS) strain using laboratory population of *D. melanogaster*, which shows extended longevity when compared with their progenitor normal lifespan (NLS) flies. The LLS flies isolated will be a useful model to study the factors involved in the longevity of organisms.

D. melanogaster (Oregon K) was obtained from the Drosophila Stock Center, University of Mysore, Mysore, India. This stock was maintained in a vivarium at 22±1°C on standard wheat cream agar medium with 12:12 light and dark cycles. The virgin females and unmated males were collected within 6 h of eclosion. Isolation of LLS was carried out using this laboratory population of *D. melanogaster*. Freshly eclosed adults from vials set up with a density of about 25 eggs per vial were collected; the virgin females and unmated males were segregated within 6 h of their eclosion. Pair mating was conducted to obtain the progeny. Flies that lived longer than the NLS flies were selected, and the progenies of the same were monitored for further generations. The LLS lines were amplified further for future studies. The adult lifespan of reproducing flies was assessed by setting up 20 vials for each strain, with each vial containing 20 males or 20 females. Freshly eclosed NLS and LLS adults from vials set up with a density of about 25 eggs per vial were collected, and unmated flies of both the sexes were segregated within 6 h of eclosion. Flies were transferred to fresh food vials every alternate day and mortality was recorded daily; any flies dying were not replaced during the course of the assay, and that was continued until all flies died (Bharathi *et al.*, 2003).

Marked differences in the rate of survivability of *D. melanogaster* flies were found. The LLS flies lived longer when compared with NLS flies. We also observed gender differences in survivability in NLS flies (male, 12 wk; female, 14 wk); whereas there was no such difference in LLS flies, wherein both males and females lived for 18 weeks (Figures 1 and 2).

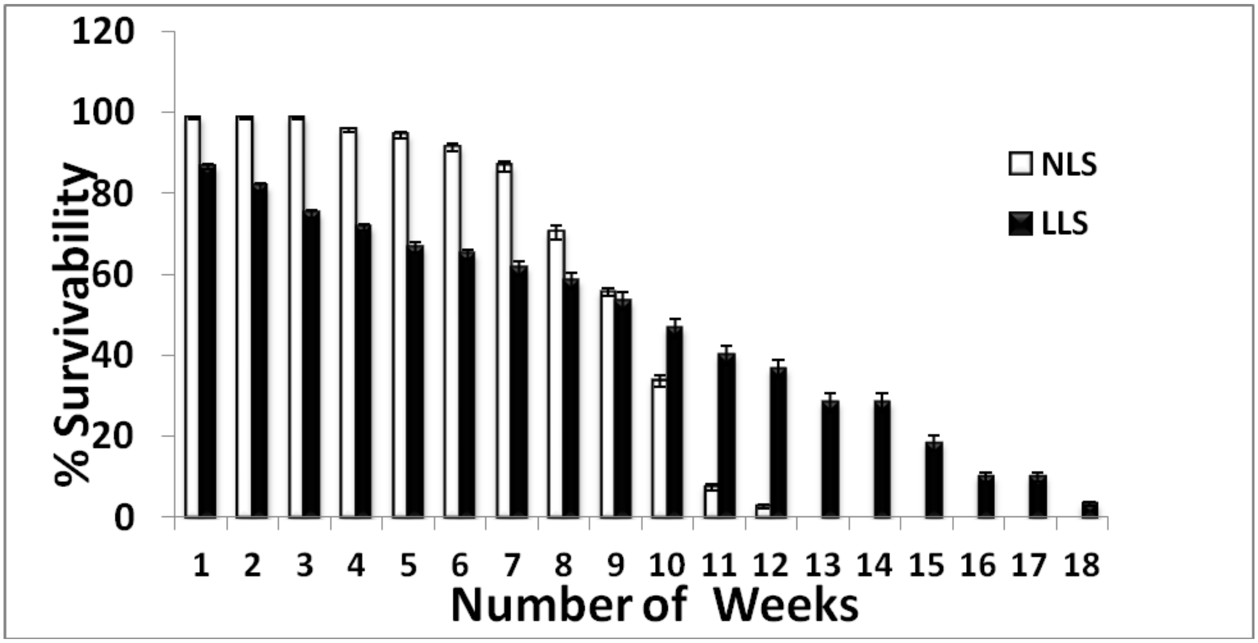

Figure 1. Mean lifespan of normal (NLS) and long lifespan (LLS) male *D. melanogaster* flies.

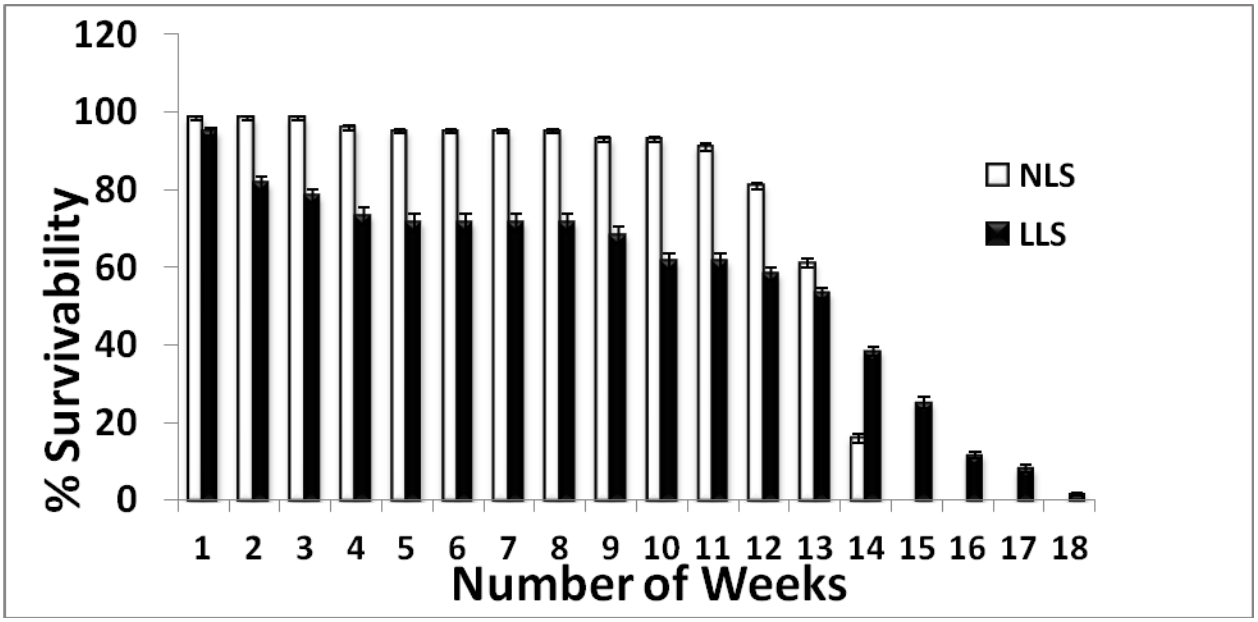

Figure 2. Mean lifespan of normal (NLS) and long lifespan (LLS) female *D. melanogaster* flies.

Various laboratories have reported long-lived strains of *Drosophila* using natural and artificial selection experiments. The long lifespan strains isolated in different laboratories are useful in unraveling the basic mechanisms of aging. According to free radical theory of aging (Harman, 1956), the "aging results from the deleterious effects of free radicals produced in the course of cellular metabolism." Luckinbill *et al.* (1984) and Arking (1987) made artificial selection to generate long-lived strains of *Drosophila* and compared them with the normal-lived progenitor strains. Selection for altered longevity was carried out by permitting reproductive success to occur at different times during the lifespan. Caloric restriction has been known for the past 75 years to be involved in extension of lifespan in rodents and *Drosophila*, and this attribute is conserved from yeast to rats (McCay *et al.*, 1989). Longevity strains have also been isolated through direct selection for delayed female fecundity (Luckinbill *et al.*, 1984; Arking, 1987; Partridge and Fowler, 1992). There are also reports of direct selection for desiccation resistance (Hoffmann and Parsons, 1989) or starvation resistance (Rose *et al.*, 1992) and thereby indirectly selected for extended longevity. Many transgenic experiments have been carried out to test antioxidant theory of aging, which exhibited resistance to stress-inducing compounds and showed increased lifespan. Several laboratories have shown various characteristics of the long-lived strains, which include increased lipid content, increased starvation and desiccation resistance, longer flight duration, high glycogen content, higher stress resistance, and so forth (*c.f.*, Arking, 2005). Therefore, longevity is associated with many factors. The LLS strain we have isolated will be useful to investigate the factors that influence extended lifespan when compared with the NLS strain, particularly with reference to oxidative stress and antioxidant enzymes.

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Importance of non genetic factor (male age) in mating success of *Drosophila bipectinata.*

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