ABSTRACT

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Senescence and the Y Chromosome
(Under the Direction of Kelly Dyer)

The Y chromosome is a non-recombining, patrilineal chromosome that is comprised of a few male specific genes, repetitive sequences, and transposable elements (Graves 2006). It is well known for its role in sex determination and male fertility, but recent research revealed a new activity for the Y chromosome: control of gene expression on other chromosomes in the genome. In 2008 Benardo Lemos discovered that, in *Drosophila melanogaster,* polymorphisms on the Y chromosome have differential effects on expression of autosomal and X-linked genes. Many of these genes are involved in pathways related to cellular stability and repair, which are mechanisms that have a role in the aging process. This led to the hypothesis that the Y chromosome may have an influence on aging. To study this possibility we created an isogenic line of *D. melanogaster*, and introduced eighteen Y chromosomes to this line: nine from African populations and nine from North American populations. We measured senescence in these flies by monitoring death rate over a 60 day period. We found significant variation in rate of aging in both male and female flies, hinting at the complex nature of studies of the aging process.

INDEX WORDS: Senescence, Y chromosome, *Drosophila melanogaster*, aging

SENESCENCE AND THE Y CHROMOSOME

by

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INTRODUCTION:

Senescence is the age related decline in physiological function. Senescence occurs in almost all living things, but limits the life span of animals in particular (reviewed in Kirkwood and Austad 2000). There have been two main approaches to the study of why aging occurs: research done on the level of the molecular biology, and investigation from an evolutionary point of view.

The molecular approach examines changes that occur within cells as they age. Telomere shortening, insulin stress, and free radical exposure are a few of the molecular changes that have been studied as causes of aging. Linear chromosomes cannot fully replicate themselves using the 5'-3' template strand so they have repetitive sequences at their ends called telomeres. Telomeres protect the chromosome from losing important information, by losing the telomeres instead (Alberts 2008). The telomere theory of aging states that as an organism ages the telomeres are lost, and this limits the lifespan of the organism (Olovnikov 1973). Another theory is that insulin and insulin-like growth factors may influence aging in a way that is related to the theory of aging due to oxidative damage in the mitochondrial genome (Hansen 2005). Oxidative damage to the mitochondrial genome is another molecular theory of aging which states that the mitochondrial genome is especially vulnerable to oxidative damage since oxygen free radicals are produced in the mitochondria as part of cellular respiration. Research has also been done on the possibility that nuclear chromosomes also incur damage due to free radical exposure. These theories state that as an organism ages, the damage from free radicals accumulates, impairing cellular function and causing senescence (Harman 1972, Miquel 1980, Larsen 1993).

The evolutionary approach focuses on two main theories. The first is the mutation accumulation theory. This idea is similar to the accumulation of DNA damage idea, however, instead of over the lifetime of an individual, this idea involves heritable mutations that are passed from one generation to the next. Deleterious mutations are usually removed from a population by natural selection, but selection against a mutation weakens the later in an individual's lifetime that mutation manifests. If a harmful mutation affects an organism well after it reaches reproductive age it can remain in the population in spite of its negative effects because the organism has most likely already reproduced and passed on that mutation. In this way, mutations that act late in life are invisible to the pressure of natural selection (reviewed in Promislow and Bronkowski 2006, original idea by Medawar 1952).

The second evolutionary theory of aging is antagonistic pleiotropy. Pleiotropy is when a single gene influences multiple phenotypic traits. Antagonistic pleiotropy describes when a gene controls traits that are beneficial and other traits that are detrimental. The antagonistic pleiotropy theory of aging is the idea that genes which cause senescence may also control other traits that are beneficial such as higher reproductive success early in life (Williams 1957). Therefore, as with the theory of accumulation of mutations, these genes do not experience negative selective pressure since they do not act until late in life, or, alternatively, are subject to positive selective pressure due to their beneficial effects in an organism's early life outweighing their contribution to the detrimental effects of aging.

Bernardo Lemos and colleagues published evidence that the Y chromosome can alter expression of autosomal and X-linked genes using isogenic lines of *Drosophila melanogaster* that differed only in the Y chromosome (Lemos 2008). The Y chromosome in *Drosophila* is a non-recombining chromosome that has few genes, most of which function in male fertility, and

large heterochromatic regions of transposable elements and repetitive sequences. These characteristics are typical for Y chromosomes in many species, including humans (Chippindale and Rice 2001). The dearth of genes on the Y chromosome led to the hypothesis that it has little effect on fitness outside of as it relates to male fertility. However, Lemos' findings suggest another way that the Y chromosome may influence an organism's fitness, particularly since many of the genes whose expression was altered were related to important biological processes, such as cytoskeletal stability and lipid metabolism. Both of these processes play important roles in cellular stability (Alberts 2008). Cellular stability and repair are characteristics that decline with age due to senescence (Hayflick 1965, reviewed in Adams 2009). These findings led to our hypothesis that the Y chromosomes may influence the overall aging process. The mutation accumulation theory, and Y chromosome's non-recombining nature provide a sound theoretical foundation for this hypothesis. Recombination breaks up deleterious mutation in population, but, since the Y chromosome does not recombine, it can serve as a reservoir for deleterious mutations including mutations that contribute to senescence. We tested this hypothesis by measuring lifespan in male flies possessing Y chromosomes from two geographic regions.

MATERIALS AND METHODS:

Creating Isogenic Lines

The first step was to create a genetic background that Y chromosomes could be introduced into. We created a lab stock with the genotype *y; bw; e; sv*^{n}. This genotype represents a visible recessive trait on each of the four chromosomes in the *Drosophila* genome (2 autosomal chromosomes, X chromosome, and dot chromosome): yellow body color (*y*); brown eyes (*bw*); ebony body color (*e*); and singed bristles (*sv*^{n}). These four mutations were used to be sure that the stock flies were of the right genotype. This began with laboratory stock flies from the

Bloomington Drosophila Stock Center at Indiana University of the genotype $y^1; bw^1; e^4; ci^1, ey^R$ (stock number 4361). The eyeless mutation (*ey)* may have an effect on aging (Clements *et al.* 2008). Thus, we eliminated this mutation by crossing $y^1; bw^1; e^4; ci^1, ey^R$ females with males that were wildtype except for a specific mutation sv^n on the fourth chromosome (+;+;+,+, sv^n, stock number 663). The male offspring were back-crossed to the original female, and the male and female *y; bw; e;* +++ offspring were collected and crossed together. We collected males and females of phenotype *y; bw; e;* +,+, sv^n from this cross. Finally, three generations of single pair brother/sister crosses were performed to ensure high genome-wide homozygosity.

Next, we introduced eighteen different Y chromosomes, from two distinct geographic areas, into the stock to create eighteen lines of the flies that were isogenic except for on the Y chromosome. Five *y; bw; e;* sv^n females were crossed to a single wildtype male from each of nine isofemale lines of *Drosophila melanogaster* collected in Malawi, Africa, and from each of nine isogenic wildtype lines collected in Raleigh, North Carolina. Only M-type North Carolina lines were used to control for the effects of hybrid dysgenesis (Mackay 1989). African lines, however, were not characterized for P elements. We back-crossed the male offspring to lab stock *y; bw; e;* sv^n females, and sorted the offspring of this second cross based on phenotype. We crossed male and virgin female *y; bw; e;* sv^n to create eighteen lines of flies that varied only on the Y chromosomes in the males. This experimental design resulted in females that were identical genetically both within and among lines, and males that were genetically identical within lines, and genetically identical across lines except in the Y chromosome (Figures 1 and 2).

Testing Senescence

Flies for the senescence assay were grown at 24 degrees C on a 12 hour light/dark cycle in 100 mL plastic bottles containing molasses food. We collected twenty-five non-virgin females

and fifteen males per line and placed them in bottles with molasses food and yeast paste. Flies were left to lay eggs in the bottle for about 4 days then removed. Before removing the flies, the bottles were inspected under a dissection microscope to ensure the number of eggs in each bottle were approximately the same. Once the larvae grew and began to eclose from pupal casings as adults, we collected male and female flies as virgins over a period of two days. We separated one hundred males and fifty females per-line into groups of ten and placed them into vials containing molasses food. Vials were numbered 1 to 270, and then renumbered using a random number scrambler (www.random.org/sequences). The renumbering ensured that longevity was measured blindly, and controlled for micro-climate effects in the incubator. The vials were stored in a controlled environment at 24 degrees C and 60% relative humidity on a 12 hour light/dark cycle. Every other day we transferred the flies to new molasses food vials, and recorded the number dead.

RESULTS:

The mean survival time for males and females from each line is shown in Tables 1 and 2. All of the flies died within 60 days, with an average lifespan for females of 27.67 days and an average lifespan for males of 20.35 days. Females live significantly longer than males (Log-Rank χ^2 =229.4521, df=1, P=<0.0001; Wilcoxon: χ^2 =177.2755, df=1, P=<0.0001) (Figure 3). When we compare African and North American flies, mean survival time was 19.5 days for African males, vs. 21.2 days for North American males (Figure 4). This difference was significant using both Log-Rank (P=0.0035) and Cox Hazard analysis (P=0.0010). Females from North American lines also live longer than females from African lines (mean survival time: 25.2 days for African females, and 30 days for North American females). This difference was

also significant using both Log-Rank (P=<0.0001) and Cox hazard analysis (P=<0.0001) (Figure 5).

We also examined variation among lines within each region, and found greater variation in survival time among lines for females than for males. The greatest variation was among the female African lines ((Log-Rank χ^2 =34.0459, df=8, P=<0.0001; Wilcoxon: χ^2 =37.5221, df=8, P=<0.0001)), we found virtually the same amount of variation between female North American lines (Log-Rank χ^2 =51.2476, df=8, P=<0.0001; Wilcoxon: χ^2 =33.4126 df=7, P=<0.0001). Overall, there was much less variation among lines for males than for females, and variation among North American males was only marginally different than among African males (North American males: Log-Rank χ^2 =15.7557, df=8, P=0.0162; Wilcoxon: χ^2 =20.0667, df=8, P=0.0101. African males: Log-Rank χ^2 =15.1348, df=8, P=0.0566; Wilcoxon: χ^2 =16.8079, df=8, P=0.0322)

DISCUSSION:

Our experiment measured lifespan as an indicator of senescence. We tested the longevity of both males and females from each Y line. The females are identical genetically both within and across Y-lines, whereas males are genetically identical within lines and differ among lines in their Y chromosome. The females, therefore, serve as a control group since they are genetically identical across lines. Our longevity experiment yielded several interesting results, among which include that females have a longer lifespan than males, that lines with a North American Y chromosome live longer than lines with an African Y chromosome, and that within each region there is variation among lines in longevity, among both males and females.

We found a significant difference in death rate between geographic regions for both males and females. These results were contrary to the hypothesis that aging rate between males would

vary due to variation in the Y chromosome, but not between females since females across lines have identical genotypes. A possible explanation for this result is that the African flies were not screened for P-elements. The greater variation between female African flies supports the possibility that the variation between African females was caused by P-elements. However, the North American females we used were all M-type, so this explanation does not account for all of the variation found in females. In addition to the biological explanation of hybrid dysgenesis, the greater variation between female flies may also be due to the fact that we screened half as many females and males. At lower sample size, there is greater potential for variation that is actually due to chance appearing significant.

On average, the flies in our study had shorter lifespans than observed in other studies of *Drosophila* (Leips and Mackay 2002, Ayroles 2008). The most probable reason for this result is that we used a genetic background that was highly inbred and heavily mutated. This background may have led to weakened lines of *Drosophila*. Indeed, while handling the flies during experiments, we observed that both African and North American wildtype flies were faster and more difficult to anesthetize using carbon dioxide than labstock flies, suggesting a general lack of vigor in the labstock. As a test of this hypothesis we will include control groups of labstock flies without introduced wildtype Y chromosomes in the next longevity experiment. If the mutated chromosomes are the cause of the shorter lifespans the complete labstock flies should have even shorter lifespans than flies from the Y extraction lines.

Another notable result was that, for both sexes, North American flies lived longer than African flies. A possible explanation for this may be that the temperature inside the incubator (24 degrees C) was more similar to a temperate environment than a tropical environment, therefore North American flies may be better adapted to living in those conditions. This idea may also

explain why variation among lines for males was similar for lines from North America and Africa. Because *D. melanogaster* generally has higher genetic diversity in Africa relative to North America (Andolfatto and Przeworski 2001), we would have predicted more variation among African males than among North American males. To test this idea we will run another experiment monitoring longevity with flies held at a warmer temperature. If adaptation to different environmental temperatures is the cause of the longer survival time of North American flies, we would expect opposite results, African flies living longer at a warmer temperature.

Repetitive elements such as transposable elements are especially common on the Y chromosome, and previous work in plants has shown that transcription elements increase activity during stressful conditions (reviewed in Grandbastien 1998). Therefore, it may be that the Y chromosome has more influence on senescence under stressful conditions, because TEs are more active and prone to affect non-Y linked genes in a stressful environment. To investigate this possibility we will test the Y extraction lines for heat shock tolerance, chill coma recovery, oxidative stress resistance, and male fertility. Evidence has been found for variation in heat shock tolerance and male fertility related to the Y chromosome (Lemos 2008). These processes, as well as cold shock and oxidative stress, are also related to the aging process (Morrow and Tanguay 2003, Le Bourg 2007).

Finally, in future experiments we will investigate whether Y chromosome based regulation correlates to genetic polymorphism on the Y chromosome. We will do this by sequencing a Y chromosome gene *kl-5*. Polymorphism in the *kl-5* gene will represent polymorphism on the Y chromosome as a whole. High polymorphism in the *kl-5* gene would suggest that variation in aging is related to genetic variation on the Y chromosome. Little polymorphism, however, may indicate either that variation in rate of senescence involves

variation in epigenetic modifications to the Y chromosome rather than differences in nucleotide sequence, or that if there is a genetic basic to Y-linked regulation, it is not associated with genetic variants at *kl-5*.

In conclusion, our project explores one of the fundamental life processes, and the results we obtained illustrate the biological complexity of the aging process. Although further study is needed to conclude whether the Y chromosomes does or does not influence the aging process, our work will lead to a deeper understanding of the aging process. It may also have implications for the human aging process because the Y chromosome is male specific in humans as well as *Drosophila*. This project may help answer the question of why certain people live longer than others, and why we age at all.

TABLES:

Table 1: Mean lifespan of female African (MM) and North American (stock number) lines.

Line	**Lifespan in Days**
MM 1-1	24.8
MM 10-1	27.81
MM 3-5	23.24
MM 4-2	25.73
MM 5-4	25.82
MM 6-4	26.71
MM 7-1	26.67
MM 8-3	29.68
MM 9-3	16.71
25176	31.2
25181	35.82
25185	31.57
25187	26.21
25188	28.02
25190	36.67
25191	27.04
25204	28.6
25208	26.14

Table 2: Mean lifespan of male African (MM) and North American (stock number) lines.

Line	**Lifespan in Days**
MM 1-1	19.47
MM 10-1	17.84
MM 3-5	18.21
MM 4-2	18.43
MM 5-4	18.29
MM 6-4	19.3
MM 7-1	19.47
MM 8-3	21.55
MM 9-3	22.91
25176	22.09
25181	24.26
25185	20.87
25187	21.67
25188	18.14
25190	23
25191	19.86
25204	21.84
25208	19.33

FIGURES:

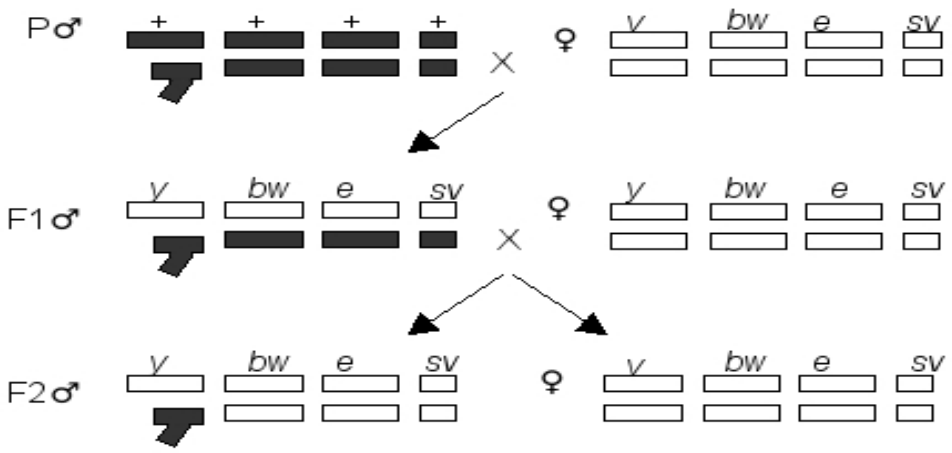

Figure 1: Introducing a wildtype (grey) Y chromosome onto the labstock (white) genetic background.

African Y

North American Y

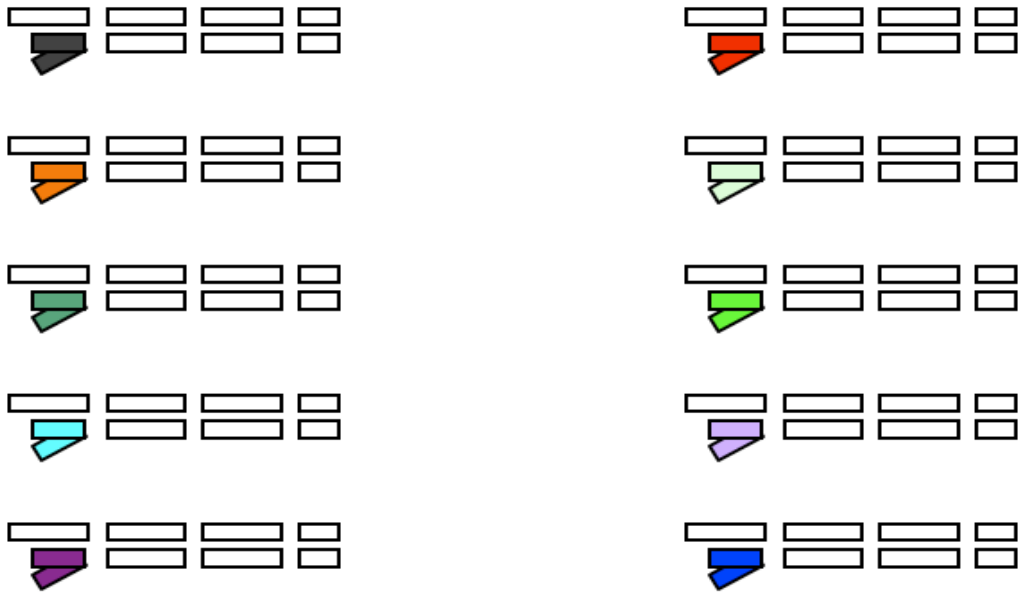

Figure 2: Isogenic Lines: Different Y chromosomes on an identical genetic background.

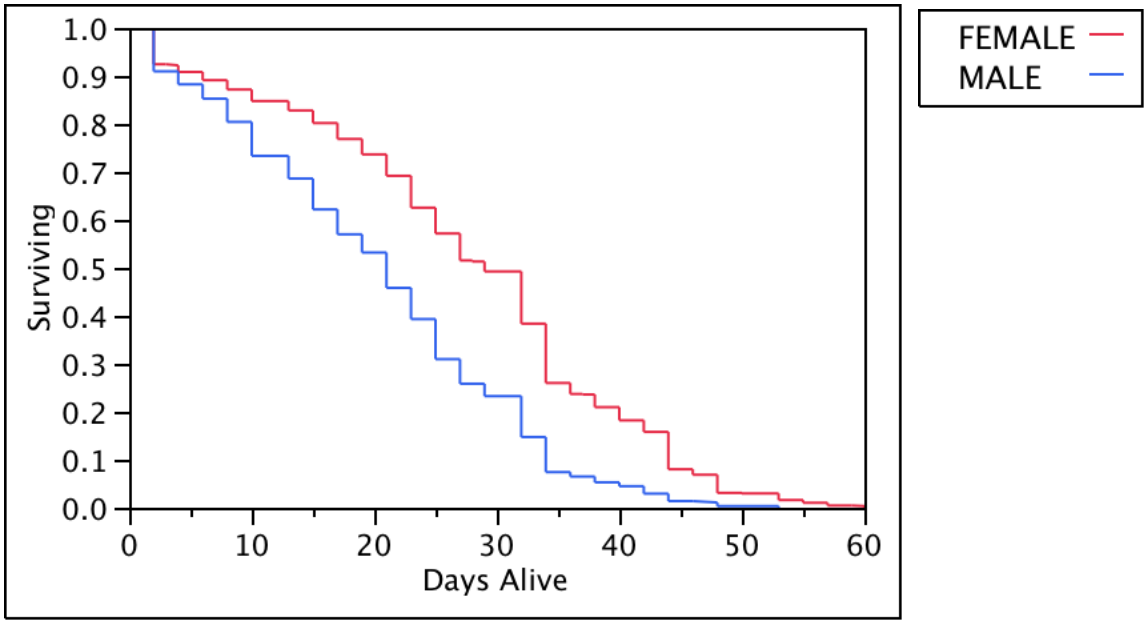

Figure 3: Survival plot for female vs. male flies.

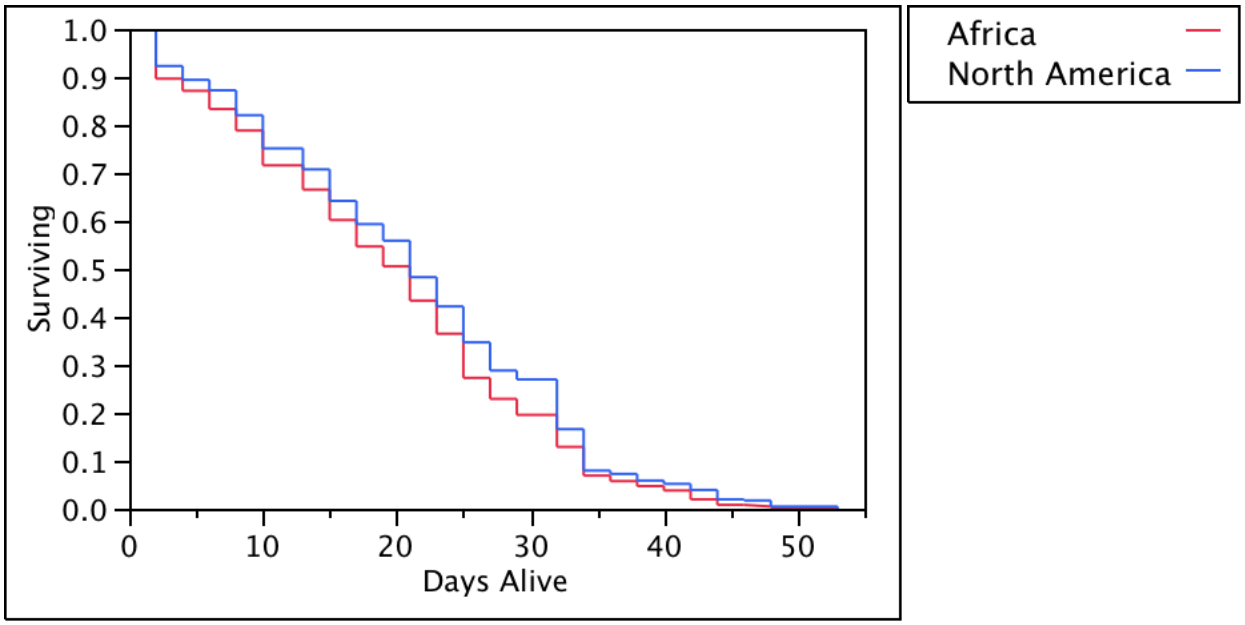

Figure 4: Survival plot African males vs. North American males.

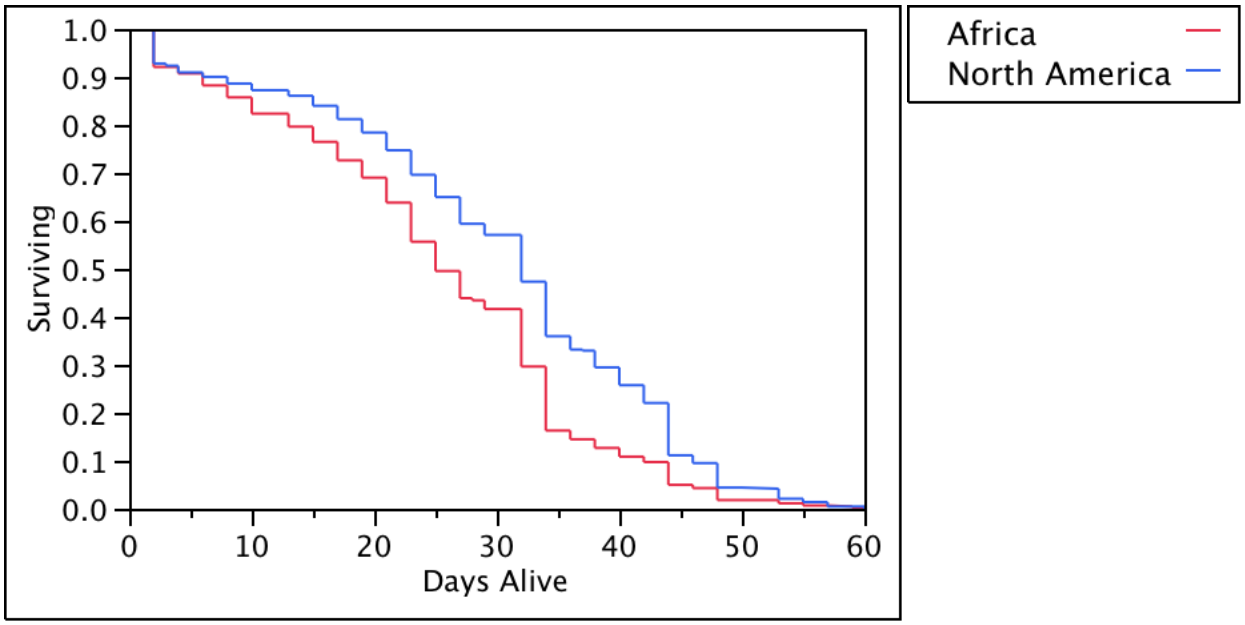

Figure 5: Survival plot African females vs. North American females.

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