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Aging in the planarian, *Schmidtea mediterranea*

A Thesis submitted in partial satisfaction of the requirements for the degree
Master of Science

in

Biology

by

David Hoang Nguyen

Committee in charge:

Professor Lin Chao, Chair
Professor Carolyn Kurle
Professor Scott Rifkin

2012

The Thesis of David Hoang Nguyen is approved and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

University of California, San Diego

2012

DEDICATION

Dedicated to my parents, Michelle and Hoa Nguyen, for their love and support throughout my education.

Special recognition also goes to my best friend, Linh Truong, and my two canine companions, Biscuit and Emma.

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ABSTRACT OF THE THESIS

Aging in the planarian, *Schmidtea mediterranea*

by

David Hoang Nguyen

Master of Science in Biology

University of California, San Diego, 2012

Professor Lin Chao, Chair

Planarians, free-living flatworms, are able to regenerate complete individuals from miniscule fragments. However, little is known about the effects of regeneration on aging, or senescence, in planarians. Using an asexual strain of the planarian, *Schmidtea mediterranea*, we studied long-term differences in aging between the two fragments of fission, the anterior (head) and posterior (tail), by tracking fecundity and viability. Fecundity was quantified by measuring patterns in the time interval between fissions. Viability was tracked as survival rates over time. We created separate lines of

successive heads and successive tails for observation. We present the first demonstration that the head and tail products of fission are not identical with respect to aging. Lines of successive heads showed aging through reduced fecundity over time; and all lines ultimately died out. Conversely, lines of successive tails showed no aging based on uniform fecundity and no recorded deaths. Furthermore, we found that all tails were equally rejuvenated regardless of the age of the original planarian at the time of fission. Thus, asexual fission can act as a mechanism for rejuvenation in tails at the cost of aging in heads.

Introduction

Ageing, also known as senescence, is the progressive degradation of physiological function with time. In fact, deteriorating fecundity and viability are hallmark characteristics of increasing age in nearly all forms of life. The topic of senescence in organisms which reproduce by asexual fission remains controversial. Studies of asexual organisms ranging from single-celled bacteria (Rang, Peng, Chao, 2011) to the multicellular *Stenostotum* have demonstrated that the products of fission are not necessarily identical (Sonneborn, 1930). Some strains of planarians, free-living flatworms, also exhibit modes of asexual fission into two fission fragments which each have the capacity to regenerate complete individuals (Morgan, 1898) due to neoblasts (planarian totipotent stem cells). Such regenerative ability has caused some researchers to speculate that planarians do not senesce at all.

Indeed, for nearly 100 years numerous investigators have studied regeneration in planarians; which were considered to be “immortal under the edge of the knife” (Reddien, 2004; Dalyell 1814). However, despite the well-documented phenomena of regeneration, the potential longevity of planarians without intervention by cuts of the knife is poorly understood. Unaided asexual fission in planarians produces anterior and posterior fragments that may illustrate differences in regenerative ability over time. Still, no studies have been conducted to compare long term viability of anterior versus posterior fission fragments in planarians.

To investigate the difference between anteriors (heads) and posteriors (tails) we use the asexual strain of the planarian, *Schmidtea mediterranea*. We observe the

difference between heads and tails by following lines of successive heads and lines of successive tails over time. Fission occurs in the posterior two-thirds of the planarian and the complex organ structures are retained in the head (Newmark, 2002); whereas the tail is reborn, initially lacking mobility, size, and digestion. Asymmetry between the heads and tails manifests as measureable differences in reproduction and viability. Tracking and analyzing patterns in the fission interval, or the time elapsed between fissions, provided a means to quantify senescence in *S.mediterranea*.

Materials and Methods

Culture Conditions

A group of 25 asexual specimens of *S.mediterranea* was obtained from the clonal line, CIW4, established by Phillip Newmark and Sánchez Alvarado (Sánchez Alvarado, 2006). The asexual, diploid ($2n=8$) strain of *S. mediterranea* was found over 40 years ago in an artificial pond at Montjuïc near Barcelona, Spain. This species was initially identified by J. Baguña and R. Ballester as *Dugesia lugubris* s.l. (Benazzi et al. 1970) and would ultimately be reclassified as *Schmidtea mediterranea* (Baguña 1999).

The cultures were maintained in dark incubators at 21°C and only exposed to ambient light during feeding for brief periods (~1hr). Culture water was mixed to simulate Montjuïc salt conditions (Cebrià and Newmark, 2005) using 18 MΩ resistance Milli-Q H₂O supplemented with 1.0mM CaCl₂, 0.1 mM KCL, 1.6 mM NaCl, 1.2 mM NaHCO₃, 0.1 mM MgCl₂, and 1.0 mM MgSO₄ and adjusted to the pH of 7.0 with HCL and/or NaOH. Animals were fed twice weekly with homogenized baby beef liver. Approximately 1 pound of fresh baby beef liver was trimmed of visible veins, connective tissue, and fat, then cubed and blended into a homogenous purée. The purée was transferred and sealed into Ziploc bags after residual air bubbles were eliminated and frozen at -20°C. In order to ensure consistent nutritional quality, small amounts of fresh liver were prepared every two months. All individuals were maintained under uniform protocols and conditions for feedings and water.

The original 25 animals from Newmark were kept in 300ml of prepared water in a plastic Tupperware container as a communal pool. For individual observation during experiments, each specimen was isolated in its own well with 5ml of prepared culture water using 6-well petri dishes. Immediately following each feeding, worms were transferred via disposable pipet to a clean well containing new culture water with matching temperature and pH.

Results

Experiment 1: Aging in Heads.

To determine whether heads age, experiment 1 established lines of successive heads to monitor patterns of fission interval over time. Two cohorts were observed, 1A and 1B. In Experiment 1A, 7 planarians measuring 8mm in length were isolated in individual Petri wells. After the first fission from each of the original 7 planarians, the head was retained and transferred to a new individual Petri dish and the newborn tail was removed for later use (experiment 2). For subsequent fissions, the head was again isolated and the time interval between fissions was recorded. Experiment 1A lasted from October 2009 to May 2012; at which point all 7 lines had died out. Experiment 1B contained the second cohort of 10 planarians initially measured at 7mm in length; and was established as a line of successive heads in the same manner as part 1A above. Experiment 1B lasted from January 2011 to April 2012.

The results of experiment 1A (Fig 1a) and 1B (Fig 1b) show that the mean fission interval of both cohorts 1A and 1B increased over time. In experiment 1A, the mean fission interval in lines of heads increased by a factor of approximately 8 (19 to 154 days, Fig 1a). Over the course of 935 days, all lines of heads in experiment 1A ultimately died out. In experiment 1B, the mean fission interval in lines of heads increased over the course of 450 days by a factor of approximately 4 (16 to 65 days, Fig 1b).

The data for experiment 1A and 1B were also presented as a cumulative curve to understand patterns of fission rate through time (Fig 2). This alternative analysis depicts the fission rate (slope of the curve) decreasing over time as the cumulative number of fissions rises. Both Fig 2a and 2b show a continuous decline in slope (Fig 2a: -2.4×10^{-5} ; Fig 2b: -4.0×10^{-5}): and thus, in fission rate, with escalating number of fissions in a line of heads.

Experiment 2: Aging in Tails.

To determine whether tails age, experiment 2 established lines of successive tails to monitor patterns of fission interval. The same original 7 planarians that started the lines of heads in experiment 1A also gave rise to the lines of tails in experiment 2. However, after the first fission, the newborn tail was transferred away from the head used in experiment 1A and isolated in a new individual Petri dish. For subsequent fissions, the tail was again retained while the head was discarded; and the time interval between fissions was recorded. Experiment 2 lasted from October 2009 to May 2012. Lines of successive tails did not exhibit a significant difference in fission interval over 935 days ($F_{1,99}=0.42$, $p=0.52$, Fig 3).

Experiment 3: Aging in tails born from old heads

To determine if tails born from old heads were rejuvenated, we measured the time interval from birth to first fission of tails born at 3 periods during experiment 1A (aging heads). The length of each period was determined by the time required for all 7 of the lines of heads to produce a tail. The 3 periods were (1) 10/29/2009 to

12/16/2009, (2) 3/11/2011 to 5/31/2011, and (3) 8/22/2011 to 1/31/2012. Tails did not exhibit a significant difference in the time interval to first fission regardless of the age of the head from which fission occurred. ($F_{1,120}=1.4$, $p=0.25$, Fig 4).

Experiment 4: Aging in heads started from a tail.

To observe the pattern of aging in a planarian starting from birth as a tail, we isolated 10 tails born on 9/13/2010 in individual petri wells. After each fission, the head was retained and transferred to a new individual Petri dish and the tail was discarded. The time interval between fissions was recorded. Therefore, the lines in experiment 4 were continued as successive heads similar to experiment 1; with the caveat that in experiment 4, lines of heads started from “young” 1.5mm tails rather than from “old” 7 or 8mm adults. Experiment 4 was conducted from September 2010 to May 2012.

A trendline of the mean fission interval over time (Fig 5) shows a U-shaped curve. Initially, the interval between fissions is high but decreasing with time. The lowest point of the curve denotes the shortest fission interval and the peak period of reproduction. In the period following the vertex, the mean fission interval begins to rise.

The data for experiment 4 was also presented as a cumulative curve to understand patterns of fission rate with time (Fig 6). The slope of the curve is positively correlated with the rate of fission and is sigmoid in shape. However, unlike the continually decreasing fission rate of old heads (Fig 2, experiment 1), the fission

rate of young heads first increases (Fig 6, experiment 4), then decreases after the peak fission rate at the inflection point. Therefore, we find that over time, and with an escalating number of fissions, the behavior of the slope for young heads begins to resemble the decreasing slope of the old heads. Using slope, we merge the cumulative curves to construct a life history of total fissions and total lifespan in lines of successive heads.

Merging data from birth to death

Initially uncertain of the role of senescence in *S.mediterranea* as well as the time scale necessary to measure aging, we designed the first experiment to maximize the probability of observing aging while minimizing the time required. Therefore, we began experiment 1 with old heads, as large in size as possible. Upon finding evidence of decreasing fecundity in old heads, we sought to record the pattern of fission in young heads starting from birth as a tail. The resulting two fragments of life history needed to be fused together. To quantify the expected total lifespan and total number of fissions in a line of successive heads, we were able to merge the three cumulative curves from Fig 2a, Fig 2b, and Fig 6 and predict average life expectancy and fecundity.

The cumulative curves were aligned by sliding the first derivatives of the original cumulative functions against one another along the x-axis to determine the intersection with minimum squared difference in slope. Fig 7 shows the range of slope intersections for each pair of functions. The lowest point on the curve corresponds with the best match (smallest squared difference) between functions.

The final result is a complete picture of the fission interval pattern in lines of successive heads from birth to death (Fig 8). The accompanying cumulative graph (Fig 9) shows the mean total number of fissions in the lifespan of *S. mediterranea* under the specific conditions of these experiments. The result of merging the cumulative curves shows that the average total lifespan of a line of successive heads under the specific conditions of these experiments is approximately 1376 days, or 3.8 years. The average total number of fissions during this period is approximately 23 fissions.

Discussion

An investigation of the long-term differences between the head and tail fragments of fission requires the establishment of separate lines of successive heads and lines of successive tails. Lines of heads and tails generate data on the interval between fissions. The patterns of fission intervals are a metric of fecundity in asexual organisms such as *S. mediterranea* and decreasing fecundity is a characteristic of senescence. Therefore, senescence can be quantified if fission intervals increase; indicating deterioration of fecundity.

The absence of prior studies comparing long-term fecundity of heads versus tails led our first experiments to investigate the existence of observable senescence in order to validate further study. We hypothesized that signs of aging would appear most rapidly in the largest individuals already at the prime of physiological development. Though planarians are capable of degrowth in response to starvation (Newmark, 2002), the factor of size plasticity due to nutritional availability was negated by consistency in the timing and nutritional quality of feedings across all individuals. Therefore, in order to maximize chances of observing senescence, the largest individuals, measuring 7mm (Fig 1b) and 8mm (Fig 1a) in length, were selected to begin lines of successive heads.

In lines of old heads, which we began to observe around mid-life as large individuals, senescence is evident not only through the manifestation of increasing fission intervals (Fig 1) but also in the ultimate demise of all 7 lines in experiment 1A.

However, does the pattern of fission intervals look different for a line of heads that started young from a newborn tail? Indeed, there are major differences due to phases of development. Though fission intervals in lines of older heads only ever increase over time (Fig 1), lines of heads started from a tail exhibit a U-shaped curve (Fig 5). The early (left) phase of Fig 5 shows high fission intervals which decrease with time due to an initial period of developmental growth. The later (right) phase of the experiment shows increasing fission intervals indicating reproductive deterioration, reminiscent of the older heads in Fig 1.

In addition, a comparison can be made between fission intervals and the cumulative number of fissions. It is clear from Fig 2 and Fig 6 that the fission intervals do not behave linearly to a rising cumulative number of fissions. The slope of this relationship is directly correlated to the fission rate and is useful to determine the junction point that joins the data from old heads and young heads. The smallest value for the squared difference of slopes reveals the intersection (Fig 7) where the young and old heads had nearly equivalent fission rates and were the same age. In this way, the functions depicting reproductive patterns for lines of heads can be connected. Thus, the merged data is a life history of reproductive rate for lines of heads, starting from birth as a tail until death (Fig 9). We demonstrate that over time, the fission rate in lines of successive heads of *S.mediterranea* slows and approaches zero. Furthermore, the survivability of the eldest planarians drops to zero. This confirms the progressive degradation of both fecundity and viability with time in lines of successive heads.

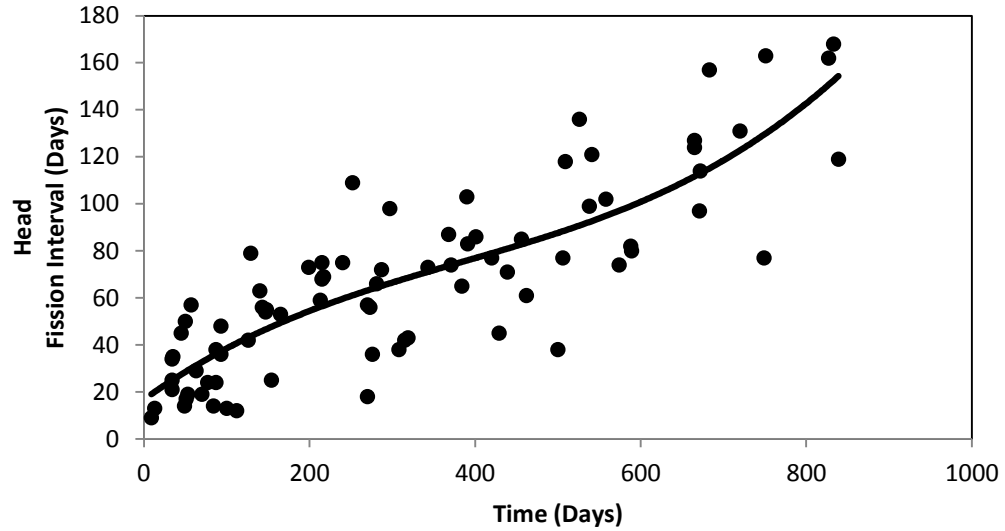
Sonneborn made a relevant remark in his work on the asexual *Stenostotum*, a member of a more basal order of Platyhelminthes than *S.mediterranea*. He states that a line of successive heads show the characteristics of a single individual that develops, grows old, and finally dies while giving rise to new young individuals at the tail end (Sonneborn, 1930). Thus, an illustrative metaphor for the relationship between head and tail fragments is that of a mother (head) and daughter (tail), in that the tail is rejuvenated. A tail, much like a germ line, is regenerated due to the process of rebuilding new tissues from pluripotent stem cells, called neoblasts in planarians (Newmark, 2002).

Therefore, the asymmetric allotment of tissue from the original individual plays a major factor in the aging differences between the head and tail. Immediately following fission of an individual, the tail (~1.5 - 2mm) is small relative to the head (~5 - 7mm) and lacks specialized tissues. The size discrepancy may be relevant to the ability of successive tail lines to remain immortal in terms of unchanging reproductive frequency. Fission occurs in the posterior two-thirds of the animal; meaning that the head fragment retains the majority of the original tissue, including the complex organs. On the other hand, the tail must regenerate these differentiated structures anew from its pluripotent neoblast cell populations. It is possible that the formation of a brain, pharynx, and other complex organs *de novo* can revitalize the tail and prevent senescence (Newmark, 2002). Lines of successive tails, unlike heads, did not show degradation of fecundity or viability over time, with neither increased fission intervals nor recorded deaths (Fig 3).

Rejuvenation of the tail not only occurs in lines of successive tails, but also in the tails produced by lines of successive heads (Fig 4). In fact, tails maintained a constant average time interval to first fission, approximately 60 days (Fig 1, 4), regardless of the age of the individual producing the tail. New tails are unhindered by the history of their origins. They are reborn. The implications of these findings suggest that *S. mediterranea* is capable of immortality through asexual fission and regeneration. However, both products of fission are not identical in this immortal ability; and the cost is the ultimate aging of the head.

Figures

a)



b)

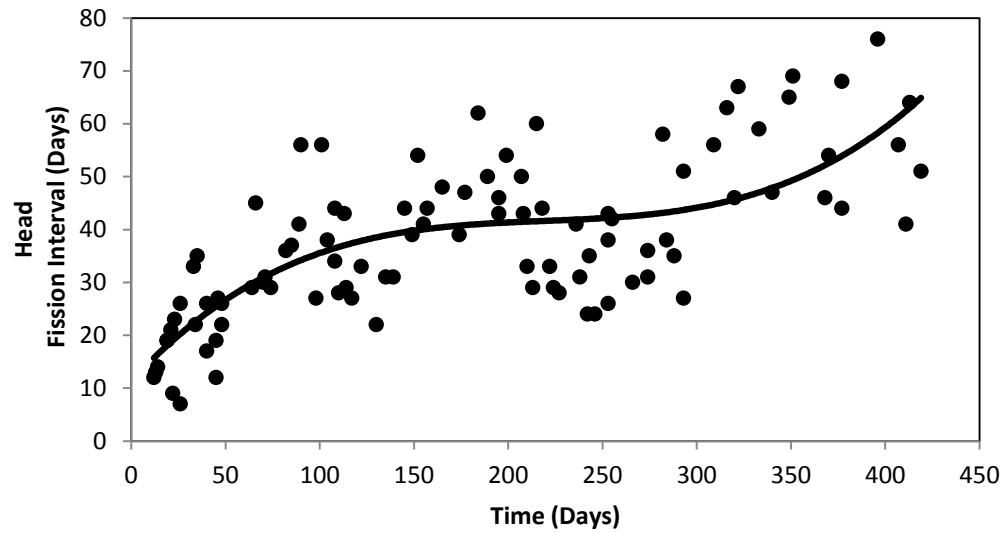
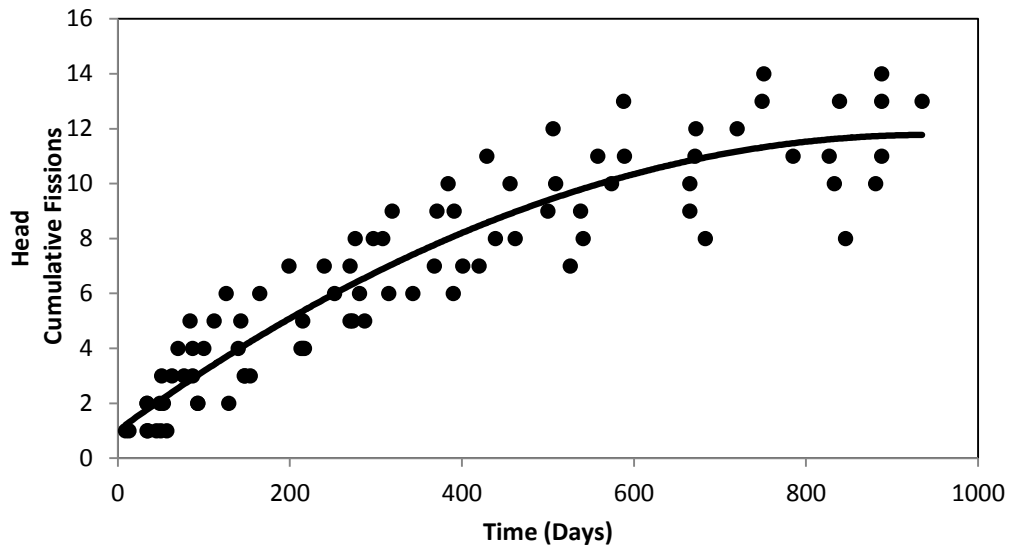


Fig.1: Results from experiment 1 plot interval between fissions over time. Each marker represents time of a fission and the interval since the previous fission of that line. 3rd order polynomial trendlines show the mean. a) Data from 7 lines of successive heads starting at 8mm in length. b) Data from 10 lines of successive heads starting at 7mm in length.

a)



b)

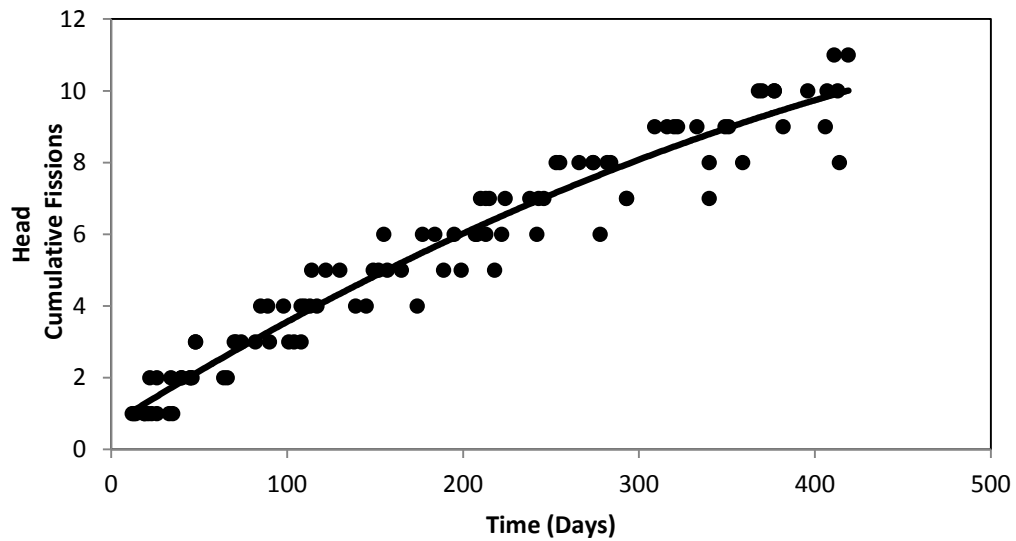


Fig. 2: Results from experiment 1 (continued). Cumulative curve plots fission rate (slope) over time. Fig. 1 data was plotted against cumulative number of fissions on the y-axis. Each marker represents the time of a fission and the cumulative number of past fissions from that line. A 2nd order polynomial trendline shows the mean. a) Data corresponds with points from Fig 1a. b) Data corresponds with points from Fig 1b.

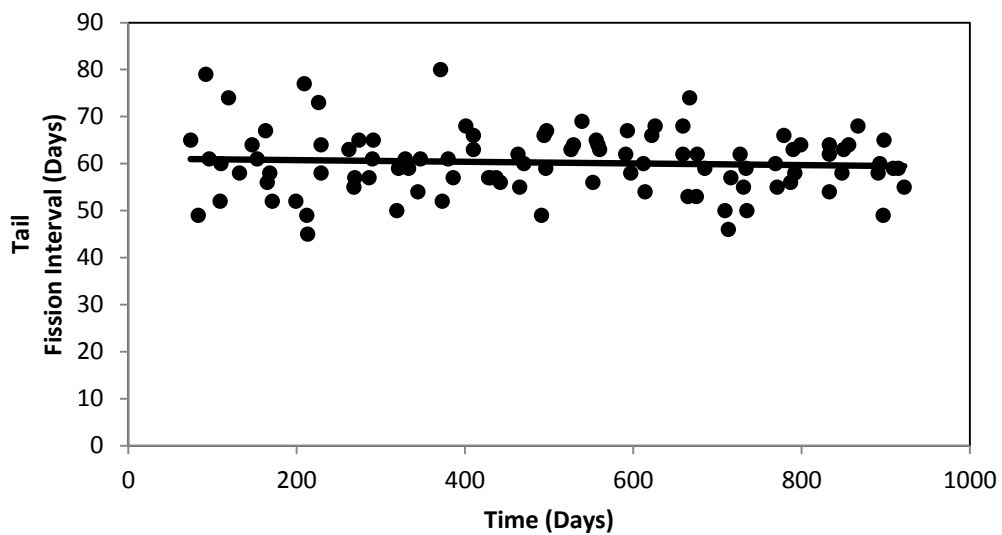


Fig. 3: Results of experiment 2 plot interval between fissions over time for 7 lines of successive tails. A linear trendline shows the mean. Each marker represents time of a fission and the interval since the previous fission of that line.

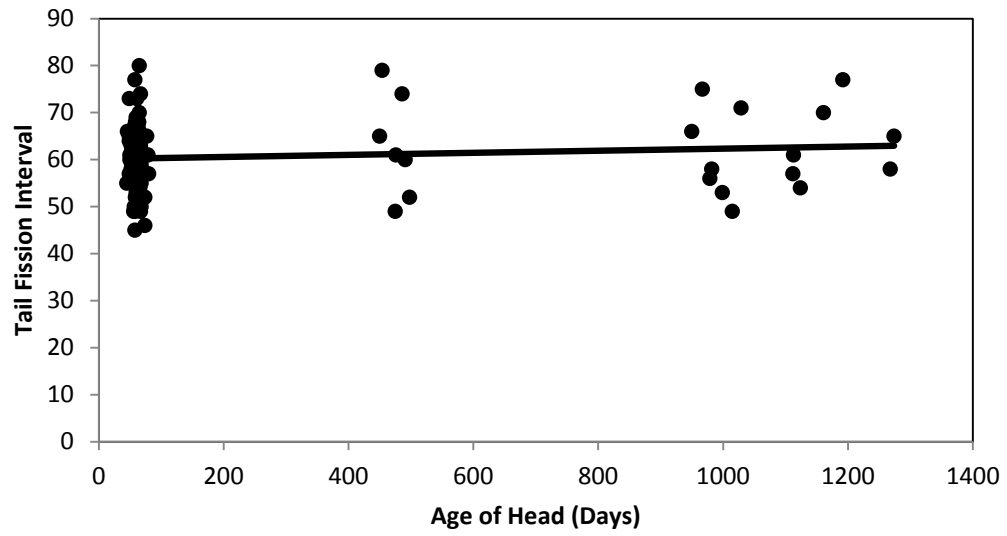


Fig. 4: Results of experiment 3 plot the interval to first fission of tails from heads of increasing age. A linear trendline shows the mean. Each marker represents the time interval to the first fission of a tail and the age of the head from which that tail was born.

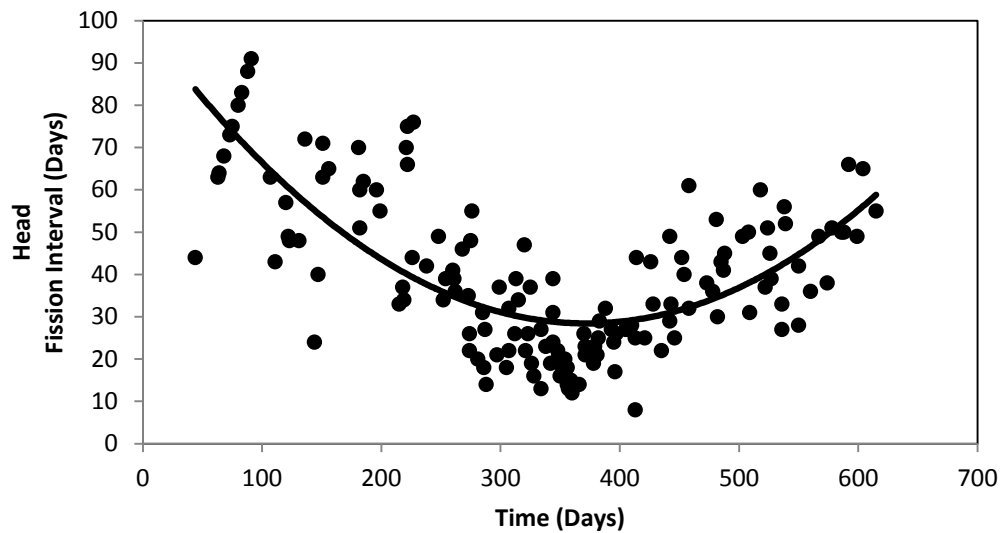


Fig. 5: Results of experiment 4 plot interval between fissions over time for heads that started from a tail. Each marker represents time of a fission and the interval since the previous fission of that line. A second order polynomial trendline shows the mean.

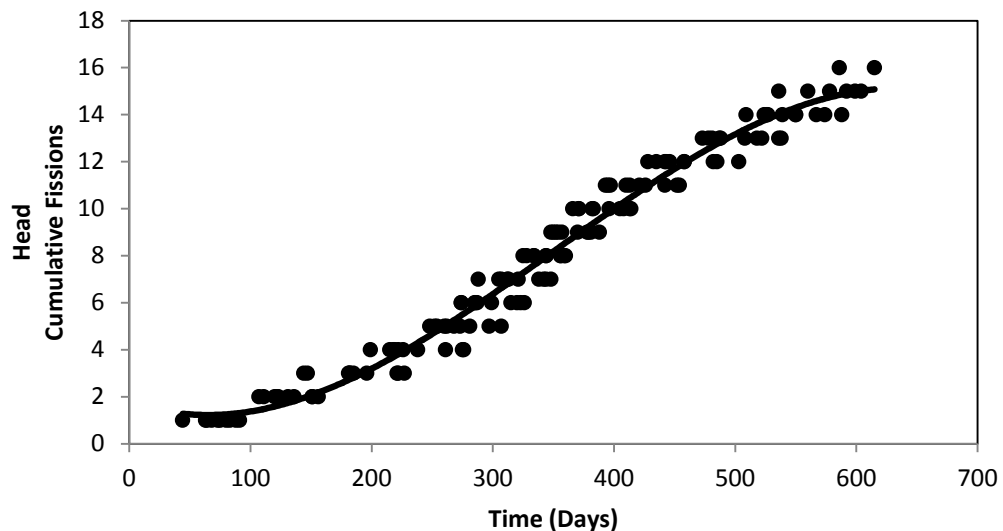
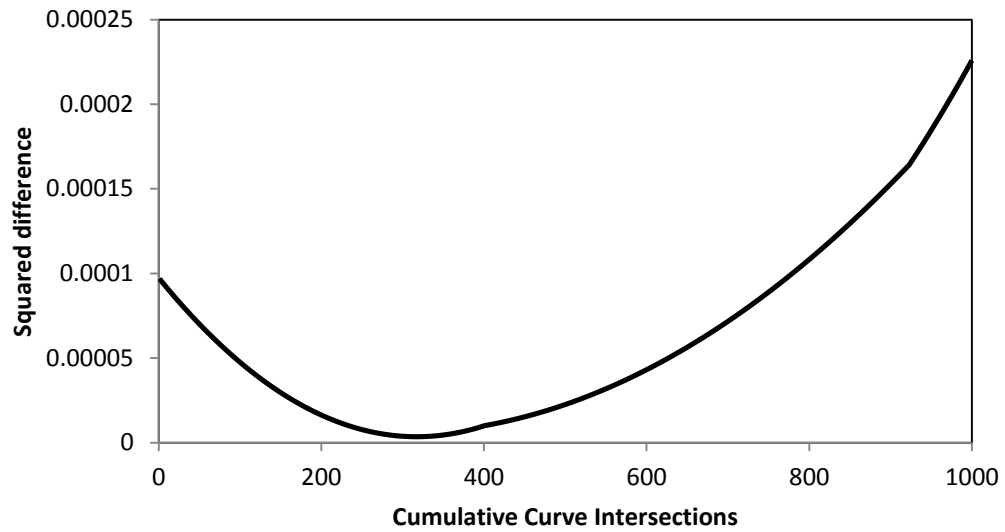


Fig. 6: Results from experiment 4 (continued). Cumulative curve plots fission rate over time. Fig. 5 data was plotted against cumulative number of fissions on the y-axis. Each marker represents the time of a fission and the cumulative number of past fissions from that line. A 3rd order polynomial trendline shows the mean. Data corresponds with points from Fig.5.

a)



b)

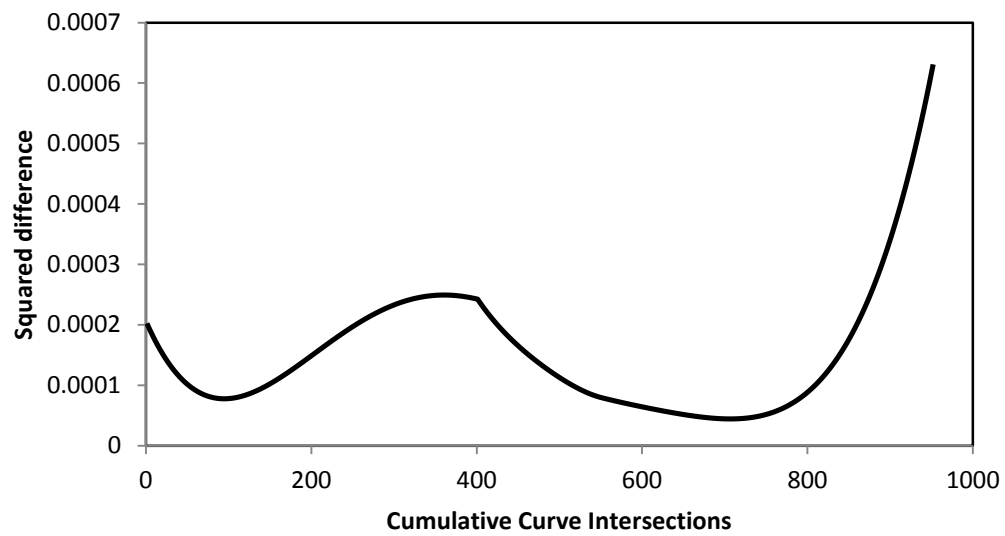


Fig.7: Range of intersections between cumulative curves (Fig 2 and 6) and corresponding values of squared difference of slopes. Lowest value of squared difference shows the intersection with the best match between slopes. a) Intersections between Fig.2a and 2b. b) Intersections between Fig.2b and Fig.6.

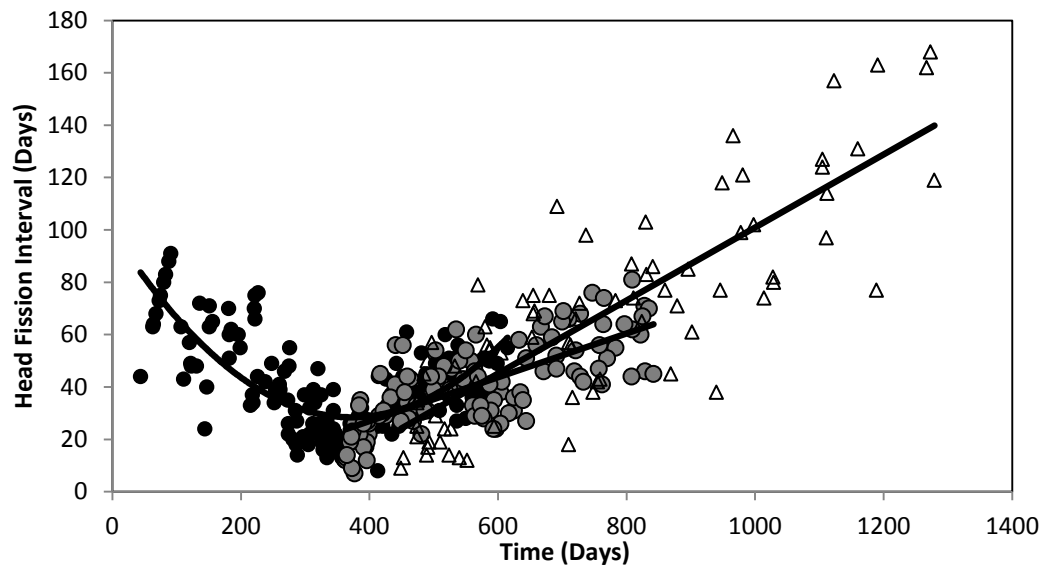


Fig.8: Pattern of fission intervals in lines of successive heads from birth to death. Black circles show data from Fig 5 (heads started from tails). Grey circles show data from Fig 1b (7mm heads). Open triangles show data from Fig 1a (8mm heads).

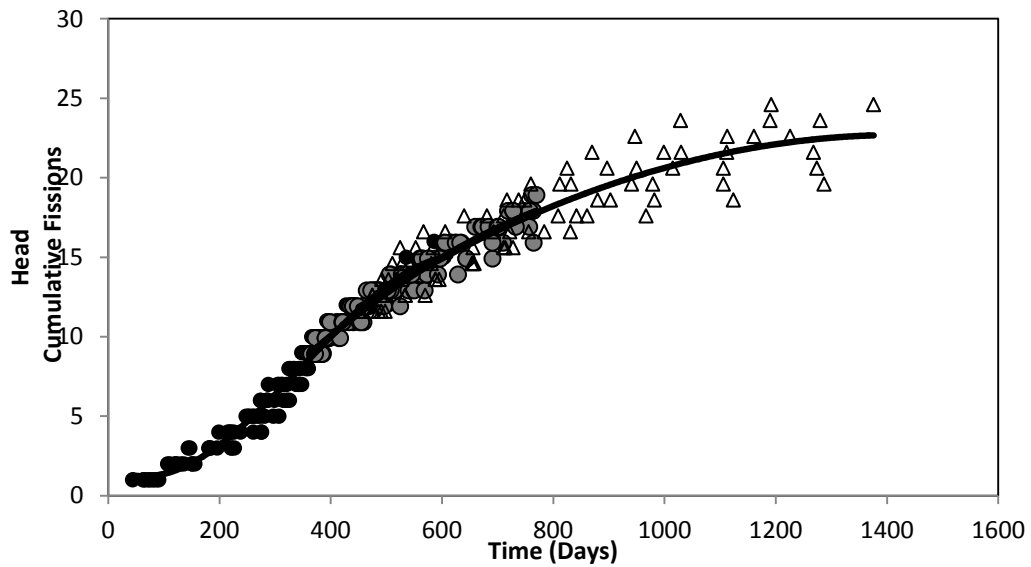


Fig.9: Cumulative number of divisions in lines of successive heads from birth to death. Black circles show data from Fig 6 (heads started from tails). Grey circles show data from Fig 2b (7mm heads). Open triangles show data from Fig 2a (8mm heads).

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