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Effect of Maternal Age on Recombination Rate in Drosophila melanogaster

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Introduction

Recombination during meiosis is a significant source of genetic variation, which is directly correlated to increased fitness in the next generation (Roeder, 1997). Genetic diversity is achieved during crossing over, where the position of alleles can be altered affecting genetic expression. This can lead to increases or decreases in fitness. This process, however, can also go awry, leading to severe negative effects, such as an increase in the rate of nondisjunction. This has the potential to cause serious defects in the offspring. (Hunt & Hassold, 2001). A common misconception is that recombination is difficult to influence with outside factors. On the contrary, many organisms experience differential recombination rates due to outside factors, including age (Rodgers-Melnick, et al., 2014). Some of these organisms commonly studied in laboratory settings are yeast (Mancera, Bourgon, Brozzi, Huber, & Steinmetz, 2008), nematodes (Barnes, Kohara, Coulson, & Hekimi, 1995) and fruit flies (Brooks & Marks, 1986).

Recombination rates are generally accepted as being phenotypically plastic, meaning a given genotype may result in several different phenotypes depending on the environment (Hunter, Robinson, Aylor, & Singh, 2016). Thus phenotypic plasticity is essential to increase fitness, as with the case of the human pathogen *Streptococcus pneumoniae*, that can show many different capsule types (Claverys, Prudhomme, Mortier-Barriere, & Martin, 2000). The

ability to show many different capsule types gives the pathogen the ability to survive in multiple environments with different stressors, which may include antibiotics. Phenotypical plasticity can also be caused by many different genetic stressors, such as nutrition (Neel, 1941), parasitism (Singh, et al., 2015) and temperature (Plough, 1921).

It is commonly understood that fitness will decrease with age (Partridge & Barton, 1993), leading to an overall decrease in progeny numbers (Stearns, 1992). Essentially, as age increases the likelihood for genes to be passed to new generations decreases. Many studies using *D. melanogaster* also support the hypothesis that recombination rate can be affected by maternal age (Plough, 1917; Plough, 1921; Redfield, 1966; Hunter & Singh, 2014; Hunter, Robinson, Aylor, & Singh, 2016). Other studies, examining mice, hamsters, (Sugawara & Mikamo, 1983) and humans (Kong, et al., 2004; Campbell, Furlotte, Eriksson, Hinds, & Auton, 2015) suggest maternal age affects recombination rate in other species as well. Significant decreases in progeny, related to maternal age, were seen in many organisms. Hence, something as small and seemingly insignificant as a fruit fly, may correlate and possess similarities to mechanisms of human genetics.

Despite extensive research into this topic, several disagreements still exist as to whether recombination rates increase (Martin, et al., 2015; Hunter, Robinson, Aylor, & Singh, 2016) decrease, (Kong, et al., 2004), have nonlinear effects (Neel, 1941), or present no significant changes in recombination rates (Stevison, 1992). With age disagreements arise among these theories due to four main reasons. First, different strains of *Drosophila* were used in these studies, making it difficult to determine if the effects of maternal age on recombination is due to genetic background. Second, an inconsistency in the procedure followed by genome studies,

where repeated matings are allowed to occur. This could result in the possibility of a direct increase of recombination rates in the tested species (Hunter, Robinson, Aylor, & Singh, 2016). Many studies also have differing experimental designs, where some of the experiments focus on the progeny from single females, to determine recombination rates, where others focus on the progeny from groups of females. Finally, the influence of maternal age on recombination rates is not uniform across the entire genome (Hunter, Robinson, Aylor, & Singh, 2016). This means that certain regions of a species genome have potentially higher rates of recombination, due to maternal age, whereas other genome regions may possess lower recombination rates, again due to maternal age.

In this study, we investigated the hypothesis that the recombination rate in the X-chromosome genomic region was positively correlated to maternal age. The *Drosophila* genome contains many different loci where eye color can be determined (Morgan, 1911). In this study, the loci examined were the white eye (*w*) mutation of the white gene and the singed bristle (sn^3) mutation of the singed gene located on the X chromosome. We predicted that recombination rate for these loci will increase as maternal age increases.

Materials and Methods

Two fly stocks, possessing different genetic markers, (white eyes (*w*) and singed small bristles (sn^3)) were used in the study, plus CS(BU), a wild type stock possessing red eyes and straight bristles. Virgin females from the *w* sn^3 group were crossed with males from the CS(BU) group. The F1 progeny from this cross was then mated to its siblings, resulting in an interbred cross of a *w* sn^3/+ + virgin female, crossed with two *w* sn^3/Y males per vial. The F2 progeny were then scored for recombination. The female recombinants had the genotypes of *w* +/*w* sn^3

(white eyes with straight bristles) or + sn^3/*w* sn^3 (red eyes with singed bristles). The recombinant F2 males will have a genotype of *w* +/Y or + sn^3/Y, whereas + + stands for red eyes and straight bristles (the CS(BU) line for the F1 generation). Non-recombinant females possess a genotype of *w* sn^3/*w* sn^3 or + +/+ +, while non-recombinant males will have *w* sn^3/Y or + +/Y genotypes. The phenotypes of both non-recombinant males and females will be red eyes with straight bristles or white eyes with singed bristles. These crosses are detailed in Figure1.

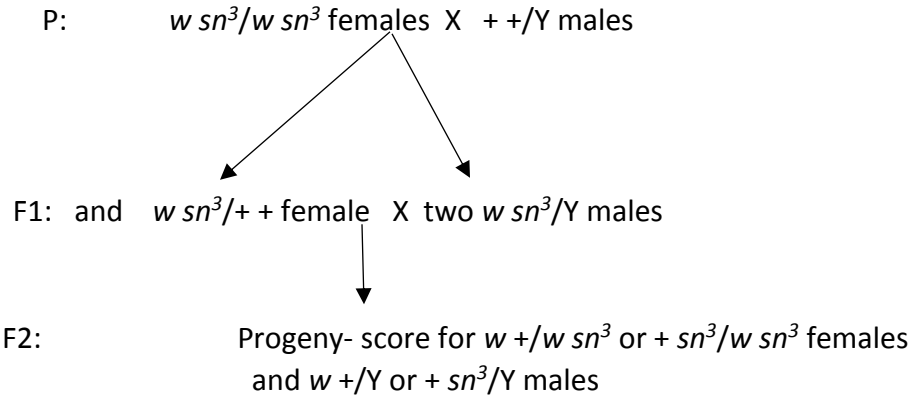

Figure 1: The crosses that were performed in the experiment. During the P cross, large numbers of virgin females were crossed with large numbers of males in a bottle. For the F1 cross, one virgin female was crossed with two males in a single vial.

There was 31 lines of flies established in this experiment, each line consisting of 10 vials. For the first vial in each line, one virgin female was mated to two males. After two days the female was transferred into a new vial, and males were discarded. The female was given two days to lay eggs and was then transferred into another vial. This process continued for twenty days and was completed for all 31 lines. The statistical analysis was conducted using the PRISM system. In the PRISM system a regression analysis was conducted and the slope of the best fit

line of the regression was obtained, to determine if the slope of this line was significantly different from zero.

Results

In total 5239 progeny were scored for recombination (4289 non-recombinants and 950 recombinants). Table 1 details the number of progeny (non-recombinants and recombinants) for the 20 days of the experiment. Table 2 shows the percent recombination for each two day period.

Table 1: The number of recombinant and non-recombinant progeny obtained in this experiment.

Days	Total Progeny	Non-Recombinants	Recombinants
2	817	690	127
4	717	625	92
6	463	379	84
8	386	320	66
10	519	429	90
12	379	301	78
14	650	509	141
16	620	465	125
18	424	334	90
20	264	207	57

Table 2: The recombination rate for each two day period. Recombination rate was determined by dividing the number of recombinant flies from a two day span by the total number of flies from the same two day span. For example, there was a total of 817 flies from days 1-2 and there was 127 recombinant flies from days 1-2. Resulting in 127/817=.1554 or 15.54%.

Day	Recombination Rate
2	15.54
4	12.83
6	18.14
8	17.10
10	17.34
12	20.58
14	21.69
16	21.16
18	21.23
20	21.59

As seen in Figure 2, the slope of the best-fit line is significantly different from zero ($p=0.001$). This means that the observed data has a 0.1% chance of being from random chance. Since there is such a low probability of random chance, our data is considered to be significant. The recombination rate increased from 15.54% in days 1-2 to 21.59% in days 19-20, resulting in an overall increase of approximately 6% ($p<0.001$). These results support our hypothesis that recombination rate increases with maternal age.

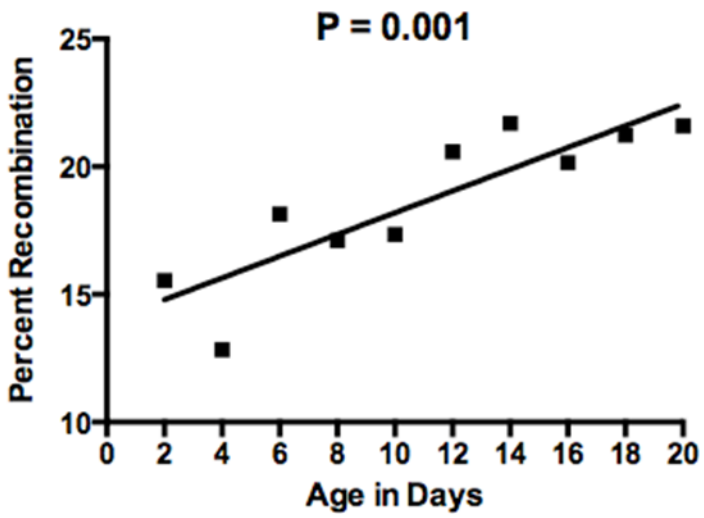

Figure 2: Recombination rates graphed with a line of best fit.

An unusual trend was observed in some data from the experiment. Some female flies would lay fertilized eggs in one vial, refrain from laying any in the next vial, and then begin laying fertilized eggs again in the subsequent vial. Table 3 and Figure 3 detail this pattern in two different vial lines.

Table 3: The number of progeny in each vial from two lines. Of particular interest is series 1 (line 2). The progeny number drops to zero and then recovers multiple times. In series 2 (line 5) the number of progeny drops to one or zero across several vials and then increased.

Vial	Series 1	Series 2
1	24	29
2	9	30
3	33	5
4	0	1
5	19	1
6	0	21
7	0	54
8	31	64
9	0	0
10	23	11

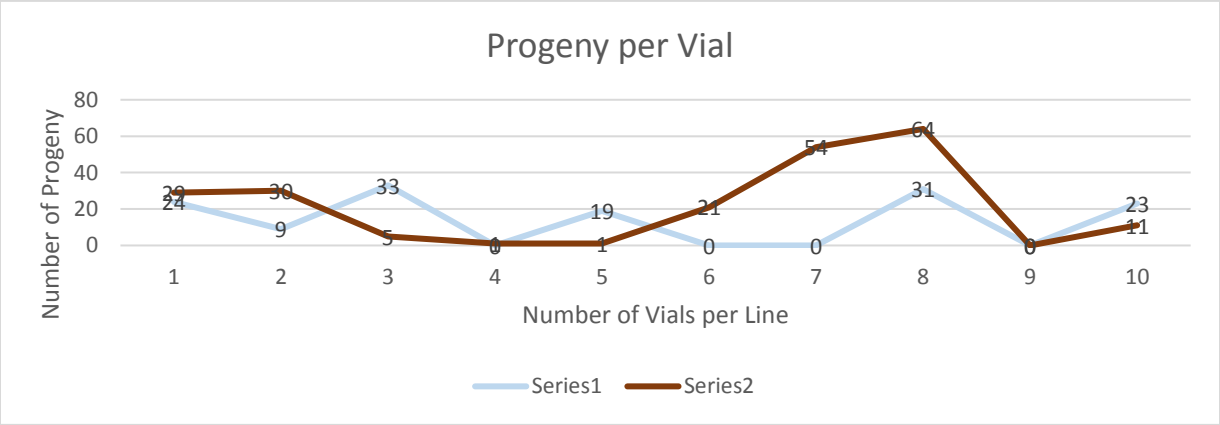

Figure 3: This graph shows the number of progeny per vial, in two lines of vials. The two lines were run for a total of twenty days. Therefore, a total of ten vials existed in each line (X-axis), with each number representing a vial that the female fly was in for two days. Series 1 represents line 2 while series 2 represents line 5 from the study.

As Figure 3 displays, the number of progeny per vial is highly variable. Noticeably, there are no progeny observed for two to four days, before appearing again. This is observed in vials six and seven for line 2 and four and five for line 5. This is of particular interest because it differs from what is expected. What is expected is that the number of progeny would decrease over time and untimely go to zero. However, that is not what is observed. This divergence from what is expected has several potential causes, including females conserving nutrients or that the vials the females were stored in did not have a sufficient amount of nutrients.

Discussion

The primary goal of this study was to determine if maternal age affects recombination rates. It is well known that as maternal age increases, nondisjunction also increases (Subramanian & Bickel, 2008). However, less research exists on how maternal age affects recombination rates specifically. As mentioned before, studies relating to maternal age and recombination rates have been very inconsistent (Hunter, Robinson, Aylor, & Singh, 2016). Some find decreasing rates of recombination and others show consistent, never changing, recombination rates. The article we based our experimental design on presented data showing increasing recombination rates under similar conditions as seen in this study (Hunter, Robinson, Aylor, & Singh, 2016). The data presented in that study supported the idea that as maternal age increases, recombination rates also increases. A consequence of increased recombination rates may be an increase in DNA mutations. Correlations observed in other studies support the idea that recombination is mutagenic (Lercher & Hurst, 2002). These genetic mutations, caused by recombination, hold the potential to be beneficial, neutral or deleterious. If recombination does cause a deleterious mutation it would be adding genetic diversity, but at the cost of

fitness to the organism. Other mutations, however, are considered neutral or beneficial (Haag-Liautard, et al., 2007). Another consequence of an increased recombination rate is an increase in positive selection (Anisimova, Nielsen, & Yang, 2003). This positive selection can lead to advantageous genes being passed to the next generation. Therefore, if recombination increases, mutation rate and positive selection would also increase. This means recombination can either be extremely beneficial or extremely detrimental.

Another factor that might affect recombination rates, other than maternal age, is genetic background. Genetic background has been shown to affect recombination rate in a positive manner, by increasing the frequency of recombination (Hunter, Robinson, Aylor, & Singh, 2016). Different genetic backgrounds cause different recombination rates. Some genetic backgrounds in *D. melanogaster* have recombination rates around 12-14%, while others have recombination rates ranging from 16% to 38% (Brooks & Marks, 1986). Such a wide range makes it optimal to test different genetic backgrounds, such as CS(BU) and *w sn*^{3} as used in this study. Flies with one genetic background would be crossed with flies that have a different genetic background. Once the data from the initial cross was collected new genetic background would be used. The changing of genetic background would make it possible to test how recombination is affected by genetic background.

The data from this study brought an interesting trend to light. In almost every line of vials the female flies would refrain from laying eggs for two to four days, before resuming egg-laying. It has proven challenging to discover a reason for this unusual behavior. One would expect *D. melanogaster* females, living in vials with ample nutrients for survival, to lay eggs continuously in a decreasing number every day. However, this was not the case. One

explanation of the different pattern observed may be that the females were attempting to conserve nutrients, during the metabolically expensive process of laying an egg (Partridge, Fowler, Trevitt, & Sharp, 1986; Partridge, Green, & Fowler, 1987; Chapman & Partridge, 1996). This few-day gap in egg laying may have given the female time to build up necessary nutrients and regain strength to resume egg-laying. Another potential explanation for why the females may have stopped laying eggs for a few days could be that the vials did not provide an ideal environment for egg laying. This would have caused the females to stop laying eggs for a few days until transferred to a new vial. This hypothesis, however, is not well supported because all the vials contained the same food source; if a female was laying eggs in one vial they should have been able to lay eggs in every vial. This interesting phenomenon should be researched more in depth and independently.

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