Teaching Notes

Effect of maternal age on recombination rate in *Drosophila melanogaster.*

Schimmoeller, Christopher, Michael A. Balinski, and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403

Abstract

Fitness is defined as the reproductive success of an organism in producing offspring and passing their genes to the next generation (Hedrick, 2011). Many genetic factors can affect fitness, including selection, mutation, and migration, but the most ubiquitous is recombination, which is caused by the exchange of genetic material between chromosomes during meiosis. Numerous studies have examined the role of recombination in evolution, including conflicting reports on the interaction of age and recombination rates (see discussion in Hudson and Kaplan, 1985; Brooks, 1988; Otto and Michalakis, 1998; Hunter *et al*. 2016). Here, we examine how maternal age affects the rate of X-chromosome recombination in *Drosophila melanogaster*. Our hypothesis is that recombination rates are positively correlated with maternal age. If this is true we would expect to see an increase in the rate of recombination as maternal age increases. Such a correlation was observed in this study.

Introduction

Recombination, which involves exchanges of genetic material between homologous chromosomes during meiosis, is a significant source of genetic variation that can influence fitness (Roeder, 1997; McDonald, Rice and Desai, 2016). Changes in positions of gene alleles on a chromosome caused by recombination could affect gene expression and lead to increases in fitness, which can assist organisms in adapting faster to changing environments (Badyaev, 2005; Presgraves *et al*., 2005). Negative effects can also arise, however, such as increases in the rate of chromosomal rearrangements if recombination occurs by unequal crossing over (Lupski, 1998). In addition, it is sometimes reported that recombination is not always influenced by environmental or biological factors (Otto and Michalakis, 1998). On the contrary, many organisms, including yeast, nematodes, and fruit flies, experience alterations in recombination rates due to environmental and biological factors, including temperature, nutrition, and age (Plough, 1917, 1921; Neel, 1941; Brooks and Marks, 1986; Parsons, 1988; Barnes *et al*., 1995; Mancera *et al*., 2008; Rodgers-Melnick, *et al.* 2014; and references in Dollard *et al*., 2016).

Fitness is usually assumed to decrease with age, leading to a reduction in progeny numbers over time (Stearns, 1992; Partridge and Barton, 1993). As age increases, therefore, the likelihood for genes to be passed to new generations decreases. Many studies using *D. melanogaster*, mice, hamsters, and humans also support the hypothesis that recombination rates can be affected by maternal age (Plough, 1917, 1921; Redfield, 1966; Kong, *et al.* 2004; Bleazard *et al*., 2013; Hunter and Singh, 2014; Campbell, *et al*., 2015; Martin *et al*., 2015; Hunter *et al.*, 2016). Despite extensive research on this topic, however, disagreements still exist as to whether recombination rates increase, decrease, or do not change with increased maternal age (see a discussion of this topic and references in Hunter *et al*., 2016).

Four issues among multiple studies have led to little consensus as to how recombination rates change with increasing maternal age in *D. melanogaster*. First, different strains were used in these studies, making it difficult to determine if the effect of maternal age on recombination is correlated with age or genetic background. Second, some studies allowed repeated matings, which could result in an increase in the rate of recombination unrelated to age (Priest *et al*., 2007; Hunter *et al*., 2016). Third, many studies focused on progeny from single females, while others counted progeny from groups of females. Finally, the influence of

maternal age on recombination rates is not uniform across the entire genome; certain regions of the genome or chromosomes have higher frequencies of recombination compared to other regions (Lercher and Hurst, 2002; Fiston-Lavier *et al.*, 2010).

In this study, we investigated the hypothesis that X-chromosome recombination rates in *D. melanogaster* are positively correlated with maternal age, with older females having higher recombination rates compared to younger flies.

Materials and Methods

A fly stock possessing two X-linked genetic markers, [white eyes (*w*) and singed small bristles (sn^3)], was used in this study, plus the Canton-S (CS) wild type stock, which has red eyes and straight bristles. As shown in Figure 1, parental virgin females from the *w* sn^3 stock were crossed with males from the CS stock, and single F1 *w* sn^3/+ + virgin females were crossed with two *w* sn^3/Y sibling males per vial. The F2 progeny were then scored for recombination. Female F2 recombinants would be *w* +/*w* sn^3 (white eyes with straight bristles) or + sn^3/*w* sn^3 (red eyes with singed bristles) genotypes. Recombinant F2 males would have a genotype of *w* +/Y (white eyes with straight bristles) or + sn^3/Y (red eyes with singed bristles). In addition non-recombinant females are *w* sn^3/*w* sn^3 (white eyes with singed bristles) or + +/ *w* sn^3, (red eyes with straight bristles), while non-recombinant males are *w* sn^3/Y (white eyes and singed bristles) or + +/Y (red eyes and straight bristles). These crosses are detailed in Figure 1.

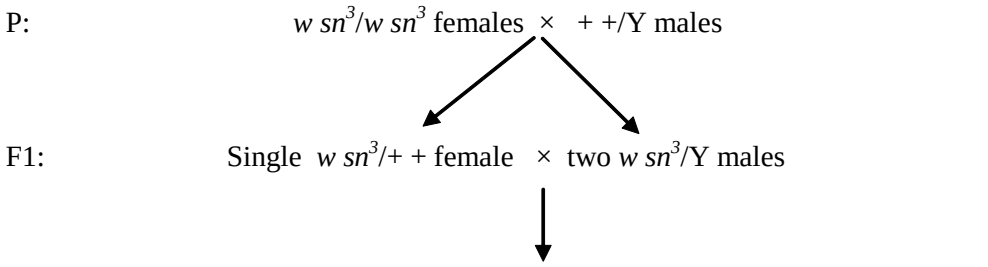

Figure 1. Crossing scheme for this experiment.

In this study, 31 total runs of flies were carried through the crossing scheme of Figure 1, with each run consisting of 10 aging vials each established two days apart. For the first aging vial in each run, one virgin F1 female was mated to two F1 males. After two days females were transferred to new vials and males were discarded. Hence, the F1 females were given two days to lay eggs and were then transferred to a fresh vial. This procedure continued for twenty days (10 vials for each run) for all 31 runs, with no additional matings beyond the first two days. Regression analysis was conducted using the PRISM statistical program to determine if recombination rate does significantly increase with maternal age.

Results

A total of 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 twenty days of the 31 runs. Table 2 shows the percent recombination for each two-day period.

As seen in Figure 2, the slope of the best-fit line is significantly different from a slope of zero ($p = 0.001$). The average recombination rate increased from 15.54 percent in days 1-2 to 21.59 percent in days 19-20, resulting in an overall significant increase in recombination rate of approximately six percent. These results support our hypothesis that recombination rates increase with maternal age.

Table 1. The number of recombinant and non-recombinant progeny obtained from 31 lines.

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

Discussion

The objective of this study was to determine if maternal age affects the rate of X-chromosome recombination. Although it is well known that as maternal age increases nondisjunction events increase (Hunt and Hassold, 2001), it is less clear how maternal age affects recombination. As previously stated, the results of studies relating to maternal age and recombination rates have been inconsistent, with reports of decreasing, increasing, or non-changes in rates with increased age. The results of this study clearly support the hypothesis that as maternal age increases, recombination rate also increases in *D. melanogaster*. Similar results were reported by Bridges (1915), Priest *et al*. (2007), Hunter and Singh (2014), and Hunter *et al.* (2016) for *Drosophila* and by Bleazard *et al*. (2013), Campbell *et al*. (2015), and Martin *et al*. (2015) for humans.

Table 2. The recombination rate for each two day period of the 31 runs. 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 127 recombinants (see Table 1), resulting in 127/817 = 15.54 percent recombination.

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

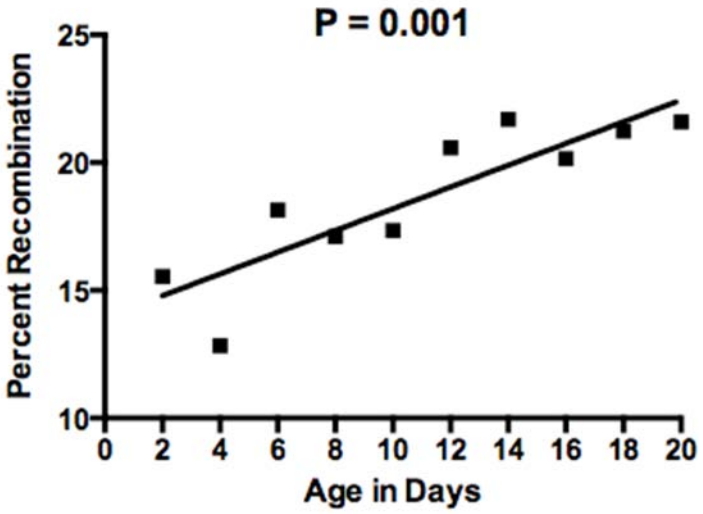

Figure 2. Recombination rates graphed with a line of best fit.

One possible consequence of increased recombination rates with age is an increase in positive selection for advantageous combinations of gene alleles on a chromosome with age. Positive selection could then lead to advantageous gene interactions in future generations. Therefore, if recombination rates increase with age, positive selection could also increase, allowing organisms to adapt more quickly to changing environments. Yet, recombination is also known to be mutagenic (Lercher and Hurst, 2002). For example, unequal recombination can lead to extra or missing base pairs that cause genetic disorders in humans (Nakamoto *et al*., 2002). Recombination can, therefore, be beneficial or detrimental to fitness.

Another factor that might affect recombination rates is the genetic background. Genetic backgrounds have been shown to affect recombination, by decreasing or increasing recombination rate by up to two-fold (Brooks and Marks, 1986; Stevison, 2011; Hunter *et al*., 2016). Hence, it would be important to test additional genetic backgrounds, other than the CS and *w sn*^{3} stocks that were used in this study, on the effect of aging on

recombination rates. Such experiments could assist future studies of how recombination rates and aging are influenced by genetic background.

One other interesting trend was observed in this study. In almost every run females refrained from laying eggs for two to four days, before resuming oviposition. One explanation for this observation may be that the females needed additional nutrients for the metabolically expensive process of oviposition (Chapman and Partridge, 1996). This gap in oviposition may give females time to build up the necessary nutrients to resume oviposition. This interesting phenomenon should be studied in more depth.

A class discussion of the results of this study might include: 1) Why was recombination and aging only tested in females in this study? There is no recombination in male *D. melanogaster* (Morgan, 1914). 2) Are there genes that are known to directly affect rates of recombination? Yes, including RAD51 in yeast, mice, *Drosophila*, and humans (for a discussion of this topic, see Baker and Hall, 1976; Shinohara *et al*., 1993; Staeva-Vieira *et al.*, 2003).

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Reversion of the *Bar* (*B*) mutation in the Basc X chromosome of *Drosophila melanogaster* by unequal crossing over.

Buettner, Kelsey Allyse, Rachel Ann Crowl, Ashley M. Everett, Cameron Drew Friedman, Marissa Rachelle Gittrich, Joseph Hasseltine, Nathaniel P. Locke, Griffith M. Saunders, Matthew Alec Schaefer, Christopher J. Schimmoeller, Kayla Christina Schwartz, Michael A. Balinski, and R.C. Woodruff. Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio 43403.

The dominant, X-linked, *Bar* (*B*) mutation was isolated in 1914 by Sabra Colby Tice as a change in the structure of the eye of *D. melanogaster* from round (wild type) to a narrow bar of eye tissue in homozygous females and hemizygous males, and as less extreme *Bar*-eyes in heterozygous, B/B^{+}, females