4-1-2013

Locating the Modifier of Segregation Distorter in Drosophila Melanogaster

Samuel Craven
Olivet Nazarene University, scraven1@olivet.edu

LOCATING THE *MODIFIER OF SEGREGATION DISTORTER* IN

DROSOPHILA MELANOGASTER

By

Samuel Craven

Honors Scholarship Project

Submitted to the Faculty of

Olivet Nazarene University

for partial fulfillment of the requirements for

GRADUATION WITH UNIVERSITY HONORS

March, 2013

BACHELOR OF SCIENCE

in

Biochemistry & Biology

Janna R. McLean		3/19/13
Scholarship Project Advisor (printed)	Signature	Date
CHARLES W. CARRIGAN		4/26/13
Honors Council Chair (printed)	Signature	Date
Pamela S. Greenlee	Pamela S. Greenlee	4/10/2013
Honors Council Member (printed)	Signature	Date

ACKNOWLEDGEMENTS

I would like to extend a special thanks to Dr. Janna McLean, who was my research mentor throughout the duration of this project. It was thanks to her training and guidance in all matters relating to this project that I was able to complete my research, and I am especially appreciative of her patience in regards to my procrastination.

I would also like to thank the Elbert Pence and Fanny Boyce Undergraduate Summer Research Experience for providing extra funding for my research. This funding enabled me to continue my research over the summer of 2012, which allowed me to obtain a much more comprehensive set of data.

Finally, I would like to thank the Bloomington Stock Center for supplying the various fly stocks that I used throughout this project.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

The *Drosophila melanogaster* meiotic drive system *Segregation Distorter* (*SD*) has been a topic of great interest over the past decades due to its implications for fertility issues in fruit flies and other species as well. Several genes have been associated with this system; however, little research has focused on a particular one of these genes—the *Modifier of SD*. The location of this modifier gene is still unknown, so multiple deleted segments of DNA that compose a suspect area along the 2nd chromosome were tested here to see if some level of distortion is established in the absence of these segments. The DNA region from chromosomal segments 43E9 to 43E18 showed the highest level of distortion (*k* value = 0.828), so genes within this segment were tested for a possible correlation to *Modifier of SD*. The three genes *torso*, *saxophone*, and *CG30497* were examined for this correlation, and *torso* showed a significant *k* value (0.689), indicating a possible relation to *Modifier of SD*.

Keywords: biology, genetics, spermiogenesis, *Drosophila melanogaster*, *Segregation Distorter*, *Modifier of SD*, Ran transport

INTRODUCTION

According to Mendelian genetics, gametes produced during meiosis receive one of two possible alleles from the parent organism and each allele has an equal chance of being selected. This concept is fundamental to the theory of natural selection, which requires separate traits having equal opportunities to be passed on to offspring so that the offspring that happen to receive the more advantageous trait can survive to pass their own traits on to future generations. The general concept is quite simple in these regards, but what would happen if this law does not always hold true? What would be the consequences if one allele is preferentially selected over the other? Such a phenomenon could be extremely detrimental to the viability of the species if the allele in question is a harmful mutation. Natural selection would fail to sort out the weak traits because the stronger ones would never be given a chance in the first place.

Several such meiotic drive systems have been discovered in various organisms from plants and fungi to insects and mammals (Kusano, Staber, & Ganetzky, 2001; McElroy, McLean, & McLean, 2008), but possibly the most extensively studied of these is the *Segregation Distorter* (*SD*) system in *Drosophila melanogaster*, the common fruit fly. Early research on *SD* was spearheaded by Yuichiro Hiraizumi and Larry Sandler (Hiraizumi, Sandler, & Crow, 1960; Hiraizumi & Nakazima, 1967; Sandler, Hiraizumi, & Sandler, 1959), whose work laid the basis for the past fifty years of research on this topic. Although the original work on *SD* focused mainly on theoretical implications of meiotic drive and locating the source of distortion (Ganetzky, 1999), major strides have been taken recently in elucidating the cellular and biochemical mechanisms by which *SD* acts.

Segregation Distorter causes a dysfunction in sperm chromatin condensation of males that are heterozygous for the distorting chromosome (*SD*) and the non-distorting, wild-type chromosome (SD^+). Interestingly enough, the chromatin fails to condense in SD^+-bearing

spermatids, which would normally be expected to function properly (Ganetzky, 2000). The distorting *SD* chromosome actually prevents spermatids containing the wild-type homolog from maturing into viable gametes! By killing off the competition, the *SD* chromosome greatly increases its own chances of being selected for fertilization. *SD* has been shown to be extremely efficient in this regard, with nearly 100% of offspring receiving the *SD* chromosome in multiple experiments (Hiraizumi & Nakazima, 1967; Kusano, Staber, Chan, & Ganetzky, 2003; Sandler et al., 1959). Many questions still remain as to exactly how the distorting chromosome achieves such an unfair advantage over its non-distorting counterpart, but recent experiments have provided us with some crucial insights into the extremely complex mechanisms of the *Segregation Distorter* system.

Two distinct genes have been found to be of particular importance to the action of *SD*. The *Sd* gene, which is located on the 2nd chromosome in *D. melanogaster*, has been shown to be largely responsible for distortion because normal segregation is established if the *Sd* locus is deleted (Ganetzky, 1977). The *Responder* (*Rsp*) locus, on the other hand, acts as the target for distortion by the *Sd* gene (Brittnacher & Ganetzky, 1989). *Rsp* is also located on the 2nd chromosome near the *Sd* locus (Ganetzky, 1977); however, if the *Sd* gene were to target the *Rsp* locus on the same chromosome, the distorting chromosomes would not be passed on and *Segregation Distorter* would have been eliminated a long time ago. Instead, as mentioned earlier, the *SD* chromosome causes distortion only in the SD^{+} homolog.

This phenomenon was accounted for by the discovery that *Rsp* exists in multiple variations (Lyttle, Brittnacher, & Ganetzky, 1986; Martin & Hiraizumi, 1979). The *Rsp* locus present on the *SD* chromosome is insensitive to distortion (Rsp^{i}), which prevents the *SD* chromosome from being affected by its own *Sd* gene. Although some SD^{+} chromosomes are also protected by an Rsp^{i} allele, most contain the distortion-sensitive version (Rsp^{s}) or even a

supersensitive version (*Rsp*^{ss}), both of which allow for the chromosome to be affected by distortion (Brittnacher & Ganetzky, 1989). The exact biochemical explanation for why *Sd* ignores *Rsp*^{i} but targets *Rsp*^{s} and *Rsp*^{ss} is still unclear, but the recent discovery of the functional product of the *Sd* gene has shed some light on this mystery.

The primary difference between the *Sd*$^{+}$ and *Sd* alleles is that the mutant version is longer than the wild-type due to a duplication of a 5kb segment of DNA (McLean, Merrill, Powers, & Ganetzky, 1994; Powers & Ganetzky, 1991). The *Sd*$^{+}$ gene normally codes for a protein called RanGAP (Ran GTPase-activating protein)—an essential component of the Ran transport system, which controls the import and export of different compounds between the cell cytoplasm and nucleus. The mutant *Sd* gene, however, produces a truncated version of RanGAP (Sd-RanGAP) (Merrill, Bayraktaroglu, Kusano, & Ganetzky, 1999), which throws the extremely complex mechanisms of Ran transport into disarray and ultimately results in the failure of the *SD*$^{+}$ chromatin to condense (McElroy et al., 2008). Our understanding of the biochemical basis behind Ran transport is still far from complete, but our knowledge of the specific role that RanGAP fills has greatly improved our understanding of *Segregation Distorter*.

The protein Ran is the central component of Ran transport, and it cycles between a GDP-bound and a GTP-bound form. GDP-GTP conversions are associated with energy transfer in numerous cellular processes, and it is this mechanism that supplies energy for Ran transport. If a compound needs to be imported from the cytoplasm to the nucleus, certain proteins called karyopherins form a complex with the compound and transfer it through the nuclear pores and into the nucleus. RanGTP exists in high abundance in the nucleus, and it is responsible for breaking apart the karyopherin complex to release the desired import cargo. RanGTP can also bind to compounds designated for export from the nucleus and transfer them to the cytoplasm. This entire process is only possible because RanGTP is localized to the nucleus, whereas RanGDP

is more abundant in the cytoplasm. If RanGTP was present in large quantities in the cytoplasm, the karyopherin complex would be broken down as soon as it was formed and the import cargo would never reach the nucleus. For this reason, RanGAP is sequestered to the cytoplasm where it converts RanGTP to RanGDP, thereby disassembling export complexes and preventing import complexes from being broken down outside the nucleus. RanGDP then cycles back to the nucleus where RanGAP's counterpart, the protein RCC1 (regulator of chromosome condensation 1), replaces the RanGDP with RanGTP in order to keep the high RanGTP concentration gradient between the nucleus and the cytoplasm (Bilbao-Cortes, Hetzer, Längst, Becker, & Mattaj, 2002; Dasso, 2001; Dasso, 2002; Görlich, Panté, Kutay, Aebi, & Bischoff, 1996; Hao & Macara, 2008; Joseph, 2006; Macara, 1999; Smith, Brownawell, & Macara, 1998; Smith, Slepchenko, Schaff, Loew, & Macara, 2002).

In contrast to wild-type RanGAP, which is kept mostly to the cytoplasm, the truncated Sd-RanGAP is mislocalized to the nucleus (Kusano et al., 2001; Kusano et al., 2002; Kusano et al., 2003). The current reasoning behind this aberration is that Sd-RanGAP lacks a nuclear export signal, which normally acts as a marker to identify RanGAP as export cargo (Kusano et al., 2001). The missing export signal hinders removal of Sd-RanGAP from the nucleus, allowing it to accumulate there and convert nuclear RanGTP to RanGDP. Since Ran transport depends heavily on the large RanGTP-RanGDP gradient between the nucleus and the cytoplasm, the reduction of the RanGTP concentration in the nucleus impairs intracellular export and import (Kusano et al., 2003). This same effect occurs even when wild-type RanGAP is forcefully overexpressed in the nucleus, which indicates that it is not some new function of Sd-RanGAP that causes distortion, but merely its location (Kusano et al., 2002).

Although *Sd* and *Rsp* are generally considered to be the most important genes involved in SD, the modifier genes *Enhancer of SD* [*E*(*SD*)], *Modifier of SD* [*M*(*SD*)], and *Stabilizer of SD*

[*St*(*SD*)] have also been shown to affect distortion (Ganetzky, 1977; Hiraizumi, Martin, & Eckstrand, 1980; Brittnacher & Ganetzky, 1984; Kusano et al., 2003). Not much research has been performed on these genes over the past fifty years, but the little that has been done focused on *E*(*SD*) in particular. In contradiction to Brittnacher and Ganetzky (1984) who concluded that *E*(*SD*) is only capable of intensifying the distorting effects of *Sd*, Temin (1991) found that a double dose of *E*(*SD*) by itself can produce levels of distortion comparable to that produced by a single dose of *Sd*. A possible explanation for these results is that *E*(*SD*) could code for some factor that increases the concentration of wild-type RanGAP in the nucleus (Kusano et al., 2002). This hypothesis has yet to be proven, but it is a major possibility because, as explained previously, distortion can be caused by either wild-type RanGAP or the truncated, *Sd* version as long as large concentrations of RanGAP are directed to the nucleus.

As of yet, no concrete evidence has been found concerning how mislocalization of RanGAP causes the distortion in chromatin condensation associated with *SD*, but a few studies have speculated on the involvement of defective histone-protamine transition during spermiogenesis, the final stage of sperm production (Hauschteck-Jungen & Hartl, 1982; McElroy et al., 2008). This transition is essential to the viability of sperm cells which require extensive chromatin condensation before spermiogenesis is complete (Aoki & Carrell, 2003). Prior to spermiogenesis, the chromatin of the developing spermatids is organized around bundles of structural proteins called histones (Peterson & Laniel, 2004). As the spermatids mature, these histones are replaced by other proteins called protamines, which results in a more condensed chromatin structure (Rathke, Baarends, Jayaramaiah-Raja, Bartkuhn, Renkawitz, & Renkawitz-Pohl, 2007). This compact form allows for increased motility of the spermatids and may even provide some form of protection (Jayaramaiah-Raja & Renkawitz-Pohl, 2005; Oliva, 2006). It is unclear how Ran transport affects this process, but there is a strong possibility that the

disruption caused by the mislocalization of Sd-RanGAP prevents protamines or other factors related to histone-protamine transition from being imported into the nucleus (McElroy et al., 2008). If these transition factors are unable to reach the nucleus, the developing spermatid will not be able to achieve the condensed chromatin state and will die off before fertilization takes place.

In summary, *Segregation Distorter* is a meiotic drive system that affects the viability of developing sperm cells in *SD*/*SD*$^{+}$ males. The *Sd* gene induces distortion in *SD*$^{+}$ spermatids bearing a sensitive *Rsp* locus, causing the preferential transfer of *SD* chromosomes to the offspring. This is accomplished by the production of a truncated RanGAP by the *Sd* gene which disrupts Ran transport and is thought to inhibit the import of protamines and other vital cellular factors into the nucleus. Without these factors, the histone-bound structure of the sperm chromatin is not replaced by the protamine-bound form, and the chromatin fails to condense. Since the chromatin does not achieve a more compact form, the spermatid never reaches maturity and is unable to be selected for fertilization.

Our understanding of how these separate processes intersect has improved much over the past fifty years, but there are still numerous questions that remain unanswered. Among the most important of these are why the *SD* chromosome targets only *Rsp*^{s} and *Rsp*^{ss} alleles while leaving *Rsp*^{i} alleles unaffected, how exactly Ran transport correlates to the failure in histone-protamine transition, and what roles the different modifier genes fulfill. In particular, my research focuses on finding possible locations for one of these genes, the *Modifier of SD*, which was first described for its enhancing effects on distortion by Hiraizumi, Martin, and Eckstrand (1980) but was never located. The *M*(*SD*) locus is thought to lie somewhere along the 2nd chromosome (J. McLean, personal communication, October 25, 2011), so a specific area along that chromosome (42A-44E) became my focus. Discovering the location of this gene could lead

to a better understanding of the complex mechanisms of *SD* and potentially reveal to us more about fertility issues, various genetic problems, and cellular function as a whole, and it is for this reason that I undertook this research.

MATERIALS AND METHODS

We purchased all fly stocks from the Bloomington Drosophila Stock Center and kept them at 18°C on a standard cornmeal and molasses media. We moved stocks to a 25°C incubator in preparation for crosses, which we performed at 25°C as well. Each experimental stock contained a different deletion of some segment of DNA along the second chromosome (Table 1), and these deletions overlapped to cover the entire chromosomal region of 42A13 to 44E3. Along with each experimental stock, we used 2 standard stocks for crosses. The *cnbw* standard stock provided distortion-insensitive females that contained white eyes and straight wings as markers. The $Rsp^{s}B^{s}$; $SD\text{-}5^{r7}/Cy$ standard stock contained an Rsp^{s} allele that was translocated along with a bar-stone eye (B^{s}) marker onto the Y chromosome. The $SD\text{-}5^{r7}$ chromosome was a revertant of *SD-5*, which is a strongly distorting chromosome consisting of *Sd*, Rsp^{i}, *E*(*SD*), *St*(*SD*), and *M*(*SD*) (Sandler et al., 1959). The revertant chromosome ($SD\text{-}5^{r7}$) contained an Sd^{+} allele instead of the mutated *Sd* allele, which prevented this chromosome from causing significant distortion by itself (McElroy et al., 2008). Instead, we considered it to be "primed" for distortion, and the lack of the chromosomal region corresponding to the *M*(*SD*) locus could potentially mimic the effects of a second mutant form of *M*(*SD*) and therefore reestablish some level of distortion.

We were unable to directly purchase males containing the revertant $SD\text{-}5^{r7}$ chromosome (distortion-primed) as well as the individual deletion of focus, so instead we set up crosses for each stock to obtain the desired genotype. First, we collected approximately 10 virgin females from each stock. All stocks contained flies that were heterozygous for the particular deletion and a curly-winged (*Cy*) marker, and since flies that are homozygous for either the deletion or the *Cy* allele are non-viable, all females collected could be assumed to be heterozygotes (barring contamination of the stock). We then crossed these virgin females with approximately 5 males

that we collected from the *Rsp^{s}B^{s}; SD-5^{r7}/Cy* standard stock. From this cross, we then collected the appropriate offspring (*Rsp^{s}B^{s}; SD-5^{r7}/deletion*) 10-19 days after initiating the cross, which ensured that only the first generation of offspring was available for collection, since fruit flies take a minimum of ten days to eclose into adults after eggs are laid.

We collected males from these crosses that contained the *Rsp^{s}B^{s}; SD-5^{r7}* chromosome and the specific deletion and then discarded the rest. The Rsp^{s} and B^{s} alleles were connected to the Y-chromosome, so all male offspring contained these alleles (this was confirmed by the males having the bar-stone eye phenotype). Both the *SD-5^{r7}* allele and the specific deletion, on the other hand, were present together in a smaller portion of the offspring; however, we confirmed the presence of both by the lack of the curly wing phenotype (both autosomal genes came from stocks that were heterozygous with the *Cy* allele). In summary, we only collected straight-winged, bar-stone males from these crosses and then used them for individual *k* tests (we collected a maximum number of 20 males for each *k* test but used fewer if necessary).

We set up a *k* test for each of the deletion-containing stocks to determine if the deleted segment caused an aberrant female-to-total-offspring ratio when introduced to distortion-primed males. To set up a *k* test, we put each of the *Rsp^{s}B^{s}; SD-5^{r7}/deletion* males (F1, or first generation) collected from the previous cross in separate vials (labeled 1-20) along with 2 *cnbw* standard virgin females (collected at regular intervals before they are mature enough to mate) and left them at 25°C for 4 days. We then transferred the flies to a new set of vials (labeled 1'-20') and left them for another 4 days at 25°C, after which we discarded the flies. We then kept the 40 vials at 25°C, and exactly 2 weeks after setting up each *k* test, we counted and recorded the number of male offspring (F2, or second generation) and the number of female offspring present in each vial of the first set (vials 1-20). After another 4 days, we discarded these flies and repeated a second count on the first set of vials and an initial count on the second set of vials

(this corresponded to exactly 2 weeks after the flies were transferred to the second set of vials). Following another 4 days, we concluded the *k* test with a second count on the second set of vials (vials 1'-20').

We entered the data from each *k* test into an Excel file which calculated the ratio of female offspring to total offspring (called the *k* value) for each deletion-containing F1 male. The importance of the *k* value was that it represented the ratio of distortion-insensitive flies (all females contained only an Rsp^{i} allele) to distortion-sensitive flies (males contained an Rsp^{s} allele on the Y chromosome). If the particular deletion in the F1 male had caused distortion, the associated *k* value would be raised because fewer male offspring would be viable. For this reason, the *k* value was a direct measurement of the level of distortion associated with a particular F1 male. We combined the *k* values for each of the F1 males for a particular *k* test to give both an average *k* value (along with standard error) and a total *k* value for each *k* test. The average *k* value represented the average of each *k* value recorded for the separate F1 males of a *k* test, whereas the total *k* value represented the female/total offspring ratio for the combined offspring from each vial associated with the *k* test. We excluded the results of any vial that did not bear offspring from these calculations since we could not determine a *k* value in such a case. We assumed these occurrences to be caused by non-distortion related problems (i.e. old food or random mutations resulting in infertility), so including such data would obscure the calculated *k* values.

We compared the average *k* values for each *k* test to *k* values from a control *k* test to determine the extent of distortion. The males for this control test contained a *Cy* allele instead of a particular deletion, and we crossed them to standard *cnbw* virgin females as in the experimental *k* tests. We kept all other procedures the same for the control test, and the resulting *k* values provided a reference point for the experimental *k* tests that would follow.

We also performed *k* tests on 3 specific genes (*torso*, *saxophone*, and *CG30497*) to test for distortion-causing effects of these genes and therefore a possible correlation to *Modifier of SD*. We introduced these genes into the distortion-primed *SD-5*^{r7} stock through the same crosses that were involved with setting up *k* tests for the individual deleted regions, and we kept all other procedures for these *k* tests identical to those for the previous *k* tests. The only difference was that each of these 3 stocks contained loss-of-function alleles for the corresponding gene instead of a deleted segment of DNA.

RESULTS

A summary of the locations of the individual deletions for each stock used in the initial *k* tests is presented in Table 1. *k* values for each of these *k* tests as well as for the *k* tests performed on the specific genes *torso*, *saxophone*, and *CG30497* (stocks 1764, 8785, and 11062, respectively) are given in Table 2.

Deletion Locations					
Stock #	**Deleted Segment**	**Stock #**	**Deleted Segment**	**Stock #**	**Deleted Segment**
control	none	23163	43B2 to 43C5	8941	43E4 to 44B5
8045	42A13 to 42E6	6142	43C1 to 43D3-7	24335	44A4 to 44C4
9062	42E1 to 43D3	7535	43D3 to 43E9	7860	44B3 to 44C2
8931	43A4 to 43F1	7536	43E9 to 43E18	7539	44B8 to 44C4
8889	43A1-2 to 43B2	23164	43E16 to 43F4	9276	44B8 to 44E3

Table 1—Deleted segments for each of the individual stocks. Arranged by deletion order.

***k* Values**							
Stock #	**Avg. *k* value**	**Std. error**	**Total *k* value**	**Stock #**	**Avg. *k* value**	**Std. error**	**Total *k* value**
control	0.612	0.024	0.591	23164	0.706	0.015	0.704
8045	0.601	0.022	0.601	8941	0.569	0.019	0.592
9062	0.546	0.022	0.548	24335	0.640	0.021	0.623
8931	0.657	0.075	0.551	7860	0.673	0.016	0.675
8889	0.550	0.017	0.551	7539	0.725	0.025	0.716
23163	0.633	0.018	0.631	9276	0.595	0.017	0.596
6142	0.595	0.017	0.587	1764	0.689	0.017	0.695
7535	0.629	0.020	0.618	8785	0.629	0.017	0.606
7536	0.828	0.020	0.818	11062	0.568	0.026	0.588

Table 2—Average and total *k* value results for the control stock and each experimental stock. Average *k* value is determined by averaging the individual *k* values for each F1 male used in the *k* test. Total *k* value is determined by combining the number of male and female offspring for each F1 male in the *k* test and then calculating the female/total offspring ratio from the combined offspring. Stocks that showed a significant level of distortion are highlighted.

Since fruit flies normally produce offspring in a 1:1 male-to-female ratio, one would expect the average and total *k* values for the control *k* test to be near 0.500. The resulting values, however, were closer to 0.600, which could possibly be due to a minor distortion effect

being caused by the distortion-primed *SD-5*^{r7} allele or by a reduced viability of male offspring caused by the presence of the bar-stone eye (*B*^{s}) marker (McLean et al., 1994). For this reason, we only considered *k* value results for the experimental stocks that were significantly greater than 0.612 to be involved with distortion. We determined significance by subtracting the standard error for each experimental *k* test from the average *k* value and comparing that to the standard error range of the average *k* value for the control test (0.612 ± 0.024). If the ranges of the control *k* value and an experimental *k* value did not overlap, we considered the experimental *k* value to be significantly greater than the control *k* value.

The first interesting results that we obtained from the *k* tests did not actually show any noticeable distortion but instead drew attention for another reason. When we set up the *k* test for stock 8931 for the first time, we recorded no offspring in any of the 20 vials used in this test. To determine if this was due to an infertility problem with the parents or a bad batch of food, we repeated the test once again. The second time through, 12 of the 20 vials produced offspring (Appendix, Table A4), but the numbers were dramatically reduced as compared to *k* tests for other stocks. This indicates that, although not completely infertile, the male parent in each vial showed a reduced level of fertility (standard *cnbw* females were used in each vial, so the issue had to lie with the male).

Since the deletion for stock 8931 encompassed a rather large segment of DNA (43A4 to 43F1), we purchased new stocks (8889, 23163, 6142, 7535, 7536, and 23164) that each contained a smaller deletion within that segment in order to narrow down the problem-causing area, and we performed *k* tests on each of them to determine if a significant level of distortion was associated with any of the deleted DNA regions. From these new stocks and the original stocks obtained at the start of the experiment, we found several DNA regions that showed significant levels of distortion based on their average *k* values. Stock #7536 (0.828 ± 0.020),

stock #23164 (0.706 ± 0.015), stock #7860 (0.673 ± 0.016), and stock #7539 (0.725 ± 0.025) each had *k* values that were significantly greater than the control *k* value and are therefore suspect for a possible correlation to the *M*(*SD*) locus. Among these stocks, 7536 showed the highest level of distortion (average *k* value of 0.828 ± 0.020), so specific genes within the deleted section of DNA became the next focus for testing. These genes were *torso* (stock #1764), *saxophone* (stock #8785), and *CG30497* (stock #11062). From the *k* tests performed on these genes, only *torso* showed a significant level of distortion (average *k* value = 0.689 ± 0.017); however, this value is relatively low when compared to the *k* value when the entire 43E9 to 43E18 region (stock #7536) was deleted.

DISCUSSION

Any deletion that results in significantly high levels of distortion could potentially coincide with the location of the *M*(*SD*) gene due to the possibility that deleting part or all of this modifier gene could mimic the effects of the mutated, distortion-promoting form. Since the distortion-causing form of *M*(*SD*) could potentially involve a gain-of-function mutation (as is the case with *Sd*), the possibility exists that deleting the segment of DNA corresponding to *M*(*SD*) will not give the same results as the mutant form; however, the procedures required to account for this are outside the scope of this experiment, and any negative results (non-highlighted stocks in Table 2) are therefore not conclusive for the absence of *M*(*SD*) in the associated area (J. McLean, personal communication, February 27, 2013).

As seen by the highlighted stocks in Table 2, certain stocks gave a significant level of distortion when we introduced their associated deletion into distortion-primed males. Due to time constraints, it was not possible to look through each of these areas for genes that could potentially correspond to *M*(*SD*), so the stock that showed the highest level of distortion (#7536) was chosen for further inspection under the assumption that the region associated with the greatest distortion would more likely contain a major contributor to the *Segregation Distorter* system [e.g. *M*(*SD*)]. The loci for approximately 50 known or suspected genes are found within the 43E9 to 43E18 region, and each has the potential to correspond to *M*(*SD*); however, due to time constraints, we selected only 3 of these genes for further *k* tests.

The first of these genes is the protein-coding gene *torso*, which is thought to be involved with several processes relating to embryogenesis and other kinase-involving pathways (Grillo, Furriols, de Miguel, Franch-Marro, & Casanova, 2012; Helman et al., 2012; Sprenger, Stevens, & Nüsslein-Volhard, 1989). We selected this gene due to the large number of suspected functions for this gene as well as the relative abundance of background literature for it. Stock 1764

contained a loss-of-function allele of *torso* (McQuilton, St. Pierre, Thurmond, & the FlyBase Consortium, 2012), which allowed us to use this stock to test for a possible correlation between *torso* and *M*(*SD*). The resulting *k* value for this stock (0.689 ± 0.017) indicates a significant level of distortion, although it does not account for the entire distortion created by deletion of the 43E9 to 43E18 region (0.828 ± 0.020). For this reason, it is likely that there is some other nearby gene or group of genes that accounts for the remainder of this distortion. Even so, further studies into the function of *torso* may provide a link to the action of *M*(*SD*) or some other *SD*-related gene.

The second gene, *saxophone*, is thought to encode a growth factor receptor, and has suspected involvement in numerous cellular processes (Twombly, Blackman, Jin, Graff, Padgett, & Gelbart, 1996; Xie, Finelli, & Padgett, 1994). We chose this gene for further study based on its role in gamete formation (known to be fundamental to the mechanisms of *Segregation Distorter*) as well as for the abundant literature on this gene and related genes (Casanueva & Ferguson, 2004). Stock 8785 contained a loss-of-function allele of *saxophone* (McQuilton et al., 2012), we used this stock to test for correlation to *M*(*SD*). The resulting *k* value for this stock (0.629 ± 0.017), however, was too low to indicate such a correlation.

We chose the final gene, *CG30497*, not for its function within *Drosophila* development but merely for its size in proportion to other genes in the 43E9 to 43E18 region. The molecular function of *CG30497* is unknown; however, it spans a region of approximately 40,000-50,000 base pairs (McQuilton et al., 2012). Stock 11062 contains a transposable P-element inserted into the *CG30497* gene, which is thought to result in a complete loss-of-function allele, although this is not certain (J. McLean, personal communication, February 27, 2013). Again, the low *k* value for this stock (0.568 ± 0.026) does not indicate a positive correlation to *M*(*SD*).

CONCLUSION

Modifier of Segregation Distorter is a poorly studied gene that is in some way involved with the *Drosophila melanogaster* meiotic drive system *Segregation Distorter*. Since the overall system has been shown to affect spermatid development (Hauschteck-Jungen & Hartl, 1982; McElroy et al., 2008), a better knowledge of the related genes like *M*(*SD*) could allow for deeper insights into the complex mechanisms involved with spermiogenesis and fertility as a whole. Since even such basic information as the location of *M*(*SD*) is still uncertain, it is important that further research be done to locate this gene as well as to determine its function. To accomplish this, we propose that the remainder of the genes within the DNA regions that were associated with a significant level of distortion be analyzed for a possible correlation to *M*(*SD*). In particular, *CanB2* and *cathD* would be good candidates due to their suspected roles in meiosis and apoptosis, respectively (McQuilton et al., 2012).

REFERENCES

Aoki, V. W., & Carrell, D. T. (2003). Human protamines and the developing spermatid: Their structure, function, expression and relationship with male infertility. *Asian Journal of Andrology, 5*, 315-324.

Bilbao-Cortes, D., Hetzer, M., Längst, G., Becker, P. B., & Mattaj, I. W. (2002). Ran binds to chromatin by two distinct mechanisms. *Current Biology, 12*(13), 1151-1156.

Brittnacher, J. G., & Ganetzky, B. (1984). On the components of segregation distortion in *Drosophila melanogaster*. III. Nature of enhancer of SD. *Genetics, 107*(3), 423-434.

Brittnacher, J. G., & Ganetzky, B. (1989). On the components of segregation distortion in *Drosophila melanogaster*. IV. Construction and analysis of free duplications for the responder locus. *Genetics, 121*(4), 739-750.

Casanueva, M.O., & Ferguson, E.L. (2004). Germline stem cell number in the *Drosophila* ovary is regulated by redundant mechanisms that control *Dpp* signaling. *Development, 131*(9), 1881-1890.

Dasso, M. (2001). Running on Ran: Nuclear transport and the mitotic spindle. *Cell, 104*(3), 321-324.

Dasso, M. (2002). The Ran GTPase: Theme and variations. *Current Biology, 12*(14), 502-508.

Ganetzky, B. (1977). On the components of segregation distortion in *Drosophila melanogaster*. *Genetics, 86*(2), 321-355.

Ganetzky, B. (1999). Yuichiro Hiraizumi and forty years of segregation distortion. *Genetics, 152*(1), 1-4.

Ganetzky, B. (2000). Tracking down a cheating gene. *American Scientist, 88*(2), 128-134.

Görlich, D., Panté, N., Kutay, U., Aebi, U., & Bischoff, F. R. (1996). Identification of different roles for RanGDP and RanGTP in nuclear protein import. *The EBMO Journal, 15*(20), 5584-5594.

Grillo, M., Furriols, M., de Miguel, C., Franch-Marro, X., & Casanova, J. (2012). Conserved and divergent elements in Torso RTK activation in *Drosophila* development. *Scientific Reports, 2*(762).

Hao, Y., & Macara, I. G. (2008). Regulation of chromatin binding by a conformational switch in the tail of the Ran exchange factor RCC1. *The Journal of Cell Biology, 182*(5), 827-836.

Hauschteck-Jungen, E., & Hartl, D. L. (1982). Defective histone transition during spermiogenesis in heterozygous *Segregation Distorter* males of *Drosophila melanogaster*. *Genetics, 101*(1), 57-69.

Helman, A., Lim, B., Andreu, M. J., Kim, Y., Shestkin, T., Lu, H., Jiménez, G., Shvartsman, S. Y., & Paroush, Z. (2012). RTK signaling modulates the Dorsal gradient. *Development, 139*(16), 3032-3039.

Hiraizumi, Y., Sandler, L., & Crow, J. F. (1960). Meiotic drive in natural populations of *Drosophila melanogaster*. III. Populational implications of the segregation-distorter locus. *Evolution, 14*(4), 433-444.

Hiraizumi, Y., & Nakazima, K. (1967). Deviant sex ratio associated with segregation distortion in *Drosophila melanogaster*. *Genetics, 55*(4), 681-697.

Hiraizumi, Y., Martin, D. W., & Eckstrand, I. A. (1980). A modified model of segregation distortion in *Drosophila melanogaster*. *Genetics, 95*(3), 693-706.

Jayaramaiah-Raja, S., & Renkawitz-Pohl, R. (2005). Replacement by *Drosophila melanogaster* protamines and Mst77F of histones during chromatin condensation in late spermatids and role of Sesame in the removal of these proteins from the male pronucleus. *Molecular and Cellular Biology, 25*(14), 6165-6177.

Joseph, J. (2006). Ran at a glance. *Journal of Cell Science, 119*(17), 3481-3484.

Kusano, A. Staber, C., & Ganetzky, B. (2001). Nuclear mislocalization of enzymatically active RanGAP causes segregation distortion in *Drosophila*. *Developmental Cell, 1*(3), 351-356.

Kusano, A., Staber, C., & Ganetzky, B. (2002). Segregation distortion induced by wild-type RanGAP in *Drosophila*. *PNAS, 99*(10), 6866-6870.

Kusano, A., Staber, C., Chan, H. Y. E., & Ganetzky, B. (2003). Closing the (Ran)GAP on segregation distortion in *Drosophila*. *BioEssays*, *25*(2), 108-115.

Lyttle, T. W., Brittnacher, J. G., & Ganetzky, B. (1986). Detection of *Rsp* and modifier variation in the meiotic drive system *Segregation Distorter* (*SD*) of *Drosophila melanogaster*. *Genetics, 114*(1), 183-202.

Macara, I. G. (1999). Nuclear transport: Randy couples. *Current Biology, 9*(12), 436-439.

Martin, D. W., & Hiraizumi, Y. (1979). On the models of segregation distortion in *Drosophila melanogaster*. *Genetics, 93*(2), 423-435.

McElroy, J. M., McLean, R. A., & McLean, J. R. (2008). Defects in nuclear transport enhance segregation distortion. *Fly, 2*(6), 280-290.

McLean, J. R., Merrill, C. J., Powers, P. A., & Ganetzky, B. (1994). Functional identification of the *Segregation distorter* locus of *Drosophila melanogaster* by germline transformation. *Genetics, 137*(1), 201-209.

McQuilton, P., St. Pierre, S. E., Thurmond, J., & the FlyBase Consortium. (2012). FlyBase 101 – the basics of navigating FlyBase. *Nucleic Acids Res*., *40*(Database issue): D706-14.

Merrill, C., Bayraktaroglu, L., Kusano, A., & Ganetzky, B. (1999). Truncated RanGAP encoded by the segregation distorter locus of *Drosophila*. *Science*, *283*(5408), 1742-1745.

Oliva, R. (2006). Protamines and male infertility. *Human Reproduction Update, 12*(4), 417-435.

Peterson, C. L., & Laniel, M. A. (2004). Histones and histone modifications. *Current Biology, 14*(14), 546-551.

Powers, P. A., & Ganetzky, B. (1991). On the components of segregation distortion in *Drosophila melanogaster*. V. Molecular analysis of the *Sd* locus. *Genetics, 129*(1), 133-144.

Rathke, C., Baarends, W. M., Jayaramaiah-Raja, S., Bartkuhn, M., Renkawitz, R., & Renkawitz-Pohl, R. (2007). Transition from a nucleosome-based to a protamine-based chromatin configuration during spermiogenesis in *Drosophila*. *Journal of Cell Science, 120,* 1689-1700.

Sandler, L., Hiraizumi, Y., & Sandler, I. (1959). Meiotic drive in natural populations of *Drosophila melanogaster*. I. The Cytogenetic basis of segregation distortion. *Genetics, 44*(2), 233-250.

Smith, A., Brownawell, A., & Macara, I. G. (1998). Nuclear import of Ran is mediated by the transport factor NTF2. *Current Biology, 8*(25), 1403-1406.

Smith, A. E., Slepchenko, B. M., Schaff, J. C., Loew, L. M., & Macara, I. G. (2002). Systems analysis of Ran transport. *Science, 295*(5554), 488-491.

Sprenger, F., Stevens, L., & Nüsslein-Volhard, C. (1989). The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. *Nature*, *338*, 478-483.

Temin, R. G. (1991). The independent distorting ability of the *Enhancer of Segregation Distortion*, *E(SD)*, in *Drosophila melanogaster*. *Genetics, 128*(2), 339-356.

Twombly, V., Blackman, R.K., Jin, H., Graff, J.M., Padgett, R.W., & Gelbart, W.M. (1996). The TGF-beta signaling pathway is essential for *Drosophila* oogenesis. *Development, 122*(5), 1555-1565.

Xie, T., Finelli, A., & Padgett, R.W. (1994). The *Drosophila saxophone* gene: A serine-threonine kinase receptor of the TGF-superfamily. *Science, 263*(5154), 1756-1759.

Appendix—Vial Counts

The individual vial counts for the control *k* test are recorded in Table A1. Individual counts for each experimental *k* test are recorded in Tables A2-A15.

Control							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	26	17	0.605	11	0	0	NA
2	42	20	0.677	12	29	18	0.617
3	45	42	0.517	13	29	6	0.829
4	15	5	0.750	14	29	12	0.707
5	17	15	0.531	15	31	35	0.470
6	35	27	0.565	16	33	14	0.702
7	34	27	0.557	17	21	15	0.583
8	25	13	0.658	18	3	1	0.750
9	15	19	0.441	19	21	17	0.553
10	34	34	0.500	20	56	36	0.609

Table A1—Control *k* test with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial. NA indicates the vial was not included in final results due to a lack of offspring.

8045							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	34	45	0.430	11	46	34	0.575
2	34	18	0.654	12	29	13	0.690
3	18	20	0.474	13	45	20	0.692
4	28	22	0.560	14	53	32	0.624
5	17	8	0.680	15	18	16	0.529
6	43	13	0.768	16	17	23	0.425
7	32	15	0.681	17	37	33	0.529
8	23	14	0.622	18	21	23	0.477
9	42	23	0.646	19	39	16	0.709
10	41	26	0.612	20	41	22	0.651

Table A2—*k* test for stock 8045 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial.

9062							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	48	31	0.608	8	28	34	0.452
2	30	20	0.600	9	84	63	0.571
3	46	38	0.548	10	45	21	0.682
4	30	23	0.566	11	25	22	0.532
5	39	32	0.549	12	8	14	0.364
6	37	36	0.507	13	5	3	0.625
7	82	82	0.500				

Table A3—*k* test for stock 9062 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial.

8931							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	4	0	1.000	11	0	0	NA
2	13	8	0.619	12	1	0	1.000
3	17	17	0.500	13	15	13	0.536
4	12	11	0.522	14	2	0	1.000
5	0	0	NA	15	3	0	1.000
6	1	1	0.500	16	3	5	0.375
7	0	0	NA	17	0	0	NA
8	0	0	NA	18	2	3	0.400
9	3	4	0.429	19	0	0	NA
10	0	0	NA	20	0	0	NA

Table A4—*k* test for stock 8931 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial. NA indicates the vial was not included in final results due to a lack of offspring.

8889							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	47	37	0.560	7	42	27	0.609
2	46	24	0.657	8	55	57	0.491
3	89	66	0.574	9	23	21	0.523
4	42	44	0.488	10	55	47	0.539
5	17	18	0.486	11	27	25	0.519
6	35	23	0.603				

Table A5—*k* test for stock 8889 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial.

23163							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	66	40	0.623	11	36	21	0.632
2	0	0	NA	12	49	42	0.538
3	0	0	NA	13	64	32	0.667
4	70	37	0.654	14	81	51	0.614
5	61	37	0.622	15	46	43	0.517
6	75	32	0.701	16	36	36	0.500
7	57	27	0.679	17	22	7	0.759
8	78	45	0.634	18	0	0	NA
9	73	50	0.593	19	107	32	0.770
10	79	58	0.577	20	49	23	0.681

Table A6—*k* test for stock 23163 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial. NA indicates the vial was not included in final results due to a lack of offspring.

6142							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	62	50	0.554	11	71	43	0.623
2	9	5	0.643	12	26	16	0.619
3	35	25	0.583	13	63	31	0.670
4	40	35	0.533	14	31	28	0.525
5	10	3	0.769	15	29	19	0.604
6	24	20	0.545	16	11	9	0.550
7	79	57	0.581	17	19	24	0.442
8	31	22	0.585	18	40	19	0.678
9	4	2	0.667	19	46	43	0.517
10	82	50	0.621				

Table A7—*k* test for stock 6142 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial.

7535							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	48	29	0.623	11	55	22	0.714
2	41	28	0.594	12	55	36	0.604
3	100	62	0.617	13	0	0	NA
4	0	0	NA	14	54	44	0.551
5	0	0	NA	15	58	44	0.569
6	0	0	NA	16	43	32	0.573
7	11	2	0.846	17	37	27	0.578
8	14	9	0.609	18	71	30	0.703
9	9	6	0.600	19	0	0	NA
10	68	50	0.576	20	68	32	0.680

Table A8—*k* test for stock 7535 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial. NA indicates the vial was not included in final results due to a lack of offspring.

7536							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	13	6	0.684	11	52	24	0.684
2	64	9	0.877	12	45	1	0.978
3	19	5	0.792	13	116	22	0.841
4	27	7	0.794	14	110	23	0.827
5	28	5	0.848	15	99	18	0.846
6	44	4	0.917	16	20	0	1.000
7	61	6	0.910	17	36	9	0.800
8	52	26	0.667	18	48	8	0.857
9	60	15	0.800	19	42	7	0.857
10	18	3	0.857	20	87	33	0.725

Table A9—*k* test for stock 7536 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial.

23164							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	76	24	0.760	11	77	34	0.694
2	81	44	0.648	12	106	18	0.855
3	66	24	0.733	13	53	23	0.697
4	115	40	0.742	14	95	45	0.679
5	38	16	0.704	15	0	0	NA
6	79	16	0.832	16	30	12	0.714
7	82	38	0.683	17	67	30	0.691
8	89	51	0.636	18	88	54	0.620
9	94	52	0.644	19	48	25	0.658
10	84	24	0.778	20	49	26	0.653

Table A10—*k* test for stock 23164 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial. NA indicates the vial was not included in final results due to a lack of offspring.

8941							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	61	38	0.616	9	38	28	0.576
2	0	0	NA	10	41	26	0.612
3	88	50	0.638	11	23	16	0.590
4	0	0	NA	12	3	3	0.500
5	17	10	0.630	13	43	24	0.642
6	72	54	0.571	14	30	29	0.508
7	32	26	0.552	15	54	41	0.568
8	2	3	0.400				

Table A11—*k* test for stock 8941 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial. NA indicates the vial was not included in final results due to a lack of offspring.

24335							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	49	44	0.527	11	31	11	0.738
2	93	74	0.557	12	21	6	0.778
3	90	22	0.804	13	92	70	0.568
4	3	3	0.500	14	89	64	0.582
5	75	67	0.528	15	35	22	0.614
6	90	56	0.616	16	45	29	0.608
7	117	84	0.582	17	45	25	0.643
8	15	4	0.789	18	86	27	0.761
9	47	19	0.712	19	76	57	0.571
10	107	47	0.695	20	96	56	0.632

Table A12—*k* test for stock 24335 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial.

7860							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	73	34	0.682	11	42	22	0.656
2	53	30	0.639	12	19	12	0.613
3	92	32	0.742	13	49	31	0.613
4	57	12	0.826	14	46	18	0.719
5	37	23	0.617	15	22	12	0.647
6	58	23	0.716	16	29	6	0.829
7	52	28	0.650	17	59	22	0.728
8	39	17	0.696	18	64	42	0.604
9	35	27	0.565	19	48	25	0.658
10	60	30	0.667	20	24	16	0.600

Table A13—*k* test for stock 7860 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial.

7539							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	4	3	0.571	11	14	0	1.000
2	37	5	0.881	12	72	33	0.686
3	10	2	0.833	13	39	16	0.709
4	50	15	0.769	14	68	13	0.840
5	23	6	0.793	15	50	31	0.617
6	18	12	0.600	16	33	15	0.688
7	29	13	0.690	17	24	10	0.706
8	20	14	0.588	18	53	35	0.602
9	32	11	0.744	19	13	7	0.650
10	40	12	0.769	20	46	15	0.754

Table A14—*k* test for stock 7539 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial.

9276							
Vial #	**insensitive females**	**sensitive males**	***k* value**	**Vial #**	**insensitive females**	**sensitive males**	***k* value**
1	16	7	0.696	8	29	18	0.617
2	59	26	0.694	9	15	12	0.556
3	74	42	0.638	10	42	38	0.525
4	28	31	0.475	11	23	22	0.511
5	50	30	0.625	12	37	24	0.607
6	49	28	0.636	13	59	43	0.578
7	24	15	0.615	14	66	51	0.564

Table A15—*k* test for stock 9276 with number of distortion-insensitive female and distortion-sensitive male offspring and individual *k* values for each vial.