5-2012

Possible Distorting Effects of Gef26 in Drosophila Melanogaster

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POSSIBLE DISTORTING EFFECTS OF Gef26 IN *Drosophila melanogaster.*

By

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Honors Capstone Project

Submitted to the Faculty of

Olivet Nazarene University

for partial fulfillment of the requirements for

GRADUATION WITH UNIVERSITY HONORS

March 13, 2012

BACHELOR OF ARTS

in

Biology

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ACKNOWLEDGEMENTS

Special thanks to Dr. Janna McLean for continuing support and guidance as a phenomenal research mentor who not only provided endless critical editing support and guidance throughout the project, but also was a spiritual example through a very difficult couple of years.

The Bloomington Stock Company was vitally important in providing the stock tested in the research outlined within this paper.

Special thanks to the Honors Program at Olivet, which despite its young age is giving a great opportunity to undergraduates helping prepare them for a better, well rounded future in both graduate studies and the work place.

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ABSTRACT

Segregation distortion is a meiotic drive system that results in the favorable inheritance of the *SD* chromosome over the SD^{+}counterpart. *SD* produces a malfunctioning form of RanGAP, instead of being able to move in and out of the nucleus this truncated RanGAP is unable to be exported and therefore aggregates within the nucleus. This appears to affect Rsp^{S} during the condensation phase of spermatogenesis, but the exact mechanism for this is unknown. In order to further understand the working components of this system, specific deletions within the second chromosome were studied. The study was conducted systematically by beginning with larger deletions that had previously shown distortion and slowly reducing the size of the deletion. When these deletion stocks were combined with $SD\text{-}5^{r7}$, with Rsp^{S} on the Y chromosome it was found that a deletion of the region 26C1;26D1 displayed distortion whereas deletion of the region 26C3;26D1 did not. When a deletion of the 26C2;26C3 region was tested distortion was observed. Within this region the genes *Cpr* and *Gef26* are of particular interest. Stocks containing a mutation in *Cpr* did not demonstrate distortion when combined with $SD\text{-}5^{r7}$. However, when a stock containing a mutation in the gene *Gef26* was combined with $SD\text{-}5^{r7}$, distortion was noted. These results suggest that better understanding of the gene *Gef26*, and its function within the cell during spermatogenesis, would shed some light as to how segregation distortion takes place on a molecular level.

INTRODUCTION

The fruit fly, *Drosophila melanogaster,* has been a central part of scientific investigation for a number of years, drawing specific attention in the field of genetic research. *Drosophila melanogaster* has been used as a suitable genetic model for organisms as simple as *Mycobacterium marinum* (Dionne et al., 2003) to even one as complex as *Homo sapiens* (Pandy & Nichols, 2011, Guarnieri & Heberlein, 2011). There are multiple reasons for the organism's usefulness in a wide variety of experiments. For instance, the fruit fly is a small creature that can be bred easily and in small spaces, it has a short generation time, the females present a high fecundity, morphological traits are relatively easy to distinguish and use as markers, and the list goes on; all of these examples, and other reasons, made *Drosophila melanogaster* the ideal candidate for the Capstone Research Project at Olivet Nazarene University.

While it is one of the best genetic models for research commonly used today, *Drosophila melanogaster* also possesses some genetic peculiarities of its own. One such oddity is the concept of segregation distortion, and the effect that a specific gene (*Sd*) has on the reproductive success of affected males. The purpose of this research was to extend our knowledge of the mechanism thought to cause this interesting shift in inheritance patterns.

REVIEW OF LITERATURE

There are a few vital genetic laws that allow for the careful analysis and prediction of inheritance of traits in a population. One of these laws is Mendel's First Law, the Law of Equal Assortment, which states when a trait is determined by the presence of two alleles then each of these alleles has an equal opportunity to be passed on to the offspring. However, in nature there are systems that violate this principle and these are termed meiotic drive systems, which are systems in which one allele is preferentially passed on to the offspring over the alternative (McElroy et al., 2008). One such system that has been studied to great lengths is the system of *SD*, *Segregation Distorter*. In this system one chromosome, *SD*, is passed on nearly 100% of the time over its counterpart, the SD^{+}chromosome (Ganetzky, 2000).

The *SD* chromosome is present in roughly 3-5% of any given wild population of *Drosophilamelanogaster* (Ganetzky, 2000). However, when taking the population demographics into consideration it is important to note that the *SD* chromosome as a whole does not cause the distortion but rather specific genes within that chromosome. The most vital gene in the process is the *Sd* gene itself. In SD flies the *Sd* gene is 12,000 base pairs long, while that same segment in normal flies is only half that length (McLean et al., 1994).

Figure 1 is a visual representation of faulty spermatogenesis due to *Sd*. Note the location of the *Sd* and *Sd+* genes, they are on homologous chromosome. Therefore, only one or the other will be passed on to a specific offspring. This diagram also shows how only the homolog possessing the *Sd* gene forms sperm capable of functioning properly,

and therefore the *SD* chromosome is passed on with a high frequency due to the inability of the *SD+* sperm to form.

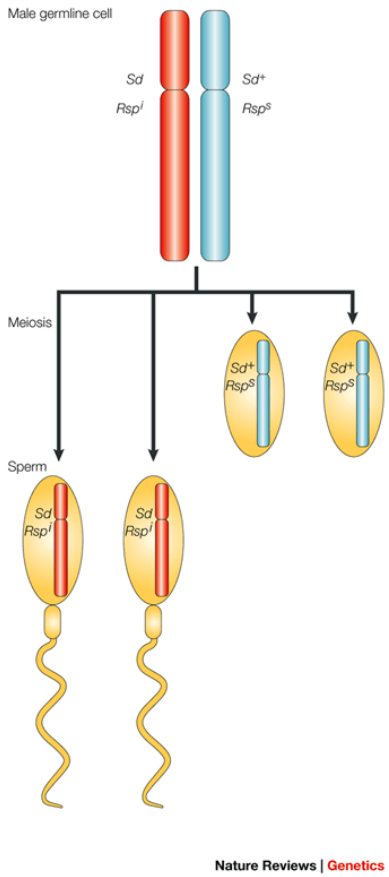


Figure 1: Comparison of spermatogenesis between *SD* and *SD+* chromosomes. Hurst, Gregory D.D. & Werren, John H. (2001) Figure 3 | Model of Segregation Distorter (Sd) in Drosophila melanogaster. http://www.nature.com/nrg/journal/v2/n8/fig_tab/nrg0801_597a_F3.html

The *Sd* allele is actually a mutant form, or allele, of a gene, here named Sd^{+}. The Sd^{+} gene codes for the enzyme RanGAP. This enzyme is vital to cell function, specifically to the process of nuclear transport. Eukaryotic cells, like those in humans and other higher organisms, have cellular divisions that are called membrane bound organelles. One of these organelles, the nucleus, is a region within the cell that is responsible for containing the cell's genetic information and is separated from the cytoplasm by a nuclear membrane. When functioning as intended RanGAP is actually present in two

forms, one of which cycles in and out of the nucleus helping while another pool of RanGAP is attached to the outside of the nuclear membrane, facing the cytoplasm. Both of these pools, in their correct placement and concentration, are necessary in order to shuttle substances back and forth between the nucleus and the cytoplasm.

The *Sd* allele also produces a form of RanGAP, however Sd-RanGAP is slightly truncated and contains only one of the two necessary nuclear export signals, NESs, which the normal RanGAP contains (Kusano et al., 2003). This alteration causes Sd-RanGAP to have an abnormally high concentration in the nucleus in contrast to normal RanGAP that usually has a higher net concentration in the cytoplasm, despite movement in and out of the nucleus. Other genes present on the *SD* chromosome that are vital to the process include *Enhancer of SD* [*E(SD*)], *Modifier ofSD* [*M(SD*)], and *Stabilizer ofSD* [*St(SD*)]. All of these extra genes are required in order to cause the full distortion effect. Deletion or recombination of these portions can cause the transmission of the *SD* chromosome to be reduced to 60-80% (McLean et al., 1994), although the specific function of these genes is not well understood.

However, the presence of *Sd* and the other genes mentioned are not able to cause distortion on their own. The portion of DNA that reacts to the distorter effects is called *Responder* (*Rsp)*. *Responder* is a gene that has many alleles. These alleles include *Responderinsensitive* (*Rsp^{i})*, *Respondersemi-insensitive* (*Rsp^{si})*, *Respondersensitive* (*Rsp^{s})*, and *Respondersupersensitive* (*Rsp^{ss};* Ganetzky, 2000). *Responder* is thought to be a piece of heterochromatin that is a roughly 120 base pair sequence, rich in adenine and thymine that repeats. Interestingly enough, the level of sensitivity of the allele is

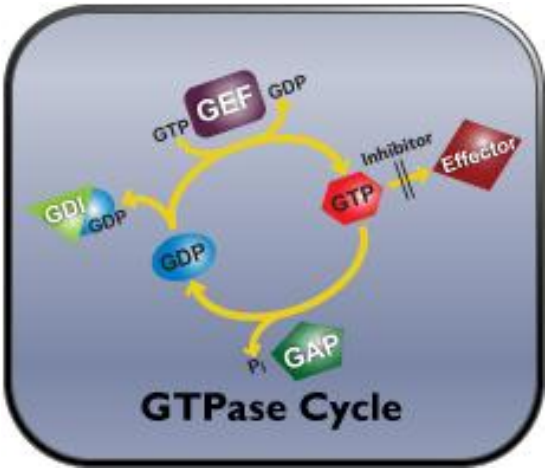
dependent upon the number of repeats of the sequence. For example *Rsp*^{i}contains fewer than fifty copies of the sequence, where as *Rsp*^{s} has several hundred copies, and *Rsp*^{ss} has around 1,000 (Ganetzky, 2000). It should be noted that *Rsp*^{i} is present on *SD* chromosomes, theoretically giving the chromosome immunity to the processes that the gene products implement.

The overall process of segregation distortion that is understood up to this point is relatively straight forward. The *SD* chromosome produces Sd-RanGAP which, due to its incorrect number of NESs, is mislocalized to the nucleus in abnormally high concentrations. This causes the RanGTP gradient over the nuclear envelope to be disturbed resulting in a malfunction of nuclear transport (Kusano et al., 2003). During spermatogenesis this proves fatal to certain developing sperm cells as chromosomes containing *Rsp*^{s}, *Rsp*^{si}, and *Rsp*^{ss} alleles fail to complete spermatogenesis due to lack of chromatin condensation. At this point there is a lot left to be understood. For instance: Why is only the *Rsp*^{s} bearing chromosome affected by the abnormal concentration gradient of RanGTP that is caused by Sd-RanGAP? Why does this failure of chromosome condensation occur in late spermatogenesis but not in mitosis and meiosis, when chromosomes also condense? Are there only particular parts of nuclear transport that are affected or can the entire system be faulty due only to the altered RanGTP gradient?

The first part of the system that is important to understand is the Ran family of enzymes that are involved in nuclear transport. In simple terms Ran has the ability to alternate between its GTP-bound and GDP-bound forms. In the nucleus there is a high concentration of RanGTP. RanGTP binds to cargo that needs to be transported out of the

nucleus and passes through nuclear protein complexes, NPCs, taking the cargo into the cytoplasm. Once in the cytoplasm, RanGTP is broken down to RanGDP by Ran, with the help of RanGAP. This hydrolysis causes RanGTP to release its cargo into the cytoplasm and causes a high concentration of RanGDP in the cytoplasm forming the RanGTP gradient across the nuclear membrane. In the cytoplasm RanGDP is bound to proteins that mediate its translocation back into the nucleus, such as NTF2. Once in the nucleus RanGEF, Ran Guanine Exchange Factor, removes the GDP and replaces it with a GTP to create the high concentration of RanGTP in the nucleus which drives the whole cycle over again (Steggerda & Paschal, 2002).

Figure 2 shows a daigram of the GTP:GDP cycle, which is vital to proper nuclear transport. It is easy to see how disruption of RanGAP, which can be seen in the lower half of the cycle, would cause a major upset in the GTP:GDP gradient due to the inability for the cell to properly convert GTP to GDP. The loss of the proper gradient across the nuclear membrane is thought to be one of the causes, if not the cause, of segregation distortion.



RanGAP is present in the cell in two specific “pools”: one of these is the high concentration of RanGAP in the cytoplasm that was mentioned earlier that actually is able to cycle in and out of the nucleus as needed due to the presence of both NLSs and NESs in its structure. The NLSs, or nuclear localization signals, are the portions of the RanGAP molecule required to identify RanGAP as a molecule that is allowed to pass through the nuclear membrane and enter the nucleus. In contrast, NESs are nuclear export signals that do the opposite, and signal that the molecule is to be exported from the nucleus and returned to the cytoplasm. The truncated form of RanGAP has an error in its NESs, and therefore the ability of the compound to exit the nucleus is greatly diminished. Therefore, instead of having a higher concentration in the cytoplasm than the nucleus, cells inflicted with *Sd* have a higher concentration of RanGAP in the nucleus, altering the cell’s ability to function properly.

The other pool of RanGAP, as previously mentioned, is actually physically attached to the filaments present on the cytoplasmic side of NPCs; it is these RanGAPs that are deemed vital to hydrolysis of RanGTP and termination of nuclear export (Steggerda & Pascha, 2002). Since Sd-RanGAP is mislocalized to the nucleus it hydrolyzes RanGTP before it is able to export the necessary cargo, thereby disrupting nuclear transport. Although it is unsure how exactly this takes effect the experiments by McElroy et. al. (2008) showed that other nuclear transport mutations resulted in a similar pattern of distortion and therefore it is concluded that the abnormal transport system does, in some way, cause distortion.

It has long been concluded that distortion is caused by loss of spermatogenic maturation due to failure of chromatin condensation in *SD*$^{+}$ spermatids (Ganetzky, 2000). The chromatin in sperm is condensed in a different form than that of normal cells that undergo mitosis and even different from the spermatocytes that undergo meiosis. The basic difference lies in the proteins around which the DNA is wound. During a period of spermatogenesis that is called the "late canoe stage" by some (Awe & Renkawitz-Pohl, 2010), and by others referred to as stage V (Popłonska, 2009), the chromatin actually first decondenses and releases the histones around which it is wound and recondenses as sperm-specific proteins called protamines take their place. It has been determined that it is over the course of this histone to protamine exchange that the *SD*$^{+}$ chromosomes fall victim to spermatogenic failure and die off resulting in the survival of only *SD* chromosomes. Protamine irregularities have actually been linked to infertility in men, one of the most common issues being that of protamine 1: protamine 2 ratios that vary drastically from 1. In cases where this is either a high or low ratio the men were found to have greater occurrences of misshapen sperm and DNA fragmentation (Carrell et al., 2007). Research has shown that acetylation of histone prior to its separation from the DNA is vital to the transition from a histone-based to a protamine-based structure. Also, protamines are positively charged proteins that must be phosphorylated in order to properly bind with the DNA (Awe & Renkawitz-Pohl, 2010; Carrell et al., 2007). However, most interesting was the research that yielded a hypothesis stating that protamines are likely produced in the ER and transported into the nucleus during late spermatogenesis right before they are needed (Popłonska, 2009). Based on previous

research, it seems reasonable to hypothesize that the inability of *SD* cells to transport protamines across the nuclear membrane at the point of chromatin condensation could cause faulty spermatogenesis.

In response to the question as to why *SD* chromosomes are able to survive spermatogenesis while SD^{+} chromosomes are not, Dej (2004) brought to light some interesting new hypotheses. Although the article was not related to *SD* specifically, the basic idea was that the IMP alpha family of proteins, which is vital for chromatin condensation in mitosis, seems to have a sort of backup system in case of mutations. Dej (2004) observed that cells containing a mutation in the dCAP-g protein were able to reach full chromatin condensation before reaching metaphase regardless of the dangerous mutation in a protein complex vital for the process. It was observed that the cell was able to lengthen the period of time spent in premetaphase so that the chromatin was given ample time to condense regardless of its handicap. In relationship to *SD* chromosomes, because the *Sd* segment is twice as long as the normal segment coding for normal RanGAP, *Sd* actually codes for both RanGAP and Sd-RanGAP. Thus, during normal cell life the *Sd* gene is able to produce enough RanGAP to at least survive and transport proteins and mRNA and necessary cell functions. Nuclear transport becomes vitally important to the haploid cells as spermatogenesis continues due to the large amount of protamines being produced in the ER outside of the nucleus that need to be transported to the nucleus (Poplanska, 2009). It is possible that something within the *SD* haploid cell triggers genetic regulation and results in more production of RanGAP (as well as Sd-RanGAP since the two genes are connected and assumed to be transcribed

together) in order to either correct or at least adjust the RanGTP gradient to the extent that nuclear transport does not prove fatal to the cells. It is possible the *SD* cell reacts similarly to the cells that Dej (2004) tested and compensate for their own mutation by either lengthening the amount of time spent in Stage V of spermatogenesis and/or supplementing its RanGTP gradient by turning on some series of genetic regulation factors in order to produce more efficient RanGAP, and this might explain why the *SD* cells survive whereas the SD^{+} cells do not.

It is also important to note that during the course of spermatogenesis if the maturing spermatocytes are taking “too long” as determined by the cells' biological time table the cells that have not yet reached maturation would be discarded (Gotoh & Durante, 2006). Therefore, although it is possible that *SD* cells are lengthening their maturation cycle, the *SD+* cells which lack this form of compensation are slower still and may therefore be discarded more readily. This could be, in part, due to the change of histones to protamines in the condensed chromosomes structures. Perhaps if protein binding factors are involved in the transfer of histones to protamines in chromatin condensation then protein binding factors could also be a cause of the distortion (Th’ng et al., 1994).

Past work by Dr. McLean has involved testing specific deletions within the left arm of the second chromosome and noting any areas that, when deleted, caused an increase in distortion (McLean, 2000). Several areas of interest were identified, and so those areas were further explored in this research project. If, after running statistical analyses, there are any deletions that appear to show distortion it is then possible to

determine what genes are known to be present in those areas and see if they align with any of the hypotheses listed above or if there are even more factors affecting segregation distortion than are currently mentioned. This way, it may be possible to take known genetic information and apply it to a genetic system that is barely understood, thereby working backwards and hopefully resulting in a clearer picture of how *Sd* truly functions.

METHODS

All stocks were ordered from the Bloomington Drosophila Stock Center. Stocks were maintained in an incubator that held a constant temperature of 18^{o}C. These stocks were transferred roughly every three weeks. Stocks that were being prepped for a k-test, or participating in a k-test were kept in a separate incubator that was held constant at 25^{o}C (McLean et al., 1994).

Before an actual k-test could be set up, each stock had to first be crossed with the stock *SD-5*^{r7}, in order to obtain the desired genome. In order to properly set up a cross it was necessary to obtain virgin flies so that we could ensure that the offspring to later be counted were, in fact, a result of the cross we desired rather than the result of some other genotypic combination. Since *Drosophila melanogaster* are not sexually active for roughly eight to ten hours after they hatch from the larvae form, if stocks are checked frequently and females are collected and set aside before they have reached the eight hour mark then it is reasonable to conclude that they are virgins and well suited for the experimental cross.

Therefore, virgins were selected from the stock to be tested, and crossed with males from the *SD-5*^{r7} stock that possessed curly wings (the homolog to the *SD-5*^{r7} chromosome and therefore the marker for when *SD-5*^{r7} is not present), and bar stone eyes (the phenotypical marker for *Rsp*^{s}). It was previously mentioned that in wild populations that display distortion, the *Rsp*^{s}gene is found on the second chromosome; the stocks used for this experimentation were specifically chosen do to their unique genotype in which *Rsp*^{s} was located on the Y chromosome (Lyttle et. al., 1989).

After roughly a week the parents of the initial cross were discarded, and two weeks after the initial cross offspring began to eclose. The offspring were anesthetized with ether, and placed under a dissecting microscope. From there it was necessary to locate the male offspring that possessed straight wings and bar stone eyes. By this process the offspring selected were known to have *SD-5*^{r7}, the deletion specific to the current stock, as well as the *Rsp*^{s} gene. These collected males were noted as stock#/*Rsp*^{s}.

It was important, for the sake of timing, to be collecting virgins from the *cnbw* stock at the same time that the *SD-5*^{r7}x Stock # cross was being conducted, so that upon collection of the stock#/*Rsp*^{s} males a k-test could be conducted.

Standard k-tests were performed for each deletion stock according to the method of McLean et al. (1994). In summary, a cross was set up with anywhere from 10-20 vials, depending on the number of virgins and stock#/*Rsp*^{s}males obtained, and in each vial one stock#/*Rsp*^{s} male was placed with two *cnbw* virgins (Day 0). On Day 4 each of the vials was transferred. Therefore, on Day 4 when the vials were transferred vial 1a was transferred to a vial number 1b, vial 2a was transferred to a vial number 2b, and so on. On Day 8 the crosses were “cleared”, meaning that the parent flies used in the crosses were discarded. By this method each vial was exposed to a cross set of flies for four days, during which mating could occur and eggs could be laid, before the vial was emptied and the eggs were allowed to mature. Also, by using a 1a/1b numbering system it was possible to identify which offspring came from the same cross set of flies.

Counts of offspring occurred on Days 14 and 18 for each set of vials. On Days 14 and 18 the “a” vials were counted, and on Days 18 and 22 the “b” vials were counted. In

this way each vial was counted both 14 and 18 days after it had been exposed to a cross set of flies.

On count days the offspring were anesthetized by exposure to ether, and placed under a dissecting microscope. The offspring were then separated, male from female, and the number of each was counted and recorded. After counting, the offspring were discarded, and the next vial to be counted was selected. In this fashion each vial was counted two times, on different days, and there were no overlapping offspring between the two counts, meaning none of the offspring from the first count participated in the second count.

The counts were carefully catalogued. After completion of the K-test all of the counts were added together to determine the total number of males and females produced from the K-test of a particular stock. Excel software was used to determine standard error for all sets of crosses. The k-values were determined by dividing the number of females counted and dividing it by the total number of offspring in the k-test. After both the k-value and the standard error of each k-test was calculated it was then possible to note whether or not there was a significant difference based on the presence, or absence, of overlap between the values.

The k-tests were run in a specified order that resulted in the largest deletion being selected first for experimentation, followed by a deletion slightly smaller from the last, and continuing in that fashion. That way, if one k-test displayed distortion, and the next did not, then it would be the non-overlapping segments of the two deletions that were of interest and could then be further studied.

RESULTS

Prior research conducted by McLean (unpublished data) noted a number of sections within the second chromosome that yielded particularly high k-values. One such stock tested contained a deletion from 26B1-2;26D1-2 and yielded a k-value of 0.757. It was this result that led to the previously outlined course of action determined for the study described in this paper.

The first k-test that was conducted was a control that included the *SD-5*^{r7} stock alone which in further experiments was crossed with the deletion stocks to produce the desired males that possessed both the specific deletion and *Rsp*^{s} on the Y chromosome. For the control k-test *SD-5*^{r7} was crossed with virgins from the stock *cnbw*,as was done for all other k-tests. The results of the k-test can be seen in Table 1.

Control (SD-5^{r7} x cnbw)		
	Female	*Male*
1	33	19
2	54	45
3	23	12
4	1	1
5	61	38
6	24	17
7	31	19
8	7	5
9	0	0
10	0	0
TOTAL	*234*	*157*

Table 1: Counts and totals from control k-test

K-value is calculated by dividing the number of offspring of the desired genotype, in this case the females, by the total number of offspring. The k-value for the control experiment was 0.5985 $\pm$ 0.018.

It was interesting to note that the control test had a relatively high k-value. It is likely that the high female to male ratio was due to the slightly less viable males due to presence of the *barstone* allele on the Y chromosome. Since this is the control for the cross, all future k-values were compared to this value rather than a perfect 0.5, which is what is expected when no distortion is observed.

The first experimental k-test involved stock 7502. This stock had the largest deletion that ran from 26C1;26D1. The results of the k-test can be seen in Table 2.

7502 (26C1;26D1)		
	Female	*Male*
1	15	6
2	0	0
3	23	3
4	0	0
5	7	3
6	45	11
7	0	0
8	0	0
9	34	11
10	27	5
Total	*151*	*39*

Table 2: Counts and totals from the k-test for stock 7502

The totals of the male and female offspring resulted in a calculated k-value of 0.7947 $\pm$ 0.030.

Since the 7502 k-test suggested that this region enhanced distortion, the next stock to be tested had a deletion from 26C3;26D1, narrowing down the area tested. The results of that k-test can be seen in Table 3.

8903 (26C3;26D1)		
	Female	*Male*
1	19	17
2	10	11
3	2	0
4	34	13
5	37	3
6	53	37
7	117	72
8	2	0
9	38	30
10	91	65
Total	*403*	*277*

The totals of the male and female offspring resulted in a k-value of 0.5927 ± 0.064.

The results of this k-test most closely compared to the results of the control k-test; therefore the deleted region of stock 8903 was not responsible for enhancing distortion.

The combination of the results of the first two k-tests suggested that a small region from 26C1;26C3 was responsible for causing the enhanced distortion. To verify this conclusion, deletions that were within the parameters of the original, large deletion that McLean (unpublished data) had made note of previously but did not include the 26C1;26C3 section, were selected. The first of these was stock 23633, which possessed a deletion from 35E1;35F1. The result of the K-test can be seen in Table 4.1.

23633 (35E1;35F1)																
	Count 1a				Count 2a				Count 1b				Count 2b			
	F		**M**		**F**		**M**		**F**		**M**		**F**		**M**	
VIAL	*R*	*W*	*BS*	*W*	*R*	*W*	*BS*	*W*	*R*	*W*	*BS*	*W*	*R*	*W*	*BS*	*W*
1	25	0	9	1	5	0	2	0	9	0	14	0	0	0	0	0
2	11	0	6	1	3	0	0	0	12	1	13	0	0	0	0	0
3	5	1	6	0	1	3	1	1	7	0	1	0	0	0	0	0
4	3	0	1	1	9	0	1	0	4	0	6	0	1	2	3	0
5	5	0	5	0	4	0	8	0	26	0	32	0	0	0	0	0
6	20	0	15	0	17	0	18	0	0	0	0	0	3	0	2	0
7	3	2	3	2	3	2	4	3	0	0	0	0	1	0	0	0
8	10	5	2	2	13	8	21	3	17	1	11	0	3	0	0	0
9	15	10	10	0	2	0	22	0	3	0	2	0	3	0	1	0
10	0	0	0	0	0	0	0	0	50	0	32	0	0	0	0	0
11	23	0	12	0	0	0	0	0	20	0	12	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	**110**	**18***	**69**	**7***	**57**	**13***	**77**	**7***	**148**	**2***	**123**	**0***	**11**	**2***	**6**	**0***

Table 4.1: Counts and totals from the k-test for 23633. R-designates red eyes, W-designates white eyes and BS-designates bar stone eyes.

For this K-test there appeared to be some sort of contamination of either the *SD-5*^{r7} stock or the *cnbw* stock. The normal phenotypes seen were red eyed females and males with bar stone eyes. However, in this k-test some individuals of both genders displayed white eyes. White eyed flies had to have been the result of non-virgin parents, or contamination in one of the stocks. Although all flies were used to calculate the k-value it made the results unreliable, since the phenotypes displayed did not match up the genotypes tested.

The totals of the male and female offspring included for calculation and the k-value that resulted can be seen in Table 4.2.

23633	
Female Total: 361	Male Total: 289
K-value: 0.5554± 0.024	

Table 4.2: Calculated k-value for k-test 23633

Since the deletion tested in this k-test was outside the parameters of the original region determined by McLean (unpublished data), 26B1-2;26D1-2, these results should have been insignificant. However, since the k-test was contaminated and the phenotypes displayed inconsistent with those of other k-tests, this portion of the experiment was inconclusive.

Similarly, a k-test was run for stock 9714 which contained a deletion from 26B4;26B11. This deletion was specifically chosen because it was within the parameters previously mentioned in Dr. McLean's work and yet did not include the 26C1;26C3 region. The results from this k-test can be seen in Table 5.

9714 (26C1;26C3)		
	Female	*Male*
1	0	0
2	0	0
3	15	8
4	0	0
5	25	13
6	2	2
7	10	8
8	0	0
9	15	14
10	0	0
Total	*67*	*45*

Figure 1: Comparison of spermatogenesis between *SD* and

The totals of the male and female offspring resulted in a k-value of 0.5982 $\pm$ 0.033. These results were important to take note of because they reaffirmed that the region likely responsible for enhancing distorting effects remained entirely within the previously tested section 26C1-26D1, as seen in k-test 7502, and that the genetic material within the 7502 deletion was likely the only material causing the distortion enhancement within the large reason previously tested by McLean (unpublished data).

Having experimentally reaffirmed past conclusions, and having narrowed down the region of importance, a k-test was run for stock 7800 which had a deletion from 26C2;26C3. The results from the k-test can be seen in Table 6.

7800 (26C2;26C3)		
	Female	*Male*
1	0	2
2	1	1
3	25	8
4	49	13
5	63	41
6	50	20
7	79	34
8	25	5
9	40	24
10	65	19
11	16	8
12	2	0
13	10	8
14	26	4
15	25	7
Total	*476*	*194*

Figure 2: Diagram of the GTP:GDP cycle. Cytoskeleton Inc. (2012)

The totals of the male and female offspring resulted in a k-value of 0.7105 $\pm$ 0.058. Although this k-value was less than that of the 7502 k-test, it was still interesting that such a small region appeared to be responsible for enhancing distortion. When the standard error is taken into account the low end of the k-value for the 7800 k-test was still higher than the high end of the 7502 k-test. The lack of overlap means there is a significant difference in the results. Also, while the average number was slightly lower than that observed with 7502, there is no significant difference between those k-values, supporting the fact that the observed enhancement is caused by something in this region.

Having narrowed down the region it was now a goal to determine the specific gene within this region that, upon deletion or mutation, resulted in distortion. A k-test was run for stock 27811 which contained a breakpoint in 26C1, this meant that in some way a gene in this region was interrupted rendering it nonfunctional. The results of the k-test can be seen in Table 7.

27811 (Breakpoint 26C1)		
	Female	*Male*
1	35	17
2	21	13
3	42	14
4	70	51
5	24	10
6	47	43
7	14	10
8	22	11
9	27	30
10	1	0
11	23	18
12	10	7
13	0	0
14	4	3
15	9	2
16	6	5
17	34	26
18	3	4
19	0	0
20	0	0
TOTAL	*392*	*264*

Table 1: Counts and totals from control k-test

The totals of the male and female offspring resulted in a k-value of 0.5976 $\pm$ 0.033. The k-value and standard error for this k-test and that of 7502 have a large overlap and therefore this breakpoint likely does not have an effect on distortion.

The next k-test was run with stock 21848 which contained a breakpoint in 26C3, specifically mutating the gene *Cpr, Cytochrome p450 reductase,* thought to have a NADPH-hemoprotein reductase activity. The results of the k-test can be seen in Table 8.

21848 (Breakpoint 26C3)		
	Female	*Male*
1	18	1
2	25	22
3	41	25
4	24	14
5	36	20
6	35	22
7	12	6
8	20	18
9	25	15
10	15	7
TOTAL	*255*	*191*

Table 2: Counts and totals from the k-test for stock 7502

The totals of the male and female offspring resulted in a k-value of 0.5717 ± 0.037. This made this specific region also unlikely to have an impact on distortion.

The final k-test involved the stock 11102, which also contained a breakpoint in 26C3. However, this breakpoint resulted in a specific mutation within the gene *Gef26*. The results of the k-test can be seen in Table 9.

11102 (Breakpoint 26C3)		
	Female	*Male*
1	93	25
2	14	4
3	50	21
4	15	6
5	18	10
6	0	0
7	51	23
8	23	9
9	3	9
10	0	0
TOTAL	277	107

Table 3: Counts and totals from the k-test for 8903

The totals of the male and female offspring resulted in a calculated k-value of 0.7214 ± 0.061. Once again, when taking into consideration both the k-value of the k-tests and their associated standard error, the results of the k-test for 11102 are statistically significant from those seen in the k-test for 7502, making it highly likely that *Gef26* was the cause for the enhancement of distortion found in the large region, 26B1-2;26D1-2, originally marked as interesting by McLean (unpublished data).

The results of all the k-tests are summarized in Table 10.

Stock	Genotype	Affected Region	Total Males	Total Counted	K-value
Control	SD-5^{r7} with Rsp^{S} on Y	NONE	157	391	0.5985 ± 0.018
7502	**w^{1118}; Df(2L)Exel6016, P{XP-U}Exel6016/CyO**	**26C1;26D1**	**39**	**190**	**0.7947 ± 0.030**
8903	w^{1118}; Df(2L)ED369, P{3'.RS5+3.3'}ED369/SM6a	26C3;26D1	277	680	0.5927 ± 0.064
23633***	w^{1118} Mi{ET1}CG1889^{MB03604}	35E1;35F1	275	601	0.5554± 0.024
9714	w^{1118}; Df(2L)BSC239/CyO	26B4;26B11	45	112	0.5982 ± 0.033
7800	**w^{1118}; Df(2L)Exel9038, P{XP-U}Exel9038/CyO**	**26C2;26C3**	**194**	**670**	**0.7105 ± 0.058**
27811	w^{1118}; Mi{ET1}CG13983^{MB09845}	Breakpoint 26C1	264	656	0.5976 ± 0.033
21848	y^{1} $w^{67}c^{23}$; P{Mae-UAS.6.11}Cpr^{DP01397}	Breakpoint 26C3 (mutation *Cpr*)	191	446	0.5717 ± 0.037
11102	**y^{1} $w^{67}c^{23}$; P{lacW}Gef26^{k13720}/CyO**	**Breakpoint 26C3 (mutation *Gef26)***	**107**	**384**	**0.7214 ± 0.061**

Table 4.1: Counts and totals from the k-test for 23633. R-designates red eyes, W-designates white eyes and BS-designates bar stone eyes.

DISCUSSION

Segregation distortion is not a condition that has been limited to *Drosophila melanogaster*. Recent research, within the last two years, has resulted in better understanding of similar meiotic drive systems in eggplant (Barchi, 2010), barley (Vaillancourt, 2010), congenic mice (Casellas, 2010), and chicken (Axelsson, 2010). Each possesses its own mechanisms that result in the disproportionate inheritance of specific alleles, and many details are still not well understood.

The research detailed in this paper is only a hint at one very small piece to the much larger puzzle that is the *Sd* gene in *Drosophila melanogaster.* Through systematic experimentation it was determined that a specific region, in particular the gene *Gef26*, may shed some light as to the mechanism by which *Sd* causes its effects.

It is understood that *Sd* alters the RanGTP gradient between the nucleus and the cytoplasm, thereby affecting nuclear transport. This thought was confirmed in research done by McLean (2000) that showed that specific mutations that caused faulty nuclear transport resulted in distortion even when *Sd* was absent, but $Rsp^{s}$was present (McElroy et al., 2008). The possible distorting effect of *Gef26* could shed further light on the mechanism of distortion.

FlyMine describes itself as an "Integrated database for *Drosophila* genomics." Their database possesses information on the *Gef26* gene. The length of the gene is 7786bp, and it produces three proteins (RA, RB, and RC) which are 1573, 1569, and 1422 amino acids in length respectively (Ensembl). It is determined to be an intracellular component with a variety of functions ranging from wing development to cell structure.

However, there are a number of functions that are of interest due to their possible relation to distortion. Some of these include developmental functions such as: germ-line stem cell maintenance, cyclic nucleotide-dependent guanyl-nucleotide exchange factor activity, guanyl-nucleotide exchange factor activity and regulation of small GTPases that mediate signal transduction.

Wang et al. (2008) connected mutations in the *Gef26* gene with malfunctions in germline stem cell maintenance. They also found that *Gef26* is required for somatic stem cell maintenance. The main reason for this is a malfunction in the Rap-GEF/Rap signal transduction pathway that is responsible for the regulation of stem cell maintenance. Specifically, malfunctions took place in the testis of the *Drosophila* in which adherens junctions necessary for proper development were not made due to the disruption of the earlier mentioned pathway. Specifically, when adherens junctions are not properly formed within the testis, cellular components break away from the “hub cells” and result in spermatogenic cysts. *A*lthough interesting from a developmental point of view, this information was not vital to the understanding of *Gef26*’s distorting effects because stem cells are diploid and also undergo different processes in order to regulate their duplication. This does not apply to spermatogenesis, which is haploid and undergoing different processes, and therefore could not be the reason that distortion results when the *Gef26* gene is mutated within a genome in which Rsp^{S} is present. Similarly, although it was interesting to note that one of the major malfunctions of the *Gef26* mutants in Wang’s research was development of the testis, the evidence so far collected against

*Sd*suggests that it is an intracellular problem occurring late in spermatogenesis, rather than a malfunction resulting from the testis organ.

As was mentioned earlier, it is understood that *Sd* affects the RanGAP cycle within the cell. This, in turn, affects the nuclear transport cycle by affecting the RanGTP-RanGDP gradient that drives the movements of vital components in and out of the nucleus. It was also mentioned earlier that *Gef26* is known to have functions relating to the regulation of GTPase pathways. Specifically, the main function of *Gef26* is guanyl-nucleotide exchange factor activity. It is stated within the FlyMine database that this means *Gef26* is capable of stimulating and exchanging guanyl nucleotides, such as those associated with GTPase. Therefore, a fault in the *Gef26* gene has the ability to impact the RanGTP:RanGDP gradient, thereby affecting nuclear transport. How these functions, the Rap-GEF pathway, and the concentration gradient of RanGTP to RanGDP all interplay in order to result in distortion is still unclear.

However, it seems reasonable to hypothesize that *Sd* and *Gef26* are both affecting vital components of the same cycle, albeit in ways unknown. With *Sd*, a vital NES is missing from the truncated RanGAP and as a result RanGAP maintains a high concentration in the nucleus and is less capable of performing its function of converting RanGTP→ RanGDP in the cytoplasm. Is the Rap-GEF pathway, located in the cytoplasm, possibly causing the same effects by affecting the RanGTP to RanGDP conversion in the cytoplasm? Further research will need to be conducted, but this could explain why the level of distortion between malfunctions in *Sd* and malfunctions in *Gef26* differ, because

although they appear to be attacking the same system, they are doing so in different ways.

Figure 3 shows a simplified version of parts of the RanGAP cycle, take particular not of the red “X” that signifies that point at which it is hypothesized that both *Sd* and *Gef26* may exert their effects, causing some form of distortion.

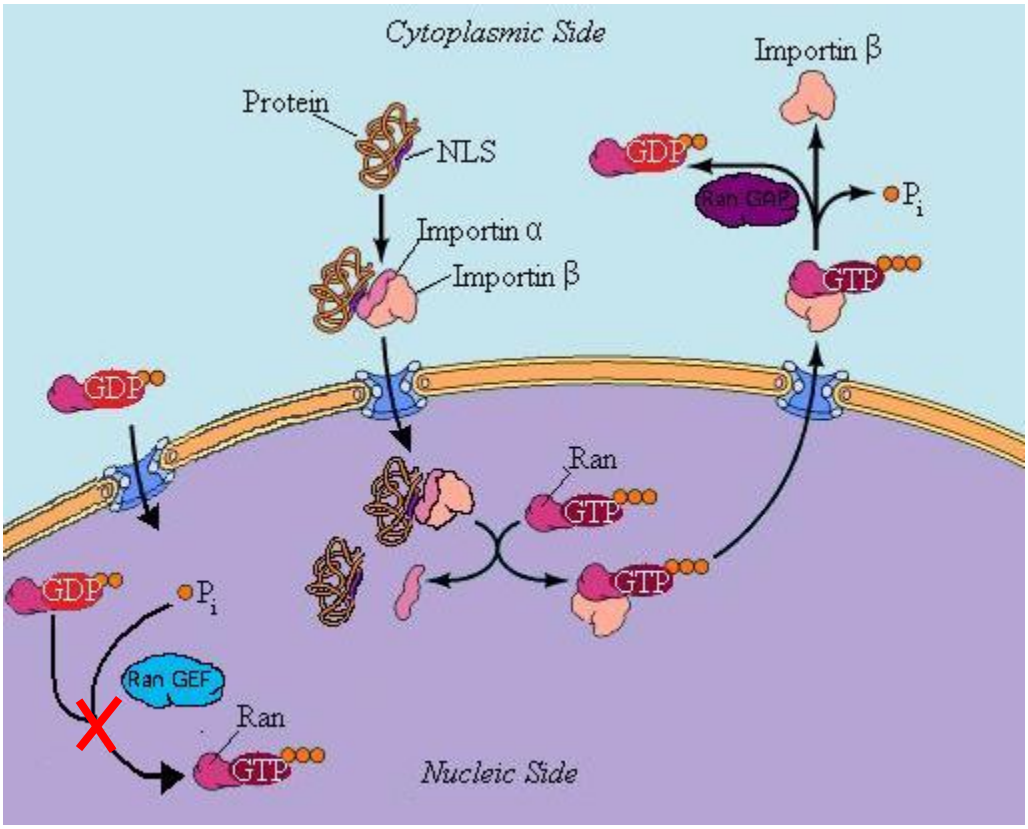


Table 4.1 Results from control K-test of 23633 males x cnbw virgin females.

*__TOTAL:__ These total numbers are from the white specimens, and were not used in

The results of this research suggest that disruption of the GTP-GDP cycle causes distortion, and it seems reasonable to conclude that *Segregation distortion* has to do with a malfunction of nuclear transportthat results from the irregular GTP-GDP gradient. The gene *Sd* and the truncated form of RanGAP that it produces appear, so far, to be the most efficient way to cause distortion and are known to result in a k-value of roughly

0.99. However, other nuclear transport malfunctions can cause the same variance to smaller degrees, such as *Gef26* with its k-value of roughly 0.71. Based on this observation, perhaps the answer to how segregation distortion occurs is not so much in the understanding of *Sd* itself, but in the response that Rsp^{s} has to cell systems that possess a faulty nuclear transport system. Future research should focus on the gene Rsp^{s} and try to determine why it is subject to distort the processes of the cell only at a specific time and in a specific way.

WORKS CITED

1. Axelsson, E., Albrechtsen, A., van, A. P., Li, L., Megens, H. J., Vereijken, A. L. J., Crooijmans, R. P. M. A., Groenen, M. A. M., Ellegren, H., Willerslev, E., & Nielsen, R. (2010). “Segregation distortion in chicken and the evolutionary consequences of female meiotic drive in birds.” *Heredity*. *105*(3), 290-298.

2. Awe, S. & Renkawitz-Pohl, R. (2010). "Histone H4 acetylation is essential to proceed from a histone- to a protamine-based chromatin structure in spermatid nuclei of Drosophila melanogaster." *Systems Biology in Reproductive Medicine*. 56.1: 44-61.

3. Barchi, L., Lanteri, S., Portis, E., Stàgel, A., Valè, G., Toppino, L., & Rotino, G. (2010). “Segregation distortion and linkage analysis in eggplant (Solanum melongena L.).” *Genome*. *53*, 805-815.

4. Carrell, D., Emery, B., & Hammoud, S. (2007). "Altered protamine expression and diminished spermatogenesis: what is the link?" *Human Reproduction Update.* 13.3: 313-327. *MEDLINE with Full Text*. EBSCO. Web.

5. Carrell, D., Emery, B., & Hammoud, S. (2008). "The aetiology of sperm protamine abnormalities and their potential impact on the sperm epigenome." *International Journal Of Andrology.* 31.6: 537-545. *MEDLINE with Full Text*. EBSCO. Web.

6. Casellas, J., Farber, C., Verdugo, R., & Medrano, J. (2010). “Segregation Analysis of a Sex Ratio Distortion Locus in Congenic Mice.” *Journal of Heredity*. *101*(3), 351-359.

7. Dej, K., Ahn, C., & Orr-Weaver, T. (2004). "INVESTIGATIONS - Mutations in the drosophila condensin sub-unit DCAP-G: Defining the role of condensin for chromosome condensation in mitosis and gene expression in interphase." *Genetics*. 168.2: 895.

8. Dionne, M., Ghori, N., & Schneider, D. (2003). "BACTERIAL INFECTIONS - Drosophila melanogaster Is a Genetically Tractable Model Host for Mycobacterium marinum." *Infection and Immunity*. *71*(6), 3540.

9. Ensembl. (2012). "Database:*Gef26*." http://useast.ensembl.org/Drosophila_melanogaster/Search/Details?species=Drosophila_melanogaster;idx=Gene;end=1;q=Gef26.

10. FlyMine. (2012). "Gene: *Gef26.D. melanogaster."* http://www.flymine.org/release-33.1/report.do?id=1093460.

11. Ganetzky, B. (2000). "Articles - Tracking down a cheating gene - Some genes will play dirty to gain a selective advantage." *American Scientist*. 88.2: 128.

12. Gotoh, E., & Durante, M. (2006). "Chromosome condensation outside of mitosis: Mechanisms and new tools." *Journal of Cellular Physiology*. 209.2: 297.

13. Guarnieri, D., & Heberlein, U. (2003). "Drosophila melanogaster, A Genetic Model System for Alcohol Research." *International Review of Neurobiology*. (54), 200-228.

14. Kusano, A. (2001). "Molecular and cellular analysis of segregation distortion in Drosophila melanogaster." *Ph.D. dissertation*. The University of Wisconsin -

Madison, United States -- Wisconsin. Retrieved October 5, 2010, from Dissertations & Theses: Full Text. (Publication No. AAT 3020683).

15. Kusano, A., Staber, C., & Ganetzky, B. (2002). "BIOLOGICAL SCIENCES - GENETICS - Segregation Distortion induced by wild-type RanGAP in Drosophila." *Proceedings of the National Academy of Sciences of the United States of America*. 99.10: 6866.

16. Kusano, A., Staber, C., Chan, H. & Ganetzky, B. (2003). "Closing the (Ran) GAP on segregation distortion in Drosophila." *Bioessays: News And Reviews In Molecular, Cellular And Developmental Biology.* 25.2: 108-115. *MEDLINE with Full Text*. EBSCO. Web.

17. Lyttle T., Walker, E., & Lucchesi, J. (1989). “Transposition of the Responder Element of the Segregation Distorter System (Sd) to the X Chromosome in Drosophila Melanogaster” *Department of Biology and Curriculum in Genetics, University of North Carolina, Chapel Hill.*

18. McElroy, J., McLean, R., & McLean, J. (2008). "Defects in nuclear transport enhance segregation distortion." *Fly* 2.6: 280-290. *MEDLINE with Full Text*. EBSCO. Web.

19. McLean, J., Merril, C., Powers, P., & Ganetzky, B. (1994). "Functional identification of the segregation distorter locus of *Drosophila melanogaster* by germline transformation." *Genetics*. 137.1: 201-209.

20. McLean, Janna R. (2000). “Enhancers of segregation distortion” *Department of Biology, Colorado State University*.

21. Pandey, U. & Nichols, C. (2011). “Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery” *Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center.*

22. Popłonska, K., Kwiatkowska, M., Wojtczak, A., & Polit, J. (2009). "Immunogold evidence suggests that endoplasmic reticulum is the site of protamine-type protein synthesis and participates in translocation of these proteins into the nucleus during Chara vulgaris spermiogenesis." *Biology Of Reproduction.* 80.3: 572-580. *MEDLINE with Full Text*. EBSCO. Web.

23. Steggerda, S., & Paschal, B. (2002). "Regulation of nuclear import and export by the GTPase Ran." *International Review Of Cytology.* 217. : 41-91. *MEDLINE with Full Text*. EBSCO. Web.

24. Th'ng, J., Guo, X., Swank, R., Crissman, H., & Bradbury, E. (1994). "Inhibition of histone phosphorylation by staurosporine leads to chromosome decondensation." *The Journal Of Biological Chemistry.* 269.13: 9568-9573. *MEDLINE with Full Text*. EBSCO. Web.

25. Vaillancourt, R. (2010). “Construction of a high-density composite map and comparative mapping of segregation distortion regions in barley.” *Molecular Genetics and Genomics*. *284*(5), 319-331.

26. Wang, H., Singh, S., Zheng, Z., Oh, S., Chen, X., Edwards, K. & Hou, S. (2006). “Rap-GEF Signaling Controls Stem Cell Anchoring to Their Niche through Regulating DE-Cadherin-Mediated Cell Adhesion in the Drosophila Testis.

Developmental Cell. Volume 10, Issue 1, January 2006, Pages 117-126, ISSN 1534-5807, 10.1016/j.devcel.2005.11.004.