EFFECTS OF SIALYLTRANSFERASE MUTATION ON *DROSOPHILA MELANOGASTER* VIABILITY, FERTILITY, AND LONGEVITY

A Senior Scholars Thesis

by

JARED DAVID PITTS

Submitted to the Office of Undergraduate Research
Texas A&M University
In partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2006

Major: Genetics

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Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

Effects of Sialyltransferase Mutation on *Drosophila melanogaster* Viability, Fertility, and Longevity (April 2006)

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Sialyltransferase and other glycosylation enzymes are emerging as important players in crucial regulatory post-translational modifications. These enzymes transfer sugars to glycoproteins and glycolipids, and may aid in activation and suppression of these molecules. Drosophila Sialyltransferase (DSiaT) shows expression during Central Nervous System development in larvae. This leads us to believe it plays an important role in not only this process but also the overall health of the flies. We report that mutations to this gene show significant reductions in fly longevity, fertility, and viability. Immunohistochemical staining experiments revealed DSiaT expression in ovaries, which implies a possible correlation between the sialyltransferase protein and oogenesis. Attempts to rescue this mutant phenotype back to wild type, using mutant flies that also possessed a functional copy of DSiaT downstream of an upstream activating sequence (UAS), were successful in most cases.

ACKNOWLEDGEMENTS

I would like to that Stacey Whitman and Kate Koles for their help with this very time consuming project. They were a great help when I was not able to be in the lab. I would also like to thank Dr. Panin for giving me this great opportunity in his laboratory.

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INTRODUCTION

It is well known that nucleic acids (genome) are the first language of life, while proteins (proteome) make up the second. One emerging theory is that glycosylation (glycome) of macromolecules comprises the "third language of life". Glycosylation is the covalent modification of proteins and lipids by the addition of sugar residues. It is a highly specific process that introduces modifications often required for viability.

Sialic acids (Sia) are negatively charged monosaccharides terminally bound to glycan chains of glycoproteins and glycolipids. Functionally they can act either as recognition masks or recognition sites for glycoproteins (1). It has been shown that sialic acids are also important in vertebrate neuronal development, as sialylation is a modulator of cell adhesion and neural plasticity. In the human blood stream the lifespan of erythrocytes is also regulated by sialylation (2). In addition, many malignant tumors show alterations in their sialic acid biosynthetic pathway.

Although it is known that *Drosophila* possess a sialyltransferase (the class of enzymes that bind sialic acids to the terminal positions of glycan chains of glycoproteins and glycolipids) in the central nervous system, it is very difficult to detect sialic acids (3), and there is still some controversy in the field about amount and distribution of sialic acids in Drosophila tissues.

This thesis follows the style and format of Proceedings of the National Academy of Sciences of the United States of America (PNAS).

We have however shown that an active sialyltransferase is present in *Drosophila* central nervous system (CNS) and reproductive system, which implies that sialic acids should also be present (4). We believe that sialyltransferase expression in CNS and reproductive system indicates a specific function of sialic acids in these organs.

Drosophila is so far the only protostome species that has been proven to possess a functional sialyltransferase. Much of the variation present in Sialyltransferases in vertebrates is believed to have evolved from this early sialyltransferase (5). Higher organisms contain up to twenty variations of sialyltransferase proteins, which leads to a great deal of complexity and functional redundancy and makes studies on vertebrates quite difficult (6). In contrast, *Drosophila*, which possesses only one sialyltransferase (with high amino acid sequence homology to the human counterpart enzyme), presents itself as an ideal model organism in which to study the effects of mutations to the sialyltransferase gene.

MATERIALS AND METHODS

Fly Strains

All fly stocks were kept on cornmeal agar food kept at 25°C and 36% relative humidity in accordance with common Drosophila husbandry protocols. The incubator in which these stocks and crosses were stored was on a 12:12 hour light-dark cycle. Sialyltransferase allele S23a3.1 (one stock with w^{1118} and one with Canton-S background) was the mutant allele being evaluated. The rescue stock contained the S23a3.1 mutant allele but also UAS-DSiaT on the third chromosome. Other strains used in these studies included KI-48.12, the sialyltransferase mutant allele with an HA tag knocked into the endogeneous locus of *DSiaT* gene, and y[1]; P{y[+mDint2] w[BR.E.BR]= SUPor-P} KG00484/SM6a; ry[506], which is a P element insert on the second chromosome that is zero map units away from the DSiaT gene. All wild type controls in this study refer to the w^{1118} stock.

Viability

The viability assay consisted of a self-cross between S23a3.1/P heterozygous flies. The S23a3.1 mutant is of w^{1118} background, which allows for differentiation of the progeny based upon eye color, as homozygous mutant flies will have white eyes and any fly containing the P element will have red/orange eyes. All progeny emerging from these crosses were scored as mutants or P element containing based on their phenotype. It is assumed that the P element mutation has no effect on viability and thus behaves as wild type; it is also assumed that flies containing only one copy of S23a3.1 behave as wild type. Controls crosses were set up to justify these

assumptions. Rescue viability assays were conducted and measured against DSiaT mutant flies in a setup identical to the procedure noted above

Longevity

The longevity study was conducted using two types of assays. The first type measured the lifespan of a single male fly in a vial, while the second measured the percent survivorship of a population consisting of 10 male *Drosophila* in a vial. Males are used in these assays, as females can have complications with mating, which affects their lifespan (7). At least 20 vials were set up for each assay giving us population sizes of 200 for the population assay and 25 for the lone fly assay. In both assays the vials were stored horizontally (to prevent sticking to the food) and flies were transferred to new food every three days. The number of flies alive in each vial was monitored daily until the last fly in the vial died. The parameters used to measure lifespan are the median (50% survivorship), the mean lifespan, and the maximum lifespan, all of which are taken in days.

Fertility

Fertility was monitored in both male and female *Drosophila*. A competitive assay, which has one mutant fly and one wild type fly, with 4 wild type flies of the opposite sex was used for both sexes. The flies were allowed to mate for seven days, transferring to fresh vials every 2-3 days. After seven days the parental generation was removed and all progeny that emerged from the vial were scored. In this assay eye color was again used to determine the genotype of each fly. However, in this experiment red-eyed mutants were used, and thus red-eyed flies were progeny of the

mutant fly while white-eyed flies were progeny of w^{1118} wild type. Rescue crosses were also set up for the female fertility assay. In the male fertility assay, only female progeny can be scored since the gene that gives them the red eye color lies on the X chromosome. Progeny in this experiment that possess the mutant allele are heterozygous and thus viability of these flies is equal to that of wild type flies. This statement is shown to be true by controls measuring the viability of flies heterozygous for the mutant alleles compared to wild type flies. A non-competitive assay was also used to measure fertility, since mating behavior can play a large role in the fecundity of male *Drosophila.* This could make a decrease seen in the competitive assay entirely behavioral, so we set up vials, which had one male of the measured population (mutant, rescue, or wild-type) and four wild type females. Again the crosses were allowed to mate for seven days and all progeny emerging from the crosses were scored.

Immunohistochemistry of *Drosophila* Ovaries

Virgin KI 48.12 flies which possessed an HA tag construct in the DSiaT gene were collected and reared on a high protein (yeast) diet for 3 days. The HA tag inserted into the DSiaT gene allowed the detection of sialyltransferase without sialyltransferase specific antibodies. The ovaries were then dissected from these flies and fixed in Ringer's solutions. The fixed ovaries were stored in methanol to help remove any background that may appear in the staining procedure. Rat anti-HA monoclonal antibody was used as the primary antibody and stained with Rat CY3 attached to a fluorescent tag as the secondary antibody at a 1:250 dilution. The stained ovaries were then visualized on a Zeiss fluorescent microscope.

RESULTS

Longevity

As displayed in Figure 1, the population longevity assay showed median lifespan for DSiaT mutants (S23a3.1) flies to be between 26 and 27 days with a terminal (maximum) life of 55 days. This is a drastic reduction from the wild type, which showed a median life of 57 days with a terminal life of 81 days. The longevity of the mutant flies was rescued when the UAS-DSiaT construct was present, as we saw the median lifespan restored to 57 days and the terminal lifespan increase to 77 days. The mean life was also calculated for each genotype and found to be 26.8 days for DSiaT mutants, 64.4 days for wild type, and 53.7 for UAS-DSiaT flies.

The lone fly longevity assay displayed values very similar to those of the population assay, as seen in Figure 2. The median lifespan for each genotype was shown to be between 26 and 27 days for sialyltransferase mutants, 63 days for wild type, and 57 days for the UAS-DSiaT flies. The maximum for the wild type fly was found to be much longer at 95 days; while the rescue and mutant max longevity remained nearly the same at 77 and 55 days respectively. The mean lifespans for this study also were consistent with those of the population study for wild type, 27.6 days, and mutant flies, 65.5 days. The mean for the rescue flies however, was markedly lower than in the population study with its value at 44.1 days.

The longevity assay was performed in both a population and lone fly conditions to determine if a difference between the two assays could be found. However, both S23a3.1 mutants and w^{1118} exhibited no change in median lifespan. Rescue flies, on the other hand showed a large decrease in longevity when in an isolated system, but a smaller population for these flies was used, which does not

make the difference statistically conclusive. More studies must be done on this to determine if the rescue flies truly have a 10-day decrease in median lifespan in the lone assay when compared to the population study.

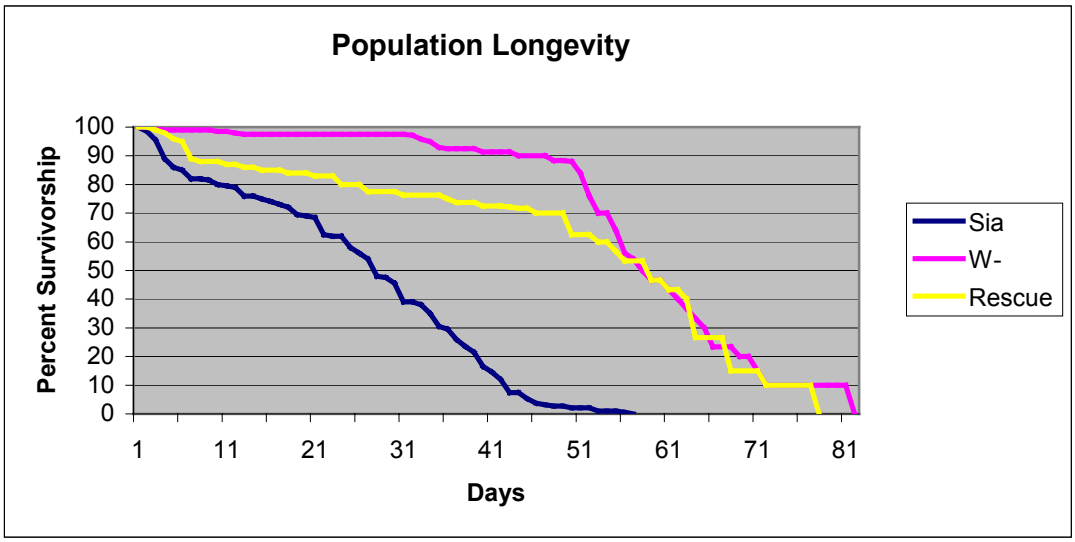

Fig. 1: Male Population Longevity Assay measured in percent of population in each individual vial still alive versus time in days. Wild type and rescue are shown to have significantly longer life spans than mutant S23a3.1 flies. ($p<0.01$)

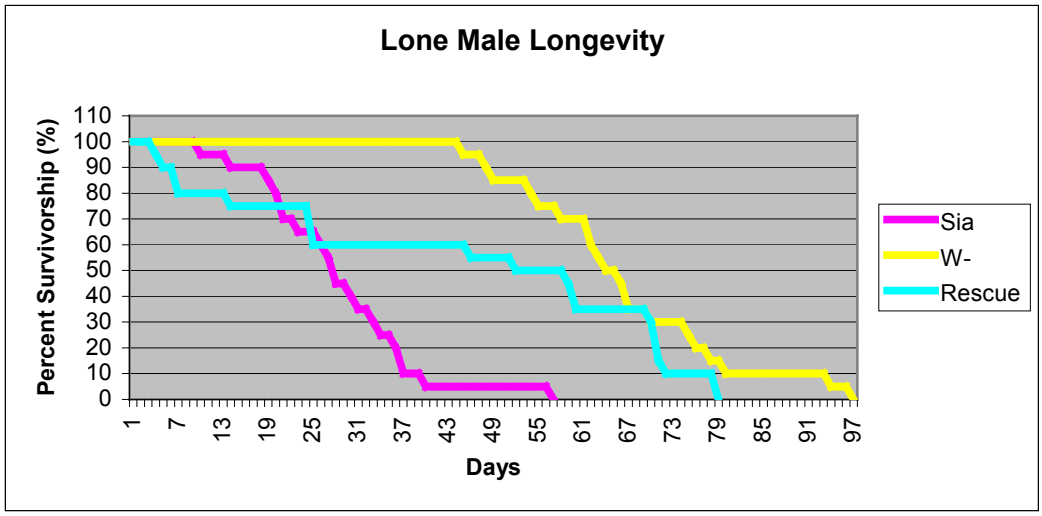

Fig. 2: Male lone fly longevity assay measured in percent survivorship of total population under study versus time in days. Wild type flies have significantly longer lives than mutant flies ($p < 0.01$). Rescue flies in this assay are not significantly differentiated from the mutant flies ($p < 0.16$)

Viability

Viability of S23a3.1 mutant flies and UAS-DSiaT rescue flies were measured in comparison to P element flies. P element flies were chosen over wild type (Canton S or w1118) because they the P element is essentially zero map units away from the DSiaT gene. This allows us to measure the progeny of each phenotype in the cross with certainty as to what genotype they possess. The two genes will assort independently and recombination is negligible at this short distance. The number of progeny scored ideally would be in a 3:1 ratio of P element (red-eyed) flies to DSiaT mutant (white-eyed) if the mutants were as viable as the P element flies. The number of P element progeny was then divided by 3, which would create an ideal 1:1 ratio and allow for the value to be normalized to the P element. A similar procedure was taken in order to achieve the values for the rescue viability, since not all can be measured simultaneously in one cross.

Sialyltransferase mutant viability was reduced by 19.5 percent when compared to progeny containing the P element insertion. UAS-DSiaT viability was actually increased over the wild type condition. The viability of these flies was 31.1 percent greater than the viability of mutant flies and 11.1 percent greater than wild type, the latter of which is not statistically significant ($p < 0.18$).

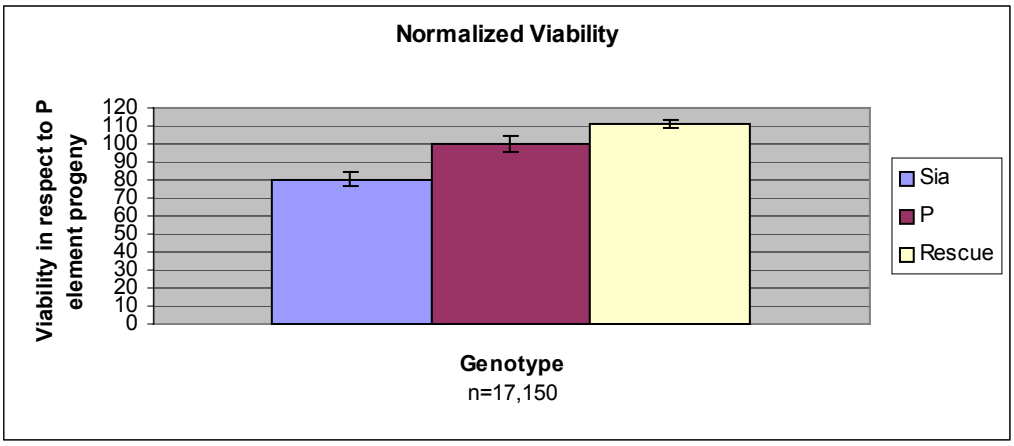

Fig. 3: The normalized viability assay measures the viability of each genotype with respect to the P element progeny, which is wild type for the purpose of this experiment. P element containing flies and rescue flies both possess significantly greater viabilities that mutants ($p < 0.01$). The error bars in this graph represent one standard deviation.

Viability in this assay is measured under a few basic assumptions about the stocks we are using. The first assumption is that homozygous and heterozygous P element flies both possess viabilities equal to that of wild type flies (w^{1118}). A second assumption is that flies that are heterozygous for the S23a3.1 mutation also act as wild type, since two-thirds of the P element flies also contain a single copy of the mutant allele. A third assumption is that recombination between the P element insertion and the DSiaT gene is negligible. These assumptions were justified in a series of control experiments.

A short recombination test was performed crossing flies with the P element and S23a, a precursor construct to the S23a3.1 mutation. A self-cross of these flies would produce white flies only in the event that a recombination occurs. Over 4,000 flies were scored for this assay and no white-eyed flies were found. Thus, recombination between the P element and the DSiaT gene is sufficiently negligible.

The first control cross was to justify the assumption that P element containing flies were as viable as wild type flies. The cross in this control should produce a 3:1 ratio of P element flies to wild type (+/+) flies, if the assumption is to hold true. Figure 4 clearly shows the ratio of the progeny produced from this cross fit the expected ratio almost exactly as P element containing flies made up 75.17% of the progeny from the cross. Thus, the other 24.83% of the progeny were made up of wild type flies and this assumption has been justified.

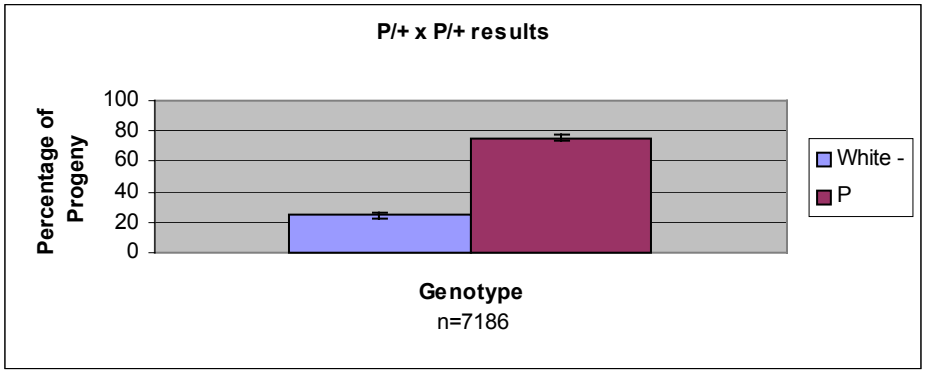

Fig. 4: Control Cross #1 (P/+ x P/+) tests the assumption that P element containing flies are as viable as wild type. The graph gives each phenotype as a percentage of the total population of progeny. The size of the total population of progeny counted is 7,186 flies. The error bars present represent one standard deviation.

The second control cross is designed to justify the assumption that *Drosophila* containing a single copy of the S23a3.1 mutant allele is acting as wild type. They are being compared to flies that contain one copy of the P element, which was shown to possess wild type viability in the first control cross. This cross should yield a 1:1 ratio of P element heterozygous flies to S23a3.1 heterozygous flies. Heterozygous DSiaT mutant flies made up 49.88% of the progeny of this cross and the other 50.12% consisted of P element heterozygotes. These results confirm that

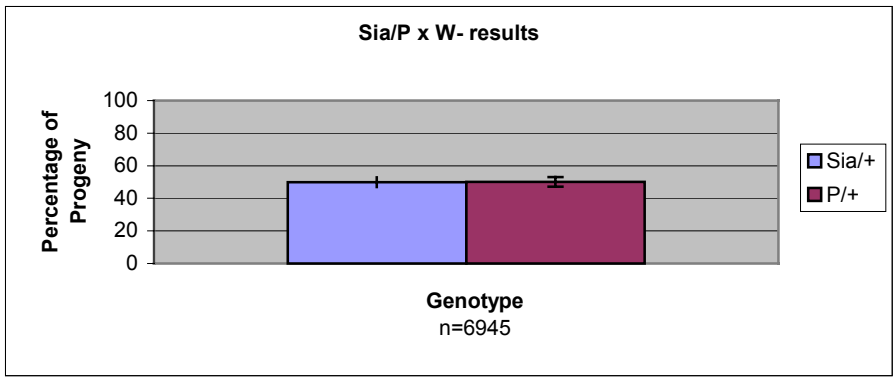

Fig. 5: Control cross # 2 (Sia/P x W-) is testing the assumption that flies containing the S23a3.1 mutant allele in the heterozygous situation are acting as wild type. The graph gives a representation of each genotype as a percentage of the total number of progeny scored (6945 flies). The error bars are represented by one standard deviation away from the mean.

the assumption that mutant heterozygous flies have viabilities equal to wild type flies (Fig. 5).

The final control cross is validating that flies possessing both one copy of the mutant allele and one P element are comparable to wild type. This cross will yield four different genotypes, three of which possess the P element and will have red eyes and one that is heterozygous for the S23a3.1 allele (Sia/+). Three of these genotypes (P/P, P/+, Sia/+) have been confirmed to behave as wild type in the first two control crosses. Therefore, any deviation from what is expected is observed it can be assumed to result from the Sia/P flies. This control is also expected to yield a 3:1 ratio of red-eyed progeny to white eyed progeny. At 24.91%, DSiaT heterozygotes represented just under one-fourth of the flies produced from these crosses, the other 75.09% were made up from the three P element containing genotypes (Fig 6). The ratio again is almost a perfect 3:1 ratio, which indicates that each of the assumptions made in the viability assay held true.

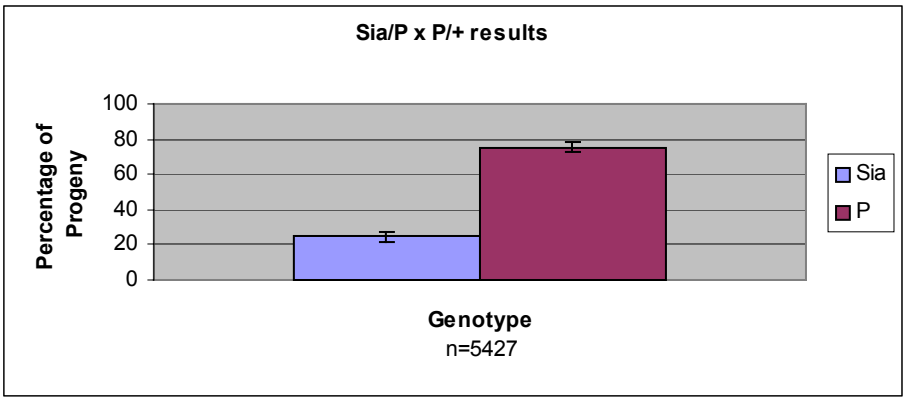

Fig. 6: Control Cross #3 (Sia/P x P/+) analyzed justifies the assumption that Sia/P flies act as wild type. The three P element genotypes are grouped together and represented as a percentage of the total number of progeny scored (5427). The error bars signify one standard deviation from the mean.

Female Fertility and Immunohistochemistry of Ovaries

Female fertility was measured in comparison to w^{1118} wild type flies. This assay was one of the few assays in which the rescue did not fully recover the phenotype to the wild type condition. Figure 7 below indicates a 91.8 percent decrease of fertility in Sialyltransferase mutants when compared to wild type females. The rescue females in this experiment produced three times the amount of progeny as the mutant females but were still 75.4 percent less fertile than wild type females.

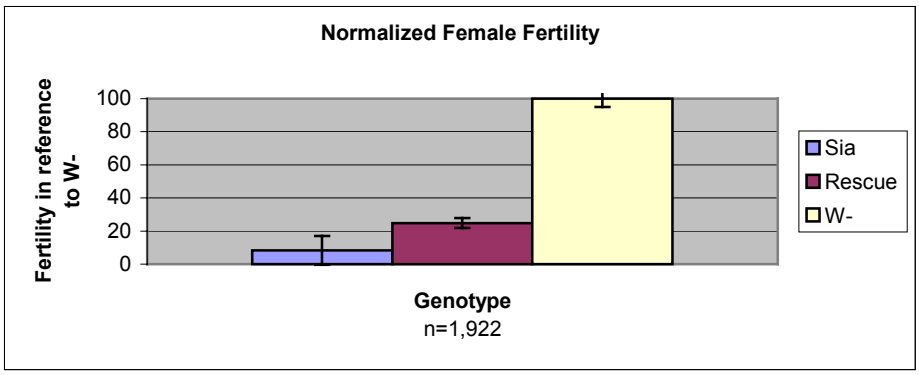

Fig. 7: Female fertility assay in respect to wild type flies. In order to normalize the data, the number of flies produced by each genotype was divided by number produced in seven days by the wild type females. 1,922 were scored in the calculation of this data. Error bars represent one standard deviation from the mean. DSiaT mutants and rescue flies are significantly less fertile than wild type ($p < 0.001$)

Immunohistochemical Stain of *Drosophila* Ovaries

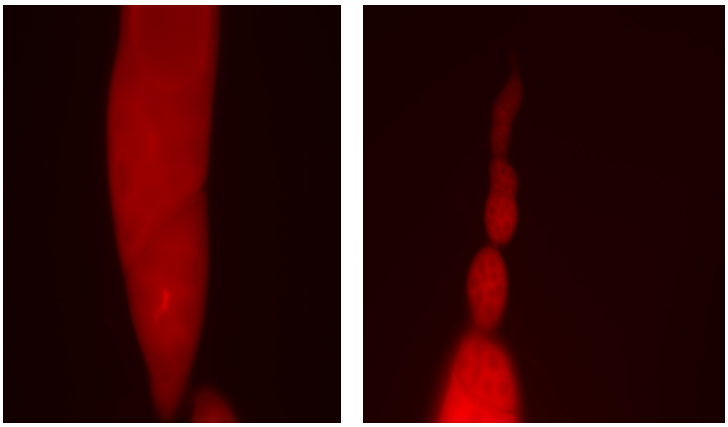

Fig. 8: Immunohistochemical staining of Drosophila ovary using KI 48.12 stocks (containing and HA tag in the DSiaT gene), is the picture on the left, and w^{1118}, which is pictured at left. Ovaries dissected in Ringers and stained with Rat anti-HA as the primary antibody and Rat CY3 attached to a fluorescent tag as the secondary antibody.

Immunostaining of the KI-48.12 stock ovary reveals expression of Sialyltransferase in the oocyte (Fig 8). Expression of the protein is seen in the more developed oocytes and generally appears to be localized in the center of the cell. The expression pattern appears to be localized to a specific organelle, but at this time it is uncertain what that organelle is. Staining also revealed several morphological abnormalities in the ovaries (i.e. dead oocytes, odd number of nuclei, etc). However,

more analysis is required before any significant morphological differences can be characterized.

Male Fertility

The competitive male fertility assay was difficult to perform, as there are many behavioral aspects associated with male fertility. Any reduction in male activity could reduce in affect alter the ability for flies to quickly mate with the females. In other words, more active flies will have the competitive advantage. In this assay it was noted that in most trials (16 of 22) the mutant males flies did not produce any offspring. In two of the trials however the S23a3.1 mutants took produced 100% of the offspring in the vial, while the remaining vials had some of each phenotype. This assay showed a reduction in fertility of 82.4% for the mutant flies when compared to the wild type (Fig 9). However, due to the large range of percentage of progeny by the mutant (0%-100%) this data is not statistically significant ($p = 0.25$). Since so many behavioral aspects are involved in this, we believed a better measure of the ability of males to mate would be a non-competitive assay. Rescue males were only tested in this non-competitive assay because of the inconclusive results for the competitive assay.

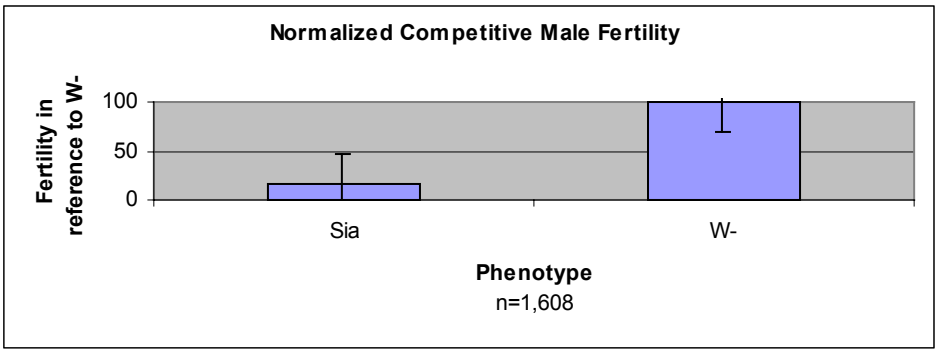

Fig. 9: Competitive Male Fertility Assay – This assay is normalized to the average number of wild type progeny produced per vial. The population size that was scored in this assay was 1,608 flies. Error bars in this graph represent one standard deviation from the mean. ($p = 0.25$)

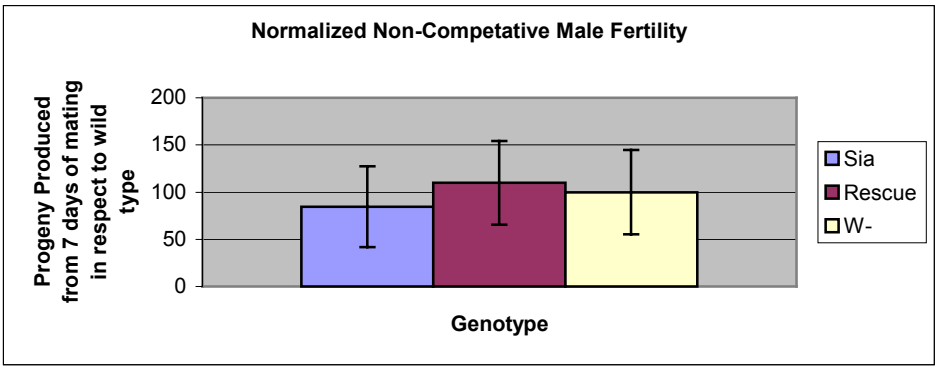

Fig. 10: Non-competitive Male Fertility Assay - This assay shows the number of progeny produced from one male of each phenotype in a vial with four wild type females, allowed to mate for seven days. The value was normalized according to the wild type. These value show no significant difference in the number of progeny produced by males of each phenotype ($p = 0.46$)

Although it was expected that non-competitive male fertility would yield better results we found that the number of progeny produced in the seven-day mating period to be so varied that our results bear no statistical significance. Therefore despite the small 18% decrease in the average number of flies produced by S23a3.1 mutants we cannot conclude that there is a real difference between them and wild type flies (Fig 10). It appears that there are too many factors that determine the number of progeny that a single male *Drosophila melanogaster* can produce to achieve an accurate count in a non-competitive male fecundity assay.

DISCUSSION AND CONCLUSIONS

The results obtained by these assays clearly show significant reductions in longevity, female fertility, and viability in Sialyltransferase mutants. The observed phenotypes in all of the cases except female fertility have the ability to be rescued by a UAS-DSiaT construct inserted on the third chromosome. This result is somewhat surprising, since UAS constructs normally require a driver to drive expression of the gene downstream of it. However, in this case it appears that expression takes place despite the absence of this driver. Observing full rescues in longevity and viability but only partial recovery in female fertility can possibly provide some valuable insight as to how sialyltransferase functions throughout the life of a *Drosophila*.

The immunohistochemical staining of the ovary also may be able to help account for the fact that only partial rescue is observed in female fertility. This stain showed sialyltransferase to be present in the oocyte, which may imply that sialyltransferase, plays important roles in the fertilization process (i.e. receptiveness to the sperm). Since sialyltransferase expression is also seen during the development of the central nervous system it is possible that other mechanisms may be responsible for the decrease in fertility as well. For example, if the central nervous system has some functional abnormalities it is possible that laying fertilized eggs is more difficult for DSiaT mutants. Hence, the decrease in fertility is due to the inability to lay as many eggs at wild types flies. These are just possible explanations for the observed phenotypes and need to be looked at in greater detail.

A fully developed central nervous system is vital component of health in all higher organisms. The reductions in phenotype in viability, fertility and longevity help to support the claim that sialyltransferase play significant roles in larval

development in *Drosophila*. The results that were acquired from these experiments will help to provide insight as to how *Drosophila* are affected by sialyltransferase mutations. These results should be also useful for better understanding the function of the human counterpart sialyltransferase. These findings could yield valuable insight as to the biochemical abnormalities that arise and produce the observed phenotypes. Hopefully this research has provided several potential targets research on sialyltransferase and its functions in fruit flies and humans to continue to advance.

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