1992

Methods to screen for mutations in a putative sodium channel gene in Drosophila

Usha Upadhyayula
San Jose State University

Order Number 1350122

Methods to screen for mutations in a putative sodium channel gene in *Drosophila*

Upadhyayula, Usha Suresh, M.A.

San Jose State University, 1992

U·M·I

300 N. Zeeb Rd.
Ann Arbor, MI 48106

METHODS TO SCREEN FOR MUTATIONS IN A PUTATIVE SODIUM CHANNEL GENE IN *DROSOPHILA*

A Thesis

Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

By

Usha Upadhyayula

August, 1992

Approved For The Department Of Biological Sciences

Susan Germeraad

Dr. Susan Germeraad

Robert Fowler

Dr. Robert Fowler

Sally Veregge

Dr. Sally Veregge

Approved For The University

Serena M. Stanford

Abstract

Methods To Screen For Mutations In A Putative
Sodium Channel Gene In *Drosophila*

Usha Upadhyayula

The two *Drosophila* genes that have sequence homology to the vertebrate sodium channel alpha subunit are *para* and *DSC*. The aim of the research was to isolate mutations in the second chromosome gene *DSC*. To find lethals of *DSC*, 1321 chromosomes were scored by making the chromosomes heterozygous with a deletion that included the *DSC* gene. Subsequently, the feasibility of using the neurotoxin veratridine, known to affect sodium currents directly, to screen for mutants was tested. At 140 μM $para^3$ conferred the greatest resistance to veratridine. A sex-specific difference in sensitivity was observed for most genotypes. This toxin sensitivity could be a good way to screen for animals with reduced numbers of sodium channels. These results suggest another way to look for mutants in *DSC*. In three trial runs no mutation of *DSC* was isolated. The methods are still under investigation.

Acknowledgements

The author is indebted to Dr. Susan Germeraad for her guidance and counsel during the course of this study. Thanks are also expressed to Professors Robert Fowler and Sally Veregge for their help and valuable suggestions and for agreeing to be on the committee. Acknowledgements are extended to Robin Isbell and technicians in the Micro Service Facility for their support.

Table of Contents

List of Tables

List of Figures

Introduction

In 1987, a putative sodium channel gene, *DSC*, was isolated from *Drosophila* based on its sequence homology to a known vertebrate gene (Salkoff et al., 1987a). A second gene *para* has also been identified as homologous (Loughney et al., 1989). This gene was previously described by a large number of behavioral and lethal mutations whose phenotypes suggested that it was involved in sodium channel function. No such mutants currently exist for the *DSC* gene. Molecular tools reveal that the *DSC* gene is expressed in embryos and pupae (> 10 Kb mRNA) with two different transcripts (Salkoff et al., 1987a). It is localized to the tip of the chromosome 2R at 60E 5/6 (Germeraad et al., 1992). The gene has not been characterized genetically or physiologically.

There are several mutations in *Drosophila* which are known to affect ion channel genes. The *para* gene, for instance, is a sodium channel gene and the *Shaker* mutation affects potassium channels directly. Finding mutations of *DSC* would provide new tools for studying ion channels. The mutants, once isolated, would be useful in looking at the channel gene's expression and also the expression of other genes that influence the ion channel expression.

This study examines two different ways of looking for mutations in the *DSC* gene. The first is isolating lethal mutations of the *DSC* gene. This was done by making mutagenized chromosomes heterozygous with chromosomes bearing a deletion that included the *DSC* gene. If *DSC* is essential, it can be mutated to exhibit a lethal phenotype.

The second part of this study investigates the sensitivity of wild type and mutant flies to the toxin veratridine which binds directly to sodium channels.

Previous studies have shown that *seizure* and *tipE*, mutations which reduce sodium currents, are resistant to veratridine (Jackson et al., 1985, 1986; O'Dowd and Aldrich, 1988). Five different mutations of the *para* sodium channel gene, $para^{1}$, $para^{2}$, $para^{3}$, $para^{ST76}$, and $para^{ST109}$, and wild type were tested on veratridine at different concentrations. If a mutant's sensitivity to the toxin is different enough from that of the wild type and provided that *DSC* is a sodium channel gene, this might be an additional approach to finding *DSC* mutants. The main purpose of these experiments was to develop genetic tools to permit functional analysis of the channel gene product and characterization of the expression of the *DSC* gene.

Literature Review

Voltage dependent ion channels

The control and transport of ions through the lipid bilayer is accomplished by ion channels within the cell membrane. These channels are integral membrane glycoproteins which open and close due to changes in the membrane potential or changes in the ligand concentration. They maintain ion balance in the cell, control ion sensitive processes and are responsible for propagating electrical signals in excitable tissues (Hille, 1984). Sodium and calcium channels provide for inward ion flow while potassium channels conduct ions out of the cell under normal physiological conditions. Voltage-gated ion channels conducting sodium, calcium, and potassium ions are found in a variety of vertebrate tissues. Using specific pharmacological properties several sodium and calcium channels were purified from tissues where they are highly concentrated. The similarity between all these voltage dependent ion channels suggests similar functional mechanisms and common evolutionary origin (Catterall, 1988).

Sodium channels

The depolarization of the electric field across a nerve cell's membrane during an action potential is caused by the opening of voltage dependent sodium channels which conduct current inwards, flowing down the electrochemical gradient into the cell. The function of sodium channels is similar in vertebrate muscle cells. Sodium channels have been purified and reconstituted from eel electroplax, mammalian skeletal muscle and mammalian brain. The sodium channels were purified by high affinity binding of tetrodotoxin (TTX), a molecule which binds specifically to sodium channels. They are composed of a single

large glycoprotein (260 KD) and some in the native state are found as a complex (more than one subunit) of the large protein called the alpha subunit and several smaller proteins called beta subunits (for review see Catterall, 1986, 1988). The evolution of ion channels apparently occurred before the differentiation of vertebrates and invertebrates because the alpha subunits are highly conserved between the vertebrates and *Drosophila* (Salkoff et al., 1987 a, b). The alpha subunit is responsible for ion conductance in the intact channel. The alpha subunits of vertebrate sodium channels represent a class of closely related proteins as demonstrated by biochemical characterization (Catterall,1986). These sodium channels act as pathways through the membrane, opening due to depolarization and closing and or inactivating with time at higher potentials. Sodium channel kinetics were first described by voltage clamp studies performed by Hodgkin and Huxley and have been further characterized by the patch clamp technique used to detect the flow of ions through a single channel (Hille, 1984). The first model of the secondary and tertiary structures of sodium channels was proposed using hydropathy profiles developed when the first gene was cloned (Noda et al., 1984).

The presence of multiple subtypes of sodium channel in the same tissue was determined by cloning experiments (Noda et al., 1986). Rat brain cDNA's were used to clone four sodium channel subtypes (Noda et al., 1986; Kayano et al., 1988). Subtypes I, II and III are distributed in the central nervous system (Gordon et al., 1987; Beckh et al., 1989) and also in the peripheral nervous system (Beckh, 1990). The sequence conservation is very high between II and IIA which vary only by six amino acids. Existence of additional neuronal subtypes is suggested by the presence of three additional transcripts in the

central and peripheral nervous system (Beckh, 1990). Rat skeletal muscle cDNA was used to clone genes structurally related to the neuronal gene (Trimmer et al., 1989). This was also done with cardiac muscle (Rogart et al., 1989). These studies identified two sodium channel sequences that are expressed exclusively in skeletal muscle and heart, respectively. Though the nucleotide sequences of cloned cDNA's are similar throughout the major regions of the coding zone, they arise from separate genes. Sodium channel genes comprise a gene family which has at least five similar members in mammals.

Diseases caused by dysfunction of the ion channels

Several human diseases are caused by interference in the function of ion channels. The autosomal dominant disorder, Hyperkalemic Periodic Paralysis (HYPP) with myotonia is caused by a mutation in a sodium channel alpha subunit gene (Cannon et al., 1991). Using restriction fragment length polymorphism (RFLP) it was determined that the disease was caused by a defect in the alpha subunit of the adult muscle sodium channel gene (Fontaine et al., 1990).

Paramyotonia Congenita (PC), another autosomal dominant disorder, is caused by mutations in the S4 region of the skeletal muscle sodium channel (NaCh) gene. S4, one of the putative transmembrane domains, is highly conserved and has regularly spaced positive charges (between 4 and 8) (Noda et al., 1984). The S4 segments have arginines, positively charged residues, in every third position. Structural criteria suggest this may be the channel's voltage sensor. The activation of the channel is thought to use a mechanism called "helical screw" where the S4 helices rotate around their axes due to the change in the electric field causing a movement towards the extracellular surface (Guy

and Seetharamulu, 1986). Functional analyses of mutations in this region of the gene show that it plays an important role in channel activation (Auld et al., 1990; Stuhmer et al., 1989). Electrophysiological studies led to the demonstration of genetic linkage between the PC disease allele and skeletal muscle NaCh gene. Sequencing of two different alleles shows that base pair changes within the same codon result in two distinct amino acid substitutions in the S4 segment of the adult skeletal muscle NaCh (Ptacek et al., 1992). Cystic fibrosis is caused by mutations in a chloride channel gene. The sodium channel distribution is affected in patients suffering from multiple sclerosis. To develop cures for these diseases it is important to understand the molecular and genetic structures of sodium ion channels. Such studies will contribute to an understanding of protein structure and function and will help in the development of therapeutic remedies to the diseases. To design accurately targeted therapeutic strategies it is important to understand the channel heterogeneity.

Neurotoxins and their effects on sodium channels

Sodium channels have high affinity for specific neurotoxins. The neurotoxins' association with sodium channels plays an important role in the molecular characterization of channel proteins, since it helps to identify the protein after solubilization and to modulate its conductance state after reconstitution. The toxins are used to determine the number and distribution of sodium channels in various tissues. They are also useful for activating purified sodium channels after reconstitution (Barchi, 1988). The sodium channel neurotoxins are classified based on the binding sites they occupy on the channel protein. Site 1 recognizes tetrodotoxin and saxitoxin and site 2 recognizes lipid soluble toxins, including batrachotoxin, veratridine, aconitine, and grayanotoxin.

Veratridine is a steroidal plant alkaloid which binds to the sodium channels resulting in their persistent activation which leads to depolarization and hyperexcitability of the excitable membrane (Catterall, 1980). Depolarization by veratridine is caused by a block of channel inactivation and a shift of activation to more negative potentials. Persistent channel activation occurs even at normal membrane potentials. If we assume that the sensitivity to the toxin is proportional to the number of channels in an animal's nervous system, then treatment with toxin may be a good way to screen for animals with reduced numbers of sodium channels. It could be a good way to screen for sodium channel mutations because it would be easy to screen large number of mutagenized flies. In vertebrates multiple sodium channel subtypes were identified based on sensitivity to toxins (Barchi, 1988). Electrophysiological and pharmacological studies have placed the binding site for the neurotoxins tetrodotoxin and saxitoxin at the extracellular "mouth" of the channel (Catterall, 1980, 1986). A substitution in a putative extracellular domain, changing a negatively charged, conserved amino acid to a neutral amino acid, lowers the affinity for the toxins by three orders of magnitude (Noda et al., 1989). This result is consistent with the position of the binding site at the channel "mouth." Neurotoxins were used to count the number of sodium channels in intact membranes, in reconstituted membranes and host cell to study the properties and for purifying channels.

Drosophila as a model system

The fruit fly *Drosophila Melanogaster* is used as a model system to study the voltage dependent sodium channel since powerful techniques exist for analyzing genomic structure and organization of gene families and also for returning channel genes to the organism in defined genetic backgrounds

(Papazian et al., 1988). In *Drosophila* excellent techniques for probing genomic structure and organization exist. For biochemical and molecular biological research, *Drosophila* is a suitable organism since it is easy to grow large quantities of adults, larvae and embryos. Growth requirements are minimal. Optimal conditions are humidity 68%, temperature 22°C and a constant light-dark cycle for maximum yield of eggs. Genetic manipulation of this organism makes it possible to remove channel genes from the genome and replace them with related genes or *in vitro* modifed genes. Analysis of genomic sequences that determine the observed patterns could be extended in the future by defining the tissue specificity and timing of sodium channel gene expression in the fly. The genetic potential of *Drosophila* will be a major contributing factor in building an understanding of channel structure, control of channel distribution and the functional basis of channel heterogeneity.

Paralytic mutants

***para* gene**

The nervous system function in *Drosophila* has been studied and manipulated extensively by characterization of behavioral mutations (Hall, 1982). In *Drosophila* two putative sodium channel genes have been identified with sequence similarity to the vertebrate sodium channel alpha subunit. One of the genes, *para*, was originally identified by looking at temperature-sensitive paralytic phenotypes in the adult (Suzuki et al., 1971). As determined by behavioral phenotypes at different developmental stages, *para* is active in larvae and adults. At high temperatures the excitation threshold for action potential increases (Siddiqi and Benzer, 1976; Wu and Ganetzky, 1980). The flies rapidly (<1 min) paralyze when raised to higher temperatures (29°C-35°C) and recover

quickly when returned to room temperature (22°C). *para* may be the primary gene coding for sodium channels in embryonic neurons. This was demonstrated by voltage clamp experiments on cultured neurons. Three of the *para* alleles alter the tetrodotoxin-sensitive sodium current differentiated in the neurons of primary embryo cultures (O'Dowd et al., 1989).

The *para* gene transcripts are derived from a genomic region of 60Kb and encode a protein of 1820 + amino acids (aa). The genomic DNA from the *para* locus was cloned and found by partial sequence analysis of cDNAs to have 26 exons. The primary transcript is subject to extensive splicing and cDNA analysis reveals alternate splicing involving several small exons. The *para* polypeptide amino acid sequence is highly homologous to the vertebrate sodium channel alpha subunit and has a higher degree of sequence similarity with the rat neuronal channel than with the *DSC* sequence from *Drosophila* (Loughney et al.,1989). The characterization and molecular analysis of the noncoding sequences at the ends of the transcripts is not yet available.

***DSC* gene**

The *DSC* gene, which is not yet described by mutation, resides at the tip of the right arm of the second chromosome. This gene was retrieved from a gene library, because of its homology to vertebrate sodium channel genes (Salkoff et al., 1987a; Okamoto et al., 1987). The protein, the gene codes for, is at least 1680 amino acids long (Salkoff et al., 1987b). The coding sequence is expressed in pupae, in early and late embryos and weakly in adults (Salkoff et al., 1987a). The 2R sequence has homology to the vertebrate skeletal muscle sequence by removing the 200 aa sequence that links two of the structural motifs. Its relationship to neuronal channels is less close (Trimmer and Agnew,

1989). The recent report of tetrodotoxin sensitive inward current in *Drosophila* larval muscle suggests another possible role for the protein as a muscle sodium channel (Yamaoka and Ikeda, 1988). The function of this gene has not been directly tested, but it is presumed to code for a sodium channel protein. Theoretically, it may be a member of the ion channel family which conducts a different ion because the sequences for channels with different ion selectivities are closely related. It may be coding for a calcium or potassium channel.

There are a number of other genes in *Drosophila* which affect sodium currents either directly or indirectly. Two other paralytic mutants, *seizure (sei)* and *tipD,* having phenotypes similar to *para,* map to the general region 60 on 2R, near the location of *DSC* (Jackson et al., 1985; Kulkarni and Padhye, 1982). Some genes like *sei* and *tipE* reduce sodium currents and toxin binding sites as shown by pharmacological and physiological data (Jackson et al., 1985,1986; O'Dowd and Aldrich, 1988). *sei* and *tipE* may be alpha subunit coding genes or genes coding for factors which regulate the activity or function of the channel genes.

The interaction between paralytic mutants and hyperexcitable mutants (example: *Shaker,* a potassium channel gene) provides another way to study the roles of these genes. The relationships between the genes is studied by altering the dosage (up or down) of different known and putative channel genes. The molecular studies have confirmed the synergistic effects of certain combinations and offsetting effects of other combinations, the basis for many hypotheses (Ganetzky, 1984, 1986; Stern et al., 1990). It should be noted that the region where *DSC* is located does not show the same effect as *para* when its dose is altered (Stern et al., 1990).

Materials and Method

Drosophila phenotypes

The mutations and rearranged chromosomes used in this study are described in Lindsley and Grell (1968) and Lindsley and Zimm (1985, 1987). Their phenotypes are described briefly below.

bw (2-104.5) *brown* eyes are not as orange and bright compared to the wild type.

sp (2-107.0) *speck* is a dot of darkly pigmented cuticle in the wing axil.

para (1-53.9) Alleles of the *paralytic* gene cause rapid, reversible temperature dependent paralysis. At room temperature the mutants appear wild type. The alleles used in this study are $para^{1}$, $para^{2}$, $para^{3}$, $para^{ST76}$, and $para^{ST109}$.

In(2LR)O This chromosome is marked with the dominant marker *Curly* which causes the wings to be upturned. It is lethal in two doses. It also carries the recessive markers *bw* and *sp*.

In(2LR)SM1 This chromosome does not carry *bw* and *sp*, but otherwise has the same phenotype as *In(2LR)O*.

In(2LR) Gla Glazed eyes are smaller and darker than average and the eye surface is smooth.

$Df(2R)gsb^{ESI}$ *Gooseberry* is a lethal phenotype seen in homozygous embryos. The chromosome contains *Minute* (another recessive lethal) which slows development in a single dose. The chromosome also carries an eye color mutation which makes eye color lighter than wild type.

bwsp/Df(2R)gsb This genotype looks like wild type. It has straight wings and normal eyes. In the crosses A and B it is the only genotype that makes the wings

straight. In crosses C and D it is the only genotype that is neither *Curly* or *Glazed*.

Drosophila stocks

Most stocks were obtained from the Mid American *Drosophila* Stock Center, Bowling Green and the Cal Tech stock center. The *Df(2R)gsb* ^{ESI} was provided by Steve Cohen.

Media

The *Drosophila* food consisted of cornmeal, yeast, dextrose, sucrose and agar. Propionic acid (bacterial inhibitor) and Tegosept (mold inhibitor) were also added. Bakers yeast was sprinkled on top of the food to attract flies and promote egg-laying. Commercially available dehydrated food (Carolina Biological Supply) was also used when there was a problem with the prepared medium. The instant food was made by measuring out equal volumes of water and flakes. The medium supported the growth of a single generation of flies.

Stock maintenance

The flies were maintained in an incubator at 22°C. The behavioral *para* mutants were very sensitive to higher temperatures. The rate of fly development depends on nutritional and temperature conditions. Egg to adult development took 10-12 days. The flies were moved to fresh medium every two weeks. Two bottles of each stock were maintained and backup bottles were kept as insurance in case something happened to the stock bottles.

The flies (male, virgin and non virgin females) were sorted with a very fine brush. The abdomen of the female is elongated and has seven segments, whereas the male abdomen has only five segments and is more rounded. The males have a sex-comb of about ten stout black bristles on the distal surface of

the basal tarsal joint of the foreleg. Young virgin females are very pale and have a dark spot in the abdomen. Carbondioxide (CO_2) was used to anaesthetise the flies. This method is very effective and causes no damage to the flies. The flies recovered quickly and mated immediately. The CO_2 was regulated by pressure valves from a cylinder into polyethylene tubing and was bubbled out through water in a conical flask. The moisture prevents dessication of the flies. CO_2 passes into the dish and puts the flies to sleep. Sorting was done within 2 minutes to minimize damage to the flies. The flies were sorted by sex and phenotype under a dissecting microscope.

Mutagenesis procedure

1. *bwsp* males collected over a short interval were mutagenized 0-4 days after emergence. The mutagenesis was done by collecting 20-30 flies in each glass test tube. They were exposed to gamma rays from the cesium source in the Nuclear Facility (SJSU). They were given a dose of 4000 rads.
2. Virgin *Cy/Gla* females were collected for mating with the mutagenized males.
3. The mated flies were put on well yeasted food and they were changed to fresh bottles every two or three days.
4. After a week all the males were removed. This prevented the testing of the identically mutated chromosomes. Eggs continued to be collected from the females.
5. The F1 flies were mated individually with flies from the *Df(2R)gsb^{ESI}/Cy* stock. Since there were four different types of flies in the F1 four different F1 crosses were made.

Cross A: *bw sp/Cy* F1 male X *Df (2R)gsb/Cy* female.

Cross B: *bw sp/Cy* F1 female X *Df (2R)gsb/Cy* male.

Cross C: *bw sp/Gla* F1 male X *Df (2R)gsb/Cy* female.

Cross D: *bw sp/Gla* F1 female X *Df(2R) gsb/Cy* male.

6. In crosses A and B, the F2 were scored for the absence of straight-winged offspring. In the crosses C and D, F2 were examined for the absence of both *Cy* and *Gla*. If the females used in crosses B and D were not virgins, they could give rise to *bwsp* flies (straight-winged, non-glazed) complicating the scoring. If females in crosses A and C were not virgins, the F1 could include additional *Cy* flies which do not interfere with scoring. A record was kept of the number of vials scored. Vials that had no offspring were not included in the count.

7. Possible lethals were checked by collecting virgin females heterozygous for a mutagenized *bwsp* chromosome and *Curly* and mating to *bwsp/Cy* males from the same vial. If the *bwsp* chromosome carries a lethal, the next generation will consist entirely of heterozygotes. If it does not carry a lethal, homozygous *bwsp* flies will occur. If non virgin F2 females are used some of the *Curly* offspring would carry the deficiency chromosome rather than *bwsp* .

Feeding veratridine

The veratridine sensitivity was quantitated by placing 30-40 adults in a vial containing one filter paper circle that was saturated with 70 µl of 20 mM citrate buffer (pH 4.8) containing 50-140 µM veratridine. The 70 µl was chosen after trials at higher volumes of 100 µl where there was little or no survival of either the wild type or the mutants. There was high variability at 100 µl and so data collected using that protocol has not been included in this study. The veratridine solution was prepared from a 10 mM stock solution in 95% ethanol. The flies were collected one day before the experiment and put on fresh food for a 24 hr period. All the flies were separated into males and females and fed the toxin at

room temperature (21°C-22°C) for 24 hrs. After 24 hrs survival was scored.

Preparation and dilution of solutions

Veratridine (Sigma; mw = 673.8) solutions were prepared by dilution of a 10 mM stock solution made in 95% ethanol with a 20 mM sodium citrate buffer (pH 4.8).

Statistical analysis

The chi-square contingency test was used to test the null hypothesis in analysis of the veratridine sensitivity data. Where the expected values approached zero and /or sample size was small, the binomial distribution was used to determine 95% confidence intervals.

Results

Mutagenesis

Recessive lethals are normal when heterozygous, lethal when homozygous and also heterozygous with a deficiency (no wild type gene present). The *para* gene which is on the first chromosome is essential since it can be mutated to lethality. If *DSC* is essential, it too can be mutated to lethality. Gamma rays which cause chromosomal breaks and deletions causing major disruption to a gene, were used to generate lethals in the search for a *DSC* lethal mutation. Once a lethal is found, further analysis would determine if it was in *DSC* or another essential gene uncovered by the *gsb* deficiency. The F2 generation of the crosses A, B, C and D, when mutagenized second chromosomes were heterozygous with the *Df(2R)gsb* ^{ESI}, was scored for lethals (Table 1). 1321 separate chromosomes were scored and none of them was lethal when heterozygous with the deficiency (Table 2).

Some of the problems faced during the mutagenesis experiment are listed below:

1. There was contamination of *Cy/Df (2R) gsb* in some of the bottles found while collecting *bwsp* males. The bottles were separated and discarded.
2. Bacterial and fungal infection were observed in *Cy/ Gla* bottles. All the contaminated bottles were discarded.
3. The incubator fan broke down, resulting in drastic cooling of the flies. Many were killed.

Table 1:

Mutagenesis Crosses

bwsp/bwsp males (irradiated) X *Cy/Gla* virgin females

F1 *bwsp/Cy, bwsp/Gla*

Cross A : *bwsp/Cy* males X *Df (2R) gsb/Cy* virgin females

Cross B : *bwsp/Cy* females X *Df (2R) gsb/Cy* males

Cross C : *bwsp/Gla* males X *Df (2R) gsb /Cy* virgin females

Cross D : *bwsp/Gla* females X *Df (2R) gsb/Cy* males

Table 2:

Number of chromosomes scored in the crosses A, B, C, and D.

Cross A	Cross B	Cross C	Cross D	Date
20	5	20	5	7/1/90
73	12	112	14	8/13/90
18		25		10/23/90
34		40		11/6/90
16		15		10/17/90
73		21		1/7/91
57		58		1/14/91
39		27		1/18/91
30		19		2/17/91
74	22	52		2/18/91
55		38		2/20/91
85		14		2/24/91
55				2/26/91
45		32		4/1/91
61	3	11		4/3/91
22		19		4/15/91
757	42	503	19	Subtotal

Total chromosomes scored = 757+42+503+19 = 1321

4. There were not enough virgins to look for lots of progeny. It was difficult to get a high yield from the *Cy/Gla* stock and the *Df(2R)gsb/Cy* stock. This made virgin collection difficult and limited the number of chromosomes tested.
5. The screen was very time consuming.

Veratridine sensitivity of the *para* alleles and wild type

Another possibly simpler and more efficient way to look for mutants might be to use the neurotoxin veratridine. The sensitivity of wild type and *para* mutants to veratridine was tested by exposing them to different concentrations of toxin as described in the methods section. All the mutants, ($para^1$, $para^2$, $para^3$, $para^{ST76}$, and $para^{ST109}$) and wild type were scored for survival after 24 hrs. The sensitivity of each *para* allele and wild type is described below.

***para*^{1}**

There is a gradual decrease of viability from 90 to140 μM in $para^1$ flies. At 140 μM there is a significant difference in the viability between the wild type and the $para^1$ (Table 3 and Figure 1). This difference is significant for both male and female survival.

***para*^{2}**

Above 100 μM veratridine, viability of $para^2$ decreased steadily. At 140 μM its survival is the same as that of wild type. This allele of *para* does not exhibit any resistance to veratridine in contrast to the others (Table 4 and Figure 2). Chi-squares values indicate p values between 0.5 and 0.7. Both males and females are as sensitive as wild type (for females, $0.8<p<0.9$).

Table 3:

Survival of *para*^{1} at different concentrations of veratridine

Date	Type	Total	Time	Conc.	Survival	Survival
			Hrs	µM		
7/20/91	*para*^{1} *(F)*	80	24	50	75	94%
7/20/91	*para*^{1} *(M)*	40	24	50	20	50%
7/20/91	*para*^{1}	120	24	50	95	79%
7/23/91	*para*^{1} *(F)*	80	24	60	72	90%
7/23/91	*para*^{1} *(M)*	80	24	60	38	48%
7/23/91	*para*^{1}	160	24	60	110	69%
7/26/91	*para*^{1} *(F)*	35	24	70	33	94%
7/26/91	*para*^{1} *(M)*	34	24	70	7	21%
7/26/91	*para*^{1}	69	24	70	40	58%
7/29/91	*para*^{1} *(F)*	69	24	80	63	91%
7/29/91	*para*^{1} *(M)*	35	24	80	7	20%
7/29/91	*para*^{1}	104	24	80	70	67%
7/31/91	*para*^{1} *(F)*	27	24	90	19	70%
7/31/91	*para*^{1} *(M)*	23	24	90	10	43%
7/31/91	*para*^{1}	50	24	90	29	58%
8/2/91	*para*^{1} *(F)*	66	24	100	60	91%
8/2/91	*para*^{1} *(M)*	57	24	100	24	42%
8/2/91	*para*^{1}	123	24	100	84	68%
8/5/91	*para*^{1} *(F)*	60	24	110	46	77%
8/5/91	*para*^{1} *(M)*	28	24	110	10	36%
8/5/91	*para*^{1}	88	24	110	56	64%
8/8/91	*para*^{1} *(F)*	105	24	120	64	61%
8/8/91	*para*^{1} *(M)*	57	24	120	12	21%
8/8/91	*para*^{1}	162	24	120	76	47%
5/22/92	*para*^{1} *(F)*	50	24	130	26	52%
5/22/92	*para*^{1} *(M)*	50	24	130	20	40%
5/22/92	*para*^{1}	100	24	130	46	46%
8/11/91	*para*^{1} *(F)*	40	24	140	20	50%
8/11/91	*para*^{1} *(M)*	34	24	140	6	18%
8/11/91	*para*^{1}	74	24	140	26	35%

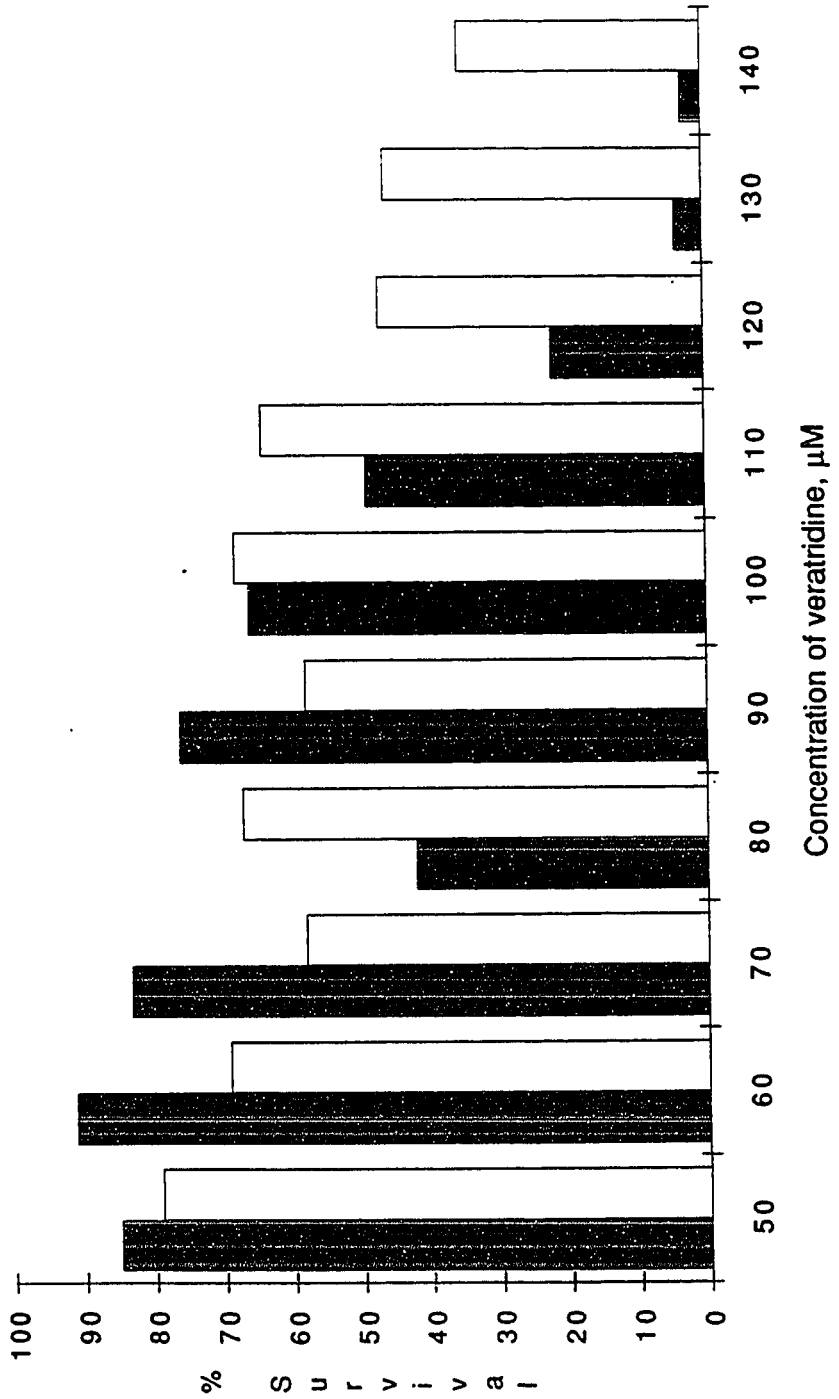

Figure 1: Survival of *para*[1] and wild type at different concentrations of veratridine.

Table 4:

Survival of *para*^{2} at different concentrations of veratridine

Date	Type	Total	Time	Conc.	Survival	Survival
			Hrs	μM		
7/20/91	*para*^{2} *(F)*	40	24	50	28	70%
7/20/91	*para*^{2} *(M)*	40	24	50	22	55%
7/20/91	*para*^{2}	80	24	50	50	63%
7/23/91	*para*^{2} *(F)*	35	24	60	24	69%
7/23/91	*para*^{2} *(M)*	17	24	60	10	59%
7/23/91	*para*^{2}	52	24	60	34	65%
7/26/91	*para*^{2} *(F)*	50	24	70	26	52%
7/26/91	*para*^{2} *(M)*	30	24	70	16	53%
7/26/91	*para*^{2}	80	24	70	42	53%
7/29/91	*para*^{2} *(F)*	23	24	80	17	74%
7/29/91	*para*^{2} *(M)*	21	24	80	10	48%
7/29/91	*para*^{2}	44	24	80	27	61%
7/31/91	*para*^{2} *(F)*	23	24	90	15	65%
7/31/91	*para*^{2} *(M)*	23	24	90	10	43%
7/31/91	*para*^{2}	46	24	90	25	54%
8/2/91	*para*^{2} *(F)*	25	24	100	18	72%
8/2/91	*para*^{2} *(M)*	19	24	100	9	47%
8/2/91	*para*^{2}	44	24	100	27	61%
8/5/91	*para*^{2} *(F)*	40	24	110	20	50%
8/5/91	*para*^{2} *(M)*	40	24	110	6	15%
8/5/91	*para*^{2}	80	24	110	26	33%
8/8/91	*para*^{2} *(F)*	80	24	120	28	35%
8/8/91	*para*^{2} *(M)*	40	24	120	5	13%
8/8/91	*para*^{2}	120	24	120	33	28%
5/21/92	*para*^{2} *(F)*	30	24	130	5	17%
5/21/92	*para*^{2} *(M)*	30	24	130	2	7%
5/21/92	*para*^{2}	60	24	130	7	12%
8/11/91	*para*^{2} *(F)*	15	24	140	1	7%
8/11/91	*para*^{2} *(M)*	15	24	140	0	0%
8/11/91	*para*^{2}	30	24	140	1	3%

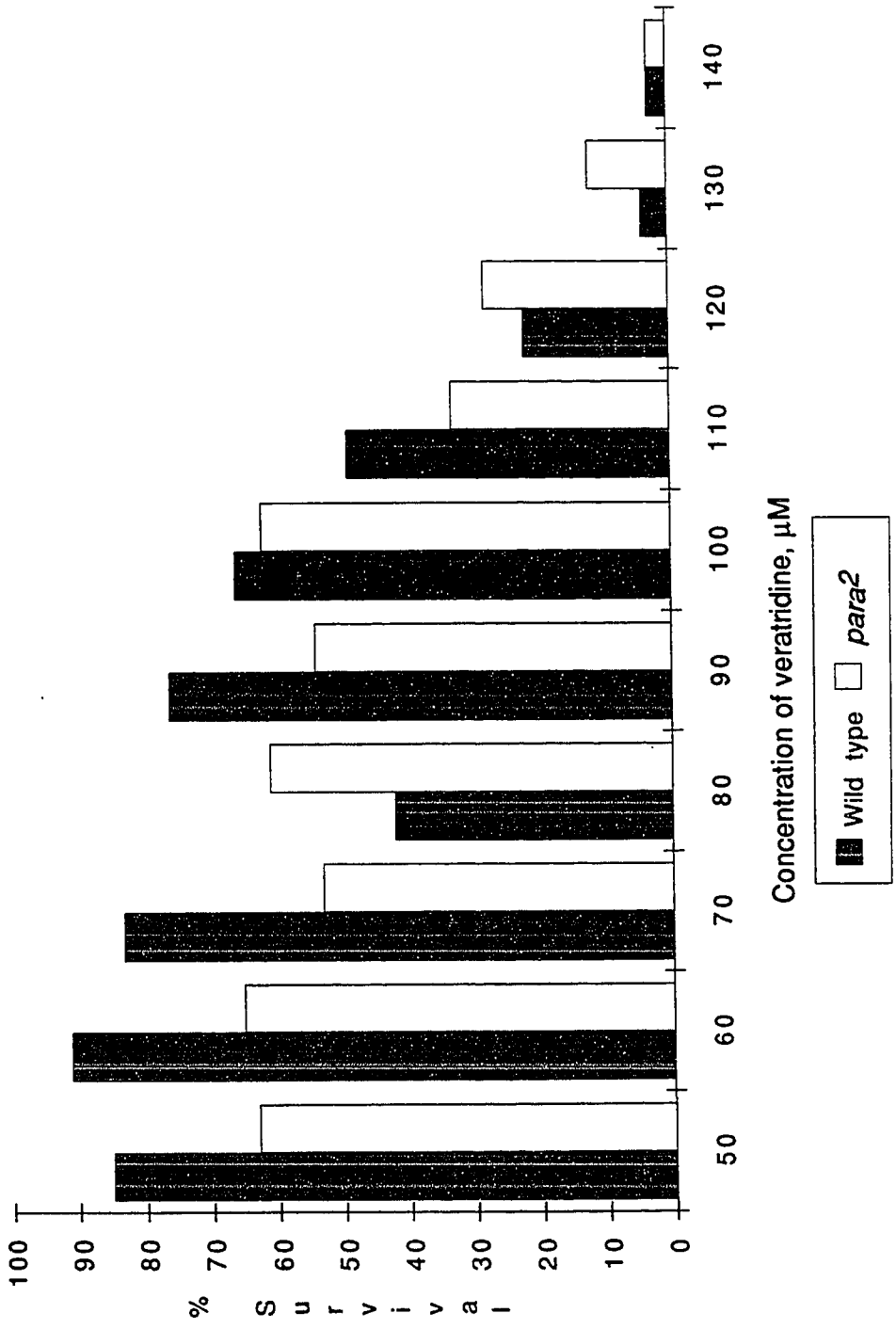

Figure 2: Survival of *para* 2 and wild type at different concentrations of veratridine.

para^{ST109}

para^{ST109} is fairly resistant to veratridine at concentrations above 110 μM (Table 5 and Figure 3). The difference between its survival and wild type is significant at 140 μM veratridine for both sexes.

para^{ST76}

When compared with wild type, *para^{ST76}* exhibits resistance to veratridine at concentrations above 110 μM (Table 6 and Figure 4).

para^{3}

para^{3} is the most resistant allele tested. It showed no sensitivity to veratridine at concentrations less than 130 μM. The males and the females were equally resistant (Table 7 and Figure 5). Chi square analysis of males, females and the combined sexes establish that the differences with wild type are highly significant ($p << 0.01$)

Wild type

The survival rate dropped at 80 μM and picked up at 90 μM. From 90-140 μM there was a steady drop to near zero. The drop in survival at 80 μM could be due to day to day variations in the assay (Table 8).

Four of the five *para* alleles confer significant resistance to veratridine at 140 μM (Table 9). The *para^{3}* allele was most resistant to veratridine as shown in the summary histogram (Figure 6). There was significant difference in the survival between wild type and *para^{3}* at 140 μM. At 140 μM *para^{3}* was the most resistant with a survival rate of 60% while the wild type survival rate dropped to 2% (Figure 5).

Female survival was high compared to that of males in the wild type and the mutants *para^{1}*, *para^{2}*, *para^{ST109}*, *and para^{ST76}* (compare Figures 7 and 8).

Table 5:

Survival of *para*^{ST109} at different concentrations of veratridine

Date	Type	Total	Time	Conc.	Survival	Survival
			Hrs	μM		
7/20/91	*para*^{ST109} *(F)*	60	24	50	58	97%
7/20/91	*para*^{ST109} *(M)*	30	24	50	24	80%
7/20/91	*para*^{ST109}	90	24	50	82	91%
7/23/91	*para*^{ST109} *(F)*	39	24	60	25	64%
7/23/91	*para*^{ST109} *(M)*	36	24	60	15	42%
7/23/91	*para*^{ST109}	75	24	60	40	53%
7/26/91	*para*^{ST109} *(F)*	30	24	70	26	87%
7/26/91	*para*^{ST109} *(M)*	30	24	70	20	67%
7/26/91	*para*^{ST109}	60	24	70	52	87%
7/29/91	*para*^{ST109} *(F)*	49	24	80	40	82%
7/29/91	*para*^{ST109} *(M)*	45	24	80	27	60%
7/29/91	*para*^{ST109}	94	24	80	67	71%
7/31/91	*para*^{ST109} *(F)*	40	24	90	37	93%
7/31/91	*para*^{ST109} *(M)*	40	24	90	14	35%
7/31/91	*para*^{ST109}	80	24	90	51	64%
8/2/91	*para*^{ST109} *(F)*	58	24	100	41	71%
8/2/91	*para*^{ST109}*(M)*	11	24	100	2	18%
8/2/91	*para*^{ST109}	69	24	100	43	62%
8/5/91	*para*^{ST109} *(F)*	75	24	110	58	77%
8/5/91	*para*^{ST109} *(M)*	30	24	110	20	67%
8/5/91	*para*^{ST109}	105	24	110	78	74%
8/8/91	*para*^{ST109} *(F)*	40	24	120	28	70%
8/8/91	*para*^{ST109} *(M)*	40	24	120	20	50%
8/8/91	*para*^{ST109}	80	24	120	48	60%
5/20/92	*para*^{ST109} *(F)*	50	24	130	40	80%
5/20/92	*para*^{ST109} *(M)*	50	24	130	38	76%
5/20/92	*para*^{ST109}	100	24	130	78	78%
8/11/91	*para*^{ST109} *(F)*	34	24	140	8	24%
8/11/91	*para*^{ST109} *(M)*	30	24	140	7	23%
8/11/91	*para*^{ST109}	64	24	140	15	23%

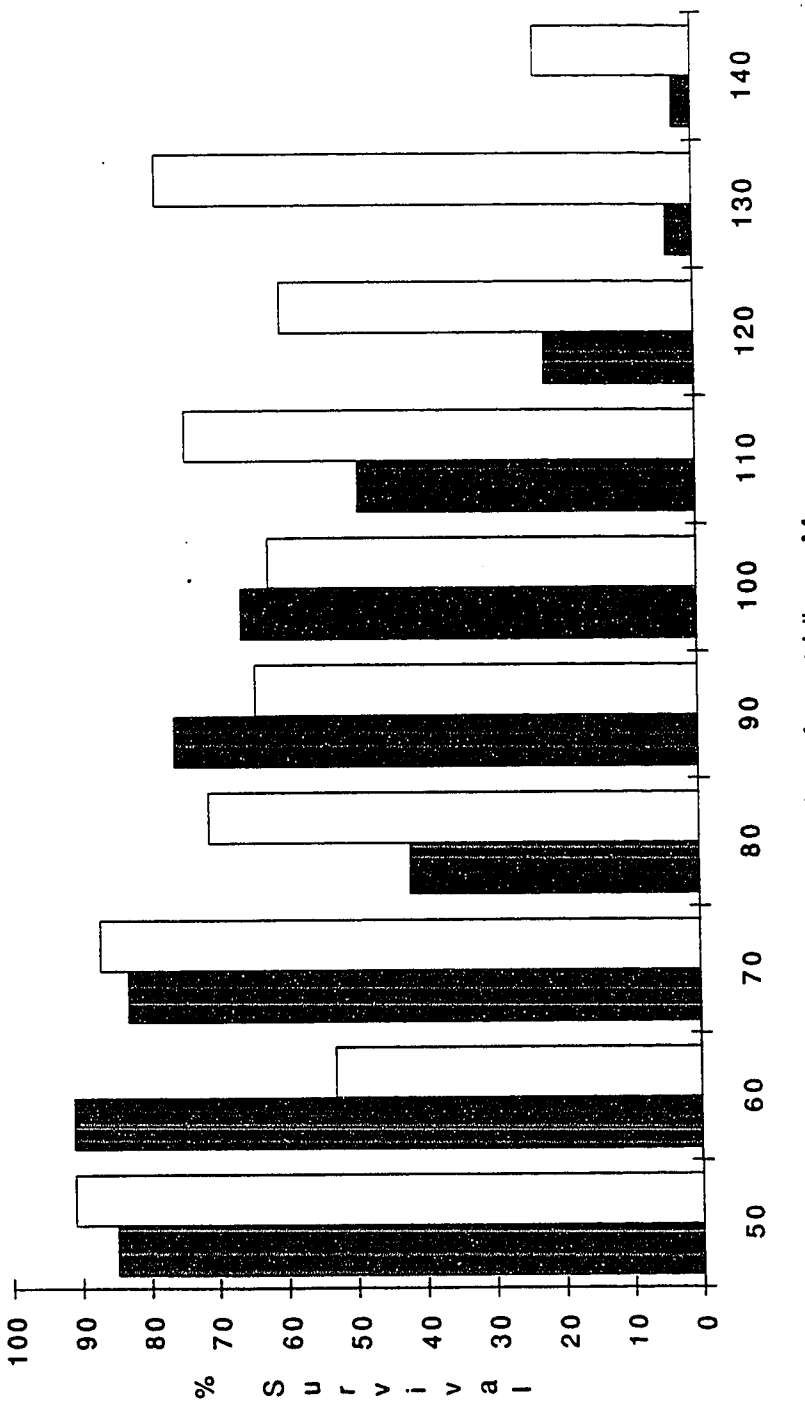

Figure 3: Survival of *para ST109* and wild type at different concentrations of veratridine.

Table 6:

Survival of *para*^{ST76} at different concentrations of veratridine

Date	Type	Total	Time	Conc.	Survival	Survival
			Hrs	µM		
7/20/91	*para*^{ST76} *(F)*	40	24	50	37	93%
7/20/91	*para*^{ST76} *(M)*	40	24	50	35	88%
7/20/91	*para*^{ST76}	80	24	50	72	90%
7/23/91	*para*^{ST76} *(F)*	95	24	60	87	92%
7/23/91	*para*^{ST76} *(M)*	51	24	60	40	78%
7/23/91	*para*^{ST76}	146	24	60	127	87%
7/26/91	*para*^{ST76} *(F)*	69	24	70	63	91%
7/26/91	*para*^{ST76} *(M)*	28	24	70	7	25%
7/26/91	*para*^{ST76}	97	24	70	70	72%
7/29/91	*para*^{ST76} *(F)*	216	24	80	190	88%
7/29/91	*para*^{ST76} *(M)*	180	24	80	107	59%
7/29/91	*para*^{ST76}	396	24	80	297	75%
7/31/91	*para*^{ST76} *(F)*	26	24	90	25	96%
7/31/91	*para*^{ST76} *(M)*	41	24	90	30	73%
7/31/91	*para*^{ST76}	67	24	90	55	82%
8/2/91	*para*^{ST76} *(F)*	119	24	100	84	71%
8/2/91	*para*^{ST76} *(M)*	117	24	100	62	53%
8/2/91	*para*^{ST76}	236	24	100	146	62%
8/5/91	*para*^{ST76} *(F)*	38	24	110	38	100%
8/5/91	*para*^{ST76} *(M)*	69	24	110	15	22%
8/5/91	*para*^{ST76}	107	24	110	53	50%
8/8/91	*para*^{ST76} *(F)*	40	24	120	28	70%
8/8/91	*para*^{ST76} *(M)*	35	24	120	7	20%
8/8/91	*para*^{ST76}	75	24	120	35	47%
5/22/92	*para*^{ST76} *(F)*	45	24	130	20	44%
5/22/92	*para*^{ST76} *(M)*	45	24	130	15	33%
5/22/92	*para*^{ST76}	90	24	130	35	39%
8/11/91	*para*^{ST76} *(F)*	119	24	140	24	20%
8/11/91	*para*^{ST76} *(M)*	120	24	140	24	20%
8/11/91	*para*^{ST76}	239	24	140	48	20%

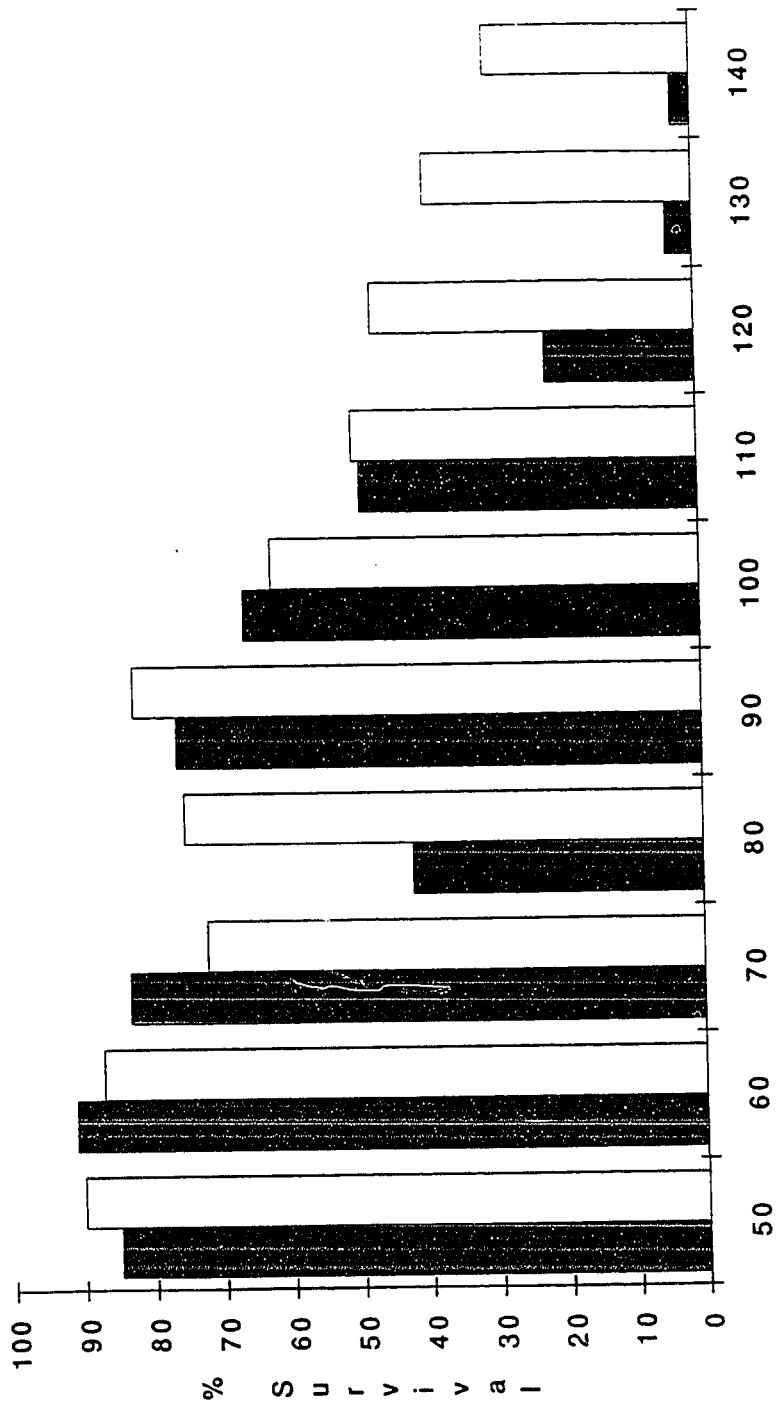

Figure 4: Survival of *paraST76* and wild type at different concentrations of veratridine.

Table 7:

Survival of *para*^{3} at different concentrations of veratridine

Date	Type	Total	Time	Conc.	Survival	Survival
			Hrs	μM		
7/20/91	*para*^{3} *(F)*	25	24	50	25	100%
7/20/91	*para*^{3}*(M)*	25	24	50	25	100%
7/20/91	*para*^{3}	50	24	50	50	100%
7/23/91	*para*^{3} *(F)*	25	24	60	25	100%
7/23/91	*para*^{3} *(M)*	25	24	60	25	100%
7/23/91	*para*^{3}	50	24	60	50	100%
7/26/91	*para*^{3} *(F)*	24	24	70	24	100%
7/26/91	*para (M)*	25	24	70	24	96%
7/26/91	*para*^{3}	49	24	70	48	98%
7/29/91	*para*^{3} *(F)*	199	24	80	197	99%
7/29/91	*para*^{3} *(M)*	169	24	80	168	99%
7/29/91	*para*^{3}	368	24	80	365	99%
7/31/91	*para*^{3} *(F)*	65	24	90	65	100%
7/31/91	*para*^{3} *(M)*	57	24	90	57	100%
7/31/91	*para*^{3}	122	24	90	122	100%
8/2/91	*para*^{3} *(F)*	114	24	100	112	98%
8/2/91	*para* ^{3} *(M)*	40	24	100	40	100%
8/2/91	*para* ^{3}	154	24	100	152	99%
8/5/91	*para*^{3} *(F)*	36	24	110	36	100%
8/5/91	*para* ^{3} *(M)*	30	24	110	30	100%
8/5/91	*para*^{3}	66	24	110	66	100%
8/8/91	*para*^{3} *(F)*	62	24	120	61	98%
8/8/91	*para*^{3} *(M)*	30	24	120	30	100%
8/8/91	*para*^{3}	92	24	120	91	99%
5/26/92	*para*^{3} *(F)*	80	24	130	73	91%
5/26/92	*para*^{3} *(M)*	80	24	130	32	40%
5/26/92	*para*^{3}	160	24	130	105	66%
8/11/91	*para*^{3} *(F)*	105	24	140	87	83%
8/11/91	*para*^{3} *(M)*	104	24	140	38	37%
8/11/91	*para*^{3}	209	24	140	125	60%

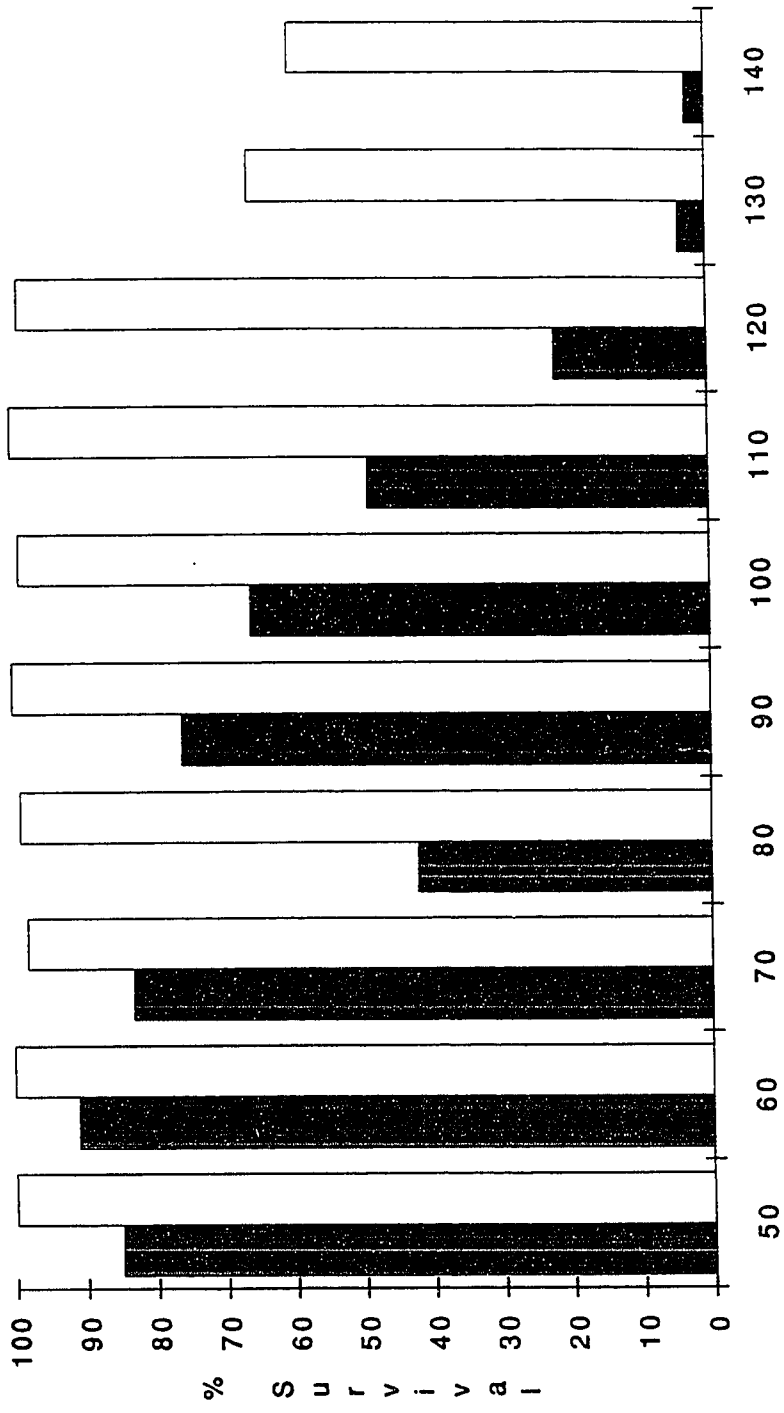

Figure 5: Survival of *para*^{3} and wild type at different concentrations of veratridine.

Table 8:

Survival of wild type at different concentrations of veratridine

Date	Type	Total	Time	Conc.	Survival	Survival
			Hrs	μM		
7/17/91	Wild (F)	80	24	50	76	95%
7/17/91	Wild (M)	80	24	50	60	75%
7/17/91	Wild	160	24	50	136	85%
7/20/91	Wild (F)	114	24	60	109	96%
7/20/91	Wild (M)	30	24	60	22	73%
7/20/91	Wild	144	24	60	131	91%
7/23/91	Wild (F)	89	24	70	86	97%
7/23/91	Wild (M)	23	24	70	11	48%
7/23/91	Wild	112	24	70	93	83%
7/26/91	Wild (F)	169	24	80	82	49%
7/26/91	Wild (M)	150	24	80	49	33%
7/26/91	Wild	319	24	80	131	41%
7/29/91	Wild (F)	130	24	90	110	85%
7/29/91	Wild (M)	75	24	90	45	60%
7/29/91	Wild	205	24	90	155	76%
7/31/91	Wild (F)	30	24	100	22	73%
7/31/91	Wild (M)	75	24	100	47	63%
7/31/91	Wild	105	24	100	69	66%
8/2/91	Wild (F)	65	24	110	42	65%
8/2/91	Wild (M)	70	24	110	24	34%
8/2/91	Wild	135	24	110	66	49%
8/5/91	Wild (F)	66	24	120	17	26%
8/5/91	Wild (M)	68	24	120	12	18%
8/5/91	Wild	134	24	120	29	22%
5/28/92	Wild (F)	50	24	130	4	8%
5/28/92	Wild (M)	50	24	130	0	0%
5/28/92	Wild	100	24	130	4	4%
8/8/91	Wild (F)	125	24	140	4	3%
8/8/91	Wild (M)	70	24	140	0	0%
8/8/91	Wild	195	24	140	4	2%

Table 9:

Wild type and *para* alleles tested at 140 μM veratridine

Total *para* and wild type

	Tested	Survived	Died	Statistical Significance
wild type	195	4	191	
$para^{2}$	30	1	29	
$para^{1}$	74	26	48	Y
$para^{ST76}$	159	24	135	Y
$para^{ST109}$	64	15	49	Y
$para^{3}$	209	125	84	Y

para and wild type females

	Tested	Survived	Died	Statistical Significance
wild type	125	4	121	
$para^{2}$	15	1	14	
$para^{1}$	40	20	20	Y
$para^{ST76}$	79	20	59	Y
$para^{ST109}$	34	8	26	Y
$para^{3}$	105	87	18	Y

para and wild type males

	Tested	Survived	Died	Statistical Significance
wild type	70	0	70	
$para^{2}$	15	0	15	
$para^{1}$	34	6	28	Y
$para^{ST76}$	80	4	76	Y
$para^{ST109}$	30	7	23	Y
$para^{3}$	104	38	66	Y

"Y" indicates survival is significantly different from wild type at the 95% confidence level.

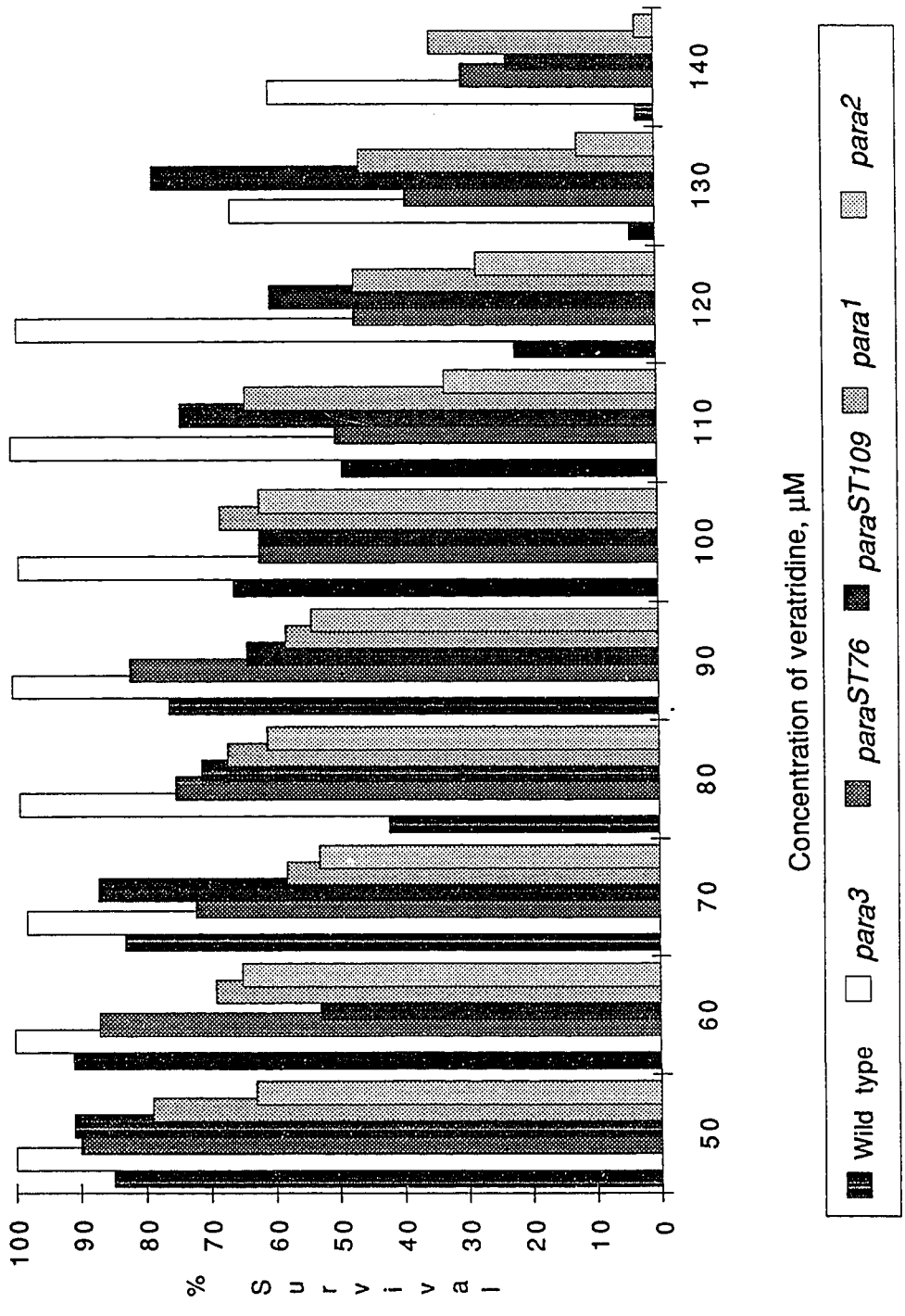

Figure 6: Survival of mutants and wild type at different concentrations of veratridine.

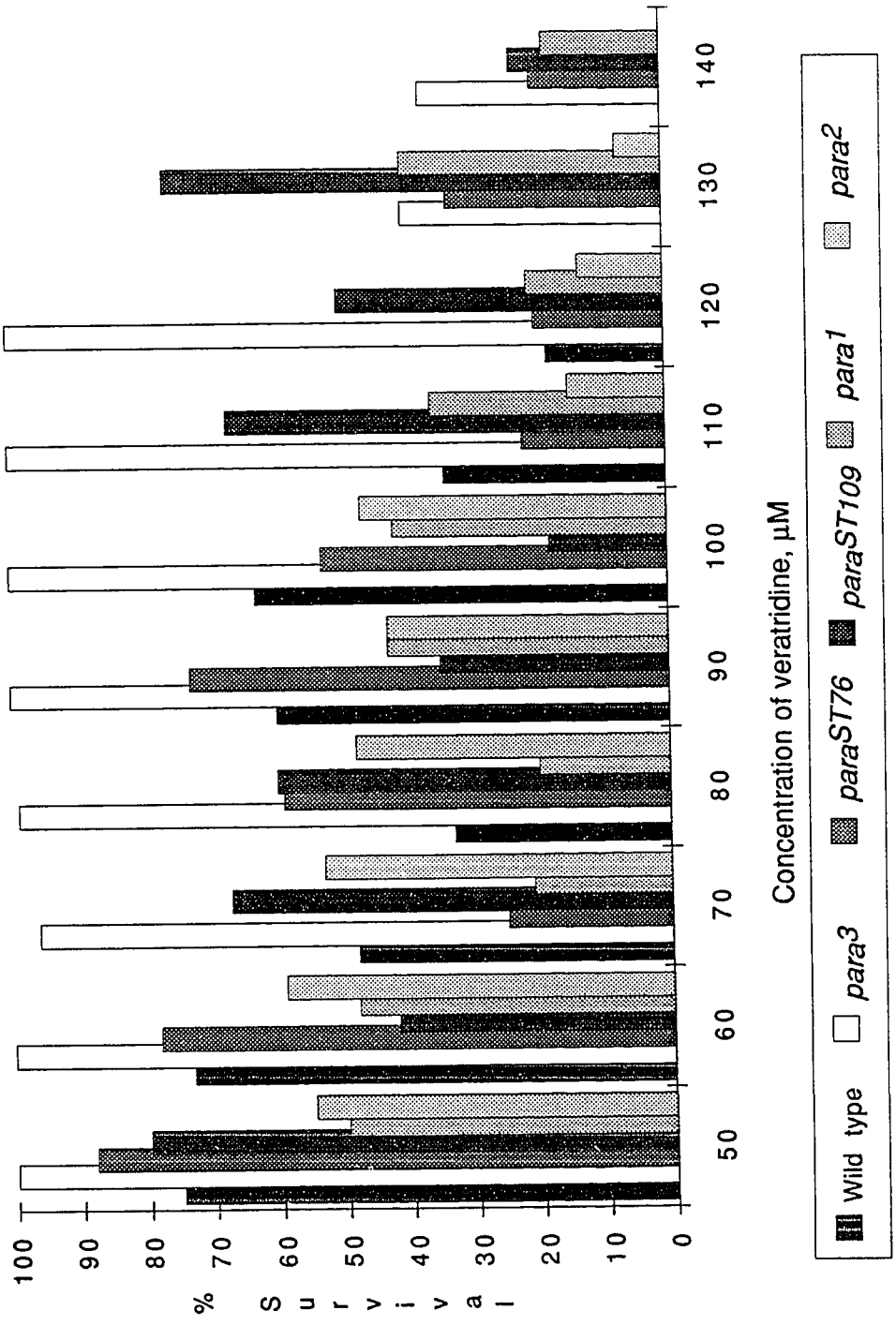

Figure 7: Survival of *para* and wild type males at different concentrations of veratridine.

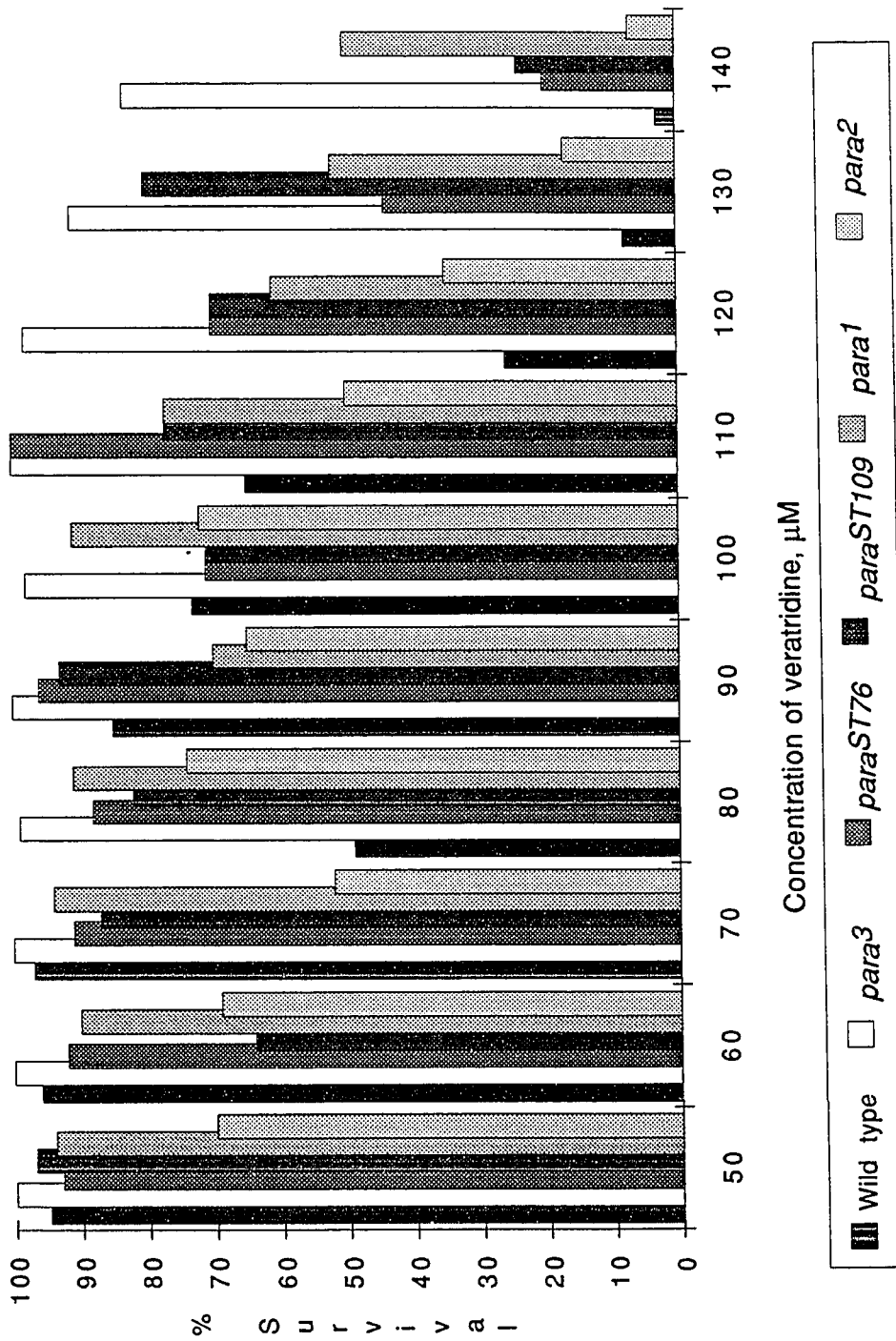

Figure 8: Survival of *para* and wild type females at different concentrations of veratridine.

This could be due to basic physiological differences between the sexes. The males with a smaller average weight give themselves larger doses of the toxin. On the other hand, there could be differences in the distribution of sodium channels between the sexes that would affect viability. The sex difference was observed consistently with wild type and all *para* mutants except $para^3$. At 140 μM veratridine survival is still high for both sexes. Perhaps at higher concentrations a sex difference would be observed. In a screen for mutants it would be more likely to detect mutation if it were carried by a female. If the mutation were similar to $para^3$, it could be detected equally well in either sex.

Veratridine screening for *DSC* mutants

To assess the usefulness of a veratridine screen for *DSC* mutations, the veratridine sensitivity of genotypes that would be used in the mutagenesis was determined. The second chromosome marker stock *bwsp* and the deficiency heterozygotes $Df(2R)gsb^{ESI}/Cy$ were tested. These experiments demonstrate that *bwsp* and *Df (2R) gsb* heterozygotes were as sensitive as wild type (Table 10). Assuming a strict dosage relationship, having half as many of the channels specified by the *DSC* gene does not alter sensitivity. However, if a fly had a mutation as well as a deficiency in the gene on the homologue, its sensitivity might be reduced, so several trial runs of the mutagenesis and screen were performed.

The *bwsp* males were irradiated and crossed to *Df (2R)gsb/Cy* virgin females. The males were removed after one week. The F1 (*bwsp/Cy, bwsp/Df (2R) gsb)* were collected and put on fresh food. They were tested on veratridine after having been on fresh food for three hours. All the flies died at 140 μM (Table 11). In three preliminary tests all the F1 flies, 256 *bwsp/Cy* and

Table 10:

Survival of *para*^{3}, *Df gsb/Cy*, *bwsp* and *Cy/Gla*.

Date	Type	Total	Time	Conc.	Survival	Survival
			Hrs	µM		
9/5-9/6/91	*para*^{3} *(F)*	44	24	140	34	77%
9/5-9/6/91	*para*^{3} *(M)*	50	24	140	6	12%
9/9-9/10/91	*para*^{3} *(F)*	100	24	140	65	65%
9/9-9/10/91	*para*^{3} *(M)*	80	24	140	15	19%
9/5-9/6/91	*Df gsb/Cy (F)*	25	24	140	0	0%
9/5-9/6/91	*Df gsb/Cy (M)*	30	24	140	0	0%
9/9-9/10/91	*Df gsb/Cy (F)*	50	24	140	0	0%
9/9-9/10/91	*Df gsb/Cy (M)*	60	24	140	0	0%
9/5-9/6/91	*bwsp (F)*	45	24	140	0	0%
9/5-9/6/91	*bwsp (M)*	45	24	140	0	0%
9/9-9/10/91	*bwsp (F)*	90	24	140	0	0%
9/9-9/10/91	*bwsp (M)*	90	24	140	0	0%
9/5-9/6/91	*Cy/Gla (F)*	38	24	140	9	24%
9/5-9/6/91	*Cy/Gla (M)*	30	24	140	5	17%
9/9-9/10/91	*Cy/Gla (F)*	80	24	140	10	13%
9/9-9/10/91	*Cy/Gla (M)*	30	24	140	4	13%

Table 11:

Veratridine Screening for *DSC* Mutants

1. *bwsp/bwsp* males irradiated X *Df (2R) gsb/Cy* virgin female

(100) (25)

F1 *bwsp/Cy* (37 F), (15 M) *bwsp/Df gsb* (15 F), (20 M)

All tested on veratridine at 140 µM and all died.

2. *bwsp/bwsp* males irradiated X *Df (2R) gsb/Cy* virgin female

(100) (25)

F1 *bwsp/Cy* (52 F), (40 M) *bwsp/Df gsb* (30 F), (21 M)

All tested on veratridine at 140 µM and all died

3. *bwsp/bwsp* males irradiated X *Df (2R) gsb/Cy* virgin female

(150) (40)

F1 *bwsp/Cy* (50 F), (62 M) *bwsp/Df gsb* (43 F), (32 M)

All tested on veratridine at 140 µM and all died.

161 *bwsp/Df(2R)gsb*, were sensitive to veratridine. None of the chromosomes was mutated to confer veratridine resistance.

Discussion

This study presents two different ways of looking for mutations in the ion channel gene at the tip of 2R. One of the methods involves the screening for lethals revealed by a small deficiency of the region. The deficiencies in this region have a *Minute* phenotype characterized by slow development and probably reduced capacity for protein synthesis (Boring et al., 1989; Kongsuwan et al., 1985). The fact that the deficiency is *Minute* introduces slowed development and lowered reproductive capacity into the screen. This compromises the success and ease of the screen. The specific localization to 60E5/6 (Germeraad et al., 1992) places the gene within a 100kb length of the chromosome represented by walks in the region in overlapping phage and cosmid clones (Cohen et al., 1989; Cote et al., 1987). Selection of mutations was based on finding chromosomes that are lethal when heterozygous with a deficiency for this region (*Df(2R)gsb* ^{ESI}). The screen requires setting up pair matings, time consuming and low success rate. The screen detects lethal mutations of other genes covered by the deficiency. These would require subsequent sorting out using genetic and molecular tools.

There are several possibilities for the function of the *DSC* gene on 2R. Previous voltage clamp studies show that if the gene is expressed in embryonic tissue, it is to a very low degree relative to *para* (Germeraad et. al., 1992). It may be a second sodium channel locus coding for a neuronally expressed sodium channel or it may encode a sodium channel expressed in another tissue, like muscle. Electrophysiological data have indicated that action potentials generated by inward currents in insect muscle were carried by calcium ions

(Yamaoka and Ikeda, 1988), but there was a small TTX sensitive current which could represent the expression of a gene such as this one. Another possibility is that the channel coded for by this gene carries an ion other than sodium. Known potassium and calcium channel structural genes are similar in sequence to the sodium channel genes. In addition Heinemann et al. (1992) demonstrated that changing a single amino acid in a sodium channel can change the ion selectivity to resemble that of a calcium channel. Characterizing the gene will help to develop genetic and molecular tools that allows functional analysis of gene products.

No lethals were observed in the test of 1321 chromosomes. Assuming one in every six deficiency-tested chromosomes carries a newly generated lethal on 2R (Ashburner, 1989) and assuming there are about 500 genes on 2R, one expects to find a lethal of *DSC* in 3000 chromosomes. Given that the selection also picks up lethals of other genes in the deficient region, the frequency of detection of a lethal should be much higher (1/600 if the deficiency has 5 genes missing, 1/300 if deficiency has 10 genes missing).

The other method takes advantage of differences observed in toxin sensitivity between wild type and animals bearing mutations known to affect sodium currents directly or indirectly. Mutations in *para* showed decreased sensitivity to veratridine compared to wild type in cultured larval neurons (Suzuki and Wu,1984). Electrophysiological data from the cultured embryos have shown that *para* exhibits different levels of sodium current expression (O'Dowd et al., 1989). Experiments conducted with veratridine in embryo cultures, counting the number of each type of cell in culture, show that the veratridine sensitivity of embryonic neurons differentiated in embryo cell culture is dramatically reduced

by *para* ^{ST76} as compared to the wild type ($p<0.01$) (Germeraad, unpublished). The studies reported here extend the effect of veratridine from embryos and larval neurons to the adult animal and provide new criteria for ranking alleles of *para*. The results clearly show the optimal conditions for distinguishing mutant and wild type genotypes is at 140µM. The wild type survival was almost zero, whereas the mutants' survival was much higher. These results provide a fast and efficient test for screening for sodium channel mutations. The effectiveness of the two screens described in this study depends on the specific function of the *DSC* gene, that it has an essential role and that it makes a veratridine-sensitive product.

There are several genes affecting the sodium currents (*nap, para, sei* and *tipE*) whose phenotypes are severe in their disruption of behavior (Ganetzky, 1984, 1986). When a mutation of *DSC* is isolated, its behavioral phenotype will be assessed to see how it compares to these mutations. The effect of mutants of the 2R gene in combination with other known mutants is potentially very iinteresting. Double mutants combinations have proven useful in understanding other physiologically defective mutants and a more extreme phenotype is observed. It may help to screen for additional mutations if there is synergism. The different portions of para and 60E5/6 screening would allow to identify new members of the gene family by analysis of existing behavioral mutants (O'Dowd and Aldrich, 1988).

References

Ashburner, N. (1989) *Drosophila*: A Laboratory Handbook. Cold Spring Harbor Laboratory Press, New York.

Auld, V. J., Goldin, A. L., Krafte, D. S., Catterall, W. A., Lester, H. A., and Davidson, N. (1990) A neutral amino acid change in segment IIS4 dramatically alters the gating properties of the voltage-dependent sodium channel. Proc. Natl. Acad. Sci. 87, 323-327.

Barchi, R.L. (1988) Probing the molecular structure of the voltage-dependent sodium channel. Ann. Rev. Neurosci. 11, 455-495.

Beckh, S., Noda, M., Lubbert, H., and Numa, S. (1989) Differential regulation of three sodium channel messenger RNAs in the rat central nervous system. EMBO J. 8, 3611-3616.

Beckh, S. (1990) Differential expression of sodium channel mRNAs in the peripheral nervous system and innervated tissues. FEBS Letters 262, 317-322.

Boring, L. F., Sinervo, B., and Schubiger, G. (1989) Experimental phenocopy of a *Minute* maternal-effect mutation alters blastoderm determination in embryos of *Drosophila melanogaster*. Develop. Biol. 132, 343-354.

Cannon,S. C., Brown Jr., R. H., and Corey, D. P. (1991) A sodium channel defect in Hyperkalemic Periodic Paralysis, Potassium- induced failure of inactivation. Neuron 6 , 619-626.

Catterall, W. A. (1980) Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. Ann. Rev. Pharm. Toxicol. 20, 15-43.

Catterall, W. A. (1986) Molecular properties of voltage-sensitive sodium channels. Ann. Rev. Biochem. 55, 953-985.

Catterall, W. A. (1988) Structure and function of voltage-sensitive ion channels. Science 242, 50-61.

Cohen, S. M., Bronner, G., Kuttner, F., Jurgens, G., and Jackle, H. (1989) Distal-less encodes a homoeodomain protein required for limb development in *Drosophila*. Nature 338, 432-434.

Cote, S., Preiss, A. Haller, J., Schuh, R., Kienlin, A., Seifert, E., and Jackle, H. (1987) The gooseberry-zipper region of *Drosophila*: five genes encode

different spatially restricted transcripts in the embryo. EMBO J. 6, 2793-2801.

Fontaine, B., Khurana, T. S., Hoffman, E.P., Bruns, G. A. P., Haines, J. L., Trofatter, J. A., Hanson, M. P., Rich, J., Mcfarlane, H., Yasek, D. M., Romano, D., Gusella, J. F., and Brown. Jr, R. H. (1990) Hyperkalemic Periodic Paralysis and the adult muscle sodium channel alpha subunit gene. Science. 250, 1000-10002.

Ganetzky, B. (1984) Genetic studies of membrane excitability in *Drosophila*: lethal interaction between two temperature-sensitive paralytic mutations. Genetics 108, 897-911.

Ganetzky, B. (1986) Neurogenetic analysis of *Drosophila* mutations affecting sodium channels: Synergistic effects on viability and nerve conduction in double mutants involving *tip-E*. J. Neurogenetics. 3, 19-31.

Ganetzky, B. and Wu, C. F. (1986) Neurogenetics of membrane excitability in *Drosophila*. Ann. Rev. Genet. 20, 13-44.

Germeraad, S., O'Dowd, D., and Aldrich, R. W. (1992) Functional assay of a putative *Drosophila* sodium channel gene in homozygous deficiency neurons. J. Neurogenetics 8, 1-16.

Gordon, D., Mettick, D., Auld, V., Dunn, R., Goldin, A. L., Davidson, N., and Catterall, W. A. (1987) Tissue specific expression of the RI and RII sodium channel subtypes. Proc. Natl. Acad. Sci. 84, 8682-8686.

Guy, H. R. and Seetharamulu, P. (1986) Molecular model of the action potential sodium channel. Proc. Natl. Acad. Sci. 83, 508-512.

Hall, J. C. (1982) Genetics of the nervous system in *Drosophila* . Quart. Rev. Biophys. 15, 223-479.

Heinemann, S. H., Terlau, H., Stuhmer, W., Imoto, K., and Numa, S. (1992) Calcium channel characteristics conferred on the sodium channel by single mutations. Nature 356, 441-443.

Hille, B. (1984) Ionic channels of excitable membranes. Sinauer Associates, Inc., Sunderland, Massachusetts.

Jackson, F. R., Gitschier, J., Strichartz, G. R., and Hall, L. M. (1985) Genetic modifications of voltage-sensitive sodium channels in *Drosophila*: Gene dosage studies of the *seizure* locus. J. Neuroscience 5, 1144-1151.

Jackson, F. R., Wilson, S. D., and Hall, L. M. (1986) The tip-E mutation of *Drosophila* decreases saxitoxin binding and interacts with other mutations affecting nerve membrane excitability. J. Neurogenet. 3, 19-31.

Kayano, T., Noda, M., Flockerzi, V., Takahashi, H., and Numa, S. (1988) Primary structure of rat brain sodium channel III deduced from the cDNA sequence. FEBS 228, 187-194.

Kongsuwan, K., Yu, Q., Vincent, A., Fisardi, M. C., Rosbash, M., Lengyel, J. A., and Merriam, J. (1985) A *Drosophila Minute* gene encodes a ribosomal protein. Nature 317, 555-558.

Kulkarni, S. J. and Padhye, A. (1982) Temperature-sensitive paralytic mutations on the second and third chromosomes of *Drosophila melanogaster.* Genet. Rresearch 40, 191-199.

Lindsley, D. and Grell, E. (1968) Genetic variations of *Drosophila melanogaster.* Carnegie Inst., Wash. Publ. 627.

Lindsley, D. and Zimm, G. (1985) The genome of *Drosophila melanogaster.* Part 1. Drosophila Inf. Serv. 62, 1-227.

Lindsley, D. and Zimm, G. (1987) The genome of *Drosophila melanogaster.* Part 3. Drosophila Inf. Serv. 65, 1-227.

Loughney, K., Kreber, R., and Ganetzky, B. (1989) Molecular analysis of the *para* locus a sodium channel gene in *Drosophila.* Cell 58, 1143-1154.

Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., TAkahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsuo, H., Raftrey, M. A., Hirose, T., Inayama, S., Hayashida, H., Miyata, T., and Numa, S. (1984) Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence. Nature 312, 121-127.

Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, T., and Numa, S. (1986) Existence of distinct sodium channel messenger RNAs in rat brain. Nature 320, 188-192.

Noda, M., Suzuki, H., Numa, S., and Stuhmer, W. (1989) A single point mutation confers tetrodotoxin and saxitoxin insensitivity on the sodium channel II. FEBS Letters 259, 213-216.

O'Dowd, D. K. and Aldrich, R. W. (1988) Voltage-clamp analysis of sodium channels in wild-type and mutant *Drosophila* neurons. J. Neurosci. 8, 3633-3643.

O'Dowd, D. K., Germeraad, S., and Aldrich, R. W. (1989) Alterations in the expression and gating of *Drosophila* sodium channels by mutations in the *para* gene. Neuron 2, 1301-1311.

Okamoto, H., Sakai, K., Goto, S., Takasu-Ishikawa, E., and Hotta, Y. (1987) Isolation of *Drosophila* genomic clones homologous to the eel sodium channel gene. Proc. Japan Acad., 63, Ser. B, 284-288.

Papazian, D. M., Schwarz, T. L., Tempel, B. L., Timpe, L. C., and Jan, L. Y. (1988) Ion channels in *Drosophila* . Ann. Rev. Physiology 50, 379-394.

Ptacek, L. J., George, A. L. Jr., Barchi, R. L., Griggs, R. C., Riggs, J. E., Robertson, M., and Leppert, M. F. (1992) Mutations in an S4 segment of the adult skeletal muscle sodium channel cause Paramyotonia Congenita. Neuron 8, 891-897.

Rogart, R. B., Cribbs, L. L., Muglia, L., Kephart, D., and Kaiser, M. (1989) Molecular cloning of a putative tetrodotoxin-resistant rat heart Na+ channel isoform. Proc. Natl. Acad. Sci. 86, 8170-8174.

Salkoff, L., Butler, A., Wei, A., Scavarda N., Giffen, K., Ifune, C., Goodman, R., and Mandel, G. (1987a) Genomic organization and deduced amino acid sequence of a putative sodium channel gene in *Drosophila* . Science 237, 744-749.

Salkoff, L., Butler, A., Scavarda, N., and Wei, A. (1987b) Nucleotide sequence of the putative sodium channel from *Drosophila*:: the four homologous domains. Nucleic Acid Research 15, 8569-8572.

Siddiqi, O. and Benzer, S. (1976) Neurophysiological defects in temperature-sensitive paralytic mutants of *Drosophila melanogaster.* Proc. Natl. Acad. Sci. USA 73, 3252-3257.

Stern, M. Kreber, R., and Ganetzky, B. (1990) Dosage effects of a *Drosophila* sodium channel gene on behavior and axonal excitability. Genetics 124, 133-143.

Stuhmer, W., Conti, F., Suzuki, N., Wang, X., Noda, M., Yahaj, N., Kubo, H., and Numa, S. (1989) Structural parts involved in activation and inactivation of the sodium channel. Nature 339, 597-603.

Suzuki, D. T., Grigliatti, T., and Williamson, R. (1971) Temperature-sensitive mutations in *Drosophila melanogaster* , VII. A mutation (*para*^{ts}) causing reversible adult paralysis. Proc. Natl. Acad. Sci. USA 68, 890-893.

Suzuki, N. and Wu, C. F. (1984) Altered sensitivity to sodium channel-specific neurotoxins in cultured neurons from temperature-sensitive paralytic mutants of *Drosophila*. J. Neurogenet. 1, 225-238.

Trimmer, J. S. and Agnew, W. S. (1989) Molecular diversity of voltage-sensitive Na channels, Ann. Rev. Physiol. 51, 401-418.

Wu, C.-F. and Ganetzky, B. (1980) Genetic alteration of nerve membrane excitability in temperature-sensitive paralytic mutants in *Drosophila melanogaster*. Nature 286, 814-816.

Yamaoka, K. and Ikeda, K. (1988) Electrogenic responses elicited by transmembrane depolarizing current in aerated body wall muscles of *Drosophila melanogaster* larvae. J. Comp. Phyiol. A 163, 705-714.