IDENTIFYING AND MAPPING

PARALLEL MUTATIONS OF GAR-3

A Senior Scholars Thesis

by

CHRISTOPHER PROMPUNTAGORN

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

UNDERGRADUATE RESEARCH SCHOLAR

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Approved by:

Research Advisor: Luis Rene Garcia
Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

Identifying and Mapping Parallel Mutations of GAR-3. (April 2009)

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This research identifies a gene that potentially works acetylcholine receptor protein GAR-3. We isolated nematodes (roundworm) *Caenorhabditis elegans* that are resistant to the drug arecoline, which stimulates behaviors such as spicule protraction (mating behavior). From the isolated worms we created about thirty recombinants, crossing arecoline resistant mutants genetically wildtype worms, and collected their DNA. Testing the chromosomes at different loci can identify a locus that contains mutant DNA; regions of the chromosome that display wildtype DNA are ruled out. An online catalog of single nucleotide polymorphisms allows us to distinguish between the wildtype (HA) and mutant (N2) DNA; there are regions that contain a single base pair difference between the N2 and HA samples. We amplified many DNA regions with polymerase chain reaction and tested each region with a restriction endonuclease that cut either the wildtype or the mutant DNA. We observed each recombinant's restriction pattern on agarose gel to determine which regions contain 100 percent mutant DNA and could thus statistically be the site of the mutation. We believe the mutation lies in a

region on the left end of the X chromosome, based on a larger proportion of N2 DNA compared to regions on the rest of the chromosomes. We believe we can use this data to narrow down the genes in this region and pinpoint which mutation is causing the nonfunctional protein.

DEDICATION

I would like to dedicate my research to my family, as well as to the people I have gotten to study with in college.

ACKNOWLEDGMENTS

I would like to acknowledge Dr. Garcia's lab for giving me a project to work on, especially an interesting one, in the first place.

Funding acknowledgements go to the Undergraduate Research Scholars program, which funded my poster printing costs, as well as any potential travel costs to present my research.

NOMENCLATURE

N2	Bristol *C. elegans*
HA	Hawaii *C. elegans*
ACh	Acetylcholine
mAChR	Muscarinic Acetylcholine Receptor
LG I	Linkage Group 1 on Chromosome 1
LG II	Linkage Group 2 on Chromosome 2
LG III	Linkage Group 3 on Chromosome 3
LG IV	Linkage Group 4 on Chromosome 4
LG V	Linkage Group 5 on Chromosome 5
LG X	Linkage Group X on Chromosome X
PCR	Polymerase Chain Reaction
SNP	Single Nucleotide Polymorphism

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CHAPTER I

INTRODUCTION

Understanding how molecules control biological pathways can help relate everyday behaviors such as completing a task. This research demonstrates how neuronal circuits can control a behavior. For experiments regarding animal behavior, a good model should be easy to manipulate and observe. A relatively simple animal with a few observable and quantifiable behaviors is the roundworm *Caenorhabditis elegans*. This worm is 1 millimeter in length, almost invisible to the naked eye. Thus behavior must be studied under a microscope. Those that have two X chromosomes develop as hermaphrodites, while those with one X chromosome develop as males. The males are posed with the task of mating to pass their genes to subsequent generations, while hermaphrodites do not require such mating for their genes to be passed on. Simple survival mechanism behaviors are easy to observe with *C. elegans*. Furthermore, because of the ease of obtaining DNA for testing, *C. elegans* makes as an excellent scientific model. Studying a behavior such as male mating behavior allows us to explore biological processes in detail. These processes can be further dissected with genetic mutations to understand the molecules that regulate how neurons and muscles work.

This thesis follows the style of The Journal of Neuroscience.

Observed behaviors

We are exploring molecular pathways that promote neuromuscular activities, which are required to complete a goal-oriented behavior. One can artificially stimulate a behavior with chemicals or drugs. When exposed to the drug arecoline, certain receptors activate. The activation leads to particular physical actions in organisms; in the case of male *C. elegans*, spicule protractor muscle contraction. Acetylcholine (ACh) receptors are respond to neurotransmitters (such as ACh) to complete activities such as synaptic transmission and muscular contraction (Marieb and Hoehn, 2006). There are two types of ACh receptors: nicotinic and muscarinic. The nicotinic ACh receptors, which are activated by nicotine, are ligand gated ion channels, whereas muscarinic ACh receptors are metabotropic G-protein coupled receptors (Sargent, 1993). The latter can be stimulated by muscarine, and also the drug arecoline.

C. elegans male mating behavior consists of multiple sensory and motor steps. One of these steps is the insertion of the male sex organ (called the spicule) into the vulva of his hermaphroditic mate. A neuromuscular circuit, consisting of spicule protractor muscles and their associated neurons, controls the movement of the spicules during mating. A molecule expressed in this circuit is the muscarinic acetylcholine receptor (mAChR). Neuronal cells in the circuit contain mAChR proteins. One example of a mAChR protein is GAR-3, which is known to be a G-protein coupled receptor on the cell membrane (Steger and Avery, 2004).

Drugs such as arecoline and oxotremorine are agonists to mAChR receptors. An agonist is drug that can combine with a receptor on a cell to produce a physiological reaction, such as muscle contraction or synaptic transmission. When mAChRs are stimulated, G-proteins activate their effectors, which cause cells to produce an output. G-protein activation involves subunit G_{α} dissociating from $G_{\beta\gamma}$, which activates other enzymes like adenylate cyclase (Aperia et al., 1994; Alberts et al., 2003) and lead to further cellular responses. In the case of males, activating GAR-3 with drugs promotes spicule protractor muscle contracting, and consequently male worms display mating behavior (Liu et al., 2007).

Experimental basis

Wild type male *C. elegans* mate by inserting their spicules into a hermaphrodite's vulva. Arecoline will make males stick their spicules out. However, if GAR-3 is mutated, fewer male worms display the extension of spicules at a concentration of arecoline that would make 100% of wildtype worms protract their spicules. Lack of GAR-3 partially impairs male *C. elegans*' ability to continuously protract their spicules (Garcia et al., 2001). However, arecoline-induced spicule protraction is not completely inhibited, suggesting that other genes contribute to alternative pathways that promote spicule muscle contraction. We identified another mutation that, in conjunction with a deletion of GAR-3, displays even more arecoline resistance. We refer to this mutation as *rg430*.

What we did not know is where this mutation maps in the genome of *C. elegans*. To find where the *rg430* mutation maps, we made hybrids between *rg430* mutants and Hawaiian wildtype variants. We homozygosed the arecoline-resistant phenotypes, and by single-nucleotide polymorphism mapping we genotyped which chromosomes did not contain the *rg430* mutation. Here I report that the *rg430* mutation maps to the *C. elegans* X chromosome, and discuss further work on identifying the molecular identity of the gene affected by *rg430*.

Scientific significance

GAR-3 is a muscarinic acetylcholine receptor found in animals, with a genetic sequence similar to the sequence in the mammalian genome. The neurological pathway circuitry of *C. elegans* is analogous to those found in higher organisms, suggesting that there is a link between the worm behavior and behavioral mechanisms in higher organisms. Therefore, mapping the gene with the mutation *rg430* may possibly identify a similar gene that is used in behaviors of other animal species.

CHAPTER II

METHODS

To create recombinant DNA, we crossed two genetically divergent lines of *C. elegans*; one from Bristol, United Kingdom (N2) and the other from Hawaii (HA). To ensure the recombined males were homozygous for *rg430*, Dr. Garcia screened each of the mutant worms with the drug arecoline; this was done in the summer of 2008, and this is where I picked up the project. Screening the N2/Hawaii hybrids for arecoline resistance identified worms with recombined Hawaiian DNA shuffled in, but still have N2 DNA at the site of the mutation.

C. elegans is a species with multiple variants, many of which display a single different nitrogenous base (single nucleotide polymorphism) in a given sequence of DNA. Many SNPs exist between the N2 variant and, according to Koch et al. in *Genome Research*, an "isolate from an island (Hawaii) that contains many unique SNPs… from the rest of the world" (Koch et al., 2000).

We wanted to homozygose the mutation to rule out the regions of a chromosome that did not have the mutation allele. SNP mapping accomplishes this by eliminating regions of the chromosome that do not contribute to the mutant phenotype. In our case, crossing in a wildtype Hawaiian genome will rule out the regions containing Hawaii-characteristic SNPs, since the mutation is homozygosed on the N2.

We homozygosed the *rg430* mutation by isolating a line of N2 worms resistant to arecoline; the Hawaii line was wildtype. We crossed these two lines together to form the F1 generation (phenotypically wildtype). After producing the N2/HA heterozygous F1 generation, we allowed them to self-reproduce to form the F2. From the F2 generation we picked out those resistant to arecoline, since the drug is the agonist for the GAR-3 protein as well as the unknown functioning unit affected by the mutation *rg430*.

We had 28 different samples of recombinant lines (five of the samples did not last for more than two weeks, so the final table on page 13 shows 23 lines). Various primers for regions on the six different chromosomes were used to amplify the *C. elegans* DNA using polymerase chain reaction (PCR) to screen those regions. I prepared the samples by lysing the worms with protease K lysis solution to obtain DNA in solution. Protease K is a serine protease that digests the cell's proteins to yield exposed DNA in solution (Ebeling et al., 1974). Once the lysis protocol was completed, I amplified the sample DNA with 30 cycles of PCR. I mixed Taq DNA Polymerase purchased from New England Biolabs, Thermopol Buffer, dNTP solution, and water. I added the appropriate primer (see Appendix B) for the segment I was amplifying as well as 5 μL of the worm DNA solution each of the samples.

To amplify the 50 μL PCR mixtures I created a program on the lab's PCR machine to start the reaction at 92 °C for two minutes. I then programmed a cycle of 92 °C for 30

seconds, annealing at 50-60 °C (depending on salt-adjusted T_m Calculations for Oligos, http://www.promega.com/biomath/calc11.htm), and 68 °C for one minute. This cycle was repeated 29 more times for 30 total cycles.

We referred to an online Single Nucleotide Polymorphism (SNP) catalogue (http://genome.wustl.edu/genome/celegans/celegans_snp.cgi) to distinguish the DNA as coming from N2 or Hawaiian parental DNA. From this catalogue I also ordered primers on either side of the SNP site that were unique to that SNP site (see Appendix B). We took advantage of these SNPs by using restriction enzymes (Figures 1 through 9) that cut one variant's sequence but not the other variant's sequence due to the single different base at the restriction site.

After cleaning the PCR product DNA, I mixed it with a restriction enzyme, determined by the information in this SNP catalogue as well as the appropriate buffer for the enzyme. I incubated the tubes of rectriction enzyme, buffer, and DNA at the appropriate temperature (usually 37 °C) for 1.5 – 2 hours. I ran the samples with a 1 kb ladder on an 8% agarose gel, usually at a voltage of around 40-60 mV, until I could observe decent separation of the DNA bands of interest.

The mutation would lie somewhere that contains only the DNA from the N2 line, so I was interested in regions with N2 restriction patterns. Finding regions of heterozygous or Hawaiian DNA on an agarose gel would rule out that region for the mutant gene of interest.

I started with linkage group 1 (LG I), which contains genes found throughout the entire DNA from chromosome 1. I sampled a region on each end of the chromosome using the SNP mapping technique described above to determine if there was Hawaii DNA present. I usually ruled out the chromosome if I found around half of the samples showing Hawaii homozygous or heterozygous DNA. I repeated this for all of the chromosomes (except LG V). Only a small segment on chromosome X displayed 100% N2 DNA, suggesting a genetic linkage to the *rg430* mutation allele.

CHAPTER III

RESULTS

Screening linkage groups

We used data from restriction sites on DNA regions with single nucleotide polymorphisms to screen the chromosomes of *C. elegans*. Chromosomes 1 through 5 had no SNPs with N2-rich regions that were common to all the recombinants. I did not screen LG V; Dr. Garcia informed me that he had extensively studied the genes on chromosome 5 already when studying GAR-3 in a past experiment, and that the mutation *rg430* cannot be linked to the fifth chromosome. For regions on the remaining chromosomes, most of the gels showed up with significant percentages of samples displaying heterozygous or homozygous Hawaiian DNA, thus ruling out the chromosome. As mentioned earlier, I disregarded as possible candidates the linkage groups where I had gels with ≈ 50% of the samples displaying N2 DNA. Figures 1 through Figure 6 shows some of the restriction bands from each linkage group:

Figure 1: LG I
ZK909 (Right End)
Digest with HindIII
N2 = 450 bp (base pairs)
HA = 250 bp

Ladder	
N2 control	
1-23	Het
1-29	N2
1-7	Het
1-20	Het
1-40	N2
Ladder	
5-1	N2
7-9	Het
7-12	Het
8-12	Both
10-3	N2
11-1	Both
6-3	HA
4-21	N2

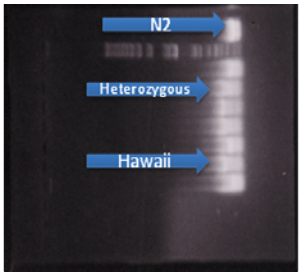


Figure 2: LG II
F45E12 (Right End)
Digest with ApoI
N2 = 250 & 130 bp
HA = 380 bp

Ladder	
N2 control	
1-23	N2
1-29	Het
1-20	HA
1-40	HA
3-42	N2
2-9	HA
3-24	N2

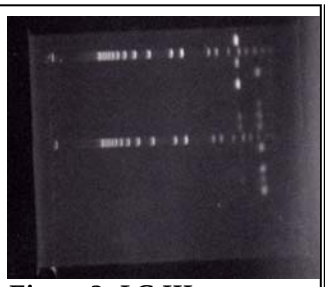


Figure 3: LG III
Y111B2A (Right End)
Digest with Mse1
N2 = 525 bp
HA = 270 bp

N2 control	
Ladder	
3-24	N2
3-31	HA
3-42	N2
3-11	none
3-13	HA
2-9	Het
2-20	Het
Ladder	
5-5	HA
5-1	N2
5-2	HA
5-9	Het
5-33	HA

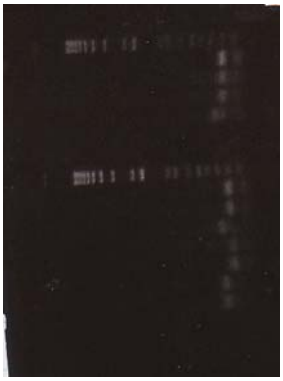
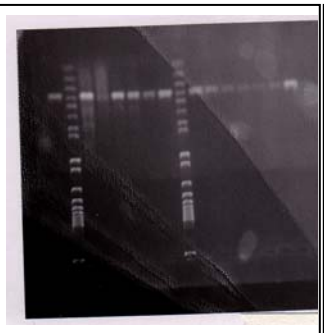


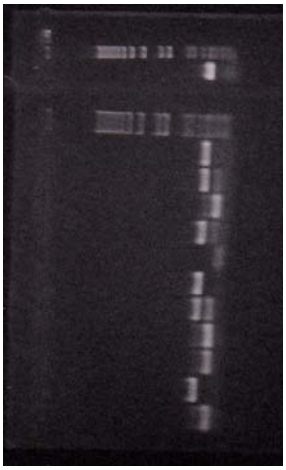
Figure 4: LG IV
Y105C5B (Right End)
Digest with DraI
N2 = 200 & 100 bp
HA = 300 bp

Ladder	
N2 control	
3-31	N2
3-42	N2
3-11	Het
3-13	HA
3-49	Het
Ladder	
7-9	N2
7-12	N2
8-12	HA
10-3	N2
11-1	N2
6-3	HA



N2 control
Ladder
3-24 N2
3-31 N2
3-42 N2
3-11 N2
3-13 N2
3-49 N2
Ladder
2-9 N2
2-20 N2
5-5 N2
5-1 N2
5-2 N2
5-9 N2
5-33 N2

Figure 5: LG X
(F49H12 Left End)
Digest with DraI
N2 = 470 bp
HA = 250 bp



Ladder
4-21 N2
Ladder
N2 control
1-12 N2
1-23 HA
1-29 N2
1-8 None
1-20 N2
1-40 Het
7-9 HA
10-3 HA
11-1 N2
6-3 HA

Figure 6: LG X
(F49H12)
Digest with DraI

However, upon initial sampling of the left end of chromosome X, at region F49H12, a significant majority of the recombinant DNA showed a restriction pattern as N2 homozygous. Upon completion of screening the region F49H12 located at the left end of region 3 of chromosome X, about two-thirds to three-quarters of the recombinant samples showed N2 homozygous DNA. I decided to focus on this region because the agarose gel of DNA restrictions had 100% N2 DNA.

I also screened the surrounding segments. To the right, on segment 3 of chromosome X, region T24D8 also had a high percentage of N2 homozygous DNA, with a few more chiasma (crossing over) regions where N2 homozygous DNA became either HA/N2 heterozygous or HA homozygous regions.

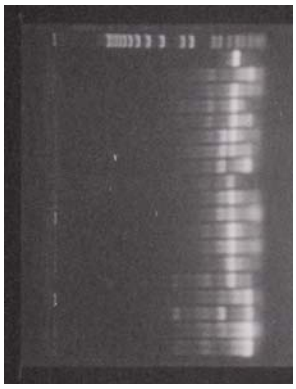


Figure 7: LG X (T24D8)
Digest with Nci1
N2 bands = 500 bp
HA = 250 bp
(Right of F49H12)

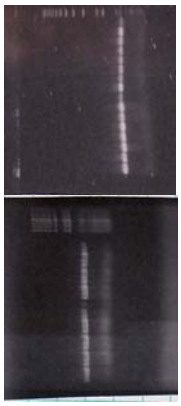


Figure 8: LG X (F09E10)
Left of F49H12
Digest with Bfa1
N2 bands = 800 bp
All bands are N2

To the left of region F49H12, region F09E10 on segment 2 of chromosome X is even more N2-rich. I have confirmed that 100% of my 23 samples in this region have N2 homozygous DNA. Furthermore, DNA bands indicative of Hawaiian DNA are present on gels analyzing segment W05H7, which is further left than F09E10 on the X chromosome; W05H7 is the left border and F49H12 is the right border for possible gene candidates. This means we can hypothesize which genes in this region contain the mutation for the nonfunctional protein.

Discussion

The results from my experiments suggest that the mutation is located between region W05H7 and F49H12, possibly close to the region F09E10, since it showed all N2 DNA. Table 1 includes the crossing over data between regions W05H7 and T24D8.

Table 1: Recombinant DNA Clones on LG X

Recombinant Number	Clone Segment								
	W05H7		F09E10 (S2R)		F49H12 (S3L)		T24D8 (S3R)		
1--23	HA | HA	x	N2 | N2	x	HA | HA		HA | HA		
1--29	N2 | HA		N2 | N2		N2 | N2		N2 | N2		
1--20	N2 | N2		N2 | N2		N2 | N2		N2 | N2		
1--40	N2 | HA	x	N2 | N2	x	N2 | HA		N2 | HA		
3--42	N2 | N2		N2 | N2		N2 | N2		N2 | N2		
2--9	N2 | HA	x	N2 | N2		N2 | N2	x	N2 | HA		
5--1	N2 | N2		N2 | N2		N2 | N2		N2 | N2		
5--2	N2 | N2		N2 | N2		N2 | N2		N2 | N2		
5--5	N2 | HA	x	N2 | N2		N2 | N2		N2 | N2		
5--9	N2 | HA	x	N2 | N2		N2 | N2		N2 | N2		
5--33	N2 | N2		N2 | N2		N2 | N2		N2 | N2		
11--1	N2 | N2		N2 | N2		N2 | N2		N2 | N2		
3--11	HA | HA	x	N2 | N2		N2 | N2		N2 | N2		
3--24	HA | HA	x	N2 | N2		N2 | N2	x	HA | HA		
1--8	HA | HA	x	N2 | N2	x	HA | HA		HA | HA		
7--9	HA | HA	x	N2 | N2	x	HA | HA		HA | HA		
7--12	HA | HA	x	N2 | N2	x	HA | HA		HA | HA		
8--12	N2 | N2		N2 | N2		N2 | N2		N2 | N2		
10--3	HA | HA	x	N2 | N2		HA | HA		HA | HA		
6--3	HA | HA	x	N2 | N2		HA | HA		HA | HA		
4--21	N2 | N2		N2 | N2		N2 | N2	x	N2 | HA		
3--31	N2 | N2		N2 | N2		N2 | N2		N2 | N2		
3--49	HA | HA	x	N2 | N2		HA | HA		HA | HA		

- X indicates chiasma (crossing over) location in between 2 SNP regions. Bold indicates double crossovers that result in a region of N2 DNA "flanked" by Hawaii DNA
- W05H7 = segment 2 center; F09E10 = segment 2 right; F49H12 = segment 3 left; T24D8 = segment 3 right
- W05H7, F09E10, F49H12, etc. are names of cloned sequences
    - The sequences that are located in regions that contain SNP
    - Genes in that region are named after the clone sequence

Genes mapped to the region between W05H7 and F49H12 are potential candidates that might be linked to *rg430*. Appendix A lists all the genes mapped in this region (W05H7 to F49H12) of the *C. elegans* genome. Since the region with the clone segment F09E10 displayed all N2 DNA, I can explore the genes in the immediate vicinity of that segment and continue screening genes outward in both directions, using the recombinants "flanked" with HA homozygous or heterozygous DNA on both sides of the regions.

CHAPTER IV

SUMMARY AND CONCLUSIONS

I have narrowed down a region where mutation *rg430* may map. This region exists between segments W05H7 and F49H12; many genes in this region have relevant molecular identities. Some of the notable genes are: **rgs-9** (ZC53.7) = regulator of G protein signaling; **rgs-8.2** (Y59E1A.2) = regulator of G protein signaling; **rgs-8.1** (F52D2.2) = regulator of G protein signaling; and **str-79** (F52D2.9) = Seven TM Receptor. Since GAR-3 (which maps to LG V) is a seven trans-membrane G-protein coupled receptor, then a parallel mutation could be something similar in nature on chromosome X.

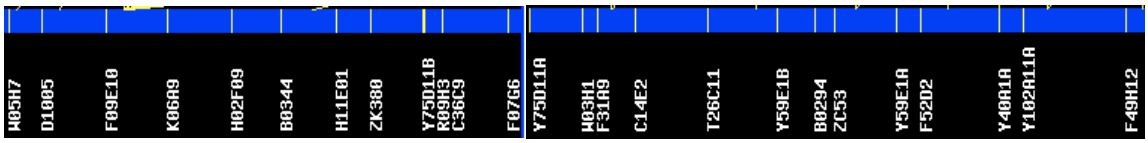


Figure 9: **Visual Map of Region with *rg430***

SNP restriction sites (areas where I could distinguish N2 and HA with PCR and restriction enzyme digestion) do not exist in this region, but SNP sites that can be distinguished by DNA sequencing exist in this interval. Therefore we must continue to sequence the genes in the samples that are N2, flanked on both sides by N2→HA chiasma points. In addition, we can also sequence candidate genes in the *rg430* mutation region and compare them with a database of mapped genes to determine if the

candidate gene contains a base pair change. Wormbase.org, a website that contains databases for *C. elegans* molecular biology, has a database of mapped genes. We may proceed to create PCR primers for the gene's sequence to be amplified in the worms. Then we can match the sequence of the gene with the recombinants' sequences to see if there is a mutated gene in any of the recombinants' genome.

This research has covered the process of identifying a phenotype linked to a neural circuit that is responsible for goal-oriented behavior, such as mating behavior. Screening chromosomes of *C. elegans* using SNP mapping has narrowed down a region on the X chromosome with several genes. We will be able to sequence within this region to determine which gene ultimately contributes to the mutation *rg430*.

REFERENCES

Alberts B, Bray D, Hopkin K, Johnson A, Lewis J, Raff M, Roberts K, Walter P (2003) Essential Cell Biology, 2nd Edition. New York: Garland Science.

Aperia A, Fredholm B, Ringertz N (1994) Signal Transduction in Cells. Stockholm: The Nobel Committee for Physiology or Medicine at the Karolinska Institute.

Ebeling W, Hennrich N, Klockow M, Metz H, Orth HD, Lang H (1974) Proteinase K from *Tritirachium album* Limber. European Journal of Biochemistry 47:91-97.

Garcia LR, Mehta P, Sternberg PW (2001) Regulation of Distinct Muscle Behaviors Controls the C. elegans Male's Copulatory Spicules during Mating. Cell 107:777-788.

Koch R, van Luenen HGAM, van der Horst M, Thijssen KL, Plasterk RHA (2000) Single Nucleotide Polymorphisms in Wild Isolates of Caenorhabditis elegans. Genome Research 10:1690-1696.

Liu Y, LeBoeuf B, Garcia LR (2007) G{alpha}q-Coupled Muscarinic Acetylcholine Receptors Enhance Nicotinic Acetylcholine Receptor Signaling in Caenorhabditis elegans Mating Behavior. J Neurosci 27:1411-1421.

Marieb EN, Hoehn K (2006) Human Anatomy And Physiology. 7th Edition. San Francisco, CA: Benjamin-Cummings Publishing Company.

Sargent PB (1993) The Diversity of Neuronal Nicotinic Acetylcholine Receptors. Annual Review of Neuroscience 16:403-443.

Steger KA, Avery L (2004) The GAR-3 Muscarinic Receptor Cooperates With Calcium Signals to Regulate Muscle Contraction in the Caenorhabditis elegans Pharynx. Genetics 167:633-643.

APPENDIX A

Table 2: **List of Genes Mapped to Region with *rg430***

W05H7 boundary	F07G6.5	mir-1018 (Y59E1B.3
D1005.3	fbxa-52 (F07G6.5)	B0294.3
D1005.2	F07G6.9	B0294.1
D1005.6	fbxa-53 (F07G6.7)	ZC53.1
D1005.5	F07G6.8	**rgs-9 (ZC53.7)**
D1005.1	F07G6.3	ZC53.6
D1005.4	F07G6.2	ZC53.2
dhs-25 (F09E10.3)	dgn-3 (F07G6.1)	ZC53.4
F09E10.5	F07G6.10	fbxa-40 (Y59E1A.1)
F09E10.6	Y75D11A.2	**rgs-8.2 (Y59E1A.2)**
F09E10.7	Y75D11A.3	fbxa-41 (F52D2.1)
F09E10.1	Y75D11A.6	**rgs-8.1 (F52D2.2)**
tts-1 (F09E10.11)	Y75D11A.1	fbxa-17 (F52D2.10)
F09E10.10	Y75D11A.5	F52D2.7
toca-1 (F09E10.8)	elc-2 (W03H1.2)	**str-79 (F52D2.9)**
K06A9.3	fbxc-37 (F31A9.4)	F52D2.5
K06A9.2	arg-1 (F31A9.3)	F52D2.6
K06A9.1	F31A9.2	fbxa-46 (F52D2.8)
H02F09.3	F31A9.1	gei-12 (F52H2.4)
H02F09.2	mir-796 (C14E2.8)	Y40A1A.2
wrt-9 (B0344.2)	C14E2.2	Y40A1A.3
H11E01.3	C14E2.7	Y40A1A.1
H11E01.2	C14E2.4	Y102A11A.9
fbxb-58 (H11E01.1)	C14E2.5	Y102A11A.2
ZK380.5	C14E2.1	Y102A11A.5
ZK380.4	C14E2.6	Y102A11A.6
tbx-32 (ZK380.1)	T26C11.2	Y102A11A.7
R09H3.1	T26C11.3	Y102A11A.8
R09H3.3	T26C11.4	Y102A11A.1
tbx-31 (C36C9.2)	ceh-41 (T26C11.5)	F49H12 boundary
fbxa-170 (C36C9.3)	ceh-21 (T26C11.6)	
C36C9.1	ceh-39 (T26C11.7)	
C36C9.6	T26C11.8	
C36C9.4	tbx-41 (T26C11.1)	
C36C9.5	Y59E1B.2	

APPENDIX B

Table 3: **Primers for SNP Mapping**

Segment	Primer	Anneals	Sequence
W05H7	FW05H7	**59** °C	TCTAAGTTGAAAAGTATGTATACTCTCGATGCACCGTACTC
	RW05H7	**59** °C	GCCGACGAGGGAATCAAGCAATTTTTCCAGGTA
F09E10	F09E10F	**56** °C	CGGGGTTTCGCATACTTTTAATGATTTAGAGTCAT
	F09E10R	57 °C calc* **56** °C used	CGGAAAGTGAGGTGGTGACAATAATCTCCT
F49H12	FF49H12	53 °C calc* **51** °C used	ATGTGAGTTTACCATCACTGGG
	F49H12R	**51** °C	TTTCGTAAAACTTACCGAGCAC

*Calculated Tm using http://www.promega.com/biomath/calc11.htm

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