A Genomic Analysis of the *C. elegans* AWC^{ON} Neuron and Its Relevance in the Olfactory Response to Isoamyl Alcohol and Butanone

Thesis by

Zhaoying Xian

In Partial Fulfillment of the Requirements for the degree of

Bachelor of Science

CALIFORNIA INSTITUTE OF TECHNOLOGY

Pasadena, California

2012

Acknowledgements

I wish to express my deepest appreciation to Professor Paul Sternberg for giving me the opportunity to conduct my thesis in his lab, as well as for his mentorship and encouragement. I also thank my postdoctoral mentor, Dr. Yen-Ping Hsueh, for introducing me to this project and allowing me to pursue this project independently whilst always giving me her support and advice. Finally, I would like to thank all the members of the Sternberg group who have welcomed me into the lab and helped me throughout this project.

Abstract

The AWC^{ON} neuron in *C. elegans* is one of a pair of bilaterally symmetrical AWC neurons that regulate the worm's olfactory response; chemosensory attraction to both IAA and butanone is attributed to this neuron. However, the genetic factors involved in AWC^{ON} mediation of *C.elegans'* chemosensory response are not very well understood. Transcriptomic analysis of the AWC^{ON} neuron gave a list of genes that are highly expressed in the AWC^{ON} neuron, from which three categories were chosen for further analysis: signaling proteins, nuclear hormone receptors, and ion channels. Nematodes carry mutations in genes of interest were tested for chemotaxis behavior in response to IAA and butanone. They were also tested for their ability to adapt to an IAA-rich environment.

Among 82 genes screened in this study, 31 genes were observed to have potential relevance to *C. elegans* chemosensation, with limited overlap between the genes. The relevant signaling proteins, *sek-1*, *rgs-9*, *ser-4*, *fshr-1*, *snf-1*, *akt-2*, *C05D10.2*, *F53C11.3*, *pkc-1* and *csb-1*, all have known functions, but only a limited number play a known role in olfaction, and even fewer have known effects in the AWC^{ON} neuron. Nuclear hormone receptors, of which *nhr-40*, *nhr-84*, *nhr-154*, *nhr-109*, *nhr-230*, *nhr-275*, *nhr-158, nhr-34, nhr-138, nhr-237, nhr-178, nhr-209,* and *nhr-243* showed potential relevance to olfaction, are a largely unstudied class of genes, and their functions in *C. elegans* are mostly not known. The genes in the ion channel category, *trpa-1*, *acr-12*, *lgc-12*, *glc-3*, *Y57G11C.44*, *cup-5*, and *mps-2*, comprise of both known and unstudied genes, with the majority of these genes having no known role in chemosensation. Thus, the discovery of the potential relevance of these 31 genes to *C. elegans* olfaction and chemosensation is a novel finding.

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List of Abbreviations

A. oligospora	*Arthrobotrys oligospora*
C. elegans	*Caenorhabditis elegans*
Ca^{2+}	calcium ion
CGC	Caenorhabditis Genetics Center
CI	chemotaxis index
ddH_2O	distilled water
ERK	extracellular-signal-regulated kinase
EtOH	ethanol
GTPase	guanosine-5'-triphosphate hydrolase
IAA	isoamyl alcohol
K^+	potassium ion
MAPK	mitogen-activated protein kinase
MAPKK	mitogen-activated protein kinase kinase
N2	wild-type *C. elegans* strain
NaN_3	sodium azide
nhr	nuclear hormone receptors
OP50	*Escherichia coli* strain OP50
SNF	sodium neurotransmitter symporter
VOC	volatile organic compound

Introduction

Background

Parasitic nematodes pose a problem to both agriculture and human health, causing significant numbers of parasitic infection and crop losses (Bundy DA et al., 2002, Nicol JM et al., 2011). Global effects of these nematodes include the destruction of pine forests in Asian countries (Futai K, 2013), devastation of the soybean crops worldwide (Miranda Vde J et al., 2013), and the infections of soil-transmitted nematodes in lakeside villages of China (Gao XH et al., 2013). The growth of these parasitic nematodes, however, is partially controlled by their natural predators (Felix MA and Braendle C, 2010). These predators include nematophagous fungi that capture nematodes through prey-induced trap formation (Saxena G et al., 1987, Xie H et al., 2010).

Whilst the superficial interactions between nematodes and their predators may be quite apparent, the molecular basis to their behavior is much less understood. Recent studies have shown that nematophagous fungi can detect and react to ascarosides, a highly conserved class of small molecules that nematodes produce (Hsueh YP et al., 2013), but nematode response to the presence of its predator fungi is not well-characterized. While it is believed that these fungi secrete nematode-attracting substances to lure their prey into the trap (Balan J et al., 1974, Yang J et al., 2011), it is unclear as to which genes are involved in the nematode olfactory response.

The mechanisms of action significant to this type of predator-prey relationship could serve as a molecular model through which scientists develop methods to combat or minimize the harmful effects of these parasitic nematodes. Presently, scientists have begun to use recombinant proteins to combat parasitic infection (Nisbet AJ et al., 2013), but such treatments are highly specific to certain nematode species. A more thorough understanding of genetic factors that are involved in the nematode response

to the trapping mechanisms of its predator fungi may aid the development of additional chemicals or drugs that can help combat parasitic nematode devastation or infection.

C. elegans as a Model Organism

The free-living nematode *C. elegans* serves as a model organism for the study of nematode behavior and attractive chemosensory response (cite Bargmann's worm book chapter). *C. elegans* could be preyed by several nematophagous fungi, including *A. oligospora* in the laboratory (Xie H et al., 2010), and has been shown to be attracted to A *A. oligospora* (Hsueh et al. unpublished). We thus to investigate the genetic factors involved in the response of *C. elegans* to *A. oligospora* attraction .

The *C. elegans* genome is fully sequenced (Hillier LW et al., 2005), so it is possible to perform a complete genomic analysis of any cells of interest. Furthermore, *C. elegans* is an ideal candidate for genetic screening and behavioral analysis due to its simple nervous system that consists of only 302 neurons (White JG et al., 1986). The worm uses its sensory neurons to assess environmental cues, including many VOCs (Bargmann CI, 2006). In particular, a pair of AWC olfactory neurons are known to be critical for *C. elegans* attraction to specific odors (O'Halloran DM et al., 2009). Through Genetic screening and laser ablation, it is shown that AWCs are involved in the worm's attraction to *A. oligospora* (Hsueh et al unpublished). The AWC neurons are bilaterally symmetrical, but their gene expression is interestingly asymmetrical (Troemel ER et al., 1999). The identities of these two neurons, known as AWC^{ON} and AWC^{OFF}, are randomly assumed by the left or right AWC neuron in the worm, and the two neurons serve different functions (Lesch BJ et al., 2009). This project analyzed the genetics of the AWC^{ON} neuron.

Determination of a Preliminary List of Genes and Chemosensory Assays

Prior to my arrival, the Sternberg lab profiled the AWC^{ON} neuron using single-cell RNA-Seq to obtain its transcriptome. More than 6000 genes were detected to express in AWCon. These genes

served a variety of intracellular and intercellular functions, but three specific categories of genes are chosen for further analyses based on their potential functions: signaling proteins, nuclear hormone receptors, and ion channels. We conducted chemotaxis assays to screen for genes that has a potential role for AWC-mediated chemosensation.

Gene-deletion mutants were tested for changes in attraction to VOCs in chemotaxis assays. *C. elegans* are known to be highly attracted to the chemicals isoamylalchohol (cite Cori's cell paper in the early 90s) and butanone (L'Etoile ND and Bargmann CI, 2000). The genes that are knocked out in mutant worms that depict a noticeable decrease in attraction to either chemical may be relevant to the chemosensory response of *C. elegans*.

As an extension of my main project, I also analyzed the ability for these mutant worms to adapt to the presence of VOCs. Adaptation is defined as a decreased chemosensory response following a prolonged exposure to a chemical (Bargmann CI, 2006), and it is a distinct process from chemical sensation (Colbert HA and Bargmann CI, 1995). A treatment that relies on an attractive chemosensory response may show a decrease in effectiveness if the nematodes are able to adapt to the presence of that treatment. To minimize such a side effect, it may also be useful to target genes that are involved in chemotaxis adaptation.

Materials and Methods

Worm Strains

C. elegans strains were grown on small 6 cm petri dishes plated with 1 cm of agar. A small section of the plate was coated with OP50 to feed the worms.

All strains were synchronized prior to chemotaxis assay. To prepare a worm strain for assay, approximately 20 eggs from the desired strain were placed on a new OP50 plate. The worms that hatch from these eggs were allowed to grow to adulthood and lay new eggs. These progeny were used for assays when they reached adulthood.

Mutant strains were obtained from CGC and Mitani lab in Tokyo, Japan.

Chemical Odorants

The VOCs selected for chemotaxis assay were IAA and butanone. IAA assays were performed at a 10^{-2} dilution in ddH_2O and butanone assays were performed at a 10^{-1} dilution in EtOH. Adaptation assays studied the ability for mutants to adapt to a prolonged exposure of concentrated IAA. All chemicals were obtained from SigmaAldrich unless otherwise noted.

IAA and Butanone Chemotaxis Assay

All assays were performed on 9 cm petri dishes containing chemotaxis agar. Prior to assay, worms were washed twice with M9 buffer and once with water. For each assay, approximately 100 worms were placed in the middle of the plate. The experimental and control chemicals were placed on opposite ends of the dish, each 0.5 cm away from the edge of the dish (see Figure 1) and at a total volume of 1 µL. The control chemical used was EtOH.

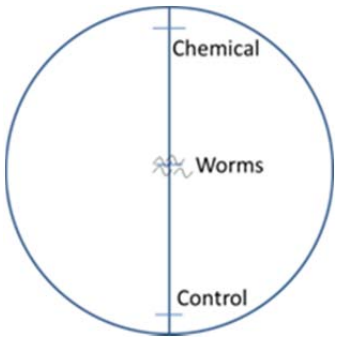

Figure 1. Layout of a single chemotaxis assay.

To paralyze the worms and facilitate counting, 1 µL of 1 mM NaN_3 is placed adjacent to both the experimental and control chemicals. Worms were allowed to move for 90 minutes. At that time, the numbers of worms within 2 cm of each chemical were counted. Worms that were not within that range were ignored. The CI, which was used to describe chemical attraction, is calculated using the following formula: $CI = \frac{\#\ worms\ at\ chemical - \#\ worms\ at\ control}{\#\ worms\ at\ chemical + \#\ worms\ at\ control}$.

A CI of 1 indicates complete attraction, of -1 indicates complete repulsion, and of 0 indicates neutrality. Each assay was initially performed in triplicate, and then repeated in triplicate at least seven days later.

Adaptation Chemotaxis Assay

Approximately 400 worms are placed on an unseeded 6 cm agar plate. The inner cap of the agar plate is coated with 10 µL of concentrated IAA. Worms were exposed to concentrated IAA for a period of 60 minutes. Worms are then washed once with M9 buffer. All remaining steps were identical to the chemotaxis assay following the initial M9 buffer wash.

Results

Threshold for Attraction

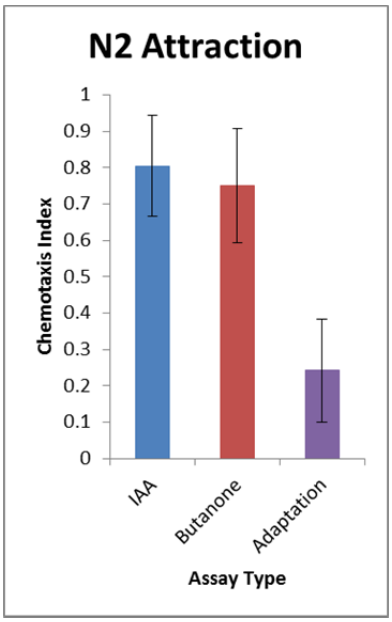

Figure 2. Wild-type *C. elegans* attraction to IAA and butanone, and IAA adaptation.

The CI for the IAA assay was 0.8, the CI for the butanone assay was 0.75, and the CI for the adaptation assay was 0.25. Given these results, the following CIs were selected as thresholds for these assays: 0.6 for the IAA assay, 0.5 for the butanone assay, and 0.5 for the adaptation assay. Any mutant worm that showed a CI of less than 0.6 in the IAA assay, less than 0.5 in the butanone assay, or greater than 0.5 for the adaptation assay was considered a positive hit.

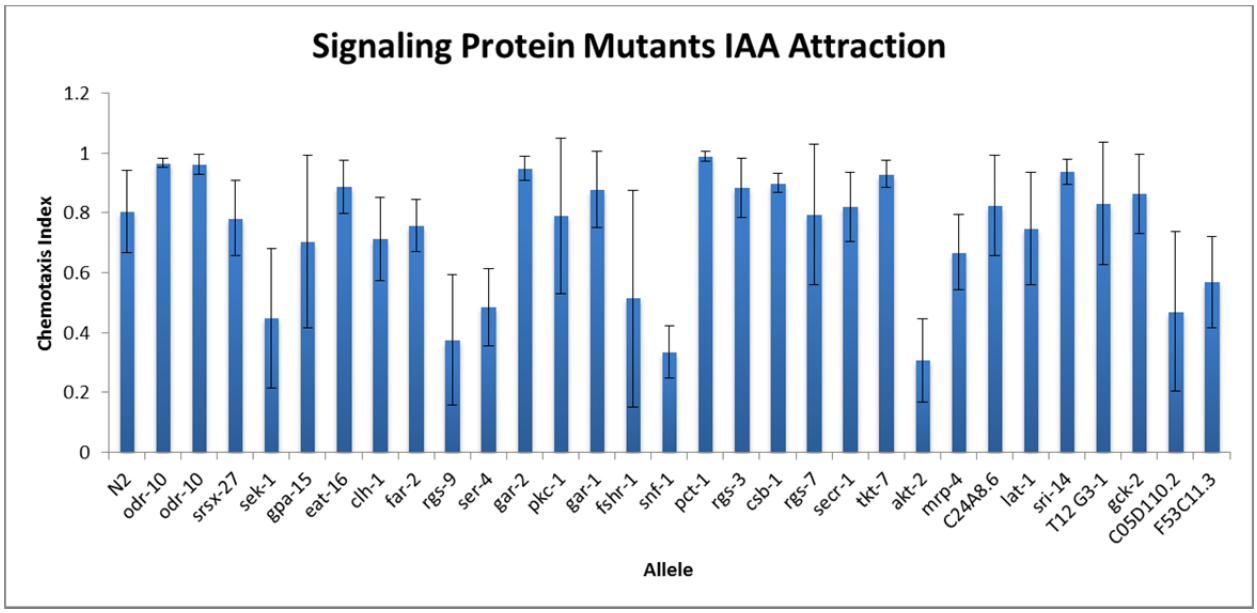

Figure 3. Attraction of signaling protein mutants to IAA.

Eight signaling proteins were observed to have CIs of less than 0.6 and thus potentially be relevant in *C. elegans* IAA attraction. These genes were *sek-1*, *rgs-9*, *ser-4*, *fshr-1*, *snf-1*, *akt-2*, *C05D10.2*, and *F53C11.3*.

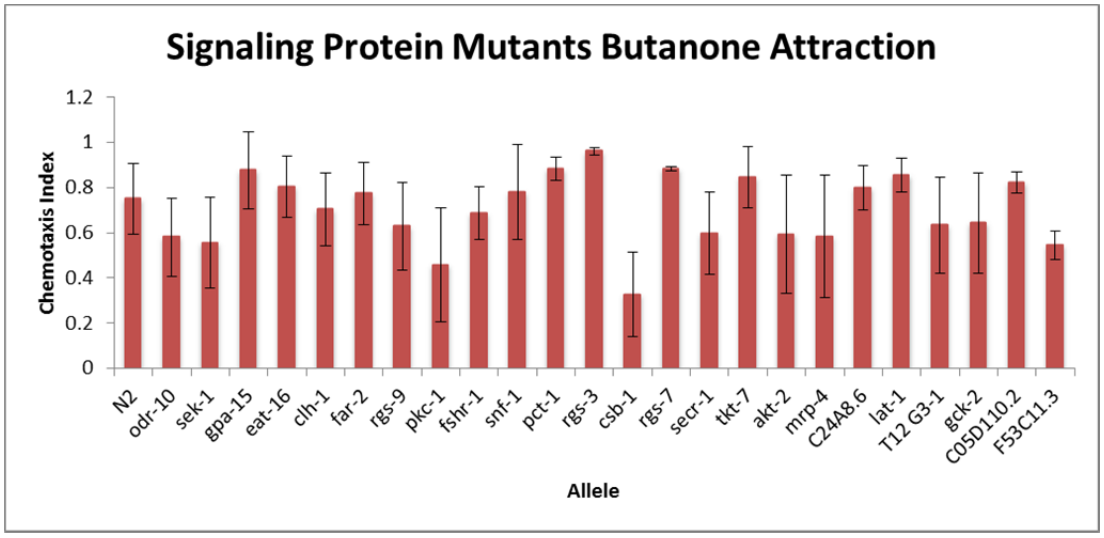

Figure 4. Attraction of signaling protein mutants to butanone.

Two signaling proteins that did not show any obvious relevance in IAA attraction appeared to be important in butanone attraction. The mutant worms for the two genes *pkc-1* and *csb-1* both showed CIs of less than 0.5.

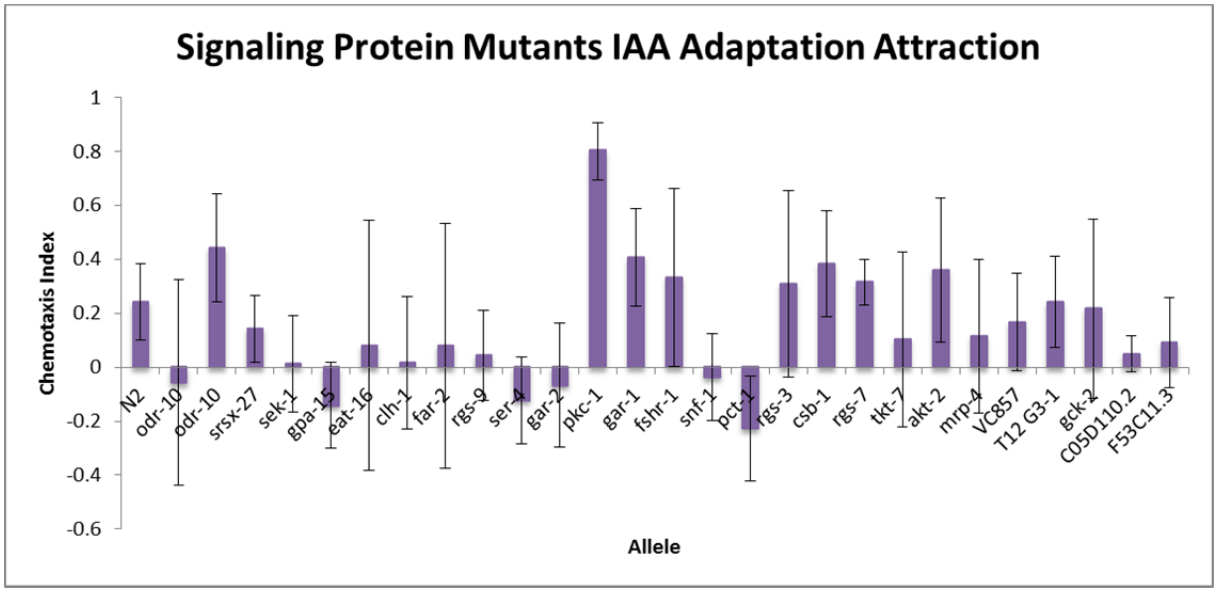

Figure 5. Attraction of signaling protein mutants following prolonged exposure to IAA.

One signaling protein appeared to be relevant in IAA adaptation. The mutant worm that had this gene knocked out showed a high CI (greater than 0.5) for IAA despite exposure to IAA. This gene, *pkc-1*, was also observed to be important in butanone attraction, but not in IAA attraction.

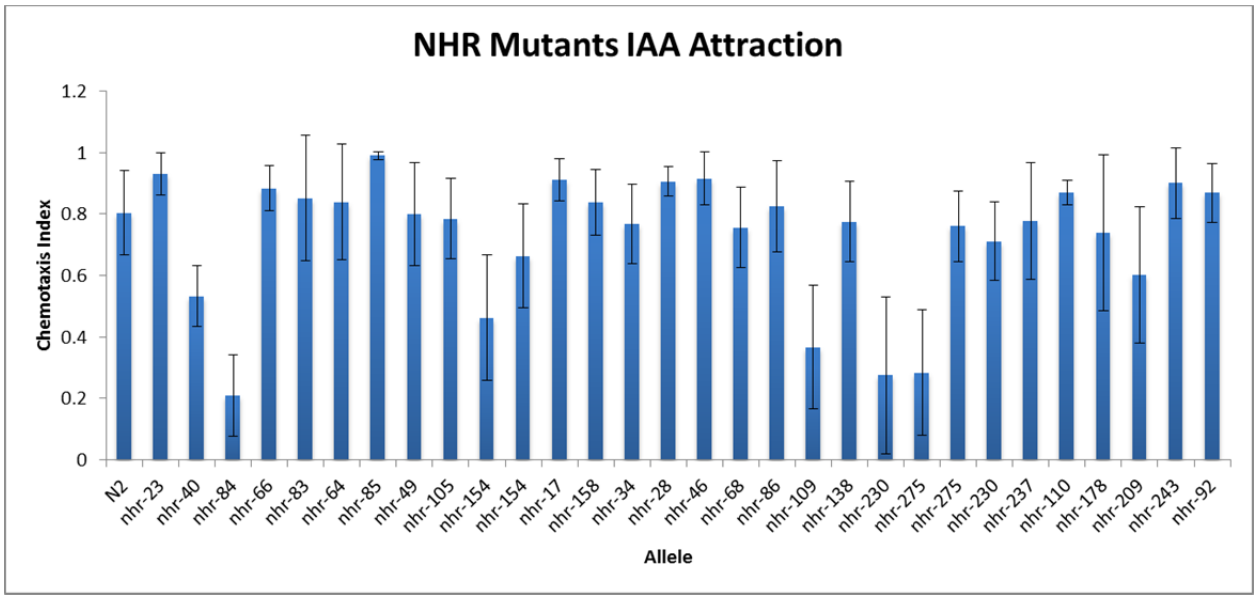

Figure 6. Attraction of NHR mutants to IAA.

Six NHR genes may play a role in *C. elegans* attraction to IAA: *nhr-40*, *nhr-84*, *nhr-154*, *nhr-109*, *nhr-230*, and *nhr-275*. The mutant worms for these NHR genes all showed CIs of less than 0.6. For *nhr-154* and *nhr-275*, only 1 of 2 mutant strains for those genes tested showed a distinctive phenotype. The *nhr-154* mutant that showed a phenotype is the result of a nucleotide insertion, whereas the mutant that showed no change in response was the result of a deletion. Both *nhr-275* mutants are the result of nucleotide deletions, but the mutant that showed a phenotype has a longer deletion by 541 nucleotides.

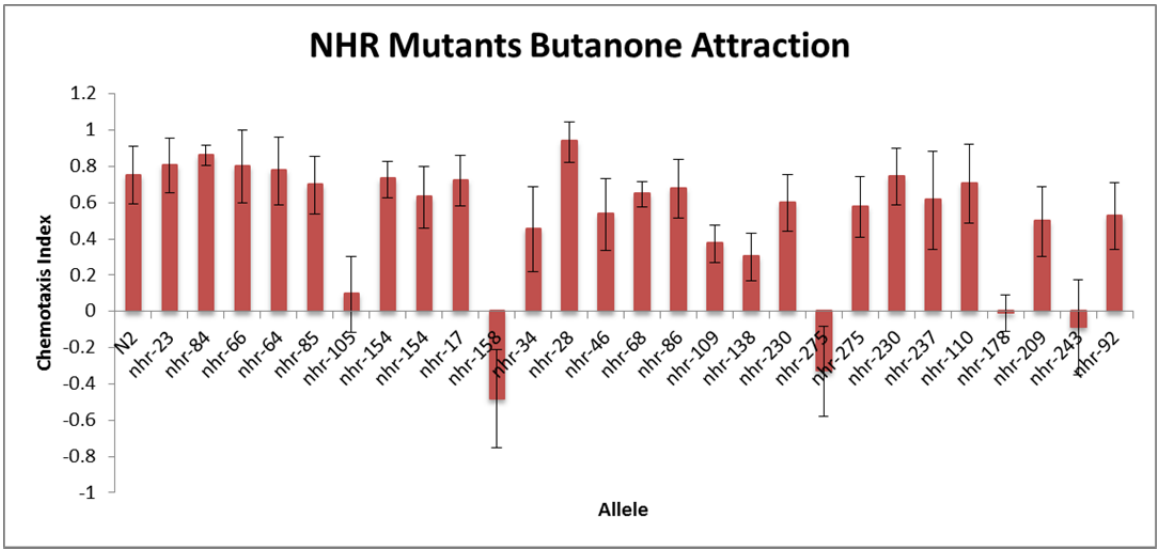

Figure 7. Attraction of NHR mutants to butanone.

Nine NHR mutant worms showed CIs of less than 0.5 for attraction to butanone. Two of these genes, *nhr-109* and *nhr-275*, were also positive hits in IAA attraction. Similarly, only 1 of the 2 *nhr-275* strains, the same one as with the IAA assay, showed a phenotype. This mutant has a nucleotide deletion that is 541 base pairs longer than the deletion in the mutant with no observed phenotype. The other seven genes are: *nhr-158*, *nhr-34*, *nhr-138, nhr-237, nhr-178, nhr-209,* and *nhr-243*.

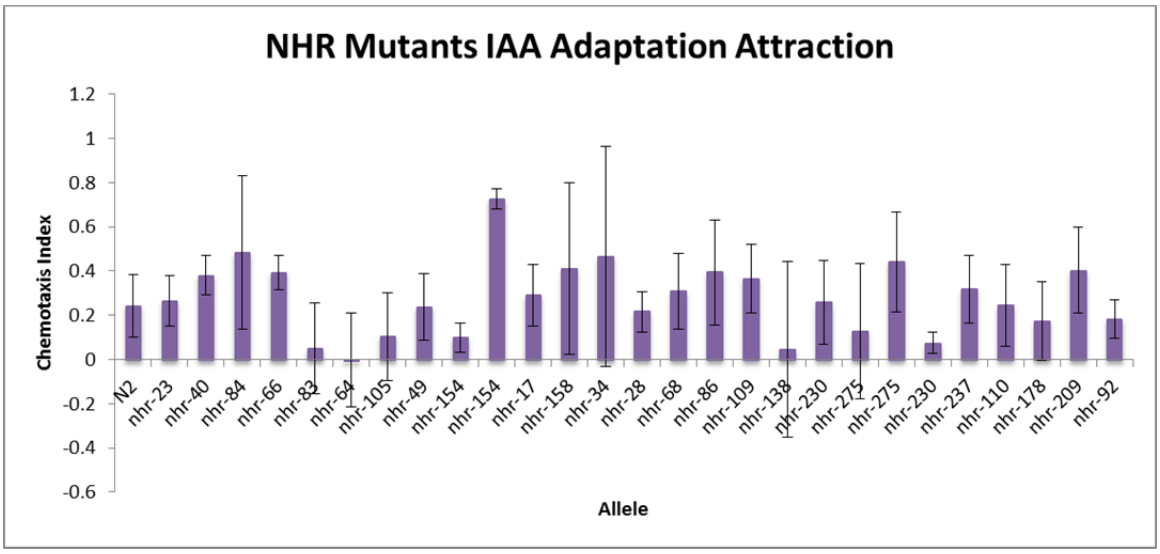

Figure 8. Attraction of NHR mutants following prolonged exposure to IAA.

One NHR gene appeared to be relevant in IAA adaptation: *nhr-154*. Although only 1 of the 2 mutant strains for *nhr-154* resulted in a CI greater than 0.5, as was observed with IAA attraction, the strain that showed a phenotype in this IAA adaptation assay is different from the strain that showed a phenotype in IAA attraction. The *nhr-154* mutant that showed a phenotype in IAA adaptation had a nucleotide deletion in its gene; the *nhr-154* mutant that showed no phenotype had insertions.

Ion Channels Relevant in C. elegans Attraction and Adaptation

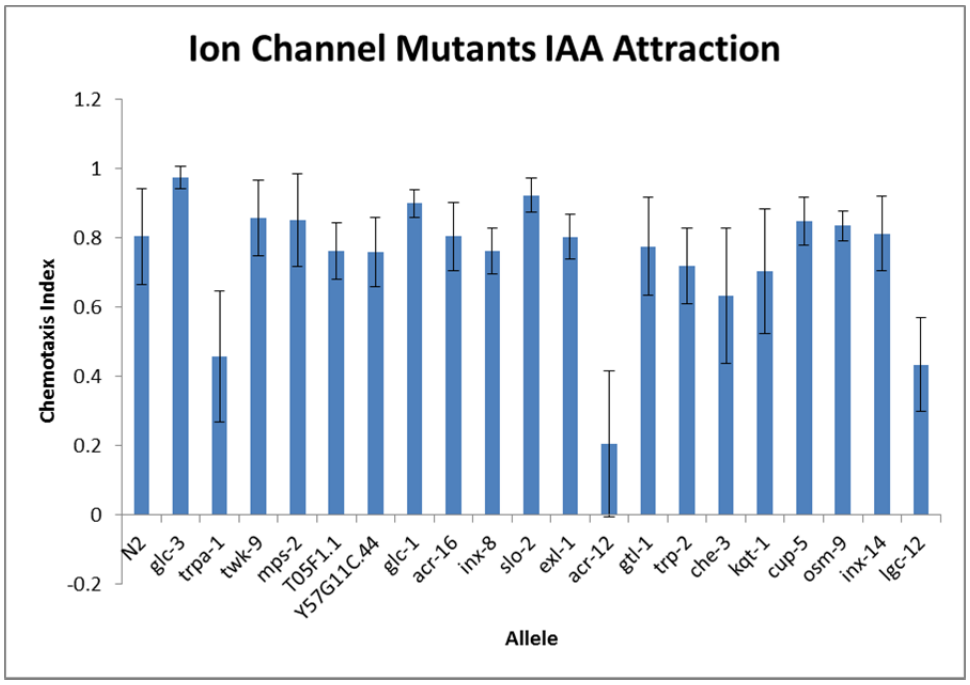

Figure 9. Attraction of ion channel mutants to IAA.

Three of the ion channel genes, *trpa-1*, *acr-12*, and *lgc-12*, appeared to play a role in IAA attraction.

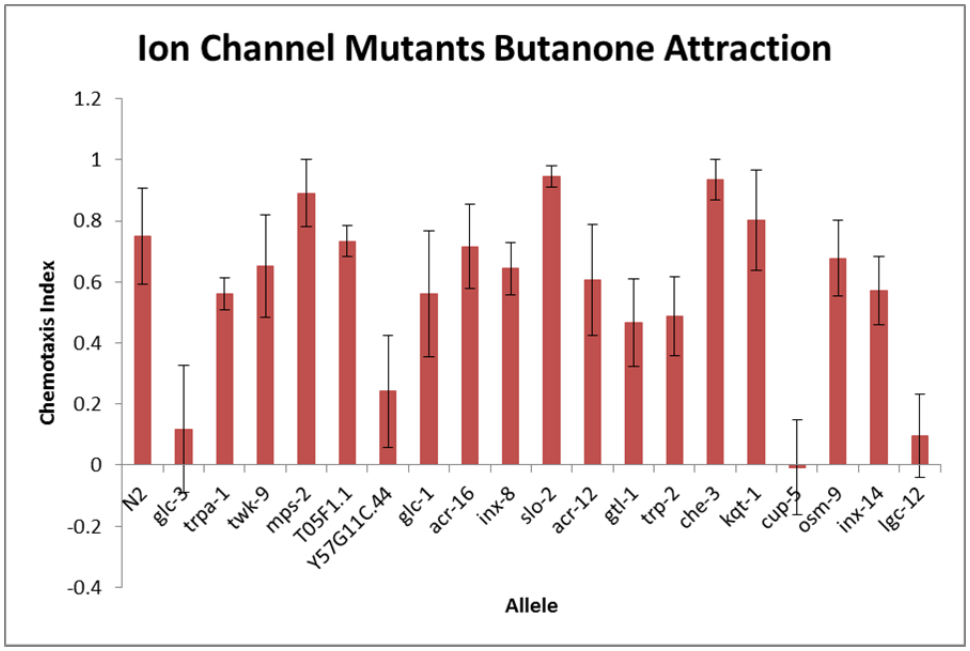

Figure 10. Attraction of ion channel mutants to butanone.

Four of the ion channel genes appeared to be relevant in *C. elegans* butanone attraction. One gene, *lgc-12*, was also observed to be important in IAA attraction. The other three genes are *glc-3*, *Y57G11C.44*, and *cup-5*.

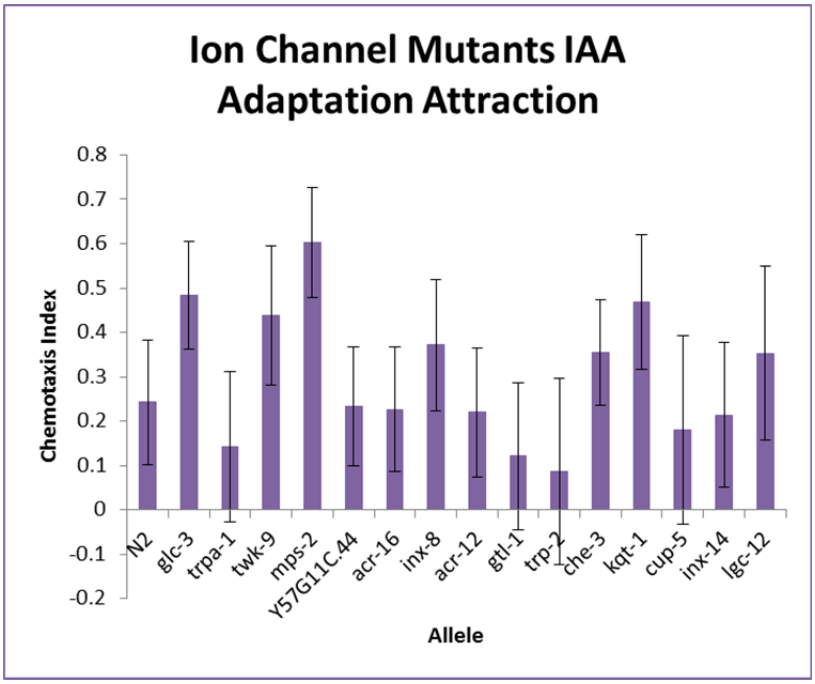

Figure 11. Attraction of ion channel mutants following prolonged exposure to IAA.

One ion channel gene showed potential significance in *C. elegans* IAA adaptation: *mps-2*. This gene showed no other obvious relevance in worm attraction.

Discussion and Analysis

The variance in the genes that appear relevant to *C. elegans* attraction to IAA or butanone and to its capacity for adaptation to IAA suggests that its response to each process is governed by a unique set of genes. While there is some repetition in the genes that are implicated for the three chemosensory behaviors studied, the overlap is minimal.

Genes Known to Regulate C. elegans Olfaction

Known Chemosensory Signaling Proteins

All of the signaling proteins that showed potential relevance in *C. elegans* olfaction have known signaling functions, but only three have been previously linked to worm chemosensation.

The following two genes, *sek-1* and *ser-4* were known to be important in the worm's attraction to IAA.

Sek-1 is a member of the MAPKK family and plays a role in *C. elegans* innate immunity against pathogens via a p38 MAPK pathway (Kim DH et al., 2002). It is also important for asymmetric expression in the AWC neurons through a Ca^{2+}-activated pathway (Tanaka-Hino M et al., 2002). Although *sek-1* is not known to affect the *C. elegans* chemosensory response to specific odorants, its role in the differential expression in the AWC neurons suggests that its signaling pathway is critical for the normal function of these neurons. Whether its role in the worm's attraction for IAA is through its MAPK or Ca^{2+} pathway, if either, is yet to be determined, but it nonetheless appears to be relevant to that olfactory response.

Ser-4 is a G protein-coupled serotonin receptor that mediates worm movement (Carre-Pierrat M et al., 2006). It is expressed in a set of sensory and interneurons (Chase DL et al., 2004, Gürel G et al., 2012), but it is not stated to have been observed in AWC^{ON} neuron. Regardless, its function in the

chemosensory response to serotonin suggests that *ser-4* has the potential to also be a signaling protein in the *C. elegans* olfaction of other chemicals, such as IAA.

The gene *pkc-1*, on the other hand, shows potential relevance to *C. elegans* butanone attraction and IAA adaptation.

Pkc-1 encodes a serine/threonine protein kinase that affects thermotaxis and chemotaxis. It is known to be involved in the worm's ability to sense VOCs (Land M and Rubin CS, 2003), and is also required for phorbolester-induced stimulation of acetylcholine release at neuromuscular junctions (Sieburth D et al., 2001). More recently, *pkc-1* is observed to regulate the secretion of neuropeptides (Sieburth D et al., 2007) and acts with the ERK MAPK signaling pathway to regulate the worm's mechanosensory response (Hyde R et al., 2011). *Pkc-1* is localized to 75 specific sensory neurons and interneurons (Tabuse Y, 2002; Sieburth et al., 2003), although not specifically to the AWC^{ON} neuron. This gene appears not only to be critical to the worm's ability to detect certain VOCs, such as butanone, but that it is also involved in the worm's mechanical response to such chemicals. Its role in *C. elegans* adaptive ability, however, appears to be a novel function.

Known Chemosensory Nuclear Hormone Receptors

The proteins of the NHR family have conserved molecular structures: an N-terminus with a DNA binding domain that consists of two zinc fingers, and a C-terminal ligand-binding domain (Antebi A, 2006). These proteins are a diverse group of transcription factors that are named because their hormonal ligands were, at the time of receptor identification, unknown (Olefsky JM, 2001). Most of the *C. elegans* NHRs, of which there are 284, remain uncharacterized (Arda HE et al., 2010), including most of the NHRs that appear relevant to this study. The NHRs that have been studied have not been analyzed in relation to *C. elegans* olfaction and will thus be discussed in the section titled "Novel Chemosensory Nuclear Hormone Receptors."

Known Chemosensory Ion Channels

Ion channel proteins studied for this project are largely known to regulate neurotransmission and the excitation of neurons in *C. elegans*. However, only the gene *mps-2* has been previously connected to chemosensation.

In particular, the *mps-2* gene appears to be relevant to IAA adaptation.

Mps-2 is a transmembrane protein that regulates K^+-channel in *C. elegans*; it enhances the nematode attraction for sodium and has been detected to function in the chemosensory activity of ADF neuron (Park KH et al., 2005). While previously undetected in the AWC neurons, it may serve a similar function in the AWC^{ON} neuron as it does on the ADF neuron. Given that it already serves a chemosensory function in the worm for one neuron, it may very well do so for other neurons as well.

Genes Previously Unlinked to C. elegans Olfaction

Novel Chemosensory Signaling Proteins

The following seven genes have not previously been observed to play a role in *C. elegans* olfaction, but their current known functions may provide insights on their potential role in that chemosensation.

The genes *rgs-9*, *fshr-1*, *snf-1*, *akt-1*, *C05D110.22*, and *F53C11.3* were observed to be important in the worm's attractive response to IAA.

Rgs-9 is a GTPase-activating regulator of G-protein signaling (Slep KC et al., 2001). While the RGS family are known to be ubiquitous regulators of neuronal G-protein signaling (Anderson GR et al., 2009), its expression in *C. elegans* neurons has previously been unobserved. However, knocking out *rgs-9* in mice has been shown to disrupt neuronal function (Papachatzaki MM et al., 2011, Mancuso JJ et al., 2010). On the other hand, *rgs-9* expression is upregulated during neuronal differentiation of mouse

embryonic stem cells (Sharma M et al., 2011), which suggests that *rgs-9* plays a role in neuron development. Given the relevance of *rgs-9* in mouse neurons, knocking down *rgs-9* in *C. elegans* may impair function or development in one or both of the AWC neurons.

Fshr-1 encodes a neuropeptide receptor that is necessary for acetylcholine secretion by synapses (Sieburth D et al., 2005), but it has not been previously localized to either of the AWC neurons. However, it regulates germline differentiation and survival in *C. elegans* (Cho S et al., 2007), which suggests that dysfunction of *fshr-1* may affect development and function of the AWC neurons, which could have led to the decrease the worm's attractive response to IAA if the AWC^{ON} neuron is implicated. This G protein-coupled receptor is also involved in *C. elegans* innate immunity through a pathway that is parallel to the p38 MAPK pathway (Powell JR et al., 2009), the latter of which involves the previously mentioned target gene *sek-1*. While this peripheral relation in immune response does not indicate any definitive relation in chemosensory behavior, it is possible that these two genes act in conjunction in *C. elegans* response to IAA.

Snf-1 is a sodium neurotransmitter symporter family gene that may be involved in embryonic development of mammals (Kertesz N et al., 2002) and sugar metabolism of plants (Chikano H et al., 2001), but its functions and localization in *C. elegans* are largely unknown. Its relevance in *C. elegans* behavior, particularly in olfaction, is thus a novel finding.

Akt-2 encodes a homolog of the serine/threonine Akt/PKB, which is necessary for the worm to enter the dauer stage of development and for regulation of the worm's life span, and it is observed to be expressed in *C. elegans* neurons (Paradis S et al., 1998). It is also involved in *C. elegans* germline apoptosis by two signaling pathways, one of which involves MAPK (Perrin AJ et al., 2013), a pathway that is related to other target genes of this project. However, the localization of *akt-2* to the AWC^{ON}

neuron or its function in chemosensation is presently unknown, so it may have a novel function in *C. elegans*.

C05D10.2 is a putative serine/threonine-protein kinase that may be involved in the MAPK cascade and plays a role in worm growth and development (Rual JF et al., 2004), but its tissue localization is unknown. *F53C11.3* is an uncharacterized oxidoreductase in *C. elegans* with unknown tissue specificity (C. elegans Sequencing Consortium, 1998). These two largely unstudied genes nevertheless appear to have strong relevance in *C. elegans* olfaction.

The gene *csb-1* appears to play a role in the worm's butanone response.

Csb-1 is a transcription-coupled repair protein that contains an SNF family DNA-dependent ATPase domain (Lans H et al., 2010) and has been observed to be involved in the UV response of somatic cells (Lee MH et al., 2002), but has not previously been detected in neurons.

Novel Chemosensory Nuclear Hormone Receptors

Given that the majority of the NHRs in *C. elegans* have not been well-studied, especially in relation to *C. elegans* chemosensation, the discovery of their localization and olfactory relevance of these fourteen genes in the AWC^{ON} neuron is quite novel.

The genes *nhr-40*, *nhr-84*, and *nhr-230* appear to be important to *C. elegans* IAA attraction only. Of these 3 genes, only *nhr-40* has previously been studied, but not in relation to chemosensation.

Nhr-40 is a supplementary nuclear receptor that is crucial to *C. elegans* embryonic and larval development, with a metabolic mutant phenotype most prominently observed in *nhr-40* null worms when they are grown at low temperatures or under food restriction (Pohludka M et al., 2008). It is expressed throughout the body, including in a subset of neurons; deletion of the gene can result in

impaired coordination and movement (Brozová E et al., 2006). However, the aforementioned defects were not observed in this project.

The genes *nhr-105*, *nhr-158*, *nhr-34*, *nhr-138*, *nhr-178*, *nhr-209*, and *nhr-243* appear to be relevant to *C. elegans* butanone attraction only. Of these seven genes, only *nhr-209* has previously been studied.

Nhr-209 is expressed throughout the body, including in some unidentified head neurons (Vohanka J et al., 2010), although not specifically in the AWC^{ON} neuron. However, it does appear to serve a wide number of functions given its extensive expression pattern.

The genes *nhr-109* and *nhr-275* is observed to play a role in both IAA and butanone attraction, although their functions are presently unstudied.

Interestingly, while two *nhr-275* strains were obtained and tested for this project, only one mutant showed a decrease in *C. elegans* attraction to IAA or butanone. Both mutants were the result of a deletion in the DNA sequence, but the deletion for the mutant showing the phenotype, dubbed *nhr-275*$^{IAA\text{-}B}$, is longer by 541 nucleotides. The exclusion of these additional nucleotides likely allowed for a more effective downregulation of the protein function, perhaps due to the result changes in protein conformation, or due to the deletion of an important active site.

The gene *nhr-154* is observed to play a role in IAA attraction and IAA adaptation.

While the function of *nhr-154* is not characterized, it is known to be expressed in *C. elegans* head neurons, although no specific neurons are reported (Vohanka J et al., 2010). Interestingly, although I tested two mutant *nhr-154* strains, only one strain showed a noticeable phenotype for IAA attraction; it is henceforth known as *nhr-154*^{IAA}. While the other strain did show a modest decrease in IAA

attraction, the decrease was much less and not beyond the threshold. However, this other strain showed a phenotype only in IAA adaptation, so it will henceforth be referred to as *nhr-154*^{ADA}. The differences between the two strains lie in the molecular change to the DNA. *Nhr-154*^{IAA} is the result of an insertion, whereas *nhr-154*^{ADA} is the result of a deletion. No other changes were reportedly made to these strains. Thus, the type of molecular change (insertion vs. deletion) may be relevant to the observed phenotype changes. It is possible that the different molecular changes led to different means of downregulating gene expression, which may have had other downstream effects.

Although all of the aforementioned NHRs are not widely studied, their role as transcription factors suggests that they may mediate chemosensation through ligand-activated transcription of other genes that result in a chemosensory response. Given the variance in the genes involved in IAA vs. butanone attraction, it would appear that different ligands and NHRs, with minimal overlap, are responsible for the worm's olfactory response to specific chemicals.

Novel Chemosensory Ion Channels

The genes *trpa-1* and *acr-12* were observed to be relevant only in IAA attraction.

Trpa-1 is the transient receptor potential ion channel that is necessary for mechanosensory behaviors such as touch-mediated foraging and nose-touch avoidance (Xiao R and Xu XZ, 2009). It is also responsible for thermosensation, specifically as a cold sensor (Chatzigeorgiou M et al., 2010) and is known to be expressed in *C. elegans* sensory neurons (Kindt KS et al., 2007). However, a role in mechanosensation does not directly suggest a role in chemosensation, nor are the two functions necessarily related, so the relevance of *trpa-1* in *C. elegans* IAA attraction is indeed novel.

Acr-12 is a nicotinic acetylcholine receptor alpha subunit that mediates fast excitatory neurotransmission, although previous research on the deletion of *acr-12* did not result in observable

defects (Mongan NP et al., 1998; Gottschalk A et al., 2005). It is expressed in *C. elegans* cholingeric motor neurons and regulates the excitation and inhibition of motor neuron activity in *C. elegans* (Jospin M et al., 2009). Specifically, loss-of-function of *acr-12* leads to increased excitation of the cholinergic motor neurons (Petrash HA et al., 2013). This excess of neuronal activity in motor neurons of *acr-12* mutants may be related to the decrease in IAA attraction. Perhaps the extra motor neuron activity caused the worms to move excessively in what appears to be a random pattern and thus not properly sense or respond to the presence of IAA.

The genes *glc-3*, *Y57G11C.44*, and *cup-5* appear to play a role in the worm's attraction to butanone.

Glc-3 is an L-glutamate-gated chloride channel subunit that is expressed in the AIY interneuron (Horoszok L et al., 2001). It is involved in the inhibition of the postsynaptic AIY interneurons, and AWC neuronal signals are known to affect AIY activity (Ohnishi N et al., 2011). Thus, it is possible that *glc-3* plays a role in both AWC and AIY signaling, although whether or not its expression in the neurons is related is unclear.

Y57G11C.44 is an amiloride-sensitive sodium channel (*C. elegans* Sequencing Consortium, 1998), but little else is known regarding this gene.

Cup-5 is polycystin cation channel in *C. elegans* that is localized to lysosomes in many cell types and may contribute to lysosomal defects (Fares H and Greenwald I, 2001) and increased cell apoptosis (Hersh BM et al., 2002) in the cell. However, it has not been specifically localized to the AWC^{ON} neuron, and its function in neurons is also unstudied. Its function in regulating lysosome function suggests that its dysfunction may lead to a disruption of lysosome activity in the AWC^{ON} neuron to interfere with its response to butanone.

Deletion of the gene *lgc-12* led to mutant phenotypes in both IAA and butanone attraction.

Lgc-12 is an acetylcholine receptor expressed in *C. elegans*, but has not been reported to be observed in neurons (*C. elegans* Sequencing Consortium, 1998). This gene is otherwise unstudied.

While these genes are largely unstudied in relation to olfaction, this does not preclude an important role in worm chemosensation. It is possible that a defect in neurotransmission affects the ability for the AWC^{ON} neuron to communicate with the rest of the worm, thus impeding the appropriate response to the presence of a chemical.

Conclusion

The genomic analysis of the AWC^{ON} neuron in *C. elegans* has led to the discovery of many genes that were previously unlinked to the worm's olfactory response to IAA or butanone. Although some of these genes were known to mediate the worm's chemosensory response, it was not to the two aforementioned chemicals specifically. Thus, the newly revealed functions of these genes will provide novel insights on their relevance to *C. elegans* chemosensation. This will perhaps aid in the understanding of chemosensory behavior in other nematodes, especially parasitic nematodes that cause devastation in agriculture or human health, which could in turn lead to the discovery of methods to combat the effects of those nematodes.

Bibliography

Anderson GR, Posokhova E, Martemyanov KA. The R7 RGS protein family: multi-subunit regulators of neuronal G protein signaling. *Cell Biochem Biophys*. 2009;54(1-3):33-46. doi: 10.1007/s12013-009-9052-9. Epub 2009 Jun 12.

Antebi, A. Nuclear hormone receptors in C. elegans. *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.64.1, http://www.wormbook.org. 2006.

Arda HE, Taubert S, MacNeil LT, Conine CC, Tsuda B, Van Gilst M, Sequerra R, Doucette-Stamm L, Yamamoto KR, Walhout AJ. Functional modularity of nuclear hormone receptors in a Caenorhabditis elegans metabolic gene regulatory network. *Mol Syst Biol*. 2010 May 11;6:367. doi: 10.1038/msb.2010.23.

Balan J, Krizková L, Nemec P, Vollek V. Production of nematode-attracting and nematicidal substances by predacious fungi. *Folia Microbiol* (*Praha*). 1974;19(6):512-9.

Bargmann CI. Chemosensation in C. elegans. *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.123.1, http://www.wormbook.org. 2006.

Bargmann CI, Hartwieg E, and Horvitz HR. Odorant-selective genes and neurons mediate olfaction in C. elegans. *Cell*. 1993; 74:515-527.

Beale E, Li G, Tan MW, Rumbaugh KP. Caenorhabditis elegans senses bacterial autoinducers. *Appl Environ Microbiol*. 2006 Jul;72(7):5135-7.

Brozová E, Simecková K, Kostrouch Z, Rall JE, Kostrouchová M. NHR-40, a Caenorhabditis elegans supplementary nuclear receptor, regulates embryonic and early larval development. *Mech Dev*. 2006 Sep; 123(9):689-701. Epub 2006 Jun 28.

Bundy DA, Guyatt HL, and Michael E. Epidemiology and control of nematode infection and disease in humans. *The Biology of Nematode*, D.L. Lee, ed. 2002. CRC Press.

C. elegans Sequencing Consortium. Genome sequence of the nematode C. elegans: a platform for investigating biology. *Science*. 1998 Dec 11; 282(5396):2012-8.

Carre-Pierrat M, Baillie D, Johnsen R, Hyde R, Hart A, Granger L, Ségalat L. Characterization of the Caenorhabditis elegans G protein-coupled serotonin receptors. *Invert Neurosci*. 2006 Dec; 6(4):189-205. Epub 2006 Nov 3.

Chase, D. L., Pepper, J. S., & Koelle, M. R. (2004). Mechanism of extrasynaptic dopamine signaling in Caenorhabditis elegans. *Nat Neurosci*. 2004; 7:1096-103.

Chatzigeorgiou M, Yoo S, Watson JD, Lee WH, Spencer WC, Kindt KS, Hwang SW, Miller DM 3rd, Treinin M, Driscoll M, Schafer WR. Specific roles for DEG/ENaC and TRP channels in touch and thermosensation in C. elegans nociceptors. *Nat Neurosci*. 2010 Jul;13(7):861-8. doi: 10.1038/nn.2581. Epub 2010 May 30.

Chikano H, Ogawa M, Ikeda Y, Koizumi N, Kusano T, Sano H. Two novel genes encoding SNF-1 related protein kinases from Arabidopsis thaliana: differential accumulation of AtSR1 and AtSR2 transcripts in response to cytokinins and sugars, and phosphorylation of sucrose synthase by AtSR2. *Mol Gen Genet*. 2001 Jan;264(5):674-81.

Cho S, Rogers KW, Fay DS. The C. elegans glycopeptide hormone receptor ortholog, FSHR-1, regulates germline differentiation and survival. *Curr Biol*. 2007 Feb 6;17(3):203-12.

Colbert HA, Bargmann CI. Odorant-specific adaptation pathways generate olfactory plasticity in C. elegans. *Neuron*. 1995 Apr;14(4):803-12.

Dayaal R, Mukerji K.G., Saxeena G. Interaction of nematodes with nematophagus fungi: induction of trap formation, attraction and detection of attractants. *FEMS Microbiology Letters*. 1987 Dec;45(6):319–327.

Fares H, Greenwald I. Regulation of endocytosis by CUP-5, the Caenorhabditis elegans mucolipin-1 homolog. *Nat Genet*. 2001 May;28(1):64-8.

Fares H, Greenwald I. Genetic analysis of endocytosis in Caenorhabditis elegans: coelomocyte uptake defective mutants. *Genetics*. 2001 Sep; 159(1):133-45.

Félix MA, Braendle C. The natural history of Caenorhabditis elegans. *Curr Biol*. 2010 Nov 23;20(22):R965-9.

Feng Z, Li W, Ward A, Piggott BJ, Larkspur ER, Sternberg PW, Xu XZ. A C. elegans model of nicotine-dependent behavior: regulation by TRP-family channels. *Cell*. 2006 Nov 3; 127(3):621-33.

Futai K. Pine Wood Nematode, Bursaphelenchus xylophilus. *Annu Rev Phytopathol*. 2013 May 6.

Gao XH, Zeng XJ, Hong XL, Jiang WS, Ge J, Hu SZ, Lan WM, Fan YL. [Investigation of infections of soil-transmitted nematodes in Fusheng Village of Poyang Lake area in Jiangxi Province]. *Zhongguo Xue Xi Chong Bing Fang Zhi Za Zhi*. 2013 Feb;25(1):73-5.

Gottschalk A, Almedom RB, Schedletzky T, Anderson SD, Yates JR 3rd, Schafer WR. Identification and characterization of novel nicotinic receptor-associated proteins in Caenorhabditis elegans. *EMBO J*. 2005 Jul 20; 24(14):2566-78. Epub 2005 Jun 30.

Gürel G, Gustafson MA, Pepper JS, Horvitz HR, Koelle MR. Receptors and other signaling proteins required for serotonin control of locomotion in Caenorhabditis elegans. *Genetics*. 2012 Dec;192(4):1359-71. doi: 10.1534/genetics.112.142125. Epub 2012 Sep 28.

Hersh BM, Hartwieg E, Horvitz HR. The Caenorhabditis elegans mucolipin-like gene cup-5 is essential for viability and regulates lysosomes in multiple cell types. *Proc Natl Acad Sci U S A*. 2002 Apr 2;99(7):4355-60. Epub 2002 Mar 19.

Hillier LW, Coulson A, Murray JI, Bao Z, Sulston JE, Waterston RH. Genomics in C. elegans: so many genes, such a little worm. *Genome Res*. 2005 Dec;15(12):1651-60.

Hsueh YP, Mahanti P, Schroeder FC, Sternberg PW. Nematode-trapping fungi eavesdrop on nematode pheromones. *Curr Biol*. 2013 Jan 7;23(1):83-6. doi: 10.1016/j.cub.2012.11.035. Epub 2012 Dec 13.

Hyde R, Corkins ME, Somers GA, Hart AC. PKC-1 acts with the ERK MAPK signaling pathway to regulate Caenorhabditis elegans mechanosensory response. *Genes Brain Behav*. 2011 Apr;10(3):286-98. doi: 10.1111/j.1601-183X.2010.00667.x. Epub 2011 Jan 10.

Jiang G, Zhuang L, Miyauchi S, Miyake K, Fei YJ, Ganapathy V. A Na+/Cl- -coupled GABA transporter, GAT-1, from Caenorhabditis elegans: structural and functional features, specific expression in GABA-ergic neurons, and involvement in muscle function. *J Biol Chem*. 2005 Jan 21; 280(3):2065-77. Epub 2004 Nov 12.

Jones AK, Sattelle DB. Functional genomics of the nicotinic acetylcholine receptor gene family of the nematode, Caenorhabditis elegans. *Bioessays*. 2004 Jan; 26(1):39-49.

Jospin M, Qi YB, Stawicki TM, Boulin T, Schuske KR, Horvitz HR, Bessereau JL, Jorgensen EM, Jin Y. A neuronal acetylcholine receptor regulates the balance of muscle excitation and inhibition in Caenorhabditis elegans. *PLoS Biol*. 2009 Dec;7(12):e1000265. doi: 10.1371/journal.pbio.1000265. Epub 2009 Dec 22.

Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J. Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. *Nature*. 2003 Jan 16; 421(6920):231-7.

Kertesz N, Samson J, Debacker C, Wu H, Labastie MC. Cloning and characterization of human and mouse SNRK sucrose non-fermenting protein (SNF-1)-related kinases. *Gene*. 2002 Jul 10;294(1-2):13-24.

Kim DH, Feinbaum R, Alloing G, Emerson FE, Garsin DA, Inoue H, Tanaka-Hino M, Hisamoto N, Matsumoto K, Tan MW, Ausubel FM. A conserved p38 MAP kinase pathway in Caenorhabditis elegans innate immunity. *Science*. 2002 Jul 26;297(5581):623-6.

Kindt KS, Viswanath V, Macpherson L, Quast K, Hu H, Patapoutian A, Schafer WR. Caenorhabditis elegans TRPA-1 functions in mechanosensation. *Nat Neurosci*. 2007 May; 10(5):568-77. Epub 2007 Apr 22.

Kwan, C. S. M., Ragnauth, C. D., & Baylis, H. A. TRPm channel function in the defecation rhythm of Caenorhabditis elegans. *European Worm Meeting*. 2002.

Land, M., & Rubin, C. S. Protein kinase C2 (KIN-11) mediates thermotactic and chemotactic signaling in C.elegans. Presented in International Worm Meeting. 2003.

Lans H, Marteijn JA, Schumacher B, Hoeijmakers JH, Jansen G, Vermeulen W. Involvement of global genome repair, transcription coupled repair, and chromatin remodeling in UV DNA damage response changes during development. *PLoS Genet*. 2010 May 6; 6(5):e1000941. doi: 10.1371/journal.pgen.1000941.

Lee, MH, Ahn, B, Choi, IS, & Koo, HS. The gene expression and deficiency phenotypes of Cockayne syndrome B protein in Caenorhabditis elegans. *FEBS Lett*. 2002; 522:47-51.

Lesch BJ, Gehrke AR, Bulyk ML, Bargmann CI. Transcriptional regulation and stabilization of left-right neuronal identity in C. elegans. *Genes Dev*. 2009 Feb 1;23(3):345-58. doi: 10.1101/gad.1763509.

Liu, XZ, Xiang, MC, and Che, YS (2009). The living strategy of nematophagous fungi. *Mycoscience*. 2009. 50:20-25.

Maeda I, Kohara Y, Yamamoto M, Sugimoto A. Large-scale analysis of gene function in Caenorhabditis elegans by high-throughput RNAi. *Curr Biol*. 2001 Feb 6; 11(3):171-6.

Mancuso JJ, Qian Y, Long C, Wu GY, Wensel TG. Distribution of RGS9-2 in neurons of the mouse striatum. *J Neurochem*. 2010 Feb;112(3):651-61. doi: 10.1111/j.1471-4159.2009.06488.x. Epub 2009 Nov 11.

Miranda Vde J, Coelho RR, Viana AA, de Oliveira Neto OB, Carneiro RM, Rocha TL, Grossi de Sa MF, Fragoso RR. Validation of reference genes aiming accurate normalization of qPCR data in soybean upon nematode parasitism and insect attack. *BMC Res Notes*. 2013 May 13;6:196. doi: 10.1186/1756-0500-6-196.

Mongan NP, Baylis HA, Adcock C, Smith GR, Sansom MS, Sattelle DB. An extensive and diverse gene family of nicotinic acetylcholine receptor alpha subunits in Caenorhabditis elegans. *Receptors Channels*. 1998; 6(3):213-28.

Nicol JM, Turner SJ, Coyne DL, den Nijs L, Hockland S, and Maafi aZT. Current nematode threats to world agriculture. *Genomics and Molecular Genetics of plant-nematode interaction*. 2011. Springer.

Nisbet AJ, McNeilly TN, Wildblood LA, Morrison AA, Bartley DJ, Bartley Y, Longhi C, McKendrick IJ, Palarea-Albaladejo J, Matthews JB. Successful immunization against a parasitic nematode by vaccination with recombinant proteins. *Vaccine*. 2013 May 21. pii: S0264-410X(13)00604-X. doi: 10.1016/j.vaccine.2013.05.026.

Ohnishi N, Kuhara A, Nakamura F, Okochi Y, Mori I. Bidirectional regulation of thermotaxis by glutamate transmissions in Caenorhabditis elegans. *EMBO J*. 2011 Apr 6;30(7):1376-88. doi: 10.1038/emboj.2011.13. Epub 2011 Feb 8.

Olefsky JM. Nuclear receptor minireview series. *J Biol Chem*. 2001 Oct 5;276(40):36863-4. Epub 2001 Jul 17.

Orton RJ, Sturm OE, Vyshemirsky V, Calder M, Gilbert DR, Kolch W. Computational modelling of the receptor-tyrosine-kinase-activated MAPK pathway. *Biochem J*. 2005 Dec 1;392(Pt 2):249-61.

Papachatzaki MM, Antal Z, Terzi D, Szücs P, Zachariou V, Antal M. RGS9-2 modulates nociceptive behaviour and opioid-mediated synaptic transmission in the spinal dorsal horn. *Neurosci Lett*. 2011 Aug 21;501(1):31-4. doi: 10.1016/j.neulet.2011.06.033. Epub 2011 Jun 29.

Paradis S, Ruvkun G. Caenorhabditis elegans Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. *Genes Dev*. 1998 Aug 15; 12(16):2488-98.

Park KH, Hernandez L, Cai SQ, Wang Y, Sesti F. A family of K+ channel ancillary subunits regulate taste sensitivity in Caenorhabditis elegans. *J Biol Chem*. 2005 Jun 10;280(23):21893-9. Epub 2005 Mar 30.

Patton A, Knuth S, Schaheen B, Dang H, Greenwald I, Fares H. Endocytosis function of a ligand-gated ion channel homolog in Caenorhabditis elegans. *Curr Biol*. 2005 Jun 7; 15(11):1045-50.

Perrin AJ, Gunda M, Yu B, Yen K, Ito S, Forster S, Tissenbaum HA, Derry WB. Noncanonical control of C. elegans germline apoptosis by the insulin/IGF-1 and Ras/MAPK signaling pathways. *Cell Death Differ*. 2013 Jan;20(1):97-107. doi: 10.1038/cdd.2012.101. Epub 2012 Aug 31.

Petrash HA, Philbrook A, Haburcak M, Barbagallo B, Francis MM. ACR-12 ionotropic acetylcholine receptor complexes regulate inhibitory motor neuron activity in Caenorhabditis elegans. *J Neurosci*. 2013 Mar 27;33(13):5524-32. doi: 10.1523/JNEUROSCI.4384-12.2013.

Pohludka M, Simeckova K, Vohanka J, Yilma P, Novak P, Krause MW, Kostrouchova M, Kostrouch Z. Proteomic analysis uncovers a metabolic phenotype in C. elegans after nhr-40 reduction of function. *Biochem Biophys Res Commun*. 2008 Sep 12;374(1):49-54. doi: 10.1016/j.bbrc.2008.06.115. Epub 2008 Jul 9.

Portman DS, Emmons SW. Identification of C. elegans sensory ray genes using whole-genome expression profiling. *Dev Biol*. 2004 Jun 15; 270(2):499-512.

Powell JR, Kim DH, Ausubel FM. The G protein-coupled receptor FSHR-1 is required for the Caenorhabditis elegans innate immune response. *Proc Natl Acad Sci U S A*. 2009 Feb 24;106(8):2782-7. doi: 10.1073/pnas.0813048106. Epub 2009 Feb 5.

Pramer, D., and Stoll, N.R. Nemin - morphogenic substance causing trap formation by predaceous fungi. *Science*. 1959; 129:966-967.

Rual JF, Ceron J, Koreth J, Hao T, Nicot AS, Hirozane-Kishikawa T, Vandenhaute J, Orkin SH, Hill DE, van den Heuvel S, Vidal M. Toward improving Caenorhabditis elegans phenome mapping with an ORFeome-based RNAi library. *Genome Res*. 2004 Oct; 14(10B):2162-8.

Salkoff L, Butler A, Fawcett G, Kunkel M, McArdle C, Paz-y-Mino G, Nonet M, Walton N, Wang ZW, Yuan A, Wei A. Evolution tunes the excitability of individual neurons. *Neuroscience*. 2001; 103(4):853-9.

Scheffzek K, Ahmadian MR. GTPase activating proteins: structural and functional insights 18 years after discovery. *Cell Mol Life Sci*. 2005 Dec;62(24):3014-38.

Sharma M, Celver J, Kovoor A. Regulator of G protein signaling 9-2 (RGS9-2) mRNA is up regulated during neuronal differentiation of mouse embryonic stem cells. *Neurosci Lett*. 2011 Sep 20;502(3):123-8. doi: 10.1016/j.neulet.2011.05.021. Epub 2011 May 14.

Sieburth, D., Dittman, J., Nurrish, S., & Kaplan, J. M. Analysis of kin-13 PKC and dgk-1 DAG kinase suggests that phorbol esters regulate a late stage of synaptic vesicle exocytosis. Presented in International C. elegans Meeting. 2001.

Sieburth, D. S., Madison, J., Mori, I., & Kaplan, J. Kin-13 PKC is a phorbol ester target that regulates acetylcholine release at the NMJ. Presented in International Worm Meeting. 2003.

Sieburth D, Ch'ng Q, Dybbs M, Tavazoie M, Kennedy S, Wang D, Dupuy D, Rual JF, Hill DE, Vidal M, Ruvkun G, Kaplan JM. Systematic analysis of genes required for synapse structure and function. *Nature*. 2005 Jul 28; 436(7050):510-7.

Sieburth D, Madison JM, Kaplan JM. PKC-1 regulates secretion of neuropeptides. *Nat Neurosci*. 2007 Jan;10(1):49-57. Epub 2006 Nov 26.

Simmer F, Moorman C, van der Linden AM, Kuijk E, van den Berghe PV, Kamath RS, Fraser AG, Ahringer J, Plasterk RH. Genome-wide RNAi of C. elegans using the hypersensitive rrf-3 strain reveals novel gene functions. *PLoS Biol*. 2003 Oct; 1(1):E12. Epub 2003 Oct 13.

Slep KC, Kercher MA, He W, Cowan CW, Wensel TG, Sigler PB. Structural determinants for regulation of phosphodiesterase by a G protein at 2.0 A. *Nature*. 2001 Feb 22;409(6823):1071-7.

Tabuse, Y. Protein kinase C isotypes in C. elegans. *J Biochem*. 2002; 132, 519-22.

Tanaka-Hino M, Sagasti A, Hisamoto N, Kawasaki M, Nakano S, Ninomiya-Tsuji J, Bargmann CI, Matsumoto K. SEK-1 MAPKK mediates Ca2+ signaling to determine neuronal asymmetric development in Caenorhabditis elegans. *EMBO Rep*. 2002 Jan; 3(1):56-62. Epub 2001 Dec 19.

Troemel ER, Kimmel BE, and Bargmann CI. Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in C. elegans. *Cell*. 1997; 91:161-169.

Troemel ER, Sagasti A, Bargmann CI. Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in C. elegans. *Cell*. 1999 Nov 12;99(4):387-98.

Van, G. M., Gissendanner, C. R., & Sluder, A. E. Diversity and function of orphan nuclear receptors in nematodes. *Crit Rev Eukaryot Gene Expr*. 2002; 12:65-88.

Vohanka J, Simecková K, Machalová E, Behenský F, Krause MW, Kostrouch Z, Kostrouchová M. Diversification of fasting regulated transcription in a cluster of duplicated nuclear hormone receptors in C. elegans. *Gene Expr Patterns*. 2010 Sep;10(6):227-36. doi: 10.1016/j.gep.2010.05.001. Epub 2010 May 10.

White JG, Southgate E, Thomson JN, and Brenner S. The structure of the nervous system of the nematode Caenorhabditis elegans. *Philosophical transactions of the Royal Society of London Series B, Biological sciences*. 1986; 314:1-340.

Whitehouse I, Flaus A, Cairns BR, White MF, Workman JL, Owen-Hughes T. Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature*. 1999 Aug 19;400(6746):784-7.

Xiao R, Xu XZ. Function and regulation of TRP family channels in C. elegans. *Pflugers Arch*. 2009 Sep;458(5):851-60. doi: 10.1007/s00424-009-0678-7. Epub 2009 May 8.

Xie H, Aminuzzaman FM, Xu L, Lai Y, Li F, Liu X. Trap induction and trapping in eight nematode-trapping fungi (Orbiliaceae) as affected by juvenile stage of Caenorhabditis elegans. *Mycopathologia*. 2010 Jun;169(6):467-73. doi: 10.1007/s11046-010-9279-4. Epub 2010 Feb 10.

Yang J, Wang L, Ji X, Feng Y, Li X, Zou C, Xu J, Ren Y, Mi Q, Wu J, Liu S, Liu Y, Huang X, Wang H, Niu X, Li J, Liang L, Luo Y, Ji K, Zhou W, Yu Z, Li G, Liu Y, Li L, Qiao M, Feng L, Zhang KQ. Genomic and proteomic analyses of the fungus Arthrobotrys oligospora provide insights into nematode-trap formation. PL*oS Pathog*. 2011 Sep;7(9):e1002179. doi: 10.1371/journal.ppat.1002179. Epub 2011 Sep 1.

Appendix A: Function of Proteins and Protein Families in List of Abbreviations

ERK	A pathway that acts in conjunction with MAPK to bring a signal from a cell-surface or transmembrane receptor into the nucleus of the cell (Orton RJ et al., 2005).
GTPase	A family of hydrolase enzymes that bind and hydrolyze guanosine triphosphate (GTP) and play many important roles in protein transport and signaling, and cell division (Scheffzek K and Ahmadian MR, 2005).
MAPK/MAPKK	A pathway that acts in conjunction with ERK to bring a signal from a cell-surface or transmembrane receptor into the nucleus of the cell (Orton RJ et al., 2005).
SNF	A family of proteins involved in DNA remodeling and packaging (Whitehouse I et al., 1999).