CHEMOSENSORY GENE EXPRESSION ANALYSIS IN THE ARGENTINE ANT, *LINEPITHEMA HUMILE*

BY

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THESIS

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ABSTRACT

This experiment was conducted to determine expression patterns of a subset of chemosensory genes, including odorant receptors (Ors) and odorant binding proteins (OBPs), in the invasive Argentine ant, *Linepithema humile*. Fragments of two Ors were identified from a whole ant EST project, which suggested their potential importance in Argentine ant ecology. An initial RT-PCR experiment demonstrated that OrA and OrB were expressed throughout the body. The newly available genome sequence was used to manually build additional Or gene models to test in RT-PCR. OBP and opsin genes were also tested in the RT-PCR to serve as positive and negative controls. The RT-PCR results were unclear; therefore qPCR was conducted for a semi-quantitative analysis of their gene expression in the tissue samples.

It was hypothesized that the expression levels for the Ors should be high in the head/antennal samples, and low in the other body samples assayed. We also hypothesized that the OBPs would have variable of expression levels in all the body regions analyzed and that opsins would be highly expressed in the head/antennal region since they are photoreceptors in the eyes. The qPCR results demonstrated that the Ors and opsins were highly expressed in the head/antennal samples compared to the thorax/legs and abdominal tissue samples. The OBPs did not have a pattern that suggested a bias toward one tissue, but were highly expressed in all body regions.

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Chapter 1: Introduction

Linepithema humile, commonly known as the Argentine ant, is an invasive pest species that is common in the United States of America. This species of "house ant" is native to South America (Suarez et. al. 2001), is about 1/8 inch long, dull brown in color, and has an uneven thorax shape with a petiole with one erect node (UC IPM 2009). The species is important for scientific study because it can create "supercolonies", in which intraspecific aggression has been averted creating large scale colonies in the New World (Thomas et. al. 2007 and Brandt et. al. 2009). They are starting to disrupt the native ecosystem by displacing the native insects and other native organisms causing them to relocate or decrease (Mitrovich et. al. 2010).

Being able to perceive the surroundings is important to all animals. For ants, the antennae are the primary source for interactions with the environment. Ants use their antennae for tactile sensation, detection of hydrocarbons, and identification of objects as a threat or resource. A large number of chemoreceptors are expressed in chemosonsory neurons within the head and antennae of insects (Ishida et. al. 2002 and de Bruyne and Baker 2008). Insects use receptor proteins, called olfactory receptors, that detect odors (Clyne et. al. 1999 and Vosshall et. al. 2000) and they play a major role in the way insects navigate, locate mates, and obtain resources present in their environment (Wang et. al. 2008). They are also part of an old superfamily with many members (Robertson et. al. 2003).

One way to control insect populations is to interfere with breeding and chemical communications (Li et. al. 2008). For example, in mosquitoes, host odors are used to bait and trap females to collect eggs and disrupt mating behaviors (Olanga et. al. 2010). Research on Argentine ant Ors might identify key receptors used for colony communication, such as a trail pheromone receptor. Identifying Or expression patterns may help scientists synthesize compounds and administer them in such a way that they could intentionally disrupt colony communication and ultimately control certain pest species populations such as *Linepethema humile*.

As a first step in this direction, I investigated the expression patterns of two Ors from an Argentine ant EST project, as well as the expression patterns of other Ors, OBPs, and opsins.

Chapter 2: Methods and Materials

Gene Annotation

Two candidate Or fragments were identified by my adviser Dr. Hugh M. Robertson, in a whole ant body EST project, and they were designated OrA and OrB. The cDNA library was built from RNA of adult workers and drones, virgin and mated queens, worker pupae, and embryos from South American and Californian populations. PCR primers in the forward and reverse direction were designed for OrA and OrB using conserved amino acid sequences (Table 1).

Once *L. humile* genomic sequences were available from the genome project, additional Ors were annotated. Honey bee representatives were selected from Dr. Hugh M. Robertson's large bee/wasp Or phylogenetic tree for each major clade (Robertson et. al. 2009). Honey bee Or protein sequences were used as a query for TBLASTN searches of the *L. humile* genome assembly. Genomic DNA scaffolds from the Argentine ant were obtained, and gene models were manually built in the PAUP (Phylogenetic Analysis Using Parsimony)*V4.0b10 (David L. Swofford, Illinois Natural History Survey, Champaign, IL) text editor. PCR primers were manually designed to the ends of two exons spanning an intron near the 3' end of the gene selected such that the final PCR product from cDNA was around 200-500 base pairs long (Table 1). The primers were checked in Amplify v3.0 for GC content, melting temperature, primer dimers, and length.

Primers were similarly designed to manual gene models of OBPs and opsins (Table 1) built from the available *L. humile* genome assembly using honeybee orthologs as TBLASTN queries. Primers were suspended to 500uM and 20uM in 1xTE, and stored at -80^{o}C and -20^{o}C respectively.

Ant colony

L. humile colonies were shipped by Neil Tsutusi at the University of California at Berkeley in 50mL tubes containing eggs, workers, and queens. The colony was maintained in a container coated in liquid Teflon and fed diced cockroaches and crickets mixed with water and honey. Small glass tubes were filled with water and plugged with a cotton ball for humidity. The colonies were stored in an

incubation chamber that maintained 12 hours of light and 12 hours of dark and kept between 25°C to 20°C.

Tissue Dissection

Ants were placed live on a chilled dissection table and separated using forceps, into three parts: head and antennae, thorax and legs, and abdomen. Body regions were placed into labeled 1.5mL microcentrifuge tubes on dry ice and were stored at -80°C. A total of 80 ants were used for each RNA extraction.

RNA Isolation

All glassware used for this experiment was baked overnight at 240°C. The body regions were manually ground in 200uL of Trizol (Invitrogen) in 1mL glass tissue grinders. After the solution was semi-homogeneous, 800uL of Trizol was added. Samples were incubated for 5 minutes at room temperature and then filtered through a QIAshredder column (Qiagen) by centrifuging at 12,000Xg for 2 minutes. The aqueous layer was pipetted off, transferred to a new 1.5mL tube, and then centrifuged again at 16°C for 5 minutes. The aqueous layer was transferred to a new 1.5mL tube and 200uL of chloroform was added and mixed for 15 seconds by hand. The tubes were incubated at room temperature for 2-3 minutes, and then centrifuged at 12,000Xg at 4°C for 15 minutes. The aqueous layer was transferred to a new tube. To remove the excess eye pigments, an equal volume of chloroform was added to the tubes. The tubes were mixed by hand for 15 seconds and then centrifuged at 12,000Xg at 4°C for 10 minutes. The aqueous layer was pipetted off and placed in a new tube, and a second chloroform extraction was performed. An equal volume of isopropanol was added to the vials and then mixed by hand. The tubes were incubated at room temperature for 10 minutes, and then centrifuged at 12,000Xg at 4°C for 15 minutes. The isopropanol was decanted off, and 1mL of 80% ethanol was added, vortexed briefly, then centrifuged at 7500Xg for 5 minutes at 4°C. The aqueous layer was decanted, and the RNA pellet was air-dried. The pellet was resuspended in 25uL of RNase-free water and stored at -80°C.

Spectrophotometry

RNA samples were analyzed on a Spectronic Genesys 5 Spectrophotometer at 260nm and 280nm. 2.5uL RNA was diluted in 78.5uL RNase free water. The concentration and 260:280 ratios were calculated for each sample.

cDNA Synthesis

1ug total RNA was reverse transcribed in a 25uL reaction containing 1.25uL oligo-dT_{16} (500ng/uL), 2uL ArrayScript 10x Buffer (Ambion), 1uL 10mM dNTPs, 0.2uL RNase inhibitor, and 0.2uL ArrayScript reverse transcriptase (200U/uL). The negative control syntheses substituted RNase-free water for the reverse transcriptase. The reactions were initially denatured at 70^{o}C for 10 minutes on a MJR Thermal Cycler and then quenched on ice. Reactions were incubated at 45^{o}C for 1 hour and then terminated at 95^{o}C for 5 minutes. Reactions were stored at -80^{o}C.

RT-PCR

For each PCR, 0.75uL forward and reverse primers each at 20uM were added to the 0.6mL thin walled PCR tubes. 18.1uL water (Sigma), 2.5uL 10x PCR buffer, 1.0uL $MgCl_2$, 0.5uL 10mM dNTP, 0.3uL Taq polymerase (Invitrogen), 0.1uL Pfu polymerase (Stratagene), and 1uL cDNA were added to the primer mix. The PCR tubes were incubated for 40 cycles of 94^{o}C - 1 minute; 54^{o}C – 1 minute; 72^{o}C – 1 minute on the MJR Thermal Cycler. 10uL aliquots of the PCR products were ran on 2% agarose gels in TBE buffer plus ethidium bromide at 84V for 1.5 hours. Gels were visualized on a UV light table and photographed with a CCD camera on black and white thermal paper.

Semi-quantitative qPCR

Oligonucleotides were designed within 3’ exons in Primer Express v3.0 (Applied Biosystems) using the default settings for Taqman/Probe Assay Design (Table 1). Primers were resuspened in forward-reverse combinations to a final concentration of 5uM each in Sigma water. Reactions were pipetted into a 384-well plate and ran on an Applied Biosystems ABI PRISM Sequence Detection System

7900HT using Sequence Detection Software V2.2. Each well contained 10uL consisting of 1uL genomic DNA or cDNA, 3uL Sigma water, 5uL Power SYBR Green PCR Master Mix (Applied Biosystems), and 1uL 5uM primer mix. Genomic DNA from whole *L. humile* (provided by K. Walden) was diluted serially to 10^{-4} for standard curve analysis. All reactions were preformed in triplicate wells, including no-template controls, 10^{-4}, 10^{-3}, 10^{-2} genomic DNA standards, minus RT (-RT) reactions and positive RT (+RT), using 1uL cDNA for -RT and +RT reactions. The 384-well plate was sealed with transparent film and centrifuged at 3000Xg for 3 minutes before loading on the instrument. The plate was incubated at 50°C for 2 minutes, then it was incubated at 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. An optional dissociation curve was generated at the end of the profile by incubating the samples at 95°C for 15 seconds, 60°C for 15 seconds, and then 95°C for 15 seconds.

Data Analysis

The raw C_T values were exported into Microsoft Excel. Data points where the dissociation curves indicated spurious products were omitted. Triplicate C_T values were averaged, and the standard curves were created for each primer set using the mean C_T values for the genomic DNA serial dilutions. The raw input cDNA (=X) was calculated using the standard curve equation of the line and the mean C_T as Y (solving for X = input). The log cDNA input was calculated by raising 10 to the raw input power. Then the background genomic DNA contamination was roughly subtracted out by calculating the log input cDNA values of the -RT reactions and subtracting it from the +RT reactions. The expression level of RPS7 was used to normalize the input cDNA amount. The normalized cDNA amounts were plotted on a bar graph grouped by primer sets on a log 10 scale for the Y axis to visualize qualitative fold differences in expression patterns.

Chapter 3: Results

The first RT-PCR experiment that was conducted analyzed OrA and OrB (later renamed Or2 and Or3), two Ors identified from a whole body Argentine ant EST project. Our hypothesis was that Or gene expression would be restricted to the head/antennae cDNA sample. The RT-PCR results however, suggested expression in all three tissue samples analyzed. Table 1 shows that PCR products of the correct size were observed in all body regions for Or2 and Or3. In some cases, PCR products corresponding to expected genomic DNA amplification sizes were observed showing that some genomic DNA contamination was present. Sequencing was not performed to confirm a particular PCR product's identity. Additional Ors were analyzed by RT-PCR, and of the 16, two primer sets for LhOr3 and LhOr9, failed to amplify any product in abdominal tissues (Table 2). In a number of cases, a PCR product was identified as similar to, but not identical to the expected genomic DNA site. This is denoted with a question-mark in Table 2.

Observing the cDNA RT-PCR results, it seemed that an appropriate sized band was present in all three body regions for most of the genes assayed (Table 2). Out of the 16 Or genes tested, only four were not expressed in all three tissue samples, and they included LhOr1, LhOr20, LhOr23, and LhOr25 (Table 2). It was unusual to see so many Ors showing expression in the different tissues tested. It was hypothesized that the Ors would show high expression in the head/antennal regions, and low to no expression levels in all the other body regions assayed.

To test our ideas of where chemosensory genes should be expressed further, we examined the odorant binding proteins (OBPs), which are genes that are expressed in many different tissues in insects (Forêt and Maleszka 2006). Overall, the experiment produced results that agreed with this hypothesis. Of the 12 OBPs tested, 10 of them were expressed in all three body regions. The reaction for LhOBP5 failed, so no relevant data was observed, and LhOBP8 showed no expression in any of the three tissue types (Table 2).

Opsins were also examined for expression in the three body regions, as an additional negative

control for thorax and abdominal cDNA samples. Opsins are photoreceptors expressed in eye tissue (Friedrich 2008). Opsin PCR products were observed in all three body regions (Table 2). To confirm these unexpected results, a second round of dissections, RNA isolation, and cDNA syntheses were performed. Similar results were obtained from the second round in that the PCR product bands were almost identical in intensity and presence/absence.

To further investigate the unusual results, a qPCR experiment was designed. Using SYBR Green analysis, the cDNA expression patterns of the Ors, OBPs, and Opsins were semi-quantified during each cycle of PCR. All of the opsins, but only a subset of the Ors and the OBPs from the earlier RT-PCR work, were selected for qPCR.

While the opsins were still detected in the thorax and abdomen cDNA, the majority of them were expressed 100X or greater in the head cDNA compared to the other body regions (Figure 1). The BLop opsin was expressed roughly 100X greater in the head when compared to thorax/legs and abdomen. The Lop1 and Lop2 were expressed around 50,000 fold, and slightly less than 100 fold greater in magnitude in the head when compared to thorax/legs and abdomen. The last opsin tested was UVop, and when compared to thorax/legs and abdomen, it was expressed around 5 fold greater in the head (Figure 1).

When a subset of the Or genes were analyzed with qPCR, they were expressed at higher levels in the head relative to the thorax and abdominal body regions. The Or expression levels relative to each other were surprisingly uniform (Figure 1). Even OrA, initially found in an EST project, was expressed at about the same level as the other Or genes assayed.

A subset of the OBPs were also analyzed by qPCR, and the results varied between the OBPs observed. Of the five OBPs assayed, four of the OBPs analyzed displayed relatively high expression levels in all three body regions relative to RPS7 (Figure 1).

Chapter 4: Discussion

This experiment investigated the expression patterns of a subset of Ors, OBPs, and opsins present in *L. humile*. The initial RT-PCR experiment on OrA and OrB suggested they were expressed in head/antennae, legs/thorax, and abdominal body regions at similar levels, which is very unusual (Table 2). Our hypothesis is that most Ors are highly expressed in the head/antennal regions because the Ors are usually restricted to the antennal region. Additional Or genes were built from the genome assembly, and they too, had similar RT-PCR results (Table 2).

OBPs were then investigated by RT-PCR because they are also usually highly expressed in the antennae. These types of proteins help the insect with chemical communication (Wang et.al 2008 and Forêt and Maleszka 2006), thus these proteins might be restricted to the antennal region. However, some OBPs are present in other insect tissues (Forêt and Maleszka 2006). The results suggested widespread expression of the OBPs with only LhOBP5 failing to amplify from any tissue. Again it was surprising that the OBPs were expressed in all body regions (Table 2). In this RT-PCR experiment, opsins were also annotated and used as a negative control for the thorax and abdominal body regions, since opsins should only be expressed in the head (Friedrich 2008). The results for the RT-PCR confirmed expression in the head/antennal regions, but also all other body regions tested (Table 2). This was unusual, and a SYBR Green analysis was conducted in order to gain insight to these results. The SYBR Green analysis provided results that support the high expression in the head/antennal regions, with very low expression in the remaining body regions tested (Figure 1). This supports our original hypothesis that opsin expression should be greater in magnitude in the head/antennal regions compared to the other body regions analyzed.

The expression patterns of the Ors, OBPs, and opsins seemed suspicious, so semi-quantitative qPCR (using SYBR Green) was used to confirm the previous results. The results from the qPCR confirmed at least low level expression of the genes in all body regions examined, but demonstrated that expression levels of the Ors and the opsins were higher in the head/antennal region (Figure 1). This

supports our hypothesis that Or and opsin expression should be greatest in the head/antennal region. The OBP expression levels were in general higher than the Ors and opsins in the different body regions assayed, confirming the previous results that OBPs are expressed throughout the insect body (Forêt and Maleszka 2006).

The RT-PCR experiment determined presence or absence of an amplification product, not giving much data on how highly expressed a gene was in a given tissue. Therefore, the qPCR was a better choice for this experiment because the PCR products were monitored in real time, giving insight into the expression levels in each of the body regions.

Although this is just preliminary data, further investigation would be needed to determine if indeed the opsins are being expressed in all tissue samples. One technique that could help shed some light on this is *in situ* hybridization. Tissue sections of *Linepithema humile* attached to glass slides would be hybridized with RNA-labeled probes to opsins.

After reviewing the qPCR results, it was not determined why OrA was present in the whole body Argentine ant EST project. Considering OrA was present in an EST project, one would expect to see higher expression levels in qPCR. When compared to the other Ors assayed, the expression was uniform (Figure 1). Some reasons why this could have occurred might be because of random sampling or the ant colonies used for the EST project may have been isolated under different conditions. Also, the EST project had a bias toward males, queens, and pupae, while in this project, only workers were dissected and analyzed. This could be a reason why OrA and OrB turned up in the EST library, and showed no obvious differences in the qPCR experiment. It might be that OrA and OrB are expressed at higher levels in queens, males, and pupae relative to workers.

Chapter 5: Tables and Figures

Table 1: *L. humile* primer sets used for the RT-PCR and qPCR reactions. The first set of primers were manually built in PAUP* v4.0B10, and then checked in Amplify v3.0. The second set of primers were designed in Primer Express v3.0 (Applied Biosystems) using the default settings for Taqman primer/probe sets. Primers are listed in 5'to 3'orientation.

Name	Forward	Reverse
LhOrA	F1-AGCTGCGTACTCGAGTGGCTGGTTCTC	AAAAATCGAGTAAGACGTCCGTAGTAT
LhOrA	F2-CTCCGGCACGGAGAGATTCAAACGCAG	
LhOrB	F1-TGCGGAGGTAGCATATATGACCGAATG	ATTTAAATATACCACTGAAGTTTTGAC
LhOrB	F2-AATGGTACAAATTGCCTTATACAATAG	
LhOr01	TCCACGATCGGCTACATACTCT	CGAAGCGAAGAGATCCAAAGATAC
LhOr02 (OrA)	GTTTATATCCCGGCGGTACTGA	CTGTACGAAAGTGTCCAAAGA
LhOr03 (OrB)	AAATGGCATCATATATTTGCGTAT	CAAAAGTTGCGATGGACAGTTG
LhOr04	GAATAAAATTGATACAAAGAGCATAG	CTGAATGTCGCAATAGAAAGTTG
LhOr05	AAGCGAGAAACACGAAGACTTT	ATCACCAAATGTCACTAAAGAGAG
LhOr07	GAGTGGAAAAATAGTGACGCC	GAAGAACTTGCCAGCTGTAAG
LhOr10	GAATGGAAAGATAACAATGCGG	AGCACCGAAGCTGTTAAGAGATAG
LhOr11	AGCTATGGGCTCGTATCTCG	ATCGCCGAAGGTTCTGATAGAAAG
LhOr14	TGATAGACGAAGCAAATGAACT	TGATGTGTAATGGTGATCGAG
LhOr15	TTGATCACAGAAGAGGATAAAG	TAGCCAATGTCATGGGAAACAC
LhOr18	TCAGAGGCCAGAGCTCCAACAC	CCCGCTTTCAGATTCAATGTTC
LhOr19	GATTCCGTCGATGCGAATAC	GCTTGTAAAACCCTCCAGAGATAA
LhOr20	TCTATGATAATAGCCGCTTTGC	CTAGAGTAAGAGAAGGTAATAAGCC
LhOr21	GTTTGCTCGCTTAGTAAATGA	CGAGATTCATAGAGATTACGTATGC
LhOr23	TACTATTGCTATTCAATTTGCAG	ATCGAGATTCATAGAGATAATGTAAG
LhOr25	GGTCCACGACTTTTGTGGATG	CAGAGATAAATTAAATTTATCACACG
LhOBP1	CCCTTATCGATGAAGTTAACGATG	TTCTGGTACTGTACCTTGGACAC
LhOBP2	AAACATTTATGGAAATGAAGAGTG	ATGAAGCAGAGTATTAATTCTAGTG
LhOBP3	ATGAAAAATTCTAAGCTGGTTG	TCTTTTGCCTCCATAATTACTGAC
LhOBP4	CTGATTTGATTACAAACGTGGAAATA	AGATCGAGAGACTTGTCGCACAT
LhOBP5	TCGCAGCAATACAGAATGGTGA	AAGTGATTTGGATCTGTCTGATAGC
LhOBP6	AGTTGATCGATAACGCAAACAAAGG	GGATCATATTCGTAGTAGCACTTGG
LhOBP7	GTAGAAAATGCAAAACGCGGAGA	GAAAATTCTTGTTCTTCAAGAAAC
LhOBP8	CACAAGTGTCAATGATGATTTTGC	CTTGCCTACTTCAGAAAAAAGGAA
LhOBP9	ATTTACTTTATTTTGCATGTTCGAG	AAATTGATTTGCATCGGTTAAGAGTTC
LhOBP10	CTGACATAGAAGCAGTAAGAAATG	ATTGCTCCGCGTAACATTTGTTGA
LhOBP11	GATATAGATTGGTCTACTATGCATGA	CTATAGTTTTGCATTCATTGAGCAT
LhOBP12	ATCAAACATTAATGAAATAAGAATGC	TTCAGGTTTCGTTTCACTTTCTTC
LhBLop	GCCAAGAAGATGAACGTCAAGTC	TCTAGGATGGTTAATTGCGTATATC
LhUVop	CTGCGATTACAATCTGCTTCCTCTTTG	GGCAGTCACAATTTCAGTTGTAGTGC
LhLop1	GTAGCTCTGATGACCATTTCTTTGTGG	TGCAGCAGCTTTTTCGCCTTCTGTGA
LhLop2	GTAGCTTTAATGACGATTTCCTTGTG	CGTCGTCGTTGTAACAGATGTCGTGT
LhRPS7	GATCATCATATATGTGCCCATGC	CTTGTGTTCAATGTTTGTCTGTTC
LhOr2Tqmn	GTGGCTGGTTCTCCGGC	GGACGATGGGCTCTGATCA
LhOr8Tqmn	GATTGGTATCGCATACCGCA	TTTGATTGTGGTATTGGACATGG
LhOr20Tqmn	GCGACTTTGTTGGTCAGTTTATAGC	TTTGAAGCATGAGGAAAACATCA
LhOr30Tqmn	CATCAGGCGAACTTGAATGGTA	CGACATAATCATAACCAACACGATC
LhOBP2Tqmn	CATATCTTTTTTCTCCAGCATGCA	GGAAATGAAGAGTGAAAACGATCTC
LhOBP4Tqmn	GTCAGAGATTCAGAAAGACAAGCTGT	CGGCCTGAGTACCAGGATTTAA
LhOBP10Tqmn	TCGGTCTGGTCGACGACAA	GGCGGGTATTCTTTGGAAAAA
LhOBP12Tqmn	TCGACATAATCATAACCAACACGAT	CATCAGGCGAACTTGAATGGTA
LhBLopTqmn	CGTCAAGTCGCTCGTATCGA	GGCGGCTTTCGCGATT
LhUVopTqmn	AAAGCCTTATTAACTCCTGGCATC	TCCAAGCAGGCCACAAATTT
LhLop1Tqmn	CGCGGGCATCTTCGAG	GCAAAGAGCGAGCCCCA
LhLop2Tqmn	TTAATGACGATTTCCTTGTGGTTTAT	CCAAATGGTGAAAATGGGACTAA
LhRPS7Tqmn	AATATTGGAGGATTTAGTGTACCCTGTT	TTTAATAAGCTGCGAGCCATCA

Table 2: *L. humile* RT-PCR results for Ors, OBPs and opsins with expected PCR product sizes. The + signs represent an identifiable band at the expected length. The – sign represents the absent of a band at the expected length. The question marks represent the presence of bands that were similar to, but not identical to the expected size.

	Head/Antennae		**Thorax/Legs**		**Abdomen**		**Length of Sequence**	
Name	**cDNA**	**Genomic**	**cDNA**	**Genomic**	**cDNA**	**Genomic**	**Genomic**	**cDNA**
LhOr1	+	-	-	-	+	-	390	234
LhOr2	+	?	+	?	+	?	319	258
LhOr3	+	+	+	+	Fail	Fail	500	267
LhOr4	+	+	+	+	+	+	346	258
LhOr5	+	?	?	+	?	-	402	268
LhOr7	+	?	+	?	+	?	638	240
LhOr10	+	+	+	+	+	-	335	267
LhOr11	+	?	+	?	+	?	378	244
LhOr14	+	-	?	-	?	-	1149	223
LhOr15	+	+	+	-	+	+	356	267
LhOr18	+	-	+	+	+	+	628	224
LhOr19	+	?	+	?	Fail	-	445	264
LhOr20	+	-	-	-	-	?	345	214
LhOr21	+	?	+	-	+	-	423	332
LhOr23	+	-	-	-	-	-	469	311
LhOr25	-	?	-	?	-	-	474	264
LhOBP1	+	-	+	-	+	-	410	257
LhOBP2	+	-	+	-	+	-	850	274
LhOBP3	+	-	+	+	+	-	416	192
LhOBP4	+	-	+	-	+	-	857	270
LhOBP5	Fail						810	271
LhOBP6	+	-	+	-	-	-	733	268
LhOBP7	+	?	+	-	+	-	441	255
LhOBP8	-	-	-	-	-	-	444	215
LhOBP9	+	?	+	?	+	?	344	271
LhOBP10	+	?	+	-	+	-	607	265
LhOBP11	+	-	+	-	+	-	541	286
LhOBP12	+	+	+	-	+	-	473	241
LhBLop	+	+	+	-	+	-	326	254
LhUVop	+	-	+	-	+	-	573	276
LhLop1	+	-	+	-	+	-	404	294
LhLop2	+	+	+	-	+	-	370	285
LhRPS7	+	-	+	-	+	-	491	332

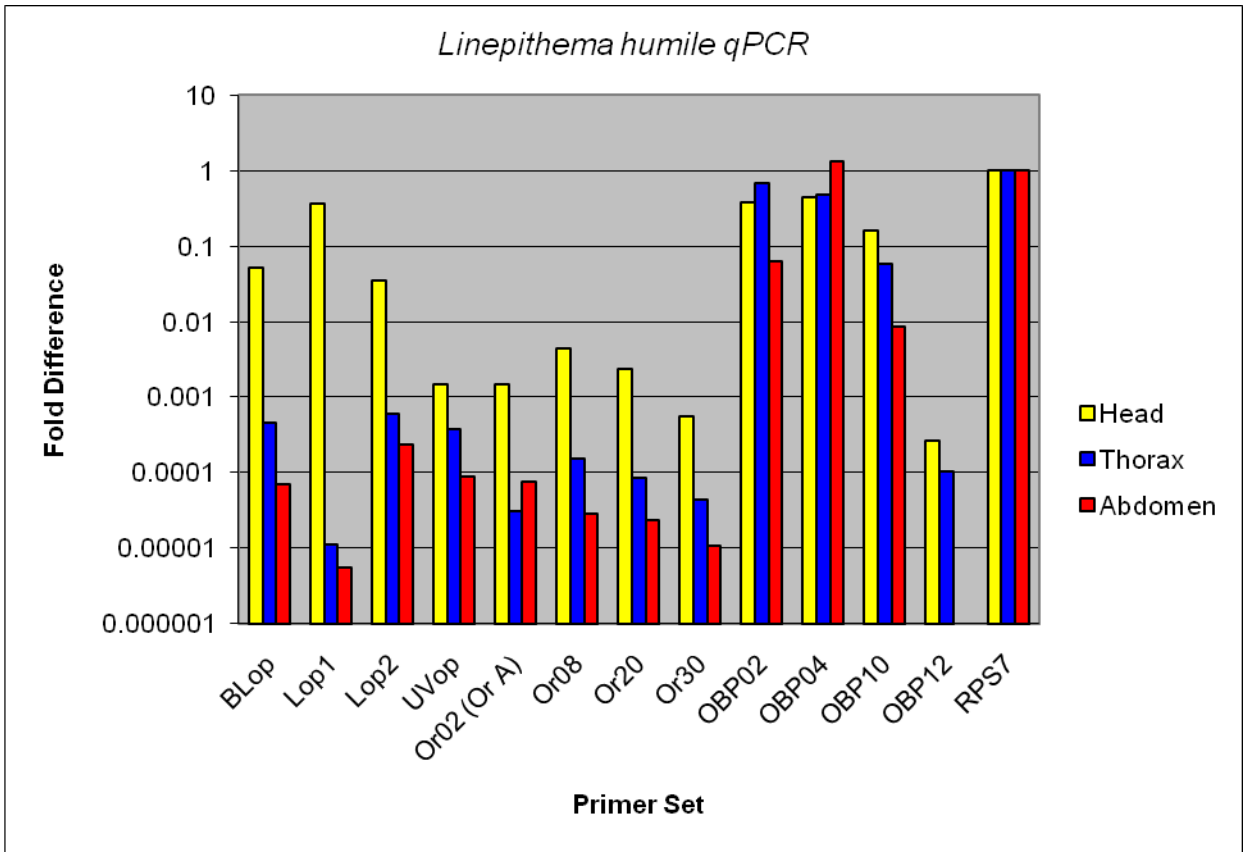

Figure 1: qPCR of *L. humile* Ors, OBPs, and opsins. This figure displays the expression levels on a log scale in the specific body regions assayed. This experiment contains only a subset of the genes that were used in the RT-PCR experiment. Note that OBP12 was not assayed against an abdominal sample. RPS7 is a housekeeping gene that was used to normalize the data across tissue types.

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