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Gene expression changes in response to field-to-lab transition in the Argentine ant, *Linepithema humile*

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ABSTRACT

Gene expression research is a valuable tool for investigating how gene regulation and expression control the underlying behaviors that structure a eusocial insect colony. However, labs that focus on ant research frequently keep ant colonies in the lab for ease of sampling. It is typically impractical to accurately emulate the field conditions where ants are collected from, so laboratory colonies can be exposed to drastically different environmental conditions and food sources than they are naturally exposed to in the wild. These shifts in diet and environment can cause changes in the gene expression of the ants, affecting downstream behavioral and physiological systems. To examine the nature of these changes, colonies of the Argentine ant, *Linepithema humile* (Mayr, 1868), were excavated from North Carolina and transferred to the lab, where they were sampled monthly. Illumina and qPCR analyses were conducted on forager samples to detect any changes in gene expression. Approximately six percent of the Argentine ant genome, which represents 765 genes, showed changes in gene regulation after six months in the laboratory environment. The subset of these genes examined via qPCR show that the expression of many genes are correlated with each other, indicating that these genes might be a part of a regulatory network. These findings showed that ant colonies kept in the lab experience changes in gene expression, resulting in downstream effects. Therefore, lab ant colonies are not necessarily representative of wild colonies when conducting experiments on the gene expression, behavior, and physiology of these colonies.

1. Introduction

Eusocial insect societies are characterized by three things: cooperative brood care, overlapping generations of individuals, and a reproductive division of labor (Wilson, 1971). Much research has been conducted in order to understand the behavioral and genetic systems underlying eusocial behavior (Toth and Robinson, 2007; Wheeler, 1986). A major part of eusociality is the reproductive division of labor between gynes and sterile workers. The way these divisions are created and maintained in the colony can vary (Anderson et al., 2008). Genes can control caste differentiation through developmental pathways at the larval stage, expression differences in adults, or through differential zygosity restricting developmental pathways (Drapeau et al., 2006; Smith et al., 2008; Toth and Robinson, 2007). Hormonal cues are also used to maintain these separate developmental pathways (Robinson, 1987). Larvae can be pushed into different developmental pathways through behaviors such as different diet and nutrition levels provided by workers in the colony (Drapeau et al., 2006; Patel et al., 2007). Behaviors such as egg policing by workers, queen activity, or aggression by fertile workers can also be used to directly maintain the reproductive division in primitively eusocial colonies, where there is minimal physiological difference between the fertile and sterile castes (Gamboa and Breed, 1977; Monnin and Ratnieks, 2001; Ratnieks, 1988). However, until recently large scale studies into the gene networks that regulate these mechanisms has been impractical due to having no feasible way to generate and analyze large quantities of genomic and transcriptomic data. However, technological progress has rendered practical the analysis of expression profiles of an entire genome, allowing for comparisons between different eusocial insect castes, subcastes, and sexes (Friedman and Gordon, 2016).

When doing long-term studies on insects, many researchers find it beneficial to establish laboratory populations for ease of collecting samples and running experiments. This is especially useful for the study of ant colonies, as it provides easy access to reproductive and immature life stages, which are normally difficult to find in the field. However, prior research has shown that keeping insects in the lab over multiple generations can cause shifts in their behavior and physiology resulting in loss of genetic variation or changes to tolerance of heat, desiccation, or UV exposure (Hoffmann and Ross, 2018; Jandt et al., 2015). In regards to gene expression, laboratory adaptation has been shown to

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decrease amylase and cytochrome P450 expression and increase hexamerin expression in housefly populations (Højland et al., 2014a). However, very little research has been done on the effect of laboratory rearing on the expression of genes, and the research that has been done is typically conducted on short lived species like mosquitos and houseflies (Aguilar et al., 2011; Højland et al., 2014b, 2014a). Since gene expression is primarily used for protein production, calculating gene expression levels and how they change can serve as a proxy for determining how modifications in protein production are affecting the physiology and behavior of lab specimens. This is especially important information when considering the plethora of gene expression research being conducted on ants and the fact that the lack of generational changes leaves gene expression the only avenue through which laboratory adaptation can occur.

The purpose of this research was to examine how the Argentine ant transcriptome changes when colonies were taken from the field and raised in a laboratory environment. Two methods were used to discover any potential changes. First, the entire transcriptome was compared between field and lab ants after six months to determine if there were any particular gene families or functional categories that changes are focused in. Second, a subset of the genes found to be differentially expressed in the first objective were analyzed on a month-by-month basis to see at what point gene expression starts to change.

2. Materials and methods

2.1. Illumina transcriptome analysis

2.1.1. Colony retrieval and sampling

Four nests of the Argentine ant, *Linepithema humile* (Mayr, 1868), were dug up from soil around Forsyth Technical Community College in Winston-Salem, North Carolina. Nests were collected within 30 m of each other, ensuring that they were part of the same supercolony and thus functionally treat each other as nestmates. The nests were transported from the site and placed in Fluon-coated plastic trays in Smith Hall at Purdue University. Foraging worker samples were taken from each nest immediately upon arrival at Purdue. The nests were extracted from the soil and moved to test tubes wrapped in aluminum foil in the same plastic trays. Nests were kept in the lab for a period of six months, provided water and sugar water *ad libitum*, and fed on a diet of cockroaches and modified Bhatkar-Whitcomb diet (Bhatkar and Whitcomb, 1970). Every month, a sample of foragers were taken from each colony and kept in RNAlater at −80 °C until RNA extraction was done. All samples taken consisted of a single aggregate sample of foragers for each colony replicate weighing in the 20–40 mg range. Identical collection and maintenance procedures were performed for colonies used in later qPCR analyses, with the exception that the aggregate forager samples taken from each colony were restricted to 20 mg in weight.

2.1.2. RNA extraction and quantification

Ant samples were homogenized using a motorized micropestle apparatus. The homogenized ant samples had their RNA extracted using an SV Total RNA Isolation System Kit (Z3101, ProMega Corporation, 2800 Woods Hollow Road, Madison, WI 53711). RNA was quantified using a NanoDrop 2000 spectrophotometer (ND-2000, Thermo Fisher Scientific, 168 Third Avenue, Waltham, MA 02451). Aliquots of RNA suspension for all 1-month and 6-month samples containing 200 ng of RNA were submitted to the Purdue University Genomics Core Facility for Illumina HiSeq analysis. Further aliquots of 200 ng RNA were converted to cDNA using a SensiFAST cDNA Synthesis Kit (BIO-65053, Bioline USA Inc, 305 Constitution Drive, Taunton, MA 02780).

2.1.3. Transcriptomic analysis using Illumina HiSeq

RNA aliquots were analyzed for RNA integrity via RIN scoring (Mueller and Schroeder, 2004). RNA was subjected to ribodepletion to remove unwanted rRNA from the samples using a TruSeq Stranded Total RNA Library Prep Gold kit (20020598, Illumina, 5200 Illumina Way, San Diego, CA 92122). Ribodepleted RNA samples were processed using a Library Preparation Kit (KR1139, Kapa Biosystems, 200 Ballardvale St, Suite 350, Wilmington, MA 01887) and analyzed using an Illumina HiSeq 2500. FASTQ read data were submitted to NCBI Sequence Read Archive under BioProject PRJNA539943. Data from the Illumina assay were submitted to Purdue University Bioinformatics Core Facility for analysis.

2.1.4. Bioinformatic analysis of Illumina data

Sequence data quality was determined using FastQC software (version 0.11.2). Quality trimming was performed using FASTX-Toolkit (version 0.0.14) to remove the bases with less than Phred33 score of 30, and resulting reads of at least 50 bp were retained (which comprised > 99% of total reads for most samples). Reads were sorted into rRNA and non-rRNA fractions using sortMERNA tool and non-rRNA read fraction was utilized for all downstream analyses.

Non-rRNA reads were mapped against the indexed *L. humile* reference genome using STAR aligner (version 2.5.2b) with default parameters. STAR derived mapping results and annotation (GFF) file for the reference genome were fed to the HTSeq package (version 0.7.0) to obtain read counts for each gene feature for each replicate. Counts from all replicates were merged together using custom Perl script to generate a gene counts matrix for both samples (1-month and 6-month). Genes with 0 counts across all replicates were discarded from the counts matrix. When genes had 0 counts in one sample but not in others, the counts were converted from 0 to 1 to avoid having infinite values being calculated for fold change. The final combined counts matrix was utilized for further differential gene expression (DGE) analysis by DESeq2 and edgeR packages. Additionally, DGE was calculated using the tuxedo protocol which directly used STAR mapping files (bam) instead of count matrix. The tuxedo protocol uses Fragments per kilobase of exon per million reads mapped (FPKM) which is corrected (normalized) for the length of the gene and the library size to represent the gene-expression as compared to raw counts in edgeR and DESeq2.

DGE analysis between 1-month (field) and 6-month (lab) samples was carried out using the 'R-Bioconductor' package (version 3.3.2) and two different methods (**DESeq2** and **edgeR**). Both edgeR (version 3.16.5) and DESeq2 (version 1.14.1) use the negative binomial distribution based data model and perform specific estimate variance-mean tests. Both methods determine differentially expressed genes with *P*-value and adjusted *P*-values of false discovery rate (FDR) to correct for multiple tests. The quality of counts matrix was verified by determining basic statistics such as data range and matrix size prior to statistical tests. The DEseq2 package provides methods to test for DGE by use of negative binomial generalized linear models, the estimates of dispersion (measure for sample variance) and logarithmic fold changes. DESeq2 applied Empirical Bayes shrinkage for dispersion estimation and Wald test was used for significance testing and DGE. In the **edgeR** package, an edgeR object was created using the counts matrix, and providing library sizes and experimental design. Normalization factors were calculated for the counts matrix, followed by estimation of common dispersion of counts. EdgeR package performed an 'exact' test to calculate DGE. The tuxedo protocol starts with combined mapping files for each sample which are then processed through the **Cufflinks8** (version 2.2.1) suite of programs (Cufflinks, Cuffmerge, Cuffquant and Cuffdiff) to determine DGE. Briefly, cufflinks performs the transcript assembly for each sample each replicate, cuffmerge combines the assemblies into a master transcriptome, cuffquant calculates the genes and transcript expression profiles, and cuffdiff compares these expression profiles to determine DGE. A pairwise comparison of Control and Treatment samples was performed using cuffdiff with default parameters. Each replicate was used to build a model, then these models are averaged to provide a single global model representing all conditions in the experiment and used for dispersion estimate. A *t*-test was performed to measure the DGE with *P*-values and also calculated adjusted *P*-values

of false discovery rate (FDR) to correct for multiple tests. A gene was considered as exhibiting differential gene expression when two or more methods detected differential gene expression in that gene. Average FC data, counts per million, and p-values were used to create scatterplot comparing CPM to average FC. Associated scripts and data were deposited on Github (DOI https://doi.org//10.5281/zenodo.3228443).

2.1.5. Blast2GO analysis

Genes showing a significant change in expression were separated into upregulated and downregulated categories, and each group was further broken down into high, medium, and low categories to denote the magnitude of the change. For each list of genes, protein sequence data was obtained from GenBank and loaded into BLAST2GO (Version 4.1). Sequence data were blasted on GenBank, to obtain descriptive information for genes. After sequences were identified, genes were mapped and annotated using default settings. InterPro was also searched for sequence hits and the results merged into annotation. Gene Ontology (GO) terms were then graphed for molecular function, and common GO terms were obtained for each category of genes.

2.2. Quantitative real time PCR (qPCR) analysis of selected transcripts

2.2.1. RNA extraction and quantification

Ant sample replicates from fresh Month 0–6 samples were homogenized using a motorized micropestle apparatus. Four sample replicates were taken for each time point, consisting of an aggregate sample of foragers weighing 20 mg. The homogenized ant samples had their RNA extracted using an SV Total RNA Isolation System Kit (Z3101, ProMega Corporation, Madison, WI 53711). RNA was quantified using a NanoDrop 2000 spectrophotometer (ND-2000, Thermo Fisher Scientific, Waltham, MA 02451). Aliquots of 200 ng RNA were converted to cDNA using a SensiFAST cDNA Synthesis Kit (BIO-65053, Bioline USA Inc, Taunton, MA 02780). cDNA was aliquoted into 10 µL aliquots and stored at −80 °C.

2.2.2. qPCR target selection

Illumina data were searched for genes from specific physiological and functional categories that show significant changes in gene expression. Thirteen genes showing diversity in direction and magnitude of expression change were chosen for qPCR analysis. Five genes that showed no changes in expression were included for comparison.

2.2.3. qPCR analysis

The cDNA aliquots synthesized from 200 ng RNA were diluted 1/10 to ensure enough sample was present to conduct all analyses. Sequence data for each of the chosen targets was obtained from the transcriptome sequence data, and primers were generated from these sequences using NCBI Primer-BLAST. qPCR reactions were done using a SensiFAST SYBR No-ROX Kit (BIO-98005, Bioline USA Inc, Taunton, MA 02780).

2.2.4. Statistical analysis

For each gene, ΔCt values for lab samples (Month 1 through Month 6) were compared to field samples (Month 0) on a month to month basis via Mann-Whitney U-Test. Fold change was calculated through the ΔΔCt method. In addition, the ΔC_t values for each of the genes were compared to each other in a regression analysis. Regression analysis script and associated data were deposited on Github (DOI:https://doi.org//10.5281/zenodo.3228443).

3. Results

3.1. Illumina transcriptome analysis

All samples had between 20 million and 45 million total reads mapped to transcriptome. Overall, the colony Illumina samples clustered more closely together based on time point (Month 1 vs. Month 6) rather than by colony identity (Fig. 1). Transcriptomic analysis discovered a total of 765 transcripts that were differentially expressed between lab and field samples, representing 6% of transcriptome (Fig. 2). Of those 765 transcripts, 735 were protein-coding genes while the remaining 30 transcripts were non-coding sequences (Table 1). Most of the differentially expressed genes were downregulated (79%), although most of them only showed an average log2 fold change between 0 and −1 (77%) (Table 1). There were a small amount of genes that were upregulated (21%), and a narrow majority of them (58%) had an average log2 fold change between 0 and 1 (Table 1).

Gene Ontology (GO) analysis indicated that the most common GO terms for both upregulated and downregulated genes were for binding and catalytic activity (Table 1). The binding category was often further broken down into ion binding, protein binding, heterocyclic and organic compound binding. The catalytic activity category was further

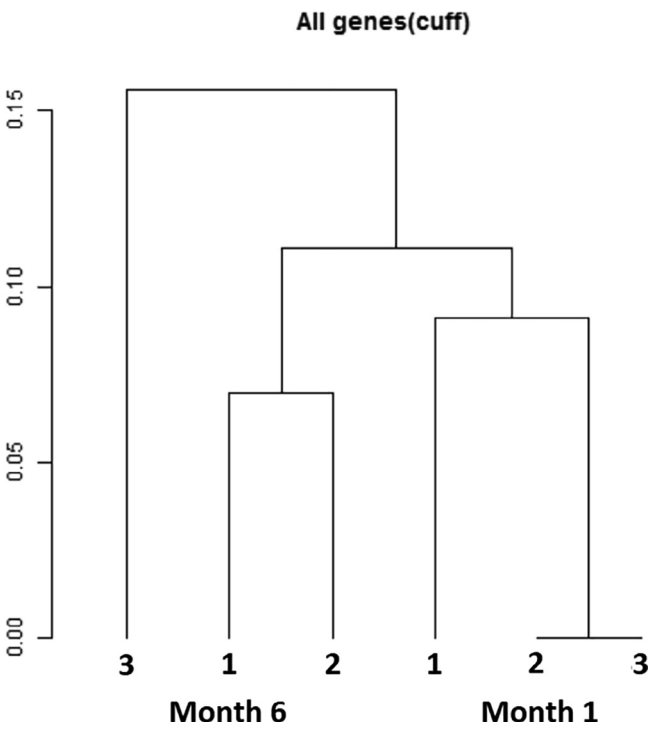


Fig. 1. Dendrogram of Month-6 and Month-1 colony samples submitted for transcriptomic sequencing and analysis. Samples cluster together by time point rather than colony number (numbers represent different colony samples).

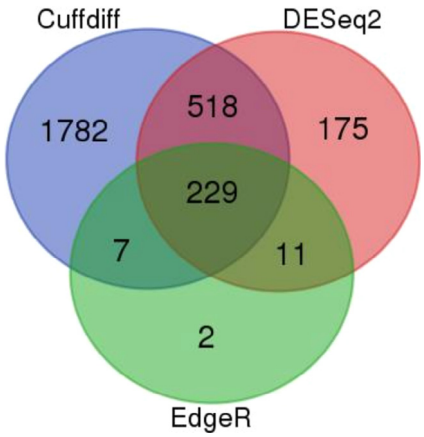


Fig. 2. Venn diagram showing the number of significant differentially expressed genes discovered by each of three transcriptome analysis methods: *Cuffdiff*, *DESeq2*, and *EdgeR*.

Table 1
Frequent GO terms for up- and downregulated genes, organized based on magnitude of change. Binding and catalytic activity GO terms show frequent expression changes.

GO #	GO Term	# of Sequences	GO #	GO Term	# of Sequences
High Downregulation (−2 < Avg Log2 FC)		33	High Upregulation (Avg Log2 FC > 2)		5
GO:0005488	Binding	12	GO:0003824	Catalytic Activity	2
GO:0005515	Protein Binding	5	GO:0016787	Hydrolase Activity	2
GO:0016787	Hydrolase Activity	4			
GO:0022892	Substrate-Specific Transporter Activity	4			
GO:0097367	Carbohydrate Derivative Binding	4			
GO:0043167	Ion Binding	4			
Unannotated		16	Unannotated		3
Medium Downregulation (−2 < Avg Log2 FC < −1)		100	Medium Upregulation (2 > Avg Log2 FC > 1)		61
GO:0005488	Binding	43	GO:0003824	Catalytic Activity	25
GO:0003824	Catalytic Activity	34	GO:0005488	Binding	21
GO:1901363	Heterocyclic Compound Binding	28	GO:0016787	Hydrolase Activity	11
GO:0097159	Organic Cyclic Compound Binding	28	GO:0005515	Protein Binding	10
GO:0043167	Ion Binding	20	GO:0016491	Oxireductase Activity	8
GO:0016787	Hydrolase Activity	16	GO:0043167	Ion Binding	8
GO:0005515	Protein Binding	15			18
Unannotated		33	Unannotated		18
Low Downregulation (−1 < Avg Log2 FC)		446	Low Upregulation (1 > Avg Log2 FC)		90
GO:0005488	Binding	242	GO:0005488	Binding	27
GO:0005515	Protein Binding	119	GO:0003824	Catalytic Activity	19
GO:0097159	Organic Cyclic Compound Binding	112	GO:0005515	Protein Binding	16
GO:1901363	Heterocyclic Compound Binding	112	GO:0005198	Structural Molecule Activity	11
GO:0003824	Catalytic Activity	96	GO:0097159	Organic Cyclic Compound Binding	11
GO:0043167	Ion Binding	88	GO:1901363	Heterocyclic Compound Binding	11
GO:0003676	Nucleic Acid Binding	69			
Unannotated		135	Unannotated		35

broken down into primarily hydrolase activity, although oxidoreductase and structural molecule activity was also represented.

The scatter plot (Fig. 3) shows that while many more genes showed upregulation in the transcriptome, a large portion of them were not significantly different between lab and field samples. Although the number of downregulated genes was more limited, a larger portion of them were found to be significant, and there were more genes that exhibited both a large downregulation and a larger CPM. Overall, the genes that are responsive to the transition tended towards reducing expression.

3.2. qPCR analysis

Results for the qPCR analysis were broken up into five graphs based on general functional categories that describe the genes. The first gene category encompasses genes involved with immune defense and detoxification of allelochemicals (Fig. 4A). In this category, genes from the cytochrome p450 6A and 6B families show changes in gene expression over months 1–6 of lab rearing. The 6A-like gene shows upregulation from month 4 onwards, while the 6B-like gene shows sudden downregulation at month 2 before returning to prior levels.

The second gene category encompasses genes involved with storage of amino acids and sugars (Fig. 4B). In this category, hexamerin shows strong and consistent upregulation after month 0, while apolipophorins and arylphorin show weaker and less consistent upregulation. Fatty acid synthase shows no change in gene expression at any time.

The third category encompasses genes that are associated with digestion (Fig. 4C). The only gene to show any kind of changes in this group is lysosomal aspartic protease, which shows a sudden and strong upregulation from month 1 onwards.

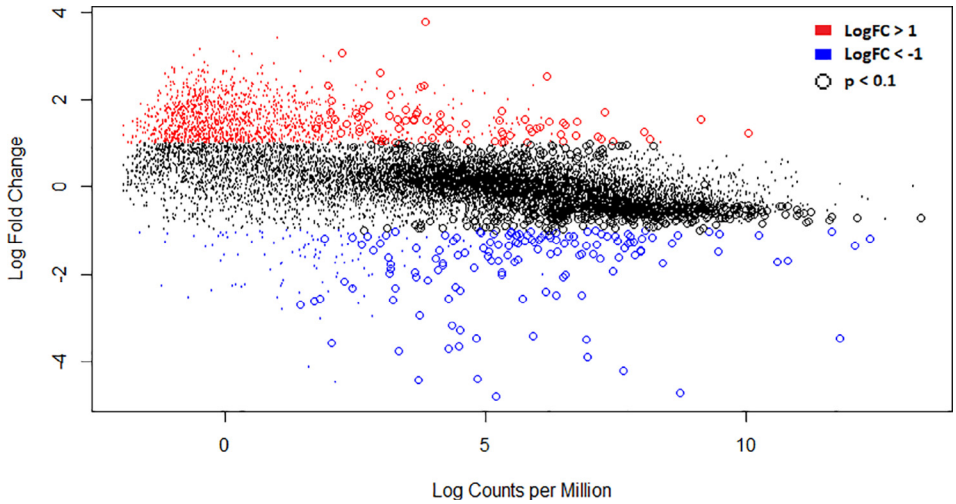


Fig. 3. Scatter plot of Log Counts/Million vs average Log2 Fold Change data calculated from the three analytical methods used in the transcriptomic analysis, Cuffdiff, DESeq2, and EdgeR. Genes were considered significantly differently expressed if 2+ analysis methods designated them as such.

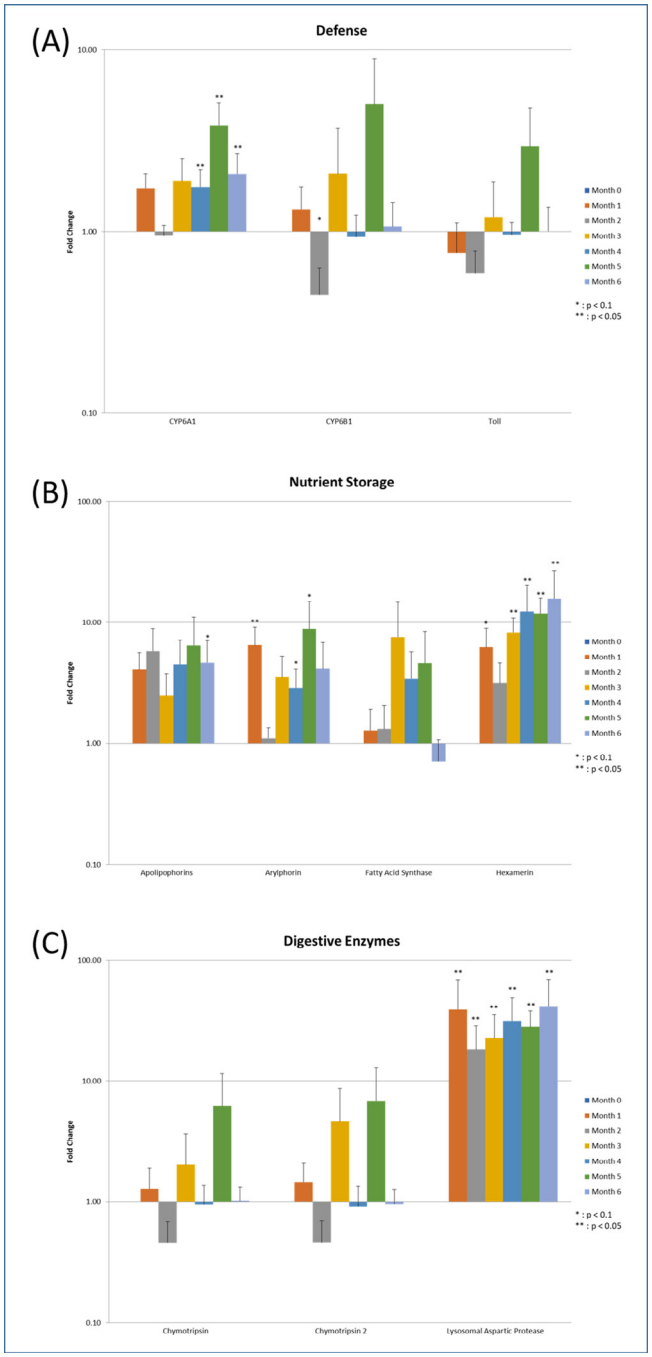


Fig. 4. Monthly fold change data for eighteen target genes as determined by qPCR analysis. Month 0 results are not visible due to their normalization to 1.0. Different panels show genes from different categories involved in: (A) defense, (B) nutrient storage, (C) digestion, (D) oogenesis, and (E) miscellaneous processes (see text for details).

The fourth category encompasses genes associated with oogenesis (Fig. 4D). Vitellogenins 1 and 3 both showed downregulation in month 2, but showed no further changes. The other two genes showed no changes in gene expression during the time points sampled.

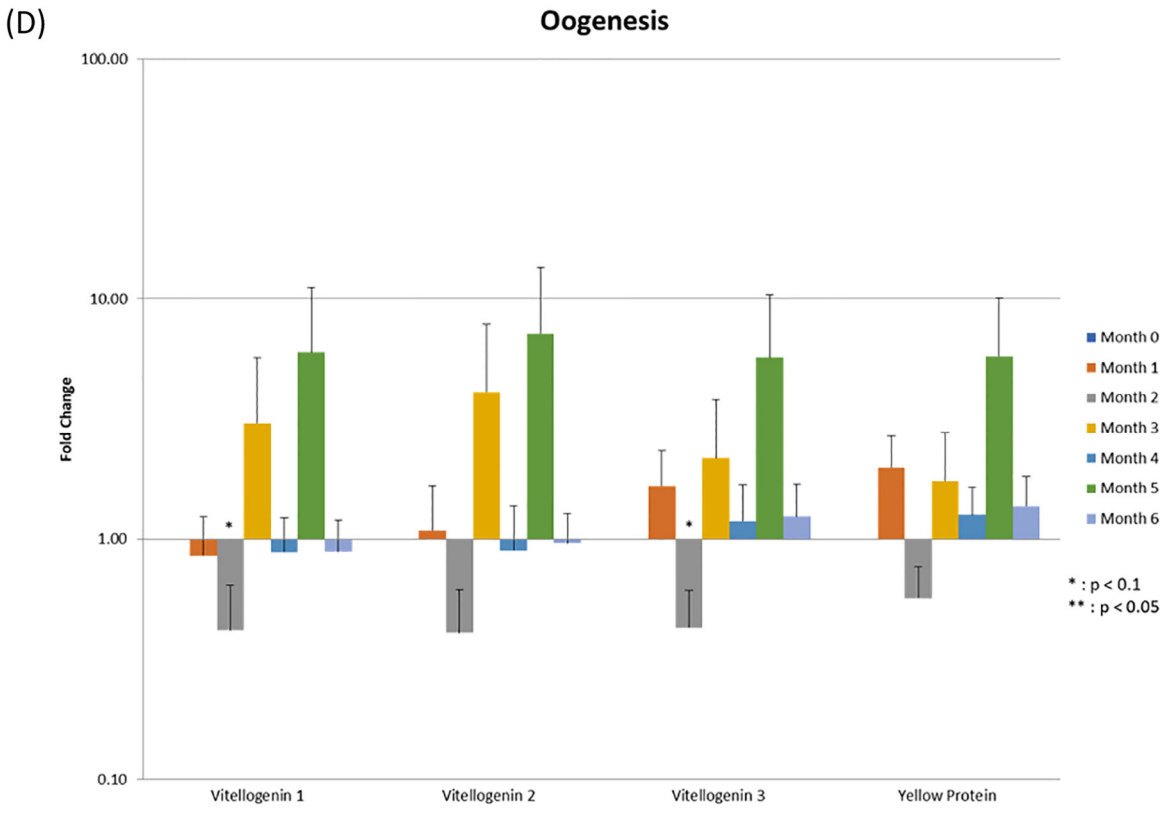
The final gene category encompasses genes that didn't fit into any specific category (see Fig. 4E). In this category, the genes sialin and clavesin both exhibited consistent upregulation from month four onwards. Aside from brief upregulation of adenosylhomocysteinase at month 5, there were no other changes in gene expression.

Finally, many of the qPCR target genes show high intercorrelation with other genes when comparing plots of their monthly qPCR ΔC_t values to one another (Fig. 5). For example, some instances of correlation are to be expected, such as vitellogenins exhibiting high intercorrelation due to their sequence homology and function within the organism. But these same vitellogenins also exhibit high correlations with a diverse array of genes such as cytochrome p450s, chymotrypsins, and desaturases. There are a number of these correlations present in the qPCR dataset, such as cytochrome p450s and chymotrypsins or adenosylhomocysteinase and digestive enzymes. These correlations suggest the presence of a network or networks of interconnected genes present within the Argentine ant genome that are involved in adaptive responses to environmental changes.

4. Discussion

While the vast majority of expressed transcripts remain at similar levels despite the environmental changes, there does appear to be a change in the expression of a portion of the Argentine ant transcriptome. Prior evidence indicates that large gene networks, spanning many diverse functional categories can influence behavioral changes in workers (Whitfield et al., 2003). For example the vitellogenins, a gene family of ancestral yolk protein precursors, have developed diverse functions within eusocial genomes, such as control of worker foraging activity in some eusocial insects (Nelson et al., 2007). However, how these networks interact and change eusocial insect phenotypes still needs to be teased apart. The question then becomes: which factors are driving which of the changes within the group of genes that are responding to this environmental transition? The transition from field to laboratory living is not a singular, easily isolated phenomenon, but a change in a number of different systems, each with their own downstream effects. The lab environment maintains a constant temperature and humidity unlike field environments, which can affect the foraging behavior of workers (Krushelnycky et al., 2005). There are no seasonal environmental changes, so the ants never experience the behavioral shifts that correspond to seasonal changes in temperature or photoperiod, such as changes in foraging patterns, queen execution, or production of reproductives (Keller et al., 1989; Rust et al., 2000). Nesting and foraging microenvironments are plastic, metal and glass, which offer much different protections from desiccation than the soil, plants and stone found in the field. Lab surfaces are sanitized and free from potentially hostile fungal, bacterial, or plant allelochemicals. Nourishment is abundant and free of contamination, which can accelerate colony growth and development, both due to the increase in available nutrients and the reduced need for detoxification genes, such as cytochrome p450s, for defense against harmful chemicals in the diet. Changes in diet composition can also affect the cuticular hydrocarbon profiles that colonies use to differentiate nestmate from conspecific, by changing the hydrocarbons produced by the insect (Liang and Silverman, 2000). Diet is also one of the major determinants of the fauna present within the ant gut microbiome, which could potentially have symbiotic interactions with its host like other insects (Hu et al., 2014; Scharf et al., 2011; Wada-Katsumata et al., 2015).

The qPCR analysis was restricted to a small set of genes, but revealed detailed changes in gene expression/transcript abundance from month 0 to month 6. Comparing each of the genes to one another via pairwise regression scatterplots revealed that many of these genes shows similar co-expression, indicating that the genes showing differential regulation as a result of the lab-to-field transition might be part of a gene network or networks. While the exact relationships between some of these seemingly co-expressed genes are difficult to ascertain, some of them show comparatively obvious relationships. The first relationship within the data shows that nutrient storage and transport proteins such as arylphorin and hexamerin are upregulated in the later months following laboratory introduction and show significant correlation in their expression with one another (Fig. 5) (Burmester, 1999; Telfer et al., 1983; Weers and Ryan, 2006). Previously, research seemed to indicate that these storage proteins were typically only present in



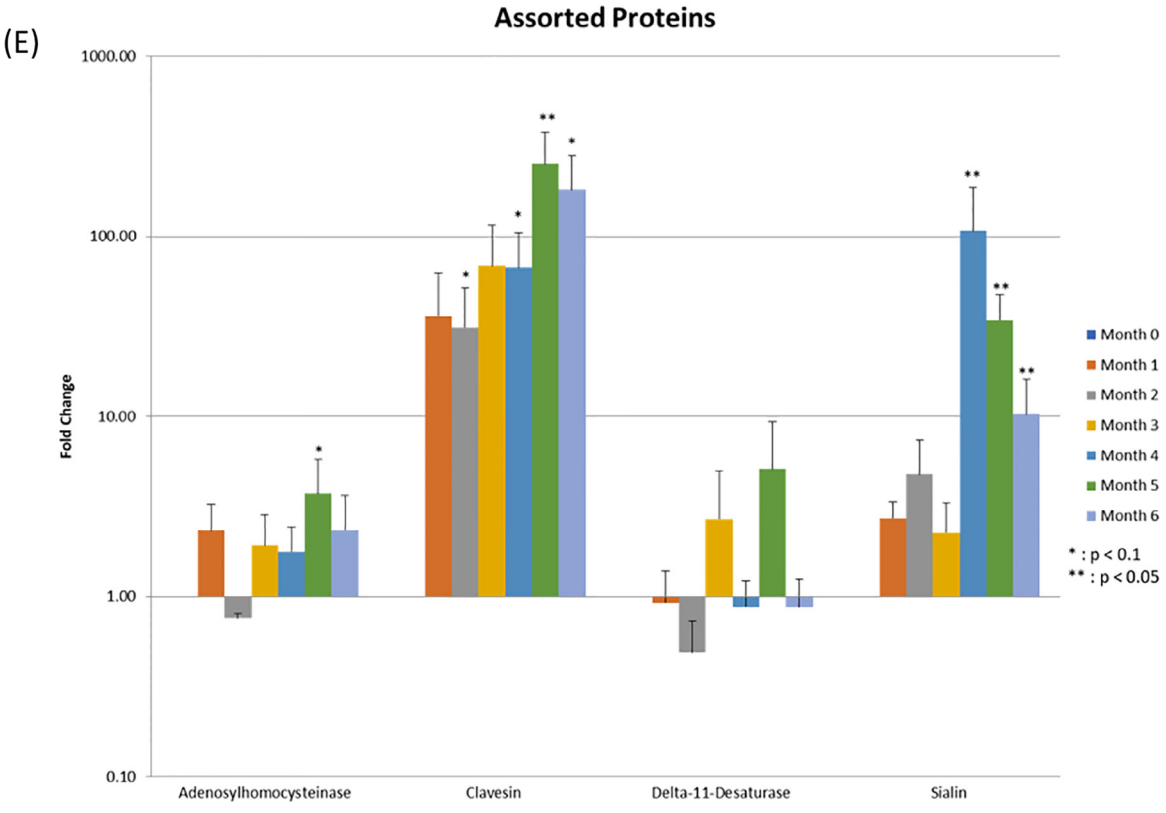


Fig. 4. (*continued*)

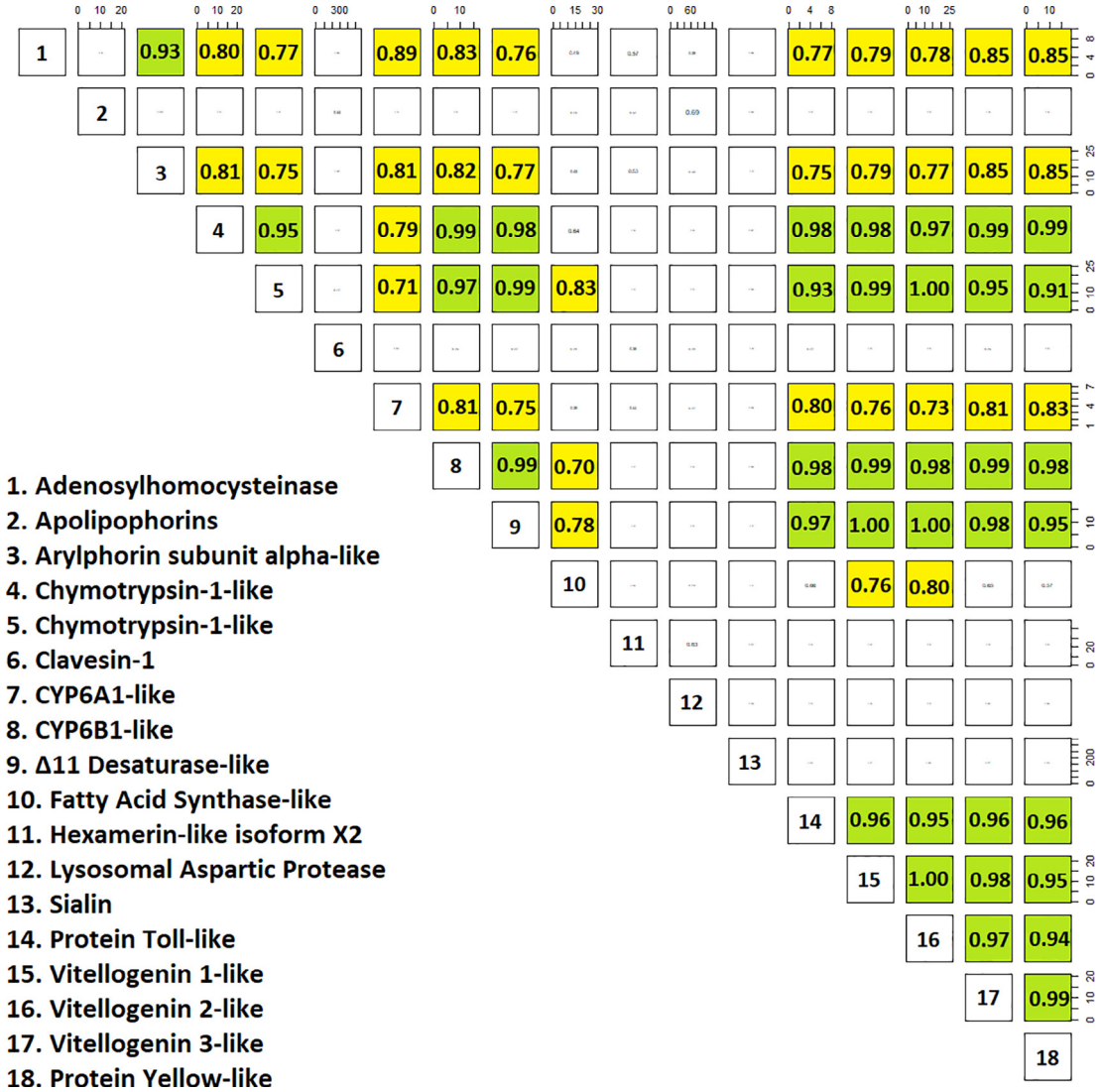


Fig. 5. LOWESS plots with r^2 values comparing qPCR-ΔCt values among eighteen target genes. The numbers 1–18 represent the gene identities as shown. Comparisons having r^2 values > 0.9 are highlighted in green and those between 0.7 and 0.9 are highlighted yellow.

immature stages, and disappear from the adult insect proteome (Burmester, 1999). However, ants have been shown to express storage hexamerins into their adult stages (Martinez and Wheeler, 1993). Ant colonies kept in the lab are typically given constant access to nutrients, whereas field colonies need to forage and thus are subject to resource scarcity, competition, and worker loss. Therefore, since nutrients are much more easily accessible in a laboratory environment (due to constant access to sugar water and regular additions of dead insect matter and artificial diet), workers are able to gather more nutrients with less energy expenditure. Lab workers appear to take advantage of this nutrient abundance by producing more proteins to adequately store and transport them within the body. However, worker ants tend to accumulate storage proteins in the hemolymph and fat body as brood production falls, typically in the fall (Wheeler and Martinez, 1995). Therefore, an alternative explanation is that the ant colonies are still able to determine seasonal changes despite being kept under stable light conditions. However, while research does indicate that circadian rhythm and clock genes are involved in seasonal shifts in gene expression, it requires a change in photoperiod as well (Koštál, 2011; Saunders, 2010). There does not appear to be any research in the literature indicating that a “circadian calendar” gene is solely responsible for seasonal behavioral or genetic shifts in Argentine ants, so the only potential evidence for this phenomenon is the storage protein gene expression patterns described in this work.

The second major relationship involves a trio of genes: *clavesin*, *sialin*, and *lysosomal aspartic protease* (LAP). Aspartic proteases are primarily involved in either digestion of food in the digestive tract or degradation of cellular debris within lysosomes (Cho et al., 1991; Dittmer and Raikhel, 1997; Terra et al., 1994). Additionally, *lysosomal aspartic protease* has been specifically implicated in the breakdown of excess vitellogenin in the fat body after oogenesis takes place (Dittmer and Raikhel, 1997; Raikhel, 1986). Clavesins are a family of genes that are found in neuron cells, where they are involved in lysosome formation and morphology (Katoh et al., 2009). *Sialin* is a transport protein that transports aspartate and glutamate into synaptic vesicles, and transports sialic acid across lysosome membranes (Miyaji et al., 2011). Sialic acids are a versatile group of molecules, with a variety of functions (Schauer, 2000). In particular, *sialin* and sialic acid have been found within neurons of *D. melanogaster* (Laridon et al., 2008; Roth et al., 1992). Together, the three genes LAP, *clavesin*, and *sialin* are all involved with lysosomes, and *clavesin* and *sialin* are both associated with lysosomes in neuron cells. These genes also show correlation in their expression with one another (Fig. 5). The function of LAP within the Argentine ant remains unclear, however. Considering the upregulation in *clavesin* expression, it could be produced for neuronal lysosomes. However, research has shown that Argentine ant workers are

functionally sterile, producing no eggs even when colonies lack queens (Passera et al., 1988). It is also possible that LAP is one of the factors keeping Argentine ants from producing eggs by degrading any vitellogenin that is produced for oogenesis in the fat body, and thus LAP is potentially involved in the maintenance of the reproductive division of labor present in the colony. Since increased diet in Argentine ant queens is correlated with increased fecundity, it is possible that the abundant diet available to the colony results in more resources available for worker oogenesis, and a commensurate increase in LAP to prevent worker egg development (Keller et al., 1989).

In addition to the upregulated genes, some of the genes that show downregulation in the transcriptome analysis have important implications as well. For instance, LOC105670541 is similar to a subunit 15 in the Mediator complex, which is involved in regulating the transcription of RNA polymerase-II dependent genes (Poss et al., 2013). *Mdt-15* has been implicated in many regulatory processes in *Caenorhabditis elegans*, such as regulating lipid metabolism and xenobiotic detoxification (Taubert et al., 2008). Given that these ants are being raised in a sterile environment, these ants are likely not being challenged by toxins as much as in a field environment, so the genes that regulate expression of detoxification proteins are being downregulated. Another gene being downregulated in the lab ants is a gene similar to vitellogenin receptors. These genes have been shown to have different functionality, such as shuttling vitellogenin into developing oocytes and influencing parental care (Roy-Zokan et al., 2015; Schneider, 1996). The only change in vitellogenin expression shown by the data is a downregulation in *vit-2* in the transcriptome data, which matches the downregulation in *vitR*, so it's unlikely that oogenesis is the cause of this change. Therefore, the downregulation shown in these two genes might have behavioral implications, such as changes in brood care or foraging, especially given the substantial role that vitellogenins play in governing worker behaviors (Corona et al., 2013; Morandin et al., 2014).

The experimental design used in this study does have a limitation. For the six month time point, field samples were not taken to compare to the lab samples, so we cannot be certain that the differences we are seeing between the one month and six month time points is not due to seasonal changes. However, this explanation would require the ants to have a method of tracking the seasons in an environment with no environmental variation that could give the organism information on the current time of year, such as photoperiod or temperature. Current knowledge regarding insect seasonal rhythmicity indicates that insects require environmental cues to drive seasonal changes in behavior or physiology (Bradshaw and Holzapfel, 2010). In addition, if we expect lab-reared Argentine ant colonies to exhibit seasonal changes in gene expression, we can expect them to exhibit typical behavioral phenotypes associated with seasonal changes as well, such as queen execution (Keller et al., 1989). Since colonies that have lived in the lab for extended periods do not exhibit these behaviors, it is reasonable to assume that they have lost the environmental and genetic cues inducing them. Therefore, they likely aren't exhibiting other, less visible, changes in gene expression that are typically associated with seasonality.

In conclusion, the results of this study have two main implications. First, ant colonies collected from the field and raised in a laboratory environment do experience changes in how their genes are expressed. These genes showing differential regulation seem to be involved in interconnected gene networks that have wide ranging effects on physiological systems such as nutrient storage and neuron function. To further elucidate what behavioral and physiological systems are changing as a result of this field-to-lab transition, experiments can be conducted in the lab by manipulating environmental variables. For instance, Argentine ant colonies that have been in the lab for long periods of time will no longer exhibit the seasonal queen execution conducted by workers to kickstart production of reproductive-destined brood (Keller et al., 1989). Creating artificial temperature and/or photoperiod variability could be done to see what cues are necessary for the performance of this behavior. Furthermore, while research has shown that separating out worker groups and feeding them different diets can trigger aggressive behaviors between workers from the same colony (via changes in hydrocarbon decomposition), we don't know the underlying mechanism causing it (Liang and Silverman, 2000). Considering gut microbiota assist in pheromone production in other insect groups like cockroaches, it is plausible that ant gut microbiota are one of the factors involved the creation of different cuticular hydrocarbon blends due to different dietary inputs. Testing this idea could be as simple as feeding worker groups antibiotics for a period of time and reintroducing them to their home colony and seeing if their nestmates will act aggressively towards them. Many other experiments can be done in this way, like testing the effects of plastic vs soil substrates, or nesting substrates and water requirements, in order to properly establish how the laboratory environment can modify the behavior and physiology of the ant colonies kept there. Second, lab and field colonies are not functionally identical in regard to patterns of gene expression. Colonies taken from the field do experience changes in how their genes are expressed as they acclimate to the laboratory environment. Since gene expression has become a popular avenue of research in examining eusocial colony structures in ants, it is important to understand that results derived from gene expression studies conducted on lab colonies are not necessarily going to be applicable to colonies in the field. Thus, field studies should be conducted concurrently whenever feasible.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jinsphys.2019.103901.

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