GENOMICS OF PERMETHRIN RESISTANCE IN THE SOUTHERN HOUSE MOSQUITO, *CULEX QUINQUEFASCIATUS* SAY

by

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Abstract

The Southern house mosquito, *Culex quinquefasciatus*, is a vector of several human diseases including West Nile fever and St. Louis encephalitis, owing to its blood feeding behavior whereby the female takes multiple blood meals. Vector control of mosquitoes has been a critical part of the current global strategy to control mosquito-associated diseases. Insecticides, especially pyrethroids, are important components in the vector-control effort. The successful management of mosquitoes, however, is negatively impacted by the development of resistance to insecticides within mosquito populations and the lack of the knowledge on the molecular basis of blood feeding behavior. The goals of our research, thus, were to gain a better understanding of insecticide resistance in *Cx. quinquefasciatus* and to identify the genes that may be involved in preparing the female for the taking of a blood meal with four specific objectives: 1) Characterize the genes up-regulated in insecticide-resistant mosquitoes, 2) Determine the genes that are differentially-expressed in response to insecticide treatment, 3) Investigate the cytochrome P450 detoxification genes in resistance, 4) Examine the changes in the gene expression profiles of newly-eclosed females as they become old enough to take a blood meal and also in response to blood feeding.

RNA-Seq was used to investigate the gene expression profiles of a highly permethrin-resistant strain of *Cx. quinquefasciatus*, and to investigate the changes in the gene expression levels after exposure to permethrin. Overall, we identified multiple genes up-regulated in insecticide-resistant mosquitoes, including genes involved in detoxification, regulation, and

proteases. We further identified that detoxification genes and proteases were up-regulated in response to permethrin exposure, while serum storage proteins were down-regulated, suggesting that fourth instar *Cx. quinquefasciatus* delay development to the pupal stage in response to insecticide challenge, possibly in response to a cessation of feeding as a means of behavioral resistance in order to reduce oral exposure to permethrin. To better understand which detoxification genes identified in *Cx. quinquefasciatus* were important in other mosquito species, we investigated the gene expression profiles of the cytochrome P450 genes in a pyrethroid-resistant field strain of the yellow fever mosquito, *Aedes aegypti* using qRT-PCR. We identified that multiple cytochrome P450 genes were up-regulated, and functional studies revealed that two of the up-regulated P450s, CYP4D4 and CYP4J15v1 could confer resistance to permethrin when over-expressed in a GAL4:UAS enhancer trap *Drosophila melanogaster* system. These cytochrome P450s were likely involved in the permethrin resistance response of mosquitoes. Examination of the genes in response to the blood feeding was conducted by determining the temporal changes in gene expression from newly eclosed female adults to those capable of taking a blood meal using RNA-Seq techniques. We found that while no females would freely take a blood meal prior to 48 h post-eclosion, the main gene expression changes in newly-eclosed females occurred within the initial 12-24 h post-eclosion, including genes encoding salivary proteins, odorant-binding proteins, proteases, and cuticular proteins. A smaller second peak of up-regulated genes was identified at 48 – 60 h post-eclosion, which coincided with the onset of the maximal time-to-mating for *Cx. quinquefasciatus.* Taken together, these results suggested that the genes needed for blood feeding in *Cx. quinquefasciatus* are primarily up-regualted within 12-24 h post-eclosion, while other later genes may be up-regulated in response to mating.

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List of Abbreviations

ANOVA	Analysis of Variance
BDRC	Bloomington Drosophila Resource Center
cDNA	copy Deoxyribo Nucleic Acid
DDT	Dichloro Diphenyl Trichloroethane
DEF	S,S,S- tributyl phosphorotrithioate
DEM	diethyl maleate
DNA	Deoxyribonucleic Acid
FDR	False discovery rate
FPKM	Fragments per kilo base of gene for every million reads mapped
GABA	gamma amino butyric acid
GAL4:UAS	Galactose 4: Upstream Activator Sequence
GEO	Gene Expression Omnibus (NCBI)
GO	Gene Ontology
GRN1	gustatory receptor neuron 1
GRN2	gustatory receptor neuron 2
HAIB	Hudson Alpha Institute for Biotechnology
IMD	immunodeficient
Kdr	knock down resistant
LC_{50}	Lethal concentration that kills 50% of the population

LC_{70}	Lethal concentration that kills 70% of the population
LPS	lipopolysaccharide
m/tr	metabolism/transport
mRNA	messenger RiboNucleic Acid
NCBI	National Center for Biotechnology Information
NONA	Non annotated
nt	nucleotide
OP	Organophosphate
P450	Cytochrome P450
PBO	Piperonyl Butoxide
PCR	Polymerase Chain Reaction
Ppm	parts per million
qRT-PCR	quantitative Real Time Polymerase Chain Reaction
Rdl	resistance to dieldrin
RNA	Ribonucleic acid
RNA-Seq	Ribonucleic acid sequencing (massively parallel)
RPKM	Reads per kilo base of gene for every million reads mapped
SCOP	Structural Classification of Proteins
SNP	Single Nucleotide Polymorphism
TOR	target of rapamycin
vitA	vitellogenin A
Vssc	Voltage sensitive sodium channel
WHO	World Health Organization

Chapter 1: Literature Review

1.1 Insects and insecticides

Insects consume or spoil roughly one-third of all food/fiber produced for human consumption and use (Johnson and Triplehorn, 2004). Insects are also direct parasites of humans, and some can vector the etiological agents of several diseases (Lounibos, 2002). In order to combat the losses of food, fiber, and to decrease the transmission rates of human pathogens by insects, chemicals (insecticides) have been developed to kill pestiferous insects (Casida and Quistad, 1998). Insecticides work by directly targeting a vital system or process of the insect, including systems such as the nervous, integument, or muscular systems, and processes such as development and molting (Yu, 2008). As a result, insecticides target specific molecules within the insect and, when applied at toxic levels, cause specific symptoms. For example, the insecticides methoprene and pyriproxyfen are mimics of juvenile hormone that disrupt the molting process of immature insects, notably killing holometabolous insects as they enter the pupal stage (Dhadialla et al., 1998). Other insecticides, such as organophosphates, target the nervous system of insects by inhibiting the action of acetylcholinesterase, which prevents the degradation of the neurotransmitter acetylcholine in insects, resulting in a sustained nervous impulse that ultimately results in death of the insect (Casida and Quistad, 1998; Yu, 2008). While insecticides provide protection to food, fiber, animals, and humans, their overuse or improper use can ultimately lead to their failure. The first documented failure of an insecticide to manage an insect pest was recorded for Paris Green, which was used for the management of the San Jose scale in the Western US (Melander, 1914). Insecticide resistance is largely believed to be a pre-adaptive phenomenon resulting from the selection of genetically-based trait(s) within a

population of insects, resulting in the ability of the target insect pest to resist the insecticide challenge. The repeated exposure of insects to insecticides led to genetic changes within the insect populations to increase the proportion of individuals within the population that harbor genetically-based traits that confer insecticide resistance (Georghiou, 1972; Liu 2008).

1.2 Insecticide resistance

Insecticide resistance is a phenomenon where an insecticide fails to kill the target insect. The resistance to insecticides within populations of insects is largely believed to be pre-adaptive, in that individuals within the population harbor traits that confer the tolerance/resistance to insecticides, which upon challenge with an insecticide results in the selection of these traits that are then passed on to successive insect generations (Georghiou, 1972; Liu 2008). As such, insecticide resistance has a genetic basis. As a consequence of the genetic basis and subsequent trait/gene selection, the repeated use of an insecticide against an insect population results in an enrichment of insecticide resistance traits/genes within the population. Thus insecticide resistance arises from the repeated use of insecticides which enriches the prevalence of these traits/genes within in the population, ultimately resulting in a failure of insect control (Georghiou and Saito 1983).

1.3 Mechanisms of insecticide resistance

Insecticide resistance can be broadly classified into two categories: physiological and behavioral (Yu, 2008). Physiological resistance can be further characterized into: target-site insensitivity, metabolic resistance and reduced uptake/acquisition. The first category, target-site insensitivity, is when there is a physical change in the target site resulting in a decreased efficacy

of the chemical. The second category, metabolic resistance, occurs due to the ability of the insect to metabolize the insecticide into less-toxic, more easily excretable forms. The third category, reduced uptake/acquisition, represents changes in the cuticular structure of the insect that results in less of the insecticide being absorbed. Finally the behavioral resistance includes behavioral adaptations of the insect that confer indirect resistance to the insect such as the ability to avoid consuming a toxin/toxicant, or other mechanisms such as the acquisition of endosymbiotic microbes capable of degrading insecticides (Yu, 2008).

1.3.1 Increased metabolic detoxification

Insects encounter multiple naturally-present toxins, for which they have a multitude of enzymes capable of mitigating the negative impact of the toxins by degrading them into less-toxic, more easily excretable forms, and are able to degrade insecticides as well. The enzymes involved in the degradation of insecticides can be broadly classified into two categories: phase I, and phase II enzymes. Phase I enzymes are the enzymes that are primarily responsible for the degradation and catalyze the oxidation, reduction, and hydrolysis reactions, such as cytochrome P450s and carboxylesterases. Phase II enzymes serve to mitigate the toxic effects of an insecticide by conjugating small molecules to the insecticide such as sugars, amino acids, or glutathione.

1.3.1.1 Cytochrome P450 monooxygenase-mediated detoxification

Of all of the phase I reactions, oxidation reactions, which are carried out by cytochrome P450s, is considered to be the most important (Yu, 2008). Cytochrome P450s are present in all divisions of life (Scott and Wen, 2001), and perform various physiological functions in insects

including the biosynthesis and degradation of ecdysteroids, cuticle formation, fatty acid synthesis and metabolism, as well as the detoxification of xenobiotics (Chavez et al., 2000; Warren et al., 2002; Petryk et al., 2003; Warren et al., 2004; Namiki et al., 2005; Ono et al., 2006; Rewitz and Gilbert, 2008; Guittard et al., 2011, Qiu et al., 2012). The numbers of P450 genes in insects ranges from as low as 37 in the human body louse *Pecidulus humanus* (Lee et al., 2010) to 204 in the Southern House Mosquito, *Culex quinquefasciatus* (Arensburger et al., 2010; Yang and Liu, 2011). Cytochrome P450s are arranged into clans, families, and subfamilies, based on sequence similarity, among which, insect cytochrome P450s are found in clans 2, 3, 4, and mito clan (Feyeresen, 1999). Many studies have investigated the role of cytochrome P450s in insecticide resistance (Strode et al., 2008; Pridgeon et al., 2009; Bariami et al., 2012; Fonseca-Gonzalez et al., 2011; Yang and Liu, 2011; Poupardin et al., 2010; Saavedra-Rodriguez et al., 2012; Strode et al., 2012) and the functionality of selected cytochrome P450s has been tested, notably among mosquito species (Boonseupsekul et al., 2008; McLaughlin et al., 2008; Muller et al., 2008; Stevenson et al., 2011; 2012).

1.3.1.2 Hydrolase-mediated detoxification

Hydrolase (esterase) mediated detoxification has been documented for nearly all insect classes, notably among organophosphorous (OP) insecticides (Li et al., 2007). Hydrolase-mediated resistance has been identified to be due to gene duplication and neo-functionalization of esterase genes in multiple insect species, whereby the amino acid sequences of carboxylesterases become modified to degrade OP compounds (Oppenoorth and Van Asperen, 1960; Campbell et al., 1998; Claudianos et al., 1999). Esterase gene duplication and amplification has also been demonstrated to serve as a means of general protection against

insecticides via sequestration of the toxicant (Field and Devonshire, 1998).

1.3.1.3 Glutathione S-transferase-mediated detoxification

Phase II reactions are conjugation reactions that include the addition of small molecules onto the insecticide, rendering it more easily excreted. Although there are multiple phase II reactions, such as the conjugation of glucose to OP compounds via uridine diphosphate glucosyl transferase (Bull and Whitten, 1972), the major enzymes involved in the phase II detoxification of insecticides are the glutathione S-transferases (Feyereisen, 1995; Hemingway and Ranson, 2000). Insect glutathione S-transferases have two subunits and may be both cytosolic and microsomal and have been proposed to serve as protection to the cell membrane (Yu, 2002). Glutathione S-transferases have been shown to confer insecticide resistance for multiple insect species against OP compounds (Huang et al., 1998), organochlorine compounds (Ranson et al., 2001; Syvanen et al., 1996), as well as pyrethroids (Syvanen et al., 1994; Vontas et al., 2002). They have also been shown to be involved in pyrethroid resistance in *Cx. quinquefasciatus*, where pretreatment of larvae with the glutathione S-transferase inhibitor diethyl maleate (DEM) resulted in a 3-fold decrease in permethrin resistance for a field-collected strain exhibiting multiple insecticide resistance (Xu et al., 2005).

1.4 Target site insensitivity

1.4.1 Insensitivity of the voltage-sensitive sodium ion channel

The voltage sensitive sodium ion channels (Vssc) of insects are the major ion channels involved in perpetuating the depolarization phase of action potentials. They are targets of DDT, pyrethroids, oxadiazines, as well as a variety of naturally-occurring neurotoxins (Dong, 2007;

Cestele and Catterall, 2000; Wang and Wang, 2003). Amino acid substitutions in the Vssc that lead to structural changes can confer insecticide resistance. The first observation of this phenomenon was in the house fly *Musca domestica*, where resistance to DDT was found to coincide with a loss of the typically rapid knock-down effect of DDT leading to the term 'knock-down resistant', or kdr for short (Farnham, 1977). Presently, the term 'kdr' is used to refer to the specific mutation in *M. domestica*, which was identified to be due to a single nucleotide change from a C to a T at position After investigating the DNA sequence of the target of DDT, which is the voltage-gated sodium channel. L1014F in *Anopheles gambiae* (Martinez-Torres et al.,1998), *Blatella germanica* (Liu et al., 2000), *Culex pipiens*, *Myzus persicae* (Martinez-Torres et al., 1999), *Culex quinquefasciatus* (Xu et al., 2006b), *Hematobia irritans* (Guerrero et al., 1997), *Leptinotarsa decemlineata* (Lee et al., 1999) and *Plutella xylostella* (Schuler et al., 1998); alternative L1014 mutations such as L1014S in *An. gambiae* (Ranson et al., 2000), *Cx. pipiens* (Martinez-Torres et al., 1999), and L1014H in *Heliothis virescens* (Park and Taylor, 1999), and *Musca domestica* (Park et al., 1997). Recent studies on *Cx. quinquefasciatus* traced the frequency of both synonymous and non-synonymous SNPs throughout the pressuring to two distinct field strains of *Cx. quinquefasciatus*. The results showed that combinations of SNPs became fixed in the population as the level of permethrin resistance increased (Li et al., 2012; Xu et al., 2012a), notably three non-synonymous SNP changes which were L982F, A109S, and W1573R, which were comparable to L1014F, A99S, and W1594R in *M. domestica*, respectively. Li et al., 2012 and Xu et al., 2012a further co-identified six synonymous that were found to be statistically correlated to permethrin resistance in *Cx. quinquefasciatus*. These correlation of these novel SNPs in the Vssc to increasing insecticide resistance suggests their involvement in insecticide resistance (Li et al., 2012).

1.4.2 Insensitivity of acetylcholinesterase

Acetylcholine is a neurotransmitter in insects that spans the synapse of two neurons to continue the nervous impulse from nerve to nerve via the nicotinic acetylcholine receptor. Following the signal transmission, acetylcholinesterase degrades acetylcholine into choline and acetic acid, terminating the nervous stimulation. Organophosphorous and carbamate compounds are both inhibitors of acetylcholine, and resistance to these two insecticides has been identified and linked to insensitivity of acetylcholinesterase in mosquitoes, houseflies, and multiple other insect species as well (Fournier and Mutero, 1994; Casida and Durkin 2013). Multiple non-synonymous mutations in the acetylcholinesterase molecule have been documented in diverse insect species including *D. melanogaster*, *Aphis gossypii*, *Lucilia cuprina*, *Ae. aegypti*, *Cx. pipiens*, *An. gambiae*, and *An. albimanus* (Mutero et al., 1994; Vaughan et al. 1997; Chen et al., 2001; Boublik et al., 2002; Weill et al., 2003; Weill et al., 2004; Menozzi et al., 2004). Not all of the non-synonymous mutations identified in these insects confer insecticide resistance, while various combinations of the other mutations, for example the combination of the two mutations S431F and A302S confers a high level of resistance to both OP compounds and carbamates in the aphids *M. persicae* and *A. gossypii* (Benting and Nauen, 2004; Andrews et al., 2004).

1.4.3 Insensitivity of the gamma-aminobutyric acid receptor

Gamma-aminobutyric acid (GABA) is the major insect neurotransmitter for inhibitory neurons, which are neurons that play an important role in nervous impulse transduction by hyperpolarizing the nerve, making an action potential less likely (Otsuka et al., 1966). The GABA receptor, itself, forms a multimeric Cl^- channel, which when bound to GABA, results in

an influx of Cl^- into the nerve cell, resulting in hyperpolarization of the membrane ((ffrench-Constant et al., 1993; Buckingham et al., 2005). The GABA channel is the target for several insecticides, inlcuding cyclodienes, phenylpyrazoles, and avermectin (Abalis et al., 1986; Gant et al., 1998). Multiple studies have identified resistance in the GABA receptor, the first of which was the identification of *rdl* (resistance to dieldrin locus), which is the result of an amino acid change from alanine to serine at position 302 (A302S) in the M2 domain of the Cl^- channel in *Drosophila melanogaster* (ffrench-Constant et al., 1993). The same A302S (or A302G in for some insects) has since been identified in a multitude of insects including *Drosophila simulans, Musca domestica, Blatella germanica, Aedes aegypti*, *Bemisia tabaci*, *Tribolium castaneum*, *Hypothenemus hampei*, and *Myzus persicae* (ffrench-Constant et al., 1993; Zhang et al., 1994; Andreev et al., 1999; ffrench-Constant et al., 1993). The *rdl* locus that has been shown to confer not only dieldrin resistance, but resistance to other insecticides that target the GABA receptor, such as phenylpyrazoles (e.g. fipronil) as well.

1.5 Other mechanisms of insecticide resistance

1.5.1 Decreased penetration

A minor increase in the resistance to insecticides as a result of changes in the cuticular structure of insect has long been proposed (Plapp and Hoyer, 1968; Terriere, 1982). Earlier work demonstrated higher protein and lipid contents in the cuticle of DDT-resistant *Heliothis virescens*, and mutiple-insecticide resistant *Musca domestica* (Vinson and Law, 1971; Patil and Guthrie, 1979). Recent work using scanning electron measurements has shown that the females of pyrethroid-resistant *Anopheles funestus* had significantly thicker cuticles than pyrethroid-susceptible females (Wood et al., 2010). Gene-based evidence for the involvement of

cuticular genes has also been demonstrated in the Colorado potato beetle *Leptinotarsa decemlineata*, where three structural glycine-rich cuticular genes were found to be highly induced upon exposure to the organophosphate aziphosmethyl (Zhang et al., 2008). More recently, tissue-specific qRT-PCR has shown that along with the up-regulation of cuticlar structural genes, the majority of the metabolic genes contributing to insecticide resistance in the common bed bug *Cimex lectularius* are expressed in the epidermal layer (Zhu et al., 2013).

1.5.2 Behavioral/Other resistance

Behavioral resistance refers to changes in the behavior of the insect that results in avoidance of the insecticide (Chareonviriyaphap et al. 2013). Behavioral resistance is broadly classified into non-contact spatial repellency (where the insect avoids the chemical without contact) and direct contact excitation (where the insect becomes hypersensitive to the chemical and moves away from the toxin/toxicant following exposure (Roberts et al., 1997). An example of behavioral resistance is that of the German cockroach *Blatella germanica* to survive a hydramethylnon-spiked corn-syrup bait by developing an aversion to D-glucose, which led to a decrease in efficacy from 90 to 39% in only five years of exposure (Silverman and Bieman, 1993), which has been recently been discovered to be the result of changes in the peripheral gustatory receptor neurons GRN1 and GRN2, which are stimulated by sugar which encourages feeding and bitter compounds which suppresses feeding, respectively (Wada-Katsamata et al., 2013). In glucose-averse roaches, GRN1 exhibits a low response to D-glucose relative to wild-type roaches, while GRN2 is stimulated in a dose dependent fashion, even though GRN2 has no response to D-glucose in wild-type *B. germanica* (Wadu-Katsamata et al., 2013). This ultimately results in glucose-averse *B. germanica* avoiding the hydramethylnon-spiked corn-syrup bait due

to the high concentration of D-glucose.

1.6 Insecticide cross resistance

The development of insecticide resistance in an insect can lead to cross-resistance to a different insecticide, even if the insect has never been exposed to the second insecticide. Examples of this are in the diamondback moth *Plutella xylostella* where selection of a strain using permethrin, conferred cross-resistance to other pyrethroids (Yu and Nguyen, 1996), the Southern House Mosquito *Culex quinquefasciatus* where resistance to fenitrothion also conferred resistance to DDT and dichlorvos (Hassall, 1990), and the house fly *Musca domestica* where a the highly pyrethroid-resistant ALHF strain was identified to have cross-resistance to type I and type II pyrethroids, as well as the carbamate propoxur due to the high activity of monooxygenases (Liu and Yue, 2001).

1.7 Interaction of insecticide resistance factors

The factors that confer insecticide resistance to insects may, independently, confer only a small level of resistance, whereas the combination of factors may confer multiplicative resistance (Georghiou, 1972). Studies using crosses of an insecticide resistant strain of *M. domestica* with an insecticide susceptible strain possessing recessive molecular markers unique to each of the five autosomes of *M. domestica* have allowed researchers to investigate the additive or multiplicative effects of insecticide resistance factors that are borne on different autosomes and their possible interaction. Georghiou (1972) identified that autosomes 2, 3, and 5 independently conferred 3.2, 1.7, and 1.0, respectively, when compared to the susceptible strain. When autosomes 2, 3, and 5 were combined, however, it resulted in a nearly complete restoration of

resistance. Other work using a similar house fly crossing strategy has shown that trans-acting factors may influence the expression of resistance genes, such as *CYP6D1*, which is located on autosome 1, but whose expression is controlled by a factor on autosome 2 (Liu and Scott, 1997). Recently, the factors involved in a multiple-insecticide resistant strain of *M. domestica* have been investigated using a combination of RNA-Seq gene expression profiling and various house fly crosses between the insecticide resistant and the insecticide susceptible strains. The results of this study indicated that factors on autosomes 2 and 5, notably cytochrome P450s and regulation genes may be largely responsible for the metabolic resistance to insecticides in *M. domestica*, with minor pesticide-metabolism factors present on autosomes 1 and 3 (Li et al., 2013).
Other studies have identified that microbes may slow the development of insecticide resistance (Broderick et al., 2006). For example, correlations have been made between the density of *Wolbachia* infection in insecticide resistant mosquitoes and have been shown to be responsible, in part to the reproductive fitness of mosquitoes (Duron et al., 2006). Finally microbes may confer insecticide resistance to insects by directly degrading them. A recent study in Japan has identified that colonies of *Burkholderia* in sugar cane fields, where fenitrothion was repeatedly and heavily applied, had developed the capacity to utilize fenitrothion as a carbon source. When the bean bug *Riptortus pedestris* feeds on the sugar cane, it acquires the bacterium which colonizes the midgut of the insect, where it confers resistance to fenitrothion by using any fenitrothion injected by *R. pedestris* as a food source (Kikuchi et al., 2012).

1.8 Culex quinquefasciatus an insect pest

The mosquito *Culex quinquefasciatus* Say is a primary vector of several disease causing agents including: West Nile virus, St. Louis encephalitis virus, Eastern Equine Encephalitis virus,

Japanese Encephalitis virus, Chikunguja virus, *Wucheria bancroftii* (Nasci and Miller, 1996; Arensburger et al., 2010). *Culex quinquefasciatus* has a global distribution, and is found predominantly in tropical, subtropical, and warmer portions of the temperate regions. In nature, *Cx. quinquefasciatus* is anautogenous, requiring a blood meal in order to provision its eggs (Gelbič and Rozsypalová, 2012). The primary hosts of *Cx. quinquefasciatus* are birds, however they also feed on humans, mammals, and amphibians (Mackay et al. 2010, Unlu et al. 2010). The degree to which *Cx. quinquefasciatus* feeds on humans in North America has been shown to vary from as low as 1% in a study in California (Reisen et al. 1990) to 50% in a study conducted in Arizona (Zinser et al. 2004). In Alabama, it is the predominant mosquito species in urban areas (Fonseca et al., 2004; Cupp et al., 2011). Current approaches to controlling mosquitoes in the state rely primarily on source reduction and the application of insecticides, primarily pyrethroids and organophosphates, for both larval and adult mosquitoes (Liu et al., 2004).

1.9 Insecticide resistance in *Cx. quinquefasciatus*

The Southern house mosquito *Cx. quinquefasciatus* has been reported to have resistance to and varying degrees of insecticide susceptibility to >20 different insecticides (Hamdan et al., 2005; Pridgeon et al., 2008; Norris and Norris, 2011), however, since pyrethroid insecticides are the most widely used insecticides for the control of mosquitoes indoors (Liu et al., 2006) and since pyrethroids represent one-quarter of the worldwide insecticide market (Hemingway et al., 2004), much of the research to investigate insecticide resistance in *Cx. quinquefasciatus* has focused on resistance to pyrethroids. Insecticide resistance mechanisms in *Cx. quinquefasciatus* can include factors sucah as increased sequestering (Scott 1991; Feyereisen 1995), however the major inseticide resistance factors pertain to target site insensitivity and metabolic detoxification

(Liu et al., 2006). Two multiple-insecticide resistant field strains of *Cx. quinquefasciatus* (HAmCq^{G0}, and MAmCq ^{G0}) have been collected from two geographically-diverse regions of Alabama (Liu et al., 2004). These field strains contained both the *kdr*-like mutation L982F (L1014F) as well as multiple metabolic-based resistance factors (Xu et al., 2005; Xu et al., 2006b). Pretreatment of the MAmCq ^{G0} and HAmCq ^{G0} strains with the cytochrome P450 inhibitor piperonyl butoxide (PBO) resulted in a 10-fold decrease in resistance to permethrin, while treatments with the hydrolase inhibitor S,S,S,-tributylphosphorotrithioate (DEF) and the glutathione S-transferase inhibitor diethyl maleate (DEM) resulted in decreases of only 3 and 2-fold, respectively (Xu et al., 2005). These results demonstrated that along with the *kdr*-like Vssc mutation, the majority of insecticide resistance in *Cx. quinquefasciatus* is attributable to cytochrome P450-mediated metabolic detoxification (Xu et al., 2005). Similar results were obtained from collections of *Cx. quinquefasciatus* worldwide from regions as diverse as Saudi Arabia (Kasai et al., 1998), California (McAbee, 2003), and West Africa (Chandre et al., 1998). Hardstone et al. (2010) further identified that there are epistatic (interactions that are non-additive) effects between the *kdr*-like *Vssc* mutation and cytochrome P450s in *Cx. quinquefasciatus* with regard to permethrin resistance. In their study, they crossed a permethrin resistant strain of *Cx. quinquefasciatus* containing the *kdr*-like mutation and cytochrome P450 metabolic detoxification (Jpal strain) with a lab susceptible strain (S-lab) to obtain a strain possessing P450-mediated resistance, but no *kdr*-like mutation. It was found that cytochrome P450-mediated detoxification alone conferred only 4% of the same resistance as the combination of the *kdr*-like mutation and the P450-mediated detoxification. They further found that if the cytochrome P450s of the Jpal strain were inhibited by PBO, the strain lost nearly all resistance to permethrin, reducing from ~28000-fold resistant to 70-fold resistant when compared to a

laboratory susceptible strain of *Cx. quinquefasciatus.* The HAmCq ^{G0} and MAmCq ^{G0} strains were further pressured with permethrin in the laboratory to obtain strains with a high level of pyrethroid resistance (Xu et al., 2006a). As a result of the permethrin pressuring, the genes involved in pyrethroid resistance were identified to be over-expressed when compared to their respective parental strains (Liu et al., 2007; Liu et al., 2011; Yang and Liu, 2011). This allowed for techniques such as suppression subtractive hybridization to be conducted to identify genes that are involved in permethrin resistance, including genes involved in cellular and molecular metabolism, signal transduction and regulation, vesicular and molecular transport, protein biosynthesis and ubiquitinization, cytoskeletal network, and others (Liu et al., 2007). Liu et al. (2007) discussed, for the first time, the possible involvement of cellular signaling in cytochrome P450 gene expression in mosquitoes and the link to insecticide resistance. Studies utilizing qRT-PCR have also been conducted to characterize the gene expression profiles of cytochrome P450s in insecticide resistant strains of *Cx. quinquefasciatus*. Liu et al. (2011) identified that four cytochrome P450 genes, *CYP6AA7*, *CYP9J40*, *CYP9J34*, and *CYP9M10* were constitutively up-regulated in the larval stages in highly permethrin-resistant *Cx. quinquefasciatus*, while only *CYP6AA7* was up-regulated in adult mosquitoes. Liu et al. (2011) further identified that three of these cytochrome P450 genes (*CYP6AA7, CYP9J34*, and *CYP9M10*) were inducted to a higher level of gene expression following a 24 h exposure to permethrin at the LC_{50} rate. The use of the two highly insecticide resistant strains of *Cx. quinquefasciatus* (HAmCq and MAmCq) also allowed for gene expression profiling of the entire compliment of the 204 cytochrome P450 genes predicted to be present in the *Cx. quinquefasciatus* genome (Arensburger et al., 2010) and to link selected cytochrome P450 over-expressed genes to permethrin resistance. In their study, Yang and Liu (2011) identified that multiple cytochrome P450 genes were up-regulated across

the two permethrin resistant lines tested when compared to their parental low insecticide resistance strains (HAmCq^{G8} to HAmCq^{G0} and S-lab, and MAmCq^{G6} to MAmCq^{G0} and S-lab, respectively). Yang and Liu (2011) further confirmed the up-regulation of *CYP6AA7*, *CYP9J40*, *CYP9J34*, and *CYP9M10* in larval permethrin-resistant *Cx. quinquefasciatus*, and further identified that *CYP6AA7* and *CYP4C52v1* were up-regulated across both strains of permethrin-resistant *Cx. quinquefasciatus* and all lifestages. This highlighted the importance of CYP6AA7 and CYP4C52v1 in permethrin resistance for all life stages, while other cytochrome P450s, notably CYP9J40, CYP9J34, and CYP9M10 may be of particular importance for permethrin resistance in the larval stage.

In addition to the deep level of knowledge of cytochrome P450 mediated resistance in *Cx. quinquefasciatus*, research has been done to investigate the sequence changes (SNPs) associated with the *Vssc* during pressuring with permethrin. SNP analyses of the successive generations of the two highly permethrin-resistant strains HAmCq^{G8} and MAmCq^{G6} as they were undergoing laboratory selection with permethrin identified a total of nine (three non-synonymous and six synonymous) SNPs in the *Vssc* that were statistically correlated to the increase in resistance to permethrin (Xu et al., 2012a; Li et al., 2012). This suggested that as permethrin resistance increases, there is a need for additional target site modifications to maintain the insensitivity of the Vssc in the presence of higher permethrin concentrations.

1.10 Next generation sequencing

With the advent of next generation sequencing, the ability to rapidly acquire large amounts of sequence information in a short period of time became available. Several technologies have been developed and are in current use as of 2012, including the Illumina-based

Solexa technology, the SOLiD technology, the Roche 454 technology, and the Ion Torrent technology (Metzker, 2010).

1.10.1 Illumina mRNA-Seq sequencing

In Illumina mRNA-Seq sequencing, the compliment of mRNA is extracted from the RNA fraction via oligo-dT hybridization or ribosomal RNA subtraction. The mRNA is then reverse transcribed into double stranded DNA, sheared into appropriate lengths (~300 nucleotides) and two unique Y-adapters are added to the ends of the molecule (Metzker, 2010). Due to the lack of complementarity of the Y-adapters at their 5'-end, a PCR generation step can be conducted that will allow for the selective amplification of only those fragments containing the two adapters (one on each end of the fragment). The fragments are then flowed across a silica slide flow cell that is coated with a sequence that is complimentary to the adapters, to which they bind and serve as the template for the extension of the covalently-bound adapters. The bound adapters are then extended using a DNA polymerase (Metzker, 2010). This step generates a second template at the end of the molecule, which can then anneal to a second covalently-bond adapter on the silica slide, where it serves as the template for the generation of the reverse strand via bridge amplification (Harris et al., 2008). This cycle is repeated to obtain localized spots or 'polonies' where single-stranded unique forward and reverse strands are present. Once the polonies have been generated, the PCR reaction proceeds in the presence of all four nucleotide bases containing a reversible allyl protecting group on the 3'-hydroxyl on the sugar and a second allyl group connecting a unique fluorophore to the nucleobase (Metzker et al., 1994; Canard and Sarfati, 1994). Once a base has been added to the growing chain, the remaining nucleotides are flushed from the flow cell and the slide is excited successively with two different wavelengths that excite

the fluorophores that are then read for each polony using a camera (Illumina, San Diego, CA). Following the recording of the flourophore (which indicates which base was incorporated), a palladium-catalysed tris(2-carboxyethyl)phosphine-mediated deallylation reaction removes the fluorophore and restores the 3'-hydroxyl on the deoxyribose sugar allowing for the next base to be incorporated and read (Bentley et al., 2008). This cycle is repeated to identify the next base and ultimately, obtain the nucleotide sequence.

1.10.2 Gene expression analysis using RNA-Seq

To determine the gene expression levels of genes using the RNA-Seq methodology, the individual reads are mapped to a nucleotide reference and the total number of times that a read matches to a sequence within a gene is recorded (Rapaport et al.,2013). Since all genes are fragmented into lengths of ~300 nt, the longer the gene is, the more fragments into which the gene will be divided. Therefore, the total number of fragments that are mapped to a gene are normalized by gene length in order to account for the additional possibility of longer genes to have mapped fragments. For this reason, the number of reads mapped is divided by the number of thousand nucleotides per gene, or reads/fragments mapped per kilobases of sequence for every million reads mapped (RPKM/FPKM) (Oshlack and Wakeffeld, 2009). For example, if 500 reads mapped to a given gene, and the gene was 2500 nt long, the 500 mapped reads would be divided by 2.5 to yield 200 and subsequently divided through by every million reads mapped, allowing genes to be compared both within, and between samples (Rapaport et al., 2013).

Chapter 2: Research Goal and Specific Objectives

2.1 Introduction

Mosquitoes serve as the vectors of several human and animal pathogens. Insecticides are used to suppress mosquito populations and protect humans and animals from disease. Insecticide resistance, however, results from the repeated use of chemical insecticides and becomes a practical problem in the management of mosquito borne diseases. The Southern house mosquito *Culex quinquefasciatus* Say is a globally-distributed mosquito and is the primary vector of West Nile virus, St. Louis encephalitis virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, Chikungunja virus, and *Wucheria bancroftii* (Nasci and Miller, 1996; Fonseca et al., 2004; Arensburger et al., 2010; Cupp et al., 2011). One strain of *Cx. quinquefasciatus*, HAmCq^{G0}, which has resistance to pyrethroids, has been further selected with permethrin for eight generations in the laboratory to produce the HAmCq^{G8} strain, and has ~300-fold higher resistance level than the HAmCq^{G0} parental strain (Xu et al., 2006a; Li and Liu, 2010). Multiple studies have identified various factors involved in insecticide resistance based on the HAmCq ^{G8} strain, including variations in the sodium channel (Xu et al., 2006b; Li et al., 2012; Xu et al., 2012a), cytochrome P450s (Liu et al., 2011, Yang and Liu, 2011; Gong et al., 2013), and other previously uncharacterized factors (Liu et al., 2007). Taken together, these results indicate that insecticide resistance in *Cx. quinquefasciatus* is the result of multiple factors. Therefore, I hypothesize that the use of RNA-Seq, which can concurrently estimate the gene expression levels of all genes, to probe the gene expression profiles of the HAmCq^{G8} strain, will elucidate novel mechanisms of insecticide resistance in *Cx. quinquefasciatus*. The questions raised are: 1) what genes are up-regulated in the HAmCq^{G8} strain, both constitutively and upon exposure to

permethrin; and 2) are the genes identified as important to insecticide resistance in *Cx. quinquefasciatus* important in other mosquitoes.

In addition to the investigation of insecticide resistance genes, we were also interested in possible new targets for the development of insecticides against *Cx. quinquefasciatus.* For this, we chose to investigate the anautogenous blood feeding requirement of *Cx. quinquefasciatus*. Adult female *Cx. quinquefasciatus* require a period of time before they are capable of taking the blood meal during which, the female mates and continues with the necessary development to be competent for the acquisition of the blood meal itself (Williams and Patterson, 1969). Many studies have shown that genes and gene up-regulation are involved in the processing of the blood meal and subsequently, vitellogenesis (Chen et al., 2004, Hansen et al., 2005, Bryant et al., 2010). Therefore I hypothesize that the use of RNA-Seq to characterize the gene expression profiles of newly-eclosed female *Cx. quinquefasciatus* will reveal genes involved in the requirements of the female for the taking of a blood meal. The two questions raised are: 1) what changes in gene expression occur during the early time points post-eclosion, and 2) are these gene changes related to vitellogenesis.

2.2 The goal of research and specific objectives

In order to answer these two questions in 2.1 and gain valuable insights into insecticide resistance and they physiology of *Cx. quinquefasciatus*, the long-term goal of my project is to first, characterize the gene expression profiles of the highly permethrin-resistant strain of *Cx. quinquefasciatus* $HAmCq^{G8}$ both in the absence of, and in presence of permethrin, and second, to characterize the gene expression profiles of *Cx. quinquefasciatus* during the early stages following eclosion in the female as they pertain to blood feeding. To achieve my long-term goals,

the following objectives will be performed: 1) characterization of the genes differentially expressed between the HAmCq^{G8} strain and its parental HAmCq^{G0} strain; 2) characterize the genes differentially expressed upon exposure to permethrin; 3) using the superfamily of cytochrome P450 genes, characterization of the up-regulation of P450 genes in a different mosquito species; 4) characterization of the changes in gene expression in newly eclosed female *Cx. quinquefasciatus*.

2.2.1 Characterization of the genes differentially expressed between the HAmCq^{G8} strain and its parental HAmCq^{G0} strain

Earlier studies have investigated the genes involved in insecticide resistance in the HAmCq^{G8} strain through various technologies including SNP reaction analysis (Xu et al., 2006b; Li et al., 2012; Xu et al., 2012b), suppression subtractive hybridization (Liu et al., 2007), and qRT-PCR (Yang and Liu, 2011). With the advent of next generation sequencing, we are now able to concurrently probe the gene expression levels of nearly all genes within *Cx. quinquefasciatus* (Metzker et al., 2010). This is further aided by the availability of the annotated genome for *Cx. quinquefasciatus* (Arensburger et al., 2010). To identify the genes that are differentially expressed between the highly permethrin-resistant strain of *Cx. quinquefasciatus* HAmCq^{G8} and its parental low-resistance strain HAmCq^{G0}, we will conduct RNA-Seq on the most pyrethroid-resistant life stage, the fourth instar (Li and Liu, 2010), then conduct tests of differential gene expression and functional enrichment of associated gene ontology (GO) terms.

2.2.2 Characterize the genes differentially expressed upon exposure to permethrin

A recent study in our lab has identified that in the HAmCq^{G8} strain, several cytochrome P450 genes are up-regulated during an exposure to permethrin (Gong et al., 2013). Following a similar approach as outlines in 2.2.1, we will expose fourth instar HAmCq^{G8} to permethrin at the LC_{50} and LC_{70} rates for 24 h, which is an exposure period that has previously been shown to result in gene up-regulation (Zhu et al., 2008b; Gong et al., 2013). In addition an acetone control at the same rate of acetone (200 ppm) will be treated for the same 24 h interval. All samples will be sequenced using RNA-Seq, mapped to the *Cx. quinquefaciatus* genome and compared to an untreated zero hour reference. Following this, tests of differential gene expression, Venn diagram analyses, and functional enrichment of associated gene ontology (GO) terms will be used to identify the changes in gene expression for a highly permethrin-resistant strain of *Cx. quinquefasciatus* during permethrin exposure.

2.2.3 Characterization of the up-regulation of P450 genes in a different mosquito species

Previous work has shown that different mosquito species have different susceptibilities to insecticides (Beckage et al., 2004; Pridgeon et al., 2008). In order to investigate the factors involved in insecticide resistance and to see if there are comparisons between different mosquito species, we will use the superfamily of cytochrome P450 genes to characterize the gene expression levels in *Aedes aegypti* to compare with the gene expression values of insecticide resistant *Cx. quinquefasciatus* (Yang and Liu, 2011). We will use qRT-PCR to proble the gene expression of all cytochrome P450 genes in the *Ae. aegypti* genome (Nene et al., 2007) to identify up-regulated P450 genes. Based on these results we will select several P450 genes for functional study using the GAL4:UAS enhancer trap methodology (Brand and Perrimon, 1993;

Bischof et al., 2007) to test the P450 gene for its functional capacity to degrade the type I pyrethroid permethrin and the type II pyrethroid beta-cypermethrin.

2.2.4 Characterization of the changes in gene expression in newly eclosed female *Cx. quinquefasciatus*

Newly-eclosed *Cx. quinquefasciatus* females require a period of time before they will freely take a blood meal (Williams and Patterson, 1969). To characterize the genes that may be involved in preparing the female for the taking of a blood meal, we will first determine the time course for which females will take a blood meal by collecting newly-eclosed adults in 12 h intervals and subsequently providing them with a blood meal at 24 h post-eclosion and every 12 h thereafter until 144 h post-eclosion. Once the time course for blood feeding has been determined, we will utilize RNA-Seq technology to probe the gene expression profiles of adult female *Cx. quinquefasciatus* from 2 h post-eclosion and every 12 h following until a time point that represents the age at which roughly half of the mosquitoes would take a blood meal. The gene expression values from the RNA-Seq sequencing will be estimated and differential gene expression will be tested as a time series to identify genes that are differentially expressed through the post-eclosion through to blooding time period. To identify if the genes detected as differentially-expressed throughout the time series by the RNA-Seq survey, we will characterize a set of genes identified as differentially-expressed in the RNA-Seq results along with selected genes known to be linked to vitellogenesis in mosquitoes including vitellogenins (Hansen et al., 2004). We will then test to see if the gene expression changes in *Cx. quinquefasciatus* during the early time point following eclosion are related to vitellogenesis by select genes identified as involved in vitellogenesis by the qRT-PCR work. We will then use 20-hydroxyecdysone as well

as ecdysone agonists to treat *Cx. quinquefasciatus* to see if we can induce the expression of *E74* which regulates the expression of vitellogenin (Guoquiang et al., 2002). This will determine if the differentially-expressed genes in the early stages of adult female *Cx. quinquefasciatus* following eclosion are involved in the vitellogenesis competency of the mosquito.

2.3 Significance

Characterization of gene expression profiles at the whole transcriptome level can elucidate patterns of gene expression that may otherwise go undetected in *a posteriori* approaches. The use of RNA-Seq to survey for uncharacterized factors involved in insecticide resistance in *Cx. quinquefasciatus* provides an ideal start point for future studies to identify the regulatory pathways involved in insecticide resistance, which may ultimately lead to an improvement of mosquito management. The same RNA-Seq technology applied to the previously uncharacterized early post-eclosion time points of *Cx. quinquefasciatus* adult females also represents the possibility for the discovery of new factors involved in host seeking and blood meal acquisition, which could represent novel targets for the development of new insecticides against *Cx. quinquefasciatus*.

Chapter 3: The Transcriptome Profile of the Mosquito *Culex quinquefasciatus* Following Permethrin Selection

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3.1 Abstract

To better understand the genetic variation in the insecticide resistant mosquito, *Culex quinquefasciatus*, and to gain valuable insights into the gene interaction and the complex regulation system involved in the development of insecticide resistance, we conducted a whole transcriptome analysis of *Culex* mosquitoes following permethrin selection. Gene expression profiles for the lower resistant parental mosquito strain HAmCq^{G0} and their permethrin-selected high resistant offspring HAmCq^{G8} were compared and a total of 367 and 3982 genes were found to be up- and down-regulated, respectively, in HAmCq^{G8}, indicating that multiple genes are involved in response to permethrin selection. However, a similar overall cumulative gene expression abundance was identified between up- and down-regulated genes in HAmCq^{G8} mosquitoes following permethrin selection, suggesting a homeostatic response to insecticides through a balancing of the up- and down-regulation of the genes. While structural and/or cuticular structural functions were the only two enriched GO terms for down-regulated genes, the

enriched GO terms obtained for the up-regulated genes occurred primarily among the catalytic and metabolic functions where they represented three functional categories: electron carrier activity, binding, and catalytic activity. Interestingly, the functional GO terms in these three functional categories were overwhelmingly overrepresented in P450s and proteases/serine proteases. The important role played by P450s in the development of insecticide resistance has been extensively studied but the function of proteases/serine proteases in resistance is less well understood. Hence, the characterization of the functions of these proteins, including their digestive, catalytic and proteinase activities; regulation of signaling transduction and protein trafficking, immunity and storage; and their precise function in the development of insecticide resistance in mosquitoes will provide new insights into how genes are interconnected and regulated in resistance.

Keywords: Pyrethroid resistance, gene expression, *Culex quinquefasciatus*, transcriptome, up-regulation

3.2 Introduction

Mosquitoes are known vectors of parasites and pathogens of both human and animal diseases and their control is an important part of the global strategy to control mosquito-associated diseases (WHO, 1957). Insecticides are the most important component of this vector-control effort, and pyrethroids such as permethrin are currently the most widely used insecticides for the indoor control of mosquitoes worldwide and the only chemical recommended for the treatment of mosquito nets, the main tool for preventing malaria in Africa (Najera and Zaim, 2001). However, the development of resistance to insecticides, especially to pyrethroids, in mosquito vectors has become a global problem (Hemingway et al., 2000; Phillips, 2001; Liu et al., 2004;

Xu et al., 2005; Liu, 2008). An improved understanding of the mechanisms governing insecticide resistance is therefore necessary to provide a knowledge base for the development of novel strategies to prevent resistance development and other tools to control resistant mosquitoes; ultimately reducing the prevalence of mosquito-borne diseases. Resistance has been assumed to be a pre-adaptive phenomenon, in that prior to insecticide exposure rare individuals already exist who carry an altered genome that results in one or more possible mechanisms (factors) allowing survival from the selection pressure of insecticides (Sawicki and Denholm, 1984;.Brattsten et al., 1986) In addition, some studies propose that resistance can also be induced by insecticide exposure (Vontas et al., 2010), and overall, the rate of development of resistance in field populations of insects depends upon the levels of genetic variability in a population (Liu and Scott, 1995; Liu and Yue, 2001). Efforts to characterize the genetic variation involved in insecticide resistance have therefore been fundamental in understanding the development of resistance and studying resistance mechanisms, as well as in practical applications such as designing novel strategies to prevent or minimize the spread and evolution of resistance development and the control of insect pests (Roush et al., 1990).

The mosquito *Culex quinquefasciatus* Say is a primary vector of West Nile virus, St. Louis encephalitis virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, Chikungunja virus, *Wucheria bancroftii*, and pathogens that cause lymphatic filariasis (Nasci and Miller, 1996; Arensburger et al., 2010). This mosquito species has a global distribution, especially throughout tropical and temperate climates of the world (Fonseca et al., 2004; Cupp et al., 2011). In Alabama, *Cx. quinquefasciatus* is the predominant mosquito species in urban areas. Current approaches to controlling mosquitoes in the state rely primarily on source reduction and the application of insecticides, primarily pyrethroids and organophosphates, for both larval and

adult mosquitoes (Liu et al., 2004). One northern Alabama *Culex* strain, HAmCq^{G0} collected from Huntsville, has demonstrated the ability to develop resistance and/or cross-resistance to not only pyrethroids and organophosphates (OPs), but also relatively new insecticides such as fipronil and imidacloprid (Liu et al., 2004). The HAmCq^{G0} mosquito strain has been further selected with permethrin for eight generations in the laboratory to produce the HAmCq^{G8} strain, which has a much higher level of resistance to permethrin than the parental strain, HAmCq^{G0} (Xu et al., 2006a; Li et al., 2009; Li and Liu, 2010). In an effort to better understand the genetic variation in resistant mosquitoes and gain valuable insights into the genes involved in the development of permethrin resistance in *Culex* mosquitoes, we chose the most resistant life stage (fourth instar larvae)(Li and Liu, 2010) and conducted a whole transcriptome analysis of the mosquito *Culex quinquefasciatus* following permethrin selection and examined the gene expression profiles between the lower resistant parental strain HAmCq^{G0} and their permethrin-selected high resistant offspring HAmCq^{G8} using Illumina RNA Seq (Morin et al., 2008).

3.3 Materials and Methods

3.3.1 Mosquito strains

Culex quinquefasciatus strain HAmCq^{G0} is a low insecticide resistant strain with a 10-fold level of resistance to permethrin compared with the laboratory susceptible S-Lab strain (Li and Liu, 2010). It was originally collected from Huntsville, Alabama in 2002 and established in laboratory without further exposure to insecticides (Liu et al., 2004). The HAmCq^{G8} strain is the 8th generation of permethrin-selected HAmCq^{G0} offspring and has a 2,700-fold level of resistance (Li and Liu, 2010). All mosquitoes were reared at 25±2^{o}C under a photoperiod of 12:12 (L:D) h. The mosquito was reared strictly under identical rearing conditions for the two mosquito

populations to enter into the fourth instar stage at the same time, which was achieved through the controlling of the egg raft collection, egg hatching, and subsequent larval development and sample collection.

3.3.2 RNA extraction

A total of 200 fourth instar larvae of the HAmCq^{G8} and HAmCq^{G8} mosquito populations were pooled, flash frozen on dry ice and immediately processed for RNA extraction. The fourth instar lifestage was selected because it is the most permethrin-resistant lifestage (Li and Liu, 2010) which should provide for the greatest differences in gene expression between the low- and highly-permethrin resistant mosquito strains. Total RNA was extracted using the hot acid phenol extraction method (Liu and Scott, 1997), after which a total of 30µg of RNA was treated with DNase I using the DNA-Free kit from Ambion (Austin, TX) to remove any contaminant DNA. Total RNA was re-extracted with two successive acid phenol: chloroform (1:1) steps followed by a final chloroform extraction to remove any residual phenol. The RNA was then precipitated over ethanol and resuspended in sterile distilled water. After a 1µg aliquot of RNA had been visually inspected for quality and for DNA contamination on a 1% agarose gel, total RNA was sent for RNA-Seq analysis (Hudson Alpha Institute of Biotechnology [HAIB]).

3.3.3 RNA library preparation, RNA Seq sequencing, Data analysis, and gene expression processing

RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA) and an Invitrogen Qubit (Invitrogen, Carlsbad, CA). Libraries were then prepared using the Illumina Tru-Seq RNA Sample Prep Kits (Illumina, San Diego, CA) for mRNA-Seq and a 3' poly A tail selection method. Samples were barcoded and run as one of four samples on a single lane of an

Illumina Hi Seq 2000 chip. Samples for the mRNA Seq were run using the PE-50 module (HAIB). Base calling, initial removal of low quality reads, and barcode parsing were conducted by the staff at HAIB. Data were sorted by coordinate using Picardtools (http://picard.sourceforge.net) and checked for mate-pair matching. Paired end reads were then mapped to the *Cx quinquefasciatus* genome from Vectorbase (Megy et al., 2009) using Tophat (Trapnell et al., 2009) with mate pair interval of 200 bases and the gtf basefeatures file. The --no-novel-juncs flag was used in the alignment to suppress the discovery of novel spliceforms in order to estimate gene expression levels based on the Vectorbase annotation of the genes. Read counts were determined using Cufflinks, and the testing of differential expression was estimated using Cuffdiff (Roberts et al., 2011). Both Cufflinks and Cuffdiff were used because these programs provide a more accurate estimation of the gene expression value by adjusting for transcript fragment biases that occur at the ends of the transcripts and fragments during the library generation protocol (Kasper et al., 2010). To adjust for the unequal coverage across a gene, Cuffdiff uses a negative binomial distribution (Anders and Huber, 2010) and applies a likelihood function to estimate gene expression that reduces bias, increases reproducibility across libraries, and gives better correlated gene expression levels as estimated by qRT-PCR and determines differentially-expressed genes at the α=0.05 false discovery rate (FDR) (Kasper et al., 2010). After analysis, only genes with expression values $\geq$1, as measured in number of fragments mapped for every thousand bases of gene length for every million fragments sequenced (FPKM), were retained for expression comparisons (Gan et al., 2010).

3.3.4 Gene expression validation using quantitative real-time PCR (qRT-PCR)

The 4th instar larvae of each mosquito population had their RNA extracted for each experiment using the acidic guanidine thiocyanate-phenol-chloroform method (Liu and Scott, 1997). Total RNA (0.5 µg/sample) from each mosquito sample was reverse-transcribed using SuperScript II reverse transcriptase (Stratagene) in a total volume of 20 µl. The quantity of cDNAs was measured using a spectrophotometer prior to qRT-PCR, which was performed with the SYBR Green master mix Kit and ABI 7500 Real Time PCR system (Applied Biosystems). Each qRT-PCR reaction (15 µl final volume) contained 1x SYBR Green master mix, 1 µl of cDNA, and a specific primer pair designed according to gene sequences (Appendix 3.1) at a final concentration of 3-5 µM. All samples, including the no-template negative control, were performed in triplicate. The reaction cycle consisted of an initial UDG glycosylase step at 50ºC for 2 min followed by a melting stage at 95°C for 10 min, followed by 40 cycles of 95ºC for 15 sec and 60ºC for 1 min. Specificity of the PCR reactions was assessed by a melting curve analysis for each PCR reaction using Dissociation Curves software. Relative expression levels for the genes were calculated by the $2^{-\Delta\Delta CT}$ method using SDS RQ software (Livak and Schmittgen, 2001). The 18S ribosome RNA gene, an endogenous control, was used to normalize the expression of target genes (Yang and Liu, 2011; Liu et al., 2011). Preliminary qRT-PCR experiments with the primer pair (Appendix 3.1) for the 18S ribosome RNA gene designed according to the sequences of the 18S ribosome RNA gene had revealed that the 18S ribosome RNA gene expression remained constant in of HAmCq^{G8} and HAmCq^{G8} mosquito populations, so the 18S ribosome RNA gene was used for internal normalization in the qRT-PCR assays. Each experiment was repeated three to four times with different preparations of RNA samples. The statistical significance of the gene expressions was calculated using a Student's *t*-test for all 2-sample comparisons and a one-way analysis of variance (ANOVA) for multiple sample

comparisons (SAS v9.1 software); a value of $P \leq 0.05$ was considered statistically significant.

3.3.5 Annotation, gene grouping, and functional gene enrichment analysis

The genes were annotated for SCOP general and detailed functions using the predicted *Cx. quinquefasciatus* annotation information available at the Superfamily website (version 1.75) supfam.cs.bris.ac.uk/SUPERFAMILY/index.html (Gough et al., 2001). Additional gene information for carboxylesterases was taken from the Vectorbase annotation for the Johannesburg strain version 1.1 (www.vectorbase.org) (Megy et al., 2009). Gene Ontology is a method of gene annotation that was introduced in 1998 (Ashburner et al., 2009). It is composed of three sets of structured gene ontology terms (GO terms) that have a carefully controlled vocabulary. These three sets represent 1) *Cellular Component*, which describe where the protein product is located at the sub-cellular and macromolecular complex level, 2) *Biological Process*, which denote gene products that are part of, or are themselves, biological processes, and 3) *Molecular Function*, which describe what the gene product does with regard to its function. Each gene may have multiple GO terms within each of the three sets of GO term ontology. Since the vocabulary of GO terms is carefully controlled, the occurrence of a given GO term can be compared between two distinct sets of genes. This allowed us to conduct an enrichment analysis of GO terms in the differentially-expressed gene sets against the entire expressed gene set using the Gene Ontology terms as annotated for the predicted genes in the *Cx. quinquefasciatus* genome using the online tool g:Profiler biit.cs.ut.ee/gprofiler/welcome.cgi (Reimand et al., 2007; Reimand et al., 2011). The g:Cocoa tool was used to test for GO term enrichment using a gSCS threshold for the significance threshold and a static background containing only genes with expression values of ≥1. This analysis took all of the GO terms associated with the differentially

down- or up-regulated gene sets and determined if a given GO term was statistically over-represented using a hypergeometric distribution to quantify the sampling probability that a given GO term is statistically more abundant in the up- or down-regulated gene set when compared to the abundance of that same GO term among the entire expressed gene set.

3.4 Results

3.4.1 Illumina RNA Seq data analysis

The maximum numbers of 51 nt paired-end reads that passed Illumina quality filtering were 32,540,882 and 37,184,673 for HAmCq^{G0} and HAmCq^{G8}, respectively (Table 3.1), which is consistent with the data typically obtained in an RNA Seq reaction that is based on an Illumina HiSeq 2000 single lane consisting of eight barcoded samples with a maximum number of reads passing filter of ~46 million (Illumina, Inc. San Diego, CA). Reads were mapped to the *Cx. quinquefasciatus* genome (version: CpipJ1.2) from Vectorbase (www.vectorbase.org) (Megy et al., 2009).

Table 3.1. Number of paired end reads from the Illumina HiSeq sequencing and the percentage of reads mapped to the *Cx. quinquefasciatus* (strain: Johannesburg) predicted transcriptome

Mosquito strain	HAmCq^{G0}	HAmCq^{G8}
Total reads	32540882[†]	37184673
Additional reads discarded	31509[‡]	16219
Reads mapped	23008772	30586459

[†]Total number of FASTQ (DNA sequence with quality scores) reads passing the Illumina quality filter
[‡]Number of reads discarded due to low quality of one or both of the paired end reads

Overall, the sequenced fragments mapped to a total of 14,440 genes, with 12,451 of these having a FPKM value of ≥1.0 in both HAmCq^{G0} and HAmCq^{G8}, which was used as the minimum value

to detect gene expression (Gan et al., 2010). All sequence traces and expression values have been submitted to the Gene Expression Omnibus at NCBI, reference accessions GSE33736 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33736 and SRA048095 (http://www.ncbi.nlm.nih.gov/sra/?term=SRA048095).

3.4.2 Transcriptome profile: SCOP general categories and detailed function categories

All expressed genes from both HAmCq^{G0} and HAmCq^{G8} were annotated for protein superfamily using the Structural Classification of Proteins (SCOP) annotations version 1.73 supplied for *Cx. quinquefasciatus* (http://supfam.cs.bris.ac.uk/SUPERFAMILY) (Hubbard et al., 1997), classified in terms of eight SCOP general categories, extra−cellular processes, intra−cellular processes, general, information, metabolism, regulation, not annotated, and other/unknown, according to the general function of the proteins. The genes expressed in both HAmCq^{G0} and HAmCq^{G8} were sorted into each of the eight SCOP general categories (Vogel et al., 2004) and then the expression values of each of these genes were summed within SCOP general category to obtain the proportion of total gene expression attributable to each of the SCOP categories (Fig. 3.1). Overall, the proportions of total gene expression were similar for HAmCq^{G0} and HAmCq^{G8}, however there were notable differences between the two mosquito strains for the metabolism category, which accounted for 32% of the gene expression in the entire HAmCq^{G8} genome compared to 26% in HAmCq^{G0}, suggesting an up-regulation of genes relating to metabolism in response to permethrin selection. Another difference in the total gene expression was in the not annotated category in HAmCq^{G0}, where it accounted for 23% of the gene expression in the entire genome compared to only 18% in HAmCq^{G8}, suggesting the down regulation of a set of genes without functional annotation in response to permethrin selection.

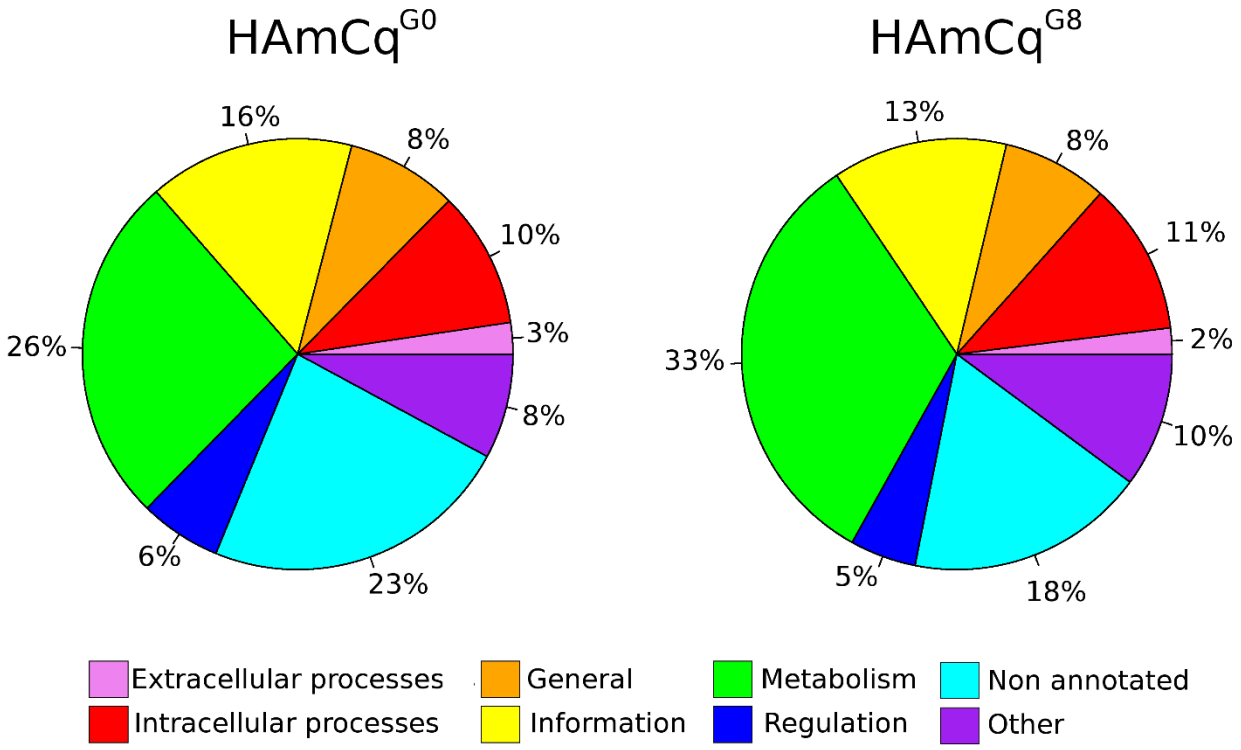

Figure 3.1. Total proportions of cumulative gene expression levels in HAmCq^{G0} and HAmCq^{G8} for the SCOP general and detailed functions using the predicted *Cx. quinquefasciatus* annotation information available at the Superfamily website (version 1.75) supfam.cs.bris.ac.uk/SUPERFAMILY/index.html.

3.4.3 Transcriptome profile: superfamily

Genes were further categorized into protein superfamilies at a gene annotation level lower than detailed function to compare the distribution of the expression levels between HAmCq^{G0} and HAmCq^{G8}. This allowed us to evaluate changes in the general gene expression within each of the superfamilies following permethrin selection. A log FPKM transformation was used to normalize the gene expression values and these were then plotted as beanplots (Fig. 3.2, Appendix 3.2). The distribution of each superfamily was broadly classified as unimodal, bimodal, or multimodal (Appendix 3.2) according to the similarities of gene expression within that superfamily. In addition, the values of skewness and kurtosis for the gene expression distribution were calculated, representing the symmetry of the gene distributions within the log normal

distributions (a positive skewness represents a gene distribution where a majority of genes have low expression levels and a negative skewness one where a majority of the genes have high expression levels) and the degree of sharpness of the curve (in leptokurtic distributions, groups of genes are expressed at similar expression levels and in platykurtic distributions, genes are expressed across a range of expression levels). Overall, all the superfamilies were comparable for HAmCq^{G0} and HAmCq^{G8}, both in terms of expression levels and in numbers of genes (as shown along the Y and the X axes, respectively, in Fig. 3.2, and in Appendix 3.2). This suggested that the permethrin selection may not have significantly influenced the overall expression levels of the genes in most superfamilies, however, in some cases the overall gene expression distribution in the two strains did differ slightly in a few superfamilies. For example, the Di-Copper containing center gene superfamily showed a multi modal distribution with three expression peaks in both HAmCq^{G8} and HAmCq^{G0}.

However, while the magnitudes of all three expression peaks were similar for a number of genes in HAmCq^{G8}, the peak with the lower mode of expression was >2-fold higher than the intermediate peak, and more than 5-fold higher than the highest mode in HAmCq^{G0}. Similar patterns were also found for the C-type lectin-like, NAP-like, and PLP binding barrel superfamilies. These slight changes in the gene expression distribution pattern may reflect the influence of up-regulated genes on the overall gene expression pattern in each of the superfamilies. The lysozyme-like superfamily, compared with HAmCq^{G0} which had a single mode, contained two modes in HAmCq^{G8} with one distribution positively and the other negatively skewed, suggesting that while some genes in this superfamily were up-regulated in HAmCq^{G8} compared with HAmCq^{G0}, the others may be down regulated.

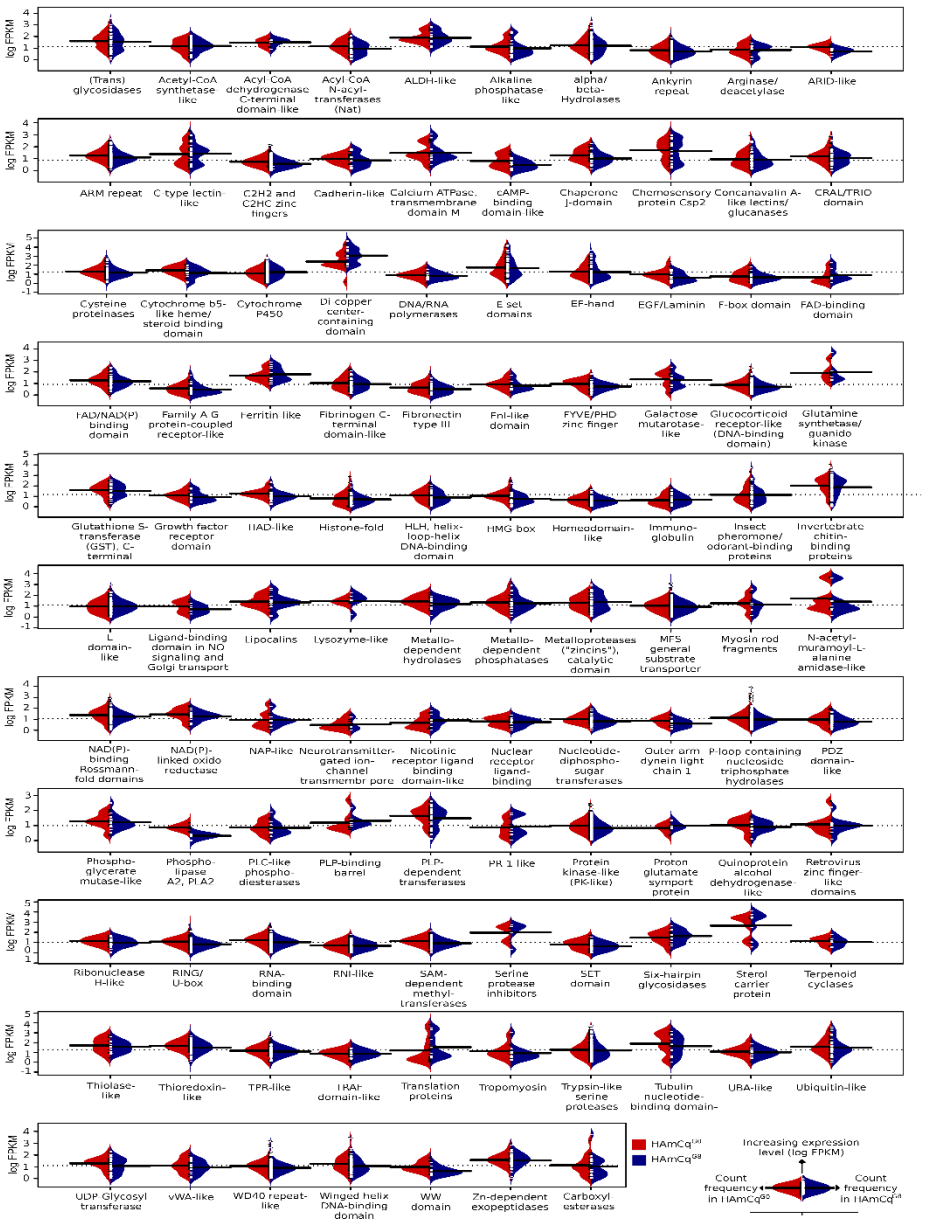

Figure 3.2. Log normal bean-plots for all expressed genes within SCOP superfamilies (SCOP version 1.75; supfam.cs.bris.ac.uk/SUPERFAMILY/index.html) in HAmCq^{G0} and HAmCq^{G8}. The distribution along the Y axis indicates a higher level of gene expression, while the distribution along the X axis indicates the proportion of genes expressed at the given level of gene expression along the Y axis. Distributions are oriented along a common central baseline so that distributions in red (HAmCq^{G0}) have more genes expressed at a given gene expression level (log FPKM) if the distribution is further to the left on the X axis, while distributions in blue (HAmCq^{G8}) are higher if they are further to the right of the X axis. The central vertical baseline for each superfamily is a mirror point for the two distributions.

3.4.4 Transcriptome profile: differential gene expression between HAmCq^{G0} and HAmCq^{G8}

Looking at the above SCOP general categories, detailed function categories and superfamily categories there is an overall similarity in the pattern of gene expression over the whole transcriptome level between the lower resistance parental mosquito HAmCq^{G0} and their permethrin selected offspring HAmCq^{G8}. We therefore went on to characterize the gene expression level between the two mosquito strains using the Cuffdiff algorithm and applying a >2-fold differential expression cut off threshold. A total of 3982 down- and 367 up-regulated genes were identified in HAmCq^{G8} (Table 3.2, Appendix 3.3;3.4) compared to HAmCq^{G0}. Overall, although there were more than 10 times the number of genes down-regulated than up-regulated, the cumulative gene expression values (FPKM) between the down- and up-regulated genes (1.43×10^5 and 1.53×10^5, respectively) were similar (Table 3.3). Interestingly, the predominant SCOP general function category for the down-regulated genes was the non-annotated category (NONA, 2016 genes), which accounted for 50% of the down-regulated genes (Table 3.2, Fig. 3.3), and represented 77% of the total cumulative expression (FPKM) of all of the down-regulated genes.

Table 3.2. Numbers of differentially-expressed genes and their cumulative gene expression level in HAmCq^{G8} sorted by the Structural Classification of Proteins general function category

	Down-regulated[†]		Up-regulated	
SCOP[‡] general function category	#genes	FPKM[*] range and (cumulative)	#genes	FPKM range and (cumulative)
Extra-cellular processes	101	0.1 - 126.4 (1.29×10^3)	20	1.0 - 121.8 (5.42×10^2)
General	355	0.1 - 192.1 (4.31×10^3)	39	1.0 - 1750.5 (5.99×10^4)
Information	171	0.1 - 344.9 (3.68×10^3)	5	4.3 - 66.0 (1.05×10^2)
Intra-cellular processes	359	0.1 - 255.4 (5.18×10^3)	58	1.7 - 5763.6 (1.28×10^4)
Metabolism	397	0.1 - 737.5 (1.15×10^4)	91	1.1 - 47162.3 (1.02×10^5)
NONA[§]	2016	0.0 - 33037.0 (1.10×10^5)	125	1.0 - 5766.3 (2.62×10^4)
Other	57	0.6 - 167.1 (1.26×10^3)	5	7.5 - 1742.1 (3.76×10^3)
Regulation	526	0.0 - 584.3 (5.56×10^3)	24	1.5 - 865.9 (1.25×10^3)
TOTAL	3982	(1.43×10^5)	367	(1.53×10^5)

[†]Down-regulated /Up-regulated genes represent those genes that differed in their expression level (FPKM) in HAmCq^{G8} by more than two fold when compared to the parental strain HAmCq^{G0}

[‡] SCOP general function categories annotated using the predicted *Cx. quinquefasciatus* annotation (version 1.75)

[*]Fragments mapped Per Kilo bases of reference sequence for every Million fragments sequenced

[§]NONA: Not annotated

Table 3.3. Gene Ontology (GO) term enrichment analysis results for differentially expressed genes in HAmCq^{G8}

GO level	GO term[†]	Term domain and name	# hits	p-value[‡]
Down-regulated genes				
Molecular function			-	-
structural molecule activity (GO:0005198)			163	1.08×10^{-11}
structural constituent of cuticle (GO:0042302)			85	3.19×10^{-18}
Up-regulated genes				
Biological process (GO:0008150)			**193**	**4.28×10^{-10}**
metabolic process (GO:0008152)			139	1.02×10^{-7}
oxidation-reduction process (GO:0055114)			38	5.39×10^{-9}
proteolysis (GO:0006508)			55	2.35×10^{-16}
Molecular function (GO:0003674)			**250**	**9.80×10^{-6}**
Catalytic activity (GO:0003824)			162	2.09×10^{-13}
oxidoreductase activity (GO:0016491)			47	1.05×10^{-10}
monooxygenase activity (GO:0004497)			29	2.84×10^{-15}
hydrolase activity (GO:0016787)			90	1.29×10^{-12}
peptidase activity (GO:0008233)			54	2.16×10^{-14}
peptidase activity, acting on L-amino acid peptides (GO:0070011)			52	1.99×10^{-15}
exopeptidase activity (GO:0008238)			10	4.66×10^{-5}
carboxypeptidase activity (GO:0004180)			7	1.03×10^{-4}
endopeptidase activity (GO:0004175)			41	1.47×10^{-12}
metallopeptidase activity (GO:0008237)			20	6.26×10^{-10}
metalloendopeptidase activity (GO:0004222)			11	2.42×10^{-6}
serine hydrolase activity (GO:0017171)			30	4.39×10^{-9}
serine-type peptidase activity (GO:0008236)			30	4.39×10^{-9}
serine-type endopeptidase activity (GO:0004252)			28	1.89×10^{-8}
hydrolase activity, acting on glycosyl bonds (GO:0016798)			15	1.23×10^{-7}
hydrolase activity, hydrolyzing O-glycosyl compounds (GO:0004553)			13	1.17×10^{-6}
Electron carrier activity (GO:0009055)			28	6.65×10^{-14}
Binding activity			-	-
tetrapyrrole binding (GO:0046906)			32	1.55×10^{-17}
iron ion binding (GO:0005506)			33	3.19×10^{-15}
heme binding (GO:0020037)			32	1.32×10^{-17}

[†]Annotation from the Gene Ontology consortium (version 1.2084; release date: 12:07:2011)
[‡] Cumulative hypergeometric p-values for GO terms of genes that were differentially up-regulated in when tested against all genes with expression levels of FPKM > 1 using the g:SCS threshold.
[*] GO Terms that do not have values for the number of hits or p-values were not statistically enriched in the functional enrichment analysis, but are included in the table to provide all parenthood connections.

This result is consistent with the results for the SCOP general categories, where a decrease in the total gene expression was found in the NONA category for HAmCq^{G8} compared to HAmCq^{G0}. In contrast, only 17% of the cumulative expression of the up-regulated genes in HAmCq^{G8} was in the NONA category. Nevertheless, the highest cumulative gene expression of up-regulated genes

was in the metabolism general function category (Table 3.2, Fig 3.3), which accounted for 67% (FPKM) of all of the up-regulated gene expression, while the cumulative expression of this category accounted for only 8% of the total cumulative expression of the down-regulated genes. Taken together, these results not only reveal equally dynamic changes in abundance for both the increases and decreases in the total gene expression for different categories in *Cx. quinquefasciatus* following permethrin selection, but also indicate an important feature of metabolic gene up-regulation in response to insecticide resistance and permethrin selection that is consistent with the data from the SCOP general category analysis, where the total expression in the metabolism general category was found to be higher in HAmCq^{G8} than in HAmCq^{G0}.

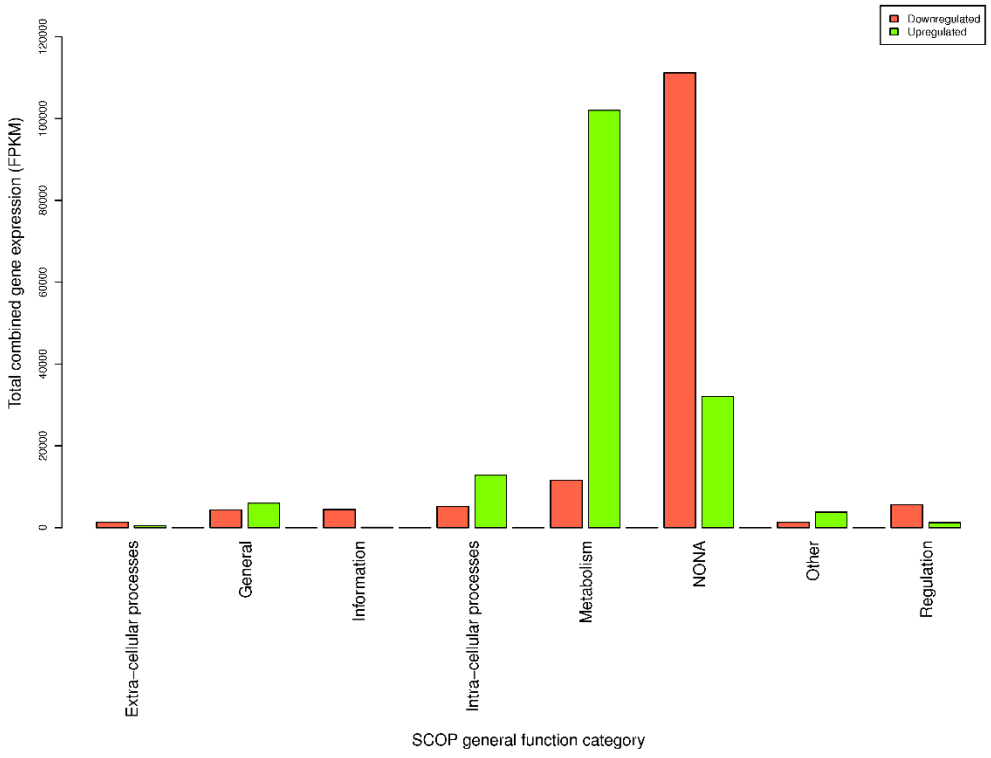

Figure 3.3. Combined gene expression levels for all up- and down-regulated genes within a general function category in HAmCq^{G8} compared to those expressed in HAmCq^{G0}.

3.4.5. Functional enrichment analysis of GO terms for differentially expressed genes

To interpret the gene expression data and gain more insight into the biological mechanisms driving the up- and down-regulated genes, Gene Ontology (GO) term enrichment or functional enrichment analysis (Castillo-Davis and Hartl, 2002; Reimand et al., 2007; Reimand et al., 2011) was performed to identify significantly enriched GO terms among the up- and down regulated genes in HAmCq^{G8}. GO terms are groups of genes sharing common biological function, regulation, or interaction (http://biit.cs.ut.ee/gprofiler/gconvert.cgi). A statistical analysis reveals which GO terms are over-represented and have hence been "enriched", or are more prevalent, within the down- or up-regulated genes in HAmCq^{G8}. Each gene can have multiple GO terms and these are part of a carefully-controlled vocabulary that allows for genes of various annotations to be grouped according to common attributes such as their cellular components, biological processes, or molecular functions (Ashburner et al., 2009). Overall, the functional enrichment analysis showed that among the down-regulated gene set in HAmCq^{G8}, the terms GO:000581 (structural molecule activity) and GO:0042302 (structural constituent of cuticle) were the only statistically over represented GO terms (P=1.08 × 10^{-11} and 3.19 × 10^{-18}, respectively) (Table 3.3). For the 3982 down-regulated genes in HAmCq^{G8}, there were 85 hits for GO:000581 and 163 hits for GO:0042302, indicating that 85 of the 3982 down-regulated genes had the structural molecule activity function and 163 the structural constituent of cuticle GO terms. Since these were the only enriched molecular function GO terms among the down-regulated gene set, there are likely to be changes of gene expression in the structural component of the cuticle in the HAmCq^{G8} mosquitoes compared to the parental HAmCq^{G0} strain.

The functional enrichment analysis of the 367 up-regulated genes in HAmCq^{G8} identified 25 statistically enriched GO terms (Table 3.3), four of which were in the categories biological process (GO:0008150), metabolic process (GO:0008152), proteolysis (GO:0006508), and

oxidation-reduction process (GO:0055114). Among these four enriched GO terms, biological process (GO:0008150) and metabolic process (GO;0008152) were the predominant GO terms, with 193 and 139 hits, respectively, suggesting that the major up-regulated genes were involved in biological and metabolic processes. The remaining 21 statistically enriched GO terms were in the molecular function category (Table 3.3) and the GO terms for catalytic activity (GO:0003824), hydrolase activity (GO:0016787), peptidase activity (GO:0008233), peptidase activity acting on L-amino acid peptides (GO:0070011), and oxidoreductase activity (GO:0016491) were the predominant GO terms, with hits that ranged from 162 to 47. Comparing the statistically enriched GO terms between the up- and down-regulated genes, these two sets of genes had obvious differences in their functions: the down-regulated genes primarily represented structural or cuticular structural activity functions, while the up-regulated genes were predominantly related to catalytic, metabolic, and proteolytic activity.

3.4.6. The molecular functional parenthood relationships of the GO terms among up-regulated genes and their interconnection

The relationships among the GO terms in the molecular function category were investigated in the up-regulated genes in $HAmCq^{G8}$ by determining whether their connection was a part of the same process or whether a parenthood process was involved (Ashburner et al., 2009). Overall, 3 functional sets of GO terms were found to be significantly overrepresented among the GO terms for molecular function (Fig. 3.4, Table 3.3), namely electron carrier activity, binding, and catalytic activity. The electron carrier activity set was mainly associated with GO terms in cytochrome P450 genes (Appendix 3.5).

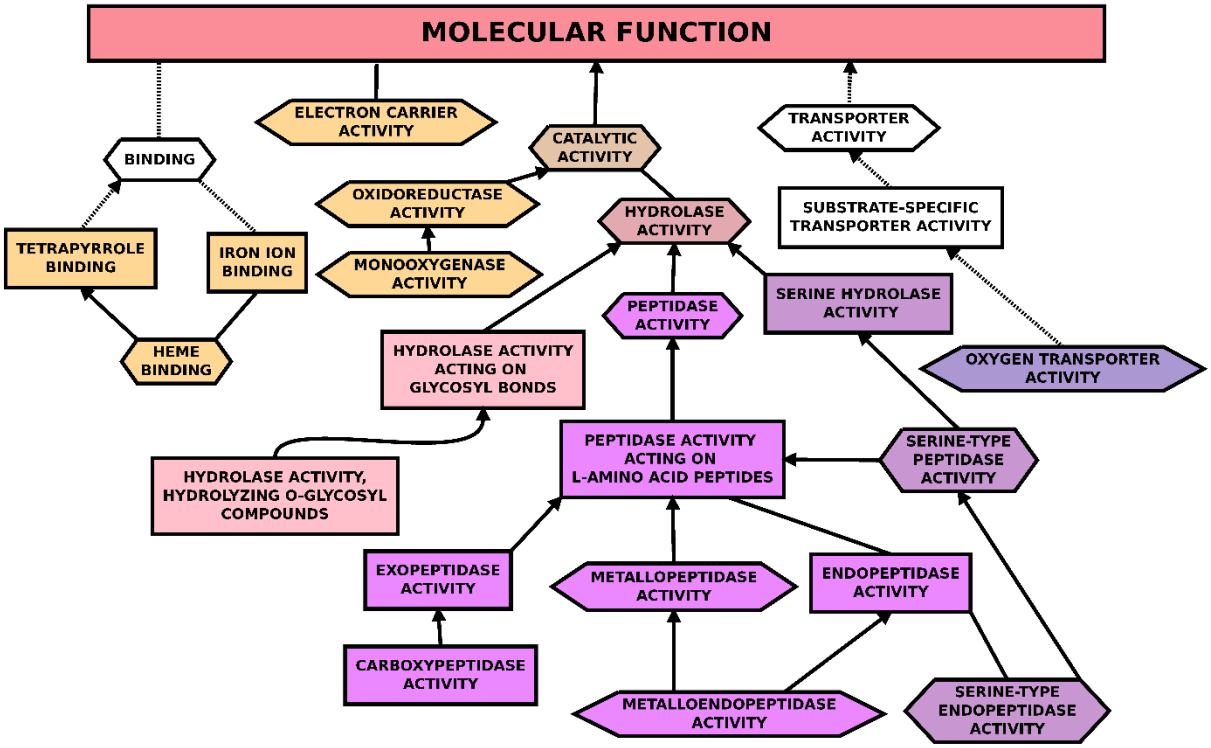

Figure 3.4. Parent-Child association for functionally enriched Gene Ontology (GO) terms among genes that were up-regulated in HAmCq^{G8}. GO terms associated with the up-regulated genes in HAmCq^{G8} were considered statistically at <0.001 using the g:SCS threshold in g:Cocoa (http://biit.cs.ut.ee/gprofiler/gcocoa.cgi). Colored boxes represent statistically functionally enriched GO terms, while the nonsignificantly-enriched GO term is marked in white and provided to display all of the parent-child relationships in the network. Lines and/or arrows represent connections between or among different GO terms. Solid lines represent relationships between two enriched GO terms. Dashed lines represent relationships between enriched and unenriched terms to connect all of the nodes on the directed acyclic graph.

The category for binding had three child branch nodes, all of which were related to metal binding: tetrapyrrole binding, iron binding, and heme binding (Fig. 3.4). These child branch nodes were again associated with the GO terms that were mainly overrepresented among cytochrome P450 genes (Appendix 3.5). The next major category was catalytic activity, which had two main child branch nodes: oxidoreductase activity with an additional branch node for

monooxygenase activity, both of which had their GO terms present in the genes annotated as cytochrome P450s (Appendix 3.5); and hydrolase activity, which contained three additional branch nodes. Of these additional hydrolase branch nodes, the first was for hydrolase activity of glycosyl bonds, with an additional sub-branch node for hydrolyzing O-glycosyl compounds. This was significantly overrepresented among the enzymes corresponding to the function of hydrolyzing glycosyl compounds such as alpha-L-fucosidases, alpha amylases and alpha glucosidases. The other two additional hydrolase branch nodes were peptidase/proteinase activity, which had an additional six sub-branch nodes relating to different peptidase/proteinase activities, and serine hydrolase activity, which had two additional sub-branch nodes for serine-type peptidase activity and serine-type endopeptidase activity (Fig. 3.4). The peptidase/proteinase and serine hydrolase activity nodes interconnected through the GO term nodes of endopeptidase activity and peptidase activity acting on L-amino acid peptides, suggesting that the GO terms associated with proteinase activity among the differentially up-regulated gene set in $HAmCq^{G8}$ were interconnected. Therefore, investigating the relationships among these enriched GO term categories of up-regulated genes revealed that functional categories were mainly overrepresented among P450s and proteases/serine proteases.

Indeed, the up-regulation of gene expression in these two categories was further confirmed by validation study of gene expression using qRT-PCR. Overall, the qRT-PCR validation data was consistent with the RNA-Seq data, showing a general trend of differential expression of genes between $HAmCq^{G8}$ and $HAmCq^{G0}$. A total of 14 up-regulated P450 genes and 24 protease related genes, which showed $\geq$ 2-fold higher expression in $HAmCq^{G8}$ compared with $HAmCq^{G8}$ in the RNA-Seq data, were selected for the study (Table 3.4).

Table 3.4. qRT-PCR validation of selected up-regulated genes in HAmCq^{G8} as identified by the RNASeq quantification.

			Fold overexpression in HAmCq^{G8†}	
Gene category	**Vectorbase**	**Annotation§**	**RNASeq**	**qRT-PCR**
Cytochrome P450	CPIJ002538	CYP6AG12‡	3.7	2.1††
	CPIJ005959	CYP6AA7‡	7.3	2.1
	CPIJ005957	CYP6AA9‡	6.6	2.8
	CPIJ010546	CYP9J34‡	13.4	2.9
	CPIJ009478	CYP4D42v1‡	2.4	3.2
	CPIJ005956	CYP6BZ2‡	3.3	3.7
	CPIJ010537	CYP9J45‡	4.8	3.8
	CPIJ012470	CYP9AL1‡	9.2	3.8
	CPIJ014218	CYP9M10‡	3.7	4.2
	CPIJ010225	CPY12F7‡	3.9	5.2
	CPIJ010227	CYP12F13‡	7.1	5.2
	CPIJ010543	CYP9J40‡	7.2	6.0
	CPIJ005955	CYP6P14‡	8.2	6.3
	CPIJ020229	CYP4D42v2‡	2.4	7.0
Protease	CPIJ002139	HzC4 chymotrypsinogen	4.3	1.1 ± 0.11
	CPIJ002130	kallikrein-7	2.4	1.5 ± 0.50
	CPIJ013319	metalloproteinase	3.5	1.5 ± 0.10
	CPIJ009106	angiotensin-converting enzyme	2.7	1.5 ± 0.84
	CPIJ001240	cathepsin B-like thiol protease	5.3	1.6 ± 0.46
	CPIJ019428	trypsin 2	3.4	1.6 ± 0.04
	CPIJ004086	angiotensin-converting enzyme	5.7	1.7 ± 0.62
	CPIJ008873	prolylcarboxypeptidase	3.5	1.7 ± 1.09
	CPIJ002135	trypsin alpha-4	5.9	1.8 ± 0.84
	CPIJ016012	tryptase-2	2.2	1.8 ± 0.10
	CPIJ002142	chymotrypsin BI	2.8	2.0 ± 0.42
	CPIJ006803	zinc metalloproteinase nas-7	4.5	2.0 ± 0.21
	CPIJ007383	endothelin-converting enzyme 1	2.5	2.1 ± 1.08
	CPIJ010224	metalloproteinase	2.9	2.4 ± 0.74
	CPIJ014523	elastase-3A	3.0	2.4 ± 0.71
	CPIJ019029	metalloproteinase	2.6	3.6 ± 0.70
	CPIJ002128	mast cell protease 2	16.1	3.6 ± 0.04
	CPIJ006542	chymotrypsin-2	19.7	5.4 ± 1.84
	CPIJ010805	carboxypeptidase A1	4.4	6.9 ± 3.72
	CPIJ006076	hypodermin-B	17.0	11.6 ± 4.96
	CPIJ001743	carboxypeptidase A2	5.4	16.3 ± 5.48
	CPIJ003623	coagulation factor XII	7.1	54.2 ± 19.79
	CPIJ001742	zinc carboxypeptidase	3.0	99.5 ± 19.35
	CPIJ009594	nephrosin	21.7	144.3 ± 13.8

§*Culex quinquefasciatus* genome, Johannesburg strain CpipJ1.2, June 2008; http://cquinquefasciatus.vectorbase.org/

†Expressed as fold change in gene expression in HAmCq^{G8} compared to HAmCq^{G0}

‡Annotations for cytochrome P450 genes were taken from the most current annotation based on: Nelson (2009) The Cytochrome P450 Homepage. Human Genomics 4, 59-65: http://drnelson.uthsc.edu/CytochromeP450.html

††Data reprinted from Yang and Liu, 2011

All 14 cytochrome P450s were up-regulated by at least 2-fold in the HAmCq^{G8} strain compared

with HAmCq^{G0}, which was consistent with the data generated using the RNAseq. Among the 23 up-regulated proteinase genes that have been identified by RNAseq, 14 of them (60%) were up-regulated by at least 2-fold in the HAmCq^{G8} strain and nine were up-regulated with a range of 1.5- to 1.8-fold compared with HAmCq^{G0} (Table 3.4). However, one of the proteinase genes had an expression level of 1.1-fold in HAmCq^{G8} compared with HAmCq^{G0}, which was significantly different from the RNAseq data.

3.5 Discussion

Based on the findings of our previous research, which has included synergism studies on the inhibition of metabolic enzymes (Xu et al., 2005), studies on the target site insensitivity of sodium channels in permethrin resistance (Xu et al., 2006b), gene expression profiles of resistance from a resistant-susceptible mosquito subtractive library (Liu et al., 2007), research into the genetic inheritance of permethrin resistance (Li and Liu, 2010), and, most recently, studies of the gene expression and characterization of P450 genes covering the entire genome sequence of resistant mosquitoes (Yang and Liu, 2011; Liu et al., 2011), it seems clear that a multiple mechanism/gene-interaction phenomenon is responsible for the development of permethrin resistance in *Culex* mosquitoes. We consider it very likely that normal biological and physiological pathways and gene expression signatures are altered in the resistant mosquitoes through changes in multiple gene expression in the resistant mosquitoes following insecticide selection that allow them to adapt to environmental or insecticide stress. While a great deal of effort has been devoted to identifying and characterizing the mechanisms and genes involved in insecticide resistance, and significant progress has been made, our previous approaches to characterizing the individual genes associated with insecticide resistance have not yet resulted in

a global understanding of the complex processes responsible for resistance. The recent genome sequencing of *Cx. quinquefasciatus* (Arensburger et al., 2010) has made direct comparisons of gene expression at the whole genome level between samples possible. The whole transcriptome analysis of the mosquito *Culex quinquefasciatus* following permethrin selection using Illumina RNA Seq reported here has allowed us to compare the cumulative gene expression in $HAmCq^{G0}$ and $HAmCq^{G8}$ mosquitoes in the SCOP general function categories and superfamilies, enabling us to evaluate major changes in the gene expression within each of the categories in the mosquitoes following permethrin selection using their median expression values. In general, similar levels of total cumulative gene expression were identified in the $HAmCq^{G0}$ and $HAmCq^{G8}$ mosquitoes in each of the general function categories, suggesting that the permethrin selection may not change the majority of the gene expression occurring in the mosquito genome, but that the changes that are found in only a select number of genes should be correlated to the permethrin selection process undergone by $HAmCq^{G8}$.

Results from our previous studies (Liu et al., 2004, Xu et al., 2005; Liu and Yue, 2001, Xu et al., 2006a, Liu et al., 2007) and from many others (David et al., 2005; Strode et al., 2006; Strode et al, 2008, Muller et al., 2008; Marcombe et al., 2009; Vontas et al., 2005) suggest that the interaction of multiple insecticide resistance mechanisms or genes may be responsible for insecticide resistance. While it is unclear whether and how these up-regulated genes are associated with insecticide resistance, the findings reported in these papers suggest that insecticide resistance in mosquitoes involves both multiple gene up-regulation and multiple complex interaction mechanisms. Taken together, the above findings suggest that not only is insecticide resistance conferred via multi-resistance mechanisms or up-regulated genes, but it is mediated through the interaction of resistance genes. The current study identified a total of 367

and 3982 genes that were up- and down-regulated, respectively, in permethrin selected offspring $HAmCq^{G8}$ compared with the parental $HAmCq^{G0}$ strain. These results provide further evidence to confirm our hypothesis that multiple gene expression in resistant mosquitoes changes following insecticide selection, thus allowing them to adapt to environmental or insecticide stress. Further, when we validated our RNAseq data using qRT-PCR, we were able to confirm that all of the cytochrome P450 genes identified as upregulated along with 60% of the proteases were indeed upregulated. Previous work using human colorectal cell lines showed that among 192 human exons , 88% of those identified as overexpressed using RNASeq could be validated as having either higher or lower expression using qRT-PCR, although the fold expression between the two strains was variable (Griffith et al., 2010). This suggested that the RNAseq methodology was suitable for the identification of genes putatively involved in insecticide resistance based on gene expression level, although some genes of interest may be overlooked due to differences in gene sequence, or genes involved in cell signaling that do not need to be more than two-fold expressed in order to be of importance to insecticide resistance.

To interpret the gene expression data and gain fresh insights into the biological mechanisms affected by the up- and down-regulated genes/proteins, we characterized the GO term enrichment, or functional enrichment, by identifying the significantly enriched GO terms among the up- and down-regulated genes in the low resistance parental strain and the high resistance eighth generation offspring. As described earlier, three categories of GO terms are used to describe gene products: biological processes, molecular functions, and cellular components (Ashburner et al., 2009). This approach facilitates efforts to understand the functional relevance of genes, allowing genes or family members that share functional and structural properties to be studied as a whole. Our comparison of the enriched GO terms in the

up- and down-regulated genes in HAmCq^{G8} revealed that the two enriched GO terms for the down-regulated genes represented primarily structural or cuticular structural functions and 50% of all the down-regulated genes, representing 77% of the total cumulative expression of those genes, were non-annotated. In contrast, the enriched GO terms for the up-regulated genes represented mainly the catalytic, metabolic, and proteolytic functions, and only 17% of the cumulative expression of the up-regulated genes was in the NONA category. Nevertheless, from an overall cumulative gene expression point of view, we saw similar expression levels between the up- and down-regulated genes in permethrin selected HAmCq^{G8}. Taken together, these results not only revealed different patterns in the enriched GO terms/functions for both the up- and down regulated genes, but also equally the dynamic changes in the abundance of both the total increased and the total decreased gene expression in *Culex* mosquitoes following permethrin selection, suggesting a homeostatic response of mosquitoes to insecticides through a balancing of up- and down-regulation of genes (Morgan 1997; 2001).

A number of mechanisms have been proposed for the balancing of up- and down-regulation, including: 1) an adaptive homeostatic response that protects the cell from the deleterious effects of oxidizing species, nitric oxide, or arachidonic acid metabolites from catalytic and/or metabolic enzymes (Morgan 2001; White and Coon, 1980); 2) a homeostatic or pathological response to inflammatory processes (Morgan, 1997); and/or 3) a need for the tissue to utilize its transcriptional machinery and energy for the synthesis of other components involved in the inflammatory response (Morgan, 1989). These hypotheses all offer reasonable explanations for our observation of both up- and down-regulation of multiple genes in the resistant mosquitoes following permethrin selection. For example, down-regulation of genes with structural or cuticular structural functions could be linked to the homeostatic response that

mosquitoes utilize to protect the cell from the toxic effects of oxidizing species derived from the extra metabolic proteolytic, and/or catalytic enzymes and metabolites that result from the up-regulated metabolic enzymes. This homeostatic response might also balance the usage of energy, O_2, and the other components needed for the syntheses of the up-regulated gene products and the catalytic or metabolic processes known to play important roles in mosquito resistance.

The functional relationships among the enriched GO terms of up-regulated genes/proteins allowed us to identify the key components involved in insecticide resistance and gain an insight into the molecular mechanisms in resistant mosquitoes as a whole. Three molecular function categories, namely electron carrier activity, binding, and catalytic activity, were significantly overrepresented among the GO terms for the up-regulated genes. Investigating the relationships among these enriched GO term categories revealed that functional categories were mainly overrepresented among P450s and proteases/serine proteases. Among these two key components, the importance of P450s has been extensively studied and it has been demonstrated that basal and up-regulation of P450 gene expression can significantly affect the disposition of xenobiotics or endogenous compounds in the tissues of organisms, thus altering their pharmacological and/or toxicological effects (Pavek and Dvorak, 2008). In many cases, increased P450-mediated detoxification has been found to be associated with enhanced metabolic detoxification of insecticides, as evidenced by the increased levels of P450 proteins and P450 activity that result from constitutive overexpression of P450 genes in insecticide resistant insects (Carino et al., 1992; Liu and Scott, 1997; Liu and Scott, 1998; Zhu et al., 2008a; Zhu et al., 2008b; Zhu and Liu, 2008; Zhu et al., 2010; Hardstone et al., 2010; Feyereisen et al., 2011; Liu et al., 2011). In addition, multiple P450 genes have been identified as being up-regulated in several individual resistant organisms, including house flies and mosquitoes (Zhu et al., 2008a; Zhu and Liu, 2008;

Marcombe et al., 2009; Itokawa et al., 2010; Yang and Liu, 2011; Liu et al., 2011), thus increasing the overall expression levels of P450 genes. Our recent studies on the characterization of P450s, their expression profiles, and their important role in the response to insecticide treatment found that multiple P450 genes were up-regulated in resistant and permethrin selected *Cx quinquefasciatus* (Yang and Liu, 2011; Liu et al., 2011). These findings together strongly suggest that overexpression of multiple P450 genes is likely to be a key factor governing the increased levels of detoxification of insecticides and insecticide resistance.

In contrast to the well-known role of P450s in insecticide resistance, apart from a few examples, less is known about the function of proteases/serine proteases in resistance. Proteases are a potent class of enzymes that catalyze the hydrolysis of peptide bonds and are known to be involved in a wide range of physiological functions, including the digestion of dietary protein, blood coagulation, immune response, hormone activation, and development (Krem et al., 2000). In addition to their digestive, catalytic, proteinase activities, proteases/serine proteases are involved in the regulation of signaling transduction (Burysek et al., 2002; Trejo, 2003; Ramsay et al., 2008; Marrs et al., 2012) and cellular protein trafficking in eukaryotic cells (Lemberg, 2011). Indeed, the up-regulation of protease genes have been identified in in DDT resistant *An. gambiae* (Vontas et al., 2005), fenitrothion resistant house flies, *Musca domestica* (Ahmed et al., 1998; Wilkins et al., 1999), as well as DDT resistant *D. melanogaster* (Pedra et al., 2004). It has been suggested that the up-regulation of proteases may enable insects to rapidly degrade proteins for their re-synthesis into detoxification enzymes as has been postulated for *M. domestica* when challenged with the insecticide fenitrothion (Wilkins et al., 1999). In addition, two serine protease genes from *Cx. pipiens pallens* have been found to be up-regulated in a deltamethrin-resistant strain (Wu et al., 2004). These reports, together with the findings reported here, suggest

the importance of the up-regulation of proteases in insecticide resistance. Whether the up-regulated proteases identified in the resistant mosquitoes play a role in the degradation of proteins for biosynthesis of the up-regulated metabolic proteins, particularly P450s and the other proteins involved in the regulation of insecticide resistance, or whether there is some form of interaction with the up-regulated genes associated with signaling transduction and protein trafficking needs further investigation.

In conclusion, this study not only provides a catalog of genes that were co-up- and down-regulated and information about their potential functions, but may also ultimately lead to a deeper understanding of transcriptional regulation and the interconnection of co-regulated genes, including metabolic genes, genes with catalytic activities, genes with proteolytic activities, and genes with, perhaps, functions involved in the regulation, signaling transduction, and protection of cells and tissues in resistant mosquitoes. It has been suggested that co-overexpressed genes are frequently co-regulated (Blalock et al., 2004; Clarke and Zhu, 2006). Therefore, characterizing these co-regulated genes as a whole will represent a good starting point for characterizing the transcriptional regulatory network and pathways in insecticide resistance, improving our understanding of the dynamic, interconnected network of genes and their products that are responsible for processing environmental input, for example the response to insecticide pressure, and the regulation of the phenotypic output, in this case, the insecticide resistance of insects (Clarke and Zhu, 2006). The new information presented here will provide fundamental new insights into precisely how insecticide resistance is regulated and how the genes involved are interconnected and regulated in resistance.

3.6 Acknowledgements

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Chapter 4: Gene Expression Profiles of the Southern House Mosquito *Culex quinquefasciatus* During Exposure to Permethrin

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4.1 Abstract

Insecticide resistance is a major obstacle to the management of disease-vectoring mosquitoes worldwide. The genetic changes and detoxification genes involved in insecticide resistance have been extensively studied in populations of insecticide-resistant, however few studies have focused on the resistance genes up-regulated upon insecticide exposure and the possible regulation pathways involved in insecticide resistance. To characterize the changes in gene expression during insecticide exposure, and to investigate the possible connection of known regulation pathways with insecticide resistance, we conducted RNA-Seq analysis of a highly-permethrin resistant strain of *Culex quinquefasciatus* following permethrin exposure. Gene expression profiles revealed a total of 224 and 146 up- and down-regulated, when compared to a blank acetone carrier treated control, respectively, suggesting that there were multiple, but

specific genes were involved in permethrin resistance. Functional enrichment analysis showed that the up-regulated genes contained multiple detoxification genes including a glutathione S-transferase and multiple cytochrome P450 genes, as well as several immune-related genes, while the down-regulated genes consisted primarily of proteases and carbohydrate metabolism and transport. Further analysis showed that permethrin exposure resulted in a decrease in the expression of serum storage proteins and likely represented a resulted in a delay in the development of the fourth instar possibly due to a decrease in feeding. This effect was more pronounced in an insecticide-resistant strain than in an insecticide-susceptible strain and may represent a behavioral mechanism of insecticide resistance in *Culex* mosquitoes.

4.2 Introduction

Mosquitoes carry and transmit parasites and pathogens, impacting human and animal health and resulting in economic losses (WHO, 1957). Insecticides, notably pyrethroids such as permethrin, are routinely applied to manage mosquito populations in order to mitigate the negative impact of mosquitoes, however, the development of insecticide resistance, especially to pyrethroids, has become a global problem (Phillips, 2001; Liu et al, 2004; Hemingway et al., 2002; Xu et al, 2005; Liu 2008; Liu et al., 2009). Insecticide resistance is presumed to be a pre-adaptive phenomenon, indicating that the genes involved in insecticide resistance and the pathways that control their regulation are already present within the insect (Sawicki and Denholm, 1984; Brattsten et al., 1986). In addition, some studies propose that resistance can also be induced by insecticide exposure (Vontas et al., 2010; Gong et al., 2013), indicating that there are underlying pathways within the insect that respond to an insecticide challenge, and that these pathways may be involved in insecticide resistance.

The mosquito *Culex quinquefasciatus* Say is a primary vector of West Nile virus, St. Louis encephalitis virus, Eastern Equine Encephalitis virus, Japanese Encephalitis virus, and lymphatic filariasis (Nasci and Miller, 1996; Arensberger et al., 2010). This mosquito species has a global distribution, especially throughout tropical and temperate climates of the world (Fonseca et al., 2004; Cupp et al., 2011). In Alabama, *Cx. quinquefasciatus* is the predominant mosquito species in urban areas, and insecticide resistance has been documented for this mosquito in the field (Liu et al., 2005). One strain of *Cx. quinquefasciatus*, collected from Alabama, has been pressured with permethrin for eight generations in the laboratory to produce the $HAmCq^{G8}$ strain, which is a highly permethrin-resistant strain that allows for the identification of genes putatively involved in insecticide resistance based on gene expression profiles (Xu et al., 2006a; Li et al., 2009, Li and Liu, 2010, Yang and Liu, 2011, Reid et al., 2012, Gong et al., 2013). The objective of our study was to characterize the expression levels of genes induced upon exposure to permethrin when compared to an acetone blank carrier and identify the possible activation of gene pathways in *Cx. quiquefasciatus*.

4.3 Materials and Methods

4.3.1 Mosquito strains

Culex quinquefasciatus strain $HAmCq^{G8}$ is a highly-insecticide resistant strain that was originally collected from Huntsville, Alabama in 2002 (Liu et al., 2004) and subsequently pressured in the laboratory with permethrin for eight successive generations to achieve a 2,700-fold level of resistance to permethrin compared with the laboratory susceptible S-Lab strain (Li and Liu, 2010). All mosquitoes were reared at 25±2^{o}C under a photoperiod of 12:12 (L:D) h.

4.3.2 Permethrin exposure treatments

A total of 400 fourth instar larvae were collected and transferred to 3 L of water in plastic containers measuring 20.5 x 35 x 11 cm for each treatment. The mosquito rearing was conducted so that the two mosquito populations entered into the fourth instar stage at the same time. This was achieved through the controlling of the egg raft collection, egg hatching, and subsequent larval development and sample collection under identical rearing conditions. Four treatments were conducted: untreated time 0h, acetone treated 24h post-application, and permethrin mixed in an acetone carrier at the LC_{50} and the LC_{70} rate (24h post-application). The treatments were considered to be representative of the LC_{50} and LC_{70} rates if the percentage of dead larvae after the 24 h permethrin exposure was 50±5% and 70±5%, respectively. The final volume of acetone was adjusted to 600 µl per 3 L for the acetone and permethrin treatments. No acetone was added to the untreated time 0h sample and no mortality was observed for either the 0h untreated treatment, or the 24 h acetone exposure treatment. A single container was used for the acetone 24 h treatment, while two pans each were used for the LC_{50} and LC_{70} treatments in order to obtain enough surviving larvae for RNA extraction.

4.3.3. RNA extraction

A total of 200 surviving fourth instar larvae of each treatment were pooled, flash frozen on dry ice and immediately processed for RNA extraction. Total RNA was extracted using the hot acid phenol extraction method as outlined by Chomczynski and Sacchi (1987), after which a total of 30 µg of RNA was treated with DNase I using the DNA-Free kit from Ambion (Austin, TX) to remove any contaminant DNA. Total RNA was re-extracted with two successive acid phenol: chloroform (1:1) steps followed by a final chloroform extraction to remove any residual

phenol. The RNA was then precipitated over ethanol and re-suspended in sterile distilled water. After a 1µg aliquot of RNA had been visually inspected for quality and for DNA contamination on a 1% agarose gel, total RNA was sent for RNA-Seq analysis (Hudson Alpha Institute of Biotechnology [HAIB]).

4.3.4. RNA library preparation, RNA Seq sequencing, Data analysis, and gene expression processing

RNA quality was assessed using a Qubit fluorimeter and an Agilent 2100 Bioanalyzer by the HAIB. Libraries were then prepared using the Illumina mRNA-Seq kit using a 3' poly A tail selection method. Samples were barcoded and run as one of four samples on a single lane of an Illumina Hi Seq 2000 chip. Samples for the mRNA Seq were run using the PE-50 module (HAIB), which results in paired end reads that are each 50 nucleotides long, spanning a 300 nt stretch of the mRNA sequence. Base calling, initial removal of low quality reads, and barcode parsing were conducted by the staff at HAIB. Data were sorted by coordinate using Picardtools (http://picard.sourceforge.net) and checked for mate-pair matching. Paired end reads were then trimmed for adapter and low quality reads were removed using Trimmomatic (Lohse et al., 2012). Surviving reads were then mapped to the *Cx quinquefasciatus* genome JHBv1.3 from Vectorbase (Arensberger et al., 2010; Megy et al., 2012) using Tophat2 (Trapnell et al., 2009; Kim et al., 2011) with mate pair interval of 200 bases and the gtf basefeatures file. The --no-novel-juncs flag was used in the alignment to suppress the discovery of novel spliceforms in order to estimate gene expression levels based on the Vectorbase annotation of the genes. Read counts were determined using Cufflinks, and the testing of differential expression was estimated using Cuffdiff (Roberts et al., 2011) for each 24h exposure treatment compared to the untreated

0h treatment. Both Cufflinks and Cuffdiff were used because these programs provide a more accurate estimation of the gene expression value by adjusting for transcript fragment biases that occur at the ends of the transcripts and fragments during the library generation protocol (Kasper et al., 2010). Genes identified as up- or down-regulated when compared to the 0h untreated sample were then subjected to Venn diagram analysis to generate a list of genes that were up- or down-regulated in the permethrin treatments, but not in the acetone treatment. Only genes with expression values ≥1, as measured in number of fragments mapped for every thousand bases of gene length for every million fragments sequenced (FPKM), were retained for expression comparisons (Gan et al., 2010).

4.3.5. Annotation, gene grouping, and functional gene enrichment analysis

The genes were annotated using the Vectorbase CpipJv1.3 annotation (Megy et al., 2012) and further annotated using the Structural Classification of Proteins (SCOP) general and detailed functions for *Cx. quinquefasciatus* available at the Superfamily website (version 1.75) supfam.cs.bris.ac.uk/SUPERFAMILY/index.html (Gough et al., 2001). Functional gene enrichment of GO terms was conducted using the online tool g:Profiler biit.cs.ut.ee/gprofiler/welcome.cgi (Reimand et al., 2007; 2011) and gene identification number discrepancies between CpipJv1.2 and CpipJ1.3 were resolved using the 'Gene annotation changes CpipJ1.2 to CpipJ1.3' file from Vectorbase (Megy et al., 2012). The g:Cocoa tool was used to test for GO term enrichment on genes that were up- or down-regulated in the permethrin treatments using a gSCS threshold for the significance threshold and a static background containing only genes with expression values of ≥1. This analysis took all of the GO terms associated with the differentially down- or upregulated gene sets and determined if a given GO

term was statistically over-represented using a hypergeometric distribution to quantify the sampling probability that a given GO term is statistically more abundant in the up- or down-regulated gene set when compared to the abundance of that same GO term among the entire expressed gene set.

4.3.6. Selected gene expression validation using qRT-PCR

Acetone and permethrin (LC_{50}) exposures were independently replicated in triplicate following the methodology for insecticide exposure previously described above. The RNA from three independent samples of 100 fourth instar larvae from both the S-lab and the $HAmCq^{G8}$ strains was obtained using the extraction method of Chomczynski and Sacchi (1987), and treated with DNase I using the DNA-Free kit from Ambion (Austin, TX) to remove any contaminant DNA. The DNase I was inactivated using the inactivation buffer from Ambion and cDNA was generated from the template RNA using the First strand cDNA synthesis kit from Roche (Indianapolis, IN). qRT-PCR was conducted on an ABI 7500 Real Time PCR system (Applied Biosystems) using the ABI SyBr Green mastermix kit (Life Technologies, Carlsbad, CA) and relative gene expression was determined by using the $2^{(-\Delta\Delta Ct)}$ method (Livak and Schmittgen, 2001) using a portion of the 18S rRNA gene as the reference gene and subtracting the acetone treatment from the LC_{50} permethrin treatment. Statistical significance between the S-lab and $HAmCq^{G8}$ strain was tested using a Welch's T-test in R (R Core Team, 2013).The primers used are provided in Appendix 4.1.

4.4 Results

4.4.1. Gene Abundance

The number of paired-end reads that passed Illumina quality filtering ranged from 25,723,783 to 30,431,848, which provided a similar depth of coverage for each of the four treatments tested (Table 4.1), which allowed us to compare the gene expression levels for all four samples tested. The adapter trimming and low-quality removal step removed an additional ~2 million paired end reads from each of the treatments resulting in a final set of reads that ranged from 24 to 28.5 million reads (Table 4.1).

Tophat2 and Cufflinks were then used to map the reads to the *Cx. quinquefasciatus* genome (version: CpipJ1.3) from Vectorbase (www.vectorbase.org) and to estimate gene abundance, which identified a total of 11,595 genes that had an FPKM ≥1.0. This was consistent with our previous study where 12,451 expressed genes were identified in the fourth instar stage of HAmCq^{G8}, (Reid et al., 2012). All sequence traces and expression values have been submitted to the Gene Expression Omnibus at NCBI, reference accession GSE51399 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE51399.

Following the determination of gene abundance, the genes were tested for differential gene expression using Cuffdiff by comparing the untreated 0 h time point with each of the 24 hour exposure treatments: acetone, permethrin LC_{50}, and permethrin LC_{70}.

4.4.2. Up-regulated Genes

The differential gene expression testing identified a total of 331 up-regulated genes, which included 107, 214, and 216 genes in the acetone, permethrin LC_{50}, and permethrin LC_{70} treatments, respectively (Fig. 4.1; Table 4.2).

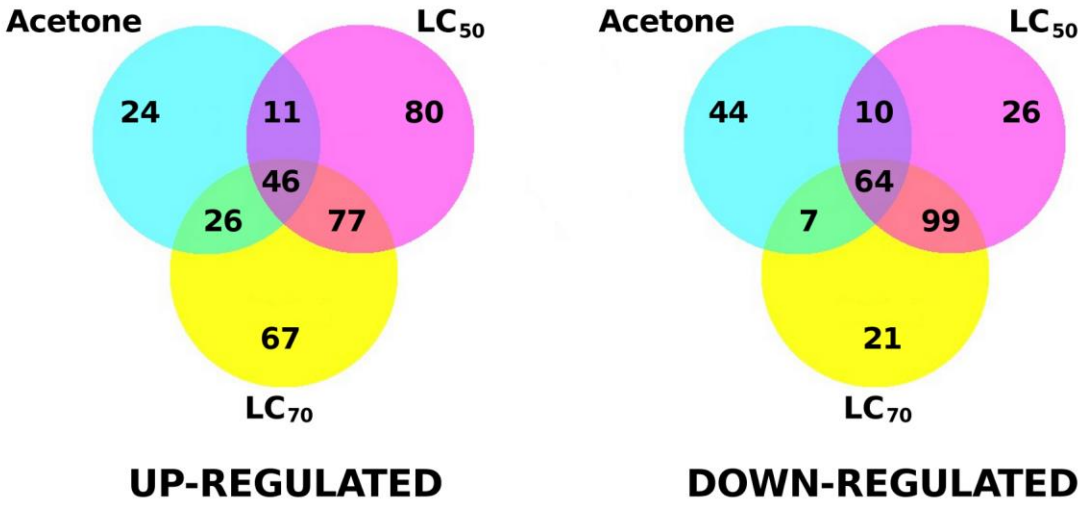

Figure 4.1. Venn diagram analysis of the total numbers of differentially-expressed up- and down-regulated genes in the fourth instar of the permethrin resistant $HAmCq^{G8}$ strain of Cx. quinquefasciatus following a 24h exposure to either an acetone control, or two rates of permethrin (LC_{50} and LC_{70}). Overlapping circles represent genes that were co-up- or co-down-regulated in two or more groups.

Among the genes up-regulated in the permethrin treatments, the majority were present within the SCOP functional categories of 'no annotation', 'metabolism', 'intra-cellular processes', and 'general' (Table 4.3).

Forty-six of the genes were up-regulated in the acetone and both permethrin treatments, with an additional 11 and 26 genes shared between the acetone and the permethrin LC_{50}, and acetone and the permethrin LC_{70} treatments, respectively. The remaining up-regulated genes were present only in the permethrin treated samples and included 80 genes up-regulated in the permethrin LC_{50} treatment, 67 genes up-regulated in the permethrin LC_{70} treatment, and 77 genes up-regulated in both the permethrin LC_{50} and LC_{70} treatments.

Table 4.2: Number of differentially-expressed genes in the highly-permethrin resistant strain of *Culex quinquefasciatus*, HAmCq^{G8}, following a 24 h exposure to either acetone, or permethrin at the LC_{50} and LC_{70} rates compared to a zero hour untreated time point.

	Down-regulated (24h post application)			Up-regulated (24h post application)		
		Permethrin			Permethrin	
SCOP† general function	Acetone	LC_{50}	LC_{70}	Ace	LC_{50}	LC_{70}
Extra-cellular processes	7	7	7	2	2	5
General	8	10	12	20	34	30
Information	0	0	3	3	4	5
Intra-cellular processes	28	42	42	17	34	47
Metabolism	50	97	91	21	47	43
No annotation	19	24	16	29	73	66
Other	2	3	4	2	3	4
Regulation	11	16	16	13	17	16
TOTAL	125	199	191	107	214	216

†Structural Classification of Proteins database (SCOP) general and detailed functions using the predicted *Cx. quinquefasciatus* annotation information available at the Superfamily website (version 1.75) supfam.cs.bris.ac.uk/SUPERFAMILY/index.html

Although several of the genes up-regulated in the permethrin treatment were present in the SCOP 'no annotation' category, there was lower-level annotation for several of these genes using the functional annotation from Vectorbase, including 10 cuticular genes, two cercropin genes, CPIJ005108 and CPIJ010699, and a high-affinity nuclear juvenile hormone (JH) binding protein CPIJ006964 (Appendix 4.2). Within the SCOP 'metabolism' category, the greatest numbers of up-regulated genes among the permethrin-treatments were contained within the redox category, including nine cytochrome P450 genes (*CYP325BF1-de1b*, *CYP325BF1v2*, *CYP6BY2*, *CYP6M14*, *CYP4H34*, *CYP6N19*, *CYP6M16*, *CYP6M13*, *CYP6M15*) (Table 4.3; Appendix 4.2). Within the SCOP 'general' category, the greatest number of up-regulated genes were contained within the small molecule binding category, which contained two fatty acyl-CoA reductases (CPIJ007244, CPIJ007245) and a glutathione S-transferase (CPIJ002679) (Table 4.3; Appendix

4.2). Finally, within the SCOP 'intra-cellular processes' category, the greatest number of up-regulated genes was present in the proteases category which contained two trypsin-like serine proteases (CPIJ002140, CPIJ016012), two metalloproteases (CPIJ002142, CPIJ002156) and nephrosin (CPIJ009594) (Table 4.3; Appendix 4.2).

4.4.3. Down-regulated Genes

A total of 271 genes were determined to be down-regulated, which included 107, 214, and 216 genes in the acetone, permethrin LC_{50}, and permethrin LC_{70} treatments, respectively (Fig. 4.1; Table 4.2). Sixty-four of the down-regulated genes were down-regulated in all of the 24 hour exposure treatments (acetone, permethrin LC_{50}, and permethrin LC_{70}), with an additional 10 genes down-regulated in both the acetone and the permethrin LC_{50} treatment, and seven genes down-regulated in both the acetone and the LC_{70} treatment (Fig. 4.1).

Table 4.3. Genes up-regulated and down-regulated 24h-post treatment following treatment with permethrin at either the LC_{50} or the LC_{70} rate.

SCOP Functional annotation[†]		Up-regulated		Down-regulated	
General function	Detailed function	Superfamilies	Genes	Superfamilies	Genes
Extra-cellular processes	Blood clotting	0	0	1	1
	Cell adhesion	3	3	2	2
	Immune response	0	0	1	1
	Toxins/defense	1	1	0	0
General	General	7	9	2	2
	Small molecule binding	5	20	4	4
Information	Chromatin structure	1	1	1	1
	DNA replication/repair	2	3	1	1
	Translation	0	0	1	1
Intra-cellular processes	Cell cycle, Apoptosis	1	1	0	0
	Cell motility	3	4	0	0
	Ion m/tr	4	10	1	2
	Phospholipid m/tr	1	3	1	2
	Proteases	7	19	5	24

	Protein modification	1	2	1	1
	Transport	3	5	0	0
Metabolism	Amino acids m/tr	0	0	1	2
	Carbohydrate m/tr	4	6	2	18
	Coenzyme m/tr	1	1	1	1
	E- transfer	1	1	1	1
	Energy	0	0	1	1
	Lipid m/tr	1	1	0	0
	Nitrogen m/tr	0	0	1	1
	Nucleotide m/tr	1	1	0	0
	Other enzymes	3	10	8	32
	Polysaccharide m/tr	1	3	1	1
	Redox	5	16	3	9
	Secondary metabolism	3	4	1	7
	Transferases	0	0	3	3
Regulation	DNA-binding	3	3	3	3
	Kinases/phosphatases	1	3	1	3
	Receptor activity	0	0	2	2
	Signal transduction	6	9	0	0
Other	Unknown function	4	4	1	2
	Viral proteins	0	0	1	1
No annotation	No annotation	1	81	1	17
	TOTAL	74	224	53	146

†SCOP general and detailed functions using the predicted *Cx. quinquefasciatus* annotation information available at the Superfamily website (version 1.75) supfam.cs.bris.ac.uk/SUPERFAMILY/index.html

Forty-four genes were down-regulated in the acetone treatment alone, while 26, 21, and 99 genes were down-regulated in the permethrin LC_{50}, the permethrin LC_{70}, and both the permethrin LC_{50} and LC_{70} treatments, respectively. Among the genes that were down-regulated only in the permethrin treatments, more than half of the genes (76) were present in the SCOP 'metabolism' category, most notably the carbohydrate metabolism/transport, other enzymes, and redox categories, which contained a carboxylesterase (CPIJ018233), four alpha amylase genes (CPIJ005062, CPIJ005725, CPIJ801597, CPIJ801761), two alpha-glucosidases (CPIJ009306, CPIJ013169), and multiple hexamerin and larval serum storage proteins (CPIJ000056, CPIJ001820, CPIJ9033, CPIJ007783, CPIJ009032) (Table 4.3; Appendix 4.3). The second

largest down-regulated SCOP general function category was 'intra-cellular processes', and contained, notably, the proteases category, which contained multiple trypsin-like serine proteases (Table 4.3; Appendix 4.3).

4.4.4. Functional enrichment of Gene Ontology (GO) terms among the differentially-expressed genes

Following the identification of the up- and down-regulation genes, we conducted a functional enrichment analysis on the gene ontology (GO) terms associated with the differentially-expressed genes. This type of analysis will identify GO terms that are statistically enriched among either the up- or the down-regulated genes in comparison to all of the genes expressed. Among the up-regulated genes, the Biological Process GO terms associated with immune response, and oxidation-reduction processes were predominant, while the predominant Molecular Function GO terms were oxidoreductase activity, monooxygenase activity, and structural constituent of cuticle (Table 4.4). Among the down-regulated genes, the Biological Process GO terms associated with carbohydrate metabolic process, lipid metabolic process, and proteolysis were identified, while the majority of the Molecular Function GO terms were associated with protease functions, phosphatidylcholine 1-acylhydrolase activity, and alkaline phosphatase activity (Table 4.4). When the functionally-enriched GO terms from the up- and down-regulated genes were connected into a network through their parent-child terms, the network revealed that the functionally-enriched GO terms among the up- and down-regulated genes shared only three higher-level nodes: metabolic process, catalytic activity, and organic substance metabolic process (Fig. 4.2). This indicated that the functionally-enriched GO terms from the up-regulated genes formed distinct clusters from the functionally-enriched GO terms

from the down-regulated genes. In particular, GO terms associated with proteolytic and primary metabolic processes were functionally-enriched among the down-regulated genes, while the GO terms structural constituent of cuticule, oxidoreductase activity, and various immune response GO terms were functionally-enriched among the up-regulated genes (Fig. 4.2).

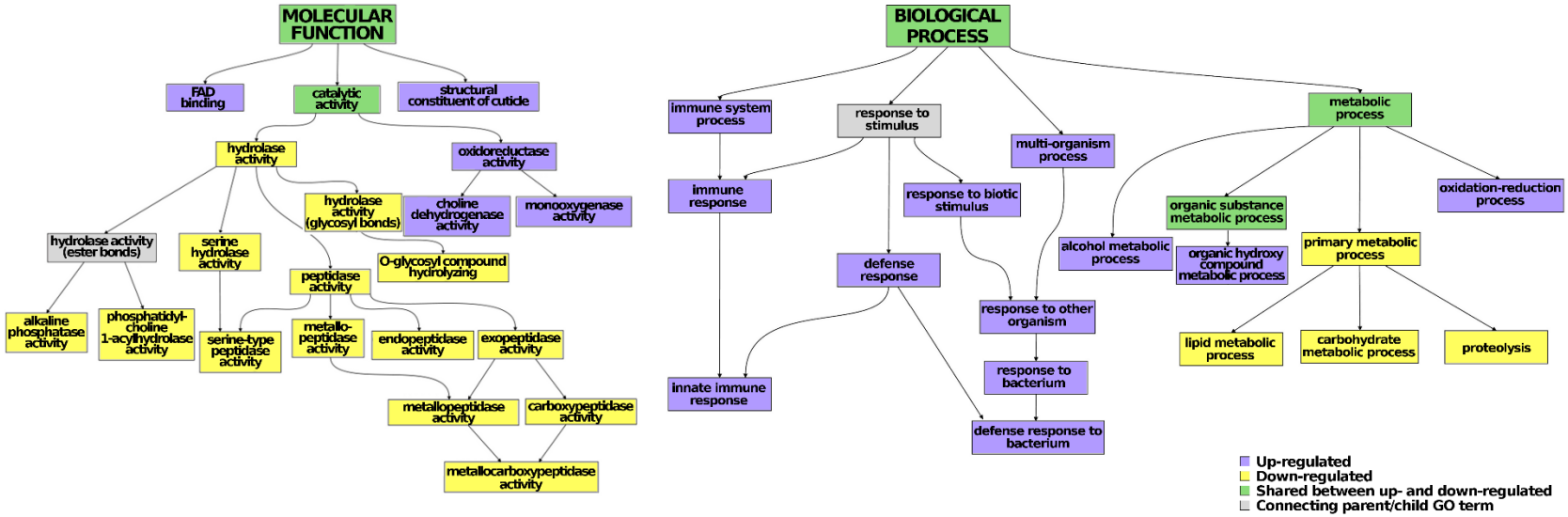

Figure 4.2. Gene ontology term functional enrichment analysis of the differentially-expressed up- and down-regulated genes identified in the fourth instar of the permethrin resistant HAmCq^{G8} strain of *Cx. quinquefasciatus* following a 24h exposure to permethrin.

When the genes associated with the functionally-enriched GO terms were compiled, a total of 29 and 200 genes were shared among the up- and down-regulated gene sets, respectively.

Table 4.4. Gene Ontology (GO) term enrichment analysis results for differentially expressed genes in the HAmCq^{G8} strain following a 24h exposure to permethrin at the LC_{50} and LC_{70} rates.

GO level	GO term[†]	Term domain and name	# hits	p-value[‡]
Up-regulated genes				
Biological Process[*]	GO:0009607	response to biotic stimulus	3	1.47×10^{-4}
	GO:0002376	immune system process	3	2.33×10^{-2}
	GO:0051704	multi-organism process	3	5.86×10^{-4}
	GO:0051707	response to other organism	3	
	GO:0009617	response to bacterium	3	1.47×10^{-4}
	GO:0006952	defense response	3	3.09×10^{-2}
	GO:0042742	defense response to bacterium	3	1.47×10^{-4}
	GO:0006955	immune response	3	2.33×10^{-2}
	GO:0045087	innate immune response	3	5.05×10^{-3}
	GO:1901615	organic hydroxy compound metabolic process	4	3.74×10^{-2}
	GO:0006066	alcohol metabolic process	4	2.55×10^{-2}
	GO:0055114	oxidation-reduction process	14	1.44×10^{-2}
Molecular Function[*]	GO:0016491	oxidoreductase activity	16	2.09×10^{-3}
	GO:0004497	monooxygenase activity	7	2.19×10^{-2}
	GO:0042302	structural constituent of cuticle	8	5.70×10^{-4}
	GO:0008812	choline dehydrogenase activity	4	1.39×10^{-3}
	GO:0050660	flavin adenine dinucleotide binding	5	3.62×10^{-2}
Down-regulated genes				
Biological Process[*]	GO:0005975	carbohydrate metabolic process	21	4.50×10^{-8}
	GO:0006629	lipid metabolic process	11	8.54×10^{-4}
	GO:0006508	Proteolysis	28	6.76×10^{-10}
Molecular Function[*]	GO:0017171	serine hydrolase activity	16	8.90×10^{-6}
	GO:0016798	hydrolase activityacting on glycosyl bonds	12	1.71×10^{-7}
	GO:0004553	hydrolase activityhydrolyzing O-glycosyl compounds	9	1.20×10^{-4}
	GO:0008233	peptidase activity	29	7.76×10^{-11}
	GO:0008238	exopeptidase activity	12	2.15×10^{-10}
	GO:0004180	carboxypeptidase activity	9	7.32×10^{-8}
	GO:0008237	metallopeptidase activity	10	3.40×10^{-4}
	GO:0004181	metallocarboxypeptidase activity	5	8.19×10^{-3}
	GO:0004175	endopeptidase activity	15	1.17×10^{-3}
	GO:0008236	serine-type peptidase activity	16	8.90×10^{-6}
	GO:0008970	phosphatidylcholine 1-acylhydrolase activity	3	1.82×10^{-3}
	GO:0004035	alkaline phosphatase activity	4	4.88×10^{-6}

[†]Annotation from the Gene Ontology consortium (version 1.2084; release date: 12:07:2011)
[‡] Cumulative hypergeometric p-values for GO terms of transcripts that were differentially upregulated in when tested against all transcripts with expression levels of FPKM > 1 using the g:SCS threshold.
[*]Higher-level GO terms have been removed

Of particular interest among the up-regulated genes with functionally-enriched GO terms were

nine cytochrome P450 genes (*CYP325BF1-de1b, CYP325BF1v2, CYP6BY2, CYP6M14, CYP4H34, CYP6N19, CYP6M16, CYP6M13, CYP6M15*), eight cuticular genes (CPIJ001839, CPIJ004289, CPIJ004293, CPIJ008231, CPIJ013788, CPIJ016318, CPIJ017925, CPIJ018582), and three genes involved in the insect immune response: two cecropin genes (CPIJ005108, CPIJ010699), and a salivary peptide gene (CPIJ010700) (Table 4.5).

Table 4.5. Differentially-expressed genes associated with functionally-enriched Gene Ontology (GO) terms in the HAmCq^{G8} strain following a 24h exposure to permethrin at the LC_{50} and LC_{70} rates.

GO term	Genes sharing a given GO term*
Up-regulated	
response to biotic stimulus	CPIJ005108, CPIJ010699, CPIJ010700
immune system process	CPIJ005108, CPIJ010699, CPIJ010700
multi-organism process	CPIJ005108, CPIJ010699, CPIJ010700
response to other organism	CPIJ005108, CPIJ010699, CPIJ010700
response to bacterium	CPIJ005108, CPIJ010699, CPIJ010700
defense response	CPIJ005108, CPIJ010699, CPIJ010700
defense response to bacterium	CPIJ005108, CPIJ010699, CPIJ010700
immune response	CPIJ005108, CPIJ010699, CPIJ010700
innate immune response	CPIJ005108, CPIJ010699, CPIJ010700
organic hydroxy compound metabolic process	CPIJ017482, CPIJ001367, CPIJ007619, CPIJ017491
alcohol metabolic process	CPIJ017482, CPIJ001367, CPIJ007619, CPIJ017491
oxidation-reduction process	CPIJ800256, CPIJ800177, CPIJ800178, CPIJ800210, CPIJ800175, CPIJ800176, CPIJ800180, CPIJ017482, CPIJ017198, CPIJ015953, CPIJ013724, CPIJ001367, CPIJ007619
oxidoreductase activity	CPIJ800256, CPIJ800177, CPIJ800178, CPIJ800210, CPIJ800175, CPIJ800176, CPIJ800180, CPIJ017482, CPIJ017198, CPIJ015953, CPIJ013724, CPIJ007244, CPIJ007245, CPIJ001367, CPIJ007619
monooxygenase activity	CPIJ800256, CPIJ800177, CPIJ800178, CPIJ800210, CPIJ800175, CPIJ800176, CPIJ800180
structural constituent of cuticle	CPIJ004293, CPIJ001839, CPIJ017925, CPIJ016318, CPIJ004289, CPIJ008231, CPIJ018582, CPIJ013788
choline dehydrogenase activity	CPIJ017482, CPIJ001367, CPIJ007619, CPIJ017491
FAD binding	CPIJ017482, CPIJ013724, CPIJ001367, CPIJ007619, CPIJ017491
Down-regulated	
carbohydrate metabolic process	CPIJ801597, CPIJ801761, CPIJ003338, CPIJ003955, CPIJ004229, CPIJ004320, CPIJ004321, CPIJ004323, CPIJ004325, CPIJ004733, CPIJ005062, CPIJ005725, CPIJ008529, CPIJ008530, CPIJ008531, CPIJ009306, CPIJ010291, CPIJ012138, CPIJ013169, CPIJ013477, CPIJ014181
lipid metabolic process	CPIJ801475, CPIJ801474, CPIJ802146, CPIJ002726, CPIJ004224, CPIJ004225, CPIJ004226, CPIJ004227, CPIJ004228, CPIJ004230, CPIJ005462
proteolysis	CPIJ011433, CPIJ801485, CPIJ801477, CPIJ801679, CPIJ801680, CPIJ802425, CPIJ002128, CPIJ002133, CPIJ002136, CPIJ002137, CPIJ002911, CPIJ002943,

	CPIJ004060, CPIJ006077, CPIJ006079, CPIJ008873, CPIJ008874, CPIJ008876, CPIJ009738, CPIJ010641, CPIJ010801, CPIJ010805, CPIJ010806, CPIJ011383, CPIJ011617, CPIJ012036, CPIJ014108, CPIJ018060
serine hydrolase activity	CPIJ011433, CPIJ802425, CPIJ002128, CPIJ002133, CPIJ002136, CPIJ002137, CPIJ002911, CPIJ006077, CPIJ006079, CPIJ008873, CPIJ008874, CPIJ008876, CPIJ010641, CPIJ011383, CPIJ011617, CPIJ018060
hydrolase activity acting on glycosyl bonds	CPIJ801597, CPIJ801761, CPIJ003338, CPIJ004229, CPIJ004320, CPIJ004321, CPIJ004323, CPIJ004325, CPIJ005725, CPIJ008529, CPIJ008530, CPIJ008531
hydrolase activity hydrolyzing O-glycosyl compounds	CPIJ003338, CPIJ004229, CPIJ004320, CPIJ004321, CPIJ004323, CPIJ004325, CPIJ008529, CPIJ008530, CPIJ008531
peptidase activity	CPIJ011433, CPIJ801485, CPIJ801477, CPIJ801679, CPIJ801680, CPIJ802425, CPIJ002128, CPIJ002133, CPIJ002136, CPIJ002137, CPIJ002911, CPIJ002943, CPIJ004060, CPIJ006077, CPIJ006079, CPIJ008873, CPIJ008874, CPIJ008876, CPIJ009738, CPIJ010641, CPIJ010801, CPIJ010805, CPIJ010806, CPIJ011383, CPIJ011617, CPIJ012036, CPIJ014108, CPIJ015407, CPIJ018060
exopeptidase activity	CPIJ801679, CPIJ801680, CPIJ002911, CPIJ004060, CPIJ008873, CPIJ008874, CPIJ008876, CPIJ010801, CPIJ010805, CPIJ010806, CPIJ012036, CPIJ015407
carboxypeptidase activity	CPIJ801679, CPIJ801680, CPIJ002911, CPIJ008873, CPIJ008874, CPIJ008876, CPIJ010801, CPIJ010805, CPIJ010806
metallopeptidase activity	CPIJ801485, CPIJ801477, CPIJ801679, CPIJ801680, CPIJ002943, CPIJ004060, CPIJ010801, CPIJ010805, CPIJ010806, CPIJ012036
metallocarboxypeptidase activity	CPIJ801679, CPIJ801680, CPIJ010801, CPIJ010805, CPIJ010806
endopeptidase activity	CPIJ011433, CPIJ801485, CPIJ801477, CPIJ002128, CPIJ002133, CPIJ002136, CPIJ002137, CPIJ002943, CPIJ004060, CPIJ006077, CPIJ006079, CPIJ010641, CPIJ011383, CPIJ011617, CPIJ012036
serine-type peptidase activity	CPIJ011433, CPIJ802425, CPIJ002128, CPIJ002133, CPIJ002136, CPIJ002137, CPIJ002911, CPIJ006077, CPIJ006079, CPIJ008873, CPIJ008874, CPIJ008876, CPIJ010641, CPIJ011383, CPIJ011617, CPIJ018060
phosphatidylcholine 1-acylhydrolase activity	CPIJ801475, CPIJ801474, CPIJ004227
alkaline phosphatase activity	CPIJ001262, CPIJ001264, CPIJ001265, CPIJ018121

[†]Annotation from the Gene Ontology consortium (version 1.2084; release date: 12:07:2011)
[*]Vectorbase: https://www.vectorbase.org/organisms/culex-quinquefasciatus; release: CpipJ1.3 2012-04-02

Among the down-regulated genes associated with the functionally-enriched GO terms, the majority of the genes were associated with proteolytic activity including two aminopeptidase N precurors (CPIJ004060, CPIJ012036), a lysosomal pro-X carboxypeptidase (CPIJ008876), and an xaa-pro aminopeptidase I (CPIJ015407). In addition, four genes associated with the GO term 'carbohydrate metabolic process' were gram-negative bacteria binding proteins (CPIJ004320, CPIJ004320, CPIJ004320, CPIJ004320), indicating that there were genes involved in the immune response of *Cx. auinquefasciatus* among both the up- and down-regulated genes,

however, these genes in the up-regulated gene set had different immune response functions than the down-regulated immune genes.

4.4.5. qRT-PCR Validation of Selected Genes

The differential up-regulation of cuticular genes in the permethrin treatments compared to the acetone treatment coincided with the up-regulation of juvenile hormone (JH) binding protein CPIJ006964 (Appendix 4.3). This led us to hypothesize that there may be a developmental delay of *Cx. quinquefasciatus*

when exposed to permethrin. To investigate if the permethrin exposure delayed the development of the fourth instar HAmCq^{G8} strain, we conducted qRT-PCR analysis on five storage serum proteins (CPIJ000056, CPIJ001820, CPIJ009033, CPIJ007783, CPIJ009032) and one carboxylesterase (CPIJ018233) previously identified as highly-expressed in the fourth instar stage of *Cx. quinquefasciatus* (Reid et al., 2012). If there is a developmental delay in *Cx. quinquefasciatus* upon exposure to permethrin, there should be a corresponding decrease in the expression of storage serum proteins in the permethrin treatment relative to the acetone treatment, moreover, if the response to insecticide exposure is related to insecticide resistance, the corresponding decrease in the expression of storage serum proteins should be more pronounced in the highly permethrin resistant HAmCq^{G8} strain, than when compared to the pyrethroid susceptible S-lab strain. We further investigated six proteases, that had been previously identified as up-regulated in the HAmCq^{G8} strain compared to its parental low permethrin resistant strain HAmCq^{G0} (Reid et al., 2012). The proteases investigated included four genes that were up-regulated upon permethrin exposure (two metalloproteases, CPIJ001240 and CPIJ009594, and two trypsin-like serine proteases, CPIJ002140 and CPIJ016012), as well as

two genes that were down-regulated upon permethrin exposure (CPIJ002142 and CPIJ002156). Following a 24h exposure to permethrin at the LC_{50} rate, one protease, CPIJ009594 was significantly up-regulated ($P<0.01$) in the HAmCqG8 strain compared to the S-lab strain, while one protease, CPIJ002142, was significantly down-regulated ($P<0.01$) (Fig. 4.3).

When the fourth instar from the two *Cx.quinquefasciatus* strains were exposed to permethrin at the LC_{50} rate for the longer 48h time period, the up-regulation of CPIJ009594 doubled in the permethrin susceptible S-lab strain, but tripled in the permethrin resistant $HAmCq^{G8}$ strain, suggesting that CPIJ009594 (nephrosin) is involved not only in response to permethrin exposure, but to insecticide resistance as well.

When the gene expression levels of the larval serum storage proteins were investigated, all six storage proteins had significantly greater down-regulation in the $HAmCq^{G8}$ strain than in the S-lab strain, with three of these genes, CPIJ000056, CPIJ001820, and CPIJ018233 being down-regulated in both mosquito strains relative to a comparable acetone exposure treatment (Fig. 4.3).

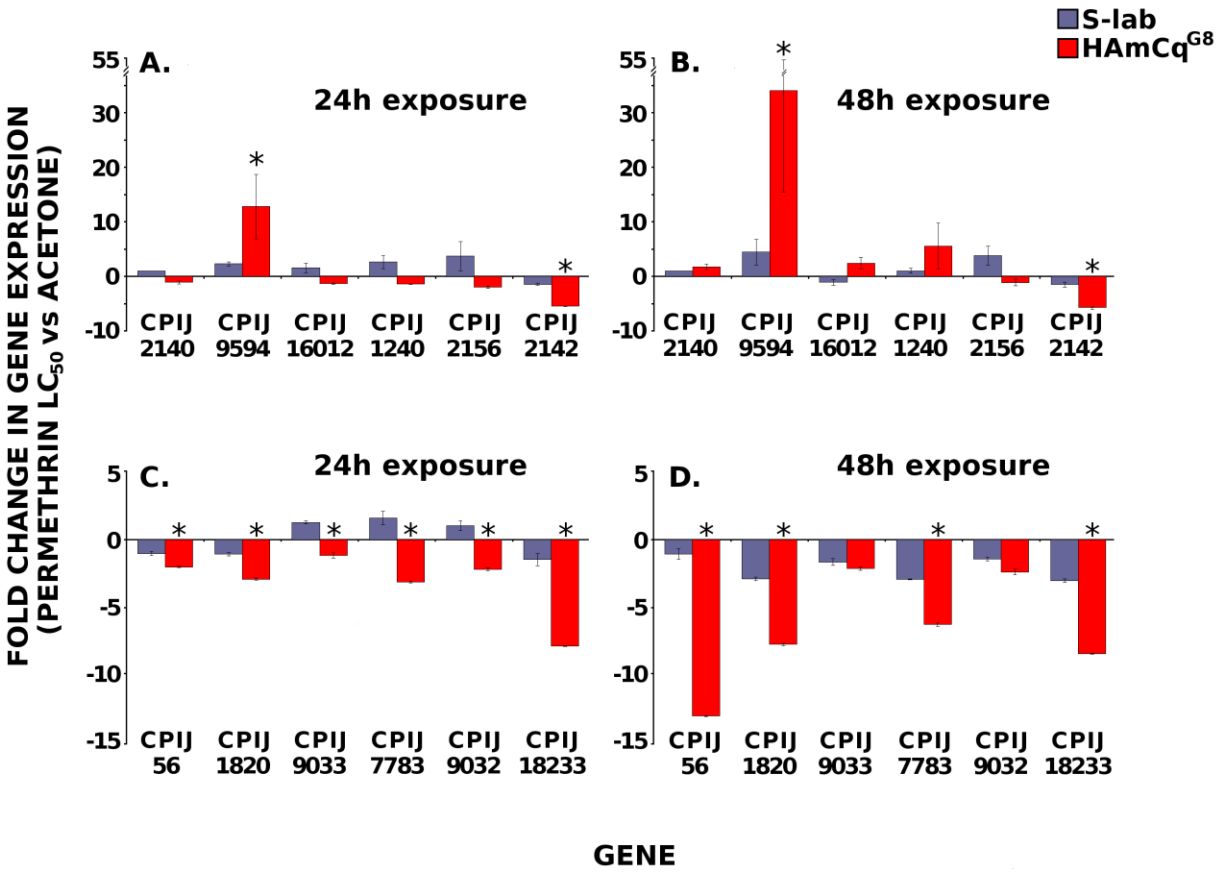

Figure 4.3. Gene expression levels for proteases (upper panels A and B) and larval storage proteins (lower panels C and D). Bars superseded with a "*" indicate that the expression level between the permethrin susceptible S-lab strain and the permethrin resistant HAmCq^{G8} strain of *Cx. quinquefasciatus* were significantly different at the α=0.01 level of significance. Bars shown superior to the dependent axis zero line indicate genes that were up-regulated relative to a comparative acetone blank treatment control, while genes inferior to the dependent axis zero line indicate genes that were down-regulated relative to a comparative acetone blank treatment control.

The down-regulation of larval serum storage proteins was more pronounced following the longer (48h) permethrin exposure, where all six genes were down-regulated in both the S-lab and the HAmCq^{G8} strains compared to the 48h acetone exposure treatment, with four of the genes, CPIJ000056, CPIJ001820, CPIJ007783, and CPIJ018233 having a significantly greater (P<0.01) down-regulation in the HAmCq^{G8} strain compared to the permethrin susceptible S-lab strain.

4.5 Discussion

Overall, a total of 224 genes were up-regulated in response to permethrin exposure and 146 genes were down-regulated. Several of the genes up-regulated were found among categories previously identified as up-regulated in response to permethrin exposure including cytochrome P450s and a glutathione S-transferase (Liu et al., 2011; Gong et al., 2013). In addition to the expected genes, we identified several novel profiles. The first was the down-regulation of multiple proteases in the permethrin treated *Cx. quinquefasciatus.* Some of the down-regulated proteases represent genes that are known to be involved in cell signaling including two aminopeptidase N precurors (CPIJ004060, CPIJ012036), a lysosomal pro-X carboxypeptidase (CPIJ008876), and an xaa-pro aminopeptidase I (CPIJ015407), while there were fewer proteases within the up-regulated genes, notably one protease, a nephrosin CPIJ009594, which was further confirmed to be up-regulated in the $HAmCq^{G8}$ strain compared to the permethrin susceptible S-lab strain. While no role for nephrosin has been proposed for mosquitoes, it is known to be involved in the immune response in fish (Boutet et al., 2006; Darawiroj et al., 2008). Darawiroj et al. (2008) further identified that nephrosin was down-regulated the common carp, *Cyprinus carpio*, when exposed to a lipopolysaccharide (LPS) treatment to mimic a gram-negative bacterium exposure. Interestingly, we identified that during the permethrin exposure, genes involved in gram-negative bacteria binding were down-regulated, while nephrosin was up-regulated. These results suggested that exposure to permethrin results in changes in the immune response status of *Cx. quinquefasicatus,* and further suggested that nephrosin is involved. The hypothesis that there is a correlation between the insect immune response and insecticide resistance was further supported by our finding that two cecropin genes (CPIJ005108, CPIJ010699) were up-regulated upon permethrin exposure. In insects, there are multiple immune

response pathways, with gram-negative bacteria activating the 'immune deficiency' (IMD) pathway and fungi and gram-positive bacteria activating the Toll pathway (DeGregorio et al., 2002; Hedengren-Olcott, 2004; Pan et al., 2012). Cecropin genes have been shown to be regulated via the Toll pathway (Hedengren-Olcott et al., 2004; Pan et al., 2012), while genes regulated by the IMD pathway (eg/ gram-negative bacteria binding) were down-regulated. Work by Ogawa et al. (2005) found a similar trend in human macrophages, where LPS-inducible genes were inhibited by nuclear receptors downstream of the Toll receptor. In addition, there are additional insect immune pathways, such as the JAK-STAT pathway, which is up-regulated in *An. aquasalis* in response to *Plasmodium vivax* infection (Bahia et al., 2011), and the prophenyloxidase (PPO) pathway, however no PPO or JAK-STAT regulated genes were identified in the up- or down-regulated genes in the permethrin exposed *Cx. quinquefasciatus*. Work by Russell and Dunn (1996) demonstrated that antibacterial proteins were up-regulated in the midgut of *Manduca sexta* during the wandering stage, while recent work on *M. sexta* by Xu et al. (2012b) has further shown that along with immune genes, other genes involved in metabolism and transport were up-regulated during the wandering stage. When fourth instar *Cx. quinquefasciatus* were exposed to permethrin at either the LC_{50} or LC_{70} rates, we identified that multiple gram-negative bacteria binding genes were down-regulated relative to the acetone control treatment, as were 18 genes involved in carbohydrate metabolism and transport (Table 4.3), suggesting that the acetone treated fourth instar were further along with development to the pupa than the permethrin treated fourth instar *Cx. quinquefasciatus*. Although *Cx. quinquefasciatus* does not exhibit a wandering stage similar to *M. sexta*, it is possible that the relatively higher expression levels of the gram-negative bacteria binding genes and the carbohydrate metabolism and transport genes indicate that the larvae in the acetone treatment

were closer to pupation than the larvae in either of the permethrin treatments, since these mentioned genes are known to be up-regulated in *M. sexta* during wandering. The differences in the gene expression profiles of immune response genes between permethrin exposed and control mosquitoes could indicate a delay in development in the fourth instar stage of *Cx. quinquefasciatus* when exposed to permethrin. A possible connection between the developmental delay and the immune response status of *Cx. quinquefasciatus* could be due to the status of the nutritional signaling target of rapamycin (TOR) receptor. TOR signaling and downstream TOR kinase is involved in the control of cellular activity in response to nutrient availability (Hansen et al., 2004) and in the larval stages of *D. melanogaster* TOR signaling in the fat body has been shown to restrict growth via a humoral mechanism, such that when nutrients are limited, the TOR pathway is suppressed (Colombani et al., 2003). In addition, connections between the TOR pathway and various immune response pathways have been identified. Turnquist et al. (2010) identified that inhibition of mTOR (mammalian TOR) induced production of Interleukin-12p70, which Lichtenegger et al. (2012) identified to be controlled by the Toll pathway. Taken together, these studies connected the TOR and Toll pathways in humans, if a similar connection exists in mosquitoes as well, it may explain the observed differences in the immune gene expression profiles between permethrin exposed and non-exposed fourth instar *Cx. quinquefasciatus*, that is: if fourth instar *Cx. quinquefasciatus* cease feeding, the resulting decrease in TOR activity could lead to an increase in Toll pathway gene expression. Feeding aversion to insecticide formulations has been documented in other insects, most notably glucose aversion in hydramethylnon baits in *Blatella germanica*, where roaches that fed on glucose-spiked hydramethylon developed an aversion to glucose (Silverman and Bieman, 1993). The underlying mechanism of glucose aversion has been identified to be due to changes in the binding properties of the gustatory

receptors of *B. germanica* to glucose, where GRN1, which detects glucose in wild type roaches became insensitive in glucose averse roaches, while GRN2, which detects bitter compounds in wild type roaches, is stimulated by glucose in glucose averse roaches (Wada-Katsumata et al., 2013). Thus the observed differences in the expression of immune-related genes in permethrin-exposed *Cx. quinquefasciatus* may be the result of changes in TOR signaling due to a decrease of nutrient availability as a consequence of a cessation of feeding in order to reduce the oral exposure of the larvae to permethrin. A delay in the development in permethrin-exposed fourth instar *Cx. quinquefasciatus* due to a cessation of feeding may represent a behavioral resistance response to limit the oral exposure of the mosquito larvae to the toxicant. To further investigate if fourth instar *Cx. quinquefasciatus* are developmentally-delayed when exposured to permethrin, we investigated the gene expression levels of serum storage proteins that are up-regulated in the fourth instar prior to pupation. The results showed that the serum storage proteins were down-regulated in the permethrin treatments relative the acetone control for both the permethrin resistant $HAmCq^{G8}$ strain, and in the permethrin susceptible S-lab strain. This indicated that the development to pupation for fourth instar *Cx. quinquefasciatus* is likely delayed in multiple strains upon exposure to permethrin. Furthermore, the level of down-regulation for the serum storage proteins was significantly greater ($P<0.01$) for the permethrin resistant $HAmCq^{G8}$ strain than for the S-lab strain, suggesting that while permethrin may cause a developmental delay in the S-lab strain, the effect is significantly greater in a permethrin resistant strain of *Cx. quinquefasciatus*. Taken together these results suggested that there may a behavioral resistance phenomenon among fourth instar *Cx. quinquefasciatus* to limit oral exposure in response to permethrin exposure mediated by a cessation of feeding.

4.6 Acknowledgements

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4.7 Disclosure

All authors declare no conflict of interest, financial or otherwise, that might potentially bias this work

Chapter 5: Gene expression analysis of a pyrethroid-resistant strain of *Aedes aegypti* and functional testing of selected family 4 cytochrome P450 genes

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5.1 Abstract

The expression levels of 164 cytochrome P450 genes in the *Aedes aegypti* genome were determined for a pyrethroid-resistant strain of *Ae. aegypti* collected from Puerto Rico. Comparison of the gene expression levels with a pyrethroid-susceptible laboratory strain confirmed the up-regulation of certain cytochrome P450 genes identified in other

geographically-diverse populations of insecticide-resistant mosquitoes as well as additional genes. To determine if the cytochrome P450s identified as up-regulated were capable of conferring metabolic resistance in mosquitoes, transgenic *Drosophila melanogaster* lines over-expressing selected cytochrome P450 genes were generated and tested for their ability to survive an insecticidal challenge of either permethrin or beta-cypermethrin. Our study adds to the knowledge-base of the gene expression profiles of cytochrome P450 genes in insecticide-resistant *Ae. aegypti* and the putative functions of these P450 genes.

5.2 Introduction

The yellow fever mosquito, *Aedes aegypti*, is a globally-distributed mosquito and the major vector of dengue virus (Gubler, 1988; Warren and Mahmoud, 1990; Gubler and Clark, 1995). Management of *Ae. aegypti* is primarily through chemical means, including pyrethroids which are preferred due to their low mammalian toxicity and high efficacy against mosquitoes (Hougard et al., 2002; Juntarajumnong et al., 2012; Manda et al., 2013). The repeated use of insecticides, however, has led to an increase in insecticide resistance among mosquitoes (Hemingway et al., 2004, ffrench-Constant et al., 2004; Liu, 2008), which makes the management of *Ae. aegypti* populations problematic since higher doses of insecticide are needed to obtain the same level of control, ultimately leading to insecticide failure (Vulule et al., 1994; Curtis et al., 1998; Liu, 2008). Insecticide resistance is a multi-faceted phenomenon for which the major mechanisms are considered to be: target site insensitivity, reduced penetration rate, and metabolic detoxification. In the latter mechanism, three major classes of enzymes degrade insecticides; they include: cytochrome P450s, hydrolases, and glutathione-S-transferases (Feyereisen, 1995; ffrench-Constant et al., 2004; Hemingway et al., 2004; Yang and Liu, 2011;

Reid et al., 2012; Gong et al., 2013). Among these three categories, studies have investigated the role of cytochrome P450s in *Ae. aegypti* (Strode et al., 2008; Pridgeon et al., 2009; Bariami et al., 2012; Fonseca-Gonzalez et al., 2011; Poupardin et al., 2010; Saavedra-Rodriguez et al., 2012; Strode et al., 2012) and the functionality of selected cytochrome P450s in *Ae. aegypti* and other mosquitoes including *Anopheles gambiae* (Boonseupsekul et al., 2008; McLaughlin et al., 2008; Muller et al., 2008; Stevenson et al., 2011; 2012). Collectively, these studies have investigated the importance of multiple cytochrome P450s in pyrethroid resistance in *Ae. aegypti* and their putative functional roles. The goal of our study was to add to this knowledge-base by determining the gene expression levels of adult females of a pyrethroid-resistant field population of *Ae. aegypti* and to estimate the functionality of selected, previously uncharacterized, cytochrome P450s for their ability to confer resistance to pyrethroids in *Ae. aegypti*.

5.3 Materials and Methods

5.3.1. Mosquito Strains

The Orlando strain of *Ae. aegypti* has been continuously reared at the Center for Medical, Agricultural, and Veterinary Entomology, ARS USDA in Gainesville, FL since 1952 (Clark et al., 2011). The Puerto Rico strain of *Ae aegypti* was collected in urban San Juan, PR in October 2008 and continuously reared in the laboratory as outlined in Clark et al., 2011. No insecticide pressuring was performed on the Puerto Rico *Ae. aegypti* strain, and each generation was tested to ensure that the level of pyrethroid resistance was present within the population.

5.3.2. Bioassays

For all bioassays, the adult female life stage was used. Adult topical assays were performed as described in Pridgeon et al., 2007. Two to five day old adults were collected by aspiration, anesthetized at 4°C for 60 minutes, then females were sorted from males and three 250cc plastic cups containing 10 adult females each were covered with two layers of tulle mesh and provided with cotton balls saturated with 10% sucrose for feeding. A total of three cups (30 insects) were used for each permethrin dose and all experiments were repeated in triplicate, with the exception of the two-point concentration testing of each generation where a single replicate was used due to the small mosquito colony size. The LC_{50} values were determined using six concentrations that resulted in mortality ranging from 10 - 90%along with an acetone (blank carrier) and untreated controls. Prior to application, females were anesthetized for 30 seconds with CO_2 and placed on a 4°C chill table (BioQuip Products, Rancho Dominguez, CA). A 0.5µl droplet of either acetone (controls), or permethrin dissolved in acetone was applied directly to the dorsal surface of the thorax using a 700 series syringe (Hamilton, Reno, NV). In the P450 inhibition assay, piperonyl butoxide (PBO), the inhibitor of P450s was applied topically to adult female *Ae. aegypti* one hour prior to the application of the permethin to allow for the PBO to inhibit the cytochrome P450 activity in the mosquitoes. The maximal concentration of PBO that could be applied to adult female *Ae. aegypti* was first tested against the Orlando strain and a rate of 0.8µg per female was determined to be the minimum concentration to result in mortality. Subsequently, a rate of 0.4 µg was used for each female for the PBO inhibitor assays and resulted in no mortality in both the Orlando and Puerto Rico strains.

5.3.3 RNA extraction, cDNA synthesis, and qRT-PCR gene expression analysis.

Total RNA was isolated using the TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol as outlined in Pridgeon et al., 2008. First strand cDNA synthesis was conducted on 5 µg of total RNA in a 20 µl reaction mixture using oligo-dT_{20} primer (Invitrogen, Carlsbad, CA). The resulting cDNA was further diluted 5-fold and a total of 1 µl was used for each 15 µl qRT-PCR reaction. Quantitative PCR was performed using the SYBR Green PCR Master Mix on an ABI 7300 quantitative PCR System (Applied Biosystems, Foster City, CA). The primers used for each of the cytochrome P450 genes were designed against the Liverpool strain genome v1.1 (Nene et al., 2007) (Table 1). The relative expression levels of each of the cytochrome P450 genes was normalized to rpL24 within mosquito strain and then the fold change in the gene expression level in Puerto Rico compared to Orlando was calculated using the equation $2^{-\Delta\Delta Ct}$ using SDS Software (Livak and Schmittgen, 2001). Testing to identify statistically significant up-regulated genes was performed using a Welch's T-test in R (R Core Team, 2013).

5.3.4 Functional testing of selected cytochrome P450 genes.

The full lengths of the up-regulated P450 genes from the Puerto Rico strain of *Ae. aegypti* were amplified from cDNA using platinum Taq DNA polymerase High Fidelity (Invitrogen, Carlsbad, CA) with specific primer pairs (Table 1) based on the 5’ and 3’ end sequences of the genes. PCR products of the full length genes were purified using a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). The purified PCR products were ligated into pCR 2.1 vector using the Original TA Cloning kit (Invitrogen, Carlsbad, CA) as described by the manufacturer. The full lengths of the genes were cloned in One Shot TOPO 10F’ cells using the One Shot TOP10F’ Chemically Competent *E. coli* kit (Invitrogen, Carlsbad, CA). Cloning and sequence analyses of the cDNAs

were repeated at least three times and three TA clones from each replication were verified by sequencing.

Table 5.1. List of primers used for the qRT-PCR determination of P450 gene expression level and the primers designed to generate the constructs for the functional testing in transgenic *Drosophila melanogaster.*

Primer	Gene†	Vectorbase‡	Forward primer (5'-3')	Reverse primer (5'-3')
Clan2 P450	CYP15B1	AAEL002067	CGGATTCGTTCCTTCGATAA	ATGGAATTCAGCACCGAAAC
	CYP18A1	AAEL004870	CAGTGAAGGTCAGCTGTGGA	CGAGACGGAGAGGTACTTGC
	CYP303A1ae	AAEL012144	GATAGCACGAGCACGACAAA	CCAAGTCCGGGTTTCATAGA
	CYP304B2xx/yy	AAEL014412	GATTGGAAGGAGCAGAGACG	CCTTTCACCGGTTAGCACAT
	CYP304B3yy/xx	AAEL014411	GGTCAGTTCTACCGGACCAA	TCAAATGCCTCAGCACAAAG
	CYP304C1	AAEL014413	GGGAGAATCTACCGGAAAGG	CTCGCGGTACATTTTGGTTT
	CYP305A6	AAEL002071	GCTCCCATTCTTCGGTAACA	TTCCCAATCTGGTTCCCATA
	CYP305A5	AAEL002043	AGCCCTCTCCAAGCAGTACA	AGCCTTGTCCCCATAGTCCT
	CYP306A1	AAEL004888	TCGTGTGATATCCGCAATGT	GCGAGGTTAGTCAGCGTTTC
	CYP307A1	AAEL009762	GCCCTGCTGAAAAGTCTACG	GCCTTTGTCGGTAACGTGAT
	CYP307A1	AAEL009768	GAGTCAGCCTCAGGAAGTGG	CCCGTTCTGATTCAACACCT
	CYP307B1	AAEL006875	ATCATGGAAGCGCTGAGACT	GGTTCTCCCAGAGGTTCTCC
Clan3 P450	CYP6F2	AAEL014678	CGTGAGTGCACCCAAGACTA	GAGCGTACGGTTTGCTCTTC
	CYP6F3	AAEL014684	GTGGCGTTTGGCATTAAGAT	CCGTACGACACCGTTTTTCT
	CYP6M5	AAEL009117	TGGATCTGCTGATCGCTATG	AGCACTTCTTGGACGCATTT
	CYP6M6	AAEL009128	AGAAAATACCCACCCGTTCC	GGTCGAATCTTTTCGGATCA
	CYP6M10	AAEL009125	TGAGTGCAACTCGATGAAGG	ATTCCACCGTGCTTTTTACG
	CYP6M11	AAEL009127	TTGTTCACGACAGGCAGAAG	CCTCGCTGCTTTTATTCTCG
	CYP6N6	AAEL009126	TTCTTCATCGGTGCTGTGAG	TTTGCGCAAGTTCGTACAAG
	CYP6N9	AAEL009121	ACCGCAACCCAAGACTACAC	AAACGCATCCCGATACAGAC
	CYP6N11	AAEL009119	CTGCCTGTCTACGCAATTCA	CAAAATTTTCAACGGGCTTG
	CYP6N11	AAEL009138	TGGGTTATCTGGCGCTATTC	ACACATCCTTGGCAAAGTCC
	CYP6N12	AAEL009124	TTCACTTGCGCGATCACTAC	TGCAGCAATTTCCTCAACAG
	CYP6N13	AAEL009137	ACAATGTCCCGAACTCGAAC	CATCATTCCGAATCGTGTTG
	CYP6N14	AAEL009133	ACGTTTTCTTCCGGGAAACT	TTTCGCCCATTTTTCTGAAC
	CYP6N15	AAEL009122	GTCAAGGCGTACCGTTCATT	CGCGATCGTGAAAGTACTGA
	CYP6N16	AAEL010151	AAAACCTCGCAAGAAAAGCA	GCCACCACCTCGTTCATACT
	CYP6N17	AAEL010158	AAGCATCACCCAGAACCAAC	ACCTTCTTGGCCAGGTTCTT
	CYP6P12v1	AAEL012491	GGCAGTTTTTGGTGGACAGT	CTGCTCGAACACCTTCTTCC
	CYP6S3	AAEL009120	AGGCAGATGGGGAAGAGAAT	TCAACAGCTGCATGAAGTCC
	CYP6Y3	AAEL009132	GCTAGTGGCTGCCGTTCTAC	CAGAGGCGAAGTCAACATGA
	CYP6Z6	AAEL009123	CGAGGTGTCTACTGCAACGA	TAACTTGTGCCCAACATCCA
	CYP6Z7	AAEL009130	GAGATCCGTTTTCTGCAAGC	GGTTCGCGATTCTCAGCTAC
	CYP6Z8	AAEL009131	CCTGAGATGATCCGATTCGT	CTCTTCGAAACCCAAAGCTG
	CYP6Z9	AAEL009129	TCCAATGGAGCAATCACGTA	ATCGTTCCGGAATGTAGCAC
	CYP6AA5v1	AAEL012492	CCAGCTTCGAGCCTTTTATG	TGAAGACTCTGTCGGCAATG
	CYP6AG3	AAEL007024	CCGAACGTTTTAACCCAGAA	CTCGTTTGCCGAAAAGTAGC
	CYP6AG5	AAEL006984	ACATTCCGCAGAAGATGGTC	TAGGTGGATAGCCCAGATCG
	CYP6AG6	AAEL006992	ACACCCGCGTTTACTACGTC	GCGCATCACAAGCAAAGATA
	CYP6AG7	AAEL006989	TGTTTCCAGCTGCATCTTTG	TTTCGCTTGCATCACGATAG
	CYP6AG8	AAEL003890	ACCAGCACACGGAAAGTACC	AACCTGCATACGACCGAAAC
	CYP6AH1	AAEL007473	CTGCGTGCTGAAAGAGTACG	ATCCGCTTACCAACGTCATC
	CYP6AH1	AAEL015641	CTGCGTGCTGAAAGAGTACG	ATCCGCTTACCAACGTCATC
	CYP6AK1	AAEL004941	AAGGATTGTACGCCGATTTG	CTCAAGGAATCCGGTTACGA
	CYP6AL1	AAEL008889	AACCGAGAATGCACCAAAAC	CGACTGTTCGTCACTGGAGA
	CYP6AL3	AAEL009656	GGCAAAGGTTCATCAGGAAA	CACTATGGGTGTGCCCTTCT

	CYP6BB2	AAEL014893	TAGTCGCTAAGGACGGAGGA	AAGTACTCCGGATCGTGGTG
	CYP6BZ1	AAEL012494	GTAGGGCAAATGCTGTGGAT	ACTCCGTTGAACGTTGTTCC
	CYP6CA1	AAEL014680	TCGAGGGACTTTCAGCACTT	AACAAATCGGCCACGTCTAC
	CYP6CB1	AAEL002046	TAAGCAGCGCACCTCCTATT	AAAATCAACGGTCAGCATCC
	CYP6CB1	AAEL009018	GAGTGCAACAGCATGAGGAA	GTCATCGTCGTTCAGCTTCA
	CYP6CB2	AAEL002872	GTTTCGGAGATGGTCCAAGA	GGTTGAAGCATCAGCAGTGA
	CYP6CC1v1	AAEL014890	GGGAACAGTTGGCAGGATAA	AAGTCCGTGTTTGGGTCTTG
	CYP6CD1	AAEL005006	TGGCCATTCTCTAGCGTTCT	TGCAGGTGGTGTAATCCGTA
	CYP9J2	AAEL006805	ACCGTTACGCCAACAAAGAC	GACGATTTTCGATCGGTTGT
	CYP9J6	AAEL002638	CAGCGTCAAACCAAGGGTAT	GTTTCAAATCCAGCCAGGAA
	CYP9J7	AAEL014606	CGGATATGGTGCACTTGTTG	GGTTCAACGCCAGTTCGTAT
	CYP9J8	AAEL006811	CCTCAACCGCAAGTACCAAT	GTCCTTGACACCGATTTGCT
	CYP9J9	AAEL006793	TGATCGCTCAGTGTTTCCTG	TTCGTAGGTCAATGGCTTCC
	CYP9J9	AAEL014605	TGATCGCTCAGTGTTTCCTG	TTCGTAGGTCAATGGCTTCC
	CYP9J10	AAEL006798	TATGGCGGAGTTTTTCAAGG	CACCGATAGCGATTGGAAGT
	CYP9J15	AAEL006795	GTACTACCCACAGCCGGAAA	ACACAACCGCTTTCATCTCC
	CYP9J16	AAEL006815	ACGATTGCCATACACAACGA	TCTGCGTCTTCTCCGTAGGT
	CYP9J17	AAEL009699	GGAGAAATTGGGGGTTGATT	TCATCCATCTCGTGCTTCAG
	CYP9J17	AAEL006784	GAAAGGGAGCACTGAAGCAC	AGGTCAAACCCGTAGACACG
	CYP9J18v1	AAEL006804	TCCCAGATCCAGATCGTTTC	AATTTGCGTCTTTTCCGTTG
	CYP9J19	AAEL006810	CCAACCTTTCTCGTTGGAAA	CTTCTACGGGTTGGTCGGTA
	CYP9J19	AAEL014611	CATCCAGAACGATCCGAAGT	GTCCGCTCCAGACTGAACTC
	CYP9J20v1	AAEL006814	ACGGAACGACATGATCAACA	AGCAACTCGTAGGCCAAGAA
	CYP9J20v2	AAEL014604	TGAGGTTGATGTTCGAGCTG	CCCATTGGTGAAGAACTCGT
	CYP9J21	AAEL014612	GTACAGCGTCTTTCCGAAGC	TGCATCAACAGGTGGATCAT
	CYP9J22	AAEL006802	TGTTGATGCAGGCAAAGAAG	CTGCCAGGAAAAAGACGAAG
	CYP9J23	AAEL014615	GTGCACCTTTCCGGAGTTTA	AAGGGTCTTCTCGAACAGCA
	CYP9J24	AAEL014613	TTCCCAACGTATGCGTTACA	GAGGAACTTCCGTCTTGCTG
	CYP9J26	AAEL014609	AGATGATCGCACAGTGCTTG	GGGCCACATTCTCAGTGTTT
	CYP9J27	AAEL014616	ACGGCAAGAAAATGATGGAC	CGGTTCCATGACTCTCCCTA
	CYP9J27	AAEL014607	CGGCACGGTAAGAAAATGAT	AGCCTTGATCGTCTCCTGAA
	CYP9J28	AAEL014617	TTTCCTCGACAAACCGATTC	AAAGTCCTTAACGGCCACCT
	CYP9J29	AAEL014610	GATGAGCAGCACAGGTCAAA	AGTGGATTGCCCATTTGAAG
	CYP9J30	AAEL014603	TCAAAGTCCTCGGGATGTTC	ATATTACGCCATCGCTGACC
	CYP9J31	AAEL002633	TTTTCAGCGATTGAGTGTCG	ATATCCTTGGTCTGGCGTTG
	CYP9J32	AAEL008846	GCCGTGACTACGTTTTGGAT	CTCGATCCAATGCAATTCCT
	CYP9M4	AAEL001320	GGTTGATCACGAAGGACGTT	AACCGTCGTAAATCCAGCAC
	CYP9M5	AAEL001288	GTCGGTTCTCAGCTTCGTTC	ATGGTCCTCGAACGTGTAGG
	CYP9M6	AAEL001312	CAGTGGCCACCTACGATTTT	CGCTGGATCGTGGATAAGAT
	CYP9M7	AAEL001292	AGGACTATGGCCGGTTTTCT	GCCCTAGAATCGGATCAACA
	CYP9M8	AAEL009591	AGCTGCCACTGTAGCCACTT	TTTGCTGCATCGATTCGTAG
	CYP9M9	AAEL001807	CACTAAGGAAATGGCCTCCA	TCCGGATCAAATCTTTCTGG
	CYP9AE1	AAEL003748	CAGTTCGGGATGGAGTTGTT	TCAACTCGTCGTCACTCCAG
	CYP329B1	AAEL003763	CTTCCTGGACGAAAGCAAAG	TAGTTCCGAATCCACGGAAG
Clan4 P450	CYP4C38	AAEL012266	GAAAAGTCCCACGGCTATGA	CTTTCGTGATGAAGGGGAAA
	CYP4C50	AAEL008017	AAATTCGGAAGCAGAAGCAA	ATGCCTCGATCAACAGATCC
	CYP4C51	AAEL008018	CAATCGACAAGCTCAGACCA	GCATTATGCGTCCGATTTCT
	CYP4C52	AAEL008023	TTCCTCGATCGGGTCATTAG	GCTTTTCTCCACCAGCTTTG
	CYP4D23	AAEL007816	GTTCAACAAGCGCAAGATCA	TTTATCGGAGTTCCCATTGC
	CYP4D24	AAEL007815	TACTTCACCCCGTACCGAAG	AGGGGTCTCCCGTCTACTGT
	CYP4D37	AAEL007795	GGAGACGGTTTGCTTCTGAG	CAAAGCGTACAGCAGCACAT
	CYP4D38	AAEL007807	CAAGCAACCCGATGAATTTT	CGAGCCAGTTGGAGAGGTAG
	CYP4D39	AAEL007808	CGTCGCAGCAATAAAACTCA	CCTCTCGATGGTGAGGAAAA
	CYP4G35	AAEL008345	GGACCGATGGCTTCAGAATA	GCATCAAGCAAAAGCAACAA
	CYP4G35	AAEL006824	GGTCGTCAAGCAGAAGAAGG	GAAATCCAGATCGTCCCTGA
	CYP4G36	AAEL004054	AACACCAATAGCGTGGAAGG	CCCATCATCGAAAGGAAGAA

	CYP4H28	AAEL003380	ACACCGAAGGTGAAACCAAG	GGGCCATCAACGAGAAGTAA
	CYP4H29	AAEL007830	TGCAGGCTGTCAAAGAAATG	GATTCTTGCCTTGCTTGCTC
	CYP4H30	AAEL003399	GCTGCTGAAAATAGCGAACC	GTCCCCCAGGAATAGGACAT
	CYP4H31	AAEL002085	ACAATTCGTGACGGTGTTCA	TTGGATTCTTCTGGGCATTC
	CYP4H32	AAEL007812	ATCCAAATGCTTGGGAACAG	CTTCCTCTCGGATGTCTTCG
	CYP4H33	AAEL013798	CAGTAATTGATGCGCGAAGA	ATGACCCTCGAACATGAAGG
	CYP4J13	AAEL013555	CAGGACCGTTGGAAGTTGAT	AGAGCGCACAGTACCGTTTT
	CYP4J14	AAEL013554	AAGTTGTCCACGGTTTCTGG	GAACGAATGAACCGGAAGAA
	CYP4J15v1	AAEL013556	GCTGGATTCGCTTTTACTCG	GAAAGCTCGGGATGACTGAG
	CYP4J15v2	AAEL014829	AAACATCGATGGGCGTTAAG	AGCCGCTTTATAGCCTGTCA
	CYP4J16	AAEL015663	AACGGATCATGAACCCTCTG	TTCCGCCAGCAGAAGACTAT
	CYP4J17	AAEL015370	AGAATTCCCCTACCGCTTGT	GGCCAGTAAGGTATCCAGCA
	CYP4J17	AAEL014019	GGAGGAGATCGAAACCATGA	ACTGTGCATCCTCCAAAACC
	CYP4K3	AAEL007798	GGAAGTGCAACGGAAATTGT	CTTCGGACTTTGAGGCGTAG
	CYP4AR2	AAEL010154	CGGAGGTTTGCAATGATTCT	CGTCTGGGTACTTTGCCATT
	CYP325E3	AAEL000338	CAATAGGGTGTTTCGGGAGA	CTGTTAAGGATGCGGGTGTT
	CYP325G2	AAEL012766	CTTATCGGTTGTGGCCATCT	CTGCATATCTTCCGGGTTGT
	CYP325G3	AAEL012772	TACCCTTTGATCGGAAGTGC	ACCGGTGAAGTGAACAGTCC
	CYP325K2	AAEL005771	ATTTTGCCCGCTATCTTCCT	TTCTCCTGGCAATAGGGATG
	CYP325K3	AAEL005788	ATTCCGAAGGGAACTGTCCT	CAAGTCGGTGCTGAGTTCAA
	CYP325L1v1	AAEL011770	TCGGTGGAAACGAAACTACC	GTTCGTCCGAGGATTGTTGT
	CYP325M1	AAEL012773	AGACGAAAAGTTCGCAGCAT	CCTCTTGATAGCAGCGTTCC
	CYP325M2	AAEL012769	TTCGTTCTTTTGGGTTCCAC	TGCTGCTTTCCAACGTATTG
	CYP325M2	AAEL015591	TTCGTTCTTTTGGGTTCCAC	TGCTGCTTTCCAACGTATTG
	CYP325M3	AAEL012765	CGATCTGTGTGGAGAGCAAA	GTTTTCGCCGTTGTTTCATT
	CYP325M4	AAEL011769	CGTTGAATCCTTCGTTTGGT	GGCCGTTGCATAGATTTGAT
	CYP325M5	AAEL011761	TGGAAAAATCAACGGAAAGC	TCGCTGCATATCGAAGTCAC
	CYP325N1	AAEL012770	GTACCTTGAAGCGCAAGAGG	TGTTCAGCATTCCTTGCTTG
	CYP325N2	AAEL012762	CTTGCCCGGATAGAAATCAA	TCCGGGTTGAAGTTTTTGAC
	CYP325P1	AAEL000340	CGTGGTTGATTTCGGAGTTT	GCCTGGGTGTGATTTCTGTT
	CYP325Q1	AAEL006044	ACCACGGAAGCTCTGAAAAA	CATCTGGTCCCCATACATCC
	CYP325Q2	AAEL015563	ACGAAAGTGCGGAAAGAAGA	CAGGTGTAGGAAACGGCATT
	CYP325R1	AAEL005775	CGGCTTACTCATGGGTTGTT	TATCCACTGGAGTCCCTTCG
	CYP325S1	AAEL000326	CCGATTTTCTTCGACAGCTC	GCAAAATTCTCCGGATCAAA
	CYP325S2	AAEL000325	GCCGAAATCATGGAACACTT	CCACATATCCGCTCTTCGAT
	CYP325S3	AAEL000357	TTGCTCGGCAGTGTATCAAG	TCCGGTAGGAAATTGTCTGG
	CYP325T2	AAEL012761	GAGTTTGCCATCCGGATCTA	CCTGCTGCAACACTTTTCAA
	CYP325T2	AAEL015475	CTCATGGCCTATGCCTGTTT	GCCATGTTTTGCCTTACGAT
	CYP325U1	AAEL000320	GGGGAAAAATGGTGAGGAAT	ATTCAGTGCCTTACGCTGCT
	CYP325X1	AAEL005695	CTGTACCGGTTTGCTGGATT	TCGTCATCTTCTCGCAACAC
	CYP325X2	AAEL005696	TGTCGTTCTACCCGGAAATC	TCGAACTCGGCCATATTTTC
	CYP325X4	AAEL005700	GGCTCAACTCCAGCTTCAAC	CGAATTCCTCTTTCCCTTCC
	CYP325Y1	AAEL006257	GAAATCGTGCTCGATGGAAT	AGATAGGCAAATGGGTGACG
	CYP325Y2v2	AAEL015362	AGGAAGCCCTCCGTATTTGT	ATGCCTTTGCTGAACTGCTT
	CYP325Y3	AAEL006246	GGCATACGCATCCCTAAAGA	TCTGCATAATCCGCAACAAA
	CYP325Y3	AAEL015361	CAATCGCTTGGTGAGGATTT	CCTCCGGGTACATTGCTAGA
	CYP325Z1	AAEL010273	CACCAAATCCAAGCCAAAGT	GTCTTTCCGCCTGTGAAGAG
	CYP325AA1	AAEL004012	TGCTTTCGTGGATCGTAGTG	CACCAGCTCTGGATGCTGTA
Mito P450	CYP12F5	AAEL001960	ACAAGGAGAAAGCTGGCAAA	CATCGAGAACTCCCAATCGT
	CYP12F6	AAEL002005	TACATCGTTGACTCCGGACA	CGAAGCGATCACTTTGTTGA
	CYP12F7	AAEL002031	CTGGAAACGATGGGTGTTCT	GATAACCGCGTCATCAACCT
	CYP12F8	AAEL006827	TGGATAAGGTTGCCCTTCAG	TCTCCAGATCGAGGGAAGAA
	CYP49A1	AAEL008638	GTGCATCAAAGAAACGCTGA	CGGTCTGGTTCTGGGAAATA
	CYP301A1	AAEL014594	CCTGGAACCGAACTTGACAT	CGTCCTTCACCCAAGTTCAT
	CYP302A1v1	AAEL011463	TTTCGATGTACGGTTGGACA	GCTTTCGATACGCTGGAGTC
	CYP302A1v2	AAEL015655	TTTCGATGTACGGTTGGACA	GCTTTCGATACGCTGGAGTC

	CYP314A1	AAEL010946	GCGGAGACAAGCAAAAGAAC	ACGATTTCGGCGATTGTATC
	CYP315A1	AAEL011850	ATTCATTGGACGCTTTTTGG	TCCCTTCGTAACCACCTTTG
Other	P450 reductase	AAEL003349	TTCCTTCCCCGCTTTTATCT	CTGTGTAGCGGTGCTTGTGT
	60S RP-L24	AAEL008329	GAGGCAGTAAAATTTCGCCA	AGGTGAAAGTCTTGCCATCG
Drosophila constructs	CYP4D24	AAEL007815	CCGCTCGAGCAAAATGCTTAT CTTATTGGCT	CTAGCTCGAGCCGCACCCTGC TTCTGATCCT
	CYP4H29	AAEL007830	CCGGAATTCCAAAATGGTGCC TCTTCTGATG	CTAGCTCGAGTCGTGGCACAA TCTTCACAAA
	CYP4J15v1	AAEL013556	CCGGAATTCCAAAATGTTGCT TATTCTAACGC	CTAGCTCGAGTCTCCTCTCAA ACCTAACCTC
	CYP4H33	AAEL013798	CCGGAATTCCAAAATGGATTT CCTAACGAAT	CTAGCTCGAGAATTCTTTCCA CTAGCTTAAT

†Nomenclature for the cytochrome P450 genes was taken for the *Ae. aegypti* cytochrome P450 database at: http://drnelson.uthsc.edu/CytochromeP450.html
‡Vectorbase *Ae. aegypti* predicted gene set v. AaegL1.1. http://aaegypti.vectorbase.org/

The clones were then sub-cloned into the pUASTattB vector (a gift from Dr. Johannes Bischof, University of Zurich) (Brand and Perrimon, 1993; Bischof et al., 2007). The plasmid of each pUASTattB-up-regulated gene was transformed into the germ line of *D. melanogaster* (Bloomington stock #24484, genotype M{vas-int.Dm}ZH-2A, M{3xP3-RFP.attP'}ZH-58A), resulting in site specific integration on chromosome 2R (Bateman et al., 2006; Rainbow Transgenic Flies Inc., Camarillo, CA). Flies were then reciprocally-crossed against a W^{1118} strain to obtain transgenic *D. melanogaster* with the orange eye phenotype. The flies were then balanced against a *D. melanogaster* balancer strain (Bloomington stock #6312, genotype: w[1118]/Dp(1;Y)y[+]; sna[Sco]/CyO, P{ry[+t7.2]=sevRas1.V12}FK1) to generate a homozygous line containing the cytochrome P450 transgene on chromosome 2R. The insertion of the up-regulated genes in the transgenic fruit fly lines were further confirmed using RT-PCR. Transgenic virgin female *D. melanogaster* were then crossed with male GAL4-expressing *D. melanogaster* (Bloomington stock #3954, genotype: P{Act5C-GAL4}17bFO1) which expresses GAL4 under control of the Act5C promoter, resulting in ubiquitous non-tissue-specific expression. The F1 generation of these crosses expressed GAL4 and contained a single copy of the Cytochrome P450 transgene, which was under control of the UAS enhancer. Permethrin

toxicity bioassays were then conducted on 2-3 day post eclosion female *Drosophila* of F1 UAS-GAL4 crosses to examine the toxicity of pyrethroids to transgenic flies. Briefly, serial concentrations of each pyrethroid solution in acetone, ranging from 25 ng/µL to 100 ng/µL that gave >0 and <100% mortality to the tested insects were prepared. Two hundred µL of each permethrin concentration solution was evenly coated on the inside of individual 20 mL glass scintillation vials. Fifteen female flies were transferred to each of the prepared vials, and three vials were used for each concentration for each bioassay replicate. The vials were plugged with cotton balls soaked with 5% sucrose and the mortality was scored after 24 hr exposure to the pyrethroids. Each bioassay was independently replicated three times using only flies that exhibited the correct morphological marker (GAL4 red eyes). The *D. melanogaster* strain (Bloomington stock #24484, genotype: M{vas-int.Dm}ZH-2A, M{3xP3-RFP.attP'}ZH-58A) containing the empty pUAST vector donated insert, but no transgene from *M. domestica* was used as the control reference strain following the identical crossing protocol of virgin control females with GAL4 expressing males to obtain the F1 generation for testing. Preliminary testing determined that vials coated with 2 and 0.3µg of permethrin and beta-cypermethrin, respectively, resulted in nearly complete mortality of the empty-vector control line. Subsequently, the lowest insecticide concentrations chosen were 5 and 0.5 µg of permethrin and beta-cypermethrin, respectively, which resulted in 100% mortality of the control mosquitoes for all bioassay replicates. All tests were run at 27^{o}C and mortality was assessed after 24 h. All *D. melanogaster* were reared on Jazz-Mix Drosophila food (Fisher Scientific, Kansas City, MO) at 25±2^{o}C under a photoperiod of 12:12 (L:D) h following standard protocols (Ashburner et al., 2005).

5.4 Results and Discussion

Topical application of permethrin revealed that the level of permethrin resistance within the Puerto Rico strain was 73 times higher than in the laboratory insecticide susceptible Orlando strain (Table 2).

Table 5.2. Resistance ratio of the permethrin-resistant strain of *Ae. aegypti* Puerto Rico compared to the laboratory permethrin-susceptible strain Orlando in the presence and absence of the cytochrome P450 metabolic inhibitor piperonyl butoxide (PBO).

Strain	LD_{50} (95% CI) ng/insect	Slope (SE)	χ^2	df	Fold resistance
Orlando	0.21 (0.12 – 0.41)	2.89 (0.44)	8.02	4	1.00
+PBO	0.14 (0.09 – 0.23)	2.08 (0.29)	6.37	5	0.68
Puerto Rico	15.20 (1.00 – 29.90)	1.48 (0.26)	1.39	7	73.07
+PBO	3.28 (2.02 - 5.85)	0.96 (0.14)	3.99	7	15.76

Following a 1 hour pre-treatment of adult mosquitoes with the cytochrome P450 inhibitor piperonyl butoxide (PBO), the resistance ratio of the Puerto Rico strain was decreased to 15-fold compared to the insecticide susceptible Orlando strain and 23-fold compared to the Orlando strain pre-treated with the PBO (Table 2). These results showed that when the cytochrome P450s in the Orlando strain were inhibited by PBO, the resistance level of adult females decreased to 0.7-fold (30% decrease), whereas when the cytochrome P450s in the Puerto Rico strain were inhibited by PBO, the resistance level of adult females decreased to 15-fold (~80% decrease). This suggested that cytochrome P450s may have a role in the level of permethrin resistance observed in the Puerto Rico strain of *Ae. aegypti*. We further tracked the level of resistance in each generation using a two-point primary component analysis to trace the attenuation of permethrin resistance in the Puerto Rico strain. The two doses of permethrin selected were 0.8 ng per female, because this rate represented the LD_{95} for the pyrethroid-susceptible Orlando strain, while the higher dose (6.25 ng per female) was intermediate to the LD_{50} for the Puerto Rico

strain and complete mortality in the Orlando strain. Overall, the level of resistance was found to decrease within the first 18 generations, suggesting that to identify which cytochrome P450s were involved in the observed resistance, all of the cytochrome P450 gene expression levels in both the Puerto Rico and Orlando strains required assessment (Fig. 1). Of the 164 cytochrome P450s investigated for their gene expression levels, a total of 33) cytochrome P450s were significantly up-regulated ($p<0.05$) with ≥ 2-fold increases, while eight cytochrome P450s were significantly down-regulated ($p<0.05$) with ≥2-fold decreases (Table 3). The remaining 123 cytochrome P450 genes showed no significant difference ($p>0.05$) between the Puerto Rico and Orlando strains of *Ae. aegypti* (Table 4). Among the up-regulated genes, the majority were in families CYP4, CYP6, and CYP9 which had 9, 11, and 7 genes, respectively (Table 3).

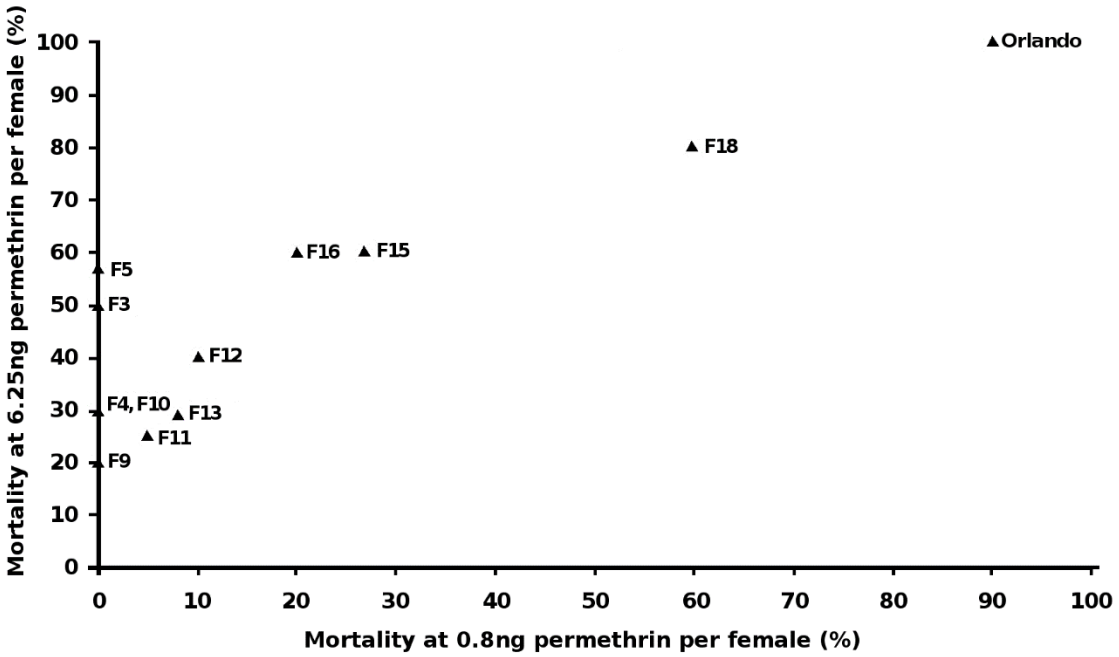

Figure 5.1. Two-point mortality for the Orlando strain of *Aedes aegypti* and the different generations of the Puerto Rico *Ae. aegypti* strain. The 0.8 ng per female (low dose) represents the maximum dose that results in survival in the pyrethroid-susceptible Orlando strain, while the 6.25 ng per female represents the high dose. Puerto Rico generations F1, F2, F6, F7, F8, F14, and F17 did not have sufficient numbers for testing.

The remaining up-regulated cytochrome P450 genes included three in clan 4 - *CYP325* genes,

two in the mito clan - *CYP12F* genes, and one in clan 2 - *CYP15B1*. Earlier studies by multiple researchers have shown up-regulation of cytochrome P450 genes in insecticide resistant *Ae. aegypti* (Strode et al., 2008; Marcombe et al., 2009; Bariami et al., 2012; Saavedra-Rodriguez et al., 2012).

Table 5.3. List of cytochrome P450 genes differentially expressed between the adult females of the pyrethroid-resistant strain of *Aedes aegypti* (Puerto Rico) and the laboratory susceptible strain (Orlando).

Gene[‡]	Name[†]	Fold expression in Puerto Rico	Studies that also identified as up-regulated in insecticide-resistant *Ae. aegypti*
Up-regulated			
Clan 2			
AAEL002067	*CYP15B1*	2.51±0.03**	
Clan 3			
AAEL007024	*CYP6AG3*	2.33±0.05**	
AAEL006989	*CYP6AG7*	2.28±0.08**	
AAEL014893	*CYP6BB2*	3.01±0.18**	(Bariami et al., 2012; Saavedra-Rodriguez et al., 2012)
AAEL002046	*CYP6CB1*	14.46±0.28**	(Strode et al., 2008; Bariami et al., 2012; Saavedra-Rodriguez et al., 2012)
AAEL009018	*CYP6CB1*	10.60±0.41**	(Strode et al., 2008; Bariami et al., 2012)
AAEL014678	*CYP6F2*	4.38±0.11**	
AAEL009127	*CYP6M11*	5.70±0.31**	(Poupardin et al., 2008; Marcombe et al., 2009; Bariami et al., 2012)
AAEL009121	*CYP6N9*	2.31±0.04**	(Bariami et al., 2012)
AAEL009124	*CYP6N12*	3.38±0.16**	(Bariami et al., 2012)
AAEL009137	*CYP6N13*	3.53±0.01**	
AAEL009123	*CYP6Z6*	3.16±0.02**	(Marcombe et al, 2009; Saavedra-Rodriguez et al., 2012)
AAEL006805	*CYP9J2*	12.17±2.83**	
AAEL006798	*CYP9J10*	2.89±0.29**	(Strode et al., 2008; Bariami et al., 2012)
AAEL006814	*CYP9J20v1*	3.29±0.20**	
AAEL014612	*CYP9J21*	3.94±0.42**	
AAEL014609	*CYP9J26*	2.76±0.22**	(Strode et al., 2008; Bariami et al., 2012)
AAEL014616	*CYP9J27*	2.85±0.13**	(Strode et al., 2008; Bariami et al., 2012)
AAEL002633	*CYP9J31*	2.30±0.07**	
Clan 4			
AAEL013556	*CYP4J15v1*	2.30±0.06**	(Marcombe et al, 2009[†])
AAEL014829	*CYP4J15v2*	3.85±0.22**	(Marcombe et al, 2009[†])
AAEL007815	*CYP4D24*	2.81±0.09**	
AAEL008345	*CYP4G35*	3.03±0.08**	
AAEL006824	*CYP4G35*	3.44±0.30**	
AAEL007830	*CYP4H29*	2.52±0.18**	
AAEL003399	*CYP4H30*	3.85±0.18**	
AAEL013798	*CYP4H33*	2.73±0.03**	
AAEL004054	*CYP4G36*	2.84±0.23**	(Saavedra-Rodriguez et al., 2012)
AAEL012766	*CYP325G2*	2.06±0.04	
AAEL012772	*CYP325G3*	3.61±0.20**	
AAEL011769	*CYP325M4*	2.72±0.11**	
Mito clan			
AAEL002005	*CYP12F6*	2.14±0.06*	(Strode et al., 2008)

AAEL006827	*CYP12F8*	2.30±0.06**
Down-regulated		
Clan 3		
AAEL009119	*CYP6N11*	0.33±0.01§
AAEL009131	*CYP6Z8*	0.06±0.01§
AAEL006784	*CYP9J17*	0.31±0.06§
AAEL014611	*CYP9J19*	0.04±0.01§
AAEL014610	*CYP9J29*	0.39±0.07§
AAEL003748	*CYP9AE1*	0.38±0.08§
Clan 4		
AAEL007812	*CYP4H32*	0.36±0.02§
AAEL014019	*CYP4J17*	0.44±0.03§
AAEL012765	*CYP325M3*	0.31±0.02§

†Nomenclature for the cytochrome P450 genes was taken for the *Ae. aegypti* cytochrome P450 database at: http://drnelson.uthsc.edu/CytochromeP450.html
‡Vectorbase *Ae. aegypti* predicted gene set v. AaegL1.1. http://aaegypti.vectorbase.org/
*significantly up-regulated (>2-fold) at the $P<0.05$ level of significance
**significantly up-regulated (>2-fold) at the $P<0.01$ level of significance
§significantly down-regulated (>2-fold) at the $P<0.01$ level of significance

In our study, we found that several of the cytochrome P450 genes that we identified as up-regulated the Puerto Rico field strain were also identified in other studies including: *CYP6BB2*, *CYP6CB1*, *CYP6M1*, *CYP6N9*, *CYP6N12*, *CYP6Z6*, *CYP9J10*, *CYP9J26*, *CYP9J27*, *CYP4J15*, *CYP4G36*, and *CYP12F6*. These results provided strong evidence that gene over-expression of cytochrome P450s was responsible for the observed level of permethrin resistance in the Puerto Rico strain of *Ae. aegypti*. However, further evidence beyond gene over-expression is necessary when characterizing cytochrome P450s for their role in insecticide resistance. Recently, Ptitsyn et al. (2011) showed that the gene expression of multiple genes within *Ae. aegypti* followed a circadian rhythym, and among these genes were 17 cytochrome P450s, including *CYP9J10*, which was identified as upregulated in our study and other studies that investigated gene up-regulation in adult female insecticide-resistant *Ae. aegypti*. In addition, insecticide metabolism studies by Stevenson et al. (2012) have shown that not all up-regulated cytochrome P450s in insecticide resistant mosquitoes can functionally metabolize insecticides. In their study,

Stevenson et al. looked at several cytochrome P450s, including two identified as up-regulated in our study, *CYP6CB1* and *CYP9J26* for which they found that CYP9J26 was functional for metabolizing both permethrin and deltamethrin, while CYP6CB1 did not metabolize either of the two pyrethroids tested.

Table 5.4. Relative cytochrome P450 gene expression values in the pyrethroid-resistant Puerto Rico strain compared with the pyrethroid-susceptible Orlando strain.

Clan[†]	P450[†]	AAEL[‡] gene number	Fold upregulated in Puerto Rico compared to Orlando
Clan 2	CYP15B1	AAEL002067	2.51±0.15[**]
	CYP18A1	AAEL004870	1.29±0.21
	CYP303A1ae	AAEL012144	1.15±0.16
	CYP304B2xx/yy	AAEL014412	1.29±0.17
	CYP304B3yy/xx	AAEL014411	1.00±0.23
	CYP304C1	AAEL014413	1.33±0.17
	CYP305A6	AAEL002071	1.41±0.26
	CYP305A5	AAEL002043	1.38±0.39
	CYP306A1	AAEL004888	0.86±0.33
	CYP307A1	AAEL009762	1.30±0.28
	CYP307A1	AAEL009768	1.48±0.28
	CYP307B1	AAEL006875	1.29±0.17
Clan 3	CYP6F2	AAEL014678	4.38±1.38[**]
	CYP6F3	AAEL014684	1.92±0.62
	CYP6M5	AAEL009117	0.80±0.38
	CYP6M6	AAEL009128	0.69±0.21
	CYP6M10	AAEL009125	0.99±0.35
	CYP6M11	AAEL009127	5.70±0.44[**]
	CYP6N6	AAEL009126	1.62±0.16
	CYP6N9	AAEL009121	2.31±0.17[**]
	CYP6N11	AAEL009119	0.33±0.01[§]
	CYP6N11	AAEL009138	1.50±0.23
	CYP6N12	AAEL009124	3.38±0.24[**]
	CYP6N13	AAEL009137	3.53±0.31[**]
	CYP6N14	AAEL009133	1.45±0.12
	CYP6N15	AAEL009122	1.61±0.17
	CYP6N16	AAEL010151	1.42±0.13
	CYP6N17	AAEL010158	1.48±0.25
	CYP6P12v1	AAEL012491	1.46±0.13
	CYP6S3	AAEL009120	1.7±0.09
	CYP6Y3	AAEL009132	1.5±0.10
	CYP6Z6	AAEL009123	3.16±0.07[**]
	CYP6Z7	AAEL009130	0.89±0.70
	CYP6Z8	AAEL009131	0.06±0.01[§]

CYP6Z9	AAEL009129	1.31±0.06
CYP6AA5v1	AAEL012492	1.61±0.09
CYP6AG3	AAEL007024	2.33±0.06**
CYP6AG5	AAEL006984	1.18±0.14
CYP6AG6	AAEL006992	1.29±0.16
CYP6AG7	AAEL006989	2.28±0.16**
CYP6AG8	AAEL003890	1.37±0.19
CYP6AH1	AAEL007473	1.51±0.11
CYP6AH1	AAEL015641	1.74±0.26
CYP6AK1	AAEL004941	1.92±0.19
CYP6AL1	AAEL008889	2.06±0.11
CYP6AL3	AAEL009656	0.83±0.16
CYP6BB2	AAEL014893	3.01±0.25**
CYP6BZ1	AAEL012494	1.67±0.15
CYP6CA1	AAEL014680	2.15±0.64
CYP6CB1	AAEL002046	14.46±0.05**
CYP6CB1	AAEL009018	10.60±0.13**
CYP6CB2	AAEL002872	0.52±0.32
CYP6CC1v1	AAEL014890	n/d
CYP6CD1	AAEL005006	1.52±0.23
CYP9J2	AAEL006805	12.17±1.23**
CYP9J6	AAEL002638	1.86±0.18
CYP9J7	AAEL014606	1.72±0.17
CYP9J8	AAEL006811	2.11±0.82
CYP9J9	AAEL006793	2.45±0.39
CYP9J9	AAEL014605	2.83±0.26
CYP9J10	AAEL006798	2.89±0.28**
CYP9J15	AAEL006795	0.70±0.53
CYP9J16	AAEL006815	0.88±0.39
CYP9J17	AAEL009699	0.78±0.24
CYP9J17	AAEL006784	0.31±0.06§
CYP9J18v1	AAEL006804	0.77±0.41
CYP9J19	AAEL006810	1.41±0.20
CYP9J19	AAEL014611	0.04±0.01§
CYP9J20v1	AAEL006814	3.29±0.44**
CYP9J20v2	AAEL014604	2.04±0.50
CYP9J21	AAEL014612	3.94±0.43**
CYP9J22	AAEL006802	2.30±0.32
CYP9J23	AAEL014615	3.77±0.31**
CYP9J24	AAEL014613	1.84±0.35
CYP9J26	AAEL014609	2.76±0.24**
CYP9J27	AAEL014616	2.85±0.16**
CYP9J27	AAEL014607	3.17±0.19**
CYP9J28	AAEL014617	1.36±0.3
CYP9J29	AAEL014610	0.39±0.07§
CYP9J30	AAEL014603	1.37±0.33

	CYP9J31	AAEL002633	2.30±0.14**
	CYP9J32	AAEL008846	1.16±0.15
	CYP9M4	AAEL001320	1.02±0.30
	CYP9M5	AAEL001288	1.18±0.34
	CYP9M6	AAEL001312	1.83±0.23
	CYP9M7	AAEL001292	1.73±0.38
	CYP9M8	AAEL009591	1.55±0.21
	CYP9M9	AAEL001807	1.01±0.14
	CYP9AE1	AAEL003748	0.38±0.08§
	CYP329B1	AAEL003763	1.51±0.13
Clan 4	CYP4C38	AAEL012266	1.09±0.15
	CYP4C50	AAEL008017	1.20±0.19
	CYP4C51	AAEL008018	0.85±0.36
	CYP4C52	AAEL008023	0.61±0.42
	CYP4D23	AAEL007816	1.68±0.17
	CYP4D24	AAEL007815	2.81±0.20**
	CYP4D37	AAEL007795	1.47±0.30
	CYP4D38	AAEL007807	1.44±0.10
	CYP4D39	AAEL007808	1.54±0.07
	CYP4G35	AAEL008345	3.03±0.07**
	CYP4G35	AAEL006824	3.44±0.20**
	CYP4G36	AAEL004054	2.84±0.16**
	CYP4H28	AAEL003380	1.27±0.71
	CYP4H29	AAEL007830	2.52±0.43**
	CYP4H30	AAEL003399	3.85±0.50**
	CYP4H31	AAEL002085	1.36±0.51
	CYP4H32	AAEL007812	0.36±0.02§
	CYP4H33	AAEL013798	2.73±0.13**
	CYP4J13	AAEL013555	1.05±0.15
	CYP4J14	AAEL013554	1.00±0.11
	CYP4J15v1	AAEL013556	2.30±0.18**
	CYP4J15v2	AAEL014829	3.85±0.46**
	CYP4J16	AAEL015663	1.06±0.29
	CYP4J17	AAEL015370	1.23±0.42
	CYP4J17	AAEL014019	0.44±0.03§
	CYP4K3	AAEL007798	1.19±0.28
	CYP4AR2	AAEL010154	1.09±0.23
	CYP325E3	AAEL000338	0.76±0.64
	CYP325G2	AAEL012766	2.06±0.31
	CYP325G3	AAEL012772	3.61±1.20**
	CYP325K2	AAEL005771	1.23±0.24
	CYP325K3	AAEL005788	2.08±0.19
	CYP325L1v1	AAEL011770	1.66±0.07
	CYP325M1	AAEL012773	1.28±0.18
	CYP325M2	AAEL012769	1.41±0.19
	CYP325M2	AAEL015591	1.54±0.19

	CYP325M3	AAEL012765	0.31±0.02[§]
	CYP325M4	AAEL011769	2.72±0.28[**]
	CYP325M5	AAEL011761	1.40±0.18
	CYP325N1	AAEL012770	0.76±0.34
	CYP325N2	AAEL012762	0.98±0.14
	CYP325P1	AAEL000340	1.66±0.13
	CYP325Q1	AAEL006044	1.16±0.13
	CYP325Q2	AAEL015563	1.44±0.30
	CYP325R1	AAEL005775	1.76±0.09
	CYP325S1	AAEL000326	0.51±0.41
	CYP325S2	AAEL000325	0.68±0.10
	CYP325S3	AAEL000357	0.56±0.05
	CYP325T2	AAEL012761	1.02±0.22
	CYP325T2	AAEL015475	0.81±0.13
	CYP325U1	AAEL000320	0.14±0.01
	CYP325X1	AAEL005695	1.19±0.13
	CYP325X2	AAEL005696	1.37±0.12
	CYP325X4	AAEL005700	1.39±0.14
	CYP325Y1	AAEL006257	1.09±0.20
	CYP325Y2v2	AAEL015362	0.50±0.23
	CYP325Y3	AAEL006246	1.21±0.20
	CYP325Y3	AAEL015361	1.31±0.22
	CYP325Z1	AAEL010273	1.30±0.13
	CYP325AA1	AAEL004012	1.51±0.21
Mito Clan	CYP12F5	AAEL001960	0.47±0.27
	CYP12F6	AAEL002005	2.14±0.19[*]
	CYP12F7	AAEL002031	1.37±0.11
	CYP12F8	AAEL006827	2.30±0.29[**]
	CYP49A1	AAEL008638	1.45±0.14
	CYP301A1	AAEL014594	1.84±0.15
	CYP302A1v1	AAEL011463	1.91±0.15
	CYP302A1v2	AAEL015655	1.46±0.22
	CYP314A1	AAEL010946	0.77±0.16
	CYP315A1	AAEL011850	1.92±0.07
Other	NADPH P450 reductase	AAEL003349	4.76±0.18[**]

[†]Nomenclature for the cytochrome P450 genes was taken for the *Ae. aegypti* cytochrome P450 database at: http://drnelson.uthsc.edu/CytochromeP450.html
[‡]Vectorbase *Ae. aegypti* predicted gene set v. AaegL1.1. http://aaegypti.vectorbase.org/
[*]significantly up-regulated (>2-fold) at the $P<0.05$ level of significance
[**]significantly up-regulated (>2-fold) at the $P<0.01$ level of significance
[§]significantly down-regulated (>2-fold) at the $P<0.01$ level of significance

Taken together, these results suggest that the cytochrome P450 genes identified as up-regulated in the Puerto Rico strain of *Ae. aegypti* may be involved in insecticide resistance, and

further investigation to test for functionality is needed to identify if they have a role in insecticide resistance. Multiple studies have investigated the functional role of insect cytochrome P450s (Joussen et al., 2008; Xu et al., 2010; Stevenson et al., 2011; Stevenson et al., 2012; Muller et al., 2008; Muller et al., 2011; Chandor-Proust et al., 2013; Yang and Liu, 2011), with the main focus on characterizing clan 3 cytochrome P450s in families CYP6 and CYP9. In order to add to the growing base of literature for cytochrome P450s involved in insecticide resistance, we selected genes from family CYP4 for further functional studies.

Four genes, *CYP4D24* (AAEL007815), *CYP4H29* (AAEL007830), *CYP4J15v1* (AAEL013556), and *CYP4H33* (AAEL013789), were tested, among which AAEL013556 had been previously identified as up-regulated in permethrin resistant *Ae. aegpyti* (Marcombe et al., 2009), while the others had not been previously linked to insecticide resistance in *Ae. aegypti*. The four P450 genes from the Puerto Rico strain of *Ae aegypti* were successfully transferred and expressed in *D. melanogaster* under control of the GAL4-UAS enhancer trap system after full length cloning and sequencing (Fig. 2). When adult female *D. melanogaster* expressing each of the transgenes were exposed to either permethrin or beta-cypermethrin (Fig. 3), the results showed a moderate increase in survival for transgenic *D. melanogaster* expressing each of the four *CYP4* genes at a rate of 5µg/vial permethrin. *CYP4D24* (AAEL007815) demonstrated survivorship at 10µg/vial suggesting that *CYP4D24* may be capable of conferring resistance to type I pyrethroids, although *CYP4D24* may not be as effective at conferring resistance to pyrethroids as other cytochrome P450s. For example, transgenic *D. melanogaster* expressing CYP6BQ9 from the QTC279 strain of *Tribolium castaneum* under the same Act5c-GAL4:UAS control conferred resistance to deltamethrin at a comparable rate (10µg/vial) (Zhu et al., 2010).

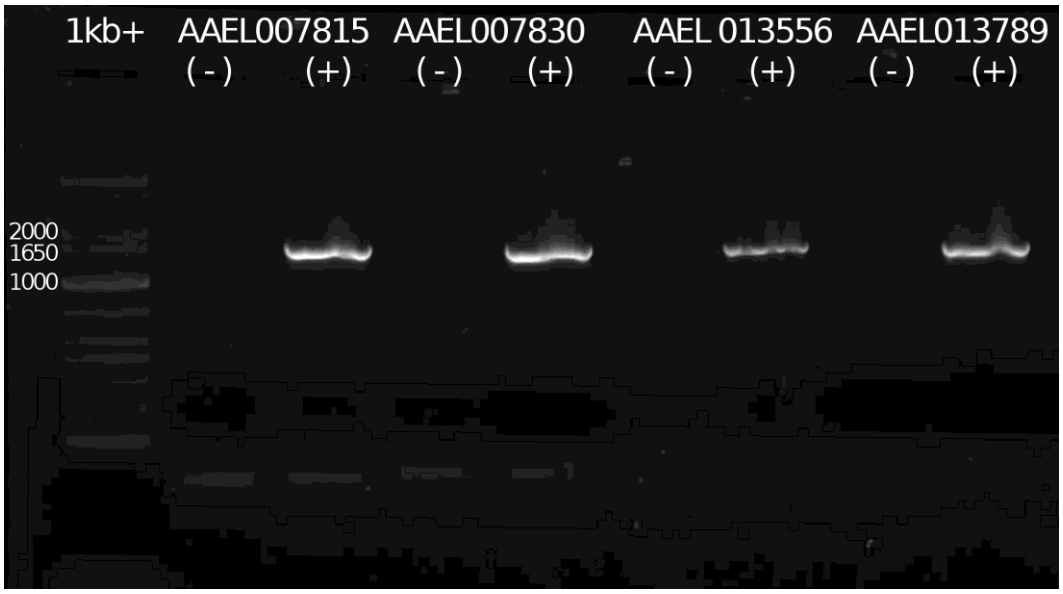

Figure 5.2. RT-PCR of transgenic *D. melanogaster* expressing *Aedes aegypti* cytochrome P450 genes. The (-) and (+) within gene represent the amplified products from the non-transgenic line (-) and the transgenic line (+) of *D. melanogaster*, respectively.

However the type II pyrethroid deltamethrin has been shown to be more toxic to mosquitoes than the type I pyrethroid permethrin (Rettich, F. 1983, Jawara et al., 2001; Allan, 2011). The transgenic *D. melanogaster* testing further showed that the over-expression of CYP4J15v1 (AAEL013556) conferred a minor level of resistance to both the type I pyrethroid permethrin and the type II pyrethroid beta-cypermethrin, while the remaining transgenic *D. melanogaster* lines were not significantly different from each other at the α=0.05 level of significance and had nearly no survival. In addition, CYP4J15v1 was identified to be up-regulated in our study and also in a previous study (Marcombe et al., 2009). Taken together, these results suggest that

CYP4J15v1 may have a minor role in conferring pyrethroid resistance to *Ae. aegypti*.

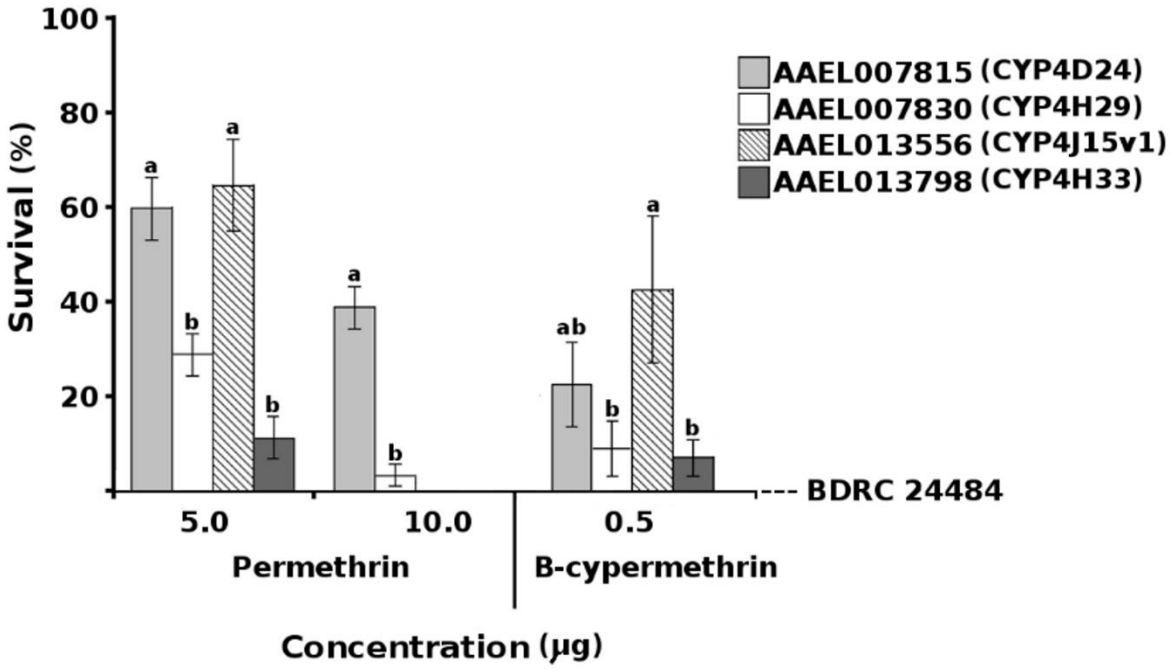

Figure 5.3. Survivorship of transgenic D. melanogaster lines following a 24h exposure to either permethrin or beta-cypermethrin. Bars within dose superceeded by the same letter are not significantly different at the α=0.05 level of significance. BDRC 24484 is the non-transgenic line of *D. melanogaster*, which had no surviving individuals at any of the doses of the insecticides tested.

5.5 Conclusions

The current study investigated the expression profiles of 164 cytochrome P450 genes in adult females of a permethrin-resistant field strain of *Ae. aegypti*. Overall, a total of 34 genes were identified to be up-regulated, several of which were also identified in other field populations of insecticide-resistant *Ae. aegypti*. Functional analysis of some of the lesser-understood cytochrome P450s suggested that two genes, *CYP4D24* and *CYP4J15v1*, were capable of conferring a low-level of resistance to insecticides when over-expressed in transgenic *D. melanogaster*. Our results add to the body of work that has investigated the gene expression profiles of cytochrome P450s in insecticide resistant field strains of *Ae. aegypti*, and identified a

possible minor role of two family 4 P450 genes, which is important to elucidate the complexity of the role of cytochrome P450 genes in pyrethroid resistance in mosquitoes.

5.6 Acknowledgements

We thank Neil Sanscrainte, Matthew Brown, Francis Golden, and Nathan Newlon for their assistance with the rearing of the mosquitoes. We also thank the Deployed War-Fighter Protection program for funding.

Chapter 6: Temporal gene expression profiles of pre and post blood-fed adult females of the Southern house mosquito *Culex quinquefasciatus*

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6.1 Abstract

Prior to acquisition of the first host blood meal, the anautogenous mosquito *Culex quinquefasciatus* requires a period of time in order to prepare for the blood feeding. In the current study, we found that adult females required a minimum of 48 h post-eclosion before they freely took their first blood meal. We hypothesized that gene expression signatures were altered in the mosquitoes before blood feeding in preparation for the acquisition of the blood meal through changes in multiple gene expression. To identify the genes involved in the acquisition of blood feeding, we quantified the gene expression levels of adult female *Cx. quinquefasciatus* using RNA Seq throughout a pre-blooding period from 2 to 72 h post eclosion at 12 h intervals. A total of 325 genes were determined to be differentially-expressed throughout the pre-blooding period, with the majority of differentially-expressed genes occurring between the 2 h and 12 h post-eclosion time points. Among the up-regulated genes were salivary proteins, odorant-binding proteins, and proteases, while the majority of the down-regulated genes were hypothetical or cuticular genes. Further analysis of the gene expression profiles following a blood meal suggested that only certain vitellogenin genes were highly expressed, and that vitellogenesis in

Cx. quinquefasciatus appears to be a longer process than in *Aedes aegypti*. Overall, this study reviewed multiple genes that might be involved in adult female competency for blood meal acquisition of mosquitoes.

Key words: Vitellogenesis, blood feeding, anautogeny

6.2 Introduction

The Southern house mosquito, *Culex quinquefasciatus* is a vector of the causative agents of several diseases including West Nile Fever, St. Louis Encephalitis, Japanese Encephalitis, and lymphatic filariasis. The pathogens vectored by *Cx. quinquefasciatus* are acquired during the blood meal acquisition, which must be taken by the adult female prior to the formation of each egg raft (Cupp et al., 2011). Newly eclosed females require a period of time before they are capable of taking the blood meal during which, the female mates and continues with the necessary development to be competent for the acquisition of the blood meal itself (Williams and Patterson, 1969). Many studies have shown that genes and gene up-regulation are involved in the processing of the blood meal and subsequently, vitellogenesis (Chen et al., 2004, Hansen et al., 2005, Bryant et al., 2010). Studies that have characterized the transcriptome expression patterns of adult *Aedes aegypti* (Price et al., 2011) and *Anopheles gambiae* (Marinotti et al., 2006) have shown that multiple genes are involved in blood feeding and that there are different expression profiles of these genes both prior to and immediately following the blood meal. The temporal characterization of the gene expression profiles of the *Cx. quinquefasciatus* transcriptome prior to the blood meal would provide valuable insight into the genes needed to prepare the female for the taking of the blood meal, and also could identify novel targets for controlling *Cx.*

quinquefasciatus by preventing the blood feeding. Characterization and identification of key genes that may be involved in the taking of a blood meal could provide novel targets for novel approaches to manage insect disease-vectors. The recent advances in next generation sequencing, including RNA Seq, allows for the characterization the gene expression profiles without requiring *a priori* knowledge of which genes to investigate. In the current study, we used RNA Seq sequencing to conduct whole transcriptome analyses of adult female mosquitoes during the post-eclosion and pre-vitellogenic stages of development to identify genes that are up- or down-regulated prior to the female freely taking a blood meal and further investigated the temporal expression of selected genes following the taking of a blood meal to identify genes that may be necessary for both taking of the blood meal, and processing of the blood meal in adult female *Cx. quinquefasciatus.*

6.3 Materials and Methods

6.3.1 Mosquito strains

Culex quinquefasciatus strain HAmCq^{G8}, whose parental line was originally collected from Huntsville, Alabama, has been established in the laboratory where it has been continuously reared since 2002 (Liu et al., 2004). All mosquitoes were reared and tested at 25±2^{o}C under a photoperiod of 12:12 (L:D) h and fed a diet of Brewer's yeast (Fleishmann, Chesterfield, MO) for the larval stages and 10% sucrose and horse blood (College of Veterinary Medicine, Auburn University) for the adult stages fed through a stretched Parafilm membrane in a 37^{o}C heated water jacketed glass holder.

6.3.2 Pre-determination of time period for mosquitoes to take their first blood meal

The adult mosquitoes (12 h post eclosion) were divided into 17 groups with a minimum of ~60 mosquitoes each in both sexes (~1:1 ratio). The mosquito groups were then independently offered pre-warmed (37^{o}C) horse blood meal (College of Veterinary Medicine, Auburn University) with an ascending order of every 12 h starting from 24 h after eclosion; i.e., 24, 36, 48, 60, and 72 84 96 108 120 132 144 h after ecolosion, respectively. Each group of mosquitoes was fed for a single blood meal for 2 h and the number of the blood fed female mosquitoes from each group were checked after blood-feeding. All blood-feeding time points were repeated in triplicate.

6.3.3 RNA extraction

All collected mosquito samples were flash frozen on dry ice and held at -80°C prior to RNA extraction. Total RNA was extracted using the hot acid phenol extraction method as outlined by Chomczynski and Sacchi (1987). A total of 30 µg of RNA was then treated with DNase I using the DNA-Free kit from Ambion (Austin, TX) to remove any contaminant DNA and re-extracted with two successive acid phenol: chloroform (1:1) steps followed by a final chloroform extraction to remove any residual phenol. The RNA was then precipitated over ethanol and re-suspended in sterile distilled water. After a 1µg aliquot of RNA had been visually inspected for quality and for DNA contamination on a 1% agarose gel. The total RNAs were subsequently used for either Seq analysis (Hudson Alpha Institute of Biotechnology [HAIB], Huntsville, AL) or gene expression analysis.

6.3.4 RNA library preparation, RNA Seq sequencing, Data analysis, and gene expression

processing

Total RNA quality was assessed by the HAIB using an Agilent 2100 Bioanalyzer and an Invitrogen Qubit to ensure quality. Libraries were then prepared using the Illumina RNA Sample Prep Kits for mRNA Seq and a 3' poly A tail selection method. Samples were barcoded and run as one of four samples on a single lane of an Illumina Hi Seq 2000 chip. Samples for the mRNA Seq were run using the PE-50 module (HAIB) which generated paired end libraries with 50 nt long sequences on each paired end. Base calling, initial removal of low quality reads, and barcode parsing were conducted by the staff at HAIB. Further cleaning of adapter was performed using Trimmomatic (Lohse et al., 2012). Paired end reads were then mapped to the *Cx quinquefasciatus* genome from Vectorbase (Megy et al., 2009, Arensberger et al., 2010) using Tophat (Trapnell et al., 2009) with mate pair interval of 200 bases and the gtf basefeatures file. The -no-novel-juncs flag was used in the alignment to suppress the discovery of novel spliceforms in order to estimate gene expression levels based on the Vectorbase annotation of the genes. Read counts were determined using Cufflinks, and the testing of differential expression was estimated using Cuffdiff as time series data (Roberts et al., 2011). After analysis, only genes with expression values ≥1, as measured in number of fragments mapped for every thousand bases of gene length for every million fragments sequenced (FPKM), were retained for expression comparisons (Gan et al., 2010). All data have been submitted to the Gene Expression Omnibus at NCBI as accession #GSE51327.

6.3.5 qPCR gene expression

The total RNA from three independent samples of 20 adult female HAmCq^{G8} mosquitoes was extracted as previously outlined above. The same methodology for obtaining even-aged females immediately following eclosion was used to obtain the non-blood-fed females for RNA extraction, using the same time points as previously indicated: 2, 12, 24, 36, 48, 60, and 72h post-eclosion. In order to obtain the material for the RNA extraction for the post-blood meal sampling time points, mosquitoes were initially reared to 7d of age (post-eclosion) prior to the offering of a blood meal. Blood meals were then offered for a 2h period at the onset of the scotophase, after which blooded females were collected at 2, 4, 8, 12, 16, 20, 24, 36, 48, 60, and 72h. Females that had not taken a blood meal were removed from the cages immediately following the blood meal and the remaining females that had taken a blood meal were held in the cages along with the males from the initial population. A total of 20 females were selected for each time point and all collections were repeated in triplicate. Total RNA was treated with DNase I using the DNA-Free kit from Ambion (Austin, TX) as previously described to remove any contaminant DNA, and the DNase I was inactivated using the inactivation buffer from Ambion. First strand cDNA was generated from the template RNA using the First strand cDNA synthesis kit from Roche (Indianapolis, IN) and an oligo dT primer. RT-qPCR was conducted on an ABI 7500 Real Time PCR system (Applied Biosystems) using theABI SyBr Green mastermix kit (Life Technologies, Carlsbad, CA) and relative gene expression was determined by using the $2^{(-\Delta\Delta Ct)}$ method (Livak and Schmittgen, 2001) using a portion of the 18S rRNA gene as the reference gene. The primers used are provided in supplementary table S1.

6.4 Results

6.4.1 Determination of the pre-blood meal time period.

To see the time period needed for mosquitoes to prepare them for the taking of their first blood meal, we conducted a time course study on blood feeding with adult *Cx. quinquefasciatus* and tested 11 groups. Each group was offered pre-warmed blood meal starting from 24 h after eclosion followed by an ascending order of every 12 h. Our results showed that *Cx. quinquefasciatus* needs a minimum of 48 h after eclosion to prepare the female for the blood meal (Fig. 6.1).

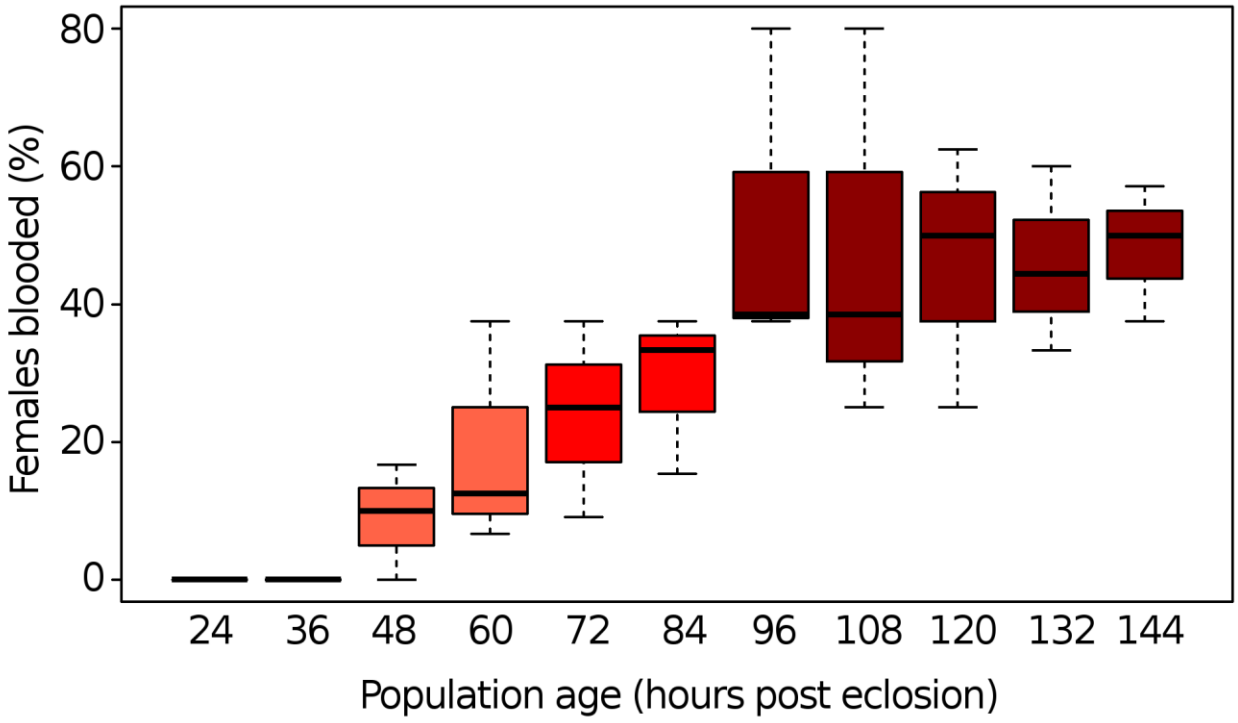

Figure 6.1. Box and whisker plot of the percentage of females from even-aged populations of *Cx. quinquefasciatus* strain HAmCq^{G8} freely taking an offered blood meal. The black lines within a bar represent the median percentage of females who freely took a blood meal. The upper and lower whiskers represent the highest and lowest observations, respectively, while the bars themselves represent the interquartile range (Q1 - Q3).

When mosquito populations had reached 96 h of age (post-eclosion), the average number of

females taking a blood meal plateaued at ~50% with no observable increase in blood meal taking by females beyond this time point. Under the rearing conditions tested, some females may remain un-mated since the sex ratio was maintained at a consistent ratio. Sebastian and DeMeillon (1967) found that mating was a pre-requisite for blood feeding in the closely-related mosquito *Culex pipiens fatigans*, and further identified that a sex ratio of 2:1 (males : females) was necessary to maximize insemination, while a sex ratio of 1:1 resulted in an insemination rate of only 83%. Since the sex ratio was held at ~1:1 in our study, this may explain why only 50% of the females took a blood meal (Craig, 1967).

Since the objective of the study was to determine the time-to-first blood meal acquisition competency, the 96 h time point represented the minimum time to reach maximum first blood meal acquisition for females in small populations of even-aged mosquitoes. This suggested that under our experimental conditions, the average pre-blood meal competency time period for females ranged ~48 h, after which females became competent to take a blood meal, reaching maximum blood meal acquisition at ~96 h post-eclosion.

6.4.2 RNA Seq characterization of the pre-blood meal and mating time periods in *Cx. quinquefasciatus*.

According to the pre-determination of the first blood meal time, we conducted RNA Seq to characterize the genes that were involved in the taking of a blood meal. Seven post-eclosion time points i.e., 2, 12, 24, 36 48, 60, and 72 h, were selected for the RNA Seq analysis, covering the time period from ecosion to the first sign of the blood feeding (i.e., by 48 h post-eclosion) and to the maximum mating period of *Cx. quinquefasciatus*, which has been shown to begin mating 24 h after ecolosion and reach a maximum by 72 h (Williams and Patterson, 1969). A total of 200

females collected from each of the time points were pooled for the RNA extraction. Except for the 2 h time point, in which mosquito pupae were allowed to eclose over the 2 h period only, pupae were allowed to eclose over a 12 h period and the females were collected at 12 24, 36, 48, 60, and 72 h time points after eclosion. Overall, the depths of sequencing for the sample time points ranged from 26 to 51 million paired-end reads (Table 6.1) and after mapping the reads to the *Cx. quinquefasciatus* genome, the genes that were identified as expressed (i.e.: those genes having an FPKM (fragments per kilo base of gene length per million reads mapped) >1 (Gan et al., 2010)), were divided among the Structural Classification Of Proteins (SCOP) general function categories of metabolism, regulation, extra-cellular processes, intra-cellular processes, information, general, other, and no annotation (Murzin et al., 1995, Andreeva et al., 2004, Vogel et al, 2004, Vogel et al., 2005).

Table 6.1. Number of paired end reads from the Illumina HiSeq sequencing and the percentage of reads mapped to the *Cx. quinquefasciatus* (strain: Johannesburg) predicted transcriptome.

Sampling time point	Total paired end reads[†]	Paired end reads discarded[‡]	Paired end reads used for mapping to the *Cx. quinquefasciatus* genome (JHB v1.2)
2 h	39890830	2950518	36940312
12 h	36030651	2405121	33625530
24 h	41173012	2979773	38193239
36 h	35519459	1894955	33624504
48 h	34587710	1909928	32677782
60 h	27128981	1276065	25852916
72 h	54895903	3819301	51076602

[†]Total number of FASTQ (DNA sequence with quality scores) reads passing the Illumina quality filter.

[‡]Number of reads discarded after adapter clipping.

When the expressed genes were pooled into their respective SCOP general function categories and their FPKM gene expression values were summed to estimate the total proportion of gene

expression within each of the SCOP general function categories, the pattern revealed that there was an overall decrease in total gene expression among the "No annotation" and the "General" SCOP general function categories occurring from 2 to the 12 h post-eclosion time points, with a respective increase in total gene expression for the categories of "regulation" and "other" for the 12 h time point (Fig. 6.2). Beyond the 12 h post-eclosion time point, the total cumulative gene expression profiles within each of the SCOP general function categories were similar up to, 72 h post-eclosion time point (Fig.6.2). These results suggested that the major global changes in gene expression for adult female *Cx. quinquefasciatus* (prior to the taking of a blood meal) occur during the initial 12 h post-eclosion.

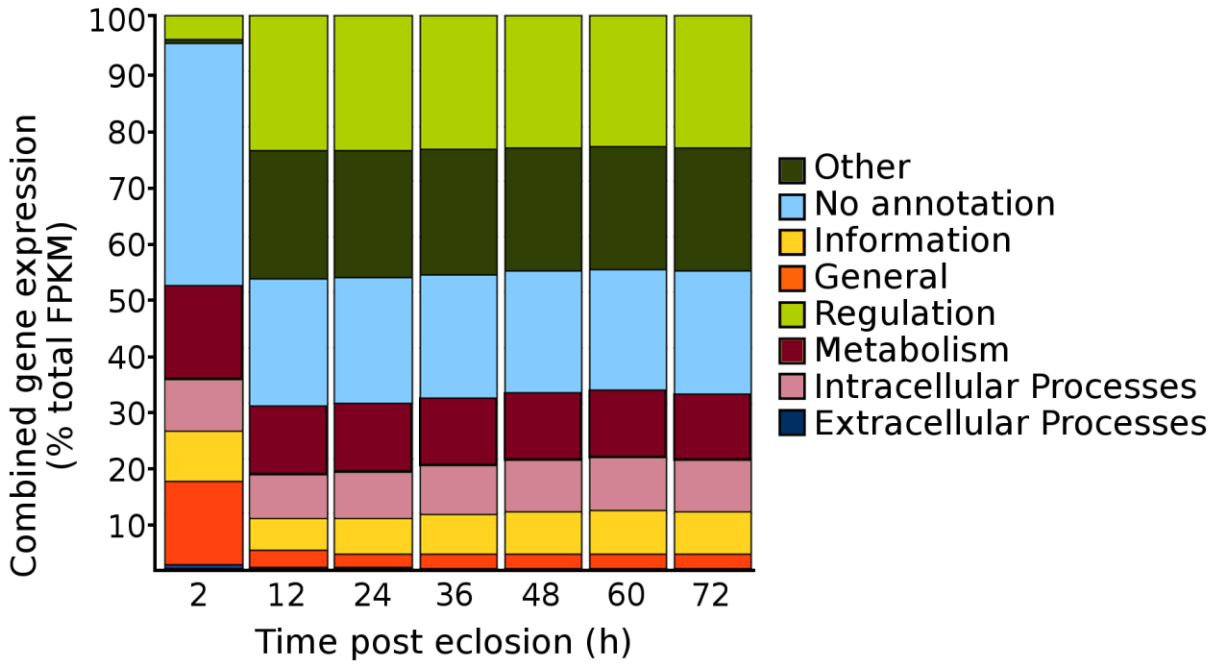

Figure 6.2. Total gene expression within the Structural Classification of Proteins (SCOP) general function categories for adult sugar-fed female *Culex quinquefasciatus*, strain HAmCq^{G8}, for the initial 72 h post-eclosion. Gene expression values expressed are summed within each SCOP category to provide an overall profile of the complete distribution of all gene expression within the mosquitoes.

To further identify candidate genes that may be involved in preparing the female for the taking of

a blood meal, we investigated the genes that were differentially-expressed throughout the time course investigated. Overall, the majority of the genes that tested as differentially up- or down-regulated occurred between the 2 and 12 h post-eclosion time period, which peaked at 12 h, after which, the number of genes that were differentially-expressed decreased (Fig. 6.3). Roughly one-third of the differentially-expressed genes identified (101 genes from a total of 325) had no functional annotation in Vectorbase, while the remaining genes had predicted functions (Appendix 6.2). At 12 h post-eclosion, the greatest numbers of differentially-expressed genes was observed with 159 genes being up-regulated, and 74 down-regulated (Fig. 6.2).

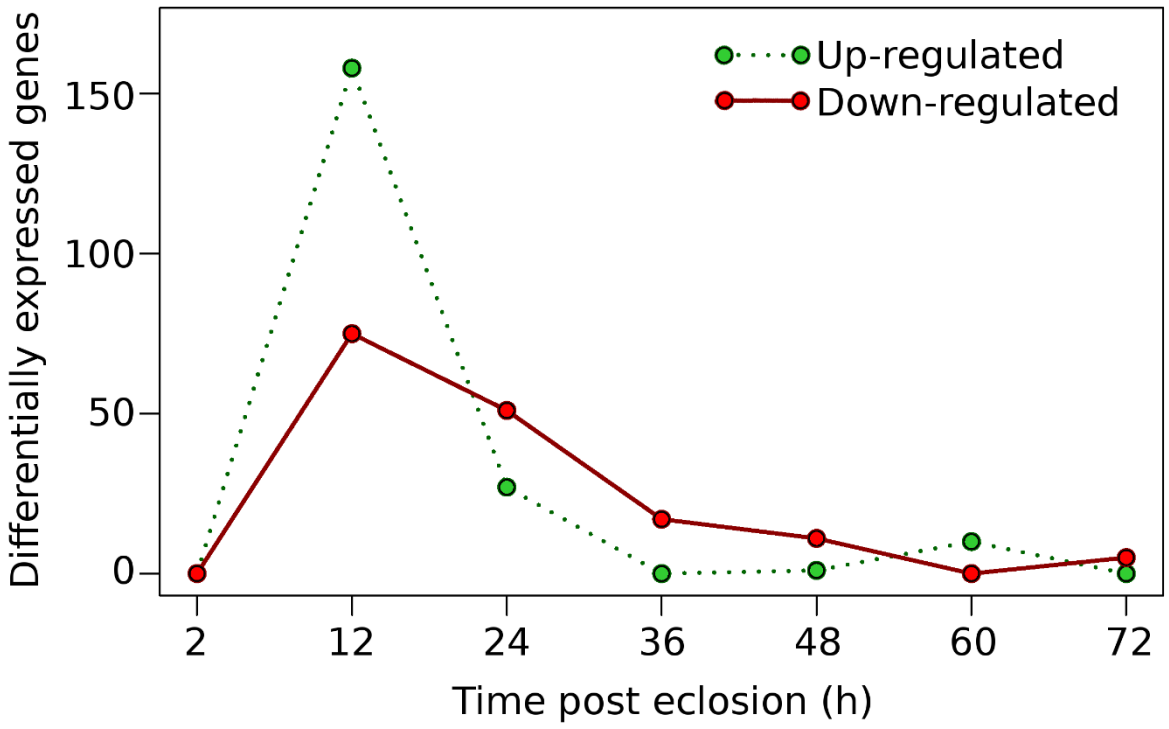

Figure 6.3. Distribution of genes tested as differentially-expressed for adult sugar-fed female *Culex quinquefasciatus*, strain HAmCq^{G8}, for the initial 72 h post-eclosion period.

Among the up-regulated genes were 32 salivary proteins and two apyrases, which may be

involved in the prevention of platelet clotting during blood feeding (Smith et al., 2002), 10 cytochrome P450s (CPIJ005952 , CPIJ011837 , CPIJ010225 , CPIJ010227 , CPIJ000294 , CPIJ019586 , CPIJ019587 , CPIJ020018 , CPIJ012470, CPIJ010546), which were distributed among families CYP4, CYP6, CYP9, and CYP325, 20 proteases, and also genes involved in embryogenesis including wnt inhibitors and oskar (Chang et al., 2011, Jaglarz et al., 2011). Among the genes down-regulated at 12 h post-eclosion were 27 hypothetical proteins, 11 cuticle proteins and five cytochrome P450 genes (CPIJ011841, CPIJ011840, CPIJ015954, CPIJ015961, and CPIJ015960), which were all in family CYP325. At 24 h post-eclosion, fewer genes were identified as differentially-expressed (27 up-regulated; 51 down-regulated). Among the up-regulated genes at 24 h post-eclosion were nine proteases and one olfactory receptor (CPIJ008023), which may be involved in preparation for the blood meal and host seeking, respectively. Among the down-regulated genes at 24 h post-eclosion were 32 hypothetical proteins, and six cuticle proteins, which may be involved in the transition from the pharate to the adult.

While the majority of differential gene expression was observed in the earlier time points, there were a few genes that were up-regulated at 48 and 60 h with a total of 18, 12, 10, and five genes for the 36 h, 48 h, 60 h, and 72 h time points, respectively. Notably at 48 h post-eclosion, CPIJ006495, a hypothetical protein possessing a ChtBD2 chitin binding motif, which is found in insect peritrophin A (Letunic et al., 2012). In addition, at 60 h post-eclosion, multiple proteases were identified as up-regulated (CPIJ000990, CPIJ002595, CPIJ003539, and CPIJ007077) as well as ficolin-3, which has been linked to the humoral lectin immune defense response in mosquitoes (Dimopolous et al., 2002).

In addition, with the exception of one hypothetical gene at the 48 h time point and the 10

genes up-regulated at the 60 h time point, all of the differentially-expressed genes beyond the 24 h time point were down-regulated. Furthermore, when the gene expression values (FPKM) were considered, there was a sharp decrease in the gene expression values of differentially-expressed genes beyond the 36 h time point, with the highest FPKM value of 60 being attributed to a hypothetical gene, CPIJ015506 (Table S2). Taken together, these results suggest that the majority of the genes that are up-regulated in preparation of the female *Cx. quinquefasciatus* for the taking of their first blood meal are up-regulated within the first 24 h post-eclosion, while a few additional genes involved in blood meal taking may be up-regulated at 48 – 60 h post-eclosion.

Recent work by Clifton and Noriega (2012) identified that nutritional status in the mosquito *Aedes aegypti* influences the expression of key vitellogenesis-related transcripts, these were: the ribosomal 60S protein rpL32, the lipophorin receptor AaLpRov, the vitellogenin receptor AaVgR, and heavy-chain clathrin (AaCHC). In our study, we identified a similar trend for these genes in *Cx. quinquefasciatus* and found that the lipophorin receptor (CPIJ018375), the 60S protein rpL32 (CPIJ001220), the pro-epidermal growth factor gene (putative vitellogenin receptor) (CPIJ020278), and heavy-chain clathrin (CPIJ014882) all gradually increased in expression over time, with the predicted genes heavy-chain clathrin and the putative vitellogenin receptor continuously increasing from 2 to 72 h post-eclosion (Table 6.2), which follows the data previously reported by Clifton and Noriega (2012).

Table 6.2. Expression levels of genes in *Culex quinquefasciatus*, strain $HAmCq^{G8}$ for genes previously identified as up-regulated in non-blood-fed female *Aedes aegypti* and linked to

nutritional status with regard to blood-feeding competency.

Gene number	Predicted function‡	Expression level (FPKM† at time point post-eclosion (h) 2	12	24	36	48	60	72
CPIJ001220	60S ribosomal protein L32	1100	1400	2200	3330	3600	4600	2900
CPIJ014882	clathrin heavy chain	100	126	154	160	200	150	210
CPIJ018375	lipophorin receptor	7	20	25	35	37	33	30
CPIJ020278*	pro-epidermal growth factor	3	12	93	130	190	200	240

†Fragments mapped Per Kilo bases of reference sequence for every Million fragments sequenced
‡Predicted function from Vectorbase, v. 1.2. https://www.vectorbase.org/organisms/culex-quinquefasciatus
*Putative vitellogenin receptor based on closest blastx match to *Anopheles gambiae*

6.4.3 Validation of selected RNA Seq genes using qPCR.

In order to estimate the accuracy of our RNA Seq results, and to determine the expression of selected genes throughout the pre blood-feeding and into the post blood-feeding time periods of *Cx. quinquefasciatus*, we selected a total of 36 genes that were predicted by the RNA Seq data to show the differential expression during the 72 h post-eclosion time period (Fig. 6.4). Among the genes selected were genes putatively involved in host finding or blood feeding behavior (odorant-binding proteins, period circadian protein), genes putatively involved in the maturation of the pharate female to the adult (cuticular proteins), genes putatively involved in the taking of the blood meal (salivary proteins), genes putatively involved in the digestion of the blood meal (trypsins, collagenase, lipases, uricase), genes predicted to be involved in the provisioning of nutrients to the egg (vitellogenins, adipophilin/perilipin), genes involved in embryogenesis (wnt inhibitor, oskar) as well as genes for proteins with other functions including: cytochrome P450s, calbindin, and oxidoreductase.

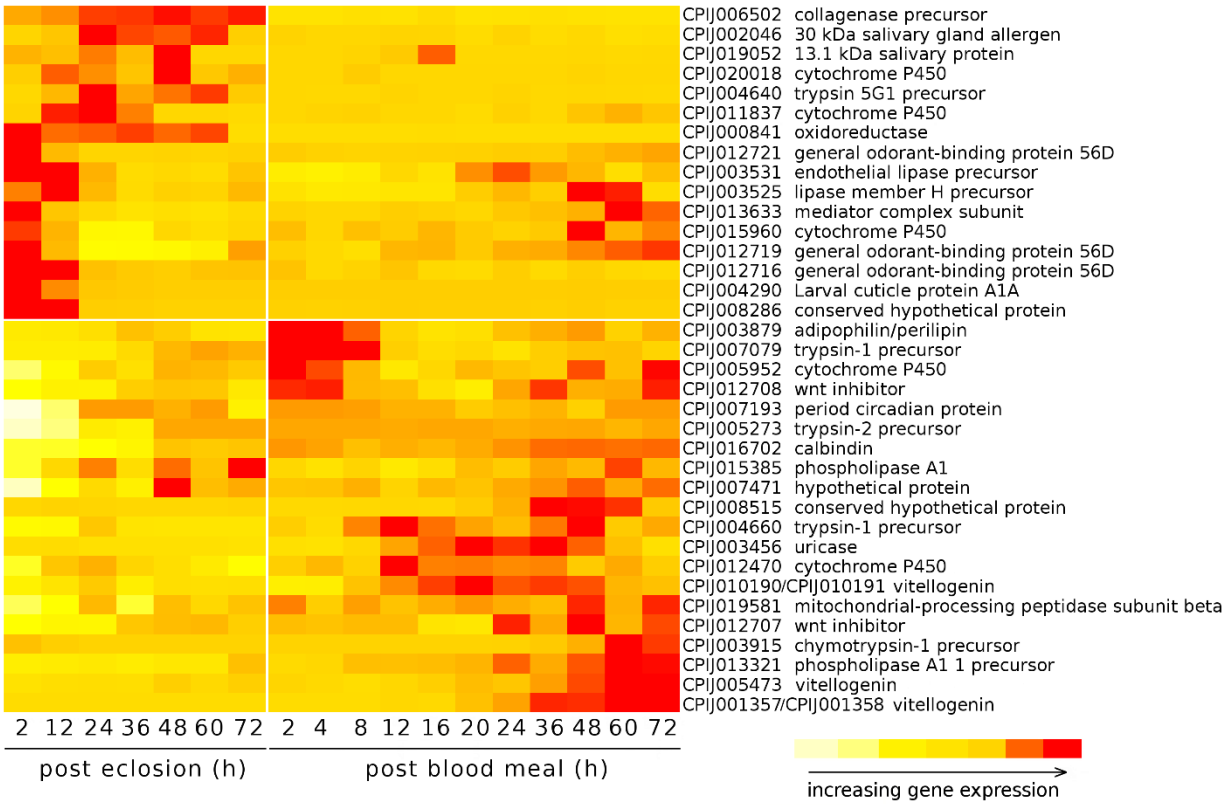

Figure 6.4. Heat map displaying the relative increases in gene expression for selected genes for the initial 72 h post-eclosion for adult female Cx. quinquefasciatus, strain HAmCqG8, and for 72 h post blood meal. Females offered a blood meal were 6 days old at the time of the blood feeding.

The expression of these 36 genes was investigated from 2 to 72 h post-eclosion using qPCR. In addition, we further investigated their gene expression after blood feeding from 2 h post blood-feeding until 72 h post blood-feeding. The results showed that among the genes that were predicted to decrease or to increase and then decrease were genes putatively involved in preparation for the blood meal, or possibly host seeking (Fig. 5 upper panels). The general odorant-binding proteins (CPIJ01716, CPIJ01719, CPIJ012721) had their highest expression at 2h post-eclosion and had a slight increase from the lowest expression values from 48h to 72h post blood-feeding. This indicated that females initially expressed the odorant-binding proteins to aid for food searching (sugar or blood) and also after the blood meal, possibly for aiding in the

identification of suitable oviposition sites. Other genes of interest were the salivary genes (CPIJ002046 and CPIJ019052) which reached maximal gene expression values at 24h and 48h, respectively, and then remained at low expression levels afterwards (Fig. 6.4).

Among the genes that were predicted to be up-regulated throughout the post-eclosion and pre-blooding time period, the vitellogenin genes CPIJ001357/CPIJ001358 reached maximal gene expression at 60h post blood-feeding, while the vitellogenin genes CPIJ010190/CPIJ010191 and CPIJ005473 reached maximal expression at 20h post blood-feeding (Fig. 6.4). The major vitellogenin gene in *Ae. aegypti* has been shown to reach maximal expression at 24h post blood-feeding, which is consistent with our findings for CPIJ010190/CPIJ010191, but not for CPIJ001357/CPIJ001358, suggesting that vitellogenesis is a slower process in *Cx. quniquefasciatus* than in *Ae. aegypti*. Following the blood meal, two trypsins (CPIJ007079, CPIJ004660) and one chymotrypsin (CPIJ003915) were up-regulated (Fig. 6.4). Trypsin CPIJ007079 was up-regulated immediately following blood feeding, while trypsin CPIJ004660 and chymotrypsin CPIJ003915 were up-regulated at 48h and 60h post blood-feeding, respectively. These proteases are likely involved in the digestion of the blood meal, with the first gene representing early trypsin, and the later two representing late trypsins. The uricase gene CPIJ003456 reached a maximal expression at 20 h post blood-feeding and decreased by 60 h post blood-feeding, suggesting that uricase may be primarily involved in ammonia metabolism as related to the processing of the blood meal (Isoe and Scaraffia, 2013).

We then further selected all of the predicted vitellogenin genes to identify the post-blood meal time point at which vitellogenin expression would reach a maximum. Since 10 genes were predicted to be vitellogenins by the Vectorbase functional annotation for the Johannesburg strain of *Cx. quinquefasciatus*, with three sets of two each of the genes having nearly identical

sequence, we designed a total of seven qPCR primers to determine which vitellogenins were the most likely to be involved in egg provisioning in *Cx. quinquefasciatus*, and when they reached maximal expression. In addition, since we were interested in understanding if the genes identified as differentially-expressed in pre blood-fed *Cx. quinquefasciatus* were involed post blood-feeding, we first characterized when vitellogenin expression was at a maximum in blood-fed *Cx. quinquefasciatus* (Fig. 6.5).

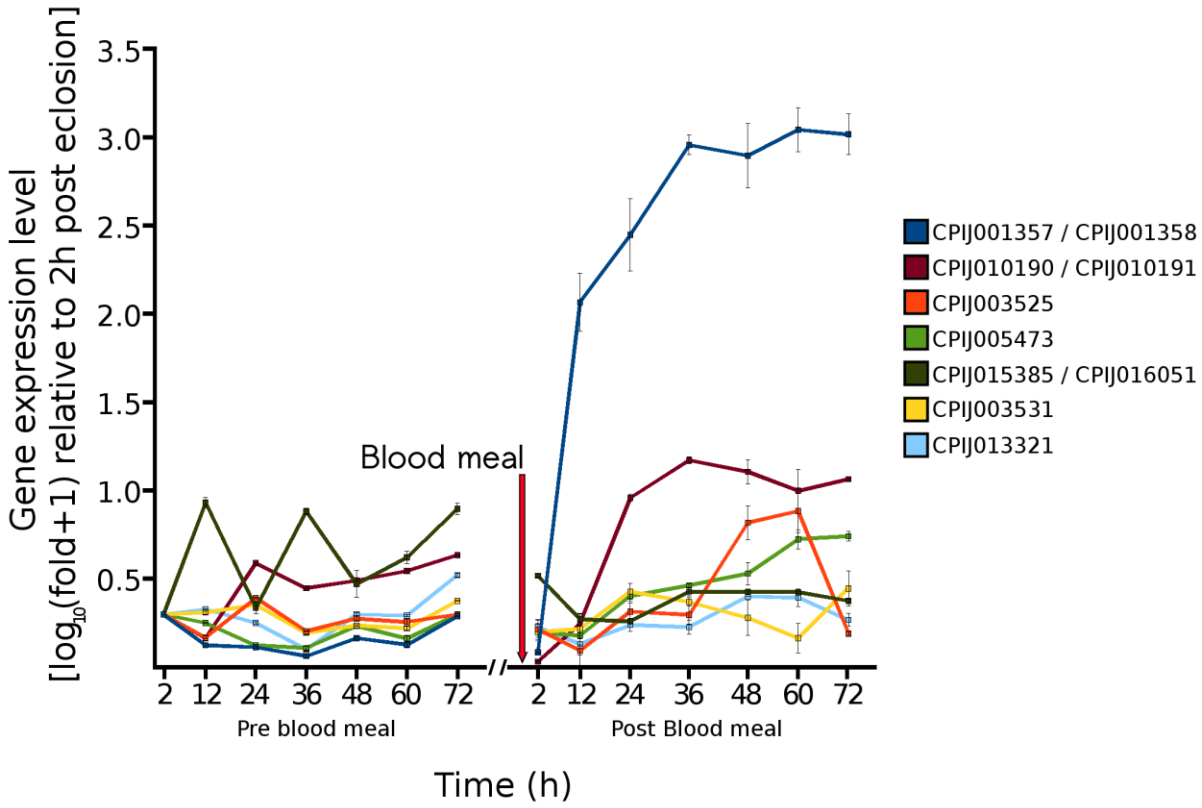

Figure 6.5. Temporal gene expression of vitellogenin genes in adult female *Cx. quinquefasciatus* for the 72 h time period immediately following eclosion and for the 72 h time period immediately following the blood meal.

To accomplish this, we selected six of the 10 predicted vitellogenin genes in *Cx. quinquefasciatus*. In order to identify which genes were involved in vitellogenesis, we initially investigated the expression profiles of the vitellogenins in female *Cx. quinquefasciatus*. Overall, we identified that the vitellogenins, CPIJ001357/CPIJ001358 were the highest expressed of the predicted vitellogenin genes, with more than two logs higher fold gene expression than the next highest expressed vitellogenin genes CPIJ010190/CPIJ010191 (Fig. 6.5). In addition, we found that these two pairs of vitellogenin genes were up-regulated 12 h post blood-feeding and continued to increase, reaching a maximal expression value at 36 h post blood-feeding.

6.5 Discussion

Males of *Cx. quinquefasciatus* have been shown to need a minimum of 24 h of post-eclosion development in order to mate, with mating reaching a maximum by 72 h (Williams and Patterson, 1969). In our study, the 72 h post-eclosion time points indicated the mean age for females to become competent to take a blood meal, reaching a maximum by 96 h. This may indicate that a male accessory gland factor transferred from the male during mating is necessary in order to induce the female to take a blood meal (Craig, 1967). Recently, egg development in *Anopheles gambiae* has been demonstrated to be controlled, in part, by exogenous ecdysone transferred to the females from the male accessory gland secretions following mating (Baldini et al., 2013). The contribution of the ecdysone was found to be a strong inducer of the *mating-induced stimulator of oogenesis* (*miso*) gene (AGAP002620) (Holt et al., 2012; Baldini et al., 2013), however in *Cx. quinquefasciatus*, the two closest homologs of *miso*, CPIJ018252 and CPIJ006083 were found to have low expression throughout the first 72 h post-eclosion time period, having their highest expression at 2 h post-eclosion (FPKM = 2 and 3, respectively)

(GEO: #GSE51327). In addition, Baldini et al. (2013) found that ecdysone was not present in the male accessory glands of *Ae. aegypti* or *Anopheles albimanus*. Taken together, this suggested that the role of the male accessory gland factors with respect to mating, egg development, and female blood meal competency differ among the various mosquito species. Since mating would have occurred after 24 h post-eclosion (Williams and Patterson, 1969), any genes that might be up-regulated in response to mating should be up-regulated in the time points >24 h post-eclosion. Genes up-regulated at the 48 and 60 h time points were related to chitin binding, proteases, and a ficolin. These genes may be involved in the preparation of the peritrophic matrix for the taking of the blood meal and for providing immunity to pathogens that may be contained within the blood. The up-regulation of these genes at 48 - 60 h may be necessary to prepare the female for the taking of a blood meal, possibly up-regulated in response to mating.

In addition to the genes up-regulated at 48 - 60 h, the differentially expressed genes from the 2 to 24 h time points are also likely involved in preparing the female for blood feeding since several of these genes could be attributed to genes that aid in the feeding and digestion of the blood meal, such as trypsins, apyrase, and salivary proteins (Barillas-Mury, 1995, Chamopagne et al., 1995, Sim et al., 2012), while genes having functions related to continued development after eclosion, such as cuticle structure were down-regulated (Riehle et al., 2002).

The previously un-characterized genes that changed their expression levels over time in our study represent novel genes for the investigation of the genetic events that occur prior to blood-meal acquisition in *Cx. quinquefasciatus*. Our finding that heavy-chain clathrin in *Cx. quinquefasciatus* was expressed throughout the entire post-eclosion time period, is likely involved with aiding in the provisioning of the egg with vitellogenins. Clathrin is involved in multiple endocytotic processes, thus the expression of heavy-chain clathrin throughout the entire

post-eclosion time period was expected (Clifton and Noriega, 2012). In contrast, the putative vitellogenin receptor, CPIJ020278 had low expression until 24h post-eclosion, suggesting that the putative vitellogenin receptor gene is developmentally regulated during the adult stage and is switched on prior to the time period when the females freely take an offered blood meal. The increase in gene expression for the vitellogenin receptor at 24 h is consistent with observations of egg provisioning in an autogenous strain of *Cx. quinquefasciatus*, where 1-day-old females have been shown to be capable of provisioning their eggs in the absence of a blood meal if they were exposed to the ecdysone agonist tebufenozide in the immature stages (Gelbič and Rozsypalová, 2012). This demonstrates that 24 h old *Cx. quinquefasciatus* females are capable of vitellogenesis if provided with the appropriate hormonal stimulation (Gelbič and Rozsypalová, 2012). Vitellogenesis and successful completion of egg formation in mosquitoes, however, requires not only ecdysone, but specific amino acids, juvenile hormone, and the activation of various signaling pathways and miRNAs as well (Hansen et al., 2004, Hansen et al, 2005, Shiao et al., 2008, Bryant et al., 2010, Gulia-Nuss et al., 2011). Vitellogenesis has also been shown to terminate, resulting in the re-absorption of vitellins, if the nutritional status of *Ae. aegypti* is not sufficient for complete egg maturation (Clifton and Noriega, 2012). Therefore since female *Cx. quinquefasciatus* are capable of vitellogenesis as early as 24 h post-eclosion (Gelbič and Rozsypalová, 2012), but do not freely take a blood meal until at least 48 - 96 h post-eclosion, the genes that were identified as up-regulated at 48 and 60 h may be essential for the taking and processing of the blood meal, but not vitellogenesis. Furthermore, the up-regulation of these genes may be in response to mating, which begins at 24 h post-eclosion in *Cx. quinquefasciatus* (Williams and Patterson, 1969).

Overall, our study found that the genes identified in the post-eclosion, but pre-blood-meal-taking time period represent genes that may be necessary for the female to freely take a blood meal. We further identified that it was possible to induce vitellogenesis and egg provisioning in *Cx. quinquefasciatus* prior to the age, at which, mosquitoes would freely take a blood meal, and further identified that the induction of vitellogenesis was independent of mating status, however none of the vitellogenesis-induced females were capable of laying eggs. Our study suggested that in *Cx. quinquefasciatus*, there is likely a complex of factors necessary to prepare the female for the taking of a blood meal that include not only the control of vitellogenesis, but the processing of the blood meal and preparation of the female for egg development and oviposition as well.

6.6 Acknowledgements

The authors are grateful to Drs. Peter W. Atkinson, Peter Arensburger and the *Culex quinquefasciatus* genome community for the efforts they have devoted to determining the genome sequence and making the information available in VectorBase. We would also like to thank the Hudson Alpha Institute of Biotechnology for their expertise in conducting the RNA sequencing work and for all of their help and support with this study.

Chapter 7: Research Summary and Future Studies

7.1 Research Summary

In this project, we used RNA-Seq to investigate the transcriptome-wide gene expression profiles of the fourth instar stage of a highly permethrin-resistant strain of *Culex quinquefasciatus* HAmCq^{G8} and its parental low permethrin-resistant strain HAmCq^{G0}. Overall, we identified 367 differentially up-regulated genes in the HAmCq^{G8} strain, namely cytochrome P450 genes and proteases. We further utilized qRT-PCR to validate the RNA-Seq results and confirmed the up-regulation of 14 cytochrome P450 genes in the HAmCq^{G8} strain as well as 14 proteases that had >2-fold up-regulation. Among the up-regulated proteases, one gene, nephrosin CPIJ009594, was more than 100 times over-expressed when tested using qRT-PCR validation. We proposed that the proteases that are differentially-expressed between the HAmCq^{G0} and HAmCq^{G8} strains may be involved in the signaling response that controls insecticide resistance in *Cx. quinquefasciatus.* We then investigated the genes that were up-regulated in the fourth instar stage of the HAmCq^{G8} strain following a 24 h chronic exposure to permethrin at the LC_{50} and LC_{70} rates, and identified 224 and 146 genes that were up- and down-regulated solely in response to permethrin, respectively. Following Gene Ontology (GO) enrichment of the differentially-expressed genes, the GO terms that were statistically enriched among the up-regulated genes included those GO terms associated with cytochrome P450 activities and immune response. Further investigation among the up-regulated genes showed that the up-regulated immune genes were genes that are controlled by the Toll pathway. Among the GO terms that were functionally-enriched among the down-regulated genes, the majority related to protease activity and carbohydrate metabolic process. Among the down-regulated genes

associated with carbohydrate metabolic process were immune genes involved in the IMD pathway, suggesting that the immune pathways that were active in permethrin and control treated *Cx. quinquefasciatus* differed. We further investigated the larval serum storage proteins as well as selected proteases that had been previously identified as up-regulated in HAmCq^{G8} in our earlier study in order to 1) determine if exposure to permethrin resulted in a decrease in serum protein expression, which could represent a developmental delay, and 2) investigate the connection of proteases that are up-regulated in the HAmCq^{G8} strain to see if they may be involved in the signaling response in permethrin resistance. Our results showed that during exposure to permethrin all larval storage proteins decreased in expression, suggesting that larval *Cx. quinquefasciatus* delay development to the pupal form during chronic permethrin exposure, possibly due to a redirection of the insect's metabolic processes to detoxification as demonstrated by the functional enrichment of GO terms associated with cytochrome P450 activities. We also identified that nephrosin, CPIJ009594 was induced during permethrin exposure, and further identified that the induction of nephrosin was significantly higher in the HAmCq^{G8} strain than when compared to the insecticide-susceptible S-lab strain and also higher than in the HAmCq^{G0} strain (Chapter 3). We proposed that upon exposure to permethrin, insecticide resistant *Cx. quinquefasciatus* cease feeding to reduce oral exposure to the toxicant as a means of behavioral resistance. It is possible that insecticide resistant *Cx. quinquefasciatus* are capable of better-sensing the permethrin exposure and cease feeding to reduce the oral exposure to the insecticide, and that through the cessation of feeding, the TOR pathway is turned off, resulting in a loss of repression of the Toll pathway. The rapid response to cease feeding by the HAmCq^{G8} strain would represent an example of behavioral resistance to permethrin oral exposure. We then diverged from *Cx. quinquefasciatus*, to investigate the cytochrome P450s up-regulated in a

permethrin-resistant strain of *Aedes aegypti* in order to expand the knowledge-base of known cytochrome P450s involved in permethrin resistance in mosquitoes. Through a combination of qRT-PCR and transgenic *Drosophila melanogaster* enhancer-trap techniques, we confirmed the up-regulation of several cytochrome P450s identified in previous studies on insecticide resistance in *Ae. aegypti*, and further demonstrated that AAEL007815 (CYP4D24) and AAEL13556 (CYP4J15v1) were functionally capable of degrading permethrin. Finally, we investigated gene expression changes in *Cx. quinquefasciatus* using RNA-Seq throughout the period of time immediately post-eclosion up to the time when females freely took a blood meal. We found that while females would not freely take a blood meal prior to 48-72 h post-eclosion, the predominance of the changes in gene expression occurred within the first 24 h post-eclosion, which coincided with the onset of egg provisioning in autogenous *Cx. quinquefasciatus*. This suggested that the physiological requirements of the female for host seeking and blood meal acquisition are independent of vitellogenesis. We further identified that the *VitA* gene (CPIJ001357/CPIJ001358) is the predominant vitellogenin gene in *Cx. quinquefasciatus*. In conclusion, our project proposes a novel model of behavioral resistance in *Cx. quinquefasciatus*, possibly mediated by the protease nephrosin, identified the functional capacity of two cytochrome P450s in *Ae. aegypti* to degrade pyrethroids, and elucidated the gene expression profiles of *Cx. quinquefasciatus* throughout the early part of the adult stage.

7.2 Edsysteroid UDP-glucosyltransferase in *Culex quinquefasciatus* as a novel target for mosquito management

The predicted genome of *Cx. quinquefasciatus* possesses two genes that are predicted to be ecdysteroid UDP-glucosyltransferases, CPIJ003694 and CPIJ016641. The latter gene, CPIJ016641 was found to have no expression in adult female *Cx. quinquefasciatus* during any pre-blooding or post-blooding stage, while CPIJ003694 was identified to be induced at 36 h post-blooding, which preceded the maximal expression of *E74* by 24 h (Fig. 7.1).

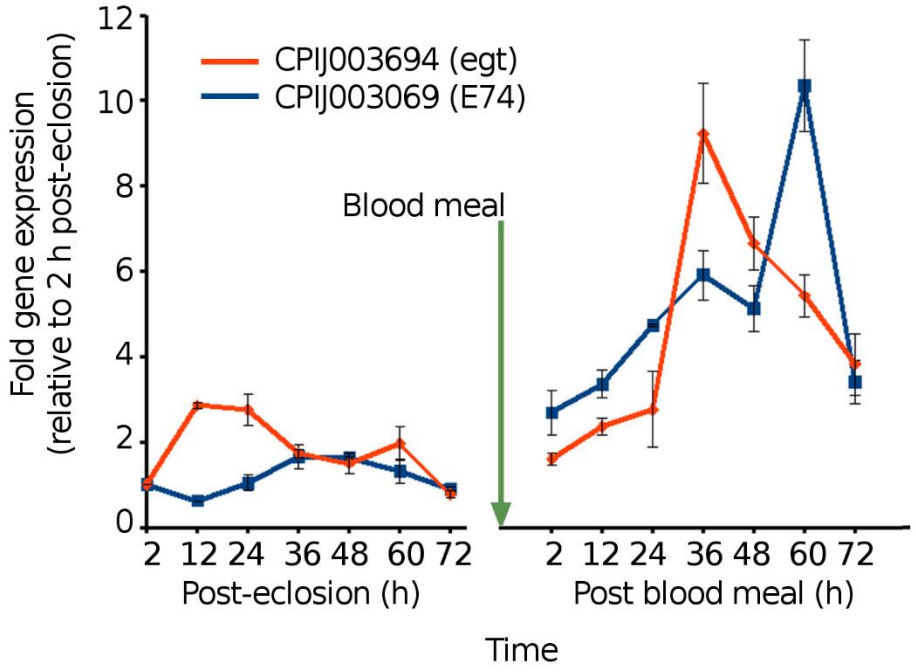

Figure 7.1 Temporal expression of the ecdysone-inducible gene *E74* and the ecdysteroid glucosyltransferase gene CPIJ003694.

The gene *E74* is inducible by ecdysone and required for the initiation of vitellogenesis in mosquitoes (Guoqiang et al., 2002), thus the expression of *E74* at 60 h post-blood meal suggests that there is a second burst of ecdysone, as is found in *Ae. aegypti* and other insects as well (Shirk et al., 1990), which has been proposed to be necessary for proper egg development. Thus, mosquitoes use ecdysone for both the initiation, and the maturation of eggs at different intervals. The observed increase in *egt* expression at 36 h (Fig. 7.1) suggests that there is a need for the mosquito to regulate the activity of ecdysone, likely through glucosylation, which has been

shown to inactivate the activity of 20-hydroxyecdysone in *Mamestra brassicae* (Clarke et al., 1996). Since ecdysone is likely necessary for proper egg maturation, it is also likely that *egt* is expressed in a tissue-specific fashion in order to protect specific mosquito tissues from the effects of ecdysone. Disruption of the role of *egt* could be a novel means of mosquito control. Future work to investigate the role of *egt* would be to conduct topical application of ecdysone agonists that cannot be glucosylated by egt. The maximal non-toxic rates for both tebufenozide, and methoxyfenozide have already been determined in Chapter 5, and along with application of 20-hydroxyecdysone as a control, the effect of egt could be estimated. That is, the activity of egt should be able to negate the effects of externally-applied 20-hydroxyecdysone, but not those of the ecdysone agonists. The measureable outcomes for this work would be adult survival, the numbers of eggs laid, the numbers of viable larvae, as well as the possible use of RNA-Seq to investigate unexpected changes in gene expression profiles. Further confirmation of the role of egt could be conducted using RNAi.

7.3 Use of *VitA* gene as a screening tool for bisacylhydrazines against mosquitoes

Non-steroidal ecdysone agonists have been developed for the control of Lepidopteran and Coleopteran pests, leading to the potential of these chemicals for the effective control of disease-vectoring insects, including mosquitoes (Smagghe et al., 2001; Retnakaran et al., 2003; Beckage et al., 2004). In Chapter 6, we found that the expression of the *vitA* gene (CPIJ001357 / CPIJ001358) was highly up-regulated (>10,000-fold) following a blood meal compared to the *vitA* gene expression in females that were not given a blood meal. Similar up-regulation patterns of *vitA* expression occurred in non-blood-fed females when these mosquitoes were treated with the ecdysone agonists, tebufenozide and methoxyfenozide, and we further found that the dose

required for induction of v*itA* expression was lower for methoxyfenozide compared with tebufenozide, suggesting a more specific effect of methoxyfenozide in *Cx. quinquefasciatus* (Fig. 7.2).

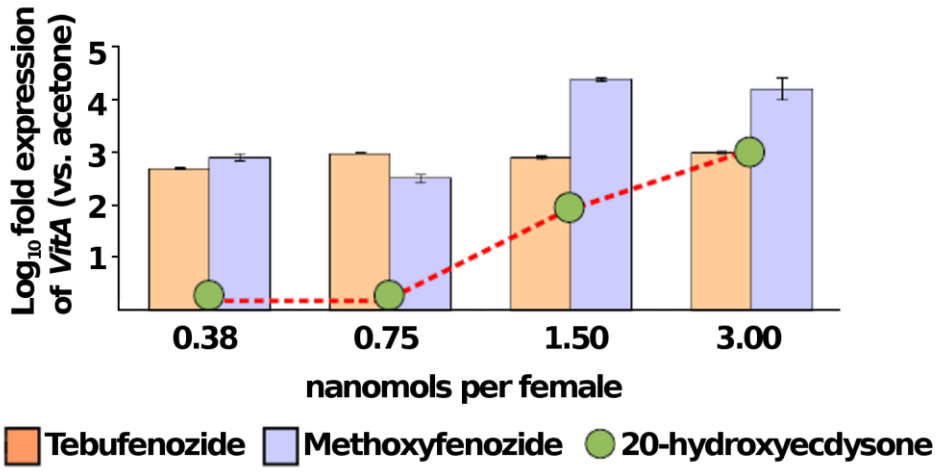

Figure 7.2. Dose-dependent effect of ecdysone agonists on the gene expression of the *VitA* gene (CPIJ001357 / CPIJ001358) against adult female *Cx. quinquefasciatus.*

Beckage et al. (2004) found that among three mosquito species, methoxyfenozide was more active than tebufenozide, suggesting that there may be a correlation between the larvicidal activity of ecdysone agonists and their ability to induce *VitA* expression. In this case, the level of *VitA* gene expression could be used as an indicator of the biological activity of ecdysone agonists. Such a system would be advantageous in a large-scale chemical screening program because only a few nanomols of test material would initially be needed to determine biological activity. In addition, the finding that topical treatment with tebufenozide and methoxyfenozide resulted in egg provisioning in *Cx. quinquefasciatus* would provide an efficient initial screen whereby mosquitoes need only be dissected at 48 h post application to determine activity.

Subsequent studies, however, would need to be conducted to optimize a chemical screening system, including characterizing the percentage of false negatives due to insufficient chemical absorption or females possibly incapable of vitellogenesis.

7.4 Determination of gene copy number in the highly pyrethroid-resistant HAmCq^{G8} strain of *Cx. quinquefasciatus*

In addition to testing the gene expression profiles in Chapter 3, we also investigated the SNPs that differed between the HAmCq^{G0} and HAmCq^{G8} strains. For this, we first mapped all of the RNA-Seq reads to the reference genome for *Cx. quinquefasciatus*, which is the Johannesberg strain (Arensburger et al., 2010). This was done so that the RNA-Seq reads could be overlapped to a reference genome, allowing for positions with different nucleotides to be determined and SNPs to be called (Neilsen et al., 2011). However since we were interested only in SNPs that differed between the HAmCq^{G0} and the HAmCq^{G8} strain, and not SNPs between the HAmCq^{G8} strain and the Johannesberg strain, we further sorted all of the identified SNPs, and retained only the SNPs that were different between the HAmCq^{G0} and HAmCq^{G8} strains. A minimum coverage of 20 reads for each SNP (Neilsen et al., 2011) was required for both the HAmCq^{G0} and HAmCq^{G8} strains in order to determine the SNPs in the HAmCq^{G8} strain,. which ensured that the SNPs called in the HAmCq^{G8} strain were different between the permethrin-selected HAmCq^{G8} strain and its parental HAmCq^{G0} strain. In total, >130,000 SNPs were determined between the HAmCq^{G0} and HAmCq^{G8} strains (Table 7.1). More than 85% of the SNPs identified (112,112 SNPs) were synonymous SNPs, suggesting that the majority of the SNPs between the HAmCq^{G0} and HAmCq^{G8} strains do not result in a change to the amino acid sequence of the proteins. Among all SNPs determined, ~58% (75,229 SNPs) of them were homozygous SNPs and ~42%

were heterozygous (Table 7.1), indicating the more than half of total SNPs had gone to fixation, that is, they were completely selected within the HAmCq^{G8} population (Barreiro et al., 2003).

Table 7.1. Single nucleotide polymorphisms in the pyrethroid-resistant strain of *Cx. quinquefasciatus*, HAmCq^{G8}, compared to the parental reference strain HAmCq^{G0}.

Region[§]	Total SNPs	Homozygous	Heterozygous
synonymous SNPs	112112	64252	47860
non-synonymous SNPs	17732	10412	7320
splice site donor	273	234	39
splice site acceptor	305	245	60
synonymous stop	57	38	19
TOTAL	**130479**	**75181**	**55298**

[§] As defined for the gene coding regions for the *Cx. quinquefasciatus* Johannesberg strain v1.3 Vectorbase, www.vectorbase.org

Other SNPs within the HAmCq^{G8} strain included the addition of 273 predicted splice site donors and 305 splice site acceptors, suggesting that alternative splicing could also play a role in the regulation of the up-regulated genes in the HAmCq^{G8} (Table 7.1). In addition, a total of 57 synonymous stop SNPs were identified in the HAmCq^{G8} strain, which are not predicted to result in any change to protein function (Table 7.1). To pinpoint the possible importance of the SNPs in insecticide resistance, we investigated the SNPs that were present in the up-regulated genes of the pyrethroid-resistant HAmCq^{G8} strain identified in Chapter 3. Among the up-regulated genes in HAmCq^{G8}, 132 of them contained SNPs, with a total of 1010 SNPs among the genes (Table 7.2). The number of SNPs per gene were compared among their Structural Classification of Proteins (SCOP) functional categories, which showed that the genes containing SNPs were present in the categories of extracellular processes, general, intracellular processes, metabolism, regulation, and no annotation (Fig. 7.3).

Table 7.2. List of genes containing SNPs/indels in the HAmCq^{G8} strain of *Cx. quinquefasciatus* when compared to the HAmCq^{G0} strain for genes shown to be up-regulated in the HAmCq^{G8} strain.

Sequence	SCOP[‡] general	SCOP[‡] detailed

variant	function	function	Gene function	Gene number[§]
Insertion	General	Small molecule binding	glutathione S-transferase	CPIJ006160
	Metabolism	Other enzymes	AMP dependent CoA ligase	CPIJ000791
			fumarylacetoacetate hydrolase	CPIJ017110
	Intra-cellular processes	Proteases	serpin B6	CPIJ014719
	Metabolism	Redox	short chain type dehydrogenase	CPIJ005656
Deletion	General	Small molecule binding	glutathione S-transferase	CPIJ006160, CPIJ002663
		General	sarcoplasmic calcium-binding protein	CPIJ001560
SNP	Extra-cellular processes	Blood clotting	ficolin-1 precursor	CPIJ012830
		Cell adhesion	cadherin	CPIJ014101
	General	General	asporin precursor	CPIJ017510
			sarcoplasmic calcium-binding protein	CPIJ001560
			troponin C, isoform	CPIJ012250, CPIJ016821
		Protein interaction	TPR repeat-containing protein T20B12.1	CPIJ007346
		Small molecule binding	D-2-hydroxyglutarate dehydrogenase	CPIJ001318
			glucose oxidase	CPIJ007620
			glutathione S-transferase	CPIJ002663, CPIJ006160
			multidrug resistance-associated protein	CPIJ001520
			myosin heavy chain, striated muscle	CPIJ000853
			NADP-dependent leukotriene B4 12-hydroxydehydrogenase	CPIJ003802
	Intra-cellular processes	Phospholipid m/tr[*]	alpha-tocopherol transfer protein	CPIJ009746
			cathepsin B precursor	CPIJ001239, CPIJ001240
		Proteases	carboxypeptidase A1 precursor	CPIJ010805
			chymotrypsin-1	CPIJ006543, CPIJ002135, CPIJ002137
			collagenase precursor	CPIJ002142, CPIJ016012
			fxna	CPIJ009738, CPIJ014110
			peptidase family	CPIJ801477
			prolylcarboxypeptidase	CPIJ008873
			serine proteases 1/2 precursor	CPIJ002139
			serpin B6	CPIJ014719
			transmembrane protease, serine	CPIJ001111
			trypsin-3 precursor	CPIJ019428
			zinc carboxypeptidase	CPIJ801679, CPIJ801680, CPIJ801685, CPIJ801686
		Transport	5'-nucleotidase, C-terminal	CPIJ800110
			ammonium transporter	CPIJ013531
			blastula protease 10 precursor	CPIJ002943
			zinc metalloproteinase	CPIJ002941, CPIJ002942,

			CPIJ019029
Metabolism	Carbohydrate m/tr	1, 4-alpha-glucan-branching enzyme	CPIJ006166
		alpha-amylase A precursor	CPIJ005725, CPIJ005060
		maltose phosphorylase	CPIJ008853
	Energy and E-transfer	acyl-coenzyme A oxidase 1, peroxisomal	CPIJ003059
		cytochrome b5	CPIJ004595
		vacuolar ATP synthase subunit C	CPIJ002067
	Lipid m/tr	lipoprotein amino region	CPIJ801684
	Other enzymes	AMP dependent CoA ligase	CPIJ000791, CPIJ006459
		chitinase class	CPIJ800112
		esterase B1	CPIJ007824, CPIJ016336
		fumarylacetoacetate hydrolase domain-containing protein	CPIJ017110
		luciferin 4-monooxygenase	CPIJ010716, CPIJ015088
		lysosomal pro-X carboxypeptidase	CPIJ008876
		phospholipase A1 member A precursor	CPIJ004222
		secreted chitinase	CPIJ019598
		selenium-binding protein 1-A	CPIJ013577
		WD-repeat protein	CPIJ002052
	Polysaccharide m/tr	2-hydroxyacylsphingosine 1-beta-galactosyltransferase	CPIJ000226
		UDP-glucuronosyltransferase 2B17 precursor	CPIJ006508
	Redox	basic juvenile hormone sensitive hemolymph protein	CPIJ005187
		cytochrome b561 domain-containing protein	CPIJ010934
		cytochrome P450	CPIJ800194 (CYP6AA7)**, CPIJ800196 (CYP6AA9), CPIJ800216 (CYP6BZ2), CPIJ800222 (CYP9J33), CPIJ800229 (CYP9J40), CPIJ800249 (CYP4D42v1), CPIJ800254 (CYP4H30), CPIJ800259 (CYP4H37v1), CPIJ015681 (CYP4H37v2), CPIJ020229 (CYP4D42v2)
		hexamerin 1.1 precursor	CPIJ000056, CPIJ006537, CPIJ006538, CPIJ018824, CPIJ018825
		larval serum protein 2 precursor	CPIJ001820
		NADH dehydrogenase	CPIJ018667
		short chain type dehydrogenase	CPIJ005656
	Secondary metabolism	beta-1, 3-glucan-binding protein precursor	CPIJ004320, CPIJ004323
		venom allergen 3 precursor	CPIJ004029

	Transferases	cystathionine gamma-lyase	CPIJ006619
Regulation	DNA-binding	sterol regulatory element-binding protein	CPIJ018167
	Signal transduction	Neuronal acetylcholine receptor subunit alpha-2	CPIJ801870
NONA[§]	not annotated	acidic mammalian chitinase precursor	CPIJ000008
		actin, muscle A2	CPIJ012574
		astacin precursor	CPIJ013319
		beta-galactosidase precursor	CPIJ003338
		cecropin-A precursor	CPIJ010699
		class VII unconventional myosin	CPIJ000852
		fibrinogen C domain-containing protein	CPIJ005841
		galactoside-binding lectin	CPIJ802228
		leucine-rich transmembrane protein	CPIJ006150, CPIJ004947, CPIJ006515
		liver carboxylesterase 1 precursor	CPIJ018231
		low choriolytic enzyme precursor	CPIJ010224
		peroxisomal membrane protein 11C	CPIJ009744
		sarcalumenin precursor	CPIJ013085
		SITS-binding protein	CPIJ008904
		translocator protein	CPIJ009683
		conserved hypothetical protein	CPIJ002103, CPIJ002117, CPIJ003223, CPIJ003485, CPIJ007785, CPIJ010305, CPIJ012702, CPIJ014226, CPIJ014892, CPIJ017149, CPIJ017150, CPIJ018791, CPIJ001427, CPIJ002070, CPIJ002744, CPIJ009034, CPIJ010757, CPIJ012287, CPIJ012899, CPIJ013195, CPIJ013296, CPIJ013736, CPIJ017076, CPIJ018002, CPIJ020308

[§]*Cx. quinquefasciatus* Johannesberg strain v1.3 Vectorbase, www.vectorbase.org
[‡] SCOP general function categories annotated using the predicted *Cx. quinquefasciatus* annotation information available at the Superfamily website (version 1.75) supfam.cs.bris.ac.uk/SUPERFAMILY/index.html

[*]m/tr= metabolism and transport
[**]Annotation of Cytochrome P450s taken from: http://drnelson.ut.mem.edu/CytochromeP450.html
[§]NONA= No Annotation

Among the SCOP categories, the categories for no annotation, intracellular processes (including proteases) and metabolism (including detoxification enzymes such as cytochrome P450s) had the greatest numbers of genes that contained SNPs, with the majority of the SNPs in these

catergories being heterozygous (Fig. 7.3). The extracellular processes and regulation categories each contained two genes that had SNPs within their sequences (Table 7.2). A total of 12 genes in the general function category contained either heterozygous or homozygous SNPs, with one gene, CPIJ006160 - glutathione-S-transferase, containing both homozygous and heterozygous SNPs. Within the metabolism category genes including two esterases and 10 cytochrome P450 genes contained both homozygous and heterozygous SNPs (Table 7.2). The median number of heterozygous SNPs per gene in metabolism category was six, which was twice the number of homozygous SNPs (Fig. 7.3).

In the remaining categories of intracellular processes and no annotation, a similar pattern of roughly twice as many heterozygous as homozygous SNPs per gene was identified, while the genes within the intracellular processes category were predominantly annotated as having proteolytic/peptiditic activities, which have been previously linked to insecticide resistance in insects as well as gene regulation and cell signaling (Pedra et al., 2004). Of particular interest was that among the up-regulated genes in the HAmCq^{G8} strain, one-third (132 of 367) contained SNPs, the majority of which were a combination of both heterozygous and homozygous SNPs. In addition, the majority of SNPs were synonymous, suggesting no change in function. As a result of the high correlation of both heterozygous and homozygous synonymous SNPs in the up-regulated genes of HAmCq^{G8}, and since we mapped to the genome of the Johannesburg strain, and not to the genome of HAmCq^{G0} or HAmCq^{G8}, we propose that the high occurrence of SNPs in the HAmCq^{G8} up-regulated genes is due to the cross-mapping of genes duplicated in the

HmCq^{G8} strain, but not the HAmCq^{G0} strain.

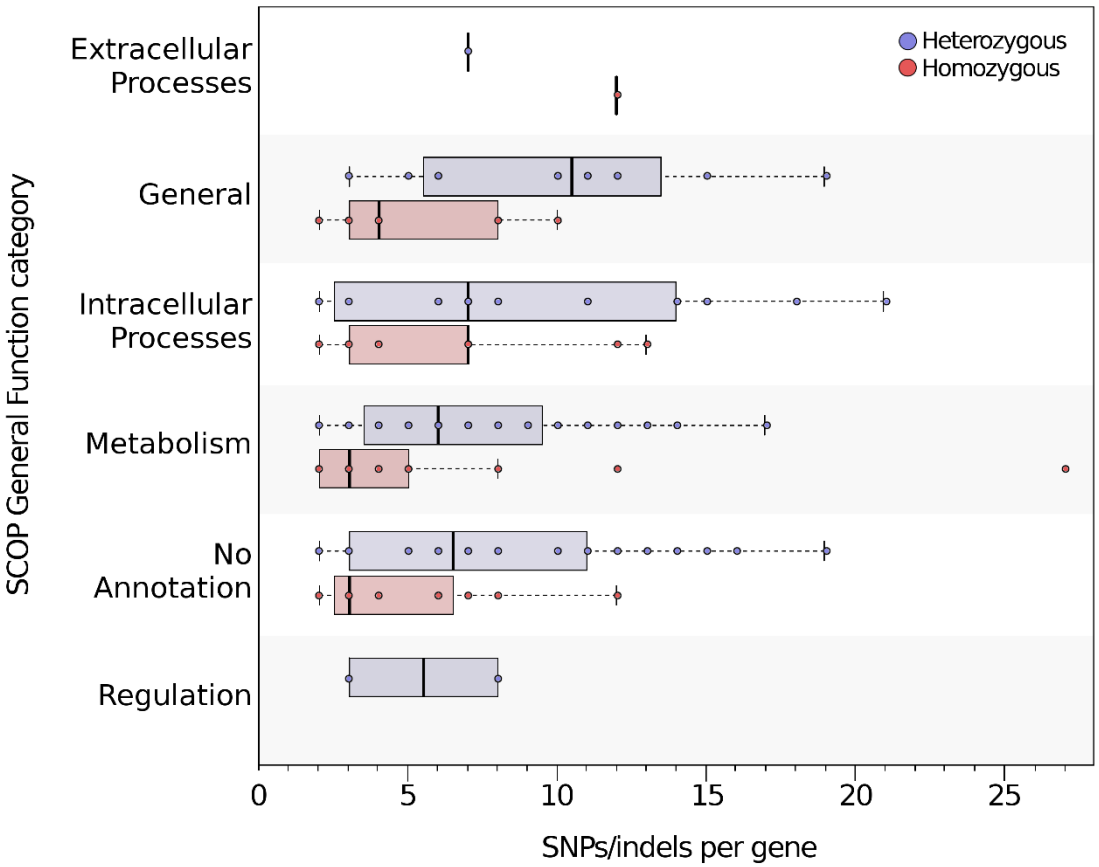

Figure 7.3. Distribution of SNPs and indels identified within the up-regulated genes in the pyrethroid-resistant HAmCq^{G8} strain of *Cx. quinquefasciatus* within the general function categories of the Structural Classification of Proteins (SCOP) datatbase. Individual points within category represent the actual number of SNPs/indels for a given gene, while the box-whisker plots represent the quartile values, where the internal black bar within each box represents the median value of SNPs/indels per gene within SCOP general function category.

In order to test this, future work using copy number qRT-PCR (Solomon et al., 2008) on the DNA of the HAmCq^{G0}, HAmCq^{G8}, and insecticide susceptible S-lab strains would identify if gene duplication is responsible for the high occurrence of SNPs in the up-regulated genes of HAmCq^{G8} strain, and may, in part, explain their up-regulation.

7.4 References

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Appendix 3.1. List and sequences of the qRT-PCR primers used.

Gene*	Sense primer (5' to 3')	Antisense primer (5' to 3')
18S rRNA	*CGCGGTAATTCCAGCTCCACTA*	*GCATCAAGCGCCACCATATAGG*
CPIJ002802	CTGCATGAAGCTCCGTTGT	AGGTGCTCTCGTGGGTAGC
CPIJ002795	ACGCTCCAGCTCTGTCGTA	GTGTAGCTGGTGGCGTGAG
CPIJ011785	AGCTGGTGTTCCAGGTGTTC	AGCTTTTGTTGGGGATTGTG
CPIJ002943	ATGAGTAACGAGTTCCAGGAGTTG	TTCTCGAACAAAATATCGACAAAC
CPIJ001979	CGAGTCTACCTACACTGGGAAGAT	TAATTCCAGCTTGATGGTTCACTA
CPIJ014110	CAACGGTACTACATCTTTCACCAA	ATGTTTGTTTCCAAATGGGTACTT
CPIJ004594	GGATTTCGGTAAATTTGAAGATGT	TTTTCGTACGTCATTTTAAACAGC
CPIJ014719	TTCAAGGGAACCTGGAAGC	ACGCGTTGAGCTCTTCAAA
CPIJ004323	AAAAGTTCCGACCGGTGAC	CGAGGTGTGTCCCATTGAC
CPIJ002139	TAATCTGTCGTGTCAATTGTCGTA	GGAAGCTATGTATTCCGATGAGAT
CPIJ002942	GACTATGGCAGTGTGATGCACTA	CAAGCACCCATACATCAAGTTTAG
CPIJ001111	ACTGGTTATTCAGCGGTGTACTTT	TTTGATCCAAGGAAGATACGTTTT
CPIJ018037	GGATGTGGTCAGACTGGGTAGTAT	AAACGCTACATCTCTCTCCAACTT
CPIJ006543	AGGTTGATGAGGAGGAGAATACAG	GGATAGATATGCTCATCGTGGAAC
CPIJ002130	GTGGAGGTTCTAAAATTTCCAGTG	TCAGGACAAACGTAGACGAAATTA
CPIJ013319	CTACTACGGTAGCGTGATGCACTA	ACATGTAGTTGACTGCAAGGATGT
CPIJ009106	CAAACCAGAAGAGTACAACTGTCG	GTAGGACACAAAGTAGCGCAGATA
CPIJ001240	AGGACGTGAATATCGTTCTGAAAT	GTTCTGATAGATCTCGGCTTTCAT
CPIJ019428	GGTTCAATAATATCCGAACGATG	GGATGCACGAAGATACTACGAAC
CPIJ004086	TTGAGACGTACAATCAGCAATTTT	TTGTTGTAATTCTCCTGCACAAAT
CPIJ008873	CAGTGAGCTGTTCAATACCTGTTC	GAGTTTGACAGAGCCTATCGGTAT
CPIJ002135	TTCAACGACTATGTTCAACCAATC	CGTATACCTCACCATGTCAGATTC
CPIJ016012	GGGAGTTATGTTGAGGACTTGAAA	GAAGGGTGGCACAGTTATTTATTC
CPIJ002142	TGAAATCCTTAGTAGTGCTTGCAG	TGACCAGAGAGAAGGATGTTGATA
CPIJ006803	GGTAATCTGGTGGAGAGTGACAT	AATGAAGTGACGTTCGGTTTTATT
CPIJ007383	TTTGGTTGACATTGAAAACACTCT	AGCTCTCGTTTCATCTTCTTGATT
CPIJ010224	GAACTATTCGAGACCGGTAGTGAT	GTGAAATTTGCTCCTCAAACACTT
CPIJ014523	TTTTAACGATTACGTTCAACCTGT	AATCTCTGGCGCCATAATAGTAAC
CPIJ019029	CTACACCAACAACAAGTGGAACA	GGTTCACGTAGATGTACTCGTCAC
CPIJ002128	TTGTTATTCTTCTCACAGCAGCTC	CATAGTCATACCATTGGTCCAGTC
CPIJ006542	TGTCGACGAATCAGTTCACTTTAT	CACTTCAAAGTGGGTAGCTGAAC
CPIJ010805	ATTGGAACTACTCATGAGGGAAGA	AGATGCATAATGGTCATAATGGTG
CPIJ006076	CTGAAAACAACACAACCTATGGTC	ATTCTCGGAAACCTCTCCACTAAC
CPIJ001743	GCCTTTGGATGGACTGACTACTAC	CTTGTGGGATAGTTTTACCACCTT
CPIJ003623	CAGTCGAGTAAACATCACCGATAG	GACCAAATGAAGTTATGCCGTACT
CPIJ001742	TGATTTTGAGGAACTTACAACGAA	AGATTTCAGCCGTGGAGTAGTAGT
CPIJ009594	GAAGTATCAGACAACCGCATTCTA	TTTCAAGTTGTTCATCACTGGTCT
CPIJ018233	GTCTGCTTGGGTTCTTCAGC	CGTCACATTGTTCGGATCAC
CPIJ006166	AAGGGAACGTCGGATGAAG	CCTTGTCCATCAGCCAGAA
CPIJ001820	GTTGAATTCTACAAGCACGGTATG	CGTAGTAGAAAACGTGGAACAGAG
CPIJ000056	GAGCTACCTGCCATACTACACCTT	GAAGAAGTCAAAGTACGTGAGCAG
CPIJ009033	ATCGACTTCAGCTATTTCTTCACC	GTCGGTAGTGTTTAGTACGACGTG
CPIJ009032	AGTTGAGATCAAGGAGTTTTCCAG	GGGAGTTCTTGTAGTTGAAGGGTA
CPIJ007783	ACTACCAATTCAAGGATCACCTTC	AGTATGTGACCAACTTGTCAATGG

**Culex quinquefasciatus* genome, Johannesburg strain CpipJ1.2, June 2008; http://cquinquefasciatus.vectorbase.org/

Appendix 3.2. Lognormal distributions for expressed genes in HAmCq^{G0} and HAmCq^{G8} by superfamily.

Superfamily[†]	Strain	N[‡]	median	mean	modality	kurtosis	skewness
(Trans)glycosidases	HAmCq^{G0}	46	1.64	1.60	bimodal	-0.51	0.14
	HAmCq^{G8}	45	1.53	1.55	bimodal	-0.42	0.29
Acetyl-CoA synthetase-like	HAmCq^{G0}	38	1.17	1.17	unimodal[*]	-0.86	-0.31
	HAmCq^{G8}	34	1.16	1.19	unimodal	-0.50	-0.17
Acyl-CoA dehydrogenase C-terminal domain-like	HAmCq^{G0}	5	1.54	1.46	unimodal[*]	-2.96	-0.32
	HAmCq^{G8}	5	1.67	1.50	unimodal	4.06	-2.01
Acyl-CoA N-acyltransferases	HAmCq^{G0}	41	1.17	1.18	unimodal[*]	-0.70	-0.05
	HAmCq^{G8}	43	1.04	0.95	unimodal[*]	-1.04	0.02
ALDH-like	HAmCq^{G0}	12	1.81	1.90	bimodal	-0.17	0.70
	HAmCq^{G8}	14	1.82	1.85	unimodal[*]	-0.71	0.29
Alkaline phosphatase-like	HAmCq^{G0}	21	1.04	1.13	bimodal	-0.07	0.60
	HAmCq^{G8}	21	0.86	0.99	bimodal	0.30	1.07
alpha/beta-Hydrolases	HAmCq^{G0}	109	1.17	1.26	unimodal	-0.07	0.54
	HAmCq^{G8}	98	1.17	1.20	unimodal[*]	0.41	0.70
Ankyrin repeat	HAmCq^{G0}	85	0.79	0.82	unimodal	-0.53	0.29
	HAmCq^{G8}	78	0.70	0.73	unimodal	-0.38	0.44
Arginase/deacetylase	HAmCq^{G0}	9	0.69	0.88	unimodal[*]	-0.78	0.25
	HAmCq^{G8}	7	1.02	0.85	bimodal	-0.60	-0.90
ARID-like	HAmCq^{G0}	6	1.12	1.09	unimodal	1.77	-1.05
	HAmCq^{G8}	6	0.82	0.74	unimodal	0.04	-1.04
ARM repeat	HAmCq^{G0}	136	1.22	1.25	unimodal	0.07	0.14
	HAmCq^{G8}	133	1.03	1.10	unimodal	0.57	0.71
C-type lectin-like	HAmCq^{G0}	33	1.17	1.39	multimodal	-1.06	0.40
	HAmCq^{G8}	28	1.50	1.41	multimodal	-0.72	0.20
C2H2 and C2HC zinc fingers	HAmCq^{G0}	515	0.71	0.73	unimodal	0.06	0.46
	HAmCq^{G8}	495	0.50	0.56	unimodal	0.55	0.71
Cadherin-like	HAmCq^{G0}	14	1.07	1.00	unimodal[*]	-1.05	-0.10
	HAmCq^{G8}	14	0.80	0.86	bimodal	-0.91	0.49
Calcium ATPase, transmembrane domain M	HAmCq^{G0}	14	1.36	1.48	bimodal	-0.81	0.33
	HAmCq^{G8}	12	1.20	1.47	bimodal	0.75	1.18
cAMP-binding domain-like	HAmCq^{G0}	15	0.69	0.81	bimodal	-1.49	0.28
	HAmCq^{G8}	14	0.49	0.48	unimodal[*]	-1.30	0.35
Chaperone J-domain	HAmCq^{G0}	20	1.20	1.29	unimodal[*]	-0.97	0.12
	HAmCq^{G8}	21	1.07	1.02	unimodal[*]	0.10	0.54
Chemosensory protein Csp2	HAmCq^{G0}	28	1.81	1.71	bimodal	-0.94	-0.20
	HAmCq^{G8}	23	1.66	1.64	bimodal	-1.52	-0.04
Concanavalin A-like lectins/glucanases	HAmCq^{G0}	45	0.85	0.95	unimodal[*]	0.58	0.77
	HAmCq^{G8}	39	0.73	0.92	unimodal	0.04	0.89
CRAL/TRIO domain	HAmCq^{G0}	48	1.17	1.18	unimodal	0.56	0.54
	HAmCq^{G8}	43	1.08	1.04	unimodal[*]	1.11	0.73
Cysteine proteinases	HAmCq^{G0}	62	1.27	1.31	unimodal	0.37	0.65
	HAmCq^{G8}	60	1.08	1.17	unimodal	0.57	0.76
Cytochrome b5-like heme/steroid binding domain	HAmCq^{G0}	11	1.50	1.42	unimodal	-0.60	-0.35
	HAmCq^{G8}	12	1.17	1.15	bimodal	-0.18	-0.51
Cytochrome P450	HAmCq^{G0}	143	1.10	1.11	unimodal	-0.68	0.08
	HAmCq^{G8}	136	1.21	1.22	unimodal	-0.70	0.20
Di-copper center-containing domain	HAmCq^{G0}	14	2.13	2.41	multimodal	1.25	-0.22
	HAmCq^{G8}	13	3.01	3.06	multimodal	-0.91	0.19
DNA/RNA polymerases	HAmCq^{G0}	11	0.81	0.89	unimodal	1.07	-0.10

	HAmCq^{G8}	10	0.69	0.80	unimodal	-0.25	0.84
E set domains	HAmCq^{G0}	51	1.56	1.74	unimodal	-0.26	0.62
	HAmCq^{G8}	49	1.39	1.69	unimodal	-0.40	0.65
EF-hand	HAmCq^{G0}	51	1.19	1.30	unimodal	-0.40	0.60
	HAmCq^{G8}	48	0.88	1.24	unimodal	-0.14	0.94
EGF/Laminin	HAmCq^{G0}	15	0.96	1.00	unimodal	1.73	0.97
	HAmCq^{G8}	14	0.49	0.62	unimodal*	-1.10	0.58
F-box domain	HAmCq^{G0}	19	0.72	0.76	unimodal*	-0.78	0.45
	HAmCq^{G8}	18	0.57	0.66	unimodal	1.10	1.00
FAD-binding domain	HAmCq^{G0}	7	0.36	0.65	bimodal	0.93	1.32
	HAmCq^{G8}	7	0.59	0.90	bimodal	0.63	1.24
FAD/NAD(P)-binding domain	HAmCq^{G0}	51	1.28	1.27	unimodal	-0.20	-0.03
	HAmCq^{G8}	48	1.15	1.18	unimodal*	-0.13	0.53
Family A G protein-coupled	HAmCq^{G0}	31	0.59	0.56	unimodal*	0.96	0.86
receptor-like	HAmCq^{G8}	21	0.27	0.47	unimodal	2.06	1.50
Ferritin-like	HAmCq^{G0}	14	1.69	1.67	bimodal	-0.02	0.54
	HAmCq^{G8}	14	1.69	1.79	unimodal*	-1.02	0.45
Fibrinogen C-terminal domain-	HAmCq^{G0}	42	0.99	1.03	unimodal*	-0.66	0.41
like	HAmCq^{G8}	38	0.94	0.94	unimodal*	-0.65	0.27
Fibronectin type III	HAmCq^{G0}	32	0.55	0.64	unimodal*	-0.30	0.54
	HAmCq^{G8}	26	0.53	0.51	unimodal*	-0.51	0.46
FnI-like domain	HAmCq^{G0}	9	0.95	0.93	unimodal	0.70	0.41
	HAmCq^{G8}	7	0.57	0.80	unimodal*	1.47	1.46
FYVE/PHD zinc finger	HAmCq^{G0}	32	1.09	0.95	unimodal*	-0.55	-0.64
	HAmCq^{G8}	32	0.76	0.74	unimodal	1.24	0.44
Galactose mutarotase-like	HAmCq^{G0}	7	1.68	1.36	multimodal	-0.57	-0.71
	HAmCq^{G8}	7	1.38	1.31	multimodal	-0.04	0.72
Glucocorticoid receptor-like	HAmCq^{G0}	154	0.85	0.87	unimodal	0.99	0.63
(DNA-binding domain)	HAmCq^{G8}	147	0.66	0.72	unimodal	1.54	1.02
Glutamine synthetase/guanido	HAmCq^{G0}	5	1.75	1.92	bimodal	1.20	1.04
kinase	HAmCq^{G8}	5	1.56	1.99	bimodal	2.73	1.69
Glutathione S-transferase	HAmCq^{G0}	29	1.66	1.57	unimodal	-0.20	-0.59
(GST), C-terminal domain	HAmCq^{G8}	27	1.54	1.52	unimodal*	-0.96	-0.07
Growth factor receptor domain	HAmCq^{G0}	11	1.18	1.09	unimodal	0.14	0.30
	HAmCq^{G8}	11	0.77	0.92	unimodal*	-0.35	0.88
HAD-like	HAmCq^{G0}	21	1.23	1.26	unimodal	1.37	0.59
	HAmCq^{G8}	22	0.97	1.01	unimodal	1.76	0.90
Histone-fold	HAmCq^{G0}	43	0.74	0.80	unimodal*	1.28	1.01
	HAmCq^{G8}	32	0.64	0.70	unimodal	1.86	1.16
HLH, helix-loop-helix DNA-	HAmCq^{G0}	32	1.19	1.08	unimodal*	-0.64	0.06
binding domain	HAmCq^{G8}	28	0.81	0.90	unimodal*	-0.88	0.33
HMG-box	HAmCq^{G0}	23	0.98	1.06	unimodal	1.80	0.63
	HAmCq^{G8}	23	0.60	0.74	unimodal*	1.52	0.99
Homeodomain-like	HAmCq^{G0}	85	0.66	0.66	unimodal	-0.52	0.35
	HAmCq^{G8}	57	0.54	0.57	unimodal	0.12	0.69
Immunoglobulin	HAmCq^{G0}	61	0.54	0.64	unimodal*	-0.53	0.69
	HAmCq^{G8}	30	0.65	0.68	unimodal	-0.90	0.32
Insect pheromone/odorant-	HAmCq^{G0}	47	0.97	1.14	unimodal	0.01	0.76
binding proteins	HAmCq^{G8}	39	1.00	1.14	unimodal*	1.31	1.06
Invertebrate chitin-binding	HAmCq^{G0}	103	2.19	2.03	unimodal*	-0.44	-0.40
	HAmCq^{G8}	102	2.05	1.87	unimodal*	-0.63	-0.38
L domain-like	HAmCq^{G0}	102	0.97	0.99	unimodal	-0.68	0.27
	HAmCq^{G8}	87	0.99	1.01	unimodal	0.55	0.62
Ligand-binding domain in NO	HAmCq^{G0}	6	1.12	0.99	bimodal	-2.30	-0.34
signaling and Golgi transport	HAmCq^{G8}	6	0.70	0.72	unimodal*	-1.82	0.24
Lipocalins	HAmCq^{G0}	15	1.30	1.42	unimodal*	-0.60	0.25

	HAmCq^{G8}	15	1.36	1.36	multimodal	0.55	-0.05
Lysozyme-like	HAmCq^{G0}	4	1.40	1.48	unimodal	3.22	1.70
	HAmCq^{G8}	5	1.58	1.49	bimodal	2.67	-1.43
Metallo-dependent hydrolases	HAmCq^{G0}	11	1.41	1.44	unimodal	-0.59	-0.09
	HAmCq^{G8}	11	1.21	1.20	unimodal*	0.02	-0.60
Metallo-dependent	HAmCq^{G0}	23	1.28	1.34	unimodal	0.38	0.51
phosphatases	HAmCq^{G8}	22	1.16	1.26	unimodal*	0.09	0.63
Metalloproteases ("zincins")	HAmCq^{G0}	49	1.40	1.33	unimodal*	-0.91	0.11
	HAmCq^{G8}	47	1.35	1.38	unimodal*	-1.01	0.25
MFS general transporter	HAmCq^{G0}	148	1.08	1.05	unimodal	0.41	0.42
	HAmCq^{G8}	140	0.87	0.96	unimodal	0.94	0.66
Myosin rod fragments	HAmCq^{G0}	5	1.31	1.26	multimodal	0.88	-0.20
	HAmCq^{G8}	5	1.12	1.16	bimodal	1.51	0.95
N-acetylmuramoyl-L-alanine	HAmCq^{G0}	9	1.56	1.70	multimodal	-0.05	1.18
amidase-like	HAmCq^{G8}	10	0.90	1.42	bimodal	0.56	1.33
NAD(P)-binding Rossmann	HAmCq^{G0}	119	1.45	1.39	unimodal*	-0.16	-0.11
	HAmCq^{G8}	115	1.23	1.25	unimodal	0.51	0.22
NAD(P)-linked oxidoreductase	HAmCq^{G0}	15	1.47	1.45	unimodal*	-0.22	-0.55
	HAmCq^{G8}	14	1.28	1.29	unimodal	0.22	-0.30
NAP-like	HAmCq^{G0}	8	0.73	0.95	multimodal	0.22	0.96
	HAmCq^{G8}	8	0.85	0.94	bimodal	2.98	1.36
Neurotransmitter-gated ion-	HAmCq^{G0}	10	0.34	0.50	unimodal	0.17	0.99
channel transmembrane pore	HAmCq^{G8}	3	0.32	0.53	bimodal	0.00	1.38
Nicotinic receptor ligand	HAmCq^{G0}	11	0.62	0.69	unimodal*	-0.93	0.66
binding domain-like	HAmCq^{G8}	7	0.94	0.90	unimodal*	-0.53	0.09
Nuclear receptor ligand-bind	HAmCq^{G0}	18	0.94	0.81	bimodal	-1.57	-0.21
	HAmCq^{G8}	12	0.83	0.74	unimodal*	-0.56	-0.36
Nucleotide-diPO4-sugar transf	HAmCq^{G0}	38	0.96	1.03	unimodal	-0.38	0.50
	HAmCq^{G8}	39	0.81	0.84	unimodal	-0.01	0.61
Outer arm dynein light chain 1	HAmCq^{G0}	7	0.96	0.86	unimodal*	-0.83	-0.18
	HAmCq^{G8}	8	0.62	0.61	unimodal	-1.21	0.20
P-loop nucleotide hydrolases	HAmCq^{G0}	427	1.09	1.14	unimodal	1.89	0.89
	HAmCq^{G8}	407	0.90	0.99	unimodal	3.57	1.41
PDZ domain-like	HAmCq^{G0}	44	0.92	0.97	unimodal	-0.50	0.07
	HAmCq^{G8}	36	0.74	0.79	unimodal	-0.41	0.48
Phosphoglycerate mutase-like	HAmCq^{G0}	15	1.32	1.29	multimodal	0.08	-0.35
	HAmCq^{G8}	14	1.14	1.24	unimodal	2.17	1.23
Phospholipase A2, PLA2	HAmCq^{G0}	6	0.85	0.87	unimodal	1.33	0.29
	HAmCq^{G8}	7	0.34	0.31	unimodal	4.53	-1.96
PLC-like phosphodiesterases	HAmCq^{G0}	9	0.79	0.87	unimodal*	-0.94	0.10
	HAmCq^{G8}	8	0.67	0.86	bimodal	1.14	1.18
PLP-binding barrel	HAmCq^{G0}	7	0.92	1.20	bimodal	4.16	2.04
	HAmCq^{G8}	6	1.14	1.33	bimodal	3.56	1.86
PLP-dependent transferases	HAmCq^{G0}	36	1.74	1.65	bimodal	-0.52	-0.40
	HAmCq^{G8}	38	1.61	1.49	bimodal	-0.76	-0.39
PR-1-like	HAmCq^{G0}	7	0.80	0.86	multimodal	-1.39	0.29
	HAmCq^{G8}	8	0.70	0.95	bimodal	-1.91	0.32
Protein kinase-like (PK-like)	HAmCq^{G0}	254	0.97	0.98	bimodal	0.52	0.53
	HAmCq^{G8}	247	0.79	0.84	bimodal	0.27	0.58
Proton glutamate symporter	HAmCq^{G0}	5	0.95	0.84	unimodal	0.51	-0.85
	HAmCq^{G8}	4	0.92	1.00	unimodal	2.51	1.59
Quinoprotein ADH-like	HAmCq^{G0}	14	1.10	1.02	bimodal	0.20	-0.93
	HAmCq^{G8}	13	0.82	0.91	unimodal*	0.12	-0.53
Retrovirus zinc finger-like	HAmCq^{G0}	10	1.06	1.08	bimodal	4.49	1.83
	HAmCq^{G8}	10	0.99	1.00	bimodal	3.18	0.86
Ribonuclease H-like	HAmCq^{G0}	27	1.12	1.13	unimodal	-0.71	-0.26

	HAmCq^{G8}	27	0.89	0.98	unimodal	-0.39	-0.37
RING/U-box	HAmCq^{G0}	90	1.12	1.08	unimodal	1.17	0.41
	HAmCq^{G8}	92	0.82	0.83	unimodal	0.97	0.52
RNA-binding domain	HAmCq^{G0}	131	1.25	1.23	unimodal	-0.02	-0.06
	HAmCq^{G8}	120	1.01	1.04	unimodal	0.46	0.30
RNI-like	HAmCq^{G0}	83	0.71	0.73	unimodal	-0.80	0.18
	HAmCq^{G8}	80	0.63	0.68	unimodal	-0.78	0.21
SAM-methyltransferases	HAmCq^{G0}	68	1.14	1.13	unimodal	-0.16	-0.18
	HAmCq^{G8}	67	0.93	0.93	unimodal	0.26	0.26
Serine protease inhibitors	HAmCq^{G0}	5	2.39	1.97	bimodal	-2.70	-0.59
	HAmCq^{G8}	5	2.55	2.03	bimodal	3.08	-1.77
SET domain	HAmCq^{G0}	40	0.91	0.82	unimodal*	-1.05	-0.13
	HAmCq^{G8}	43	0.71	0.63	unimodal	-0.92	0.16
Six-hairpin glycosidases	HAmCq^{G0}	11	1.46	1.48	unimodal*	-0.06	-0.56
	HAmCq^{G8}	10	1.71	1.64	unimodal*	-0.99	-0.01
Sterol carrier protein	HAmCq^{G0}	7	3.32	2.65	bimodal	-1.04	-0.97
	HAmCq^{G8}	7	3.40	2.70	multimodal	-1.12	-0.82
Terpenoid cyclases	HAmCq^{G0}	6	1.30	1.13	unimodal*	-1.69	-0.86
	HAmCq^{G8}	6	1.10	1.07	unimodal*	-0.53	0.27
Thiolase-like	HAmCq^{G0}	12	1.73	1.70	unimodal	-1.03	-0.14
	HAmCq^{G8}	12	1.58	1.58	unimodal	-1.11	0.22
Thioredoxin-like	HAmCq^{G0}	58	1.64	1.69	unimodal	0.62	-0.40
	HAmCq^{G8}	58	1.52	1.50	unimodal	-0.50	-0.20
TPR-like	HAmCq^{G0}	74	1.17	1.17	unimodal	0.17	-0.14
	HAmCq^{G8}	67	1.07	1.10	unimodal	0.75	0.37
TRAF domain-like	HAmCq^{G0}	16	1.00	0.88	unimodal	-0.17	-0.73
	HAmCq^{G8}	15	0.87	0.86	unimodal	-1.04	0.20
Translation proteins	HAmCq^{G0}	13	0.51	1.19	bimodal	0.03	1.15
	HAmCq^{G8}	9	0.92	1.54	bimodal	-1.02	0.87
Tropomyosin	HAmCq^{G0}	9	0.93	1.12	bimodal	2.29	1.40
	HAmCq^{G8}	11	0.68	0.92	bimodal	5.73	2.21
Trypsin-like serine proteases	HAmCq^{G0}	217	1.22	1.27	unimodal	0.06	0.66
	HAmCq^{G8}	205	1.07	1.20	unimodal	0.18	0.85
Tubulin nucleotide-binding	HAmCq^{G0}	10	1.97	1.90	multimodal	-1.00	-0.51
	HAmCq^{G8}	11	1.80	1.63	unimodal*	-1.07	-0.12
UBA-like	HAmCq^{G0}	11	1.03	1.09	unimodal	2.27	0.17
	HAmCq^{G8}	10	1.05	1.01	unimodal	-0.08	0.42
Ubiquitin-like	HAmCq^{G0}	33	1.42	1.60	unimodal	-0.22	0.47
	HAmCq^{G8}	32	1.36	1.48	unimodal	0.11	0.66
UDP-Glycosyltransferase	HAmCq^{G0}	29	1.38	1.29	unimodal	0.32	-0.52
	HAmCq^{G8}	33	1.08	1.08	unimodal*	-1.08	-0.09
vWA-like	HAmCq^{G0}	13	1.02	1.11	unimodal*	-0.86	0.10
	HAmCq^{G8}	14	0.92	0.96	unimodal*	-0.94	-0.15
WD40 repeat-like	HAmCq^{G0}	183	1.06	1.06	unimodal	1.91	0.65
	HAmCq^{G8}	173	0.89	0.91	unimodal	3.28	1.06
Winged helix DNA-binding	HAmCq^{G0}	57	1.20	1.26	unimodal*	0.89	0.55
	HAmCq^{G8}	56	0.92	1.07	unimodal*	1.41	0.96
WW domain	HAmCq^{G0}	10	0.86	0.99	unimodal*	-0.28	0.62
	HAmCq^{G8}	11	0.67	0.64	unimodal	1.01	1.11
Zn-dependent exopeptidases	HAmCq^{G0}	40	1.54	1.58	unimodal	0.86	-0.01
	HAmCq^{G8}	41	1.54	1.54	unimodal	0.15	-0.38
**Carboxylesterases	HAmCq^{G0}	17	1	1.12	unimodal*	1.19	0.94
	HAmCq^{G8}	16	0.71	1.03	bimodal	3.22	1.51

†Superfamilies from Structural Classification of Proteins (v1.73)

‡Total number of genes detected within the superfamily

*unimodal, but shouldered distribution

[**]Not a SCOP superfamily classification. Genes were grouped based on Vectorbase annotation as carboxylesterases.

Appendix 3.3. Complete list of all differentially upregulated genes[†] in HAmCq^{G8}.

General function[*]	Detailed function	Superfamily	Gene accession[**]	CpipJ_1.2 annotation	HAmCq^{G8} FPKM	Fold FPKM relative to HAmCq^{G0} [¤]
Extra-cellular processes	Cell adhesion	C-type lectin-like	CPIJ000449	galactose-specific C-type lectin	52.0	38.6
			CPIJ015401	galactose-specific C-type lectin	54.1	42.6
			CPIJ017075	galactose-specific C-type lectin	2.2	-
			CPIJ019507	salivary C-type lectin	23.8	5.2
		Cadherin-like	CPIJ014101	conserved hypothetical protein	56.6	2.7
		EGF/Laminin	CPIJ014886	conserved hypothetical protein	5.2	4.4
	Blood clotting	Fibrinogen C-terminal domain-like	CPIJ010089	microfibril-associated glycoprotein 4	28.8	2.9
			CPIJ012830	fibrinogen and fibronectin	121.8	6.7
			CPIJ013294	fibrinogen and fibronectin	11.1	-
			CPIJ015014	conserved hypothetical protein	9.4	8.3
			CPIJ018159	fibrinogen and fibronectin	15.1	3.8
	Cell adhesion	Fibronectin type III	CPIJ000838	conserved hypothetical protein	2.7	7.6
		FnI-like domain	CPIJ013195	conserved hypothetical protein	53.0	2.4
		RNI-like	CPIJ002173	conserved hypothetical protein	16.0	3.2
			CPIJ004947	leucine-rich repeat-containing protein 1	58.7	3.5
			CPIJ011874	predicted protein	1.3	-
			CPIJ014115	conserved hypothetical protein	10.7	4.0
			CPIJ014953	membrane glycoprotein LIG-1	10.5	4.0
			CPIJ016528	conserved hypothetical protein	1.0	-
			CPIJ019556	predicted protein	8.9	-
General	Protein interaction	Ankyrin repeat	CPIJ003373	predicted protein	15.5	12.0
			CPIJ008490	ankyrin repeat domain-containing protein 44	1.3	-
			CPIJ009398	conserved hypothetical protein	4.6	3.4
	General	ARM repeat	CPIJ005388	conserved hypothetical protein	4.0	2.8
		EF-hand	CPIJ001560	calcium-binding protein	1097.9	4.4
			CPIJ012250	troponin C	567.7	6.3
			CPIJ016821	troponin C	497.5	4.5
			CPIJ019636	EF-hand calcium-binding domain-containing protein 1	1.5	-
	Protein interaction	F-box domain	CPIJ019555	predicted protein	11.4	34.5
	Small molecule binding	FAD-binding domain	CPIJ001318	d-lactate dehydrognease 2	40.5	2.2
			CPIJ013647	alkyldihydroxyacetonephosphate synthase	4.3	3.3

			CPIJ016321	alkyldihydroxyacetonephosphate synthase	3.7	3.1
			CPIJ016322	alkyldihydroxyacetonephosphate synthase	3.9	3.3
		FAD/NAD(P)-binding domain	CPIJ007620	choline dehydrogenase	132.3	2.9
			CPIJ008048	peroxisomal N1-acetyl-spermine/spermidine oxidase	14.5	2.6
			CPIJ008445	amine oxidase	21.4	2.8
			CPIJ017813	spermine oxidase	8.8	2.7
		Glutathione S-transferase (GST), C-terminal domain	CPIJ002663	glutathione S-transferase 1-1	354.4	2.2
			CPIJ006160	glutathione s-transferase	173.7	2.4
			CPIJ018631	glutathione-s-transferase theta, gst	8.3	3.7
	General	L domain-like	CPIJ000315	conserved hypothetical protein	1.0	-
			CPIJ003143	conserved hypothetical protein	14.0	2.9
			CPIJ004946	leucine-rich repeat-containing protein 15	11.0	2.5
			CPIJ017510	conserved hypothetical protein	914.6	7.3
	Small molecule binding	NAD(P)-binding Rossmann-fold domains	CPIJ003802	NADP-dependent leukotriene B4 12-hydroxydehydrogenase	169.2	4.0
			CPIJ004379	steroid dehydrogenase	10.5	12.7
		P-loop containing nucleoside triphosphate hydrolases	CPIJ000853	myosin heavy chain	1750.5	3.8
			CPIJ001520	multidrug resistance-associated protein 1	34.6	2.7
			CPIJ003262	zinc finger protein	1.2	-
			CPIJ004695	dynein-1-beta heavy chain	1.9	2.3
			CPIJ009034	conserved hypothetical protein	27.0	2.3
			CPIJ009593	conserved hypothetical protein	8.0	2.9
			CPIJ015649	DNA-binding protein smubp-2	1.8	4.0
			CPIJ019948	myosin vii	5.9	2.5
	Protein interaction	TPR-like	CPIJ007346	TTC27 protein	37.5	2.7
		UBA-like	CPIJ011358	conserved hypothetical protein	27.9	2.8
	General	Ubiquitin-like	CPIJ014273	conserved hypothetical protein	1.1	-
		WD40 repeat-like	CPIJ006339	receptor of activated protein kinase C 1	6.9	5.3
			CPIJ012294	conserved hypothetical protein	2.0	3.5
Information	DNA replication/repair	FYVE/PHD zinc finger	CPIJ002070	conserved hypothetical protein	66.0	4.0
			CPIJ011117	conserved hypothetical protein	4.3	19.5
	Chromatin structure	NAP-like	CPIJ007782	nucleosome assembly protein	7.5	3.2
	DNA replication/repair	RING/U-box	CPIJ000388	ubiquitin conjugating enzyme 7 interacting protein	17.4	2.9
	Chromatin structure	Smc hinge domain	CPIJ018617	structural maintenance of chromosomes protein 3	10.4	2.8

Intra-cellular processes	Transport	Ammonium transporter	CPIJ013531	ammonium transporter	31.9	3.0
	Phospholipid m/tr	CRAL/TRIO domain	CPIJ001321	conserved hypothetical protein	1.7	-
			CPIJ003223	conserved hypothetical protein	43.4	2.2
			CPIJ009746	conserved hypothetical protein	54.3	2.6
			CPIJ014226	cellular retinaldehyde-binding protein	838.3	2.9
	Proteases	Cysteine proteinases	CPIJ001239	cathepsin B	190.2	9.7
			CPIJ001240	cathepsin B-like thiol protease	99.7	5.3
	Ion m/tr	Ferritin-like	CPIJ014287	ferritin heavy chain	202.2	4.0
	Transport	Glycolipid transfer protein, GLTP	CPIJ003328	conserved hypothetical protein	29.9	2.5
		Lipocalins	CPIJ013296	conserved hypothetical protein	29.2	2.7
			CPIJ015725	apolipoprotein D	457.6	4.6
	Proteases	Metallo-dependent phosphatases	CPIJ018314	5' nucleotidase	43.6	2.8
		Metalloproteases ("zincins"), catalytic domain	CPIJ001050	protease m1 zinc metalloprotease	266.9	3.6
			CPIJ002941	high choriolytic enzyme 1	273.8	3.5
			CPIJ002942	zinc metalloproteinase nas-12	1025.0	2.9
			CPIJ002943	conserved hypothetical protein	202.6	4.3
			CPIJ002945	zinc metalloproteinase dpy-31	101.1	3.8
			CPIJ004086	angiotensin-converting enzyme	292.0	5.7
			CPIJ006803	zinc metalloproteinase nas-7	22.5	4.5
			CPIJ007383	endothelin-converting enzyme 1	20.1	2.5
			CPIJ009106	angiotensin-converting enzyme	209.4	2.7
			CPIJ012036	aminopeptidase N	30.6	3.1
	Ion m/tr	MFS general substrate transporter	CPIJ001774	synaptic vesicle protein	4.9	3.4
			CPIJ001812	sugar transporter	3.3	4.8
			CPIJ005300	sugar transporter	2.9	9.3
			CPIJ005372	endogenous retrovirus A receptor	3.1	4.4
			CPIJ008813	sodium-dependent phosphate transporter	4.6	2.8
			CPIJ014925	solute carrier family 2	20.7	2.3
	Phospholipid m/tr	PLC-like phosphodiesterases	CPIJ002103	conserved hypothetical protein	78.7	2.4
	Transport	Proton glutamate symport protein	CPIJ000673	glutamate transporter	22.5	2.4
	Proteases	Serine protease inhibitors	CPIJ012287	hypothetical protein	89.0	4.8
	Cell motility	Tropomyosin	CPIJ008188	conserved hypothetical protein	3.3	5.4
	Proteases	Trypsin-like serine proteases	CPIJ000616	clip-domain serine protease	23.8	3.4
			CPIJ000617	clip-domain serine protease	18.1	3.6

			CPIJ001979	conserved hypothetical protein	22.0	3.0
			CPIJ002128	mast cell protease 2	94.1	16.1
			CPIJ002133	trypsin epsilon	47.8	7.9
			CPIJ002135	trypsin alpha-4	61.8	5.9
			CPIJ002137	serine protease1/2	122.4	3.4
			CPIJ002139	HzC4 chymotrypsinogen	559.8	4.3
			CPIJ002140	chymotrypsin BI	205.9	3.5
			CPIJ002142	chymotrypsin BI	5763.6	2.8
			CPIJ002156	chymotrypsin BI	194.8	2.8
			CPIJ003623	coagulation factor XII	15.0	7.1
			CPIJ004594	conserved hypothetical protein	11.0	5.9
			CPIJ005272	trypsin 3A1	2.4	-
			CPIJ006543	urokinase-type plasminogen activator	84.5	8.2
			CPIJ014656	coagulation factor XII	5.8	3.3
			CPIJ016102	transmembrane protease	29.2	2.7
			CPIJ018037	serine protease	15.7	3.9
			CPIJ019428	trypsin 2	36.9	3.4
		Zn-dependent exopeptidases	CPIJ001742	zinc carboxypeptidase	175.1	3.0
			CPIJ001743	carboxypeptidase A2	91.2	5.4
			CPIJ001744	zinc carboxypeptidase	195.5	3.5
			CPIJ001745	zinc carboxypeptidase	80.8	7.4
			CPIJ009738	conserved hypothetical protein	73.2	2.3
			CPIJ010805	carboxypeptidase A1	180.8	4.4
			CPIJ014110	conserved hypothetical protein	35.1	2.4
Metabolism	Carbohydrate m/tr	(Trans)glycosidases	CPIJ002104	plasma alpha-L-fucosidase	25.6	3.4
			CPIJ005060	alpha-amylase B	3837.1	2.3
			CPIJ005725	alpha-amylase A	171.1	3.1
			CPIJ006166	deltamethrin resistance-associated NYD-GBE	98.8	2.5
			CPIJ008528	glycoside hydrolase	7.6	3.9
			CPIJ009306	neutral alpha-glucosidase ab	10.0	3.7
	Other enzymes	3-carboxy-cis,cis-mucoante lactonizing enzyme	CPIJ013577	selenium-binding protein 2	164.4	2.7
		Acetyl-CoA synthetase-like	CPIJ000791	conserved hypothetical protein	124.8	2.8
			CPIJ006459	long-chain-fatty-acid-CoA ligase	114.8	3.5
			CPIJ010716	luciferin 4-monooxygenase	31.1	2.2
			CPIJ015088	4-coumarate-CoA ligase 1	34.2	2.2
	E- transfer	Acyl-CoA dehydrogenase C-terminal domain-like	CPIJ003059	acyl-CoA oxidase	47.2	4.0
	Transferases	Acyl-CoA N-acyltransferases (Nat)	CPIJ011827	conserved hypothetical protein	1.8	-

Redox	ALDH-like	CPIJ009438	aldehyde dehydrogenase	95.0	2.6
Other enzymes	alpha/beta-Hydrolases	CPIJ002715	lipase 3	10.4	3.2
		CPIJ004222	pancreatic triacylglycerol lipase	1790.9	4.7
		CPIJ007461	epoxide hydrolase	18.3	2.5
		CPIJ007824	esterase B1	34.4	2.2
		CPIJ008876	lysosomal pro-X carboxypeptidase	901.2	3.4
		CPIJ016336	esterase B1	32.3	2.2
		CPIJ019917	triacylglycerol lipase	23.0	2.8
Secondary metabolism	Concanavalin A-like lectins/glucanases	CPIJ004320	gram-negative bacteria-binding protein 1	221.8	5.6
		CPIJ004323	gram-negative bacteria binding protein	293.4	4.1
		CPIJ006421	conserved hypothetical protein	8.8	2.5
		CPIJ013048	conserved hypothetical protein	2.3	-
E- transfer	Cytochrome b5-like heme/steroid binding domain	CPIJ004595	cytochrome b5	91.7	2.3
		CPIJ005308	conserved hypothetical protein	1.4	-
Redox	Cytochrome P450	CPIJ002538	*CYP6AG12* £	575.0	3.7
		CPIJ005952	*CYP6BB4* £	4.0	2.8
		CPIJ005953	*CYP6BB3* £	74.9	2.6
		CPIJ005955	*CYP6P14* £	126.6	8.2
		CPIJ005956	*CYP6BZ2* £	460.1	3.3
		CPIJ005957	*CYP6AA9* £	84.9	6.6
		CPIJ005959	*CYP6AA7* £	98.3	7.3
		CPIJ006721	*CYP4H37v* £*1*	56.7	2.3
		CPIJ007188	*CYP4H30* £	27.9	2.3
		CPIJ008566	*CYP6Z15* £	5.4	3.3
		CPIJ009085	*CYP6AG13* £	5.9	3.4
		CPIJ009478	*CYP4D42v1* £	59.5	2.4
		CPIJ010225	*CYP12F14* £	30.8	3.9
		CPIJ010227	*CYP12F13* £	67.8	7.1
		CPIJ010537	*CYP9J45* £	109.4	4.8
		CPIJ010538	*CYP9J46* £	35.3	7.5
		CPIJ010542	*CYP9J38* £	19.6	8.3
		CPIJ010543	*CYP9J40* £	297.6	7.2
		CPIJ010544	*CYP9J33* £	58.9	3.1
		CPIJ010546	*CYP9J34* £	50.9	13.4
		CPIJ011127	*CYP4H34* £	9.9	2.7
		CPIJ012470	*CYP9AL1* £	86.2	9.2
		CPIJ014218	*CYP9M10* £	771.2	3.7
		CPIJ015681	*CYP4H37v2* £	42.8	2.6

		CPIJ015958	*CYP325BC1* [£]	2.6	8.1
		CPIJ017243	*CYP304B4* [£]	73.4	3.8
		CPIJ017244	*CYP304B5* [£]	2.7	18.9
		CPIJ020229	*CYP4D42v2* [£]	38.1	2.4
	Di-copper centre-containing domain	CPIJ000056	larval serum protein 1 beta chain	5111.7	4.9
		CPIJ001820	larval serum protein 2	9964.0	4.3
		CPIJ005187	phenoloxidase subunit 1	147.6	2.6
		CPIJ006537	larval serum protein 1 beta chain	986.8	9.7
		CPIJ006538	larval serum protein 1 beta chain	1170.3	10.3
		CPIJ007783	arylphorin subunit alpha	3379.4	2.8
		CPIJ009032	larval serum protein 2	574.1	3.3
		CPIJ009033	arylphorin subunit C223	47162.3	2.4
		CPIJ018824	larval serum protein 1 beta chain	1024.6	7.6
Coenzyme m/tr	Dihydropteroate synthetase-like	CPIJ003752	ficolin-2	14.8	4.1
Other enzymes	FAH	CPIJ017110	fumarylacetoacetate hydrolase	46.7	2.6
	Fumarate reductase respiratory complex transmembrane subunits	CPIJ004125	succinate dehydrogenase	10.6	13.5
	Galactose mutarotase-like	CPIJ004867	conserved hypothetical protein	4.7	3.2
Amino acids m/tr	Glutamine synthetase/guanido kinase	CPIJ007538	arginine kinase	4762.7	3.5
Other enzymes	HydA/Nqo6-like	CPIJ018869	NADH dehydrogenase iron-sulfur protein 7, mitochondrial	206.2	2.7
Carbohydrate m/tr	Invertebrate chitin-binding proteins	CPIJ014999	conserved hypothetical protein	36.4	4.9
Lipid m/tr	Lipovitellin-phosvitin complex, superhelical domain	CPIJ001746	conserved hypothetical protein	1918.0	4.6
Other enzymes	Lysozyme-like	CPIJ018802	endochitinase A	177.0	2.6
		CPIJ019598	basic endochitinase CHB4	77.3	4.0
	N-acetylmuramoyl-L-alanine amidase-like	CPIJ006560	peptidoglycan recognition protein-lc	4.6	-
Transferases	Nucleotide-diphospho-sugar transferases	CPIJ001091	lactosylceramide 4-alpha-galactosyltransferase	1.1	-
Other enzymes	Phosphoglycerate mutase-like	CPIJ014577	phosphoglycerate mutase 2	330.2	2.7
Amino acids m/tr	PLP-binding barrel	CPIJ009094	ornithine decarboxylase 1	16.0	3.0
Transferases	PLP-dependent transferases	CPIJ006619	cystathionine gamma-lyase	120.6	3.4
Secondary metabolism	PR-1-like	CPIJ000211	cysteine-rich secretory protein-2	2.5	-
		CPIJ004029	venom allergen 5	72.0	6.1

	Other enzymes	Quinoprotein alcohol dehydrogenase-like	CPIJ002052	WD repeat protein 61	28.2	2.3
	Carbohydrate m/tr	Six-hairpin glycosidases	CPIJ008853	maltose phosphorylase	53.8	2.2
	Coenzyme m/tr	Sterol carrier protein, SCP	CPIJ012490	sterol carrier protein 2	12774.9	3.1
	Other enzymes	Thiolase-like	CPIJ003495	fatty acid synthase S-acetyltransferase	7.3	2.7
	Redox	Thioredoxin-like	CPIJ018667	NADH dehydrogenase flavoprotein 2, mitochondrial	116.0	2.8
	Polysaccharide m/tr	UDP-Glycosyltransferase/glycogen phosphorylase	CPIJ000226	glucosyl/glucuronosyl transferase	45.1	2.3
			CPIJ003692	glucosyl/glucuronosyl transferase	9.3	4.7
			CPIJ006508	UDP-glucuronosyltransferase 2B4	58.0	3.5
			CPIJ015996	ecdysteroid UDP-glucosyltransferase	3.2	7.5
	Energy	Vacuolar ATP synthase subunit C	CPIJ002067	vacuolar ATP synthase subunit C	147.7	2.4
NONA[§]	not annotated	NONA	CPIJ000008	chitotriosidase-1	64.4	3.7
			CPIJ000448	conserved hypothetical protein	5.4	6.9
			CPIJ000494	conserved hypothetical protein	1406.6	3.4
			CPIJ000665	galectin	223.6	3.6
			CPIJ000852	myosin-Id	810.6	3.9
			CPIJ000905	tetraspanin	58.7	2.4
			CPIJ001111	proacrosin	51.7	3.3
			CPIJ002056	adenylate cyclase type 2	3.5	2.9
			CPIJ002117	conserved hypothetical protein	71.4	2.3
			CPIJ002130	kallikrein-7	23.1	2.4
			CPIJ002138	chymotrypsinogen	242.3	824.5
			CPIJ002168	conserved hypothetical protein	43.2	2.7
			CPIJ002247	elongation factor-1 alpha	2.3	-
			CPIJ002359	myomesin	30.9	3.2
			CPIJ002361	sodium/solute symporter	26.5	5.3
			CPIJ002406	conserved hypothetical protein	2.0	-
			CPIJ002882	conserved hypothetical protein	7.8	2.5
			CPIJ003306	conserved hypothetical protein	1.8	12.6
			CPIJ003317	conserved hypothetical protein	15.2	3.7
			CPIJ003338	beta-galactosidase	101.7	3.8
			CPIJ003485	cuticle protein	158.6	2.6
			CPIJ004394	hypothetical protein	677.2	2.5
			CPIJ004558	conserved hypothetical protein	6.2	22.0
			CPIJ004600	oxidoreductase	1.5	-
			CPIJ004927	potassium channel kcnq	2.0	-

CPIJ004976	conserved hypothetical protein	5.4	5.1
CPIJ005090	conserved hypothetical protein	12.7	3.5
CPIJ005451	lysozyme	141.8	2.2
CPIJ005479	hypothetical protein	10.1	13.7
CPIJ005495	hypothetical protein	806.7	2.5
CPIJ005656	oxidoreductase	58.5	2.8
CPIJ005841	angiopoietin-1	118.2	8.2
CPIJ006076	hypodermin-B	11.5	17.0
CPIJ006150	Toll9	30.5	3.0
CPIJ006293	conserved hypothetical protein	16.5	2.3
CPIJ006294	conserved hypothetical protein	7.7	2.8
CPIJ006393	conserved hypothetical protein	6.4	-
CPIJ006515	Toll9	25.6	2.5
CPIJ006516	conserved hypothetical protein	17.3	9.7
CPIJ006542	chymotrypsin-2	64.8	19.7
CPIJ006585	glycoprotein	27.6	3.5
CPIJ006588	NADH dehydrogenase 1 alpha subcomplex subunit 6	6.5	-
CPIJ007033	lipase	112.0	2.7
CPIJ007035	lipase	574.2	3.9
CPIJ007382	hypothetical protein	7.7	-
CPIJ007432	sialin	6.2	2.6
CPIJ007683	adam	2.5	-
CPIJ007721	hypothetical protein	995.1	2.9
CPIJ007785	conserved hypothetical protein	143.6	2.3
CPIJ007966	conserved hypothetical protein	19.0	2.3
CPIJ008031	conserved hypothetical protein	17.7	2.5
CPIJ008110	conserved hypothetical protein	15.8	2.6
CPIJ008379	conserved hypothetical protein	583.6	2.7
CPIJ008651	solute carrier family 41	23.4	2.7
CPIJ008662	conserved hypothetical protein	7.0	8.6
CPIJ008663	conserved hypothetical protein	243.3	2.8
CPIJ008807	ficolin-1	3.7	-
CPIJ008858	conserved hypothetical protein	38.1	3.2
CPIJ008873	prolylcarboxypeptidase	83.0	3.5
CPIJ008904	alpha-glucosidase	24.3	2.1
CPIJ009556	serine threonine-protein kinase	4.7	-
CPIJ009594	nephrosin	26.2	21.7
CPIJ009609	conserved hypothetical protein	20.7	2.6
CPIJ009683	translocator protein	106.8	2.6

CPIJ009726	conserved hypothetical protein	29.9	3.1
CPIJ009744	conserved hypothetical protein	38.3	2.6
CPIJ009902	predicted protein	14.3	4.8
CPIJ009929	conserved hypothetical protein	14.9	2.4
CPIJ010224	metalloproteinase	29.4	2.9
CPIJ010247	raw	14.9	2.7
CPIJ010305	CHKov1	60.2	4.4
CPIJ010426	nucleoporin	2.4	12.7
CPIJ010563	conserved hypothetical protein	2.3	-
CPIJ010641	prostasin	104.0	24.2
CPIJ010699	cecropin A	374.4	2.7
CPIJ010757	conserved hypothetical protein	325.2	3.0
CPIJ010759	conserved hypothetical protein	1.4	-
CPIJ010761	conserved hypothetical protein	738.5	2.8
CPIJ010934	conserved hypothetical protein	252.5	2.4
CPIJ010987	conserved hypothetical protein	2.5	3.5
CPIJ011523	conserved hypothetical protein	16.0	2.8
CPIJ012458	chromatin assembly factor 1, p180-subunit	8.8	2.6
CPIJ012571	actin	1474.9	4.4
CPIJ012573	actin	4309.3	4.8
CPIJ012574	actin	97.7	2.1
CPIJ012700	CHKov1	5.8	5.8
CPIJ012899	secreted protein	91.7	2.3
CPIJ013085	sarcalumenin	167.6	2.5
CPIJ013319	metalloproteinase	132.2	3.5
CPIJ013351	hypothetical protein	2.0	-
CPIJ013355	conserved hypothetical protein	55.0	2.4
CPIJ013736	hypothetical protein	1306.7	3.1
CPIJ014184	conserved hypothetical protein	270.6	-
CPIJ014236	conserved hypothetical protein	1.1	-
CPIJ014523	elastase-3A	18.3	3.0
CPIJ014719	alaserpin	202.3	2.3
CPIJ014892	conserved hypothetical protein	29.5	2.4
CPIJ015171	hypothetical protein	1.5	-
CPIJ015328	nesprin	30.5	3.0
CPIJ015823	conserved hypothetical protein	1.3	-
CPIJ015857	NADH dehydrogenase	489.7	2.4
CPIJ016012	tryptase-2	190.8	2.2
CPIJ016374	conserved hypothetical protein	32.9	3.6
CPIJ016375	conserved hypothetical protein	3.7	-

			CPIJ016440	dihydroceramide delta (4)-desaturase	18.9	4.1
			CPIJ016762	conserved hypothetical protein	1.0	-
			CPIJ016914	hypothetical protein	1.6	-
			CPIJ017076	conserved hypothetical protein	34.4	3.0
			CPIJ017149	l(2) long form	57.7	2.9
			CPIJ017150	l(2) long form	30.0	2.6
			CPIJ017621	conserved hypothetical protein	1.1	-
			CPIJ017717	conserved hypothetical protein	27.3	4.1
			CPIJ017730	hypothetical protein	39.8	9.2
			CPIJ018002	conserved hypothetical protein	33.5	2.1
			CPIJ018092	ryanodine receptor 3, brain	9.5	2.5
			CPIJ018231	carboxylesterase	81.7	3.2
			CPIJ018233	carboxylesterase	5766.3	3.5
			CPIJ018544	conserved hypothetical protein	5.8	20.3
			CPIJ018724	conserved hypothetical protein	70.8	2.5
			CPIJ018791	conserved hypothetical protein	60.3	2.9
			CPIJ018967	conserved hypothetical protein	6.6	3.5
			CPIJ018988	phosphatidylinositol glycan, class c	1.5	-
			CPIJ019007	polyserase-2	10.4	2.9
			CPIJ019029	metalloproteinase	181.5	2.6
			CPIJ019577	alpha-actinin	361.7	2.9
Other	Unknown function	Bactericidal permeability-increasing protein, BPI	CPIJ020308	conserved hypothetical protein	1742.1	3.1
		E set domains	CPIJ002744	conserved hypothetical protein	944.9	51.0
			CPIJ018825	larval serum protein 1 beta chain	1061.3	9.4
		Ligand-binding domain in the NO signalling and Golgi transport	CPIJ004088	guanylyl cyclase receptor	7.5	3.2
	Viral proteins	Retrovirus zinc finger-like domains	CPIJ006202	conserved hypothetical protein	10.0	3.4
Regulation	DNA-binding	C2H2 and C2HC zinc fingers	CPIJ004716	zinc finger protein 266	3.8	5.5
			CPIJ009633	conserved hypothetical protein	7.6	3.2
			CPIJ011598	zinc finger protein	2.1	3.5
			CPIJ015936	hypothetical protein	2.4	-
	Receptor activity	Chemosensory protein Csp2	CPIJ002617	chemosensory protein 1	865.9	2.3
	Signal transduction	Growth factor receptor domain	CPIJ005087	cell wall cysteine-rich protein	16.2	2.8
	DNA-binding	HLH, helix-loop-helix DNA-binding domain	CPIJ018167	sterol regulatory element-binding protein 1	39.1	2.1
		Homeodomain-like	CPIJ002050	homeobox protein	21.7	2.9

Signal transduction	Insect pheromone/odorant-binding proteins	CPIJ001872	Odorant-binding protein 56a	24.7	5.0
		CPIJ002108	odorant-binding protein	17.2	2.7
		CPIJ002111	Odorant-binding protein 50d	28.8	4.0
		CPIJ004145	predicted protein	1.9	-
		CPIJ009038	odorant binding protein 1	1.8	-
	Nicotinic receptor ligand binding domain-like	CPIJ002436	neuronal acetylcholine receptor subunit alpha-2	30.1	2.5
	Nuclear receptor ligand-binding domain	CPIJ010249	retinoid X receptor alpha	24.6	2.5
	PDZ domain-like	CPIJ015336	Dlg5 protein	4.0	2.6
Kinases/phos-phatases	Protein kinase-like (PK-like)	CPIJ010307	conserved hypothetical protein	31.9	20.3
		CPIJ010319	Juvenile hormone-inducible protein	12.6	2.7
		CPIJ010324	conserved hypothetical protein	19.5	4.2
		CPIJ012702	conserved hypothetical protein	32.8	2.3
		CPIJ012763	3-phosphoinositide-dependent protein kinase 1	6.3	2.7
RNA binding, m/tr	RNA-binding domain, RBD	CPIJ001827	conserved hypothetical protein	1.5	7.8
Signal transduction	TRAF domain-like	CPIJ001427	conserved hypothetical protein	28.3	2.3
		CPIJ006152	conserved hypothetical protein	25.9	2.7

[†]Differentially expressed genes represent those genes that differed in their expression level (FPKM) in HAmCq^{G8} by more than two fold when compared to the parental strain HAmCq^{G0}.

[*]SCOP general and detailed functions using the predicted *Cx. quinquefasciatus* annotation information available at the Superfamily website (version 1.75) supfam.cs.bris.ac.uk/SUPERFAMILY/index.html

[**]*Culex quinquefasciatus* genome, Johannesburg strain CpipJ1.2, June 2008; http://cquinquefasciatus.vectorbase.org/

[£] Annotations for cytochrome P450 genes were taken from the most current annotation based on: Nelson, DR (2009) The Cytochrome P450 Homepage. Human Genomics 4, 59-65: http://drnelson.uthsc.edu/CytochromeP450.html

[§]NONA: Not annotated

[¤]Fold FPKM relative to HAmCq^{G0} indicates the ratio of the FPKM value in HAmCq^{G8} divided by the FPKM value of HAmCq^{G0}.

Appendix 3.4. List of genes downregulated by at least two-fold in HAmCq^{G8} when compared to HAmCq^{G0}.

Level of SCOP* classification			Downregulated genes	
General function	**Detailed function**	**Superfamily**	**Gene Accession****	**Vectorbase annotation‡**
Extra-cellular processes	Blood clotting	Fibrinogen C-terminal domain-like	CPIJ000868	conserved hypothetical protein
			CPIJ001260	fibrinogen and fibronectin
			CPIJ001908	fibrinogen and fibronectin
			CPIJ006392	fibrinogen and fibronectin
			CPIJ012891	scabrous protein
			CPIJ013288	fibrinogen and fibronectin
			CPIJ014543	conserved hypothetical protein
			CPIJ014544	conserved hypothetical protein
			CPIJ017657	salivary secreted angiopoietin
			CPIJ017837	angiopoietin 2
			CPIJ017877	fibrinogen and fibronectin
			CPIJ018745	zinc finger protein
			CPIJ018858	fibrinogen and fibronectin
	Cell adhesion	alpha-catenin/vinculin-like	CPIJ005773	actin binding protein
		C-type lectin-like	CPIJ000443	galactose-specific C-type lectin
			CPIJ001323	galactose-specific C-type lectin
			CPIJ004607	conserved hypothetical protein
			CPIJ005619	collagen alpha 1
			CPIJ005984	conserved hypothetical protein
			CPIJ005987	conserved hypothetical protein
			CPIJ006092	conserved hypothetical protein
			CPIJ012139	conserved hypothetical protein
			CPIJ015742	conserved hypothetical protein
			CPIJ018154	conserved hypothetical protein
		Cadherin-like	CPIJ017350	conserved hypothetical protein
			CPIJ018739	conserved hypothetical protein
			CPIJ018999	conserved hypothetical protein
		EGF/Laminin	CPIJ017322	conserved hypothetical protein
			CPIJ004682	laminin subunit beta-1
			CPIJ005569	neurogenic locus notch
			CPIJ005673	conserved hypothetical protein
			CPIJ009613	serrate protein
			CPIJ009614	serrate protein

	CPIJ009802	conserved hypothetical protein
	CPIJ011761	conserved hypothetical protein
	CPIJ015361	conserved hypothetical protein
	CPIJ019124	conserved hypothetical protein
FAS1 domain	CPIJ003831	conserved hypothetical protein
	CPIJ004689	conserved hypothetical protein
Fibronectin type III	CPIJ002017	cell adhesion molecule
	CPIJ003912	conserved hypothetical protein
	CPIJ004128	conserved hypothetical protein
	CPIJ005092	conserved hypothetical protein
	CPIJ006893	myosin light chain kinase
	CPIJ008112	cell adhesion molecule
	CPIJ009217	roundabout 1
	CPIJ014383	factor for adipocyte differentiation
	CPIJ014908	host cell factor C1
	CPIJ018836	conserved hypothetical protein
	CPIJ020251	conserved hypothetical protein
FnI-like domain	CPIJ005093	conserved hypothetical protein
	CPIJ013285	conserved hypothetical protein
	CPIJ015976	conserved hypothetical protein
	CPIJ020078	conserved hypothetical protein
Immunoglobulin	CPIJ003910	conserved hypothetical protein
	CPIJ004966	conserved hypothetical protein
	CPIJ007299	beat protein
	CPIJ009084	conserved hypothetical protein
	CPIJ009950	conserved hypothetical protein
	CPIJ012166	conserved hypothetical protein
	CPIJ012499	conserved hypothetical protein
	CPIJ014508	conserved hypothetical protein
	CPIJ015510	conserved hypothetical protein
	CPIJ017558	defective proboscis extension response
	CPIJ018083	conserved hypothetical protein
Integrin alpha N-terminal domain	CPIJ005252	T-cell immunomodulatory protein
	CPIJ017320	integrin alpha-PS2
RNI-like	CPIJ002568	f-box/leucine rich repeat protein
	CPIJ003404	conserved hypothetical protein
	CPIJ004460	tubulin-specific chaperone
	CPIJ014282	conserved hypothetical protein
	CPIJ015037	conserved hypothetical protein
	CPIJ015883	f-box/lrr protein, drome

			CPIJ016145	predicted protein
			CPIJ016147	predicted protein
			CPIJ016518	conserved hypothetical protein
			CPIJ017031	conserved hypothetical protein
			CPIJ017284	conserved hypothetical protein
			CPIJ019360	predicted protein
		SEA domain	CPIJ011474	conserved hypothetical protein
		Somatomedin B domain	CPIJ000705	conserved hypothetical protein
		Spectrin repeat	CPIJ003238	conserved hypothetical protein
			CPIJ006907	conserved hypothetical protein
			CPIJ011432	conserved hypothetical protein
			CPIJ013591	conserved hypothetical protein
		TSP-1 type 1 repeat	CPIJ000706	conserved hypothetical protein
		vWA-like	CPIJ004522	26S proteasome non-ATPase regulatory subunit 4
			CPIJ005333	transport protein sec23
			CPIJ006690	integrin beta-PS
	Immune response	Complement control module/SCR domain	CPIJ005492	conserved hypothetical protein
			CPIJ007796	conserved hypothetical protein
			CPIJ008865	conserved hypothetical protein
		Tetraspanin	CPIJ013906	platelet endothelial tetraspan antigen 3
			CPIJ017253	conserved hypothetical protein
		TNF-like	CPIJ011491	conserved hypothetical protein
	Toxins/defense	AhpD-like	CPIJ010665	P53 regulated pa26 nuclear protein sestrin
		omega toxin-like	CPIJ000956	conserved hypothetical protein
		Scorpion toxin-like	CPIJ011918	conserved hypothetical protein
		Snake toxin-like	CPIJ011561	14.5 kDa salivary peptide
			CPIJ011853	conserved hypothetical protein
			CPIJ017088	activin receptor type I
General	General	ARM repeat	CPIJ006701	26S proteasome non-ATPase regulatory subunit 1
			CPIJ000004	conserved hypothetical protein
			CPIJ000739	conserved hypothetical protein
			CPIJ001288	conserved hypothetical protein
			CPIJ001478	pre-mRNA-splicing factor cwc22
			CPIJ004189	conserved hypothetical protein
			CPIJ004193	conserved hypothetical protein
			CPIJ004613	importin subunit beta
			CPIJ004649	cell differentiation protein rcd1
			CPIJ004747	smaug protein

	CPIJ004787	FKBP12-rapamycin complex-associated protein
	CPIJ004900	armadillo repeat-containing protein 6
	CPIJ006132	importin alpha
	CPIJ006316	conserved hypothetical protein
	CPIJ006702	26S proteasome non-ATPase regulatory subunit 1
	CPIJ007322	conserved hypothetical protein
	CPIJ009961	stromal antigen
	CPIJ010395	conserved hypothetical protein
	CPIJ012523	coatomer subunit gamma
	CPIJ012812	thyroid hormone receptor interactor 12
	CPIJ013105	sorting nexin
	CPIJ013982	conserved hypothetical protein
	CPIJ014675	conserved hypothetical protein
	CPIJ018110	conserved hypothetical protein
	CPIJ018379	adaptin, alpha/gamma/epsilon
	CPIJ018684	conserved hypothetical protein
	CPIJ019706	26S proteasome non-ATPase regulatory subunit 2
BRCT domain	CPIJ011710	conserved hypothetical protein
Calponin-homology domain, CH-domain	CPIJ003234	conserved hypothetical protein
	CPIJ004533	microtubule binding protein
	CPIJ008540	muscle-specific protein 20
Cryptochrome/photolyase FAD-binding domain	CPIJ003975	deoxyribodipyrimidine photo-lyase
	CPIJ009455	DNA photolyase
	CPIJ018859	cryptochrome 2
EF-hand	CPIJ001307	predicted protein
	CPIJ001896	conserved hypothetical protein
	CPIJ002594	nadph oxidase
	CPIJ004307	supercoiling factor
	CPIJ005099	voltage-dependent p/q type calcium channel
	CPIJ008225	conserved hypothetical protein
	CPIJ009356	dynamin-associated protein
	CPIJ010178	conserved hypothetical protein
	CPIJ012251	troponin C
	CPIJ015810	calcium-binding protein E63-1
	CPIJ015812	calcium-binding protein E63-1
Kelch motif	CPIJ000983	actin binding protein
	CPIJ011586	actin binding protein
	CPIJ011613	conserved hypothetical protein
	CPIJ014909	host cell factor
L domain-like	CPIJ001272	leucine-rich transmembrane protein

	CPIJ001895	conserved hypothetical protein
	CPIJ003386	predicted protein
	CPIJ003844	leucine-rich transmembrane protein
	CPIJ004868	leucine-rich transmembrane protein
	CPIJ005354	conserved hypothetical protein
	CPIJ006822	leucine-rich transmembrane protein
	CPIJ011783	conserved hypothetical protein
	CPIJ012832	conserved hypothetical protein
	CPIJ013310	adenylate cyclase
	CPIJ013854	conserved hypothetical protein
	CPIJ015804	reticulon/nogo receptor
	CPIJ016693	ras suppressor protein 1
	CPIJ016806	leucine rich repeat protein
	CPIJ017602	conserved hypothetical protein
	CPIJ018453	conserved hypothetical protein
	CPIJ018876	leucine-rich repeat-containing protein 24
	CPIJ019625	conserved hypothetical protein
	CPIJ019816	conserved hypothetical protein
Spermadhesin, CUB domain	CPIJ003741	conserved hypothetical protein
	CPIJ015617	conserved hypothetical protein
	CPIJ018283	conserved hypothetical protein
Transthyretin (synonym: prealbumin)	CPIJ014057	conserved hypothetical protein
Ubiquitin-like	CPIJ002389	transcription elongation factor B polypeptide 2
	CPIJ003604	conserved hypothetical protein
	CPIJ007098	ubiquitin-fold modifier 1
	CPIJ011014	peptidylglycine alpha-amidating monooxygenase COOH-terminal interactor protein-1
	CPIJ011765	conserved hypothetical protein
	CPIJ014686	conserved hypothetical protein
	CPIJ019167	peptidylglycine alpha-amidating monooxygenase COOH-terminal interactor protein-1
WD40 repeat-like	CPIJ000633	conserved hypothetical protein
	CPIJ001314	WD repeat protein 46
	CPIJ001915	WD repeat domain 50
	CPIJ002199	vesicle associated protein
	CPIJ002451	WD repeat protein 57
	CPIJ003096	WD repeat protein 7
	CPIJ003211	WD repeat protein 51B
	CPIJ003605	vacuolar membrane protein pep11
	CPIJ004561	pleiotropic regulator 1

		CPIJ005067 G protein beta subunit
		CPIJ005486 autophagy-specific gene 18
		CPIJ006095 conserved hypothetical protein
		CPIJ006523 conserved hypothetical protein
		CPIJ007014 splicing factor 3B subunit 3
		CPIJ007402 guanine nucleotide-binding protein subunit beta 1
		CPIJ009226 groucho protein
		CPIJ009392 nucleoporin Nup43
		CPIJ009985 conserved hypothetical protein
		CPIJ010477 conserved hypothetical protein
		CPIJ010919 will die slowly
		CPIJ011032 conserved hypothetical protein
		CPIJ011061 WD repeat protein 51A
		CPIJ011261 cell cycle control protein cwf8
		CPIJ011322 mediator complex, 95kD-subunit
		CPIJ011395 conserved hypothetical protein
		CPIJ013687 conserved hypothetical protein
		CPIJ013831 WD repeat-containing protein srw1
		CPIJ014207 elongator complex protein 2
		CPIJ014657 vesicle associated protein
		CPIJ015003 predicted protein
		CPIJ015347 receptor for activated protein kinase C
		CPIJ015352 conserved hypothetical protein
		CPIJ015911 conserved hypothetical protein
		CPIJ017007 vesicle associated protein
		CPIJ017534 serine-threonine kinase receptor-associated protein
		CPIJ017825 wd-repeat protein
		CPIJ018264 will die slowly
		CPIJ018766 WD repeat domain phosphoinositide-interacting protein 2
		CPIJ019808 receptor for activated protein kinase C
		CPIJ020009 wd-repeat protein
Ion binding	Amyloid beta a4 protein copper binding domain (domain 2)	CPIJ008559 conserved hypothetical protein
	ArfGap/RecO-like zinc finger	CPIJ002305 arf GTPase-activating protein
		CPIJ008955 arf GTPase-activating protein
		CPIJ010923 conserved hypothetical protein
	B-box zinc-binding domain	CPIJ015175 predicted protein
Ligand binding	GYF domain	CPIJ001658 conserved hypothetical protein
		CPIJ010290 CD2 antigen cytoplasmic tail-binding protein 2

Protein interaction	Ankyrin repeat	CPIJ000121	conserved hypothetical protein
		CPIJ000884	DNA-binding protein rfxank
		CPIJ001854	conserved hypothetical protein
		CPIJ002709	conserved hypothetical protein
		CPIJ004774	developmental protein cactus
		CPIJ004983	ga binding protein beta chain
		CPIJ006925	conserved hypothetical protein
		CPIJ006926	phosphatase 1 regulatory subunit 12b
		CPIJ009794	conserved hypothetical protein
		CPIJ010734	sex-determining protein fem-1
		CPIJ013520	conserved hypothetical protein
		CPIJ014438	sex-determining protein fem-1
		CPIJ015938	conserved hypothetical protein
		CPIJ017182	forked protein
		CPIJ018599	transient receptor potential channel
		CPIJ018744	ankyrin 2,3/unc44
	BAG domain	CPIJ002809	conserved hypothetical protein
	BAR/IMD domain-like	CPIJ002500	conserved hypothetical protein
		CPIJ007107	insulin receptor tyrosine kinase substrate
		CPIJ011282	islet cell autoantigen 1
		CPIJ017697	endophilin b
	Dimerization-anchoring domain of cAMP-dependent PK regulatory subunit	CPIJ004553	predicted protein
	F-box domain	CPIJ015945	conserved hypothetical protein
		CPIJ000909	conserved hypothetical protein
		CPIJ006380	conserved hypothetical protein
		CPIJ017453	transmembrane protein 183
	Hemopexin-like domain	CPIJ001428	matrix metalloproteinase
		CPIJ010856	matrix metalloproteinase
	HIV integrase-binding domain	CPIJ003093	hepatoma-derived GF
	IP3 receptor type 1 binding core, domain 2	CPIJ012217	inositol 1,4,5-trisphosphate receptor
	POZ domain	CPIJ001236	conserved hypothetical protein
		CPIJ001455	ankyrin repeat and BTB/POZ domain-containing protein 2
		CPIJ001669	BTB/POZ domain-containing protein 7
		CPIJ003990	conserved hypothetical protein
		CPIJ005014	BTB/POZ and Kelch domain-containing protein
		CPIJ005696	serine-enriched protein
		CPIJ007217	speckle-type poz protein
		CPIJ007547	conserved hypothetical protein

	CPIJ008271	conserved hypothetical protein
	CPIJ009395	speckle-type poz protein
	CPIJ009648	conserved hypothetical protein
	CPIJ012486	conserved hypothetical protein
	CPIJ012629	conserved hypothetical protein
	CPIJ013200	conserved hypothetical protein
	CPIJ013368	microtubule binding protein
	CPIJ013627	conserved hypothetical protein
	CPIJ016082	conserved hypothetical protein
	CPIJ017663	leucine-zipper-like transcriptional regulator 1
	CPIJ018109	conserved hypothetical protein
	CPIJ018129	conserved hypothetical protein
SNARE-like	CPIJ009504	clathrin coat assembly protein AP17
	CPIJ013281	conserved hypothetical protein
	CPIJ017540	coatomer subunit delta
SWIB/MDM2 domain	CPIJ019141	brg-1 associated factor
	CPIJ019147	brg-1 associated factor
TPR-like	CPIJ001441	eukaryotic translation initiation factor 3 subunit
	CPIJ002036	transmembrane and TPR repeat-containing protein
	CPIJ003799	suppressor of forked
	CPIJ004405	conserved hypothetical protein
	CPIJ005156	conserved hypothetical protein
	CPIJ008245	conserved hypothetical protein
	CPIJ008355	transmembrane protein 1/tmem1b
	CPIJ010925	heat shock protein 70
	CPIJ011544	tetratricopeptide repeat domain 21B
	CPIJ017131	prolyl 4-hydroxylase subunit alpha-1
	CPIJ019076	tetratricopeptide repeat protein 15
UBA-like	CPIJ006055	conserved hypothetical protein
	CPIJ011493	conserved hypothetical protein
	CPIJ012192	conserved hypothetical protein
Vasodilator-stimulated phosphoprotein, VASP, tetramerisation domain	CPIJ004707	vasodilator-stimulated phosphoprotein
WW domain	CPIJ000289	conserved hypothetical protein
	CPIJ000291	conserved hypothetical protein
	CPIJ004712	conserved hypothetical protein
	CPIJ013704	conserved hypothetical protein
	CPIJ014839	conserved hypothetical protein
	CPIJ019077	conserved hypothetical protein
FAD/NAD(P)-binding domain	CPIJ001215	alcohol dehydrogenase

	CPIJ001367	glucose dehydrogenase
	CPIJ002196	lysine-specific histone demethylase
	CPIJ002643	CDNA sequence
	CPIJ005552	thioredoxin reductase 1, mitochondrial
	CPIJ007619	glucose dehydrogenase
	CPIJ007625	alcohol dehydrogenase
	CPIJ009583	glucose dehydrogenase
	CPIJ010620	conserved hypothetical protein
	CPIJ010669	rab protein geranylgeranyltransferase component A 1
	CPIJ013724	dimethylaniline monooxygenase
	CPIJ013725	dimethylaniline monooxygenase
	CPIJ017482	choline dehydrogenase
	CPIJ017488	glucose dehydrogenase
	CPIJ017490	glucose dehydrogenase
	CPIJ017491	glucose dehydrogenase
Glutathione S-transferase (GST), C-terminal domain	CPIJ002660	glutathione-s-transferase theta, gst
	CPIJ002680	glutathione S-transferase
	CPIJ003988	prostaglandin E synthase 2
	CPIJ014051	glutathione-s-transferase theta, gst
	CPIJ014053	glutathione-s-transferase theta, gst
	CPIJ018524	prostaglandin E synthase 2
	CPIJ018633	glutathione-s-transferase theta
NAD(P)-binding Rossmann-fold domains	CPIJ016763	short-chain dehydrogenase
	CPIJ000400	3-hydroxyisobutyrate dehydrogenase
	CPIJ000841	dimeric dihydrodiol dehydrogenase
	CPIJ003056	hydroxysteroid dehydrogenase
	CPIJ003801	NADP-dependent leukotriene B4 12-hydroxydehydrogenase
	CPIJ003837	short-chain dehydrogenase
	CPIJ004391	fatty acyl-CoA reductase 1
	CPIJ004392	fatty acyl-CoA reductase 2
	CPIJ005892	conserved hypothetical protein
	CPIJ006479	3-hydroxyacyl-coa dehyrogenase
	CPIJ007225	3-ketodihydrosphingosine reductase
	CPIJ007244	fatty acyl-CoA reductase 1
	CPIJ007245	fatty acyl-CoA reductase 1
	CPIJ011767	short-chain dehydrogenase
	CPIJ013219	3-hydroxybutyrate dehydrogenase type 2
	CPIJ014059	NADP-dependent leukotriene B4 12-hydroxydehydrogenase
	CPIJ014121	short-chain dehydrogenase
	CPIJ014122	dehydrogenase/reductase SDR family member 8

	CPIJ014580	dimeric dihydrodiol dehydrogenase
	CPIJ015671	glyoxylate reductase/hydroxypyruvate reductase
	CPIJ015685	3-oxoacyl-[acyl-carrier-protein] reductase
	CPIJ016656	short-chain dehydrogenase
	CPIJ016657	short-chain dehydrogenase
	CPIJ016719	alcohol dehydrogenase 1
	CPIJ016777	hydroxyacyl-coenzyme A dehydrogenase, mitochondrial
	CPIJ017297	quinone oxidoreductase
	CPIJ017713	short-chain dehydrogenase
	CPIJ018318	short-chain dehydrogenase
	CPIJ019137	dihydropteridine reductase
	CPIJ019281	conserved hypothetical protein
	CPIJ019362	dehydrogenase/reductase SDR family member 8
	CPIJ019941	conserved hypothetical protein
	CPIJ019942	conserved hypothetical protein
	CPIJ020005	UDP-glucuronic acid decarboxylase 1
Nucleotide-binding domain	CPIJ002817	d-amino acid oxidase
	CPIJ007272	d-amino acid oxidase
	CPIJ007273	d-amino acid oxidase
Obg GTP-binding protein N-terminal domain	CPIJ005917	Spo0B-associated GTP-binding protein
P-loop containing nucleoside triphosphate hydrolases	CPIJ014210	conserved hypothetical protein
	CPIJ000310	heparan sulfate 2-o-sulfotransferase
	CPIJ000320	conserved hypothetical protein
	CPIJ000874	carbohydrate sulfotransferase
	CPIJ000964	chromosome-associated kinesin KIF4A
	CPIJ001058	ADP-ribosylation factor
	CPIJ001311	multidrug resistance-associated protein 2
	CPIJ001383	kinesin-like protein KIF3A
	CPIJ001540	abc transporter
	CPIJ001695	conserved hypothetical protein
	CPIJ001702	conserved hypothetical protein
	CPIJ001756	conserved hypothetical protein
	CPIJ001842	translation initiation factor IF-2, mitochondrial
	CPIJ001988	ATP-dependent RNA helicase A
	CPIJ002335	ATP-dependent RNA helicase DDX51
	CPIJ003150	vesicular-fusion protein Nsf1
	CPIJ003814	cell cycle checkpoint protein rad17
	CPIJ003934	RNA helicase
	CPIJ003935	ATP-dependent RNA helicase p62
	CPIJ004665	chromodomain helicase-DNA-binding protein 3

CPIJ004980 ATP-binding cassette sub-family A member 3
CPIJ005169 ras-related protein Rab-10
CPIJ005172 GTP-binding protein
CPIJ005340 ATP-binding cassette transporter
CPIJ005341 abc transporter
CPIJ005366 GTP-binding protein yptV1
CPIJ005545 conserved hypothetical protein
CPIJ007064 conserved hypothetical protein
CPIJ007231 translation elongation factor
CPIJ007588 conserved hypothetical protein
CPIJ007795 guanylate kinase
CPIJ007814 myosin iii
CPIJ007889 abc transporter
CPIJ008104 ras-related protein Rab-39B
CPIJ008284 canalicular multispecific organic anion transporter 1
CPIJ008677 conserved hypothetical protein
CPIJ008800 ATP-dependent protease La
CPIJ008893 conserved hypothetical protein
CPIJ008983 ATP-dependent RNA helicase DBP8
CPIJ009005 ATP-dependent DNA helicase MER3
CPIJ009065 ras-related protein Rab-9
CPIJ009089 ras-related protein Rab-7
CPIJ009531 conserved hypothetical protein
CPIJ009998 transcriptional regulator ATRX
CPIJ010194 ras-related protein
CPIJ010818 GTP-binding protein alpha subunit, gna
CPIJ010888 origin recognition complex subunit 1
CPIJ010998 werner helicase interacting protein
CPIJ011002 bile salt sulfotransferase 1
CPIJ011328 GTP:AMP phosphotransferase mitochondrial
CPIJ011521 mitochondrial 28S ribosomal protein S29
CPIJ011567 conserved hypothetical protein
CPIJ011830 serine protease
CPIJ012284 abc transporter
CPIJ012364 abc transporter
CPIJ012365 ATP-binding cassette sub-family G member 4
CPIJ012510 ATP-dependent RNA helicase DDX24
CPIJ012512 ATP-dependent RNA helicase p62
CPIJ012614 sulfotransferase 1A1
CPIJ012621 ATP-dependent RNA helicase Ddx1

			CPIJ013250	conserved hypothetical protein
			CPIJ013393	mitochondrial chaperone BCS1
			CPIJ013525	conserved hypothetical protein
			CPIJ013876	DNA polymerase theta
			CPIJ014038	DEAD-box ATP-dependent RNA helicase 57
			CPIJ014142	nucleotide-binding protein 1
			CPIJ014150	ATP-binding cassette sub-family F member 3
			CPIJ014305	conserved hypothetical protein
			CPIJ014361	conserved hypothetical protein
			CPIJ014443	ATP-binding cassette sub-family G member 4
			CPIJ014693	transcriptional regulator ATRX
			CPIJ014902	CDC42
			CPIJ015682	translation initiation factor if-2
			CPIJ015769	myosin vi
			CPIJ015845	elongation factor tu
			CPIJ015898	conserved hypothetical protein
			CPIJ016097	peroxisomal membrane protein 70 abcd3
			CPIJ016664	CTP synthase
			CPIJ016808	ribosome biogenesis protein
			CPIJ017203	conserved hypothetical protein
			CPIJ017338	DEAD box ATP-dependent RNA helicase
			CPIJ017393	conserved hypothetical protein
			CPIJ017570	myosin IB heavy chain
			CPIJ017886	ATP-dependent RNA helicase
			CPIJ018454	chromosome-associated kinesin KIF4A
			CPIJ018540	hypothetical protein
			CPIJ019196	DEAD box ATP-dependent RNA helicase
			CPIJ019594	chromosome transmission fidelity protein 18
			CPIJ019631	kinesin heavy chain
			CPIJ019640	conserved hypothetical protein
		PEBP-like	CPIJ003429	brother of ft and tfl1
			CPIJ008654	phosphatidylethanolamine-binding protein
Information	Chromatin structure	NAP-like	CPIJ015455	nucleosome assembly protein
			CPIJ015773	nucleosome assembly protein
	DNA replication/repair	Chromo domain-like	CPIJ007340	conserved hypothetical protein
			CPIJ014352	conserved hypothetical protein
			CPIJ019929	conserved hypothetical protein

DNA polymerase III clamp loader subunits, C-terminal domain	CPIJ017857	ATPase WRNIP1
DNA/RNA polymerases	CPIJ007351	terminal deoxycytidyl transferase rev1
	CPIJ012266	DNA polymerase subunit gamma 1, mitochondrial
	CPIJ015260	DNA polymerase alpha catalytic subunit
DNase I-like	CPIJ006698	type I inositol-1,4,5-trisphosphate 5-phosphatase
	CPIJ008163	skeletal muscle/kidney enriched inositol 5-phosphatase
	CPIJ012006	conserved hypothetical protein
	CPIJ019984	sphingomyelin phosphodiesterase 2
FYVE/PHD zinc finger	CPIJ001881	conserved hypothetical protein
	CPIJ002025	conserved hypothetical protein
	CPIJ004116	zinc finger FYVE domain-containing protein 28
	CPIJ006170	CpG-binding protein
	CPIJ009232	conserved hypothetical protein
	CPIJ009396	inhibitor of growth protein 3
	CPIJ011285	fetal alzheimer antigen, falz
	CPIJ013376	phd finger protein
	CPIJ013847	conserved hypothetical protein
	CPIJ014289	conserved hypothetical protein
	CPIJ015635	conserved hypothetical protein
	CPIJ016701	inhibitor of growth protein 1
His-Me finger endonucleases	CPIJ002289	deoxyribonuclease I
	CPIJ006433	caspase-activated nuclease
HRDC-like	CPIJ009943	conserved hypothetical protein
Nucleic acid-binding proteins	CPIJ003130	replication factor A, 14kD-subunit
	CPIJ004582	mitochondrial ribosomal protein S17
	CPIJ005206	DNA-directed RNA polymeraseI
	CPIJ005868	multisynthetase complex auxiliary component p43
	CPIJ006691	insect replication protein a
	CPIJ008535	conserved hypothetical protein
	CPIJ019290	DNA ligase 4
Nudix	CPIJ014785	mitochondrial ribosomal protein, L46
Restriction endonuclease-like	CPIJ019123	conserved hypothetical protein
RING/U-box	CPIJ015192	conserved hypothetical protein
	CPIJ001165	ubiquitin conjugation factor E4 A
	CPIJ001468	RING-box protein 1a
	CPIJ003711	E3 ubiquitin-protein ligase MARCH6
	CPIJ004515	conserved hypothetical protein
	CPIJ004519	conserved hypothetical protein
	CPIJ005021	vacuolar protein sorting-associated protein 18

		CPIJ005056	conserved hypothetical protein
		CPIJ005135	peroxisome assembly factor 1
		CPIJ006036	ring finger protein
		CPIJ006232	rolling pebbles
		CPIJ007831	conserved hypothetical protein
		CPIJ010005	RING finger protein 126-B
		CPIJ010511	hypothetical protein
		CPIJ010577	conserved hypothetical protein
		CPIJ010613	zinc finger protein
		CPIJ011856	E3 ubiquitin-protein ligase MARCH5
		CPIJ012808	conserved hypothetical protein
		CPIJ014265	zinc and ring finger 2
		CPIJ015415	conserved hypothetical protein
		CPIJ016043	autocrine motility factor receptor
		CPIJ016186	conserved hypothetical protein
		CPIJ016790	zinc finger protein
		CPIJ017375	conserved hypothetical protein
		CPIJ017992	conserved hypothetical protein
	Tudor/PWWP/MBT	CPIJ003208	predicted protein
		CPIJ005604	conserved hypothetical protein
		CPIJ012664	conserved hypothetical protein
		CPIJ014035	conserved hypothetical protein
RNA processing	EPT/RTPC-like	CPIJ009234	RNA 3'-terminal phosphate cyclase
	Eukaryotic type KH-domain (KH-domain type I)	CPIJ002909	far upstream binding protein
		CPIJ010419	conserved hypothetical protein
		CPIJ010634	conserved hypothetical protein
		CPIJ011349	igf2 mRNA binding protein
		CPIJ014324	conserved hypothetical protein
		CPIJ015571	heterogeneous nuclear ribonucleoprotein
		CPIJ018107	zinc finger protein
	PAP/OAS1 substrate-binding domain	CPIJ011744	poly a polymerase
		CPIJ015488	sigma DNA polymerase
	RNase III domain-like	CPIJ007416	ribonuclease iii
		CPIJ008368	39S ribosomal protein L44
		CPIJ013579	ribonuclease iii
	Translin	CPIJ011089	translin associated factor x
		CPIJ011091	translin associated factor x
Transcription	beta and beta-prime subunits of DNA dependent RNA-polymerase	CPIJ002457	DNA-directed RNA polymerase I 135 kDa polypeptide

		CPIJ018338	DNA-directed RNA polymerase I largest subunit
	CYTH-like phosphatases	CPIJ009805	conserved hypothetical protein
	occludin/ELL-like	CPIJ004404	conserved hypothetical protein
	RBP11-like subunits of RNA polymerase	CPIJ005304	DNA-directed RNA polymerase II subunit J
	TATA-box binding protein-like	CPIJ007600	TATA-box-binding protein
Translation	Anticodon-binding domain of a subclass of class I aminoacyl-tRNA synthetases	CPIJ019163	conserved hypothetical protein
	Class II aaRS ABD-related	CPIJ007439	conserved hypothetical protein
		CPIJ011030	conserved hypothetical protein
		CPIJ017958	conserved hypothetical protein
	EF-Tu/eEF-1alpha/eIF2-gamma C-terminal domain	CPIJ000412	elongation factor-1 alpha
		CPIJ005761	elongation factor 1-alpha
		CPIJ006444	elongation factor 1-alpha
		CPIJ009508	elongation factor 1 alpha
	eIF1-like	CPIJ009942	density-regulated protein
		CPIJ013497	eukaryotic translation initiation factor 1b
	eIF4e-like	CPIJ012031	eukaryotic translation initiation factor 4e type
	Elongation factor TFIIS domain 2	CPIJ013846	conserved hypothetical protein
	Elongation factor Ts (EF-Ts), dimerisation domain	CPIJ004698	elongation factor ts
	L21p-like	CPIJ015639	39S ribosomal protein L21, mitochondrial
	L27 domain	CPIJ009529	membrane-associated guanylate kinase
	L30e-like	CPIJ005818	13 kDa ribonucleoprotein-associated protein
	L35p-like	CPIJ019136	39S ribosomal protein L35, mitochondrial
	L9 N-domain-like	CPIJ013282	39S ribosomal protein L9, mitochondrial
	Prokaryotic ribosomal protein L17	CPIJ008935	39S ribosomal protein L17, mitochondrial
	Prokaryotic type KH domain (KH-domain type II)	CPIJ013400	ribosomal protein S3
		CPIJ016018	ribosomal protein S3
	Release factor	CPIJ019188	peptide chain release factor 1
	Ribosomal L11/L12e N-terminal domain	CPIJ013673	39S ribosomal protein L11, mitochondrial
	Ribosomal protein L16p/L10e	CPIJ012999	serrate protein
		CPIJ009291	60S ribosomal protein L10
		CPIJ017073	conserved hypothetical protein
		CPIJ018417	conserved hypothetical protein
		CPIJ018847	60S ribosomal protein L10
	Ribosomal protein L20	CPIJ010000	39S ribosomal protein L20, mitochondrial
	Ribosomal protein L29 (L29p)	CPIJ010482	hypothetical protein
	Ribosomal protein L30p/L7e	CPIJ011578	mitochondrial ribosomal protein L30
		CPIJ012743	60S ribosomal protein L7
		CPIJ017899	tetratricopeptide repeat protein, tpr
	Ribosomal protein L36	CPIJ009257	mitochondrial ribosomal protein L36

Ribosomal protein S10	CPIJ011818	Ded1-like DEAD-box RNA helicase
	CPIJ017617	40S ribosomal protein S20
	CPIJ018278	40S ribosomal protein S20
	CPIJ018511	40S ribosomal protein S20
Ribosomal protein S16	CPIJ009237	28S ribosomal protein S16
Ribosomal protein S18	CPIJ002644	28S ribosomal protein S18b, mitochondrial
Ribosomal protein S3 C-terminal domain	CPIJ015427	40S ribosomal protein S3
Ribosomal protein S5 domain 2-like	CPIJ008237	40S ribosomal protein S2
	CPIJ013104	40S ribosomal protein S2
	CPIJ016812	40S ribosomal protein S2
	CPIJ018157	exosome complex exonuclease RRP46
	CPIJ018280	conserved hypothetical protein
Ribosomal protein S6	CPIJ007632	mitochondrial 28S ribosomal protein S6
Ribosomal proteins L15p and L18e	CPIJ012021	60S ribosomal protein L18
Ribosome inactivating proteins (RIP)	CPIJ009211	conserved hypothetical protein
Second domain of FERM	CPIJ017573	focal adhesion kinase
Sm-like ribonucleoproteins	CPIJ005588	small nuclear ribonucleoprotein SM D3
	CPIJ006616	small nuclear ribonucleoprotein-associated protein B
	CPIJ017424	small nuclear ribonucleoprotein E
ThrRS/AlaRS common domain	CPIJ015553	alanyl-tRNA synthetase domain-containing protein 1
Translation initiation factor 2 beta, aIF2beta, N-terminal domain	CPIJ013860	eukaryotic translation initiation factor 2 subunit beta
Translation proteins	CPIJ007631	elongation factor-1 alpha
	CPIJ009557	elongation factor 1 alpha
	CPIJ015678	conserved hypothetical protein
Translation proteins SH3-like domain	CPIJ008796	39S ribosomal protein L2, mitochondrial
	CPIJ019257	39S ribosomal protein L19, mitochondrial
Translational machinery components	CPIJ000042	40S ribosomal protein S14
	CPIJ000487	conserved hypothetical protein
	CPIJ000875	40S ribosomal protein S14-A
	CPIJ002488	40S ribosomal protein S14-B
	CPIJ002871	40S ribosomal protein S14
	CPIJ003216	40S ribosomal protein S14-B
	CPIJ003943	40S ribosomal protein S14
	CPIJ006101	40S ribosomal protein S14-A
	CPIJ007174	40S ribosomal protein S14
	CPIJ008067	40S ribosomal protein S14-A
	CPIJ009287	conserved hypothetical protein
	CPIJ010252	40S ribosomal protein S14
	CPIJ010640	predicted protein

			CPIJ011289	40S ribosomal protein S14
			CPIJ011697	40S ribosomal protein S141
			CPIJ012110	40S ribosomal protein S14-A
			CPIJ013076	40S ribosomal protein S14
			CPIJ013802	40S ribosomal protein S14-2
			CPIJ014959	40S ribosomal protein S14-A
			CPIJ015991	conserved hypothetical protein
			CPIJ016597	40S ribosomal protein S14
			CPIJ017293	40S ribosomal protein S14
			CPIJ018446	40S ribosomal protein S14-B
		Zn-binding ribosomal proteins	CPIJ014045	39S ribosomal protein L32, mitochondrial
Intra-cellular processes	Cell cycle, Apoptosis	CAD & PB1 domains	CPIJ008787	conserved hypothetical protein
			CPIJ020175	conserved hypothetical protein
		Cell cycle regulatory proteins	CPIJ006105	cyclin-dependent kinaseregulatory subunit 1
		Cullin homology domain	CPIJ003980	anaphase-promoting complex subunit 2
		Cystine-knot cytokines	CPIJ000272	Sptzle 2
			CPIJ000273	sptzle 2
			CPIJ001752	sptzle 3A
			CPIJ002281	sptzle 6
			CPIJ012748	conserved hypothetical protein
		DEATH domain	CPIJ010093	netrin receptor unc5
			CPIJ010503	ankyrin 2,3/unc44
		Inhibitor of apoptosis (IAP) repeat	CPIJ006918	conserved hypothetical protein
		RCC1/BLIP-II	CPIJ011645	hyperplastic discs protein
			CPIJ015298	regulator of chromosome condensation
		Rhodanese/Cell cycle control phosphatase	CPIJ001662	M-phase inducer phosphatase 2
			CPIJ013880	heat shock protein 67B2
	Cell motility	Actin depolymerizing proteins	CPIJ007823	glial maturation factor
			CPIJ015839	conserved hypothetical protein
			CPIJ019500	conserved hypothetical protein
		Actin-crosslinking proteins	CPIJ013211	conserved hypothetical protein
		DLC	CPIJ015622	dynein light chain 1, cytoplasmic-like protein
			CPIJ015623	predicted protein
		Formin homology 2 domain (FH2 domain)	CPIJ003134	formin 1,2/cappuccino
			CPIJ006609	conserved hypothetical protein
			CPIJ007323	formin 3
		I/LWEQ domain	CPIJ004416	huntingtin interacting protein
		Myosin rod fragments	CPIJ014522	lava lamp protein
			CPIJ017450	mushroom body defect protein

	Outer arm dynein light chain 1	CPIJ012834	conserved hypothetical protein
		CPIJ015679	conserved hypothetical protein
	Tropomyosin	CPIJ001763	Ofd1 protein
		CPIJ005452	M-type 9 protein
	Tubulin nucleotide-binding domain-like	CPIJ003263	tubulin beta chain
		CPIJ011550	tubulin alpha-2 chain
		CPIJ017383	tubulin alpha-1 chain
Ion m/tr	Band 7/SPFH domain	CPIJ001131	erythrocyte band 7 integral membrane protein
	Calcium ATPase, transmembrane domain M	CPIJ001884	cation-transporting ATPase 13a1
		CPIJ005964	Na+/K+ ATPase alpha subunit
		CPIJ005965	conserved hypothetical protein
		CPIJ013541	conserved hypothetical protein
	Clc chloride channel	CPIJ004937	chloride channel protein 3
	Cupredoxins	CPIJ010466	laccase-like multicopper oxidase 1
		CPIJ012244	multicopper oxidase
		CPIJ012357	multicopper oxidase
		CPIJ016802	laccase-like multicopper oxidase 1
		CPIJ020002	multicopper oxidase
	Ferritin-like	CPIJ003762	coenzyme q10 biosynthesis protein
	HMA, heavy metal-associated domain	CPIJ015637	antioxidant enzyme
	MFS general substrate transporter	CPIJ000765	monocarboxylate transporter
		CPIJ000988	sugar transporter
		CPIJ001970	organic anion transporter
		CPIJ001971	solute carrier organic anion transporter family member 3A1
		CPIJ001972	organic anion transporter
		CPIJ002124	hippocampus abundant 1 protein
		CPIJ002172	oligopeptide transporter
		CPIJ003413	UNC93A protein
		CPIJ003611	sugar transporter
		CPIJ004177	sodium-dependent phosphate transporter
		CPIJ005445	glucose transporter
		CPIJ006419	monocarboxylate transporter
		CPIJ007434	sodium/phosphate cotransporter
		CPIJ008117	monocarboxylate transporter
		CPIJ008119	monocarboxylate transporter
		CPIJ008274	monocarboxylate transporter 3
		CPIJ008344	sugar transporter
		CPIJ008424	integral membrane protein efflux protein efpA
		CPIJ008947	sugar transporter
		CPIJ008948	sugar transporter

		CPIJ011542	synaptic vesicle protein
		CPIJ011543	proton-associated sugar transporter A
		CPIJ012022	organic cation/carnitine transporter 1
		CPIJ014358	organic cation transporter
		CPIJ015155	adenylate cyclase
		CPIJ015621	cis,cis-muconate transport protein MucK
		CPIJ015630	conserved hypothetical protein
		CPIJ017354	adenylate cyclase
		CPIJ017478	conserved hypothetical protein
		CPIJ018460	mfs transporter
		CPIJ018461	mfs transporter
		CPIJ019487	organic cation transporter
		CPIJ019488	organic cation transporter
		CPIJ019562	conserved hypothetical protein
		CPIJ019820	sugar transporter
	Multidrug resistance efflux transporter EmrE	CPIJ009014	UDP-N-acetylglucosamine transporter
		CPIJ010400	conserved hypothetical protein
		CPIJ017962	conserved hypothetical protein
	Neurotransmitter-gated ion-channel transmembrane pore	CPIJ006949	histamine-gated chloride channel subunit
		CPIJ007636	conserved hypothetical protein
		CPIJ010616	conserved hypothetical protein
	Periplasmic binding protein-like II	CPIJ006667	glutamate receptor
		CPIJ007989	porphobilinogen deaminase
		CPIJ010822	conserved hypothetical protein
	SET domain	CPIJ008357	conserved hypothetical protein
		CPIJ010143	conserved hypothetical protein
		CPIJ013516	conserved hypothetical protein
		CPIJ013517	conserved hypothetical protein
		CPIJ013971	Mll1 protein
		CPIJ015254	conserved hypothetical protein
		CPIJ016652	histone-lysine N-methyltransferase SETDB1
		CPIJ018501	conserved hypothetical protein
	Voltage-gated potassium channels	CPIJ000215	sodium-and chloride-activated ATP-sensitive potassium channel
		CPIJ000749	conserved hypothetical protein
		CPIJ001990	conserved hypothetical protein
		CPIJ005769	calcium-activated potassium channel alpha chain
		CPIJ010846	potassium channel subfamily K member 9
		CPIJ017948	voltage and ligand gated potassium channel
Phospholipid	CRAL/TRIO domain	CPIJ001389	conserved hypothetical protein

m/tr			
		CPIJ005816	CRAL/TRIO domain-containing protein
		CPIJ008515	cellular retinaldehyde binding protein
		CPIJ008920	tyrosine phosphatase n9
		CPIJ009578	CRAL/TRIO domain-containing protein
		CPIJ013463	conserved hypothetical protein
		CPIJ013464	conserved hypothetical protein
		CPIJ013472	conserved hypothetical protein
		CPIJ013592	conserved hypothetical protein
		CPIJ013676	cellular retinaldehyde binding protein
		CPIJ014217	CRAL/TRIO domain-containing protein
		CPIJ014225	CRAL/TRIO domain-containing protein
		CPIJ016765	ganglioside induced differentiation associated protein
		CPIJ018183	conserved hypothetical protein
		CPIJ018213	CRAL/TRIO domain-containing protein
	CRAL/TRIO N-terminal domain	CPIJ013466	conserved hypothetical protein
		CPIJ014222	CRAL/TRIO domain-containing protein
		CPIJ018181	conserved hypothetical protein
		CPIJ018184	conserved hypothetical protein
	Phospholipase A2, PLA2	CPIJ001437	phospholipase A2
		CPIJ011154	secretory Phospholipase A2
		CPIJ011155	secretory Phospholipase A2
		CPIJ019557	conserved hypothetical protein
	PLC-like phosphodiesterases	CPIJ008722	conserved hypothetical protein
Proteases	BPTI-like	CPIJ004215	conserved hypothetical protein
	ClpP/crotonase	CPIJ002685	enoyl-CoA hydratase ECHA12
		CPIJ005435	peroxisomal 3,2-trans-enoyl-CoA isomerase
		CPIJ009999	methylcrotonoyl-CoA carboxylase beta chain, mitochondrial
		CPIJ013793	cuticle protein 8
		CPIJ020263	fatty acid oxidation complex subunit alpha
	Creatinase/aminopeptidase	CPIJ011945	methionine aminopeptidase 2
		CPIJ020069	methionine aminopeptidase 2
		CPIJ006323	xaa-Pro aminopeptidase 1
		CPIJ014907	xaa-pro dipeptidase
	Cystatin/monellin	CPIJ002770	cystatin-like protein
		CPIJ002771	cystatin-like protein
	Cysteine proteinases	CPIJ000133	conserved hypothetical protein
		CPIJ000575	oryzain gamma chain
		CPIJ003218	ubiquitin carboxyl-terminal hydrolase 22
		CPIJ004347	Autophagy-specific protein

	CPIJ005164	ubiquitin specific proteinase
	CPIJ005165	ubiquitin specific proteinase
	CPIJ005440	conserved hypothetical protein
	CPIJ007009	conserved hypothetical protein
	CPIJ010867	conserved hypothetical protein
	CPIJ013438	conserved hypothetical protein
	CPIJ014293	ubiquitin specific protease 2
	CPIJ014687	ubiquitin carboxyl-terminal hydrolase 14
	CPIJ014690	conserved hypothetical protein
	CPIJ015776	OTU domain-containing protein 6B
	CPIJ016134	ubiquitin carboxyl-terminal hydrolase 64E
DPP6 N-terminal domain-like	CPIJ008362	DET1 protein
Elafin-like	CPIJ006214	salivary cysteine-rich peptide
	CPIJ019874	salivary cysteine-rich peptide
HSP40/DnaJ peptide-binding domain	CPIJ006891	tumorous imaginal discs, mitochondrial
Kazal-type serine protease inhibitors	CPIJ011189	predicted protein
LuxS/MPP-like metallohydrolase	CPIJ001880	mitochondrial-processing peptidase alpha subunit
	CPIJ019576	mitochondrial-processing peptidase subunit beta
Metallo-dependent phosphatases	CPIJ000169	sphingomyelin phosphodiesterase
	CPIJ001371	serine/threonine-protein phosphatase 4 catalytic subunit
	CPIJ004547	lariat debranching enzyme
	CPIJ009664	purple acid phosphatase
Metalloproteases ("zincins"), catalytic domain	CPIJ001052	aminopeptidase 2, mitochondrial
	CPIJ001462	conserved hypothetical protein
	CPIJ001808	conserved hypothetical protein
	CPIJ005931	ADAM 17
	CPIJ006295	protease m1 zinc metalloprotease
	CPIJ011458	aminopeptidase N
	CPIJ012680	ADAM 12
	CPIJ013386	zinc metalloproteinase nas-14
	CPIJ014660	protease m1 zinc metalloprotease
	CPIJ017830	conserved hypothetical protein
PMP inhibitors	CPIJ010990	pacifastin light chain
Protease propeptides/inhibitors	CPIJ016547	proprotein convertase subtilisin/kexin type 4, furin
Rhomboid-like	CPIJ003969	stem cell tumor
	CPIJ014344	conserved hypothetical protein
	CPIJ014350	transmembrane protein 115
	CPIJ015372	rhomboid protein 1, mitochondrial
Serpins	CPIJ000915	serpin B3
	CPIJ005227	serine protease inhibitor

	CPIJ010186	conserved hypothetical protein
	CPIJ012016	serine protease inhibitor, serpin
	CPIJ016299	serine protease inhibitor, serpin
	CPIJ017784	serpin B8
Subtilisin-like	CPIJ013852	tripeptidyl-peptidase 2
	CPIJ015180	proprotein convertase subtilisin/kexin type 4, furin
	CPIJ015181	proprotein convertase subtilisin/kexin type 4, furin
Thyroglobulin type-1 domain	CPIJ001687	conserved hypothetical protein
Trypsin-like serine proteases	CPIJ000593	coagulation factor XI
	CPIJ001059	serine protease
	CPIJ001060	coagulation factor XI
	CPIJ001099	serine protease
	CPIJ001107	serine protease 27
	CPIJ001109	serine protease
	CPIJ001983	trypsin 5G1
	CPIJ002490	conserved hypothetical protein
	CPIJ002491	conserved hypothetical protein
	CPIJ002531	serine protease
	CPIJ004037	serine protease
	CPIJ004038	clip-domain serine protease
	CPIJ004091	trypsin eta
	CPIJ004093	coagulation factor XI
	CPIJ004094	serine protease
	CPIJ004095	serine protease
	CPIJ004304	serine protease
	CPIJ004990	serine protease1/2
	CPIJ005480	serine protease
	CPIJ005904	anionic trypsin-2
	CPIJ006226	serine protease
	CPIJ006544	chymotrypsinogen 2
	CPIJ006568	chymotrypsin 1
	CPIJ006869	mast cell protease 3
	CPIJ008062	conserved hypothetical protein
	CPIJ008523	serine-type enodpeptidase
	CPIJ008567	serine protease
	CPIJ008568	serine protease
	CPIJ009113	chymotrypsin BI
	CPIJ009142	coagulation factor VII
	CPIJ009480	tryptase gamma
	CPIJ009592	serine-type enodpeptidase

		CPIJ009624	serine protease
		CPIJ009792	conserved hypothetical protein
		CPIJ009890	serine proteinase stubble
		CPIJ009891	serine protease
		CPIJ009893	serine protease
		CPIJ009894	serine protease
		CPIJ010297	coagulation factor X
		CPIJ010615	proclotting enzyme
		CPIJ011477	conserved hypothetical protein
		CPIJ012017	serine protease
		CPIJ013043	chymotrypsin A
		CPIJ013044	anionic trypsin
		CPIJ013362	conserved hypothetical protein
		CPIJ013396	urokinase-type plasminogen activator
		CPIJ013616	trypsin 5
		CPIJ015405	serine protease
		CPIJ016103	serine protease
		CPIJ016220	serine protease
		CPIJ017794	220 kDa silk protein
		CPIJ017797	neurohypophysial hormones
		CPIJ017798	serine protease
		CPIJ017990	serine protease1/2
		CPIJ018529	trypsin 1
		CPIJ019031	chymotrypsin BII
		CPIJ019291	serine protease htra2
		CPIJ019781	trypsin 1
		CPIJ019952	serine protease
		CPIJ020116	conserved hypothetical protein
	Zn-dependent exopeptidases	CPIJ001174	plasma glutamate carboxypeptidase
		CPIJ009394	zinc carboxypeptidase
		CPIJ009466	conserved hypothetical protein
		CPIJ010806	conserved hypothetical protein
		CPIJ012908	glutaminyl-peptide cyclotransferase
		CPIJ015253	zinc carboxypeptidase A 1
		CPIJ019695	plasma glutamate carboxypeptidase
		CPIJ019890	carboxypeptidase D
Protein modification	ATPase domain of HSP90 chaperone/DNA topoisomerase II/histidine kinase	CPIJ011247	heat shock protein 82
	Chaperone J-domain	CPIJ007923	conserved hypothetical protein
		CPIJ008583	DnaJ domain containing protein

	CPIJ011412	M-phase phosphoprotein 11
	CPIJ017724	conserved hypothetical protein
	CPIJ018848	mitochondrial protein import protein MAS5
Cyclophilin-like	CPIJ004991	peptidyl-prolyl cis-trans isomerase
	CPIJ011947	peptidyl-prolyl cis-trans isomerase 10
	CPIJ016592	peptidyl-prolyl cis-trans isomerase
	CPIJ020068	peptidyl-prolyl cis-trans isomerase cyp8
FKBP-like	CPIJ011777	FK506-binding protein 2
	CPIJ014691	FK506-binding protein 59
	CPIJ014950	FK506-binding protein
	CPIJ014951	conserved hypothetical protein
GroEL equatorial domain-like	CPIJ008889	60 kDa heat shock protein, mitochondrial
	CPIJ018780	ribosomal protein S6
GroES-like	CPIJ000840	conserved hypothetical protein
	CPIJ007228	heat shock protein
	CPIJ017296	conserved hypothetical protein
Hect, E3 ligase catalytic domain	CPIJ004914	ubiquitin-protein ligase
	CPIJ011644	ubiquitin-protein ligase
	CPIJ012813	conserved hypothetical protein
	CPIJ017821	hect type E3 ubiquitin ligase
HSP20-like chaperones	CPIJ005642	heat shock protein 27
	CPIJ005645	heat shock protein 22
	CPIJ007348	nuclear movement protein nudC
	CPIJ013743	integrin beta-1-binding protein 2
	CPIJ019282	NudC domain containing 1
	CPIJ019713	chaperone binding protein
Peptide methionine sulfoxide reductase	CPIJ005204	peptide methionine sulfoxide reductase msrA
	CPIJ018565	peptide methionine sulfoxide reductase
Prefoldin	CPIJ004062	conserved hypothetical protein
	CPIJ008888	conserved hypothetical protein
Tubulin chaperone cofactor A	CPIJ003559	tubulin-specific chaperone A
UBC-like	CPIJ004186	ubiquitin-conjugating enzyme morgue
	CPIJ005149	ubiquitin-conjugating enzyme E2 i
	CPIJ005316	RWD domain-containing protein 4A
	CPIJ006567	ubiquitin-conjugating enzyme E2-17 kDa
	CPIJ007408	ubiquitin-conjugating enzyme m
	CPIJ007726	ubiquitin-conjugating enzyme E2 Q2
	CPIJ009955	ubiquitin conjugating enzyme E2
	CPIJ011320	Ufm1-conjugating enzyme 1
	CPIJ013524	ubiquitin-conjugating enzyme E2 g

		CPIJ014766	nedd8-conjugating enzyme nce2
Transport	ABC transporter transmembrane region	CPIJ011959	conserved hypothetical protein
		CPIJ011961	multidrug resistance protein 2
	Aquaporin-like	CPIJ016447	aquaporin transporter
	Cap-Gly domain	CPIJ003549	150 kDa dynein-associated polypeptide
	CBS-domain	CPIJ006251	AMPK-gamma subunit
	ENTH/VHS domain	CPIJ000451	conserved hypothetical protein
		CPIJ001186	conserved hypothetical protein
		CPIJ001538	hepatocyte growth factor-regulated tyrosine kinase substrate
		CPIJ002094	liquid facets
		CPIJ010606	conserved hypothetical protein
		CPIJ012987	conserved hypothetical protein
		CPIJ014748	conserved hypothetical protein
		CPIJ019897	phosphatidylinositol-binding clathrin assembly protein
	Glycolipid transfer protein, GLTP	CPIJ010828	conserved hypothetical protein
	LDL receptor-like module	CPIJ004357	conserved hypothetical protein
		CPIJ014258	conserved hypothetical protein
		CPIJ016346	conserved hypothetical protein
	Lipocalins	CPIJ015727	apolipoprotein D
		CPIJ015728	conserved hypothetical protein
		CPIJ017615	apolipoprotein D
	Mitochondrial carrier	CPIJ002253	mitochondrial carnitine/acylcarnitine carrier protein
		CPIJ006475	mitochondrial 2-oxoglutarate/malate carrier protein
		CPIJ001256	mitochondrial uncoupling protein
		CPIJ001257	mitochondrial uncoupling protein
		CPIJ005941	ADP,ATP carrier protein 2
		CPIJ007010	peroxisomal membrane protein pmp34
		CPIJ007454	small calcium-binding mitochondrial carrier
		CPIJ008580	mitochondrial carrier protein
		CPIJ011555	mitochondrial carrier protein
		CPIJ012095	mitochondrial carrier protein
		CPIJ012803	folate carrier protein
		CPIJ013684	Mitochondrial glutamate carrier
		CPIJ013697	tricarboxylate transport protein, mitochondrial
		CPIJ016473	mitochondrial solute carrier protein
		CPIJ016474	mitochondrial solute carrier protein
		CPIJ019834	mitochondrial carnitine/acylcarnitine carrier protein
		CPIJ020183	folate carrier protein
	Multidrug efflux transporter AcrB transmembrane domain	CPIJ002969	conserved hypothetical protein

			CPIJ011712	conserved hypothetical protein
			CPIJ019195	conserved hypothetical protein
		NTF2-like	CPIJ001995	conserved hypothetical protein
			CPIJ005154	nuclear transport factor 2
		Nucleoporin domain	CPIJ009141	nuclear pore complex protein nup214
		Phoshotransferase/anion transport protein	CPIJ005835	sodium bicarbonate cotransporter
		Preprotein translocase SecY subunit	CPIJ002966	transport protein Sec61 subunit alpha 2
		Sec1/munc18-like (SM) proteins	CPIJ007221	vacuolar protein sorting-associated
		Second domain of Mu2 adaptin subunit (ap50) of ap2 adaptor	CPIJ003697	clathrin coat assembly protein AP50
			CPIJ009776	AP-2 complex subunit mu
		SNARE fusion complex	CPIJ007395	conserved hypothetical protein
			CPIJ009959	conserved hypothetical protein
			CPIJ010472	conserved hypothetical protein
			CPIJ011681	synaptosomal-associated protein 29
Metabolism	Amino acids m/tr	SRP19	CPIJ012130	conserved hypothetical protein
		Alanine racemase C-terminal domain-like	CPIJ010687	ornithine decarboxylase
		Arginase/deacetylase	CPIJ011847	histone deacetylase
			CPIJ019172	histone deacetylase
		Glutaminase/Asparaginase	CPIJ008684	l-asparaginase i
		L-aspartase-like	CPIJ002702	adenylosuccinate lyase
		PLP-binding barrel	CPIJ008556	ornithine decarboxylase
			CPIJ010688	ornithine decarboxylase
	Carbohydrate m/tr	Tryptophan synthase beta subunit-like PLP-dependent enzymes	CPIJ011197	threonine dehydratase/deaminase
		(Trans)glycosidases	CPIJ002066	alpha-galactosidase A
			CPIJ003944	brain chitinase and chia
			CPIJ004564	brain chitinase and chia
			CPIJ008532	glycoside hydrolase
			CPIJ011854	CD98hc amino acid transporter protein
			CPIJ012134	brain chitinase and chia
			CPIJ013476	chitooligosaccharidolytic beta-N-acetylglucosaminidase
			CPIJ014063	glycoside hydrolase
			CPIJ015627	alpha-N-acetyl glucosaminidase
			CPIJ018222	alpha-amylase B
		Aldolase	CPIJ006003	delta-aminolevulinic acid dehydratase
		Carbohydrate phosphatase	CPIJ016359	myo inositol monophosphatase
		Galactose-binding domain-like	CPIJ003832	thioredoxin family Trp26
			CPIJ008825	conserved hypothetical protein

	CPIJ011079	discoidin domain receptor
	CPIJ014812	eph receptor tyrosine kinase
HIT-like	CPIJ005586	histidine triad protein member
Invertebrate chitin-binding proteins	CPIJ016342	conserved hypothetical protein
	CPIJ000248	conserved hypothetical protein
	CPIJ000681	obstractor B
	CPIJ003955	predicted protein
	CPIJ004334	conserved hypothetical protein
	CPIJ004728	conserved hypothetical protein
	CPIJ006133	conserved hypothetical protein
	CPIJ007317	chitin binding protein
	CPIJ007603	conserved hypothetical protein
	CPIJ007661	conserved hypothetical protein
	CPIJ007662	conserved hypothetical protein
	CPIJ008466	conserved hypothetical protein
	CPIJ008502	conserved hypothetical protein
	CPIJ008558	conserved hypothetical protein
	CPIJ009078	conserved hypothetical protein
	CPIJ009407	conserved hypothetical protein
	CPIJ009969	conserved hypothetical protein
	CPIJ011482	conserved hypothetical protein
	CPIJ012138	conserved hypothetical protein
	CPIJ012316	conserved hypothetical protein
	CPIJ012665	conserved hypothetical protein
	CPIJ013980	conserved hypothetical protein
	CPIJ014180	conserved hypothetical protein
	CPIJ014194	conserved hypothetical protein
	CPIJ014195	conserved hypothetical protein
	CPIJ014197	conserved hypothetical protein
	CPIJ014267	conserved hypothetical protein
	CPIJ015173	conserved hypothetical protein
	CPIJ015174	conserved hypothetical protein
	CPIJ015734	conserved hypothetical protein
	CPIJ016344	conserved hypothetical protein
	CPIJ018321	conserved hypothetical protein
	CPIJ018323	conserved hypothetical protein
	CPIJ018465	conserved hypothetical protein
	CPIJ020138	conserved hypothetical protein
Seven-hairpin glycosidases	CPIJ006601	conserved hypothetical protein
	CPIJ009935	mannosyl-oligosaccharide alpha-1,2-mannosidase

Coenzyme m/tr	Six-hairpin glycosidases	CPIJ008855	maltose phosphorylase
	Activating enzymes of the ubiquitin-like proteins	CPIJ013962	sumo-1-activating enzyme E1a
		CPIJ016556	ubiquitin-activating enzyme E1
	Acyl-CoA dehydrogenase NM domain-like	CPIJ008217	acyl-coa dehydrogenase
		CPIJ014783	isovaleryl-CoA dehydrogenase, mitochondrial
		CPIJ016451	crotonobetainyl-CoA dehydrogenase
		CPIJ016453	acyl-coa dehydrogenase
		CPIJ016454	acyl-coa dehydrogenase
	Class II aaRS and biotin synthetases	CPIJ019538	aspartyl-tRNA synthetase
		CPIJ001182	asparaginyl-tRNA synthetase
		CPIJ013145	prolyl-tRNA synthetase
		CPIJ016067	phenylalanyl-tRNA synthetase beta chain
	Glutathione synthetase ATP-binding domain-like	CPIJ003283	conserved hypothetical protein
		CPIJ009145	phosphoribosylamine-glycine ligase
		CPIJ013436	conserved hypothetical protein
	PCD-like	CPIJ006712	conserved hypothetical protein
	Peptide deformylase	CPIJ011016	peptide deformylase, mitochondrial
	S-adenosylmethionine decarboxylase	CPIJ005587	s-adenosyl methionine decarboxylase
	Substrate-binding domain of HMG-CoA reductase	CPIJ004077	3-hydroxy-3-methylglutaryl-coenzyme A reductase
	UROD/MetE-like	CPIJ010693	uroporphyrinogen decarboxylase
	Cytochrome b5-like heme/steroid binding domain	CPIJ000318	cytochrome b5
		CPIJ010629	membrane associated progesterone receptor
		CPIJ013832	cytochrome b5
		CPIJ018120	flavohemoprotein B5/b5r
	Cytochrome c oxidase subunit h	CPIJ012223	cytochrome c oxidase,-subunit VIb
	FMN-dependent nitroreductase-like	CPIJ012177	iodotyrosine dehalogenase 1
	Molybdenum cofactor-binding domain	CPIJ013919	xanthine dehydrogenase/oxidase
		CPIJ013920	aldehyde oxidase
		CPIJ013921	aldehyde oxidase
		CPIJ013934	xanthine dehydrogenase/oxidase
Energy	6-phosphogluconate dehydrogenase C-terminal domain-like	CPIJ008427	conserved hypothetical protein
		CPIJ012165	conserved hypothetical protein
		CPIJ013021	6-phosphogluconate dehydrogenase
	Citrate synthase	CPIJ019860	citrate synthase
	Enolase C-terminal domain-like	CPIJ013600	mandelate racemase
	Mitochondrial cytochrome c oxidase subunit VIIa	CPIJ014384	conserved hypothetical protein
	PEP carboxykinase-like	CPIJ010515	phosphoenolpyruvate carboxykinase
	Vacuolar ATP synthase subunit C		

Lipid m/tr	Acyl-CoA binding protein	CPIJ019707	conserved hypothetical protein
	Creatinase/prolidase N-terminal domain	CPIJ016993	xaa-pro dipeptidase
	Lipovitellin-phosvitin complex, superhelical domain	CPIJ002028	conserved hypothetical protein
	Thioesterase/thiol ester dehydrase-isomerase	CPIJ009653	conserved hypothetical protein
	YWTD domain	CPIJ000808	low-density lipoprotein receptor
		CPIJ017507	low-density lipoprotein receptor
Nitrogen m/tr	RmlC-like cupins	CPIJ003551	conserved hypothetical protein
Nucleotide m/tr	dUTPase-like	CPIJ005616	deoxyuridine 5'-triphosphate nucleotidohydrolase
	Nucleoside hydrolase	CPIJ008181	inosine-uridine preferring nucleoside hydrolase
		CPIJ014047	inosine-uridine preferring nucleoside hydrolase
	Nucleotidyltransferase	CPIJ010886	conserved hypothetical protein
	Nucleotidylyl transferase	CPIJ010393	cysteinyl-tRNA synthetase
		CPIJ010526	cysteinyl-tRNA synthetase
	PRTase-like	CPIJ004528	conserved hypothetical protein
		CPIJ004967	uracil phosphoribosyltransferase
		CPIJ012747	uridine cytidine kinase i
	Pseudouridine synthase	CPIJ002499	ribosomal pseudouridine synthase
		CPIJ014146	conserved hypothetical protein
	Ribonuclease H-like	CPIJ002928	conserved hypothetical protein
		CPIJ005339	ATP-binding cassette transporter
		CPIJ008015	conserved hypothetical protein
		CPIJ010267	3'-5' exonuclease
	Ribulose-phoshate binding barrel	CPIJ008100	conserved hypothetical protein
	SAICAR synthase-like	CPIJ001991	inositol triphosphate 3-kinase c
		CPIJ001992	inositol triphosphate 3-kinase c
	Tetrahydrobiopterin biosynthesis enzymes-like	CPIJ000863	6-pyruvoyl tetrahydrobiopterin synthase
		CPIJ014857	GTP cyclohydrolase i
		CPIJ018483	GTP cyclohydrolase i
	Acetyl-CoA synthetase-like	CPIJ000424	AMP dependent coa ligase
		CPIJ000425	short-chain-fatty-acid-CoA ligase
		CPIJ002867	AMP dependent coa ligase
		CPIJ007302	long-chain fatty acid transport protein 4
		CPIJ009978	AMP dependent coa ligase
		CPIJ009981	conserved hypothetical protein
		CPIJ011600	long-chain-fatty-acid coa ligase
		CPIJ015670	4-coumarate-CoA ligase 3
		CPIJ015716	4-coumarate-CoA ligase 1
		CPIJ017396	AMP dependent ligase
		CPIJ018155	luciferin 4-monooxygenase

Actin-like ATPase domain	CPIJ004484	conserved hypothetical protein
	CPIJ006534	conserved hypothetical protein
	CPIJ011081	heat shock protein 70 B2
	CPIJ011082	heat shock protein 70 B2
	CPIJ011083	heat shock protein 70 B2
	CPIJ019868	heat shock 70 kDa protein 4
Alkaline phosphatase-like	CPIJ001263	membrane-bound alkaline phosphatase
	CPIJ002095	alkaline phosphatase
	CPIJ006774	arylsulfatase B
	CPIJ010201	heparan n-sulfatase
	CPIJ010661	conserved hypothetical protein
	CPIJ011047	arylsulfatase b
	CPIJ015241	alkaline phosphatase
	CPIJ017042	conserved hypothetical protein
alpha/beta-Hydrolases	CPIJ000367	lysosomal acid lipase
	CPIJ001035	conserved hypothetical protein
	CPIJ001352	N-myc downstream regulated
	CPIJ002719	lipase 1
	CPIJ002720	lysosomal acid lipase
	CPIJ002721	lysosomal acid lipase
	CPIJ002722	lipase 1
	CPIJ002723	lysosomal acid lipase
	CPIJ002726	lipase 3
	CPIJ004066	juvenile hormone esterase
	CPIJ004226	pancreatic triacylglycerol lipase
	CPIJ004636	para-nitrobenzyl esterase
	CPIJ004802	endothelial lipase
	CPIJ006220	conserved hypothetical protein
	CPIJ007141	esterase FE4
	CPIJ007424	juvenile hormone esterase
	CPIJ007825	para-nitrobenzyl esterase
	CPIJ010991	neural stem cell-derived dendrite regulator
	CPIJ013280	lysosomal Pro-X carboxypeptidase
	CPIJ013720	conserved hypothetical protein
	CPIJ013838	lipase 1
	CPIJ013918	esterase B1
	CPIJ014154	esterase FE4
	CPIJ015386	hepatic triacylglycerol lipase
	CPIJ015557	Sn1-specific diacylglycerol lipase alpha
	CPIJ018753	juvenile hormone esterase

	CPIJ019227	pancreatic triacylglycerol lipase
	CPIJ019228	pancreatic triacylglycerol lipase
	CPIJ019996	conserved hypothetical protein
Amidase signature (AS) enzymes	CPIJ005591	indoleacetamide hydrolase
Calcium-dependent phosphotriesterase	CPIJ003362	odd Oz protein
Carbonic anhydrase	CPIJ001807	carbonic anhydrase
	CPIJ011424	carbonic anhydrase
	CPIJ011533	carbonic anhydrase
	CPIJ014280	carbonic anhydrase
Casein kinase II beta subunit	CPIJ014996	casein kinase II subunit beta
DHH phosphoesterases	CPIJ005338	PRUNE protein
DHS-like NAD/FAD-binding domain	CPIJ002993	deoxyhypusine synthase
F1 ATPase inhibitor, IF1, C-terminal domain	CPIJ000503	mitochondrial ATPase inhibitor
Folate-binding domain	CPIJ014981	aminomethyltransferase, mitochondrial
Galactose mutarotase-like	CPIJ015655	lysosomal alpha-mannosidase
	CPIJ015656	lysosomal alpha-mannosidase
Glycoside hydrolase/deacetylase	CPIJ006311	conserved hypothetical protein
	CPIJ008266	conserved hypothetical protein
	CPIJ008267	conserved hypothetical protein
	CPIJ018088	conserved hypothetical protein
HAD-like	CPIJ010121	copper-transporting ATPase 1
	CPIJ008694	conserved hypothetical protein
	CPIJ010605	conserved hypothetical protein
	CPIJ010899	conserved hypothetical protein
	CPIJ013914	dullard protein
HD-domain/PDEase-like	CPIJ000309	sam/hd domain protein
Kinase associated domain 1, KA1	CPIJ006188	conserved hypothetical protein
	CPIJ015835	conserved hypothetical protein
LysM domain	CPIJ013415	nucleolar protein c7b
Metallo-dependent hydrolases	CPIJ002583	Ampd2 protein
	CPIJ009741	N-acetylglucosamine-6-phosphate deacetylase
N-acetylmuramoyl-L-alanine amidase-like	CPIJ006558	peptidoglycan recognition protein la
	CPIJ008514	peptidoglycan recognition protein-1
N-terminal nucleophile aminohydrolases (Ntn hydrolases)	CPIJ000897	proteasome subunit alpha type 1
	CPIJ001361	proteasome subunit beta type 3
	CPIJ003586	proteasome subunit alpha type 3
	CPIJ006946	proteasome subunit alpha type 2
	CPIJ008264	proteasome subunit beta type 7
	CPIJ009861	proteasome component PRE2

		CPIJ016242	proteasome subunit beta type 5,8
		CPIJ016997	proteasome subunit beta type 5,8
		CPIJ017386	proteasome subunit beta type 8
		CPIJ017722	gamma glutamyl transpeptidase
		CPIJ019606	asparagine synthetase
	NAD kinase	CPIJ009966	sphingosine kinase a, b
	NHL repeat	CPIJ003685	tripartite motif protein trim2,3
		CPIJ003686	conserved hypothetical protein
	Peptidyl-tRNA hydrolase domain-like	CPIJ007051	immature colon carcinoma
	PFL-like glycyl radical enzymes	CPIJ005992	ribonucleoside-diphosphate reductase large subunit
	Phosphoglycerate mutase-like	CPIJ016005	acid phosphatase-1
		CPIJ002955	multiple inositol polyphosphate phosphatase
		CPIJ009604	phosphoglycerate mutase family member 5
		CPIJ011248	multiple inositol polyphosphate phosphatase 1
		CPIJ016006	conserved hypothetical protein
	Phospholipase D/nuclease	CPIJ006211	tyrosyl-dna phosphodiesterase
		CPIJ009798	conserved hypothetical protein
	PurM C-terminal domain-like	CPIJ009144	phosphoribosylamine-glycine ligase
	Quinoprotein alcohol dehydrogenase-like	CPIJ000465	conserved hypothetical protein
		CPIJ000963	kinesin family member 21A
		CPIJ007852	receptor for activated protein kinase C
		CPIJ011019	wd-repeat protein
		CPIJ014368	conserved hypothetical protein
		CPIJ019807	proliferation-inducing gene 21
	Ribokinase-like	CPIJ011108	conserved hypothetical protein
		CPIJ011111	conserved hypothetical protein
		CPIJ016881	conserved hypothetical protein
		CPIJ020058	pyridoxal kinase
	SGNH hydrolase	CPIJ011741	platelet-activating factor acetylhydrolase IB subunit beta
		CPIJ012575	phospholipase b
		CPIJ012576	phospholipase b
		CPIJ012577	phospholipase b, plb1
		CPIJ016880	phospholipase b, plb1
	Thiolase-like	CPIJ002342	3-ketoacyl-CoA thiolase
		CPIJ018065	trifunctional enzyme beta subunit
	Trimeric LpxA-like enzymes	CPIJ003121	dynactin subunit 5
Photosynthesis	PRC-barrel domain	CPIJ005871	conserved hypothetical protein
Polysaccharide m/tr	DAK1/DegV-like	CPIJ014451	conserved hypothetical protein

		CPIJ016133	dihydroxyacetone kinase
	Ricin B-like lectins	CPIJ005695	polypeptide N-acetylgalactosaminyltransferase 5
		CPIJ014647	N-acetyl galactosaminyl transferase 7
		CPIJ017873	16.7 kDa salivary peptide
	RuBisCo LSMT C-terminal, substrate-binding domain	CPIJ018263	conserved hypothetical protein
	Starch-binding domain-like	CPIJ011486	NOMO3 protein
	UDP-Glycosyltransferase/glycogen phosphorylase	CPIJ000038	UDP-glucuronosyltransferase 1-3
		CPIJ004369	glucosyl transferase
		CPIJ010412	fucosyltransferase 11
		CPIJ013202	glycoprotein 3-alpha-L-fucosyltransferase A
		CPIJ014333	glucosyl/glucuronosyl transferase
Redox	2Fe-2S ferredoxin-like	CPIJ020265	aldehyde oxidase
	Acid phosphatase/Vanadium-dependent haloperoxidase	CPIJ003606	dolichyldiphosphatase 1
	ALDH-like	CPIJ013217	glutamate semialdehyde dehydrogenase
	Aromatic aminoacid monoxygenases, catalytic and oligomerization domains	CPIJ014156	conserved hypothetical protein
	Cu,Zn superoxide dismutase-like	CPIJ000146	superoxide dismutase 2
	Cytochrome P450‡	CPIJ018854	*CYP4C50v2*
		CPIJ001754	*CYP4J6*
		CPIJ001757	*CYP4H39*
		CPIJ001810	*CPY4C38*
		CPIJ003361	*CPY6BY2*
		CPIJ003375	*CYP6BY3*
		CPIJ005899	*CYP6N26P*
		CPIJ006321	*SCOP predicted cytochrome P450
		CPIJ006322	*CYP307A1*
		CPIJ008972	*CYP6F5P*
		CPIJ010810	*CYP325BC2*
		CPIJ016355	*CYP6AK1-de1b*
		CPIJ016846	*CYP6M13*
		CPIJ016847	*CYP6CQ2*
		CPIJ016849	*CYP6M12*
		CPIJ016850	*CYP6Y4*
		CPIJ016853	*CYP6N21P*
		CPIJ016854	*CYP6N22*
		CPIJ016856	*CYP6N18*
		CPIJ017245	*CYP304B6*
		CPIJ017351	*CYP4C50v1*
		CPIJ018716	*CYP4C38*
		CPIJ019704	*CYP6N24*

	FAD-dependent thiol oxidase	CPIJ012226	augmenter of liver regeneration
	FAD/NAD-linked reductases, dimerisation (C-terminal) domain	CPIJ002642	apoptosis-inducing factor 1, mitochondrial
	Ferredoxin reductase-like, C-terminal NADP-linked domain	CPIJ003578	conserved hypothetical protein
	Formate/glycerate dehydrogenase catalytic domain-like	CPIJ006365	conserved hypothetical protein
		CPIJ011531	adenosyl homocysteinase
	Heme-dependent peroxidases	CPIJ003117	dual oxidase 1
		CPIJ016742	thyroid peroxidase
		CPIJ018105	chorion peroxidase
	Inosine monophosphate dehydrogenase (IMPDH)	CPIJ011687	inosine-5'-monophosphate dehydrogenase
	Metallo-hydrolase/oxidoreductase	CPIJ011621	conserved hypothetical protein
		CPIJ011625	conserved hypothetical protein
		CPIJ019501	hydroxyacylglutathione hydrolase
		CPIJ019503	DNA cross-link repair 1A protein
	NAD(P)-linked oxidoreductase	CPIJ003374	aldo-keto reductase
		CPIJ003393	aldose reductase
		CPIJ003722	aldo-keto reductase
		CPIJ017461	aldo-keto reductase
	PHM/PNGase F	CPIJ014202	dopamine beta hydroxylase
	Thioredoxin-like	CPIJ001856	conserved hypothetical protein
		CPIJ003089	SCO1, mitochondrial
		CPIJ003399	peroxiredoxins, prx-1, prx-2, prx-3
		CPIJ003709	thioredoxin, mitochondrial
		CPIJ003981	15 kDa selenoprotein
		CPIJ007327	disulfide-isomerase A6
		CPIJ008802	conserved hypothetical protein
		CPIJ009940	conserved hypothetical protein
		CPIJ010610	NADH-ubiquinone oxidoreductase B8 subunit
		CPIJ011296	peroxiredoxin 6
		CPIJ012568	phospholipid hydroperoxide glutathione peroxidase 1
		CPIJ015346	glutaredoxin, grx
		CPIJ016175	glutaredoxin, grx
		CPIJ017364	endoplasmic reticulum resident protein
		CPIJ017625	conserved hypothetical protein
Secondary metabolism	Clavaminate synthase-like	CPIJ014046	conserved hypothetical protein
		CPIJ014507	conserved hypothetical protein
		CPIJ017090	gamma-butyrobetaine dioxygenase
		CPIJ017091	gamma-butyrobetaine dioxygenase

		CPIJ018084	uty-prov protein
	Concanavalin A-like lectins/glucanases	CPIJ001299	keratinocyte lectin
		CPIJ004321	gram-negative bacteria binding protein
		CPIJ004683	laminin alpha-1, 2 chain
		CPIJ004919	conserved hypothetical protein
		CPIJ005988	conserved hypothetical protein
		CPIJ006598	tripartite motif protein trim9
		CPIJ012172	conserved hypothetical protein
		CPIJ012874	kinase c-binding protein nell1
		CPIJ013642	conserved hypothetical protein
		CPIJ016123	conserved hypothetical protein
	Homo-oligomeric flavin-containing Cys decarboxylases, HFCD	CPIJ019818	phosphopantothenoylcysteine decarboxylase
	Terpenoid synthases	CPIJ008089	conserved hypothetical protein
		CPIJ016309	candidate tumor suppressor protein
		CPIJ016310	candidate tumor suppressor protein
		CPIJ016311	decaprenyl-diphosphate synthase subunit 2
Transferases	4'-phosphopantetheinyl transferase	CPIJ011416	aminoadipate-semialdehyde dehydrogenase
	Acyl-CoA N-acyltransferases (Nat)	CPIJ000413	conserved hypothetical protein
		CPIJ001343	histone acetyltransferase type B catalytic subunit
		CPIJ008392	N-acetyltransferase 5
		CPIJ010396	conserved hypothetical protein
		CPIJ012930	conserved hypothetical protein
		CPIJ015282	dopamine N acetyltransferase
		CPIJ015982	N-acetyl transferase separation anxiety
	Class I glutamine amidotransferase-like	CPIJ006930	gamma-glutamyl hydrolase
	CoA-dependent acyltransferases	CPIJ001609	choline O-acetyltransferase
		CPIJ005612	carnitine o-acyltransferase
	Formyltransferase	CPIJ009143	phosphoribosylglycinamide formyltransferase
	Glycerol-3-phosphate (1)-acyltransferase	CPIJ004138	1-acyl-sn-glycerol-3-phosphate acyltransferase
		CPIJ004141	1-acyl-sn-glycerol-3-phosphate acyltransferase beta
		CPIJ013939	glycerol-3-phosphate acyltransferase
		CPIJ015965	transmembrane protein 68
	Homocysteine S-methyltransferase	CPIJ008869	homocysteine S-methyltransferase
	MIR domain	CPIJ016258	probable ER retained protein
	NagB/RpiA/CoA transferase-like	CPIJ004258	ribose-5-phosphate isomerase
		CPIJ005163	conserved hypothetical protein
		CPIJ006915	translation initiation factor 2b, delta subunit
		CPIJ008074	glucosamine-6-phosphate isomerase
		CPIJ011933	conserved hypothetical protein

		Nucleotide-diphospho-sugar transferases	CPIJ003171	UDP-n-acteylglucosamine pyrophosphorylase
			CPIJ000257	conserved hypothetical protein
			CPIJ002650	dolichol-phosphate mannosyltransferase
			CPIJ004318	galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase I
			CPIJ005229	N-acetyl galactosaminyl transferase 6
			CPIJ012815	mannose-1-phosphate guanyltransferase
			CPIJ016255	chitin synthase
			CPIJ018702	beta-1,3-galactosyltransferase brn
		PLP-dependent transferases	CPIJ003522	cysteine desulfurase, mitochondrial
			CPIJ010034	glutamate decarboxylase
			CPIJ013307	aromatic-L-amino-acid decarboxylase
		Protein prenylyltransferase	CPIJ005820	geranylgeranyl transferase type-2 alpha subunit
			CPIJ017557	smile protein
		S-adenosyl-L-methionine-dependent methyltransferases	CPIJ001152	HemK methyltransferase family member 2
			CPIJ001336	ribosomal RNA large subunit methyltransferase J
			CPIJ001402	histone-arginine methyltransferase CARM1
			CPIJ001578	conserved hypothetical protein
			CPIJ005043	conserved hypothetical protein
			CPIJ006933	conserved hypothetical protein
			CPIJ007234	AdoMet-dependent rRNA methyltransferase spb1
			CPIJ008978	conserved hypothetical protein
			CPIJ010001	tRNA methyltransferase
			CPIJ010915	arginine n-methyltransferase
			CPIJ013558	23S rRNA methyltransferase
			CPIJ016651	HemK methyltransferase family member 1
			CPIJ018143	conserved hypothetical protein
Regulation			CPIJ009244	conserved hypothetical protein
	DNA-binding	AlbA-like	CPIJ010569	conserved hypothetical protein
		AN1-like Zinc finger	CPIJ002707	zinc finger protein
			CPIJ002783	AN1-type zinc finger protein 2B
		ARID-like	CPIJ008131	receptor for activated protein kinase C
		ATP-dependent DNA ligase DNA-binding domain	CPIJ017286	conserved hypothetical protein
		Bromodomain	CPIJ007940	conserved hypothetical protein
			CPIJ012613	conserved hypothetical protein
		C2H2 and C2HC zinc fingers	CPIJ018818	conserved hypothetical protein
			CPIJ000409	conserved hypothetical protein
			CPIJ000911	zinc finger protein 383
			CPIJ001029	serendipity locus protein delta
			CPIJ001300	zinc finger protein 780B

CPIJ001471	transcription factor hamlet
CPIJ001473	conserved hypothetical protein
CPIJ001552	conserved hypothetical protein
CPIJ001985	conserved hypothetical protein
CPIJ002705	zinc finger protein 90
CPIJ002824	predicted protein
CPIJ002932	conserved hypothetical protein
CPIJ003270	broad-complex core-protein
CPIJ003609	Sp5 transcription factor
CPIJ003667	zinc finger protein 141
CPIJ003749	tRNA delta
CPIJ003796	conserved hypothetical protein
CPIJ004257	zinc finger-containing protein
CPIJ004351	zinc finger protein 92
CPIJ004384	zinc finger protein
CPIJ004667	zinc finger protein
CPIJ004785	conserved hypothetical protein
CPIJ004963	conserved hypothetical protein
CPIJ005175	transcription factor sp8,sp9
CPIJ005503	zinc finger protein 36
CPIJ005812	zinc finger protein
CPIJ005813	zinc finger protein
CPIJ006385	conserved hypothetical protein
CPIJ006765	conserved hypothetical protein
CPIJ006854	conserved hypothetical protein
CPIJ006855	zinc finger protein
CPIJ007837	zinc finger protein
CPIJ007858	conserved hypothetical protein
CPIJ008060	conserved hypothetical protein
CPIJ008297	conserved hypothetical protein
CPIJ008361	double-stranded RNA-binding protein zn72d
CPIJ008549	conserved hypothetical protein
CPIJ008696	conserved hypothetical protein
CPIJ009409	conserved hypothetical protein
CPIJ009502	zinc finger protein
CPIJ009503	zinc finger protein 583
CPIJ009524	forkhead box protein
CPIJ009647	conserved hypothetical protein
CPIJ009780	zinc finger protein 75A
CPIJ009786	zinc finger protein

CPIJ009787	zinc finger protein 582
CPIJ009989	zinc finger protein
CPIJ009990	zinc finger protein
CPIJ010551	predicted protein
CPIJ010652	conserved hypothetical protein
CPIJ010850	conserved hypothetical protein
CPIJ011015	conserved hypothetical protein
CPIJ011166	zinc finger protein
CPIJ011789	conserved hypothetical protein
CPIJ012039	conserved hypothetical protein
CPIJ012535	zinc finger protein
CPIJ012594	predicted protein
CPIJ012610	zinc finger protein 38
CPIJ013068	zinc finger protein
CPIJ013118	conserved hypothetical protein
CPIJ013246	conserved hypothetical protein
CPIJ013653	conserved hypothetical protein
CPIJ014029	conserved hypothetical protein
CPIJ014036	conserved hypothetical protein
CPIJ014135	conserved hypothetical protein
CPIJ014711	zinc finger protein
CPIJ014712	krueppel protein
CPIJ014714	zinc finger protein
CPIJ014715	zinc finger protein
CPIJ015000	zinc finger protein 250
CPIJ015265	zinc finger protein 345
CPIJ015267	zinc finger protein ZNF780A
CPIJ015425	conserved hypothetical protein
CPIJ015578	hypothetical protein
CPIJ015579	conserved hypothetical protein
CPIJ015582	conserved hypothetical protein
CPIJ016725	conserved hypothetical protein
CPIJ016726	conserved hypothetical protein
CPIJ016862	conserved hypothetical protein
CPIJ016943	conserved hypothetical protein
CPIJ016944	conserved hypothetical protein
CPIJ017035	zinc finger protein 141
CPIJ017141	conserved hypothetical protein
CPIJ017142	conserved hypothetical protein
CPIJ017259	predicted protein

	CPIJ017278	zinc finger protein 436
	CPIJ017355	conserved hypothetical protein
	CPIJ017654	conserved hypothetical protein
	CPIJ017756	conserved hypothetical protein
	CPIJ018074	conserved hypothetical protein
	CPIJ018336	conserved hypothetical protein
	CPIJ018423	zinc finger protein
	CPIJ018448	transcription factor btd
	CPIJ018505	zinc finger transcription factor
	CPIJ019181	U1 small nuclear ribonucleoprotein C
	CPIJ019417	zinc finger protein 322A
	CPIJ019621	conserved hypothetical protein
	CPIJ019710	conserved hypothetical protein
	CPIJ019775	conserved hypothetical protein
	CPIJ020207	zinc finger protein 36
CCCH zinc finger	CPIJ018734	conserved hypothetical protein
CSL zinc finger	CPIJ001284	conserved hypothetical protein
Cyclin-like	CPIJ006843	transcription initiation factor TFIIB
	CPIJ010939	conserved hypothetical protein
	CPIJ012914	cyclin T
	CPIJ013566	cyclin a
	CPIJ013567	cyclin a
Cysteine-rich DNA binding domain, (DM domain)	CPIJ004057	male-specific doublesex protein
DNA-binding domain	CPIJ004606	phd finger domain
Glucocorticoid receptor-like (DNA-binding domain)	CPIJ002376	elongation factor 1-alpha
	CPIJ002547	conserved hypothetical protein
	CPIJ002808	conserved hypothetical protein
	CPIJ002811	conserved hypothetical protein
	CPIJ005912	conserved hypothetical protein
	CPIJ006674	malate dehydrogenase
	CPIJ006684	predicted protein
	CPIJ006830	conserved hypothetical protein
	CPIJ007349	zinc finger protein 225
	CPIJ007701	predicted protein
	CPIJ008216	nuclear hormone receptor ftz-f1
	CPIJ008348	GATA transcription factor GATAd
	CPIJ008682	hypothetical protein
	CPIJ009297	conserved hypothetical protein
	CPIJ009298	conserved hypothetical protein
	CPIJ009299	four and a half lim domains

	CPIJ010100	conserved hypothetical protein
	CPIJ010408	conserved hypothetical protein
	CPIJ010866	conserved hypothetical protein
	CPIJ011684	conserved hypothetical protein
	CPIJ011743	conserved hypothetical protein
	CPIJ012188	epsilon-trimethyllysine 2-oxoglutarate dioxygenase
	CPIJ012588	predicted protein
	CPIJ013614	conserved hypothetical protein
	CPIJ014027	conserved hypothetical protein
	CPIJ014086	conserved hypothetical protein
	CPIJ014594	predicted protein
	CPIJ016033	GATA-binding factor-C
	CPIJ016621	conserved hypothetical protein
	CPIJ017547	conserved hypothetical protein
	CPIJ018862	retinoic acid receptor beta
Histone-fold	CPIJ001398	transcription initiation factor TFIID subunit 9
	CPIJ008494	histone h2a
	CPIJ010882	conserved hypothetical protein
	CPIJ011778	transcription initiation factor TFIID subunit 12
	CPIJ014768	histone 1
	CPIJ017187	histone H3.3 type 2
	CPIJ017276	suppressor of ty3
	CPIJ018900	conserved hypothetical protein
HIT/MYND zinc finger-like	CPIJ014434	predicted protein
	CPIJ017587	conserved hypothetical protein
HLH, helix-loop-helix DNA-binding domain	CPIJ002332	conserved hypothetical protein
	CPIJ003409	enhancer of split mgamma protein
	CPIJ007015	conserved hypothetical protein
	CPIJ008120	conserved hypothetical protein
	CPIJ012827	conserved hypothetical protein
	CPIJ015080	conserved hypothetical protein
	CPIJ015473	max binding protein
HMG-box	CPIJ001997	conserved hypothetical protein
	CPIJ005084	coiled-coil domain-containing protein 124
	CPIJ006395	conserved hypothetical protein
	CPIJ012202	capicua protein
	CPIJ014423	conserved hypothetical protein
	CPIJ014424	conserved hypothetical protein
	CPIJ017659	conserved hypothetical protein
Homeodomain-like	CPIJ001021	homeobox protein abdominal-B

	CPIJ002815	conserved hypothetical protein
	CPIJ005379	metastasis-associated protein 3
	CPIJ005827	predicted protein
	CPIJ006382	paired box protein pax-6
	CPIJ006390	paired box protein Pax-6
	CPIJ008039	homeobox protein extradenticle
	CPIJ009982	rest corepressor protein
	CPIJ010220	segmentation polarity homeobox protein engrailed
	CPIJ012080	conserved hypothetical protein
	CPIJ012784	zinc finger protein 1
	CPIJ014669	predicted protein
	CPIJ015889	homeobox protein
	CPIJ017153	conserved hypothetical protein
	CPIJ017214	mesoderm induction early response protein 1
	CPIJ019460	hypothetical protein
Insert subdomain of RNA polymerase alpha subunit	CPIJ003123	DNA-directed RNA polymerase I 40 kDa polypeptide
Kix domain of CBP (creb binding protein)	CPIJ005540	conserved hypothetical protein
lambda repressor-like DNA-binding domains	CPIJ003986	multiprotein bridging factor
	CPIJ014526	conserved hypothetical protein
Leucine zipper domain	CPIJ003266	CCAAT/enhancer-binding protein
	CPIJ003767	conserved hypothetical protein
	CPIJ003805	cyclic-AMP response element binding protein
	CPIJ012178	ovary C/EBPg transcription factor
	CPIJ014920	par domain protein
	CPIJ016941	conserved hypothetical protein
p53-like transcription factors	CPIJ000431	conserved hypothetical protein
	CPIJ000433	T-box protein H15
	CPIJ000721	conserved hypothetical protein
	CPIJ002764	conserved hypothetical protein
	CPIJ007738	T-box protein H15
	CPIJ016469	signal transducer and activator of transcription
Periplasmic binding protein-like I	CPIJ010082	atrial natriuretic peptide receptor
	CPIJ019599	glutamate receptor, ionotropic kainate 1, 2, 3
	CPIJ020040	conserved hypothetical protein
Putative DNA-binding domain	CPIJ000580	ladybird homeobox corepressor
	CPIJ000978	transforming protein Ski
RPB6/omega subunit-like	CPIJ018444	DNA-directed RNA polymeraseI
SAM/Pointed domain	CPIJ000845	conserved hypothetical protein
	CPIJ000860	conserved hypothetical protein
	CPIJ009010	conserved hypothetical protein

		CPIJ017757	conserved hypothetical protein
	SAP domain	CPIJ007511	conserved hypothetical protein
	SMAD MH1 domain	CPIJ009526	nuclear factor i
	SMAD/FHA domain	CPIJ000274	conserved hypothetical protein
		CPIJ006464	nuclear inhibitor of protein phosphatase 1
		CPIJ006834	kinesin-like protein KIF1B
		CPIJ009516	conserved hypothetical protein
	SRF-like	CPIJ008335	conserved hypothetical protein
		CPIJ016459	conserved hypothetical protein
	Tim10-like	CPIJ018054	mitochondrial import inner membrane translocase subunit Tim8 A
		CPIJ010382	mitochondrial import inner membrane translocase subunit Tim10
		CPIJ010840	mitochondrial inner membrane protein translocase, 9kD-subunit
		CPIJ018053	mitochondrial inner membrane protein translocase, 8kD-subunit
		CPIJ018211	mitochondrial inner membrane protein translocase, 13kD-subunit
	Winged helix DNA-binding domain	CPIJ001680	DNA-binding protein D-ELG
		CPIJ001711	conserved hypothetical protein
		CPIJ003068	conserved hypothetical protein
		CPIJ003923	conserved hypothetical protein
		CPIJ004011	rfx transcription factor
		CPIJ009522	conserved hypothetical protein
		CPIJ010292	vacuolar protein sorting-associated protein 25
		CPIJ013475	transcription initiation factor IIE subunit beta
		CPIJ014720	ets DNA-binding protein pokkuri
		CPIJ015971	26S proteasome non-ATPase regulatory subunit 11
Kinases/phosphatases	(Phosphotyrosine protein) phosphatases II	CPIJ018450	slingshot dual specificity phosphatase
		CPIJ019559	phosphatase Slingshot
		CPIJ001969	tyrosine phosphatase prl
		CPIJ003757	tyrosine phosphatase mitochondrial 1
		CPIJ008018	dual specificity protein phosphatase
		CPIJ008808	conserved hypothetical protein
		CPIJ009405	testis/ seletal muscle dual specificty phosphatase
		CPIJ011976	tyrosine phosphatase, non-receptor type nt1
		CPIJ013410	tyrosine-protein phosphatase Lar
		CPIJ014898	tyrosine phosphatase n11

FAT domain of focal adhesion kinase	CPIJ018298 tryrosine phosphatase
GHMP Kinase, C-terminal domain	CPIJ017572 focal adhesion kinase
Myosin phosphatase inhibitor 17kDa protein, CPI-17	CPIJ015185 conserved hypothetical protein
Phosphotyrosine protein phosphatases I	CPIJ004356 conserved hypothetical protein
PP2C-like	CPIJ008291 low molecular weight phosphotyrosine protein phosphatase 1
Protein kinase-like (PK-like)	CPIJ012255 pyruvate dehydrogenase
	CPIJ010275 map/microtubule affinity-regulating kinase 2,4
	CPIJ000270 tyrosine kinase
	CPIJ000288 serine/threonine-protein kinase 3
	CPIJ000816 kinase protein
	CPIJ000833 tyrosine-protein kinase btk29a
	CPIJ000891 cell division protein kinase 8
	CPIJ001155 cell division protein kinase 2
	CPIJ001273 conserved hypothetical protein
	CPIJ003568 mitosis inhibitor protein kinase
	CPIJ003599 serine/threonine protein kinase
	CPIJ003985 tyrosine-protein kinase Abl
	CPIJ003996 calcium-dependent protein kinase
	CPIJ004173 Juvenile hormone-inducible protein
	CPIJ004685 Dual specificity tyrosine-phosphorylation-regulated kinase
	CPIJ004687 Dual specificity tyrosine-phosphorylation-regulated kinase
	CPIJ004799 activin receptor type I
	CPIJ004910 serine/threonine kinase NLK
	CPIJ005276 cGMP-protein kinase
	CPIJ005290 conserved hypothetical protein
	CPIJ005449 nuclear body associated kinase
	CPIJ006283 ser/thr protein kinase-trb3
	CPIJ006284 ser/thr protein kinase-trb3
	CPIJ006704 serine/threonine-protein kinase D3
	CPIJ007227 tyrosine-protein kinase
	CPIJ007458 tyrosine-protein kinase src64b
	CPIJ008931 dual specificity mitogen-activated protein kinase kinase hemipterous
	CPIJ008953 mitogen-activated protein kinase kinase kinase
	CPIJ009012 mixed lineage protein kinase
	CPIJ009223 serine/threonine-protein kinase
	CPIJ010322 cell division control protein
	CPIJ011073 discoidin domain receptor
	CPIJ011673 cell division control protein
	CPIJ011936 S6 kinase II beta

		CPIJ012176	ribosomal protein S6 kinase, 90kD, polypeptide
		CPIJ012534	eukaryotic translation initiation factor 2-alpha kinase 1
		CPIJ012560	leucine-rich repeat serine/threonine-protein kinase 1
		CPIJ013693	serine/threonine-protein kinase rio2
		CPIJ013835	conserved hypothetical protein
		CPIJ013942	conserved hypothetical protein
		CPIJ014803	Dual specificity tyrosine-phosphorylation-regulated kinase
		CPIJ015689	serine/threonine-protein kinase rio2
		CPIJ015690	serine/threonine-protein kinase RIO2
		CPIJ015801	mitogen activated protein kinase kinase 2
		CPIJ015833	map/microtubule affinity-regulating kinase 2,4
		CPIJ015918	conserved hypothetical protein
		CPIJ015922	Juvenile hormone-inducible protein
		CPIJ016475	conserved hypothetical protein
		CPIJ016644	integrin-linked protein kinase
		CPIJ016729	serine/threonine-protein kinase vrk
		CPIJ016868	conserved hypothetical protein
		CPIJ018008	fibroblast growth factor receptor
		CPIJ018201	serine/threonine protein kinase lats
		CPIJ019408	conserved hypothetical protein
		CPIJ019493	fibroblast growth factor receptor 1
		CPIJ019683	conserved hypothetical protein
Other regulatory function	GCM domain	CPIJ006640	conserved hypothetical protein
	Mago nashi protein	CPIJ002949	mago nashi
	Mob1/phocein	CPIJ002914	conserved hypothetical protein
	N-terminal domain of adenylylcyclase associated protein, CAP	CPIJ003125	adenylyl cyclase-associated protein
	Ran BP2/NZF zinc finger-like	CPIJ003010	conserved hypothetical protein
		CPIJ007177	nucleoporin, Nup153
	Sec7 domain	CPIJ002812	guanyl-nucleotide exchange factor
		CPIJ015585	arf6 guanine nucleotide exchange factor
Receptor activity	Chemosensory protein Csp2	CPIJ019986	serine/threonine kinase
		CPIJ002601	conserved hypothetical protein
		CPIJ002605	serine/threonine kinase
		CPIJ002607	conserved hypothetical protein
		CPIJ002608	chemosensory protein
		CPIJ002609	serine/threonine kinase

		CPIJ002616	serine/threonine kinase
		CPIJ002625	chemosensory protein 1
		CPIJ002629	sensory appendage protein
		CPIJ017094	serine/threonine kinase
		CPIJ019985	sensory appendage protein
RNA binding, m/tr	Alpha-L RNA-binding motif	CPIJ016140	tyrosyl-tRNA synthetase
	dsRNA-binding domain-like	CPIJ003264	40S ribosomal protein S2
		CPIJ004832	tar RNA binding protein
		CPIJ006845	40S ribosomal protein S2
		CPIJ008332	40S ribosomal protein S2
		CPIJ009774	40S ribosomal protein S2
		CPIJ011850	double-stranded RNA-specific editase Adar
		CPIJ013506	ATP-dependent RNA helicase
		CPIJ014041	conserved hypothetical protein
	Nop domain	CPIJ002179	U4/U6 small nuclear ribonucleoprotein Prp31
	Nop10-like SnoRNP	CPIJ018833	H/ACA ribonucleoprotein complex subunit 3
	PUA domain-like	CPIJ006069	adenylsulfate kinase
		CPIJ008799	ATP-dependent Lon protease
		CPIJ012137	conserved hypothetical protein
	RNA-binding domain, RBD	CPIJ014704	splicing factor
		CPIJ019416	splicing factor
		CPIJ000025	negative elongation factor E
		CPIJ000485	conserved hypothetical protein
		CPIJ000588	polypyrimidine tract binding protein
		CPIJ000880	RNA-binding post-transcriptional regulator csx1
		CPIJ001612	heterogeneous nuclear ribonucleoprotein
		CPIJ003074	conserved hypothetical protein
		CPIJ003555	nuclear cap-binding protein subunit 2
		CPIJ003738	heterogeneous nuclear ribonucleoprotein r
		CPIJ003854	conserved hypothetical protein
		CPIJ003881	G-rich sequence factor-1
		CPIJ004538	conserved hypothetical protein
		CPIJ004892	conserved hypothetical protein
		CPIJ005653	developmentally regulated RNA-binding protein
		CPIJ006107	NONA protein
		CPIJ006979	polypyrimidine tract binding protein
		CPIJ007047	serine/arginine rich splicing factor
		CPIJ007295	conserved hypothetical protein
		CPIJ007629	splicing factor

		CPIJ007834	RNA and export factor binding protein
		CPIJ008248	conserved hypothetical protein
		CPIJ008476	conserved hypothetical protein
		CPIJ008628	predicted protein
		CPIJ008634	52K active chromatin boundary protein
		CPIJ008698	rbm25 protein
		CPIJ008786	arginine/serine-rich splicing factor
		CPIJ009773	conserved hypothetical protein
		CPIJ012174	conserved hypothetical protein
		CPIJ012515	conserved hypothetical protein
		CPIJ012662	cleavage stimulation factor 64 kDa subunit
		CPIJ012814	scaffold attachment factor b
		CPIJ012850	splicing factor u2af large subunit
		CPIJ013237	conserved hypothetical protein
		CPIJ014506	fuse-binding protein-interacting repressor siahbp1
		CPIJ015052	RNA binding motif protein 18
		CPIJ015262	heterogeneous nuclear ribonucleoprotein 27C
		CPIJ015350	eukaryotic translation initiation factor 3 subunit 4
		CPIJ015549	ribosomal biogenesis protein Gar2
		CPIJ016143	conserved hypothetical protein
		CPIJ016329	conserved hypothetical protein
		CPIJ016517	conserved hypothetical protein
		CPIJ017755	conserved hypothetical protein
		CPIJ018451	conserved hypothetical protein
	Surp module (SWAP domain)	CPIJ017179	scaffold attachment factor B
Signal transduction	C2 domain (Calcium/lipid-binding domain, CaLB)	CPIJ005008	E3 ubiquitin ligase
		CPIJ005701	kinase C alpha-polypeptide
		CPIJ011785	E3 ubiquitin-protein ligase nedd-4
		CPIJ015045	conserved hypothetical protein
		CPIJ015112	E3 ubiquitin-protein ligase nedd-4
		CPIJ016865	conserved hypothetical protein
	cAMP-binding domain-like	CPIJ004551	conserved hypothetical protein
		CPIJ005277	conserved hypothetical protein
		CPIJ005279	cGMP-dependent protein kinase
		CPIJ006213	cyclic nucleotide-gated cation channel 4
		CPIJ015446	cyclic-nucleotide-gated cation channel
		CPIJ015653	cGMP-dependent protein kinase
		CPIJ015942	c-AMP dependent protein kinase typeI-beta regulatory subunit

	CPIJ018420	conserved hypothetical protein
	CPIJ019876	cyclic nucleotide-gated cation channel beta 3
DBL homology domain (DH-domain)	CPIJ001482	pak-interacting exchange factor, beta-pix/cool-1
	CPIJ004982	rho guanine exchange factor
	CPIJ011504	RHO guanyl-nucleotide exchange factor
	CPIJ013987	conserved hypothetical protein
	CPIJ015421	Rho GEF and pleckstrin domain protein
	CPIJ017598	guanine nucleotide exchange factor
Family A G protein-coupled receptor-like	CPIJ006268	cardioacceleratory peptide receptor
	CPIJ006269	cardioacceleratory peptide receptor
	CPIJ011619	leucine-rich transmembrane protein
	CPIJ014487	beta adrenergic receptor
	CPIJ014753	conserved hypothetical protein
	CPIJ015979	conserved hypothetical protein
	CPIJ016092	adenosine A2 receptor
Frizzled cysteine-rich domain	CPIJ011799	conserved hypothetical protein
Growth factor receptor domain	CPIJ009117	conserved hypothetical protein
	CPIJ015183	proprotein convertase subtilisin/kexin type 4, furin
	CPIJ017374	conserved hypothetical protein
GTPase activation domain, GAP	CPIJ010879	conserved hypothetical protein
	CPIJ012517	cdc42 GTPase-activating protein
	CPIJ015825	conserved hypothetical protein
	CPIJ019727	conserved hypothetical protein
Insect pheromone/odorant-binding proteins	CPIJ001867	hypothetical protein
	CPIJ001871	hypothetical protein
	CPIJ001874	Odorant-binding protein 56a
	CPIJ003865	conserved hypothetical protein
	CPIJ003866	conserved hypothetical protein
	CPIJ004634	odorant-binding protein
	CPIJ004635	odorant-binding protein OBPjj7a
	CPIJ007337	conserved hypothetical protein
	CPIJ010787	conserved hypothetical protein
	CPIJ010788	conserved hypothetical protein
	CPIJ018881	tRNA delta
Insulin-like	CPIJ018049	conserved hypothetical protein
Nicotinic receptor ligand binding domain-like	CPIJ007639	acetylcholine receptor protein alpha 1, 2, 3, 4 invertebrate
	CPIJ016909	nicotinic acetylcholine receptor, beta-2 subunit
Nuclear receptor ligand-binding domain	CPIJ002963	ecdysone receptor
	CPIJ004609	nuclear hormone receptor
	CPIJ008215	nuclear hormone receptor ftz-f1

	CPIJ009588	conserved hypothetical protein
	CPIJ014945	nuclear hormone receptor ftz-f1
	CPIJ015542	nuclear receptor 3
	CPIJ016024	retinoid x receptor
Nucleotide cyclase	CPIJ004739	adenylate cyclase
	CPIJ015189	adenylate cyclase
	CPIJ017081	guanylate cyclase soluble subunit beta-1
	CPIJ017287	adenylate cyclase type
	CPIJ019946	adenylate cyclase type 5
PDZ domain-like	CPIJ001710	conserved hypothetical protein
	CPIJ003808	partitioning defective 3
	CPIJ004495	conserved hypothetical protein
	CPIJ005564	26S proteasome non-ATPase regulatory subunit 9
	CPIJ006020	conserved hypothetical protein
	CPIJ006377	conserved hypothetical protein
	CPIJ007358	conserved hypothetical protein
	CPIJ007684	conserved hypothetical protein
	CPIJ010875	conserved hypothetical protein
	CPIJ015012	conserved hypothetical protein
	CPIJ016071	golgi reassembly-stacking protein 2
	CPIJ018273	conserved hypothetical protein
	CPIJ018340	ezrin-radixin-moesin-binding phosphoprotein 50
	CPIJ019226	Glutamate receptor binding protein
	CPIJ019766	conserved hypothetical protein
PH domain-like	CPIJ000361	conserved hypothetical protein
	CPIJ004690	numb protein
	CPIJ004706	vasodilator-stimulated phosphoprotein
	CPIJ005592	decapping protein 1
	CPIJ005688	conserved hypothetical protein
	CPIJ006699	wiskott-aldrich syndrome protein
	CPIJ007927	conserved hypothetical protein
	CPIJ009368	conserved hypothetical protein
	CPIJ009993	conserved hypothetical protein
	CPIJ010369	conserved hypothetical protein
	CPIJ010878	conserved hypothetical protein
	CPIJ011071	structure-specific recognition protein
	CPIJ011748	conserved hypothetical protein
	CPIJ012737	nucleoporin 50kDa
	CPIJ013583	conserved hypothetical protein
	CPIJ013686	signal transduction protein lnk-realted

	CPIJ013709	conserved hypothetical protein
	CPIJ013938	conserved hypothetical protein
	CPIJ018995	conserved hypothetical protein
	CPIJ019612	myosin xv
	CPIJ019728	conserved hypothetical protein
	CPIJ019740	conserved hypothetical protein
	CPIJ020011	FACT complex subunit Ssrp1
PX domain	CPIJ001623	sorting nexin
	CPIJ005342	sorting nexin-6
	CPIJ006689	conserved hypothetical protein
	CPIJ010119	sorting nexin-9
	CPIJ013306	conserved hypothetical protein
PYP-like sensor domain (PAS domain)	CPIJ003682	hypoxia-inducible factor
	CPIJ013448	conserved hypothetical protein
	CPIJ014773	arylhydrocarbon receptor nuclear translocator
Rap/Ran-GAP	CPIJ018142	rap GTPase-activating protein
Ras GEF	CPIJ005593	ras GTP exchange factor, son of sevenless
	CPIJ005901	ral guanine nucleotide exchange factor 2
	CPIJ017680	c-AMP-dependent rap1 guanine-nucleotide exchange factor
Regulator of G-protein signaling, RGS	CPIJ004658	beta-adrenergic receptor kinase
	CPIJ006996	conserved hypothetical protein
	CPIJ015931	regulator of g protein signaling
SH2 domain	CPIJ000806	growth factor receptor-bound protein
	CPIJ003100	cytoplasmic protein NCK1
	CPIJ003380	suppressorsof cytokine signalling
	CPIJ010659	proto-oncogene tyrosine-protein kinase src
	CPIJ012129	conserved hypothetical protein
	CPIJ014900	corkscrew phosphatase
SH3-domain	CPIJ006389	abl interactor 2
	CPIJ002311	conserved hypothetical protein
	CPIJ002634	membrane traffic protein
	CPIJ003002	nebl protein
	CPIJ006223	Plenty of SH3s
	CPIJ010657	conserved hypothetical protein
	CPIJ016081	endophilin a
	CPIJ017698	conserved hypothetical protein
	CPIJ018548	abl interactor 2
TRAF domain-like	CPIJ001208	autoimmune regulator
	CPIJ001213	tripartite motif-containing protein 37
	CPIJ009247	E3 ubiquitin-protein ligase sina

			CPIJ010192	conserved hypothetical protein
		Transducin (alpha subunit), insertion domain	CPIJ016397	guanine nucleotide-binding protein G
		Transducin (heterotrimeric G protein), gamma chain	CPIJ005863	guanine nucleotide-binding protein gamma-1 subunit
		Ypt/Rab-GAP domain of gyp1p	CPIJ016901	conserved hypothetical protein
			CPIJ008317	TBC1 domain family member 22B
			CPIJ010266	GTPase-activating protein gyp2
			CPIJ011634	gh regulated tbc protein-1
Other	Unknown function	alpha/beta knot	CPIJ006883	conserved hypothetical protein
			CPIJ010464	conserved hypothetical protein
		Anti-sigma factor antagonist SpoIIaa	CPIJ006006	sulfate transporter
			CPIJ000611	sulfate transporter 1.2
			CPIJ005331	sulfate transporter
			CPIJ012147	sulfate transporter
			CPIJ017095	sulfate transporter
		beta-sandwich domain of Sec23/24	CPIJ008805	conserved hypothetical protein
			CPIJ009281	Sec24B protein
			CPIJ014598	conserved hypothetical protein
			CPIJ017651	conserved hypothetical protein
		BtrG-like	CPIJ005346	conserved hypothetical protein
			CPIJ008797	conserved hypothetical protein
		Crustacean CHH/MIH/GIH neurohormone	CPIJ003972	ion transport peptide
		Cysteine alpha-hairpin motif	CPIJ000336	conserved hypothetical protein
			CPIJ014050	predicted protein
			CPIJ019817	cytochrome c oxidase assembly protein COX19
		Cysteine-rich domain	CPIJ000792	myotonin-protein kinase
			CPIJ006705	protein kinase C
			CPIJ006920	conserved hypothetical protein
		Delta-sleep-inducing peptide immunoreactive peptide	CPIJ006273	conserved hypothetical protein
		E set domains	CPIJ002638	KDEL motif-containing protein 2
			CPIJ002736	conserved hypothetical protein
			CPIJ002737	MPA2 allergen
			CPIJ004282	hexamerin 2 beta
			CPIJ004652	conserved hypothetical protein
			CPIJ005750	conserved hypothetical protein
			CPIJ008373	conserved hypothetical protein
			CPIJ016072	SEC63 protein
			CPIJ016988	beta-arrestin 1
		Frataxin/Nqo15-like	CPIJ014024	frataxin, mitochondrial
		GckA/TtuD-like	CPIJ004803	glycerate kinase

	Hairpin loop containing domain-like	CPIJ005068	conserved hypothetical protein
		CPIJ005070	conserved hypothetical protein
		CPIJ018970	conserved hypothetical protein
	HCP-like	CPIJ001345	conserved hypothetical protein
	Hook domain	CPIJ018678	hook protein
	Ligand-binding domain in the NO signalling and Golgi transport	CPIJ010765	conserved hypothetical protein
		CPIJ019438	conserved hypothetical protein
	MAL13P1.257-like	CPIJ013731	conserved hypothetical protein
	PIN domain-like	CPIJ018772	conserved hypothetical protein
	PTPA-like	CPIJ017735	serine/threonine-protein phosphatase 2A regulatory subunitB'
	Pym (Within the bgcn gene intron protein, WIBG), N-terminal domain	CPIJ006916	conserved hypothetical protein
	Roadblock/LC7 domain	CPIJ002048	dynein light chain
		CPIJ011264	mitogen-activated protein-binding protein-interacting protein
		CPIJ014786	conserved hypothetical protein
	Subunits of heterodimeric actin filament capping protein Capz	CPIJ010373	F-actin capping protein subunit beta
		CPIJ011271	f-actin capping protein alpha
		CPIJ011272	conserved hypothetical protein
		CPIJ019319	F-actin capping protein subunit beta
	YggU-like	CPIJ001196	conserved hypothetical protein
	YjeF N-terminal domain-like	CPIJ011786	conserved hypothetical protein
	YjgF-like	CPIJ008399	conserved hypothetical protein
	Zinc beta-ribbon	CPIJ001855	DNA-directed RNA polymerase II 15.1 kDa polypeptide
Viral proteins	Arp2/3 complex 16 kDa subunit ARPC5	CPIJ012795	arp2/3 complex 16 kd subunit
	Retrovirus zinc finger-like domains	CPIJ001891	conserved hypothetical protein
	Tetrapyrrole methylase	CPIJ008675	diphthine synthase
	Eferin C-derminal domain-like	CPIJ003754	conserved hypothetical protein
	ERH-like	CPIJ013494	enhancer of rudimentary protein
	Expressed protein At2g23090/F21P24.15	CPIJ002299	conserved hypothetical protein

†Differentially expressed genes represent those genes that differed in their expression level (FPKM) in HAmCq^{G8} by more than two fold when compared to the parental strain HAmCq^{G0}.

*SCOP general and detailed functions using the predicted *Cx. quinquefasciatus* annotation information available at the Superfamily website (version 1.75)

**_Culex quinquefasciatus_ genome, Johannesburg strain CpipJ1.2, June 2008; http://cquinquefasciatus.vectorbase.org/

‡Vectorbase annotation taken from CpipJ1.2, June 2008; http://cquinquefasciatus.vectorbase.org/ with the exception of cytochrome P450 genes whose annotations were taken from the most current P450 annotation based on: Nelson, DR (2009) The Cytochrome P450 Homepage. Human Genomics 4, 59-65: http://drnelson.uthsc.edu/CytochromeP450.html

[§]NONA: Not annotated

Appendix 3.5. List of differentially upregulated genes in HAmCq^{G8} which contained functionally-enriched Gene Ontology terms.

Gene[†]	Annotation[*]	GO TERMS[**]																			
		catalytic activity (GO:0003824)	oxidoreductase activity (GO:0016491)	heme binding (GO:0020037)	tetrapyrrole binding (GO:0046906)	iron ion binding (GO: 0005506)	electron carrier activity (GO:009505)	monooxygenase activity (GO:004497)	hydrolase activity (GO:0016787)	peptidase activity (GO:0008233)	peptidase activity, acting on L-amino acid peptides (GO:0070011)	endopeptidase activity (G):0004175)	serine-type endopeptidase activity (GO:0004252)	serine-type peptidase activity (GO: 0008236)	serine hydrolase activity (GO:0017171)	metallopeptidase activity (GO:0008237)	exopeptidase activity (GO:0008238)	hydrolase activity, acting on glycosyl bonds (GO:0008238)	hydrolase activity, hydrolyzing O-glycosyl compounds (GO:0004553)	oxygen transporter activity (GO:0005322)
CPIJ020229	*CYP4D42v2*	+[¤]	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ017244	*CYP304B5*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ017243	*CYP304B4*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ015958	*CYP325BC1*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ015681	*CYP4H37v2*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ014218	*CYP9M10*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ012470	*CYP9AL1*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ011127	*CYP4H34*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ010546	*CYP9J34*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ010544	*CYP9J33*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ010543	*CYP9J40*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

CPIJ010542	*CYP9J38*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ010538	*CYP9J46*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ010537	*CYP9J45*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ010227	*CYP12F13*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ010225	*CYP12F14*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ009478	*CYP4D42v1*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ009085	*CYP6AG13*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ008566	*CYP6Z15*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ007188	*CYP4H30*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ006721	*CYP4H37v 1*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ005959	*CYP6AA7*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ005957	*CYP6AA9*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ005956	*CYP6BZ2*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ005955	*CYP6P14*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ005953	*CYP6BB3*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ005952	*CYP6BB4*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ002538	*CYP6AG12*	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ004088	guanylyl cyclase receptor	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ019428	trypsin 2	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ019007	polyserase-2	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ018037	serine protease	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ016102	transmembrane protease	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ016012	tryptase-2	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ014523	elastase-3A	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ010641	prostasin	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ006543	urokinase-type plasminogen activator	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ006542	chymotrypsin-2	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ006076	hypodermin-B	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ005272	trypsin 3A1	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ004594	conserved hypothetical protein	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ003623	coagulation factor XII	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ002156	chymotrypsin BI	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ002142	chymotrypsin BI	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ002140	chymotrypsin BI	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ002139	HzC4 chymotrypsinogen	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ002138	chymotrypsinogen	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ002137	serine protease1/2	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ002135	trypsin alpha-4	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ002133	trypsin epsilon	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ002130	kallikrein-7	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-

CPIJ002128	mast cell protease 2	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ001979	conserved hypothetical protein	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ001111	proacrosin	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ000617	clip-domain serine protease	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ000616	clip-domain serine protease	+	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-
CPIJ019029	metalloproteinase	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
CPIJ013319	metalloproteinase	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
CPIJ010224	metalloproteinase	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
CPIJ009594	nephrosin	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
CPIJ007383	endothelin-converting enzyme 1	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
CPIJ002945	zinc metalloproteinase dpy-31	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
CPIJ002943	conserved hypothetical protein	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
CPIJ002942	zinc metalloproteinase nas-12	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
CPIJ002941	high choriolytic enzyme 1	+	-	-	-	-	-	-	+	+	+	+	+	-	-	+	-	-	-	-
CPIJ012036	aminopeptidase N	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
CPIJ010805	carboxypeptidase A1	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
CPIJ009106	angiotensin-converting enzyme	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
CPIJ004086	angiotensin-converting enzyme	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
CPIJ001745	zinc carboxypeptidase	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
CPIJ001744	zinc carboxypeptidase	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
CPIJ001743	carboxypeptidase A2	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
CPIJ001742	zinc carboxypeptidase	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-
CPIJ008876	lysosomal pro-X carboxypeptidase	+	-	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-
CPIJ008873	prolylcarboxypeptidase	+	-	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	-
CPIJ001240	cathepsin B-like thiol protease	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
CPIJ001239	cathepsin B	+	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-
CPIJ001050	protease m1 zinc metalloprotease	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-	-
CPIJ014110	conserved hypothetical protein	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
CPIJ009738	conserved hypothetical protein	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
CPIJ010716	luciferin 4-monooxygenase	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ005187	phenoloxidase subunit 1	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ019598	basic endochitinase CHB4	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ018802	endochitinase A	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ009306	neutral alpha-glucosidase ab	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ008904	alpha-glucosidase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ008528	glycoside hydrolase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ006585	glycoprotein	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ006166	deltamethrin resistance-associated NYD-GBE	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ005451	lysozyme	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ004323	gram-negative bacteria binding protein	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-

CPIJ004320	gram-negative bacteria-binding protein 1	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ002104	plasma alpha-L-fucosidase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	-
CPIJ005725	alpha-amylase A	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
CPIJ005060	alpha-amylase B	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-
CPIJ019948	myosin vii	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ019917	triacylglycerol lipase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ018233	carboxylesterase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ018231	carboxylesterase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ017110	fumarylacetoacetate hydrolase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ016336	esterase B1	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ015649	DNA-binding protein smubp-2	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ013085	sarcalumenin	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ007824	esterase B1	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ007461	epoxide hydrolase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ007035	lipase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ006560	peptidoglycan recognition protein-lc	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ004695	dynein-1-beta heavy chain	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ004222	pancreatic triacylglycerol lipase	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ002103	conserved hypothetical protein	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ002067	vacuolar ATP synthase subunit C	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ001520	multidrug resistance-associated protein 1	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ000853	myosin heavy chain	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ000852	myosin-Id	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ003495	fatty acid synthase S-acetyltransferase	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
CPIJ014287	ferritin heavy chain	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ005308	conserved hypothetical protein	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ004595	cytochrome b5	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ004125	succinate dehydrogenase	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ018869	NADH dehydrogenase iron-sulfur protein 7, mitoch.	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ016440	dihydroceramide delta (4)-desaturase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ016322	alkyldihydroxyacetonephosphate synthase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ016321	alkyldihydroxyacetonephosphate synthase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ013647	alkyldihydroxyacetonephosphate synthase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ009438	aldehyde dehydrogenase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ007620	choline dehydrogenase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ005656	oxidoreductase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ004600	oxidoreductase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ004379	steroid dehydrogenase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ003802	NADP-dependent leukotriene B4 12-hydroxydehydrog.	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ003059	acyl-CoA oxidase	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CPIJ001318	d-lactate dehydrognease 2	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ018631	glutathione-s-transferase theta, gst	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ015996	ecdysteroid UDP-glucosyltransferase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ015088	4-coumarate-CoA ligase 1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ014577	phosphoglycerate mutase 2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ012763	3-phosphoinositide-dependent protein kinase 1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ011827	conserved hypothetical protein	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ009929	conserved hypothetical protein	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ009094	ornithine decarboxylase 1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ008853	maltose phosphorylase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ008110	conserved hypothetical protein	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ007538	arginine kinase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ006619	cystathionine gamma-lyase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ006508	UDP-glucuronosyltransferase 2B4	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ006459	long-chain-fatty-acid-CoA ligase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ006339	receptor of activated protein kinase C 1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ006160	glutathione s-transferase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ004867	conserved hypothetical protein	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ003692	glucosyl/glucuronosyl transferase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ002663	glutathione S-transferase 1-1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
CPIJ001427	conserved hypothetical protein	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ001091	lactosylceramide 4-alpha-galactosyltransferase	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ000791	conserved hypothetical protein	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CPIJ018825	larval serum protein 1 beta chain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
CPIJ018824	larval serum protein 1 beta chain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
CPIJ009032	larval serum protein 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
CPIJ007783	arylphorin subunit alpha	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
CPIJ006538	larval serum protein 1 beta chain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
CPIJ006537	larval serum protein 1 beta chain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
CPIJ001820	larval serum protein 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
CPIJ000056	larval serum protein 1 beta chain	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

[†]Genes within the functionally enriched GO terms for the differentially upregulated gene set in HAmCq^{G8} when tested by gProfiler

[*]*Culex quinquefasciatus* genome, Johannesburg strain CpipJ1.2, June 2008; http://cquinquefasciatus.vectorbase.org/ Annotations for cytochrome P450 genes were taken from the most current annotation based on: Nelson, DR (2009) The Cytochrome P450 Homepage. Human Genomics 4, 59-65: http://drnelson.uthsc.edu/CytochromeP450.html

[**]Gene Ontology consortium (version 1.2084; release date: 12:07:2011)

[¤] A "+" sign indicates GO terms within column that are assigned to the gene in the list, while a "-" sign indicates that GO terms within the column are not.

Appendix 4.1. List and sequences of the qRT-PCR primers used.

Gene*	Sense primer (5' to 3')	Antisense primer (5' to 3')
18S rRNA	CGCGGTAATTCCAGCTCCACTA	GCATCAAGCGCCACCATATAGG
CPIJ002139	TAATCTGTCGTGTCAATTGTCGTA	GGAAGCTATGTATTCCGATGAGAT
CPIJ001240	AGGACGTGAATATCGTTCTGAAAT	GTTCTGATAGATCTCGGCTTTCAT
CPIJ002156	CACTGCGTCGTTGATGCTAC	CCACATCATTTCGCACAATC
CPIJ016012	GGGAGTTATGTTGAGGACTTGAAA	GAAGGGTGGCACAGTTATTTATTC
CPIJ002142	TGAAATCCTTAGTAGTGCTTGCAG	TGACCAGAGAGAAGGATGTTGATA
CPIJ009594	GAAGTATCAGACAACCGCATTCTA	TTTCAAGTTGTTCATCACTGGTCT
CPIJ018233	GTCTGCTTGGGTTCTTCAGC	CGTCACATTGTTCGGATCAC
CPIJ001820	GTTGAATTCTACAAGCACGGTATG	CGTAGTAGAAAACGTGGAACAGAG
CPIJ000056	GAGCTACCTGCCATACTACACCTT	GAAGAAGTCAAAGTACGTGAGCAG
CPIJ009033	ATCGACTTCAGCTATTTCTTCACC	GTCGGTAGTGTTTAGTACGACGTG
CPIJ009032	AGTTGAGATCAAGGAGTTTTCCAG	GGGAGTTCTTGTAGTTGAAGGGTA
CPIJ007783	ACTACCAATTCAAGGATCACCTTC	AGTATGTGACCAACTTGTCAATGG

*_Culex quinquefasciatus_ genome, Johannesburg strain CpipJ1.3
http://cquinquefasciatus.vectorbase.org/

Appendix 4.2: Genes up-regulated in the pyrethroid-resistant HAmCq^{G8} strain of *Culex quinquefasciatus* following permethrin challenge.

SCOP functional annotation†			Vectorbase annotation‡		FPKM¤	Fold change (24h)		
							Permethrin	
General	Detailed	Superfamily	Accession	Gene	Untreated	Acetone	LC_{50}	LC_{70}
Extra-cellular processes	Cell adhesion	C-type lectin-like	CPIJ019507	salivary C-type lectin	52.8	1.7	3.9	4.2*
			CPIJ016716	conserved hypothetical protein	23.8	3.4*	2.7	8.2*
		Immunoglobulin	CPIJ015022	stretchin-mlck	0.5	2.5	3.7	6.5*
		RNI-like	CPIJ001238	conserved hypothetical protein	1.9	3.7*	4.1*	4.0*
			CPIJ018057	conserved hypothetical protein	0.6	3.5	4.1*	4.2
	Toxins/defense	Superantigen toxins	CPIJ003460	M12 mutant protein precursor, putative	0.3	4.2	4.2	6.4*
General	General	ARM repeat	CPIJ005388	conserved hypothetical protein	1	4.2*	3.5	4.4*
			CPIJ801656	armadillo repeat-containing protein 4	1	3.7*	3.9*	3.6
		EF-hand	CPIJ002594	NADPH oxidase	1.4	2	4.4*	3
			CPIJ008636	conserved hypothetical protein	1.2	3.3*	3.3	3.1
			CPIJ001307	predicted protein	0.9	4.9	9.5*	5.4
		GLA-domain	CPIJ802301	conserved hypothetical protein	29.6	5.8*	3.7	14.3*
		Kelch motif	CPIJ011586	actin binding protein, putative	5	1.6	1.8	3.8*
			CPIJ003681	ring canal kelch protein	0.7	3.6*	4.3*	3.2
		L domain-like	CPIJ802473	conserved hypothetical protein	3.4	2.1	7.1*	7.3*
			CPIJ002160	conserved hypothetical protein	1.6	1.9	4.6*	4.5*
			CPIJ802491	conserved hypothetical protein	1.3	1.8	7.0*	7.4*
			CPIJ802477	leucine rich protein	1.3	1.3	4.4*	4.1*
		Spermadhesin, CUB domain	CPIJ015617	conserved hypothetical protein	1.1	1.8	4.4*	2.5
		WD40 repeat-like	CPIJ000996	axonemal dynein intermediate chain polypeptide	0.7	3.1	4.2*	3.2
	Protein interaction	Ankyrin repeat	CPIJ801726	conserved hypothetical protein	18.9	3.2*	4.1	3.4
		POZ domain	CPIJ008757	conserved hypothetical protein	0.2	10.0*	6.1*	23.0*
	Small molecule binding	FAD/NAD(P)-binding domain	CPIJ001367	glucose dehydrogenase	3.7	1.9	4.9*	3.6
			CPIJ017482	choline dehydrogenase	2.7	1.8	3.9*	3.2*
			CPIJ017491	glucose dehydrogenase	2	1.5	5.1*	3.2
			CPIJ013724	dimethylaniline monooxygenase	1.7	1.3	2.4	4.3*
			CPIJ007619	glucose dehydrogenase	1.4	2.3	4.9*	3.2
			CPIJ017490	glucose dehydrogenase	0	1.3*§	1.7*§	2.3*§
		GST C-terminal domain-like	CPIJ002679	glutathione S-transferase theta-2	8.7	1.9	15.6	15.0*
		NAD(P)-binding Rossmann-fold domains	CPIJ017620	hypothetical protein	97.2	3.5*	5.5	8.2*
			CPIJ009105	hypothetical protein	27	2.5	7.6*	5.1*
			CPIJ007450	conserved hypothetical protein	2.2	2.8*	2.7*	5.7*
			CPIJ004391	fatty acyl-CoA reductase 1	25.6	4.4*	6	10.3*
			CPIJ011767	short-chain dehydrogenase	22.5	4.2	3.5	11.8*
			CPIJ003056	hydroxysteroid dehydrogenase	10.6	2.0*	1.6*	4.5*
			CPIJ019942	conserved hypothetical protein	9.1	4.1	5.0*	10.5*
			CPIJ019941	conserved hypothetical protein	7.1	2.2	3.2*	6.9
			CPIJ014121	short-chain dehydrogenase	3.5	1.4	4.0*	2.5
			CPIJ007244	fatty acyl-CoA reductase 1	2.4	2.3	4.8	5.1*
			CPIJ007245	fatty acyl-CoA reductase 1	1.3	3.2	7.2*	7.2*
			CPIJ004392	fatty acyl-CoA reductase 2	1.1	4.0*	7.4*	8.0*

		P-loop containing nucleoside triphosphate hydrolases	CPIJ009593	conserved hypothetical protein	1.3	4.1*	4.1*	4.6*
			CPIJ000848	myosin-2 heavy chain	0.9	2.5	3.8*	4.6*
			CPIJ012205	dynein-1-beta heavy chain	0.4	2.6	3.6*	3
			CPIJ007215	ciliary dynein heavy chain 5	0.3	2.8	4.1*	3.1
			CPIJ012527	ciliary dynein heavy chain 5	0.3	3.2*	3.9*	3.9*
			CPIJ002912	ciliary dynein heavy chain 11	0.3	3.1*	3.5	3.1
			CPIJ001823	conserved hypothetical protein	0.3	3.2*	3.2	3.3
			CPIJ013371	conserved hypothetical protein	0.2	3.7*	4.6*	3.9
			CPIJ004695	dynein-1-beta heavy chain	0.2	3.2*	4.3*	4.7*
			CPIJ017862	sulfotransferase	0.1	4.3	1.3	11.9*
			CPIJ005250	conserved hypothetical protein	0.1	6.6*	8.7*	5.8
			CPIJ011001	sulfotransferase	0.1	22.7	4.8	43.1*
			CPIJ009296	conserved hypothetical protein	0.5	4	5.5*	6.5*
		Thiamin diphosphate-binding fold (THDP-binding)	CPIJ011488	conserved hypothetical protein	0.1	11.8	41.0*	40.2*
Information	Chromatin structure	Histone H3 K4-specific methyltransferase SET7/9 N-terminal domain	CPIJ009321	conserved hypothetical protein	1.6	1.9	5.1*	4.5*
			CPIJ801398	conserved hypothetical protein	1	4.4*	3.9	4.3
	DNA replication/ repair	FYVE/PHD zinc finger	CPIJ011620	conserved hypothetical protein	1.7	4.0*	4.0*	4.0*
		Nucleic acid-binding proteins	CPIJ000529	conserved hypothetical protein	0.1	3	1.9	10.9*
		RING/U-box	CPIJ007874	predicted protein	0	0.6§	0.6§	1.2*§
			CPIJ015248	conserved hypothetical protein	0.2	7.6	9.2*	6.6
	Translation	Ribosome inactivating proteins (RIP)	CPIJ009211	conserved hypothetical protein	0.3	7.1*	7.2*	15.4*
Intra-cellular processes	Cell cycle, Apoptosis	Inhibitor of apoptosis (IAP) repeat	CPIJ006918	conserved hypothetical protein	38.8	2.1	5.5*	5.9*
	Cell motility	Outer arm dynein light chain 1	CPIJ802497	conserved hypothetical protein	0.1	7.9	18.6*	21.0*
		Phase 1 flagellin	CPIJ009539	hypothetical protein	22.5	2.2	4.8*	4.0*
		Tropomyosin	CPIJ001907	conserved hypothetical protein	2.4	1.9	3.5	3.8*
			CPIJ802332	conserved hypothetical protein	0.6	3.8	3.6	4.7*
		Tubulin C-terminal domain-like	CPIJ001353	tubulin alpha chain	4.5	5.4*	5.6*	5.3*
		Tubulin nucleotide-binding domain-like	CPIJ018045	tubulin alpha-1 chain	1.2	5.3*	5.4*	4.8*
			CPIJ012634	tubulin beta-3 chain	0.3	5.7*	6.7*	5.4*
	Ion m/tr	Cupredoxins	CPIJ010466	laccase-like multicopper oxidase 1	2.4	1.8	3.6*	3.2
		Ferritin-like	CPIJ000900	conserved hypothetical protein	16.1	2.6	5.7*	3.8
		MFS general substrate transporter	CPIJ002794	hypothetical protein	4.8	3.3	7.1*	6.4*
			CPIJ015621	cis,cis-muconate transport protein MucK, putative	3.3	2.2	1.3	5.4*

		CPIJ002172	oligopeptide transporter	2.5	2.1	4.7*	4.2*
		CPIJ008813	sodium-dependent phosphate transporter	1.1	3.5*	2.9	3.4
		CPIJ018461	mfs transporter	0.6	4.3	2.9	11.0*
		CPIJ002957	sugar transporter	0.4	3.2	1.7	7.5*
		CPIJ019487	organic cation transporter, putative	0.3	3.4	2.6	10.3*
		CPIJ002956	sugar transporter	0.3	3.4	3.8	6.7*
	Periplasmic binding protein-like II	CPIJ008094	conserved hypothetical protein	3.9	1.6	4.1*	3.3
	SET domain	CPIJ019392	conserved hypothetical protein	0.1	11.0*	5	40.9*
Phospholipid m/tr	CRAL/TRIO domain	CPIJ801421	CRAL/TRIO domain-containing protein	7.6	1.8	3.9*	5.9*
		CPIJ014225	CRAL/TRIO domain-containing protein	8.3	3.4*	3.3	8.3*
		CPIJ013676	cellular retinaldehyde binding protein, putative	4.1	1.5	4.0*	4.2*
		CPIJ013463	conserved hypothetical protein	1.8	1.6	4.1*	2.7
Proteases	Cysteine proteinases	CPIJ001240	cathepsin B precursor	28.2	-1.6	2.5*	2.2*
	LuxS/MPP-like metallohydrolase	CPIJ013977	conserved hypothetical protein	3	2.0*	3.8	2.7
	Metalloproteases ("zincins"), catalytic domain	CPIJ009594	nephrosin	14.1	-1.1	22.2*	15.6*
		CPIJ001808	conserved hypothetical protein	2.3	1.5	3.7*	3.2
		CPIJ012680	ADAM 12 precursor	0.5	2.5	3.2	5.1*
		CPIJ009594	Astacin precursor	14.1	-1.1	22.2*	15.6*
	Pyrrolidone carboxyl peptidase (pyroglutamate aminopeptidase)	CPIJ009328	conserved hypothetical protein	1.9	3.7	7.5*	8.4
	Serine protease inhibitors	CPIJ012287	hypothetical protein	52.1	-5.9	3.8*	2.1
	Serpins	CPIJ010576	endopin-1 precursor	2.9	1.2	2.5	4.1*
	Subtilisin-like	CPIJ014726	conserved hypothetical protein	0.5	2.4	5.6*	3.2
	Trypsin-like serine proteases	CPIJ010091	hypothetical protein	43.5	3.3*	3.2*	3.5*
		CPIJ801497	serine protease	1.5	2.1	2.8	4.6*
		CPIJ006544	chymotrypsinogen 2 precursor	43.6	2.5	7.4*	6.0*
		CPIJ011901	polyserase-2 precursor	13.3	1.5	3	4.0*
		CPIJ018800	ovochymase-2 precursor	8.3	1.4	3.7	4.0*
		CPIJ003994	serine collagenase 1 precursor, putative	8.2	2.3	3.4	5.3*
		CPIJ010615	proclotting enzyme precursor	2.9	1.8	4.8*	3.9*
		CPIJ009891	serine protease	1.8	1.4	3.9*	2.7
		CPIJ017797	neurohypophysial hormones	0.7	2	5.2*	4.5*
		CPIJ004092	oviductin	0.6	5.6*	8.1*	14.5*
		CPIJ015103	trypsin-5 precursor	0.6	2.3	3.2	5.9*
		CPIJ019781	trypsin 1 precursor	0.1	90.2*	25.3*	222.0*
		CPIJ003325	anionic trypsin-2 precursor	0	1.6*§	0.0§	3.2*§
		CPIJ006869	mast cell protease 3 precursor	0	0.1§	0.3§	1.0*§
		CPIJ004984	serine proteases 1/2 precursor	0	1.6*§	0.0§	5.5*§
		CPIJ018529	trypsin 1 precursor	0	0.7§	0.4§	1.3*§
		CPIJ002140	collagenase precursor	124	1	2.6*	3.2*
		CPIJ016012	collagenase precursor	85.6	1.3	1.5*	1.1
	Zn-dependent exopeptidases	CPIJ000990	cytosol aminopeptidase	12.7	3.9*	4.5*	4.4*
		CPIJ003539	cytosol aminopeptidase	12.1	3.6*	3.4	4

			CPIJ009640	cytosol aminopeptidase	3.4	3.2*	3.5	3.4
			CPIJ011999	zinc carboxypeptidase A 1 precursor	0.1	6.2	2.5	13.7*
	Protein modification	HSP20-like chaperones	CPIJ005642	heat shock protein 27	1.9	1.3	-2.1	6.3*
			CPIJ005646	alphaA-crystallin, putative	1.1	3.4	-4.7	11.4*
	Transport	Integral outer membrane protein TolC, efflux pump component	CPIJ013907	conserved hypothetical protein	1.2	2.4	4.6*	3.6
			CPIJ018992	conserved hypothetical protein	0.8	2.7	5.4*	3.6
		Lipocalins	CPIJ015730	apolipoprotein D, putative	13.5	-1.1	5.8*	5.4*
			CPIJ015727	apolipoprotein D, putative	0.9	-1.3	2.9	5.4*
		Mitochondrial carrier	CPIJ005941	ADP,ATP carrier protein 2	1.4	2.3	1.8	4.4*
		SNARE fusion complex	CPIJ009052	myosin motor, putative	1.8	3.1*	3.4	3.6
		t-snare proteins	CPIJ014869	conserved hypothetical protein	1.8	3.6*	3.9	3.7
Metabolism	Amino acids m/tr	Glutamine synthetase/guanido kinase	CPIJ002029	arginine kinase	5.4	3.9*	4.3*	3.9*
	Carbohydrate m/tr	(Trans)glycosidases	CPIJ013084	brain chitinase and chia	3.4	1.8	1.9	3.8*
			CPIJ008532	glycoside hydrolases	1.1	3.5*	1.6	12.4*
			CPIJ019693	alpha-glucosidase precursor	0.5	-1.3	2.3	5.1*
		Invertebrate chitin-binding proteins	CPIJ000248	conserved hypothetical protein	16.4	1.8	4.3*	2.3
			CPIJ006133	conserved hypothetical protein	16.2	2.1	2.7	4.2*
			CPIJ003962	conserved hypothetical protein	6.7	1.6	4.3*	3.4
			CPIJ009451	conserved hypothetical protein	6.3	6.5*	10.5*	13.3*
		Xylose isomerase-like	CPIJ005176	protein G12 precursor	0	1.3§	0.0§	2.1*§
	Coenzyme m/tr	Acyl-CoA dehydrogenase NM domain-like	CPIJ016452	acyl-coa dehydrogenase	3.7	4.2*	1.8	2.4
			CPIJ016453	acyl-coa dehydrogenase	0.3	20.0*	7.7*	13.5*
		Cell wall binding repeat	CPIJ006797	conserved hypothetical protein	7.4	1.7	3.9*	2.8
		Glutathione synthetase ATP-binding domain-like	CPIJ006090	conserved hypothetical protein	0.7	3.9*	4.2*	3.8
		Precorrin-8X methylmutase CbiC/CobH	CPIJ019685	conserved hypothetical protein	0	0.5*§	0.5*§	1.0*§
	E- transfer	Cytochrome c	CPIJ010388	cytochrome c-2	7.5	3.6*	3.7	4.1*
			CPIJ000571	conserved hypothetical protein	3.4	4.7	4.3*	4.2*
	Energy	Mitochondrial cytochrome c oxidase subunit VIIa	CPIJ014384	conserved hypothetical protein	4.4	2.5*	4.4*	4.4*
		Mitochondrial cytochrome c oxidase subunit VIIIb (aka IX)	CPIJ008751	conserved hypothetical protein	0.2	18.2*	9.7*	55.9*
	Lipid m/tr	Apolipoprotein A-I	CPIJ802074	conserved hypothetical protein	35	1.4	6.7*	6.5*
			CPIJ801556	conserved hypothetical protein	0.1	37.0*	-2.1	81.5*
	Nucleotide	Nucleoside	CPIJ012972	conserved hypothetical protein	11	1.7	5.2*	3.2

m/tr	hydrolase						
Other enzymes	Acetyl-CoA synthetase-like	CPIJ015716	4-coumarate-CoA ligase 1	0.1	20.0*	8.6	51.4*
		CPIJ003423	AMP dependent ligase	4.1	4.1*	4.8*	10.8*
	Actin-like ATPase domain	CPIJ014564	heat shock protein 70 B2	1.3	4.0*	4.6*	3.9*
	alpha/beta-Hydrolases	CPIJ019227	pancreatic triacylglycerol lipase precursor	4.7	1.1	3.2	4.5*
		CPIJ005348	lipase 3 precursor	11.7	1.1	4.0*	5.5*
		CPIJ002073	juvenile hormone esterase	9	1.3	4.1*	3.8
		CPIJ013679	alpha-esterase, putative	1.8	2.3	3.8*	2.5
		CPIJ016686	esterase FE4 precursor	1.5	-1.6	7.4*	5.0*
		CPIJ006547	hepatic triacylglycerol lipase precursor	0.4	1.1	8.5*	5.8*
		CPIJ002720	lysosomal acid lipase, putative	0.2	5.4	8.6*	11.5*
	Galactose mutarotase-like	CPIJ802328	conserved hypothetical protein	0.7	4.5*	4.4*	3.2
	Glycoside hydrolase/deacetylase	CPIJ018088	conserved hypothetical protein	4.1	2.4	4.2*	4.4*
	Lysozyme-like	CPIJ009668	conserved hypothetical protein	0.7	9.4*	1.4	3.3
		CPIJ014435	hypothetical protein	20.5	3.7*	6.2*	7.1*
	SGNH hydrolase	CPIJ012577	phospholipase b, plb1	1.2	2.4	4.8*	4.1
		CPIJ012575	phospholipase b, putative	0.4	2.3	5.9*	5.6*
	N-terminal nucleophile aminohydrolases (Ntn hydrolases)	CPIJ019795	conserved hypothetical protein	0.1	5.9*	7.4*	6.2*
Polysaccharide m/tr	UDP-Glycosyltransferase/glycogen phosphorylase	CPIJ000038	UDP-glucuronosyltransferase 1-3 precursor	0.9	-1.3	5.0*	4.9
		CPIJ000040	UDP-glucuronosyltransferase 2B1	0.1	2	13.3*	11.7*
		CPIJ009316	hypothetical protein	37.2	3.1*	6.3	7.5*
		CPIJ802191	glucosyl transferase	6.5	-1.5	3.7*	3.4
Redox	Aminoacid dehydrogenase-like, N-terminal domain	CPIJ011318	conserved hypothetical protein	0.3	2.0*	7.0*	7.6*
	Aromatic aminoacid monoxygenases, catalytic and oligomerization domains	CPIJ014156	conserved hypothetical protein	33.9	2.8	3.7	6.3*
	Cytochrome P450	CPIJ017198	CYP325BF1-de1b£	0.8	2.7	8.1*	4.2*
		CPIJ015953	CYP325BF1v2£	0.5	2.3	5.0*	3.6
		CPIJ019704	CYP6N11£	2.1	-1.0*	6.8*	10.0*
		CPIJ800210	CYP6BY2£	8.4	-1.3	13.9*	14.7*
		CPIJ800176	CYP6M14£	8.1	-1.2	4.0*	3.4
		CPIJ800256	CYP4H34£	6.9	1	4.8*	4.4
		CPIJ800180	CYP6N19£	3.3	-1.1	10.9*	12.4*
		CPIJ800178	CYP6M16£	2.6	-1.8	3.7*	4.2*
		CPIJ800175	CYP6M13£	1	-2.5	6.2*	5.6*
		CPIJ800177	CYP6M15£	0.3	0	8.9	10.2*
	Formate/glycerate dehydrogenase	CPIJ002343	conserved hypothetical protein	0.8	1.8	4.2*	3.2*

		catalytic domain-like						
		LDH C-terminal domain-like	CPIJ004727	conserved hypothetical protein	0.5	4	4.2*	4.5
		NAD(P)-linked oxidoreductase	CPIJ000901	conserved hypothetical protein	7.6	2.1	5.0*	3.5*
			CPIJ009681	translocator protein	1.8	4.5	4.1*	4.7*
		Thioredoxin-like	CPIJ010478	conserved hypothetical protein	1.1	3.4	3.8*	4.2
	Secondary metabolism	Concanavalin A-like lectins/glucanases	CPIJ001299	keratinocyte lectin, putative	1.4	1.4	5.1*	4
			CPIJ016495	thrombospondin-4, putative	0.2	1.4	4.2	5.7*
		Dimeric alpha+beta barrel	CPIJ002813	conserved hypothetical protein	3.4	1.5	5.4*	2.7
		PR-1-like	CPIJ000211	cysteine-rich secretory protein-2, putative	1.7	2.2	6.8*	9.5*
	Transferases	PLP-dependent transferases	CPIJ010034	glutamate decarboxylase	1.7	6.0*	3.2	17.7*
No annotation	No annotation	No annotation	CPIJ007504	bumetanide-sensitive sodium-(potassium)-chloride cotransporter	2.9	2.1	5.4*	4.0*
			CPIJ004245	cationic amino acid transporter	1.8	2.2	6.7*	4.5*
			CPIJ010699	cecropin	25	1.5	1.7	4.6*
			CPIJ005108	cecropin	12.5	2.5	7.7*	8.0*
			CPIJ004293	cuticle protein, putative	10	1.9	5.1*	4.7*
			CPIJ003491	cuticle protein, putative	11	5.1*	-1.5	10.9*
			CPIJ017925	cuticle protein, putative	7.7	1.8	1.8	4.0*
			CPIJ001839	cuticle protein, putative	6.5	2.6	7.3*	4.4*
			CPIJ000106	elongase, puatative	4.3	1.4	3.8*	2.4
			CPIJ013663	elongase, putative	9.3	2.2	2.1	3.7*
			CPIJ003687	elongation of very long chain fatty acids protein 4	0.3	2.5	8.9*	7.9*
			CPIJ006964	high affinity nuclear juvenile hormone binding protein, putative	3.2	2.3	5.0*	4.0*
			CPIJ016318	larval cuticle protein 8.7	2.5	1.8	4.4	5.0*
			CPIJ004289	larval cuticle protein A3A	9.5	1.8	6.6*	6.5*
			CPIJ002801	larval/pupal cuticle protein H1C precursor	36.7	1.9	5.8*	4
			CPIJ005349	lysosomal acid lipase, putative	5	1.2	4.0*	6.3*
			CPIJ004704	myosin motor, putative	0.3	4.5*	3.4	3.4
			CPIJ007819	NADH:ubiquinone dehydrogenase, putative	4.7	3.9	4.8*	6.4*
			CPIJ008136	osiris 11	3.7	3.3*	5.0*	7.3*
			CPIJ009829	osiris 18	15.5	1.4	4.1*	2.4
			CPIJ004911	osiris 21, putative	0.5	3	2	6.4*
			CPIJ008140	Osiris, putative	7.3	1.9	2.6	4.9*
			CPIJ011100	Osiris, putative	1.5	1.9	4.8*	3.6
			CPIJ001618	protofilament ribbon protein	2.5	3.1*	3.3	3.6
			CPIJ006318	proton-coupled amino acid transporter 1	2.8	3.0*	2.9	6.0*
			CPIJ009324	pupal cuticle protein 78E, putative	7.2	2.3	5.7*	5.7*
			CPIJ018582	pupal cuticle protein, putative	11.9	1.7	4.4*	3.5
			CPIJ008231	pupal cuticle protein, putative	10.8	1.7	4.6*	3.2
			CPIJ010700	putative 4.2 kda basic salivary peptide	2.5	2.3	2.4	10.3*
			CPIJ008286	serine protease, putative	6	1.6	4.3*	2.8
			CPIJ012056	sodium-dependent serotonin	2.9	2	4.1*	3

	transporter				
CPIJ801536	sodium/potassium-dependent ATPase beta-2 subunit	0.8	14.2*	32.5*	24.9*
CPIJ012066	sodium/shloride dependent amino acid transporter, putative	1.9	2.4	44.6*	42.7*
CPIJ801722	sodium/solute symporter	2	-1.1	6.0*	5.1*
CPIJ801721	sodium/solute symporter	0.5	2.4	7.5*	3.7
CPIJ015023	stretchin-mlck	0.2	2.5	5	12.2*
CPIJ013788	structural contituent of cuticle, putative	90.5	2	5.1*	4.2
CPIJ012065	tryptophan transporter	0.8	2.9	17.5*	21.2*
CPIJ802272	conserved hypothetical protein	46.8	3.5*	4.1*	3.8
CPIJ018910	conserved hypothetical protein	36.2	3.5*	3	9.3*
CPIJ007289	conserved hypothetical protein	22.1	1.8	5.1*	3.4
CPIJ014232	conserved hypothetical protein	19.8	4.4*	2.2	11.2*
CPIJ017629	conserved hypothetical protein	19.5	3.0*	3.5	3.3
CPIJ004475	conserved hypothetical protein	19.1	1.9	4.1*	3.7*
CPIJ012507	conserved hypothetical protein	16.1	2.9*	4.0*	5.4*
CPIJ008525	conserved hypothetical protein	14.2	4.3*	4.9*	5.1*
CPIJ017913	conserved hypothetical protein	13.4	3.0*	3.6	3.4
CPIJ802297	conserved hypothetical protein	12.5	2.1	2.2	5.1*
CPIJ009334	conserved hypothetical protein	12.3	1.2	4.0*	2.5
CPIJ006965	conserved hypothetical protein	10.1	2	3.8*	5.5*
CPIJ002836	conserved hypothetical protein	8.8	1.1	3.5	4.3*
CPIJ010705	conserved hypothetical protein	6.6	1.4	3.8*	3.2
CPIJ010748	conserved hypothetical protein	6	1.7	4.1*	3.5
CPIJ801448	conserved hypothetical protein	5.7	1.5	3.8*	2.6
CPIJ006959	conserved hypothetical protein	5.5	2.4	5.4*	4.0*
CPIJ009100	conserved hypothetical protein	5.3	2.3	4.9*	4.3*
CPIJ004790	conserved hypothetical protein	5	2.1	4.3*	3.6
CPIJ008211	conserved hypothetical protein	4.6	2.9	-2.8	5.5*
CPIJ007448	conserved hypothetical protein	4.1	8.2*	5.9*	19.5*
CPIJ000450	conserved hypothetical protein	4	1.8	3.8*	3.3
CPIJ016842	conserved hypothetical protein	3.7	1.6	4.1*	3.2
CPIJ011498	conserved hypothetical protein	3.7	1.4	4.0*	3.8*
CPIJ010184	conserved hypothetical protein	3.3	3.4*	4.7*	4
CPIJ007813	conserved hypothetical protein	3	3.2*	4.8*	3.4
CPIJ020250	conserved hypothetical protein	2.7	4.9*	4.2*	5.8*
CPIJ006892	conserved hypothetical protein	2.3	3.1	8.0*	4.9*
CPIJ002597	conserved hypothetical protein	2.1	2	3.9*	3.1
CPIJ013908	conserved hypothetical protein	1.9	2.5	4.2*	3.7
CPIJ010176	conserved hypothetical protein	1.8	2.1	4.2*	4.5*
CPIJ007344	conserved hypothetical protein	1.7	1.6	4.0*	3.1
CPIJ006083	conserved hypothetical protein	1.7	2.7	4.6*	3.9
CPIJ008664	conserved hypothetical protein	1.4	5.6*	13.6*	10.2*
CPIJ016283	conserved hypothetical protein	1	2.1	1.6	6.6*
CPIJ014725	conserved hypothetical protein	0.9	-1	6.1*	2.3
CPIJ011914	conserved hypothetical protein	0.9	4.2*	3.2	4.4
CPIJ018462	conserved hypothetical protein	0.8	6.1*	5.2	16.8*
CPIJ008775	conserved hypothetical protein	0.7	5.0*	2.4	2.8
CPIJ016278	conserved hypothetical protein	0.6	6.1*	5.0*	6.1*
CPIJ011487	conserved hypothetical protein	0.6	5.9	10.9*	10.7*
CPIJ018794	conserved hypothetical protein	0.6	2.3	3.5	6.3*
CPIJ005199	conserved hypothetical protein	0.4	2.6	6.2*	3.4
CPIJ006046	conserved hypothetical protein	0.4	74.4*	4.8	259.6*
CPIJ018443	conserved hypothetical protein	0.3	4.5*	4.5	5.2*
CPIJ007442	conserved hypothetical protein	0.3	3.3	9.9*	5.9
CPIJ008147	conserved hypothetical protein	0.2	5.7	5	12.4*
CPIJ017621	conserved hypothetical protein	0.2	6.6	9.2*	7.8
CPIJ002735	conserved hypothetical protein	0	2.5*§	2.2*§	2.9*§
CPIJ006215	conserved hypothetical protein	0	2.0§	0.8§	7.3*§
CPIJ014238	conserved hypothetical protein	0	0.2§	0.5§	1.3*§

			CPIJ014355	conserved hypothetical protein	0	1.3§	0.8§	3.5*§
			CPIJ802086	hypothetical protein	77.4	2.3	6.5*	4.6
			CPIJ010703	hypothetical protein	50.4	2.3	7.0*	5.0*
			CPIJ009104	hypothetical protein	43.4	1.9	6.3*	4.2*
			CPIJ012015	hypothetical protein	43	2.8*	2.6	2.8
			CPIJ009101	hypothetical protein	38.3	2.1	4.9*	4.7
			CPIJ011254	hypothetical protein	31.6	2.1	6.0*	5.2*
			CPIJ004688	hypothetical protein	23.2	3.9*	5.2*	4.8*
			CPIJ014436	hypothetical protein	21.4	2.1	5.4*	2.9
			CPIJ019329	hypothetical protein	14.6	-5.6	8.2*	9.4*
			CPIJ000531	hypothetical protein	12.9	1.4	2.4	4.2*
			CPIJ013119	hypothetical protein	9.2	3.0*	4.2*	7.1*
			CPIJ010444	hypothetical protein	2.9	3.4	7.0*	6.2*
			CPIJ018459	hypothetical protein	2.1	2.6	1.3	6.9*
			CPIJ005376	hypothetical protein	1	2.6	4.5	6.8*
			CPIJ019239	hypothetical protein	0.7	6.9	42.1*	11.5
			CPIJ015171	hypothetical protein	0	1.0§	2.2*§	0.9§
Other	Unknown function	Cysteine-rich domain	CPIJ007580	conserved hypothetical protein	0.4	3.1	4.8*	4.6
		E set domains	CPIJ020106	translocator protein	0.9	6.5*	6.8*	8.2*
			CPIJ002737	MPA2 allergen	11.4	2	1.7	3.9*
			CPIJ013180	conserved hypothetical protein	5.5	-1.1	1.1	7.9*
		Fibrinogen coiled-coil and central regions	CPIJ014143	conserved hypothetical protein	0.3	4.8*	5.6*	4.7
		SpoIIaa-like	CPIJ005331	sulfate transporter, putative	1	2.5	1.8	5.2*
Regulation	DNA-binding	beta-beta-alpha zinc fingers	CPIJ007837	zinc finger protein	8.2	-1	3.4	4.1*
			CPIJ016287	conserved hypothetical protein	1.7	3.2*	2.2	2.6
			CPIJ018516	conserved hypothetical protein	0.3	8.9*	7.3	10.6*
		Glucocorticoid receptor-like (DNA-binding domain)	CPIJ008216	nuclear hormone receptor ftz-f1	3.1	2.8	4.6*	4.2*
		Homeodomain-like	CPIJ012997	Eip93F	1.8	3.1*	4.6*	4.8*
		Leucine zipper domain	CPIJ019348	sarcolemmal associated protein, putative	0.1	3.4	8.1*	6.1
			CPIJ004502	conserved hypothetical protein	0.1	10.2*	11.4*	12.8*
		RPB6/omega subunit-like	CPIJ005881	conserved hypothetical protein	0.2	9.0*	8.2*	7.1*
	Kinases/phosphatases	Protein kinase-like (PK-like)	CPIJ801694	testis-specific serine/threonine-protein kinase 1	1.5	4.0*	4.9*	4.1*
			CPIJ005558	conserved hypothetical protein	1.8	-1.9	6.9*	6.5*
			CPIJ013214	rage-1	0.3	5.9*	4	4.8
			CPIJ007354	testis-specific serine/threonine-protein kinase 6	0.2	4.6	6.5*	5.9
			CPIJ017094	protein serine/threonine kinase, putative	16	1.7	4.2*	3.4
	Receptor activity	Cytoplasmic domain of a serine chemotaxis receptor	CPIJ009621	conserved hypothetical protein	1	3.1*	4.0*	3.5
			CPIJ006799	conserved hypothetical protein	0.3	5.6*	1.6	15.7*
	Signal transduction	cAMP-binding domain-like	CPIJ008823	conserved hypothetical protein	0.3	3.5	5.0*	4.7
			CPIJ006213	cyclic nucleotide-gated cation channel 4	0.1	3.9	6.1*	6.1*
		Insect pheromone/odor	CPIJ012719	odorant binding protein OBP20	1.9	2.2	4.6*	3

ant-binding proteins						
	CPIJ001874	Odorant-binding protein 56a, putative	0.9	31.6*	3	62.1*
	CPIJ009568	odorant binding protein OBP8	0.3	-1	37.2*	24.0*
	CPIJ801715	Odorant-binding protein 56a	0.2	36.5*	2.9	63.1*
Nicotinic receptor ligand binding domain-like	CPIJ016909	nicotinic acetylcholine receptor, beta-2 subunit, putative	2.9	1.3	3.4	5.3*
Nuclear receptor ligand-binding domain	CPIJ014945	nuclear hormone receptor ftz-f1	8.2	2.6	5.1*	4.0*
	CPIJ008215	nuclear hormone receptor ftz-f1	4.5	2	4.4*	3.1
PDZ domain-like	CPIJ001710	conserved hypothetical protein	0.3	4.4	3.6	11.0*
PYP-like sensor domain (PAS domain)	CPIJ007193	period circadian protein	0.4	3.6*	2.8	2.8
Regulator of G-protein signaling, RGS	CPIJ004658	beta-adrenergic receptor kinase	0.5	5.1	7.1*	5.3
Ypt/Rab-GAP domain of gyp1p	CPIJ001736	conserved hypothetical protein	0.7	3.7*	3.1	3.9

[†]SCOP general and detailed functions using the predicted *Cx. quinquefasciatus* annotation information available at the Superfamily website (version 1.75) supfam.cs.bris.ac.uk/SUPERFAMILY/index.html

[‡]*Culex quinquefasciatus* genome, Johannesburg strain CpipJ1.3; http://cquinquefasciatus.vectorbase.org/

[£] Annotations for cytochrome P450 genes were taken from the most current annotation based on: Nelson, DR (2009) The Cytochrome P450 Homepage. Human Genomics 4, 59-65: http://drnelson.uthsc.edu/CytochromeP450.html

[¤]Fragments Per Kilo base of gene for every Million reads mapped (FPKM)

[*]Significantly up-regulated compared to the untreated control with a false discovery rate of 0.05.

[§]Fold expression relative to the untreated sample not calculable, the values represent the actual FPKM values for the genes.

[**]m/tr= metabolism/transport

Appendix 4.3. Genes down-regulated in the pyrethroid-resistant HAmCq^{G8} strain of *Culex quinquefasciatus* following permethrin challenge.

SCOP functional annotation†			Vectorbase annotation‡		FPKM¤	Fold change (24h)		
							Permethrin	
General	Detailed	Superfamily	Accession	Gene	Untreated	Acetone	LC_{50}	LC_{70}
Extra-cellular processes	Blood clotting	Fibrinogen C-terminal domain-like	CPIJ013290	conserved hypothetical protein	4.7	-2.9	-4.3*	-3.2*
			CPIJ018159	fibrinogen and fibronectin	11.1	-3.3*	-15.1*	-12.0*
			CPIJ013538	ficolin-1 precursor	32.4	-39.7*	-95.8*	-48.6*
			CPIJ017657	salivary secreted angiopoietin, putative	13.4	-5.1*	-4.2*	-2.1
	Cell adhesion	alpha-catenin/vinculin	CPIJ018558	conserved hypothetical protein	1	-5.8*	-1.9	-1.2
		C-type lectin-like	CPIJ000449	galactose-specific C-type lectin, putative	19.4	-1.6	-3.0*	-3.7*
			CPIJ012307	galactose-specific C-type lectin, putative	62.5	-4.1*	-1.5	-1.6
			CPIJ016688	galactose-specific C-type lectin, putative	5	-8.1*	-1.3	-1.5
		FnI-like domain	CPIJ000931	conserved hypothetical protein	8.5	-2.2	-3.7*	-3.3*
		RNI-like	CPIJ004947	leucine-rich repeat-containing protein 1	33.4	-3.8*	-4.2*	-3.8*
	Immune response	Staphylokinase/streptokinase	CPIJ801956	croquemort	179.1	-1.3	-3.3	-3.7*
General	General	EF-hand	CPIJ005975	conserved hypothetical protein	482.8	-2.9	-4.1*	-5.1*
		WD40 repeat-like	CPIJ000959	conserved hypothetical protein	39.8	-4.7*	-2	-2.5*
			CPIJ004852	conserved hypothetical protein	3.2	1.3	-2.2	-2.9*
	Small molecule binding	FAD-binding domain	CPIJ802221	24-dehydrocholesterol reductase	197.8	-3.4*	-2.9	-3
		FAD/NAD(P)-binding domain	CPIJ010903	conserved hypothetical protein	101.7	-3.3*	-2.5*	-2.3
			CPIJ008048	peroxisomal N1-acetyl-spermine/spermidine oxidase	5.3	-2.6	-3.4*	-2.8*
		GST C-terminal domain-like	CPIJ018632	glutathione-s-transferase theta, gst	457.8	-6.3*	-5.0*	-4.3*
		NAD(P)-binding Rossmann-fold domains	CPIJ000372	L-xylulose reductase	29.9	-3.8*	-5.1*	-4.5*
			CPIJ005655	oxidoreductase	55.4	-3.1*	-5.1*	-4.2*
			CPIJ005656	oxidoreductase	164.6	-5.6*	-6.1*	-4.9*
		Nucleotide-binding domain	CPIJ007272	d-amino acid oxidase	31.4	-2.6	-5.1*	-3.9*
		P-loop containing nucleoside triphosphate hydrolases	CPIJ008284	canalicular multispecific organic anion transporter 1	1.8	-2.1	-3.0*	-3.8*
			CPIJ012368	lipoprotein-releasing system ATP-binding protein lolD	3.2	-5.4*	-2.1	-2.7*
		Thiamin diphosphate-binding fold (THDP-binding)	CPIJ011465	C-4 methylsterol oxidase	4.6	-1.3	-3.3*	-3.5*
Information	Chromatin structure	Histone H3 K4-specific methyltransferase SET7/9 N-terminal domain	CPIJ007018	conserved hypothetical protein	11.7	-1.2	-2.8	-3.1*
	DNA replication/repair	RING/U-box	CPIJ011986	nuclear transcription factor, x-box binding 1	2	-2.6	-1.8	-3.3*

	Translation	Ribosomal protein S5 domain 2-like	CPIJ005090	conserved hypothetical protein	10.7	-1.8	-2.1	-2.7*
Intra-cellular processes	Cell cycle, Apoptosis	CAD & PB1 domains	CPIJ011935	conserved hypothetical protein	5.5	-14.0*	-29.0*	-29.5*
	Ion m/tr	MFS general substrate transporter	CPIJ017878	permease, putative	60.2	-6.3*	-4.0*	-2.8
			CPIJ007428	Sialin, Sodium/sialic acid cotransporter, putative	35.8	-1.8	-2.1	-2.6*
			CPIJ012069	sucrose transport protein	5.9	-3.3*	-5.0*	-4.2*
			CPIJ012070	sucrose transport protein	9.7	-6.7*	-2.6*	-2.4*
			CPIJ008941	sugar transporter	8.7	-3.1*	-3.2*	-3.7*
			CPIJ012676	sugar transporter	18	-3.6*	-3.4*	-3.1*
			CPIJ017566	synaptic vesicle protein	7	-5.3*	-5.0*	-7.4*
			CPIJ003138	UNC93A protein, putative	40	-1.9	-2.9*	-2.4
	Phospholipid m/tr	CRAL/TRIO domain	CPIJ014226	cellular retinaldehyde-binding protein	394.7	-1.3	-5.1*	-5.5*
			CPIJ014224	conserved hypothetical protein	15.7	-4.0*	-3.1*	-3.9*
			CPIJ014228	conserved hypothetical protein	253.2	-2.5	-3.3*	-2.8*
			CPIJ015060	conserved hypothetical protein	24.6	-3.6*	-2.2	-2.5*
	Proteases	Creatinase/aminopeptidase	CPIJ015407	xaa-Pro aminopeptidase 1	46.6	-1.5	-2.4	-2.9*
		Cysteine proteinases	CPIJ001239	cathepsin B precursor	91.8	-3.3*	1.9	1.5
		Kazal-type serine protease inhibitors	CPIJ000733	conserved hypothetical protein	287.5	-3.2*	-2	-1.6
		Metallo-dependent phosphatases	CPIJ800110	conserved hypothetical protein	21.6	-1.7	-6.9*	-7.3*
		Metalloproteases ("zincins"), catalytic domain	CPIJ004060	aminopeptidase N precursor	28.5	-1.1	-18.5*	-24.9*
			CPIJ012036	aminopeptidase N precursor	15	-2.1	-3.2*	-3.4*
			CPIJ002943	conserved hypothetical protein	126.3	-1.2	-2.3	-3.0*
			CPIJ013316	zinc metalloproteinase	2.8	-7.8*	1.7	-1
			CPIJ801477	protease m1 zinc metalloprotease	57.5	-1.7	-5.9*	-6.7*
			CPIJ801485	protease m1 zinc metalloprotease	51.5	-1.2	-4.8*	-4.9*
		Serine protease inhibitors	CPIJ012287	hypothetical protein	52.1	-5.9*	3.8	2.1
		Serpins	CPIJ000214	serpin B10	5.6	-3.7*	-2.6*	-2.3
		Trypsin-like serine proteases	CPIJ002126	chymotrypsin-1	70.9	-2.9*	-17.3*	-16.4*
			CPIJ002138	chymotrypsinogen, putative	93.1	-4.7*	-9.0*	-7.3*
			CPIJ011617	chymotrypsinogen, putative	382.8	-2	-3.6	-3.7*
			CPIJ006076	hypodermin-B precursor	8.2	-4.4*	-2.6*	-2.1
			CPIJ002128	mast cell protease 2 precursor	65.6	-1.1	-4.2*	-4.0*
			CPIJ010641	prostasin precursor	37.9	-2.4	-2.5*	-2.5*
			CPIJ017798	serine protease	4.6	-3.6*	-2.5	-1.5
			CPIJ002136	serine proteases 1/2 precursor	179.6	-1.5	-5.4*	-5.8*
			CPIJ002137	serine proteases 1/2 precursor	47.1	1.1	-2.9*	-3.1*
			CPIJ007385	serine-type enodpeptidase, putative	32.6	-4.6*	-10.8*	-12.5*
			CPIJ011383	serine-type enodpeptidase, putative	1263.6	-2.7	-12.5*	-16.5*
			CPIJ013616	trypsin 5 precursor	18.7	-16.2*	-3.9*	-5.6*
			CPIJ002133	trypsin epsilon precursor	22	-2.2	-4.4*	-4.8*
			CPIJ006077	trypsin theta precursor	380.9	-1.8	-4.6*	-5.1*
			CPIJ006079	tryptase gamma precursor	1353.8	-1.3	-7.8*	-7.3*
			CPIJ801807	brachyurin	278.3	-1.9	-7.2*	-6.7*
			CPIJ002142	collagenase precursor	2981.5	-1.4	-3.2*	-3.6*
			CPIJ002156	chymotrypsin BI precursor	145.1	-2.0*	1.4	1.2
		Zn-dependent exopeptidases	CPIJ010805	carboxypeptidase A1 precursor	141.2	-1.6	-4.7*	-4.5*
			CPIJ010801	carboxypeptidase B precursor	11.6	1.4	-6.2*	-5.7*
			CPIJ009738	conserved hypothetical protein	44.6	-1.5	-3.1*	-3.3*
			CPIJ010806	conserved hypothetical protein	100.4	-1.1	-11.6*	-5.3*
			CPIJ014108	conserved hypothetical protein	19.3	-1.7	-2.9*	-3.4*
			CPIJ801685	carboxypeptidase A2	54.8	-2.9*	-4.7*	-5.7*
			CPIJ801679	zinc carboxypeptidase	100.5	-1.6	-4.7*	-5.6*

			CPIJ801680	zinc carboxypeptidase	41.6	-1.7	-3.6*	-3.2*
	Protein modification	GroES-like	CPIJ013379	sorbitol dehydrogenase	73.6	-4.2*	-2.1	-1.8
		HSP20-like chaperones	CPIJ005645	heat shock protein 22	4	-1.3	-9.2*	3
		Peptide methionine sulfoxide reductase	CPIJ018565	peptide methionine sulfoxide reductase	9	-3.8*	2.1	2.4
	Transport	Ammonium transporter	CPIJ013531	ammonium transporter, putative	24.4	-3.4*	1.3	-1.7
		Lipocalins	CPIJ801584	apolipoprotein D	7.8	-4.3*	1	1.3
		Mitochondrial carrier	CPIJ019873	mitochondrial brown fat uncoupling protein	42.1	-3.1*	-3.8*	-3.3*
			CPIJ013697	tricarboxylate transport protein, mitochondrial precursor	47.6	-3.1*	-2.2	-2.3
Metabolism	Amino acids m/tr	Arginase/deacetylase	CPIJ015718	arginase	30.2	-3.0*	-3.3*	-3.9*
		PLP-binding barrel	CPIJ008556	ornithine decarboxylase	4.1	-8.3*	-2.1	-1.5
			CPIJ009093	ornithine decarboxylase	6.9	-1.9	-2	-2.5*
			CPIJ009094	ornithine decarboxylase 1	13.7	-1.6	-3.4*	-3.1*
		Tryptophan synthase beta subunit-like PLP-dependent enzymes	CPIJ012752	cysteine synthase	48.5	-4.2*	-8.3*	-5.5*
	Carbohydrate m/tr	(Trans)glycosidases	CPIJ801597	alpha-amylase 1	387.1	1.1	-22.1*	-23.6*
			CPIJ801761	alpha-amylase A	346.3	-1.8	-5.8*	-5.4*
			CPIJ008663	conserved hypothetical protein	219.4	-2	-4.8*	-5.7*
			CPIJ007459	hypothetical protein	23.7	-2.5	-2.7*	-1.3
			CPIJ005062	alpha-amylase	249.1	-1.4	-3.2	-4.8*
			CPIJ013169	alpha-glucosidase precursor	13.7	-1.2	-2.6*	-2.5*
			CPIJ003338	beta-galactosidase	62.4	-1.5	-3.2*	-3.3*
			CPIJ013477	beta-hexosaminidase	1.6	1.5	-3.0*	-2.1
			CPIJ008529	lactase-phlorizin hydrolase precursor	54	-1.9	-4.2*	-4.2*
			CPIJ008530	lactase-phlorizin hydrolase precursor	242.1	-2.9	-16.7*	-17.1*
			CPIJ008531	lactase-phlorizin hydrolase precursor	138.1	-2.5	-5.0*	-5.3*
			CPIJ005725	alpha-amylase A precursor	48.7	-1.5	-4.2*	-3.4*
			CPIJ009306	neutral alpha-glucosidase ab precursor	7.6	-1.9	-5.4*	-7.1*
		Invertebrate chitin-binding proteins	CPIJ004731	conserved hypothetical protein	11.9	-5.3*	-2	-2.3
			CPIJ011623	conserved hypothetical protein	74.9	-3.1*	-2.4	-3.1*
			CPIJ012138	conserved hypothetical protein	546.8	-1.6	-4.3*	-5.0*
			CPIJ014181	conserved hypothetical protein	235.5	-2	-3.1	-3.5*
			CPIJ014194	conserved hypothetical protein	114.7	-5.1*	-1.6	-1.9
			CPIJ015734	conserved hypothetical protein	23.9	-5.3*	-1.2	-1.4
			CPIJ016342	conserved hypothetical protein	58.7	-4.7*	-1.2	-1.6
			CPIJ003955	predicted protein	211.7	-2.4	-7.9*	-4.7*
			CPIJ004733	predicted protein	50.4	-1.5	-3.7*	-4.6*
			CPIJ018945	predicted protein	13.4	-1.5	-2.9*	-4.7*
	Coenzyme m/tr	Cell wall binding repeat	CPIJ016313	endocuticle structural glycoprotein SgAbd-2	5.3	0.0*	-39.8*	0.0*
		SCP-like	CPIJ015122	sterol carrier protein-2, putative	2729.5	-3	-4.2*	-3.6
		Single hybrid motif	CPIJ016638	glycine cleavage system h protein	112.8	-3.3*	-2	-1.9
	E- transfer	CO dehydrogenase molybdoprotein N-domain-like	CPIJ000151	sodium/solute symporter	20.5	-1.3	-2.5*	-1.4
	Energy	Citrate synthase	CPIJ010291	citrate synthase, mitochondrial precursor	8.6	-2.5	-3.1*	-2.4*
		PEP carboxykinase-like	CPIJ010515	phosphoenolpyruvate carboxykinase	87.3	-9.5*	-4.2*	-2.7
			CPIJ010518	phosphoenolpyruvate carboxykinase	154.2	-6.2*	-4.0*	-3.2
	Lipid m/tr	Acyl-CoA binding protein	CPIJ011388	diazepam binding inhibitor, putative	718.5	-5.5*	-4.5*	-4.6*

	Tp47 lipoprotein, N-terminal domain	CPIJ016325	conserved hypothetical protein	359.9	-2.8*	-3.0*	-2.8*
Nitrogen m/tr	RmlC-like cupins	CPIJ020056	cysteine dioxygenase	54.1	-1.5	-2.4*	-2
Nucleotide m/tr	Adenine nucleotide alpha hydrolases-like	CPIJ007938	asparagine synthetase B	11.3	-4.4*	-1.8	-1.9
	SAICAR synthase-like	CPIJ801627	purine biosynthesis protein 6, pur6	104.8	-3.2*	-3.8*	-2
Other enzymes	Acetyl-CoA synthetase-like	CPIJ016639	acetyl-coa synthetase	48.5	-2.9*	-1.4	-1.2
		CPIJ012907	luciferin 4-monooxygenase	17.3	-3.3*	1.3	1.4
	Actin-like ATPase domain	CPIJ012574	actin	36.4	-1.3	-3.8*	-3.6*
		CPIJ006534	conserved hypothetical protein	19.7	-4.2*	-1.5	-1.8
		CPIJ011081	heat shock protein 70 B2	1.1	1.7	-5.8*	3.3
		CPIJ011082	heat shock protein 70 B2	4.2	-1.3	-7.4*	1.8
		CPIJ011083	heat shock protein 70 B2	3.4	-1.6	-7.8*	1.5
	Alkaline phosphatase-like	CPIJ001264	alkaline phosphatase	33.2	-2.4	-3.7*	-5.3*
		CPIJ001265	alkaline phosphatase	75.5	-1.3	-5.4*	-6.5*
		CPIJ001262	alkaline phosphatase	99.4	-1.6	-4.0*	-5.3*
		CPIJ015241	alkaline phosphatase	8.1	-3.9*	-2.9*	-2.6*
		CPIJ018121	membrane-bound alkaline phosphatase precursor	107.2	-2.2	-11.9*	-15.4*
	alpha/beta-Hydrolases	CPIJ018233	esterase FE4 precursor	3085	-2.1*	-7.9*	-8.0*
		CPIJ001372	bphl protein	30.6	-2.7*	-2.6*	-2.8*
		CPIJ018231	carboxylesterase	36.1	-2.2	-12.7*	-14.3*
		CPIJ018232	cholinesterase	259.7	1.2	-4.2*	-4.1
		CPIJ007461	epoxide hydrolase	21.9	-4.5*	-3.0*	-2
		CPIJ004637	glutactin precursor	41.1	-9.2*	-4.3*	-4.0*
		CPIJ001121	kynurenine formamidase	29.7	-3.4*	-2.6*	-3.0*
		CPIJ004227	lipase	631.6	-1.9	-19.4*	-16.1*
		CPIJ004228	lipase	1160	-2	-8.5*	-9.5*
		CPIJ004230	lipase	170.5	-1.8	-8.7*	-7.0*
		CPIJ002726	lipase 3 precursor	116.5	-2.3	-8.7*	-13.3*
		CPIJ008876	lysosomal pro-X carboxypeptidase, putative	439.2	-2.3	-10.8*	-11.9*
		CPIJ004224	pancreatic triacylglycerol lipase	347.8	-1.6	-4.7*	-5.0*
		CPIJ004226	pancreatic triacylglycerol lipase	22.7	-2	-7.2*	-5.6*
		CPIJ019228	pancreatic triacylglycerol lipase	7.2	-3.2*	1.4	1.6
		CPIJ004225	pancreatic triacylglycerol lipase precursor	482.6	-1.8	-6.8*	-7.9*
		CPIJ005462	pancreatic triacylglycerol lipase precursor	19.5	-1.6	-2.4*	-3.0*
		CPIJ008873	prolylcarboxypeptidase, putative	19.4	1.4	-3.1*	-3.2*
		CPIJ008874	prolylcarboxypeptidase, putative	252.8	-1.4	-4.3*	-4.5*
		CPIJ008877	prolylcarboxypeptidase, putative	17.1	-22.6*	-4.7*	-6.0*
		CPIJ008878	prolylcarboxypeptidase, putative	13.3	-139.5*	-14.4*	-22.0*
		CPIJ002911	retinoid-inducible serine carboxypeptidase precursor	48.1	-2	-6.7*	-8.1*
		CPIJ018060	thymus-specific serine protease precursor	53.7	-1.3	-4.3*	-5.7*
		CPIJ018061	thymus-specific serine protease precursor	6.6	-1.3	-7.7*	-5.0*
		CPIJ801474	conserved hypothetical protein	260	-2.6	-10.7*	-11.4*
		CPIJ801475	conserved hypothetical protein	30.5	-1.5	-30.8*	-50.7*
		CPIJ802425	dipeptidyl peptidase 4	70	-2.1	-3.3*	-3.7*
	Calcium-dependent phosphotriesterase	CPIJ007528	anterior fat body protein	116.4	-4.1*	-1.9	-1.9
		CPIJ007230	regucalcin	72	-8.2*	-5.6*	-5.1*
	Carbon-nitrogen hydrolase	CPIJ003840	aliphatic nitrilase, putative	59.4	-1.5	-2.2	-2.7*
	Carbonic anhydrase	CPIJ014281	carbonic anhydrase precursor	34.9	-1.4	-2.6*	-2.6*
	Cytidine deaminase-like	CPIJ801876	cytidine deaminase	13.3	-3.9*	-2.2	-3.0*

	HAD-like	CPIJ008977	pyridoxal phosphate phosphatase	1.6	-6.9*	-17.8*	-3.6*
	Isochorismatase-like hydrolases	CPIJ008109	conserved hypothetical protein	13.3	-1.4	-3.1*	-3.1*
	Metallo-dependent hydrolases	CPIJ003807	allantoinase	85	-5.4*	-4.7*	-7.8*
	N-acetylmuramoyl-L-alanine amidase-like	CPIJ016771	peptidoglycan recognition protein precursor	4339.8	-1.6	-15.0*	-13.6*
	N-terminal nucleophile aminohydrolases (Ntn hydrolases)	CPIJ802450	gamma glutamyl transpeptidase	7.7	-1.4	-7.2*	-7.2*
	Thiolase-like	CPIJ005595	fatty acid synthase S-acetyltransferase	80.5	-4.4*	-3	-3
	YVTN repeat-like/Quinoprotein amine dehydrogenase	CPIJ009045	conserved hypothetical protein	24	-22.6*	-3.1*	-4.3*
Photosynthesis	Light-harvesting complex subunits	CPIJ005531	conserved hypothetical protein	12.9	-3.4*	-2.5*	-2.9*
Polysaccharide m/tr	Ricin B-like lectins	CPIJ013165	16 kDa salivary peptide, putative	11.5	-2	-4.1*	-2.2
Redox	Acid phosphatase/Vanadium-dependent haloperoxidase	CPIJ013993	amino acid transporter	1.1	-1.1	-1.6	-7.0*
	ALDH-like	CPIJ009438	aldehyde dehydrogenase	84.7	-2.6	-3.1*	-2.8
	Aromatic aminoacid monoxygenases, catalytic and oligomerization domains	CPIJ002149	phenylalanine hydroxylase	34.8	-2.8*	-2.2	-1.6
	Cytochrome P450	CPIJ800155	CYP15B1	1.4	-1.9	-1.4	-3.2*
		CPIJ800249	CYP4D42	16.6	-1.5	-2.8*	-2.7*
		CPIJ800254	CYP4H30	21.4	-1.7	-6.5*	-6.8*
		CPIJ800257	CYP4H35	15.8	-2.5	-2.4*	-2.4*
		CPIJ800260	CYP4H38	20.3	-1.7	-3.2*	-3.8*
		CPIJ800227	CYP9J38	5.8	1.1	-1.9	-2.4*
		CPIJ016284	CYP4J4	1.8	-1.6	-3.3*	-2.7
	Di-copper centre-containing domain	CPIJ007783	arylphorin subunit alpha precursor	3799.6	-7.6*	-7.3	-6.8
		CPIJ006537	larval serum protein 1 beta chain precursor	68.1	-5.9*	-9.0*	-9.1*
		CPIJ006538	larval serum protein 1 beta chain precursor	96.3	-6.2*	-6.8*	-6.0*
		CPIJ018824	larval serum protein 1 beta chain precursor	203	-6.0*	-6.1*	-6.2*
		CPIJ000056	hexamerin 1.1 precursor	1590	-4.9*	-5.4*	-4.3*
		CPIJ007783	hexamerin 1.1 precursor	3800	-7.6*	-7.3*	-6.8*
		CPIJ009032	hexamerin 1.1 precursor	357	-3.7*	-3.6*	-2.8*
		CPIJ009033	hexamerin 1.1 precursor	15465.4	-1.9*	-1.5	-1.6
		CPIJ001820	Larval serum protein 2 precursor	6341.4	-5.7*	-5.4*	-6.0*
	Thioredoxin-like	CPIJ018629	glutathione-s-transferase theta, gst	96.5	-3.0*	-1.7	-1.8
		CPIJ008450	peroxiredoxin-6	183.5	-4.0*	-3.1*	-3.4*
Secondary metabolism	Concanavalin A-like lectins/glucanases	CPIJ001786	collagen alpha chain	6.8	-2.1	-3.7*	-4.0*
		CPIJ006421	conserved hypothetical protein	16.2	-2	-2.4	-2.5*
		CPIJ004229	gram negative bacteria binding protein 2	1.3	-2.7	-12.6*	-25.6
		CPIJ004321	gram-negative bacteria binding protein	199.5	1.3	-4.0*	-4.6*
		CPIJ004323	gram-negative bacteria binding protein	141.5	-1.4	-3.2*	-3.2*
		CPIJ004325	gram-negative bacteria binding	11.1	-2	-2.9*	-4.7*

				protein				
			CPIJ004320	gram-negative bacteria-binding protein 1 precursor	134.9	-2.9	-38.1*	-24.8*
	Transferases	Acyl-CoA N-acyltransferases (Nat)	CPIJ015296	retinol-binding protein	5.7	-3.6	-3.6	-5.7*
		Glycerol-3-phosphate (1)-acyltransferase	CPIJ802146	1-acyl-sn-glycerol-3-phosphate acyltransferase	25.1	-2.5	-3.2*	-3.0*
		PLP-dependent transferases	CPIJ006619	cystathionine gamma-lyase	56.9	-1.6	-4.5*	-4.0*
			CPIJ004400	ornithine aminotransferase, mitochondrial precursor	110.7	-4.9*	-5.8*	-6.5*
			CPIJ008287	phosphoserine aminotransferase	31.3	-3.5*	-2.3	-1.6
No annotation	No annotation	No annotation	CPIJ017146	2-acylglycerol O-acyltransferase 2-A	63.9	-1.6	-2.6	-2.8*
			CPIJ013992	amino acid transporter, putative	5.1	-1.6	-3.1*	-2.3
			CPIJ802440	cuticle protein CP14.6	232.4	-5.6*	-5.4*	-5.8*
			CPIJ003483	cuticle protein, putative	61.1	-3.6*	-4.5*	-3.6*
			CPIJ003484	cuticle protein, putative	59.5	-3.9*	-3.8*	-3.3*
			CPIJ003485	cuticle protein, putative	42.3	-1.9	-4.7*	-3.1*
			CPIJ007171	proton-coupled amino acid transporter 1	7.6	-1.9	-3.4*	-2.8*
			CPIJ801724	sodium/solute symporter	18.1	-3.4*	-2.7*	-2.8*
			CPIJ003879	lipid storage droplets surface-binding protein 1	60.2	-3.0*	-1.2	1.1
			CPIJ000534	hypothetical protein	17.2	-2.8	-4.1*	-1.9
			CPIJ000535	hypothetical protein	28.6	-2.7	-3.3*	-2.3
			CPIJ007722	hypothetical protein	534.9	1.1	-3.3*	-2.6
			CPIJ011256	hypothetical protein	74.8	-1.7	-2.5*	-1.5
			CPIJ019329	hypothetical protein	14.6	-5.6*	8.2	9.4
			CPIJ005479	hypothetical protein	22.2	-18.7*	-2.4	-3.8*
			CPIJ008508	predicted protein	2.2	-3.7	-7.0*	-2.7
			CPIJ009902	predicted protein	16.7	-2.3	-12.7*	-12.9*
			CPIJ017828	predicted protein	841.3	-8.2*	-5.7	-3.6
			CPIJ017829	predicted protein	78.8	-3.4*	-4.4*	-4.1*
			CPIJ801843	conserved hypothetical protein	230	-3.0*	-3.6*	-2.2
			CPIJ801846	conserved hypothetical protein	380.2	-2.4	-3.2*	-1.6
			CPIJ801847	conserved hypothetical protein	137.5	-7.3*	-10.4*	-2.5*
			CPIJ002115	conserved hypothetical protein	7	-3.5*	-1.2	-1.4
			CPIJ002309	conserved hypothetical protein	66.7	-3.8*	-3.2*	-2.5*
			CPIJ003026	conserved hypothetical protein	6	-5.0*	-4.0*	-6.4*
			CPIJ003129	conserved hypothetical protein	10.8	-3.3*	-1.6	-1.5
			CPIJ008107	conserved hypothetical protein	4.3	1	-2.5*	-1.7
			CPIJ008353	conserved hypothetical protein	55.9	-1.8	-2.2	-2.5*
			CPIJ008379	conserved hypothetical protein	466.9	-1.7	-4.3*	-2.6
			CPIJ009330	conserved hypothetical protein	12.6	-6.8*	-8.6*	-8.3*
			CPIJ010904	conserved hypothetical protein	128.4	-2.9*	-2.1	-2.5
			CPIJ011137	conserved hypothetical protein	3.9	-3.4	-3.2*	-1.5
			CPIJ011505	conserved hypothetical protein	39.6	-4.1*	-2.1	-1.6
			CPIJ017076	conserved hypothetical protein	21.6	-1.9	-3.8*	-7.8*
			CPIJ017687	conserved hypothetical protein	21.8	-3.4*	-2	-1.1
Other	Unknown function	E set domains	CPIJ018825	larval serum protein 1 beta chain precursor	154.6	-6.0*	-6.6*	-5.5*
			CPIJ002744	conserved hypothetical protein	670.9	-1.3	-7.8*	-8.6*
			CPIJ018326	conserved hypothetical protein	1192.8	-8.2*	-1.7	-2.1
			CPIJ004282	hexamerin 2 beta	44.8	-2.1	-4.3*	-5.9*
	Viral proteins	Head and neck region of the ectodomain of NDV fusion glycoprotein	CPIJ802080	conserved hypothetical protein	3	-1.8	-2.7	-3.2*
Regulation	DNA-binding	"Winged helix" DNA-binding domain	CPIJ801885	conserved hypothetical protein	9.1	-1.7	-2.6*	-2.8*
		ParB/Sulfiredoxin	CPIJ009003	conserved hypothetical protein	44.2	-2	-2.4*	-2.7*

	ROP protein	CPIJ009715	conserved hypothetical protein	1050.3	-2	-18.7*	-21.7*
Kinases/phosphatases	Protein kinase-like (PK-like)	CPIJ018315	NIMA-family kinase NERCC1	2.5	-2.5	-4.1*	-5.9*
		CPIJ010321	conserved hypothetical protein	4.9	-3.6*	-3.0*	-2.9*
		CPIJ007628	Juvenile hormone-inducible protein, putative	28.1	-2.6	-2.5*	-2.2
		CPIJ010315	Juvenile hormone-inducible protein, putative	16.5	-2.3	-8.2*	-8.4*
Receptor activity	Chemosensory protein Csp2	CPIJ801979	serine/threonine kinase	6.5	-33.4*	-4.3*	-5.0*
		CPIJ801985	serine/threonine kinase	5.2	-4.3	-7.9*	-6.4*
	SRCR-like	CPIJ006993	protein-lysine 6-oxidase, putative	6.6	-2.7	-2.7*	-2.4*
Signal transduction	Growth factor receptor domain	CPIJ005087	cell wall cysteine-rich protein	8.1	-4.2*	-2.7*	-2.6*
	Insect pheromone/odorant -binding proteins	CPIJ010787	odorant binding protein OBP51	71.2	-3.6*	-1.5	-1.5
		CPIJ004635	odorant-binding protein OBPjj7a	115.4	-3.4*	-2.3	-1.7
		CPIJ012786	predicted protein	13.3	-4.4*	-4.9*	-3.4*
		CPIJ801711	hypothetical protein	426.8	-7.3*	-5.8*	-4.7*
		CPIJ801713	hypothetical protein	22.5	-9.4*	-12.6*	-9.0*
		CPIJ801712	predicted protein	430.7	-4.9*	-4.4*	-2.9*
		CPIJ801709	predicted protein	8.8	-5.8*	-9.6*	-3.3*
	PH domain-like	CPIJ801535	sodium/potassium-dependent ATPase beta-2 subunit	16.8	-9.4*	-2.1	-3.8*

[†]SCOP general and detailed functions using the predicted *Cx. quinquefasciatus* annotation information available at the Superfamily website (version 1.75) supfam.cs.bris.ac.uk/SUPERFAMILY/index.html

[‡]*Culex quinquefasciatus* genome, Johannesburg strain CpipJ1.3; http://cquinquefasciatus.vectorbase.org/

[£]Annotations for cytochrome P450 genes were taken from the most current annotation based on: Nelson, DR (2009) The Cytochrome P450 Homepage. Human Genomics 4, 59-65: http://drnelson.uthsc.edu/CytochromeP450.html

[¤]Fragments Per Kilo base of gene for every Million reads mapped (FPKM)

[*]Significantly down-regulated compared to the untreated control with a false discovery rate of 0.05.

[**]m/tr= metabolism/transport

Appendix 6.1. List of primers used for the qRT-PCR determination of genes.

Vectorbase‡	Forward primer (5'-3')	Reverse primer (5'-3')
CPIJ000841	CTTGATCTGGGCGTGAACA	TTTTCCATGGGCTCCAAAG
CPIJ001357	TCGGGATCGTCATCTTCTTC	GACCGATCGGCAGTGTACG
CPIJ002046	AATTCCGAACCGTCGTCAC	GTAGGTCGCGGCATAGCTC
CPIJ003456	GCACATCCCCGAAGTGTC	CCAGCTGGGCGTAAATGA
CPIJ003525	GGCGTAACGTGGATGTTTCT	ATAAACTTGAACGCCGTTGG
CPIJ003531	ACGAATCGTACGACCTGGAC	CTTCTGGCCAAGCTTCAAAC
CPIJ003879	CAGCTGGCAGTGTTGCTG	TCCAAGTGGACGGCCTTA
CPIJ003915	GACGAGCACGTTACGCTCA	ACGGACCACCAGAGTCACC
CPIJ004290	AAGGCCGTCGATGACTACG	AATCCGTTGACGGGATCAG
CPIJ004640	TTCCAGGTGTCCCTCATCC	TCAGCGCGTTGTAGTTTGG
CPIJ004660	GAACCGATTCACCGTCCTG	ATCCACCGCAGAAGTGTCC
CPIJ005273	GAACCGGCTGACGAGAGTC	GCGTCCTTTCCTCCTTCCT
CPIJ005473	CCTGCCGGACAAAGACTAAG	TCGGGGGTTGTTAGTACCAG
CPIJ005952	TACGAGCTGGCCCTTAATCCGTTT	AGACTTTCCGCAGGTGGGTACTTT
CPIJ006502	GTTCCACCACATCCCAACTC	CGGCTCAAACAGATCAGACA
CPIJ007079	ATCCATCATCTCGCCCAAG	TTCAGCTCCAGCAGGGAGT
CPIJ007193	ACGACGCCCAGTATTACGC	ATACTTGCGCAGCATCACG
CPIJ007471	TGGATCTGCTGCGTCTGA	AGCGCCAGTTTGAGTTGC
CPIJ008286	GACCGGGAAGTCAGATCCA	TGCGTTCCAGATGAAGGTG
CPIJ008515	GATCCTCAGCGATCGAAGC	CAGCAGCTCGTTGCACACT
CPIJ010190	AACGCTTCTGCCAGTGTC	AAAACCACATGCCAGCATTC
CPIJ011837	TTATTCCGTTCAGTGGAGGCACGA	TTCAGCAGTGCTTCAAACCGGAAG
CPIJ012470	TGAACGTCCTTAGGGATGGCGAAA	TTGCTAGTCGCGGAAACGAACTGA
CPIJ012707	CCGACATGGGACCTGTGTA	CTGCATCGCAGCACATTC
CPIJ012708	TCCTGCGTTGCTCCAAAT	GTACCCCGCATTGCAGTC
CPIJ012716	TGCCATCATTTCCCTAGCC	AGAAGCACTGCACGAAGCA
CPIJ012719	CTGACCATCGAGCAGCAGA	CGACGGTCTTTTCCTGGAC
CPIJ012721	CCAAGTGTTTCGTGCGTTG	CCGGTTCCGATAGAAGCAC
CPIJ013321	GCAAACTCTGCTGGGCTATC	CGTGTCCAGGTGCTTGTAGA
CPIJ013633	CCAGGTCTCGTTCCTGCAT	CAGGTAGGGCTTCCACCAG
CPIJ015385	GCCAATCCGTGCTTCAAC	AGGTTGCACGGACACCAC
CPIJ015960	AGTGCATTCGGAGGTCCTTCATGT	AGACTTGTCACCAGCTTATCGGCA
CPIJ016702	CAGCAGCAGCAAAAAGTGC	GTGTTCGCACTGGAGACGA
CPIJ019052	GCCTTGATTTCCGGGACTT	CGCCCAGATCTTTGTGCTT
CPIJ019581	CAACTACGAGTGGGGCAAGT	ACTCAAGACGGCAATGATGA
CPIJ020018	TGTCCAAGTTTCGGTTCGAGGCTA	AGGTGATGGCATCCGTTGAGGTAT
rRNA	CGCGGTAATTCCAGCTCCACTA	GCATCAAGCGCCACCATATAGG

‡*Cx. quinquefasciatus* genome Johannesberg strain v1.2, www.vectorbase.org

Appendix 6.2. Complete list of up- and down-regulated genes for sugar-fed only females for the HAmCq^{G8} strain of *Culex quinquefasciatus* for the initial 72h post-eclosion.

Time interval	SCOP† general function	Gene	Vectorbase anotation‡	FPKM* (time 1)	FPKM (time 2)	Fold change
2 to 12h	General Information	CPIJ002675	glutathione S-transferase 1	1.0	21.8	22.5
		CPIJ009133	salivary endonuclease	3.2	94.6	29.4
	Intra-cellular processes	CPIJ001773	synaptic vesicle protein	0.2	6.3	29.0
		CPIJ001774	synaptic vesicle protein	0.3	5.1	17.7
		CPIJ001775	synaptic vesicle protein	0.7	21.9	31.0
		CPIJ008945	sugar transporter	2.5	25.7	10.3
		CPIJ008946	sugar transporter	3.0	34.3	11.3
		CPIJ017478	conserved hypothetical protein	3.4	28.0	8.3
		CPIJ019591	solute carrier family 2	10.8	125.7	11.6
		CPIJ001239	cathepsin B	2.0	96.0	49.2
		CPIJ001240	cathepsin B-like thiol protease	0.4	7.9	21.3
		CPIJ002595	zinc carboxypeptidase	1.3	12.5	9.4
		CPIJ004640	trypsin 5G1	5.7	354.0	61.8
		CPIJ004984	serine protease1/2	1.1	29.8	26.0
		CPIJ005273	trypsin 2	2.7	76.8	27.9
		CPIJ006502	late trypsin	3.1	324.9	104.5
		CPIJ007025	FXa-directed anticoagulant	1.7	43.0	24.7
		CPIJ008388	aminopeptidase N	0.5	20.9	40.9
		CPIJ010521	serine protease inhibitor dipetalogastin	0.5	7.4	13.6
		CPIJ011998	zinc carboxypeptidase A 1	2.5	23.8	9.6
		CPIJ012161	sphingomyelin phosphodiesterase	1.0	42.5	42.6
		CPIJ014781	cysteine-rich protease inhibitor	0.5	26.2	55.8
		CPIJ016348	serine protease1/2	2.5	243.5	97.7
		CPIJ016937	coagulation factor X	1.3	37.5	29.6
		CPIJ017414	trypsin 4	0.3	19.6	68.3
		CPIJ017964	trypsin 7	0.3	13.0	51.6
		CPIJ017965	trypsin 7	0.5	10.0	19.5
		CPIJ018871	salivary apyrase; 5' nucleotidase	0.6	15.0	24.2
		CPIJ019168	salivary apyrase	1.3	12.0	9.6
		CPIJ020192	trypsin-like salivary secreted protein	0.4	14.6	36.0
	Metabolism	CPIJ017521	alpha-amylase I	5.7	180.2	31.6
		CPIJ011388	diazepam binding inhibitor	63.4	626.6	9.9
		CPIJ001082	cat eye syndrome critical region protein 1	3.0	36.6	12.3
		CPIJ005463	salivary lipase	1.0	13.3	12.8
		CPIJ006549	lipase member I	0.1	4.4	72.7
		CPIJ008977	pyridoxal phosphate phosphatase	12.5	247.6	19.7
		CPIJ012882	argininosuccinate synthase	1.4	20.0	14.2
		CPIJ016050	hepatic triacylglycerol lipase	0.2	7.9	32.9
		CPIJ017178	myoinositol oxygenase	14.5	155.4	10.7
		CPIJ018802	endochitinase A	0.3	4.7	16.1
		CPIJ000040	UDP-glucuronosyltransferase 2B1	2.1	51.6	24.1
		CPIJ015713	conserved hypothetical protein	8.8	84.8	9.6
		CPIJ019044	15.3 kDa basic salivary protein	1.5	67.3	46.2
		CPIJ000294	cytochrome P450 4C1	3.2	25.6	8.0
		CPIJ005952	cytochrome P450	11.9	339.4	28.4
		CPIJ009032	larval serum protein 2	1.9	22.7	12.2
		CPIJ010225	cytochrome P450 12b1, mitochondrial	1.7	15.0	9.0

	CPIJ010227	cytochrome P450 12b1, mitochondrial	2.8	32.0	11.5
	CPIJ010546	cytochrome P450 9c1	0.1	7.7	106.0
	CPIJ011837	cytochrome P450	5.1	58.3	11.4
	CPIJ011996	10-formyltetrahydrofolate dehydrogenase	3.4	62.5	18.3
	CPIJ012470	cytochrome P450 9b2	5.6	60.9	10.9
	CPIJ019586	cytochrome P450 6d3	2.2	19.2	8.7
	CPIJ019587	cytochrome P450 6d3	6.3	57.8	9.1
	CPIJ020018	cytochrome P450 6d3	7.1	70.8	10.0
	CPIJ000021	salivary protein	5.9	135.2	23.0
	CPIJ004030	venom allergen	0.6	8.2	13.5
	CPIJ015956	glycine N-methyltransferase	2.2	31.1	13.9
No Annotation	CPIJ000835	chymotrypsin-2	5.6	111.7	20.0
	CPIJ001276	defensin-A	18.5	154.3	8.4
	CPIJ001685	conserved hypothetical protein	0.8	15.2	19.0
	CPIJ001686	conserved hypothetical protein	0.3	11.6	39.1
	CPIJ002046	salivary protein	1.8	84.0	46.6
	CPIJ002089	salivary protein	7.8	183.4	23.6
	CPIJ002476	hypothetical protein	2.9	50.5	17.6
	CPIJ002532	sodium-dependent multivitamin transporter	4.1	35.7	8.6
	CPIJ003019	conserved hypothetical protein	1.4	21.9	15.3
	CPIJ003054	conserved hypothetical protein	0.1	8.0	58.9
	CPIJ003129	conserved hypothetical protein	1.6	14.0	8.5
	CPIJ003456	uricase	5.1	91.6	18.1
	CPIJ003468	hypothetical protein	151.1	3496.9	23.1
	CPIJ003615	salivary protein	21.6	368.5	17.1
	CPIJ003879	lipid storage droplets surface-binding protein 1	22.9	239.8	10.5
	CPIJ004054	hypothetical protein	0.2	5.9	23.9
	CPIJ004641	trypsin	0.5	135.2	286.0
	CPIJ005906	conserved hypothetical protein	2.3	41.6	17.9
	CPIJ005910	7.8 kDa basic salivary peptide	22.7	191.1	8.4
	CPIJ006908	carboxylesterase-6	9.3	102.1	11.0
	CPIJ007079	trypsin-1	70.9	928.5	13.1
	CPIJ007333	amylase	2.9	65.2	22.4
	CPIJ007452	8.9 kDa basic salivary peptide	12.0	311.6	26.0
	CPIJ007471	oskar	2.1	44.9	21.7
	CPIJ007646	conserved hypothetical protein	0.4	200.4	458.4
	CPIJ007741	conserved hypothetical protein	5.3	42.9	8.1
	CPIJ007742	30.5 kDa secreted protein 30.5k-1	3.5	40.0	11.5
	CPIJ007838	chymotrypsin-2	3.4	36.4	10.8
	CPIJ008014	oxidase/peroxidase	3.1	101.8	33.1
	CPIJ008032	conserved hypothetical protein	0.8	7.6	9.9
	CPIJ008464	hypothetical protein	0.3	69.0	235.6
	CPIJ008471	hypothetical protein	6.8	269.7	39.5
	CPIJ008479	9.7 kDa salivary peptide	6.4	146.7	22.9
	CPIJ010046	threonine-rich salivary mucin	27.5	394.3	14.3
	CPIJ010333	conserved hypothetical protein	1.5	26.0	17.6
	CPIJ010337	hypothetical protein	2.9	1053.0	365.9
	CPIJ010338	conserved hypothetical protein	4.6	684.0	149.2
	CPIJ010339	conserved hypothetical protein	1.0	173.3	182.4
	CPIJ010699	cecropin A	12.3	190.6	15.5
	CPIJ010772	16 kDa salivary peptide	0.2	43.1	259.7
	CPIJ010773	16.8 kDa salivary peptide	0.2	29.5	183.0
	CPIJ010792	16.7 kDa salivary peptide	0.3	10.6	33.7

	CPIJ011013	apyrase	2.8	24.5	8.8
	CPIJ011505	conserved hypothetical protein	19.7	193.9	9.9
	CPIJ012056	sodium-dependent serotonin transporter	2.5	56.8	22.4
	CPIJ012254	conserved hypothetical protein	1.3	36.5	29.1
	CPIJ012707	wnt inhibitory factor 1	3.6	108.3	30.4
	CPIJ012708	wnt inhibitory factor 1	2.2	111.2	51.0
	CPIJ012783	7.7 kDa salivary cysteine-rich peptide	2.0	34.5	17.6
	CPIJ012900	als	1.4	20.5	14.8
	CPIJ013450	hypothetical protein	0.2	5.0	20.9
	CPIJ013702	17.2 kDa salivary peptide	8.7	136.2	15.6
	CPIJ013705	conserved hypothetical protein	13.1	127.3	9.7
	CPIJ013706	conserved hypothetical protein	11.0	129.7	11.8
	CPIJ014402	hypothetical protein	0.2	13.1	75.5
	CPIJ014545	short form D7clu32 salivary protein	1.0	13.3	13.7
	CPIJ014861	conserved hypothetical protein	4.8	47.8	9.9
	CPIJ015385	vitellogenin	0.3	54.3	158.5
	CPIJ015502	16.8 kDa salivary protein	0.7	41.0	60.1
	CPIJ015613	galactose-specific C-type lectin	2.4	124.0	51.8
	CPIJ015614	galactose-specific C-type lectin	3.0	208.0	68.8
	CPIJ015615	salivary C-type lectin	2.1	33.4	16.2
	CPIJ015774	34 kDa salivary secreted protein 34k-2	0.3	4.8	16.8
	CPIJ016318	larval cuticle protein 8.7	102.4	3783.8	37.0
	CPIJ016702	calbindin-32	10.9	223.0	20.5
	CPIJ016792	hypothetical protein	1.0	16.4	17.0
	CPIJ016936	Trypsin	1.3	47.5	36.7
	CPIJ016972	salivary secreted protein 62k-3	1.0	14.2	14.8
	CPIJ017043	hypothetical protein	4.9	104.4	21.2
	CPIJ017044	hypothetical protein	1.9	91.3	47.5
	CPIJ017687	conserved hypothetical protein	4.6	81.3	17.8
	CPIJ017960	hypothetical protein	4.6	117.3	25.2
	CPIJ018205	chymotrypsin-2	2.2	23.0	10.3
	CPIJ018773	hypothetical protein	8348.3	68893.7	8.3
	CPIJ018872	salivary mucin	0.5	18.6	37.9
	CPIJ019040	15.8 kDa salivary peptide	12.9	313.1	24.4
	CPIJ019051	16.7 kDa salivary peptide	0.6	63.0	104.6
	CPIJ019052	13.1 kDa salivary protein	0.3	70.6	216.7
	CPIJ019055	17.5 kDa salivary peptide	0.4	60.8	137.1
	CPIJ019252	salivary mucin	0.3	32.7	96.0
	CPIJ019253	apyrase	0.2	9.3	42.8
	CPIJ019268	calbindin-32	14.8	237.5	16.0
	CPIJ019284	hypothetical protein	6.6	116.4	17.6
	CPIJ019552	calbindin-32	9.1	198.1	21.8
	CPIJ019905	hypothetical protein	10.5	886.2	84.7
	CPIJ019944	hypothetical protein	9.5	132.9	14.0
	CPIJ019945	hypothetical protein	6.9	101.9	14.7
Regulation	CPIJ010170	conserved hypothetical protein	0.3	4.9	17.6
	CPIJ010171	conserved hypothetical protein	0.1	1.3	15.5
	CPIJ013451	zinc finger protein	0.2	6.2	26.8
	CPIJ001084	low molecular weight protein-tyrosine-phosphatase	2.4	35.9	14.9
	CPIJ010312	conserved hypothetical protein	20.2	224.9	11.1
	CPIJ009440	cytoplasmic polyadenylation element binding protein	0.1	4.7	32.5
	CPIJ004145	predicted protein	1.5	18.8	12.8
	CPIJ007193	period circadian protein	9.1	89.2	9.9

	CPIJ010788	conserved hypothetical protein	12.1	98.2	8.1
	CPIJ014546	salivary short D7 protein 4	1.0	22.9	22.3
	CPIJ014550	long form D7Bclu1 salivary protein	1.1	19.8	17.6
	CPIJ014553	salivary long D7 protein 3	5.0	57.3	11.5
	CPIJ015944	predicted protein	0.3	8.4	30.8
Extra-cellular processes	CPIJ017322	conserved hypothetical protein	46.3	5.0	-9.2
General	CPIJ011014	peptidylglycine alpha-amidating monooxygenase COOH-terminal interactor protein-1	18.9	2.3	-8.3
	CPIJ000841	dimeric dihydrodiol dehydrogenase	420.9	10.6	-39.7
	CPIJ005895	conserved hypothetical protein	15.4	1.6	-9.4
	CPIJ013724	dimethylaniline monooxygenase	117.3	0.6	-186.6
	CPIJ017482	choline dehydrogenase	76.8	3.4	-22.7
	CPIJ017483	glucose dehydrogenase	27.2	0.3	-96.4
	CPIJ017487	glucose dehydrogenase	1.7	0.1	-23.8
Intra-cellular processes	CPIJ008515	cellular retinaldehyde binding protein	190.2	17.4	-10.9
	CPIJ008722	conserved hypothetical protein	90.2	9.4	-9.6
	CPIJ003915	chymotrypsin 1	166.1	0.5	-348.2
	CPIJ004215	conserved hypothetical protein	16.5	1.2	-13.6
	CPIJ004659	trypsin 7	5.0	0.1	-57.6
	CPIJ004660	trypsin 1	339.9	17.5	-19.4
	CPIJ012643	conserved hypothetical protein	57.1	1.4	-39.5
	CPIJ017794	220 kDa silk protein	13.8	1.4	-9.5
	CPIJ019781	trypsin 1	20.4	0.4	-47.1
	CPIJ011555	mitochondrial carrier protein	105.9	11.4	-9.3
Metabolism	CPIJ002066	alpha-galactosidase A	23.8	1.6	-15.0
	CPIJ010945	acidic mammalian chitinase	43.0	3.4	-12.7
	CPIJ002725	lipase 1	58.4	0.9	-67.8
	CPIJ004802	endothelial lipase	3.1	0.2	-15.9
	CPIJ013029	esterase FE4	1.9	0.1	-16.2
	CPIJ011840	cytochrome P450	33.6	0.9	-35.9
	CPIJ011841	cytochrome P450	34.7	1.9	-17.8
	CPIJ015954	cytochrome P450	14.8	1.6	-9.4
	CPIJ015960	cytochrome P450 4A6	152.1	14.1	-10.8
	CPIJ015961	cytochrome P450	37.0	2.5	-15.1
	CPIJ017484	glucose dehydrogenase	26.2	0.4	-67.3
No Annotation	CPIJ000499	hypothetical protein	10.5	0.3	-30.6
	CPIJ001222	conserved hypothetical protein	252.2	2.0	-126.7
	CPIJ001839	cuticle protein	54.4	1.0	-56.9
	CPIJ002800	larval/pupal cuticle protein H1C	16.4	0.5	-31.9
	CPIJ002801	larval/pupal cuticle protein H1C	9.3	0.5	-17.6
	CPIJ003026	conserved hypothetical protein	13.6	0.2	-87.2
	CPIJ004287	conserved hypothetical protein	22.5	2.6	-8.6
	CPIJ004288	cuticle protein	8.8	0.7	-13.2
	CPIJ004290	cuticle protein	249.6	7.6	-32.8
	CPIJ004293	cuticle protein	88.7	2.8	-31.4
	CPIJ004475	conserved hypothetical protein	210.1	5.1	-41.3
	CPIJ005176	G12	11.3	0.3	-33.1
	CPIJ006327	metalloproteinase	29.1	0.4	-65.9
	CPIJ007055	SEC14	77.9	6.1	-12.9
	CPIJ007056	conserved hypothetical protein	71.0	4.8	-14.8
	CPIJ007448	conserved hypothetical protein	12.7	0.4	-28.8
	CPIJ008211	conserved hypothetical protein	270.1	0.8	-323.1
	CPIJ008231	pupal cuticle protein	123.2	4.3	-28.9

		CPIJ008286	serine protease	813.0	16.9	-48.2
		CPIJ008659	metalloproteinase	15.8	0.2	-95.0
		CPIJ008974	cuticle protein	28.6	0.9	-32.7
		CPIJ009098	conserved hypothetical protein	1444.0	24.9	-58.1
		CPIJ009099	conserved hypothetical protein	1853.0	57.2	-32.4
		CPIJ009207	conserved hypothetical protein	19.9	0.3	-74.2
		CPIJ009585	hypothetical protein	143.3	0.5	-265.3
		CPIJ011001	sulfotransferase	5.0	0.3	-19.1
		CPIJ012507	conserved hypothetical protein	104.1	2.0	-51.9
		CPIJ013663	elongase	179.3	9.9	-18.1
		CPIJ013764	cuticle protein 7	12.7	0.9	-14.9
		CPIJ013765	cuticle protein 18.6	44.4	2.0	-22.5
		CPIJ013931	conserved hypothetical protein	5.3	0.3	-16.0
		CPIJ014435	hypothetical protein	89.0	0.7	-120.9
		CPIJ014778	conserved hypothetical protein	39.4	3.0	-13.1
		CPIJ015291	hypothetical protein	8.3	0.1	-66.9
		CPIJ016716	conserved hypothetical protein	59.8	4.9	-12.2
		CPIJ017020	hypothetical protein	30.9	0.1	-268.6
		CPIJ017620	hypothetical protein	2100.1	8.7	-240.2
		CPIJ017806	conserved hypothetical protein	31.1	2.2	-14.3
		CPIJ017862	sulfotransferase	6.3	0.5	-12.8
		CPIJ018582	pupal cuticle protein	140.8	5.0	-27.9
		CPIJ018910	conserved hypothetical protein	64.9	5.7	-11.3
		CPIJ019396	hypothetical protein	267.8	23.7	-11.3
	Regulation	CPIJ012716	odorant-binding protein	134.0	2.8	-48.2
		CPIJ012719	general odorant-binding protein 56d	679.5	47.9	-14.2
		CPIJ012721	odorant-binding protein	59.0	1.0	-57.7
		CPIJ018956	general odorant-binding protein 56d	523.0	42.4	-12.3
12 v 24h	Extra-cellular processes	CPIJ018858	fibrinogen and fibronectin	4.1	70.7	17.2
		CPIJ002173	conserved hypothetical protein	6.2	60.0	9.6
		CPIJ014105	galactose-specific C-type lectin	1.7	24.1	13.8
	Information	CPIJ017289	conserved hypothetical protein	0.0	3.5	N/C*
	Intra-cellular processes	CPIJ000214	serpin B10	9.2	122.6	13.3
		CPIJ005273	trypsin 2	76.8	7233.8	94.2
		CPIJ011998	zinc carboxypeptidase A 1	23.8	321.9	13.5
		CPIJ014254	chymotrypsin BI	0.4	11.6	29.0
		CPIJ015161	chymotrypsin 1	0.1	24.6	172.0
		CPIJ015162	serine-type enodpeptidase	0.1	9.7	86.6
		CPIJ017414	trypsin 4	19.6	368.2	18.8
		CPIJ017964	trypsin 7	13.0	110.5	8.5
	Metabolism	CPIJ017575	low-density lipoprotein receptor	12.7	120.0	9.4
		CPIJ002715	lipase 3	0.4	9.8	23.4
		CPIJ001886	cytochrome P450 4C1	0.0	2.9	66.5
		CPIJ006840	CD109 antigen	0.4	4.7	10.9
	No Annotation	CPIJ000529	conserved hypothetical protein	0.1	3.5	60.6
		CPIJ000835	chymotrypsin-2	111.7	4177.6	37.4
		CPIJ001237	conserved hypothetical protein	3.2	32.4	10.0
		CPIJ004491	sodium/potassium/calcium exchanger 3	1.9	16.9	9.0
		CPIJ005637	conserved hypothetical protein	0.0	0.1	0.0
		CPIJ006087	sodium/solute symporter	1.3	15.1	11.9
		CPIJ008023	olfactory receptor	0.1	3.9	35.5
		CPIJ012164	conserved hypothetical protein	0.1	16.4	177.5
		CPIJ014969	caldecrin	2.4	33.1	13.7

	CPIJ015718	arginase	2.1	18.6	9.0
Regulation	CPIJ015936	hypothetical protein	0.7	6.9	10.4
Extra-cellular processes	CPIJ011368	f-box/lrr protein	69.9	2.1	-32.9
Intra-cellular processes	CPIJ000521	sodium-dependent phosphate transporter	4.2	0.3	-12.5
	CPIJ010466	laccase-like multicopper oxidase 1	6.8	0.6	-12.3
	CPIJ016802	laccase-like multicopper oxidase 1	9.7	0.9	-10.4
	CPIJ011997	zinc carboxypeptidase A 1	68.8	1.2	-56.8
	CPIJ012680	ADAM 12	23.8	2.1	-11.2
	CPIJ016937	coagulation factor X	37.5	3.8	-9.9
Metabolism	CPIJ000679	conserved hypothetical protein	142.5	14.0	-10.1
	CPIJ000680	conserved hypothetical protein	384.3	19.9	-19.3
	CPIJ007603	conserved hypothetical protein	325.4	25.3	-12.8
	CPIJ010945	acidic mammalian chitinase	3.4	0.3	-10.6
	CPIJ012316	conserved hypothetical protein	30.6	3.6	-8.6
	CPIJ005936	carbonic anhydrase II	9.9	0.2	-43.7
	CPIJ006311	conserved hypothetical protein	56.7	6.3	-9.0
	CPIJ011837	cytochrome P450	58.3	3.4	-17.0
No Annotation	CPIJ000641	salivary asparagine-rich mucin	134.3	1.7	-79.1
	CPIJ001605	pro-resilin	37.8	1.6	-23.2
	CPIJ002016	conserved hypothetical protein	19.2	2.2	-8.9
	CPIJ003019	conserved hypothetical protein	21.9	0.8	-27.8
	CPIJ003030	adult cuticle protein	13.2	0.2	-57.7
	CPIJ003473	cuticle protein	337.4	16.8	-20.1
	CPIJ003474	cuticle protein	1824.0	129.9	-14.0
	CPIJ005336	conserved hypothetical protein	23.8	1.7	-14.0
	CPIJ006195	hypothetical protein	16.2	0.5	-33.3
	CPIJ006794	conserved hypothetical protein	141.5	15.8	-9.0
	CPIJ006796	conserved hypothetical protein	124.6	13.6	-9.2
	CPIJ006797	conserved hypothetical protein	196.3	20.1	-9.8
	CPIJ008231	pupal cuticle protein	4.3	0.3	-13.7
	CPIJ008489	conserved hypothetical protein	65.9	6.4	-10.3
	CPIJ009100	conserved hypothetical protein	586.3	21.4	-27.4
	CPIJ009334	conserved hypothetical protein	113.3	2.3	-48.9
	CPIJ010338	conserved hypothetical protein	684.0	55.7	-12.3
	CPIJ010705	conserved hypothetical protein	32.8	2.2	-14.9
	CPIJ012090	actin	373.3	24.4	-15.3
	CPIJ012641	pupal cuticle protein	18.6	0.5	-34.8
	CPIJ012973	conserved hypothetical protein	5.6	0.4	-15.1
	CPIJ013278	conserved hypothetical protein	31.8	0.6	-52.8
	CPIJ013783	pupal cuticle protein	31.9	1.7	-19.3
	CPIJ013785	conserved hypothetical protein	828.9	2.6	-321.8
	CPIJ015249	hypothetical protein	12.0	1.4	-8.4
	CPIJ015250	hypothetical protein	61.0	5.3	-11.5
	CPIJ016655	conserved hypothetical protein	733.3	45.9	-16.0
	CPIJ016702	calbindin-32	223.0	22.7	-9.8
	CPIJ016842	conserved hypothetical protein	16.4	1.2	-13.6
	CPIJ017736	conserved hypothetical protein	184.0	16.8	-11.0
	CPIJ017876	cuticle protein	306.8	13.5	-22.7
	CPIJ019699	structural contituent of cuticle	145.7	11.3	-12.9
	CPIJ019849	conserved hypothetical protein	13.9	1.5	-9.5
	CPIJ019982	conserved hypothetical protein	10.7	0.9	-11.5
Regulation	CPIJ000274	conserved hypothetical protein	28.1	1.8	-15.3
	CPIJ011799	conserved hypothetical protein	15.7	1.7	-9.2

24 v 36h	Extra-cellular processes	CPIJ000931	conserved hypothetical protein	12.6	1.3	-9.7
		CPIJ011371	f-box/lrr protein	23.1	1.8	-12.8
	Information	CPIJ017289	conserved hypothetical protein	3.5	0.0	0.0
	Intra-cellular processes	CPIJ000214	serpin B10	122.6	4.4	-27.9
	No Annotation	CPIJ003470	hypothetical protein	2549.0	37.8	-67.5
		CPIJ003473	cuticle protein	16.8	0.5	-31.9
		CPIJ003474	cuticle protein	129.9	2.4	-53.9
		CPIJ003476	cuticle protein	1099.5	17.3	-63.7
		CPIJ003477	cuticle protein	1043.6	16.9	-61.7
		CPIJ009100	conserved hypothetical protein	21.4	1.7	-13.0
		CPIJ009101	hypothetical protein	80.5	6.0	-13.4
		CPIJ009111	conserved hypothetical protein	50.0	5.2	-9.6
		CPIJ012090	actin	24.4	2.5	-9.8
		CPIJ017874	hypothetical protein	490.3	9.7	-50.5
		CPIJ017875	hypothetical protein	1222.8	30.2	-40.5
		CPIJ018642	pupal cuticle protein	80.3	8.5	-9.4
		CPIJ018939	oxidoreductase	0.1	0.0	0.0
36 v 48h	Metabolism	CPIJ006495	conserved hypothetical protein	2.3	21.0	9.2
	Intra-cellular processes	CPIJ000990	cytosol aminopeptidase	6.6	0.1	-79.3
		CPIJ002595	zinc carboxypeptidase	19.0	0.1	-170.8
		CPIJ003539	cytosol aminopeptidase	6.2	0.0	-146.0
	Metabolism	CPIJ004028	venom allergen 3	14.2	0.4	-36.0
	No Annotation	CPIJ007077	trypsin-4	9.5	0.4	-26.2
		CPIJ010092	ficolin-3	15.2	0.3	-44.5
		CPIJ010778	conserved hypothetical protein	6.1	0.6	-10.5
		CPIJ011171	LWamide neuropeptides	47.7	0.3	-178.1
		CPIJ011620	conserved hypothetical protein	1.4	0.0	-63.2
		CPIJ016384	conserved hypothetical protein	12.2	0.2	-56.0
	Regulation	CPIJ015944	predicted protein	18.2	0.3	-57.2
48 v 60h	Intra-cellular processes	CPIJ000990	cytosol aminopeptidase	0.1	2.3	27.2
		CPIJ002595	zinc carboxypeptidase	0.1	18.2	163.7
		CPIJ003539	cytosol aminopeptidase	0.0	3.0	70.3
	Metabolism	CPIJ014185	conserved hypothetical protein	1.0	14.8	15.0
		CPIJ004028	venom allergen 3	0.4	12.6	31.9
	No Annotation	CPIJ007077	trypsin-4	0.4	9.6	26.5
		CPIJ010092	ficolin-3	0.3	9.0	26.5
		CPIJ011171	LWamide neuropeptides	0.3	38.8	144.9
		CPIJ016384	conserved hypothetical protein	0.2	15.0	68.8
	Regulation	CPIJ015944	predicted protein	0.3	7.7	24.1
60 v 72h	Intra-cellular processes	CPIJ002595	zinc carboxypeptidase	18.2	2.2	-8.4
	Metabolism	CPIJ009796	lipoprotein lipase	5.0	0.4	-12.0
	No Annotation	CPIJ001231	conserved hypothetical protein	10.7	0.2	-53.7
		CPIJ015506	hypothetical protein	60.2	3.0	-19.9
		CPIJ016394	nuclear pore complex protein Nup93	0.4	0.0	-23.7

†Structural Classification of Proteins (SCOP) database for the *Culex quinquefasciatus* database (v1.73). http://supfam.cs.bris.ac.uk/SUPERFAMILY/

‡Vectorbase annotation for the Johannesburg strain of *Cx. quniquefasciatus* JHBv1.2. http://www.vectorbase.org/

*[Paired end] Fragments Per Kilo bases of gene length per Million RNA-Seq reads mapped. Time 1 and 2 represent

the earlier and later time points in the comparison, respectively.
**N/C= Not calculable