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The genetic basis of cuticular hydrocarbon production in Drosophila melanogaster and D. simulans

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

THE GENETIC BASIS OF CUTICULAR HYDROCARBON PRODUCTION IN *DROSOPHILA MELANOGASTER* AND *D. SIMULANS*

(The genetics of cuticular hydrocarbon production in *Drosophila*)

(Thesis format: Monograph)

by

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Abstract

Individuals within many species have evolved complex reproductive strategies to ensure they identify appropriate mates so as to increase their fitness. Chemical signals, which are often species- and sex-specific, are one means by which many insect species communicate. Differences in cuticular hydrocarbons (CHCs) profiles can influence reproductive isolation in *Drosophila*. A previous study identified large genomic regions that contain genes responsible for the different CHC profiles observed in *D. melanogaster* and *D. simulans* females. I refined these earlier results by utilizing fine-scale mapping, focusing on the entire CHC profile, in an effort to determine which genes are responsible for the different CHC profiles of this species pair. Five genomic regions along Chromosome *3* were identified as well as a candidate gene, *Dhc93AB*. Determining the molecular mechanisms governing the differential CHC profiles in these two species could provide insight into the genes responsible for CHC formation as well as reproductive isolation.

Keywords

Cuticular hydrocarbons, *Drosophila*, deficiency mapping, pheromones, reproductive isolation

Dedication

This work is dedicated to my family, whose unending support has motivated me to see this project through to completion. I am forever in their debt.

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Firstly, I would like to thank Dr. Amanda Moehring, whose guidance and support over the years have not only shaped me as a scientist, but as a person as well. I will forever admire her creativity, attention to detail and enthusiasm. I appreciate the countless number of hours she has spent fielding my questions, editing drafts and giving me pep-talks. She is the master-juggler of all things in life and I wish her much success, she certainly deserves it.

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Many thanks to Dr. Howard Rundle, as he helped with the development of this project, and he also ran my samples on his GC.

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Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

Bal	balancer chromosome
CHC	cuticular hydrocarbon
cVA	*cis*-vaccenyl acetate
Df	deficiency chromosome
Dhc93AB	*Dynein heavy chain at 93AB*
DVM	dominant visible marker
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
mel	*Drosophila melanogaster*
sim	*Drosophila simulans*
3L	left arm of Chromosome *3*
3R	right arm of Chromosome *3*
2-MH	2-methyl hexacosane
5,9-HD	5,9-heptacosadiene
7-P	7-pentacosene
7-T	7-tricosene
7,11-HD	7,11-heptacosadiene
7,11-ND	7,11-nonacosadiene

1 General Introduction

1.1 Reproductive strategies

Individuals from many species have evolved pre- and postzygotic reproductive isolating mechanisms (Dobzhansky, 1937), which ensure they find an appropriate mate and reject those that are not, thus increasing their reproductive fitness. Postzygotic isolating mechanisms occur after the formation of the zygote and include hybrid inviability and hybrid sterility, while prezygotic isolating mechanisms include ecological, temporal, mechanical and/or behavioural isolation (Dobzhansky, 1937). Prezygotic barriers are thought to have a greater impact on reproductive isolation as they act earlier in the reproductive cycle and are more often the only isolating mechanism between species (Coyne and Orr, 1989; 1997). One form of prezygotic isolation is the behavioural differences between species that decrease the likelihood of interspecies mating (Dobzhansky, 1937).

Courtship behaviours involve auditory, visual, tactile and chemical signals that may act individually or in combination, serving both as species-specific cues to avoid heterospecific matings, as well as sex-specific cues to select the most appropriate conspecific mate. For example, in Amazonian frogs, males produce species-specific auditory calls (Boul *et al*., 2007) which allow females to avoid males of other species. Furthermore, the call may differ considerably between conspecific males, allowing the female to gauge the attractiveness of her potential mates (Boul *et al*., 2007). Males of many bird species use visual cues, using elaborate plumage to attract potential mates, with female bias generally favouring the longer, more elaborate tail feathers for males (Andersson, 1982; Pryke and Andersson, 2002). Courtship behaviours of aphid

parasitoids, such as *Aphidius ervi* and *A. nigripes*, involve several chemical cues. Virgin females emit a pheromone that acts long-distance to attract potential mates, while other chemical and auditory signals serve once the sexes are in close proximity. Once the male has mounted the female (but before copulation), he will stroke her antennae with his, resulting in an exchange of tactile and possibly chemical signals between the pair (McNeil and Brodeur, 1995; McClure *et al*., 2007). The courtship rituals of Australian redback spiders (*Latrodectus hasselti*) have been well-characterized and include all of the above signals (Forster, 1992; Stoltz and Andrade, 2010). *Latrodectus hasselti* females release a sex pheromone (chemical cue) which induces males to exhibit courtship behaviours (Jerhot *et al.*, 2010). In the first stage of courtship, males will perform a stereotypical courtship dance, which involves plucking the web (sound cue), throwing silk, waving their legs (visual cue) and grooming. The second stage involves physical contact (tactile cue) with the female in the form of tapping and probing. The final courtship stage involves continued contact with the female, particularly around her genital region. The order and timing of each courtship event is species-specific and if any step is deemed unacceptable or unattractive by the female, she will become aggressive toward the male and mating will not occur.

1.2 Chemical systems in arthropods

Arthropods have evolved complex chemical signalling systems, not only for reproductive cues, but to fulfill a number of roles. Chemical signals (infochemicals) can be separated into two main categories: pheromones, which are used for intraspecific communication or allelochemicals, used for interspecific communication (Lewis, 1984). Within species, pheromones influence many behaviours, such as aggregation, signalling danger,

establishing social castes and mate recognition (Howard and Blomquist, 2005). Between species, chemical cues can act in the avoidance of interspecific mating, location of food and defence (Howard and Blomquist, 2005). The active space over which these cues are detected will vary depending on their chemical composition, some being effective over kilometer distances, others over centimeters, while others only when there is direct contact with the source (Lewis, 1984).

Many insects produce cuticular hydrocarbons (CHCs) which are important in providing desiccation resistance by changing the permeability of the cuticle. Edney (1967) found that beetles inhabiting the desert had an impermeable cuticle whereas cockroaches living under the desert sand had a very permeable cuticle and both species had adapted to their own environments by producing more or less of these hydrocarbon compounds. CHC production has also been correlated with geographical location for *Drosophila melanogaster*. Rouault *et al.* (2001) collected 85 strains of *D. melanogaster* from various geographical locations and observed a pattern of increasing CHC chain length with increasing temperature and humidity. The results of this study lent support for work done by Gibbs *et al.* (1997), in which they concluded that longer chain lengths better protect against desiccation as they have higher melting points.

This principle of desiccation resistance has been thoroughly studied, with the common consensus being that the more abundant CHCs are on an individual, the better able that individual is at maintaining overall body water in very dry environments (Gibbs *et al.*, 1997; Gibbs *et al*., 1998; Kwan and Rundle 2010; Savarit and Ferveur, 2002).

However, it is now well established that, in addition to desiccation resistance, CHCs also provide important chemical cues in a variety of species in several insect

Orders. Individuals of a given species produce a particular number and amount of compounds known as the CHC profile, which may be important in both species- and sex-specific interactions (Pechine *et al.*, 1985). Differences in CHC profiles influence the way in which individuals interact with one another and their environments, and serve a variety of functions, including establishment of social systems, determination of an individual's social status, deception of others as well as reinforcement of reproductive isolation (Cremer *et al.*, 2002; Greene and Gordon, 2003; Stökl *et al.* 2011; Thomas and Simmons, 2011; Wagner *et al.*, 2001). The CHC profile will change with different developmental stages during the life cycle and in a number of different species in different Orders, when adults reach reproductive maturity, they produce the species- and sex- specific profiles that are important for mate recognition (Blomquist and Bagnères, 2010).

1.3 Role of CHCs in social systems, status and deception

Eusocial insect species are successful, in part, because of their high level of organization and CHCs play a significant role in the establishment and maintenance of social structure. For example, a colony of harvester ants (*Pogonomyrmex barbatus*), will contain different worker castes, including foragers, patrollers and individuals who maintain the nest, with each caste fulfilling a particular need for the colony as a whole (Gordon, 1996). Different castes have different CHC profiles, which will signal their role to other colony members (Greene and Gordon, 2003). For example, the outside workers of *P. barbatus* have a greater abundance of CHCs than those staying inside the nest, probably because they are exposed to higher temperatures and thus, are in greater need of protection against desiccation (Wagner *et al.*, 1998). *Pogonomyrmex barbatus* workers also use CHC

profiles to differentiate nest mates from individuals of other colonies (Wagner *et al.*, 2001).

Chemical signals may also serve to indicate social status within a population, which may be of considerable importance for mating success. For example, in the field cricket, *Teleogryllus oceanicus,* dominant or subordinate males, defined by male-to-male fighting ability, have different CHC profiles (Shackleton *et al.*, 2005). *Teleogryllus oceanicus* subordinate males produce a greater amount of their species-specific sex pheromone (Thomas and Simmons, 2010). Dominant males use sound signals to attract females, but this signal is also used by subordinate males, who will remain close by and try to intercept an approaching female. The higher level of sex pheromone in the subordinate individuals increases the probability that they successfully mate. However, if a female mates with a dominant and subordinate male, the dominant male will father the majority of the resulting progeny, as there is a positive relationship between dominance and the number of viable sperm produced (Thomas and Simmons, 2009). Thomas and Simmons (2011), described how *T. oceanicus* male CHC profiles changed when their social status changed (dominant male becomes subordinate or *vice versa*, through wins/losses in fighting matches).

Chemical deception, based on CHCs, has been demonstrated in a variety of processes such as parasitism, intraspecific reproductive strategies and even plant-insect interactions (Bagnères *et al.*, 1996; Blomquist and Bagnères, 2010; Cremer *et al.*, 2002; Stökl *et al.*, 2010). A well-documented example of chemical deception using CHCs has been described in the wasps (*Polistes biglumis bimaculatus*) and their obligate parasite (*P. atrimandibularis*) (Bagnères *et al.*, 1996). A fertilized queen parasite will invade a

nest and change her CHC profile to match that of the host individuals. Subsequently, she will lay eggs and her larvae are then raised by the host workers, so by using chemical deception the parasite gains reproductive fitness.

Many species will use particular CHCs as sex pheromones and these influence sexual behaviour, which plays an important evolutionary role in reproductive isolation between certain species pairs (Coyne *et al.*, 1994; Spikes *et al.*, 2010; Thomas and Simmons, 2010). Cuticular hydrocarbons are often a necessary component to identify or attract potential mates. In some species, competition for mates is so intense that they have developed reproductive strategies involving intraspecific chemical deception. In the ant *Cardiocondyla obscurior* there are wingless and winged males that compete for virgin queens. The winged males mimic the CHC profile of the virgin queens and so gain more access to the queens because the wingless males lose time courting the winged males (Cremer *et al*., 2002).

Stökl *et al.* (2011) reported a case of plants utilizing this mimicry principle to gain access to pollinators. Terrestrial orchids (*Epipactis veratrifoloa*) produce beta farnasene, which is also the alarm pheromone that aphids emit when they are attacked by their natural enemies. However, certain natural enemies use the alarm pheromone as a kairomone (chemical signal released for benefit of the receiver; Lewis, 1984), allowing them to locate aphid colonies. Thus, natural enemies of the aphids are deceptively attracted to the orchid and when searching for potential prey they pollinate the orchid. While beta farnasene is not a CHC, there are examples in which orchids mimic particular CHCs to gain access to pollinators (Schiestl *et al*., 2000). The complexity of these strategies in both plants and animals are extremely impressive, creating a need for

intensive research in this area so as to better understand the full implications chemical signals have in communication.

1.4 Biosynthesis of CHCs

Given the importance of CHCs for many intra- and interspecific interactions in insects, there is a significant body of research on the biosynthesis of these compounds (see Blomquist and Bagnères, 2010, and references therein). Biosynthetic pathways have been proposed for a number of species including house flies (*Musca domestica*), cockroaches (*Periplaneta americana* and *Blattella germanica*), and termites (*Zootermopsis angusticollis*). Enzymes such as desaturases, elongases and decarboxylases act on metabolites to form these CHC compounds (Blomquist and Bagnères, 2010). Cuticular hydrocarbons are produced in the oenocytes and throughout the fat body, and transported, through an unknown mechanism, to the cuticle (Howard and Blomquist, 2005).

1.5 Perception of CHCs

It is evident that if the remarkable diversity of infochemicals produced are to be effective as chemical signals, then there must be the appropriate sensory networks to perceive and interpret these chemicals to elicit appropriate behavioural/physiological responses. Insects have a large number of receptors, which can recognize and appropriately identify chemical signals, located on different parts of the body (Lewis, 1984). The location, type and density of sensilla will vary, not only between species, but also on the chemicals that make up a given cue and its biological function(s). Receptors may be found on sites as varied as the antennae, the tarsae, various mouth parts and the ovipositor (Blomquist and Bagnères, 2010). Insect antennae are an important site for the reception of volatile cues,

and there are many specific receptors that respond to the different components of a chemical message (Lewis, 1984). They may also detect less volatile cues through contact during antennation. Säid *et al*. (2005) demonstrated that receptors on the antennae of *Periplaneta americana* responded to the three main CHC compounds in their species-specific profile. Similarly, Ozaki *et al*. (2005) reported that contact chemical signals detected by sensillae on the antenna (or mouth parts) allowed workers of the ant *Camponotus japonicas* to discriminate between nest mates and non-nestmates.

1.6 *Drosophila* as a model system

Drosophila melanogaster has long been used as a model system in the study of reproductive isolation for a number of reasons. Firstly, because of their small size, vinegar flies are relatively easy to maintain. Secondly, *Drosophila melanogaster* has many closely related sister species, from which there is only partial reproductive isolation as interspecific crosses can be achieved in the lab for some species pairs (Coyne, 1992). Thirdly, there are a wide variety of molecular tools available for several *Drosophila* species, which include complete genome sequences and mutant stocks which can be ordered from a stock center (Matthews *et al*., 2005). Finally, mating behavior has been well-characterized for many *Drosophila* species.

1.7 *Drosophila* Courtship

Male courtship behaviour in *Drosophila* has been well-characterized and follows a series of steps: he (i) orients himself toward the female; (ii) taps the female's abdomen with his front leg; (iii) vibrates a wing, producing a species-specific courtship song; (iv) licks the female's genitalia; then finally, (v) curls his abdomen and attempts copulation with the female (Hall, 1994; Greenspan, 1995; Greenspan and Ferveur 2000). Female behaviours

during courtship have not been as well-characterized. It is known that when the female does not want to mate with a courting male, she will fully extrude her ovipositor, however, partial extrusion of the ovipositor serves as a stimulant for males (Griffith and Ejima, 2009). This partial extrusion releases a liquid droplet, thought to contain additional chemical signals that the male receives (Lasbleiz *et al*., 2006; Ferveur, 2010). At any point during this courtship, it is the female's choice in whether she mates or not. The detection of low volatile chemical signals within the CHC profile, that influence mating, is thought to occur in the "tapping" and "licking" stages in courtship.

1.8 Species isolation in *Drosophila*

Different chemical cues have been shown to influence reproductive isolation in many *Drosophila* species (Billeter *et al.*, 2009; Carracedo *et al.*, 2003; Civetta and Cantor, 2003; Cobb and Jallon, 1990; Coyne *et al.*, 1994; Coyne and Charlesworth, 1997; de la Paz Fernandez *et al.*, 2010; Rundle *et al.*, 2005; Savarit *et al.*, 1999). Within the *Drosophila melanogaster* subgroup the sibling species pair *D. melanogaster* and *D. simulans* are almost entirely homosequential and can only be distinguished from one another morphologically by the shape of the male genital arch (Coyne and Orr, 1989). Both species are generally cosmopolitan and while they are sympatric, they remain reproductively isolated (Carracedo *et al.*, 2000; Moulin *et al.*, 2004). 7-tricosene (7-T) is the most abundant CHC in both sexes of *D. simulans* and also in *D. melanogaster* males, while in *D. melanogaster* females, 7,11-heptacosadiene (7,11-HD) is the most abundant CHC (Pechine, *et al.*, 1985). *Drosophila melanogaster* females also produce 7,11-nonacosadiene (7,11-ND), which is a moderate attractant for *D. melanogaster* males (Jallon, 1984). *Cis*-vaccenyl acetate (cVA), which is not a CHC, is also important for

mating in *D. melanogaster*. This compound is found in the male ejaculatory bulb and is transferred to the female during mating, making her unattractive to other potential mates (Jallon, 1984). In addition, exposure to cVA can induce male-to-male aggression (Wang and Anderson, 2010).

This species pair demonstrates asymmetrical sexual isolation. *Drosophila melanogaster* males will court and mate with *D. simulans* females but the reciprocal cross rarely occurs (Cobb and Jallon, 1990), in part, due to the different CHC profile of *D. melanogaster* females (Coyne, 1996; Jallon, 1984; Jallon and David, 1987; Savarit *et al*., 1999). Support for this hypothesis was obtained in hydrocarbon transfer assays (Coyne *et al*., 1994). When *D. simulans* females were treated with *D. melanogaster* CHCs, they were seldom courted by conspecific males, and *D. simulans* males would court *D. melanogaster* females treated with *D. simulans* female CHCs.

1.9 CHC Biosynthesis in *Drosophila*

Several enzymes, such as desaturases and elongases, are involved in CHC biosynthesis in *D. melanogaster* (Figure 1.1). The desaturase *desat1* is expressed in the oenocytes and the fat body, acting to transform palmitic and stearic acids to palmitoleic and oleic acids, respectively, by adding a double bond at the carbon 7 position (Dallerac *et al.*, 2000; Labeur *et al.*, 2002; Marcillac *et al.*, 2005). This ω7 desaturation, when followed by elongation and decarboxylation, creates the *D. melanogaster* male pheromone 7-T. The biosynthesis pathway proposed suggests that elongation, a second desaturation followed by decarboxylation, results in the production of the *D. melanogaster* female pheromone 7,11-HD (Gleason *et al*., 2009). When *desat1* is downregulated, there is an increase in

the number of saturated CHCs and an accompanying decrease in the number of desaturated CHCs (Labeur *et al.*, 2002; Ueyama *et al.* 2005).

Another desaturase, d*esat2,* which is only expressed by females of the African (z-strain) of *D. melanogaster* (Takahashi *et al.*, 2001), adds a double bond to the carbon 5 position of myristic acid, resulting in myristoleic acid. A series of steps including elongation, decarboxylation, and another desaturation results in the z-strain *D. melanogaster* female pheromone 5,9-heptacosadiene (5,9-HD), which is not produced in cosmopolitan *D. melanogaster* females (Coyne *et al.*, 1999). Interestingly, z-strain *D. melanogaster* females produce very low amounts of 7,11-HD (Grillet *et al*., 2012).

The desaturase *desatF* (also known as *Fad2*) gene is present in all of the species within the *melanogaster* subgroup, but expressed only in *D. melanogaster* females (Chertemps *et al.*, 2006). It is responsible for adding a second double bond at the carbon 11 position to the ω7 precursors (and after decarboxylation), results in a diene, 7,11-HD (Legendre *et al.*, 2008). When *desatF* is downregulated, the production of dienes in *D. melanogaster* females is drastically reduced (Wicker-Thomas *et al.*, 2009).

An elongase, *eloF,* is also involved in the *D. melanogaster* CHC biosynthesis pathway (Chertemps *et al.*, 2007). This elongase acts to extend the carbon chains of ω7 and ω11 precursors (Chertemps *et al.*, 2007).

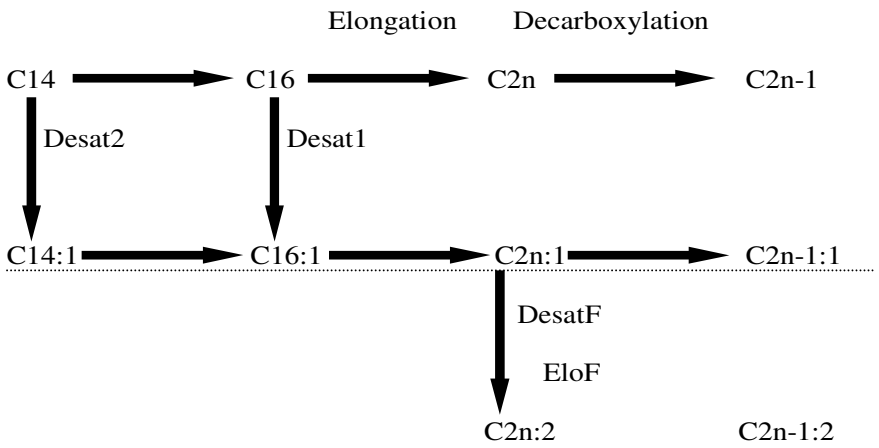

Figure 1.1. Proposed biosynthesis pathway for the production of 7-tricosene, 7-pentacosene, 7,11-heptacosadiene and 5,9-heptacosadiene in *D. melanogaster*. Steps above the dashed line are common to both males and females. Desaturation of C14 (*desat2* activity) only occurs in African *D. melanogaster* flies. Figure adapted from Legendre *et al.*, 2008.

1.10 Perception of CHCs in *Drosophila*

The advent of new molecular tools has allowed researchers to also examine how chemical signals are detected by *Drosophila* species (Bousquet *et al.*, 2012; Houot *et al.*, 2010; Wang and Anderson, 2009). Wang and Anderson (2009) described the role of the receptor, Or67d, in the perception of the compound cVA in *D. melanogaster*, showing that when this receptor was activated by direct exposure to cVA or through genetic manipulation, males exhibited aggressive behaviour.

While *desat1* is involved in CHC production by *D. melanogaster*, its role in the perception of CHCs, first suggested by Houot *et al.* (2010), had not been confirmed. Bousquet *et al.* (2012) demonstrated how both the production and perception of CHCs in *D. melanogaster* are controlled, at least in part, by a single gene. The expression patterns of five *desat1* transcripts showed that, depending on the regulatory region employed, *desat1* products can be seen in the fat body and oenocytes (for CHC biosynthesis) or in the receptors involved in the detection of CHCs.

1.11 Deficiency mapping

Deficiency mapping has been used to identify genes which contribute to quantitative traits, including mating behaviour (Moehring and Makay, 2004) and longevity (Pasyukova *et al.*, 2000). This technique employs a series of stocks, each of which contain individuals that are entirely diploid except for a deleted region on one of the homologous chromosomes (an individual is hemizygous at this region). Each stock has a different hemizygous region and there are hundreds of stocks available which span almost all of the *D. melanogaster* genome. Coyne (1996) used deficiency mapping and reported that large candidate regions on Chromosome *3* contributed to CHC production in

melanogaster/simulans hybrids, an observation confirmed in subsequent studies (Civetta and Cantor, 2003; Coyne and Charlesworth, 1997; Ferveur and Jallon, 1996; Gleason *et al.*, 2009).

While the search for genes contributing to CHC production in *D. melanogaster* has been fairly successful, this is applicable to only a few compounds within a single species. Earlier studies focused exclusively on the prominent CHC compounds in *Drosophila* (Coyne, 1996; Coyne and Elwyn, 2006; Labeur *et al.*, 2002), but the blends and ratios of CHCs are also very important factors in mate recognition and mating behaviour (Everaerts *et al.*, 2010; Savarit *et al.*, 1999). As CHCs influence reproductive isolation between *D. melanogaster* and *D. simulans*, it is desirable to know which genes are responsible for all of the differences in the CHC profiles. Therefore, in my research, I used deficiency mapping in an attempt to identify genes which contribute to the differential CHC profiles observed in *D. melanogaster* and *D. simulans*. As all genes that are known contributors to the *D. melanogaster* CHC biosynthesis pathway are located on Chromosome *3*, my research focuses solely on the 3rd chromosome. I hypothesize that there are genes located on Chromosome *3* that contribute to the different CHC profiles between *D. melanogaster* and *D. simulans*.

2 Methods

2.1 *Drosophila* Stocks

Pure *D. melanogaster* isofemale (BJS1, London, Ontario) and *D. simulans* (Florida City, USA) lines, together with each deficiency line ordered from the Bloomington *Drosophila* Stock Center (Bloomington, IN, USA), were maintained in 30 ml plastic food vials containing standard yeast-agar medium (Bloomington recipe) and housed in incubators with a 14:10 light:dark cycle, at 24°C and 80% relative humidity.

2.2 *Drosophila* Crosses

Newly emerged (0-8 h) virgin males and females from each stock were collected under light CO_2 anaesthesia, stored separately until after they reached sexual maturity (7 d for males, 14 d for females) and then used in crosses. Females from each deficiency line were crossed to either *D. melanogaster* males (intraspecific) or *D. simulans* males (interspecific: Figure 2.1). For the intraspecific crosses, five females were paired with five males. An average of three intraspecific crosses was set up for each deficiency line. The interspecific crosses contained approximately 10 females and 25 males: the interspecific crosses required a greater number of individuals because of a lower incidence of mating. An average of 30 interspecific crosses was set up for each deficiency line. Virgin F_1 hybrid females were collected using light CO_2 anaesthesia 0-8 h post-emersion and separated into the four possible genotypes used to assess CHC production (see below).

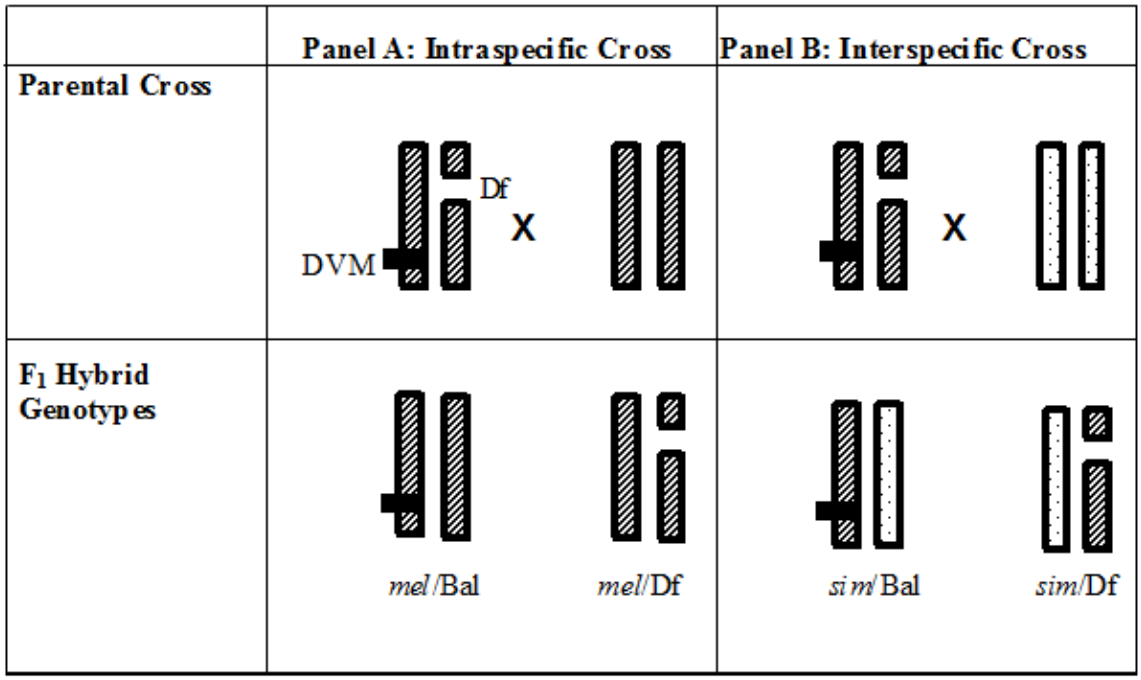

Figure 2.1. Creation of *melanogaster/simulans* F_1 hybrid females used for deficiency mapping of Chromosome *3* to locate genes potentially contributing to cuticular hydrocarbon production. The vertical hatched bars represent homologous *D. melanogaster* 3rd chromosomes; the vertical dotted bars represent *D. simulans* homologous 3rd chromosomes. The deficiency stocks, shown in top left in panels A and B, are entirely *D. melanogaster*. Each line harbours a dominant visible marker (DVM) as well as a deleted region (represented by a gap in the chromosome). Intra- and interspecific crosses with these deficiency lines will result in four F_1 genotypes: *mel/Bal*, *mel/Df*, *sim/Bal* and *sim/Df*.

2.3 Deficiency Mapping

For this study, 74 deficiency lines were tested (Table 2.1). Each deficiency line utilized in this study has a different deletion on Chromosome *3*. These deleted regions overlap in such a way that enables one to "walk" down the chromosome in an attempt to identify regions containing genes contributing to CHC production. An individual of any given deficiency line will possess a complete *D. melanogaster* genome, with the exception of the deleted region, where the individual is hemizygous. For the 3rd chromosome, the homolog with the deletion is named deficiency (*Df*), while the other homolog harbours inversions to prevent recombination, and is named balancer (*Bal*). This balancer chromosome has a dominant visible marker (DVM) which allows one to track which offspring of a cross received the balancer and which received the deficiency. Coyne (1996) confirmed that *D. melanogaster* genes for CHC production act in a dominant fashion in *melanogaster/simulans* F_1 hybrid females. This pattern of *D. melanogaster* dominance holds true for most of the CHC compounds identified in this study (Figure 2.2). Therefore, I expected that *mel*/*Bal*, *mel*/*Df* and *sim*/*Bal* would have the same CHC profile as they all contain a complete homolog set of the *D. melanogaster* genome. The *sim*/*Df* flies lack the dominant *D. melanogaster* genome in the small region that is deficient, and therefore the *D. melanogaster* genes are not present to mask the effects of the recessive *D. simulans* genes. If a deficiency region spans a gene in the *D. simulans* genome that is important in the production of its CHC profile, a more *D. simulans*-like profile would be observed in the *sim*/*Df* flies.

Table 2.1. Deficiency lines spanning Chromosome *3*, used for mapping for genes contributing to the differential CHC profiles observed in *D. melanogaster* and *D. simulans*.

Line Number	Deficiency	Cytological Location
2400	*Df(3L)R-G7*	62B7;62E5-6
3650	*Df(3L)M21*	62F;63D, 62A;64C
5877	*Df(3L)ZP1*	66A17-20;66C1-5
6460	*Df(3L)BSC13*	66B12-C1;66D2-4
24413	*Df(3L)BSC389*	66C12;66D8
3024	*Df(3L)h-i22*	66D10-11;66E1-2
4500	*Df(3L)Scf-R6*	66E1-6;66F1-6
9355	*Df(3L)ED4457*	67E2;68A7
26828	*Df(3L)BSC730*	68F7;69E6
8072	*Df(3L)ED4486*	69C4;69F6
6457	*Df(3L)BSC12*	69F6-70A1;70A1-2
3124	*Df(3L)fz-GF3b*	70C1-2;70D4-5, 66E
3126	*Df(3L)fz-M21*	70D2-3;71E4-5
2993	*Df(3L)st-f13*	72C1-D1;73A3-4
2608	*Df(3L)W10*	75A6-7;75C1-2
8082	*Df(3L)ED4782*	75F2;76A1
6754	*Df(3L)fz2*	75F10-11;76A1-5
5126	*Df(3L)XS533*	76B4;77B
3127	*Df(3L)ri-79c*	77B-C;77F-78A

Line Number	Deficiency	Cytological Location
5878	*Df(3L)ri-XT1*	77E2-4;78A2-4
4429	*Df(3L)ME107*	77F3;78C8-9
9700	*Df(3L)BSC223*	79A3;79B3
23149	*Df(3L)BSC249*	79B2;79D1
24955	*Df(3L)BSC451*	79B2;79F5
8089	*Df(3L)ED230*	79C2;80A4
25669	*Df(3L)BSC554*	80A1;80C1
8102	*Df(3L)ED5017*	80A4;80C2
1518	*Df(3R)ME15*	81F3-6;82F5-7
4787	*Df(3R)3-4*	82F3-4;82F10-11
8965	*Df(3R)ED5156*	82F8;83A4
7623	*Df(3R)Exel6144*	83A6;83B6
8103	*Df(3R)ED5177*	83B4;83B6
8685	*Df(3R)ED7665*	84B4;84E11
1968	*Df(3R)p712*	84D4-6;85B6, 25D;85B6
9077	*Df(3R)ED5330*	85A5;85D1
6756	*Df(3R)BSC24*	85B7;85D15
7080	*Df(3R)BSC38*	85F1-2;86C7-8
3128	*Df(3R)M-Kx1*	86C1;87B1-5
3003	*Df(3R)T-32*	86D9;87C3-4
3358	*Df(3R)ry85*	87B15-C1;87F15-88A1, 87C2-3;88C2-3;21-40

Line Number	Deficiency	Cytological Location
24137	*Df(3R)ED5664*	88D1;88E3
24975	*Df(3R)BSC471*	88E3;88E5
756	*Df(3R)sbd105*	88F9-89A1;89B9-10
1467	*Df(3R)P115*	89B7-8;89E7;20
4431	*Df(3R)DG2*	89E1-F4;91B1-B2
5127	*Df(3R)RD31*	89E2;90D
8104	*Df(3R)ED5780*	89E11;90C1
3011	*Df(3R)Cha7*	90F1-F4;91F5
3012	*Df(3R)Dl-BX12*	91F1-2;92D3-6
4962	*Df(3R)H-B79*	92B3;92F13
7413	*Df(3R)BSC43*	92F7-93A1;93B3-6
3340	*Df(3R)e-R1*	93B6-7;93D2
5798	*Df(3R)e-GC3*	93C6;94A1-4
8684	*Df(3R)ED6096*	94B5;94E7
8583	*Df(3R)BSC56*	94E1-2;94F1-2
7990	*Df(3R)Exel9012*	94E9;94E13
9497	*Df(3R)BSC137*	94F1;95A4
7675	*Df(3R)Exel6196*	95C12;95D8
4432	*Df(3R)crb-F89-4*	95D7-D11;95F15
2363	*Df(3R)crb87-5*	95F6-8;96A18-20
9211	*Df(3R)ED6220*	96A7;96C3
7682	*Df(3R)Exel6203*	96E2;96E6

Line Number	**Deficiency**	**Cytological Location**
24909	*Df(3R)BSC321*	96E6;96E9
5601	*Df(3R)Espl3*	96F1;97B1
25001	*Df(3R)BSC497*	97E6;98B5
25390	*Df(3R)BSC567*	98B6;98E5
430	*Df(3R)3450*	98E3;99A6-8
8925	*Df(3R)ED6316*	99A5;99C1
3547	*Df(3R)L127*	99B5-6;99E4-F1, 98F;100F
3546	*Df(3R)B81*	99C8;100F5, 99D;100F
2599	*Df(3R)tll-g*	99F1-2;100B4-5
7696	*Df(3R)Exel6218*	100B5;100C1
24516	*Df(3R)ED50003*	100E1;100F5
Gene Disruption Line	**Disruption**	**Cytological Location**
25298	*Mi{ET1}Dhc93AB[MB05444]*	93B7
35872	*Mi{y[+mDint2]=MIC}CG17278 [MI02608]*	93B4

The line numbers (Bloomington *Drosophila* Stock Center) are shown along with their cytological location in *D. melanogaster*. Chromosome *3* is cytologically numbered from 61 at the left telomere to 100 at the right telomere, each number is subdivided into A-F, then further subdivided by a variable number. The two gene disruption lines used for testing *Dhc93AB* are also listed.

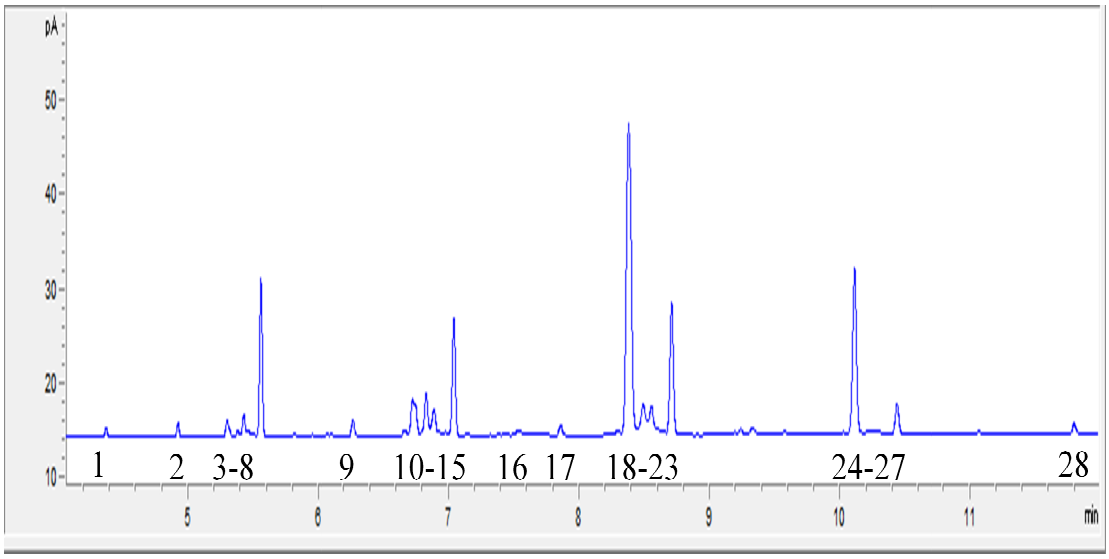

Figure 2.2. A GC trace of a *Drosophila melanogaster* female CHC profile. The x-axis represents retention time in minutes and the y-axis represents signal intensity. Compounds analyzed in this study are numbered 1-28.

2.4 Gene Disruption Lines

Two overlapping deficiency lines (*Df(3R)BSC43* and *Df(3R)e-R1*) yielded significant results (see Chapter 3) and within the overlapping region was a single gene: *Dhc93AB*. This candidate gene was tested for its role in the differential CHC profiles in *D. melanogaster* and *D. simulans*. Two gene disruption lines, obtained from the Bloomington *Drosophila* Stock Center, were used to test this candidate gene. Both lines were created through the use of p-element insertions which were made homozygous. For these lines, I had to introduce a dominant visible marker (DVM), by crossing females of the gene disruption lines to males harbouring a DVM on their 3rd chromosome. Female offspring with the DVM were collected and then used in the interspecific or intraspecific crosses as described above.

2.5 CHC Extractions and Gas Chromatography

The CHC profiles were collected from mature F_1 hybrid females 8 d after eclosion, by washing individual flies in 100 µl hexane for approximately 3 min then vortexing for 1 min. Flies were then removed and discarded. Octadecane (C_{18}) and n-hexacosane (C_{26}) were added to the extract as internal standards (10 ng/µl) for subsequent gas chromatographic analysis. For each line, 20 individuals were analyzed, five for each genotype. The sample size of five individuals per genotype was chosen because there was very little variance among samples within a group and due to the amount of work required to obtain individuals from the interspecific crosses.

Compounds in the hydrocarbon extract were separated on an Agilent Technologies 6890N chromatograph (GC) fitted with an HP5 column 30.0 m x 250.00 µm internal diameter, pulsed splitless inlets and a flame ionization detector. The

temperature program was as follows: 60°C for 0.5 min, increasing to 190°C at 120°C/min then increasing to 260°C at 7°C/min then finally to 310°C at a rate of 120°C/min, where it was maintained for 3.5 min.

2.6 Data Analysis

Data was analyzed in a similar manner as reported previously (Blows and Allan, 1998; Coyne, 1996; Kwan and Rundle, 2010). Agilent Chemstation software was used to analyze the chromatograms, as well as to determine the relative abundances of the CHCs within the samples (Figure 2.3). For each sample, 28 compounds were analyzed (Figure 2.2) as this was the number of compounds consistently identified through this particular GC method. The relative abundance of each compound was calculated by dividing the area of a particular peak in the chromatogram by the total peak area of all compounds analyzed in the chromatogram. This method of calculating the relative abundances of compounds allowed for samples of different overall concentration to be compared. This also controlled for the nonbiological variation in the extraction of CHCs and for the quantity of extract injected into the GC. For cases in which a compound was absent or undetectable, a value of 0.5 (half the lowest detection limit of 1.0) was recorded in the data file. The internal standards were used as reference points when comparing the chromatograms for each genotype. The data were analyzed using a one-way analysis of variance (ANOVA) to compare the mean relative abundance of CHCs among all four genotypes. Under the null hypothesis, the mean relative abundance of CHCs would be equal among the 4 genotypes, while the alternate hypothesis states the mean relative abundance of CHCs differs in at least 1 genotype; a *post hoc* assessment was performed to ensure that significant results were due to differences in the *sim/Df* genotype. To

account for multiple tests on the same data (28 ANOVAs per line) the *p*-value was set to 0.001 as per the Bonferroni adjustment (Samuels and Witmer, 2003). Also, all data points were arcsine transformed prior to analysis to ensure a normal distribution (Zar, 2010).

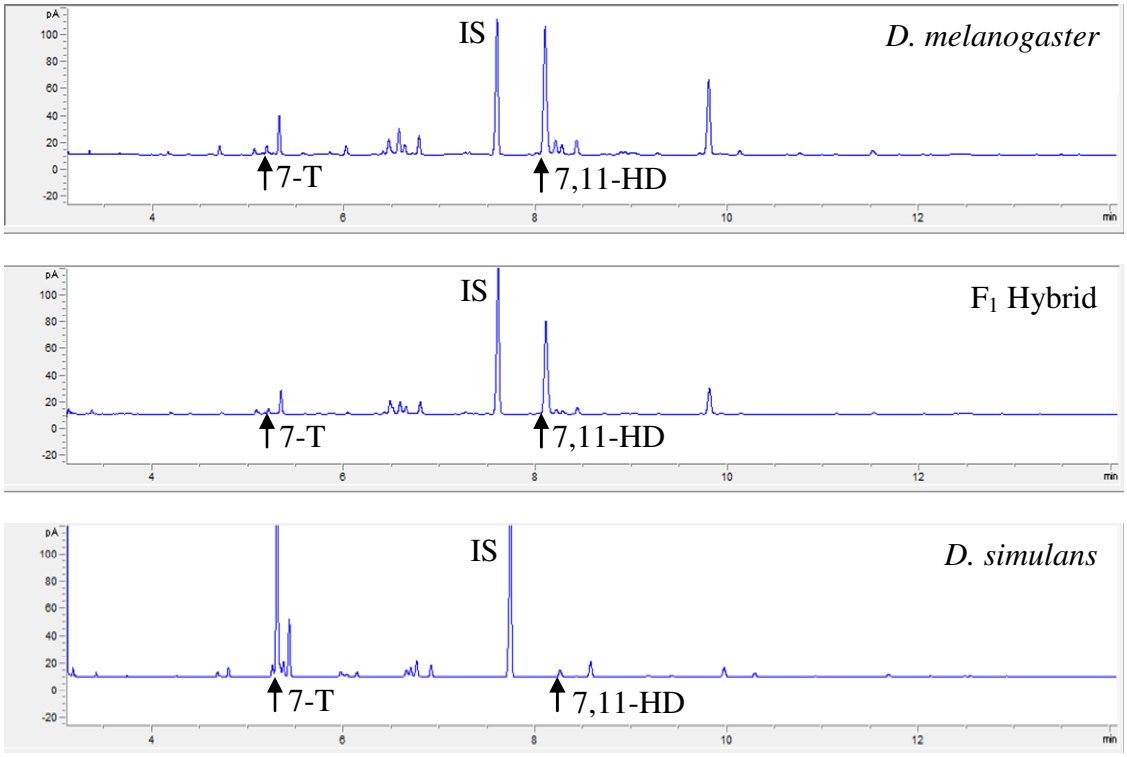

Figure 2.3. Chromatograms of female cuticular hydrocarbon profiles of *D. melanogaster*, *D. simulans* and their F_1 hybrid. For each chromatogram, the x-axis represents retention time in minutes and the y-axis represents signal intensity. The internal standard n-hexacosane is labeled as IS and appears just after 7.5 minutes; the internal standard octadecane elutes at approximately 3 minutes and is not shown. The species-specific sex pheromones 7-tricosene (7-T) and 7,11-heptacosadiene (7,11-HD) are labeled as well. *Drosophila melanogaster* genes are mostly dominant with respect to CHC production as the F_1 hybrid profile looks much more *melanogaster*-like than *simulans*-like.

3 Results

3.1 Deficiency mapping of chromosome 3L

One of the 28 deficiency lines used to map the left arm of Chromosome *3* yielded significant results and genes located within this region (cytological location 67E2-68A7) are listed in Appendix A. For this deficiency (*Df(3L)ED4457*), the *sim*/*Df* individuals had a significant increase in 7-pentacosene (7-P) and a significant decrease in 7,11-HD (Figures 3.1 and 3.2). There are 80 genes within this region.

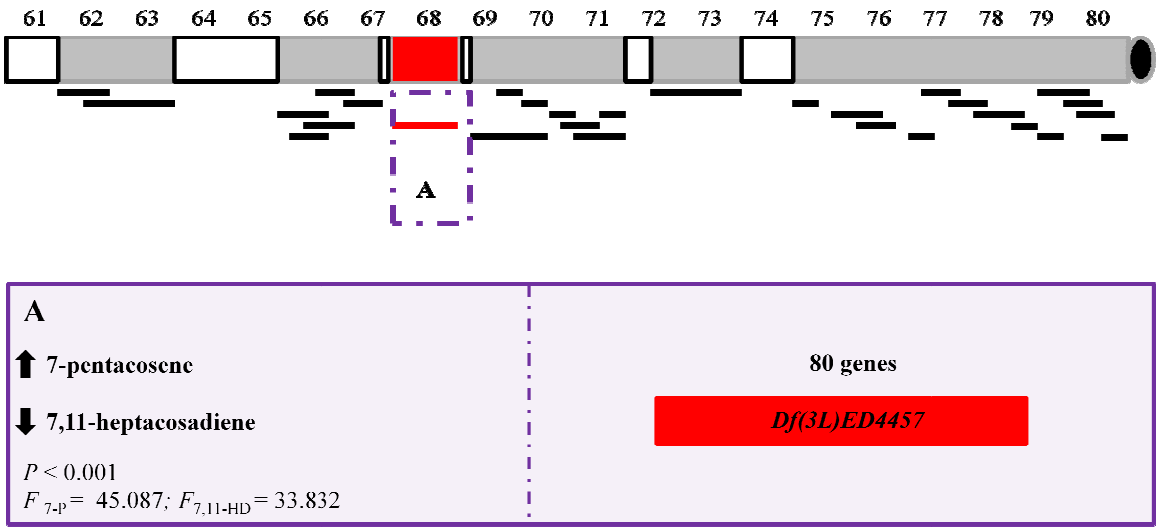

Figure 3.1. Deficiency mapping of chromosome 3L for genes contributing to CHC production in *D. melanogaster* and *D. simulans*. The grey bar represents the left arm of Chromosome *3*; the cytological locations are numbered on top, from 61 at the telomere to 80 at the centromere. The red box within the chromosome represents the approximate location of a significant region, while the white boxes represent regions of the chromosome that were not mapped. The bars underneath the chromosome represent the approximate locations of the deleted regions used for deficiency mapping; the black bars are not significant, the red bar is significant. The significant region is highlighted with a purple box and is labeled A. A detailed illustration of the significant region is found below the chromosome and follows the same colour scheme as above. The way in which particular CHCs are affected by the deletion of this region is listed, along with a statistical description of the results and the number of genes within the significant region.

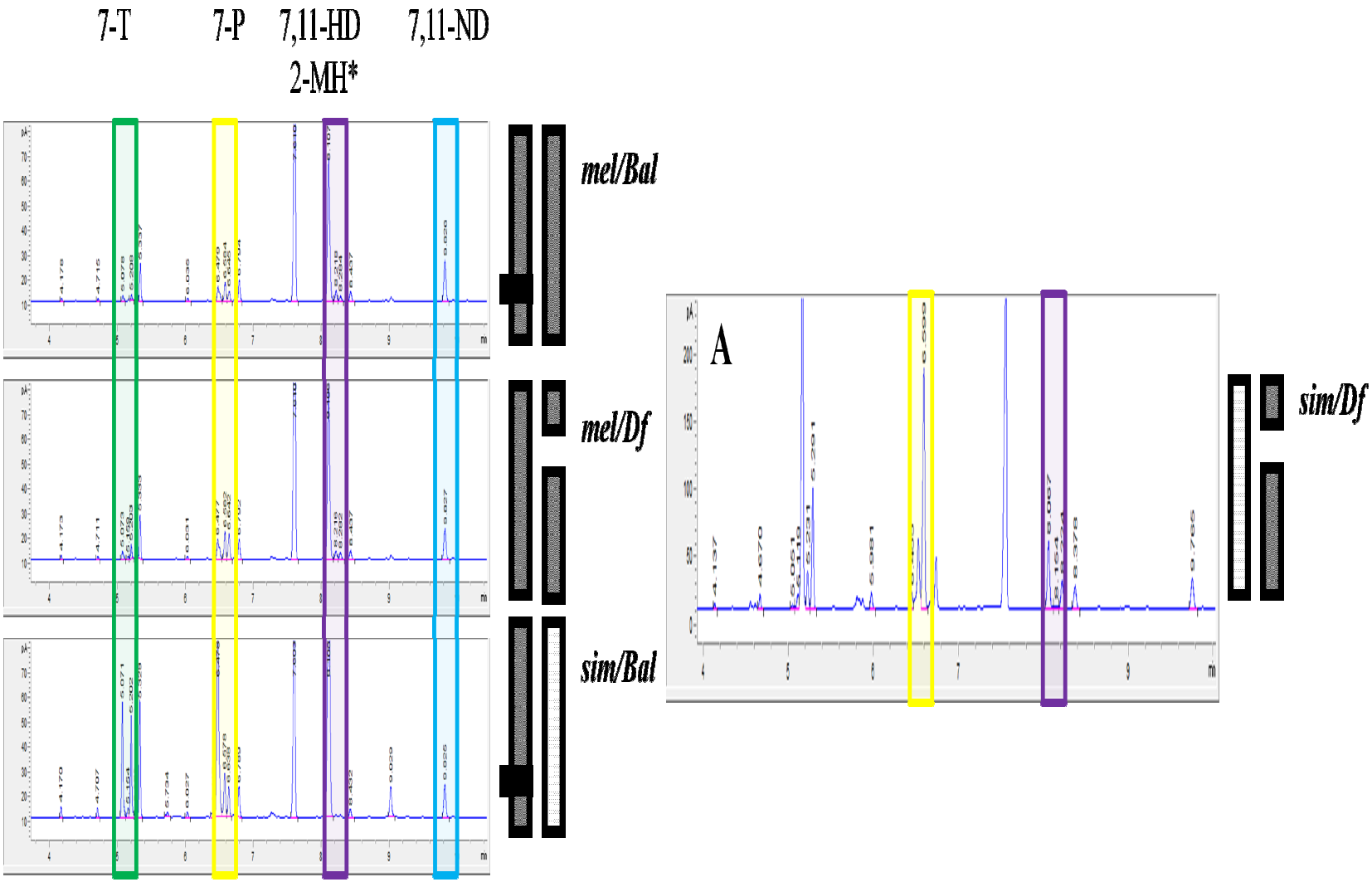
7-T
7-P
7,11-HD
2-MH*
7,11-ND
mel/Bal
mel/Df
sim/Bal
A
sim/Df

Figure 3.2. Deficiency mapping uncovers a region on chromosome 3L containing a gene(s) which contributes to the differential CHC profiles for *D. melanogaster* and *D. simulans*. GC chromatogram traces of 8-day-old females after hexane extraction. The three control genotypes (*mel*/*Bal*, *mel*/*Df* and *sim*/*Bal*) are presented on the left. The chromatogram for a *sim*/*Df* individual from the line spanning the significant region, cytological location 67E2-68A7, is presented on the right, labeled (A). The deletion of region (A) results in an increase of 7-P, highlighted in yellow, and a decrease in 7,11-HD, highlighted in purple, in *sim*/*Df* flies.

3.2 Deficiency mapping of chromosome 3R

Six of the 46 overlapping deficiency lines that were used to map the right arm of Chromosome *3* yielded significant results, uncovering four genomic regions of interest (Figure 3.3). For each of six lines, the *sim*/*Df* individuals had a compound, or compounds, within their CHC profile that differed significantly from the other three genotypes (Figure 3.4).

Within the first significant region (cytological location 87B6-88A1) (Figure 3.3 A), there is a gene (or genes) contributing to the production of 7,11-HD, as the deletion of this region resulted in a significant decrease in 7,11-HD. There are 230 genes (Appendix B) within the entire region exposed by the two deficiency lines (*Df(3R)T-32* and *Df(3R)ry85*) Within the region of overlap for *Df(3R)T-32* and *Df(3R)ry85* (cytological location 87B15-87C4) there are 15 genes (marked with asterisks in Appendix B).

The second significant region (cytological region 93A1-93D5) (Figure 3.3 B) contains genes associated with the production of 7,11-HD as well as 2-methyl hexacosane (2-MH). There are 84 genes (Appendix C) within the entire region exposed by the two significant lines (*Df(3R)BSC43* and *Df(3R)e*-R1). Interestingly, the overlapping region of deficiency lines *Df(3R)BSC43* and *Df(3R)e-R1* both affect 7,11-HD production in the *sim*/*Df* individuals, while only *Df(3R)e-R1* affects the amount of 2-MH. Thus, the region of overlap of these two deficiency lines likely contains a gene(s) that affects the production of 7,11-HD, while the region only exposed by *Df(3R)e-R1*likely contains a gene(s) that affects the production of 2-MH. The region exposed by *Df(3R)e-R1* but not *Df(3R)BSC43* (cytological region 93B7-93C6) contains 30 genes (marked with asterisks in Appendix C). Within the overlap of deficiency lines *Df(3R)BSC43* and *Df(3R)e-R1*,

there is a single gene, *Dhc93AB*. A significant decrease in the amount of 7,11-HD in the *sim*/*Df* individuals was observed for both of these lines (Figure 3.3 B). Two *Dhc93AB* gene disruption lines (*Mi{ET1}Dhc93AB[MB05444]* and *Mi{y[+mDint2]=MIC}CG17278[MI02608]*) that were tested had the same significant reduction in 7,11-HD as found for the overlapping deficiency region (Figure 3.5).

When the third significant region (cytological location 96A20-96C3) (Figure 3.3 C) is deleted from the *melanogaster/simulans* F_1 hybrid genome, there is an increase in the amount of 7-T, the *D. simulans* female sex pheromone. There are 86 genes (Appendix D) within this significant region.

The deletion of the fourth significant region along chromosome 3R (cytological location 98E5-99A5; Figure 3.3 D) resulted in a decrease in the amount of 7,11-ND in *sim*/*Df* individuals. There are 63 genes (Appendix E) within this significant region.

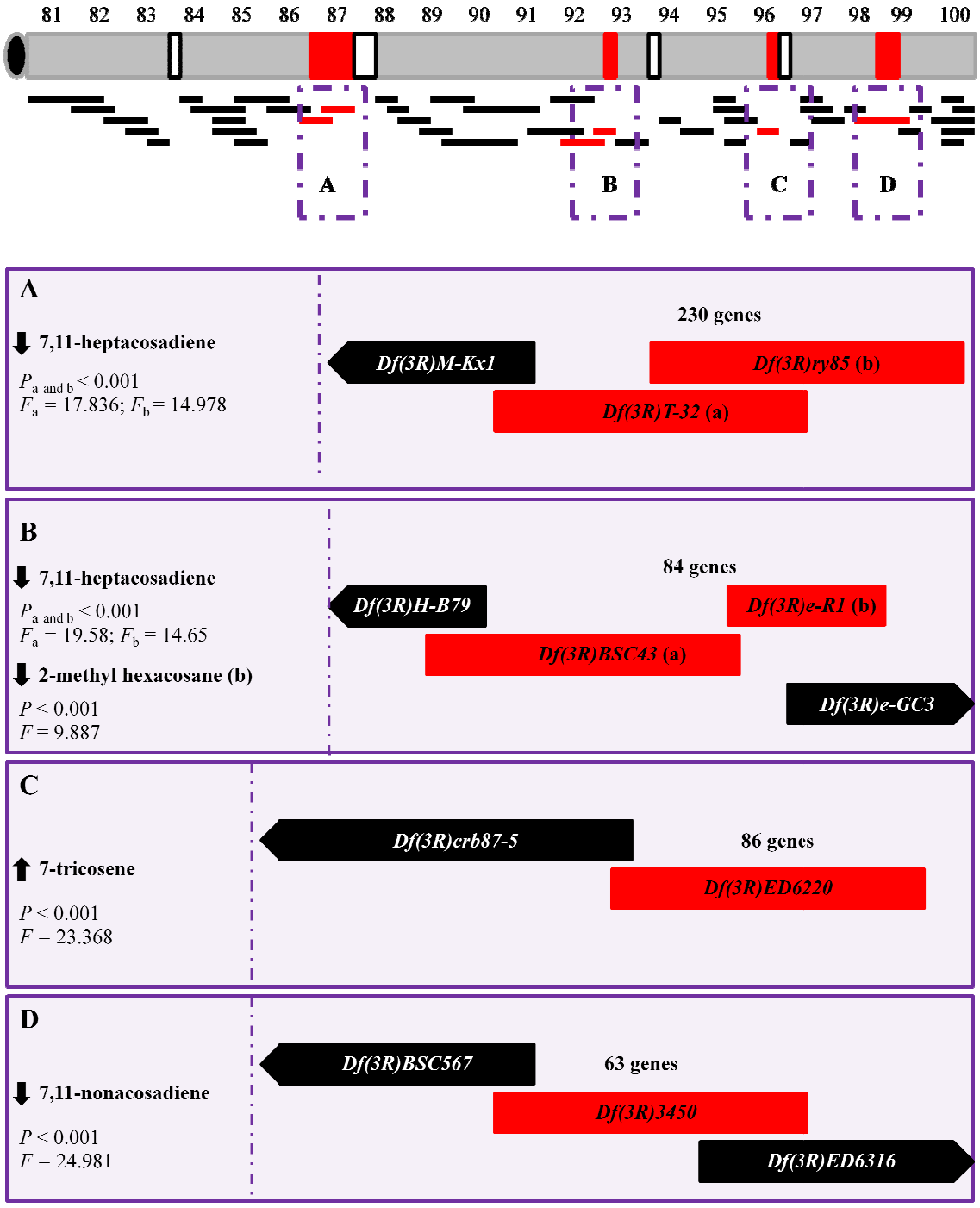

Figure 3.3. Deficiency mapping of chromosome 3R for genes contributing to CHC production in *D. melanogaster* and *D. simulans*. The grey bar represents the right arm of Chromosome *3;* the cytological locations are numbered on top, from 81 at the centromere to 100 at the telomere. The red boxes within the chromosome represent approximate locations of significant regions, while the white boxes represent regions of the

chromosome that were not mapped. The bars underneath the chromosome represent the approximate locations of the deleted regions used for deficiency mapping; the black bars are not significant, the red bars are significant. The significant regions are highlighted with a purple box and are labeled A to D. A detailed illustration of the significant regions is found below the chromosome and follows the same colour scheme as above. The way in which particular CHCs are affected by the deletion of these regions are listed, along with a statistical description of the results and the number of genes within each significant region.

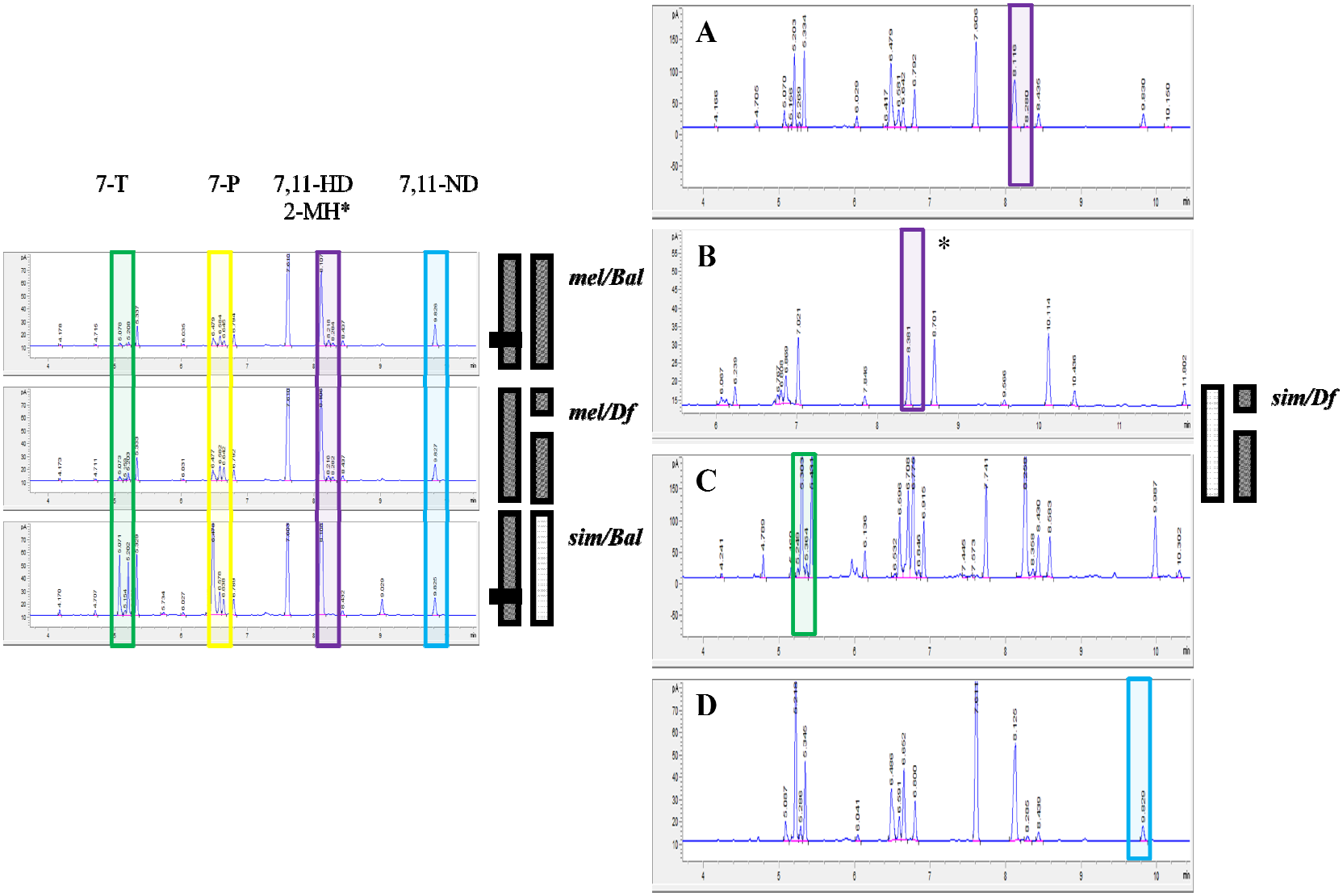
7-T
7-P
7,11-HD
2-MH*
7,11-ND
mel/Bal
mel/Df
sim/Bal
sim/Df
A
B
C
D
*

Figure 3.4. Deficiency mapping uncovers regions on chromosome 3R containing genes that contribute to the differential CHC profiles for *D. melanogaster* and *D. simulans*. GC traces of 8-day-old females after hexane extraction. The three control genotypes (*mel*/*Bal*, *mel*/*Df* and *sim*/*Bal*) are presented on the left. The chromatograms for the *sim*/*Df* individuals from the lines spanning the significant regions are presented on the right and labeled A to D (corresponding to the same designations as Figure 3.1). The deletion of region (A), cytological location 87B6-88A1, results in a decrease in 7,11-HD, highlighted in purple, in *sim*/*Df* flies. (B) represents cytological location 93A1-93D5, this deletion also reduces the amount of 7,11-HD in *sim*/*Df* individuals. * Denotes 2-methyl hexacosane (2-MH), which was significantly reduced in the deficiency line, *Df(3R)e-R1;* this compound elutes directly after 7,11-HD so is grouped into the same highlighted region. The deletion of region (C), cytological location 96A20-96C3, results in an increase in the amount of 7-T, highlighted in green, in *sim*/*Df* flies. (D) represents cytological location 98E5-99A5, this deletion results in a decrease in 7,11-ND, highlighted in blue, in *sim*/*Df* flies.

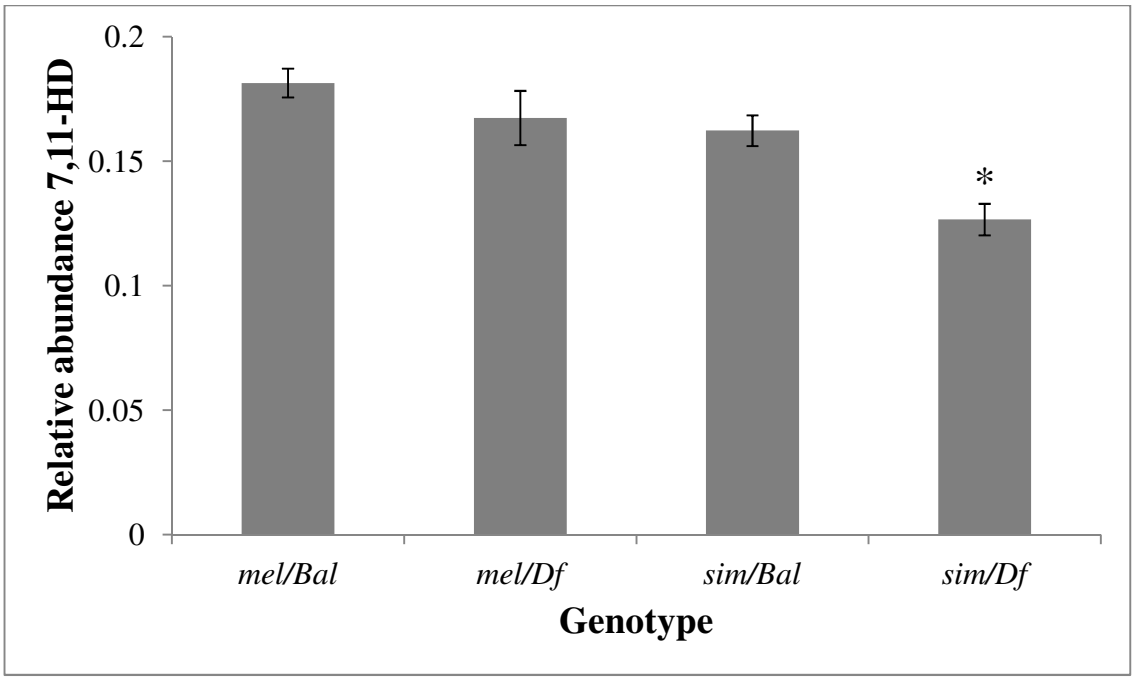

Figure 3.5. Relative abundance of 7,11-HD in females from four genotypes, testing candidate gene *Dhc93AB*. Bars represent the mean (±SEM) relative abundance of 7,11-HD in four genotypes created through both intra- and interspecific crosses with *D. melanogaster* gene disruption lines for *Dhc93AB*. *mel*/*Bal* individuals have two *D. melanogaster* copies of *Dhc93AB*; *sim*/*Bal* has one *D. melanogaster* copy and one *D. simulans* copy of *Dhc93AB*; *mel*/*Df* has one *D. melanogaster* copy and *sim*/*Df* has one *D. simulans* copy. Cuticular hydrocarbons (CHCs) were extracted eight days post-emergence. The mean relative abundance of 7,11-HD in *sim*/*Df* individuals (*) was significantly lower than the other three genotypes ($P < 0.001$, ANOVA). n=5 for each of the genotypes.

4 Discussion

4.1 The genetic basis of CHC production

Through the use of deficiency mapping, I identified several genomic regions that contain genes contributing to the differential CHC profiles between *D. melanogaster* and *D. simulans* (Appendix A-E). I was able to uncover regions in the genome that contained genes already known to influence CHC production in *D. melanogaster*: *desat1*, *desat2*, and *desatF*. Unfortunately, the cytological region where *eloF* is located was not mapped. In addition, I tested a candidate gene, *Dynein heavy chain at 93AB* (*Dhc93AB*). The results suggest *Dhc93AB* is implicated in the production of CHCs in *D. simulans* (Figure 3.5): *sim/Df* females, who possess one *D. simulans* copy of *Dhc93AB* and no *D. melanogaster* copy, have decreased amounts of 7,11-HD, which is characteristic of *D. simulans* females.

The *D. melanogaster Dhc93AB* gene has a homolog in *D. simulans*, *Dsim\GD20019*. When examining the sequences of both of these genes, the most striking difference is the length of the gene: *Dhc93AB* is 13461nt while *Dsim\GD20019* is 9585nt. The resulting proteins from the transcription and translation of these genes in *D. melanogaster* and *D. simulans* are very different in both length (*Dhc93AB* codes for 4486 amino acids, *Dsim\GD20019* codes for 3194 amino acids) and also in amino acid content as the sequences themselves differ between the two species. Also, there are numerous differences within the promoter regions of these two genes which could affect the timing or quantity of expression.

Drosophila sechellia is another species in the *melanogaster* subgroup that, like *D. melanogaster* females, have a high abundance of 7,11-HD in their CHC profile (Pechine *et al*., 1985). Comparison of the sequences for *Dhc93AB*, *Dsim\GD20019* and the *D. sechellia* homolog, *Dsec\GM15114*, revealed that the length of the protein resulting from *Dsec\GM15114* transcription and translation is more similar to *D. melanogaster* than *D. simulans*, as the *D. sechellia* copy of this gene is 13482nt long and codes for 4493 amino acids. Relative to *D. melanogaster* and *D. sechellia*, the *D. simulans* copy of *Dhc93AB* is missing nucleotides #2411-3715 (which are present in both *D. melanogaster* and *D. sechellia*). This missing portion of the protein contains several very important things: (i) Two P-loops, which are important for ATP binding; (ii) the MT domain, essential for microtubule binding. Due to the absence of these domains, *Dhc93AB* may not function properly in *D. simulans*. This gene could therefore be important for the production of 7,11-HD for many species, not just *D. melanogaster* and *D. simulans* females.

The gene product(s) of *Dhc93AB* has a predicted function, based on sequence similarity, as an ATPase involved in microtubule based movement (Rasmusson *et al*., 1994). The *Dhc93AB* gene sequence is present in both *D. melanogaster* and *D. simulans* although it is expressed at very low levels in the former species (Rasmusson *et al*., 1994). Additional work needs to be done to determine whether it is differentially expressed, for example through differing quantity or timing of expression, or if the protein products vary between the two species. While I am unable to make concrete conclusions as to how *Dhc93AB* is involved in 7,11-HD synthesis, I suggest two possibilities.

Firstly, it is possible that *Dhc93AB* is directly involved in the production of 7,11-HD, however, as the deletion of this gene has no effect on 7-T, which is a part of the

proposed biosynthesis pathway (Figure 1.1), this seems unlikely. It is however possible that the products of this gene could act on the precursors of 7,11-HD, in the latter part of the pathway through elongation, desaturation, or decarboxylation.

Another possibility is that the product(s) of *Dhc93AB* are involved in the transport of CHCs from their site of synthesis to the cuticle. It has been shown that plants use ATP pumps to move compounds from the site of synthesis (epidermal cells) to the exo-cuticle (Pighin *et al*., 2004). Due to genetic differences in *Dhc93AB*, the two species examined here could have variation in how much 7,11-HD is exported to the cuticle. As noted previously, how insects transfer CHCs from the sites of production to the cuticle is currently unknown, so examining the possibility that *Drosophila* use ATP pumps, such as those coded for by *Dhc93AB*, to transport 7,11-HD is a very exciting prospect.

The significant region (67E2 to 68A7) contains *desatF,* a gene coding for an desaturase which acts to add a second double bond to the carbon 11 position of precursors to 7,11-HD (Chertemps *et al*., 2006). *DesatF* contributes to the *D. melanogaster* CHC biosynthesis pathway (Chertemps *et al*., 2006). The deletion of this region results in a decrease in two compounds: 7,11-HD and 7-P, and there might be a gene within the 67E2-68A7 region that directly determines the *D. simulans*-specific amount of 7,11-HD. However, it is more likely that the decrease observed is due to the *sim*/*Df* individuals lacking a *D. melanogaster* copy of *desatF* for although the gene is present in *D. simulans* females, it is not expressed. *DesatF* has not been linked to the production of monoenes (compounds with one double bond), but rather with later parts of the biosynthesis pathway, the observed decrease in 7-P was unexpected and suggests that there are other genes within the 67E2-68A7 region that influence the formation of 7-P (Appendix A). 7-

P is a prominent compound in the male *D. melanogaster* CHC profile and acts as an anti-aphrodisiac (Scott and Richmond, 1988), however, the role of 7-P in females is not known.

Within the significant region 67E2-68A7 there are several genes that could be considered candidates for CHC biosynthesis. *Ir67b* is located cytologically at 67E3 and is thought to be involved in the detection of chemical stimulus. As mentioned earlier, *desat1* acts in a pleiotropic manner in the production and detection of CHCs in *D. melanogaster* so it can be proposed that other genes (such as *Ir67b*) may act in this same way. Another excellent candidate gene for CHC biosynthesis present in this significant region is *Elo68alpha*. This gene codes for an elongase which acts to extend the carbon chain of fatty acid precursors and is thought to be involved in pheromone biosynthesis processes.

The *desat1* gene is located within candidate region 87B5 to 88A1 (overlapping deficiency lines *Df(3R)T-32* and *Df(3R)ry85*: Figure 3.3 A). While the role of *desat1* in CHC biosynthesis has been well documented in *D. melanogaster*, this is the first time it has been implicated in the observed interspecific differences in 7,11-HD. The product of *desat1* acts to add a double bond at the carbon 7 position to precursors of both 7-T and 7,11-HD (*D. simulans* and *D. melanogaster* female sex pheromones, respectively), so it is possible that this gene is differentially regulated in *D. melanogaster* and *D. simulans*. However, as both of *Df(3R)T-32* and *Df(3R)ry85* affected 7,11-HD in the same way, it is more likely that a gene located within the region of overlap of these two deficiency lines is responsible for the reduction of this compound. Of the 15 genes within overlapping region 87B15-87C4, there are genes that stand out as good candidates. Genes involved in

metabolic processes are excellent candidates to consider regarding CHC biosynthesis as enzymes involved in the formation of CHCs act on metabolites (Blomquist and Bagnères, 2010). Two such genes within 87B15-87C6 are *CG6753* and *CG11600*, both of which have proposed functions as being involved in lipid metabolic processes.

The deletion of two overlapping deficiency lines [(*Df(3R)BSC43* and *Df(3R)e-R1)*, cytological location 93A1 to 93D5)] had the same effect on 7,11-HD, however, only *Df(3R)e-R1* impacted the production of 2-MH. It is possible that the non-overlapping portion of *Df(3R)e-R1* contains a gene important for the formation of 2-MH (Appendix C). There are 30 genes within the non-overlapping region (Appendix C), one of which is an odorant-binding protein, *Obp93a*, believed to be involved in the perception of chemical stimulus (Hekmat-Scafe *et al*., 2002). Much like *desat1*, mentioned above, it is possible *Obp93a* is also involved in the formation of 2-MH.

While fine-mapping needs to be done for the significant region 96A20-96C3, genes involved in olfaction (*PQBP1*) and metabolic processes (*CG10899*) stand out as great candidates for reasons mentioned above. Likewise, for significant region 98E5-99A5, there are a few good candidate genes within that region, also associated with olfaction and metabolic processes (*beta4GalNAcTB*, *CG14512* and *CG14515*). Using smaller deficiencies to fine-map these large significant regions will provide insight into which genes are influencing CHC production.

In this study, I identified candidate regions contributing to five of the 28 detectable hydrocarbon compounds present on the cuticle. These five compounds differ between the two species in a manner that would, theoretically, allow for genes

contributing to these differences to be identified using deficiency mapping methodology. For those compounds for which I was unable to uncover genes contributing to their formation, it is possible my methodology was not sufficient to identify these genes for a number of reasons. It is likely that genes contributing to the production of some compounds within the CHC profile are simply located on a different chromosome than Chromosome *3*. Previous work examining the genetic basis of the differences in CHC profiles between *D. melanogaster* and *D. simulans* reported that only regions on the 3rd chromosome were involved (Coyne, 1996). However, this work only focused on the most abundant CHCs rather than all of the compounds in the profile, as I did in this study. Therefore, it is possible that genes on other chromosomes contribute to CHC production. Or perhaps regions on the 3rd chromosome contain genes which have little effect alone, but may interact epistatically with others to influence CHC production; deficiency mapping does not detect such epistatic interactions. In addition, *D. melanogaster* genes are dominant over *D. simulans* genes with respect to CHC profiles, for some but not all compounds. Consequently, any *D. simulan*s-specific compounds that are unaffected by *D. melanogaster* genes would not be detected by deficiency mapping.

One last caveat that must be mentioned with respect to my results is that of chromosome coverage. Survival was very low in some deficiency lines before and/or after they were crossed, eliminating my ability to assay those regions. If genes important in determining the differential CHC profiles of *D. melanogaster* and *D. simulans* were in those regions, they would not be detected in this study. Perhaps by using more deficiency lines with smaller deletions, it will be possible to map these regions.

While I was able to identify and test a candidate gene, *Dhc93AB*, further work is required to determine how it influences CHC production. It would be advantageous to establish expression patterns of *Dhc93AB* in both *D. melanogaster* and *D. simulans* using q-PCR as well as *in situ* hybridization to identify in what tissue(s) this gene is expressed (Shirangi *et al*., 2009). Subsequently, to confirm the role of *Dhc93AB* in CHC production, an isolated *D. simulans* copy of this gene could be introduced into *D. melanogaster* that have a mutation at this particular locus. A mutation is necessary as *D. melanogaster* genes have dominant function at this locus. If *Dhc93AB* does influence CHC production, I would expect to see a "*D. simulans*-like" CHC profile produced by the transgenic flies and the presence of 7,11-HD confirmed by GC coupled with mass spectrometry (GC-MS).

4.2 Conclusions and future work

Individuals from *D. melanogaster* and *D. simulans* use chemical cues to attract and identify appropriate mates and discriminate against those who are inappropriate. Females from these two species have different CHC profiles and this difference contributes to the reproductive isolation in this species pair. For this reason, finding genes contributing to the production and detection of CHC profiles is of great interest as this could provide insight into the genetic basis of reproductive isolation.

The deficiency map created here is an excellent starting point for many future endeavors as there are several large genomic regions that could be refined. For these regions, fine-mapping with additional smaller deficiencies and subsequent testing of candidate genes needs to be done. Also, testing whether *Dhc93AB* is involved in the transport of 7,11-HD is extremely important. Lastly, behaviour assays could be done to

determine whether the differences in female CHC profiles for *D. melanogaster* and *D. simulans* are influencing their mating behaviour.

The research presented here identified five genomic regions that contain genes contributing to the different CHC profiles observed in *D. melanogaster* and *D. simulans*. With the molecular tools available in *Drosophila*, it was possible to refine the deficiency map of Chromosome *3* down to an individual candidate gene, *Dhc93AB*. This gene is proposed to be involved in the biosynthesis of 7,11-HD, was tested and putatively shown to affect the CHC profile.

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Appendix A: Candidate genes within region 67E2-68A7

Gene Name	Cytological Location	Base Position	Molecular Function	Biological Process
mir-276a	67E2-67E2	3L:10358328..10358425	Unknown	Unknown
CR43990	67E2-67E2	3L:10405007..10405477	Unknown	Unknown
CG12362	67E2-67E3	3L:10405918..10407874	zinc ion binding	Unknown
Ir67b	67E3-67E3	3L:10422789..10425083	ligand-gated ion channel activity	detection of chemical stimulus
Ir67c	67E3-67E3	3L:10425484..10427205	ligand-gated ion channel activity	detection of chemical stimulus
Gem3	67E3-67E3	3L:10452181..10456201	ATP-dependent RNA helicase activity; RNA helicase activity	ribonucleoprotein complex assembly; neuromuscular junction development
CG32061	67E3-67E3	3L:10456916..10457735	Unknown	Unknown
S-Lap3	67E3-67E4	3L:10458287..10462074	aminopeptidase activity	Proteolysis

A2bp1	67E4-67E5	3L:10474512..10587112	transcription factor binding; transcription regulatory region DNA binding	nervous system development; imaginal disc-derived wing vein specification; oogenesis; positive regulation of transcription, DNA-dependent
S-Lap4	67E4-67E4	3L:10483961..10486034	aminopeptidase activity	Proteolysis
CG6527	67E4-67E4	3L:10551265..10552182	structural molecule activity	Unknown
CG42521	67E5-67E5	3L:10616166..10616676	Unknown	Unknown
CG43127	67E5-67E5	3L:10617828..10618699	Unknown	Unknown
CG34238	67E5-67E5	3L:10619599..10620539	Unknown	Unknown
CG14151	67E5-67E5	3L:10620780..10621584	Unknown	Unknown
CG42536	67E5-67E5	3L:10622183..10623169	Unknown	Unknown
CG42535	67E5-67E5	3L:10623134..10624035	Unknown	Unknown

CG34001	67E5-67E5	3L:10624179..10627712	Unknown	Unknown
hay	67E5-67E5	3L:10624179..10627069	helicase activity; ATP-dependent 3'-5' DNA helicase activity	adult locomotory behavior; transcription from RNA polymerase II promoter; regulation of alternative nuclear mRNA splicing, via spliceosome; response to UV
E(z)	67E5-67E5	3L:10627675..10631230	histone methyltransferase activity (H3-K27 specific); protein binding; histone methyltransferase activity (H3-K9 specific); histone methyltransferase activity	histone methylation; axon guidance; histone H3-K27 methylation; cuticle hydrocarbon biosynthetic process; dendrite morphogenesis; histone H3-K9 methylation; muscle organ development; syncytial blastoderm mitotic cell cycle; neurogenesis

CG8009	67E5-67E5	3L:10631246..10632778	Unknown	Unknown
CG18628	67E5-67E5	3L:10632840..10633236	Unknown	multicellular organism reproduction
CG32066	67E5-67E6	3L:10633386..10654113	Unknown	Unknown
CG14154	67E5-67E5	3L:10634989..10636109	Unknown	Unknown
CG14153	67E5-67E5	3L:10636628..10637715	Unknown	Unknown
CG8003	67E6-67E6	3L:10654575..10656257	zinc ion binding	Unknown
CG32068	67E6-67E6	3L:10656476..10657535	acireductone dioxygenase [iron(II)-requiring] activity	oxidation-reduction process
simj	67E6-67E6	3L:10657839..10683834	protein binding	negative regulation of transcription from RNA polymerase II promoter
CG11811	67E6-67E7	3L:10684124..10685698	guanylate kinase activity	purine nucleotide metabolic process

CG33493	67E7-67E7	3L:10685630..10686267	Unknown	Unknown
CG6463	67E7-67E7	3L:10686284..10687208	NADH dehydrogenase activity; NADH dehydrogenase (ubiquinone) activity	mitochondrial electron transport, NADH to ubiquinone
NijA	67E7-67E7	3L:10687457..10689977	Unknown	cell adhesion
CG43245	67F1-67F1	3L:10765092..10766499	Unknown	Unknown
CG12523	67F1-67F1	3L:10794290..10795201	Unknown	Unknown
CG42831	67F1-67F1	3L:10807919..10809184	Unknown	Unknown
tna	67F1-67F1	3L:10850989..10869596	zinc ion binding	chromatin-mediated maintenance of transcription
CG6418	67F1-67F1	3L:10869415..10872137	helicase activity; ATP-dependent RNA helicase activity	Unknown
blos4	67F1-67F1	3L:10872185..10873288	protein binding	Unknown

CG6409	67F1-67F1	3L:10873799..10875154	Unknown	GPI anchor biosynthetic process
CG7949	67F1-67F1	3L:10875990..10876673	Unknown	Unknown
CG6404	67F1-67F1	3L:10877361..10879571	protein transporter activity	respiratory chain complex IV assembly
Cpr67Fa1	67F1-67F1	3L:10883090..10883800	structural constituent of chitin-based cuticle; structural constituent of chitin-based larval cuticle	Unknown
Cpr67Fa2	67F1-67F1	3L:10884814..10885616	structural constituent of chitin-based cuticle	Unknown
Cpr67Fb	67F1-67F1	3L:10887273..10887793	structural constituent of chitin-based cuticle	multicellular organism reproduction
Aps	67F1-67F1	3L:10887876..10892223	diphosphoinositol-polyphosphate diphosphatase activity	inositol phosphate metabolic process; nucleotide metabolic process
CG34050	67F1-67F1	3L:10937556..10938200	Unknown	Unknown

CG14147	68A1-68A1	3L:10965748..10966576	Unknown	Unknown
klu	68A1-68A1	3L:10974271..11001933	nucleic acid binding	larval feeding behavior; tricarboxylic acid cycle; positive regulation of compound eye retinal cell programmed cell death
snoRNA:Me18S-G962	68A1-68A1	3L:10994509..10994589	Unknown	Unknown
Fad2	68A1-68A1	3L:11016635..11017946	stearoyl-CoA 9-desaturase activity	pheromone metabolic process; courtship behavior; cytokinesis; phagocytosis, engulfment
CG32079	68A1-68A1	3L:11018661..11020432	amino acid transmembrane transporter activity	amino acid transmembrane transport
CG32081	68A1-68A2	3L:11020679..11022545	amino acid transmembrane transporter activity	Transport
CG43693	68A2-68A3	3L:11029458..11044471	Unknown	Unknown

CG7888	68A3-68A3	3L:11047135..11052759	amino acid transmembrane transporter activity	amino acid transmembrane transport
CG6321	68A3-68A3	3L:11052537..11054324	pyridoxal phosphate binding; transferase activity	biosynthetic process
blos2	68A3-68A3	3L:11054671..11055315	protein binding	Unknown
CG32069	68A3-68A3	3L:11055231..11055714	Unknown	Neurogenesis
CG32075	68A3-68A4	3L:11055826..11058041	Unknown	Neurogenesis
SuUR	68A4-68A4	3L:11058091..11062125	chromatin binding	chromatin assembly or disassembly; heterochromatin assembly; chromosome organization; DNA replication; DNA endoreduplication; positive regulation of DNA endoreduplication
CG6310	68A4-68A4	3L:11062143..11062964	Unknown	Unknown

Mocs1	68A4-68A4	3L:11063626..11066448	4 iron, 4 sulfur cluster binding; metal ion binding; catalytic activity	Mo-molybdopterin cofactor biosynthetic process
l(3)01239	68A4-68A4	3L:11066392..11067488	chaperone binding	oogenesis; phagocytosis, engulfment
CG7839	68A4-68A5	3L:11067869..11071836	sequence-specific DNA binding transcription factor activity	Neurogenesis
JIL-1	68A5-68A6	3L:11071852..11086293	histone kinase activity (H3-S10 specific); protein kinase activity	female meiosis chromosome segregation; negative regulation of chromatin silencing; chromatin organization; chromosome organization; oogenesis
CG6279	68A6-68A6	3L:11086471..11089287	oxidoreductase activity	Unknown
pncr011:3L	68A6-68A6	3L:11089757..11091660	Unknown	Unknown
CG6272	68A6-68A6	3L:11090546..11091286	protein heterodimerization activity	Neurogenesis

APP-BP1	68A6-68A6	3L:11091512..11093678	protein binding; NEDD8 activating enzyme activity	protein neddylation
Elo68beta	68A6-68A6	3L:11093775..11094798	Unknown	Unknown
Elo68alpha	68A6-68A6	3L:11094975..11096130	fatty acid elongase activity	fatty acid elongation, unsaturated fatty acid; pheromone biosynthetic process
CG32071	68A6-68A6	3L:11096521..11096973	Unknown	Unknown
CG32073	68A7-68A7	3L:11100108..11100476	Unknown	Unknown
CG12522	68A7-68A7	3L:11101335..11101832	Unknown	Unknown
CG34012	68A7-68A7	3L:11101920..11102605	Unknown	Unknown
CG7638	68A7-68A7	3L:11102790..11105217	Unknown	Unknown
Sod	68A7-68A7	3L:11105381..11106840	antioxidant activity; superoxide dismutase activity	determination of adult lifespan; response to oxidative stress

fd68A	68A7-68A7	3L:11107269..11113875	sequence-specific DNA binding transcription factor activity	regulation of transcription, DNA-dependent
mRpL2	68A7-68A7	3L:11113947..11115141	structural constituent of ribosome	Translation
Ufd1-like	68A7-68A7	3L:11115066..11116316	Unknown	positive regulation of proteasomal ubiquitin-dependent protein catabolic process; proteolysis; ubiquitin-dependent protein catabolic process
CG42575	68A7-68A8	3L:11116448..11127763	sodium-dependent phosphate transmembrane transporter activity	sodium-dependent phosphate transport

Appendix B: Candidate genes within region 87B6-88A1

Gene Name	Cytological Location	Base Position	Molecular Function	Biological Process
CG4066	87B6-87B6	3R:8137075..8138841	Unknown	chorion-containing eggshell formation
tRNA:CR31432	87B6-87B6	3R:8148285..8148356	ACA codon-amino acid adaptor activity	Translation
CG10013	87B6-87B7	3R:8149245..8150936	Unknown	Unknown
CG10038	87B7-87B7	3R:8174709..8176367	Unknown	Unknown
CG10041	87B7-87B7	3R:8176537..8177801	serine-type endopeptidase activity	multicellular organism reproduction
MBD-R2	87B7-87B8	3R:8177865..8182024	DNA binding; zinc ion binding	mitotic cell cycle G2/M transition DNA damage checkpoint; neurogenesis
CG4115	87B8-87B8	3R:8185733..8188975	binding; carbohydrate binding	Unknown
snmRNA:419	87B8-87B8	3R:8188638..8188711	Unknown	Unknown
Tim17a1	87B8-87B8	3R:8189389..8190402	P-P-bond-hydrolysis-driven protein transmembrane transporter activity; protein transporter activity	protein import into mitochondrial inner membrane; protein targeting to mitochondrion

GstD10	87B8-87B8	3R:8190496..8191440	glutathione transferase activity	Unknown
GstD9	87B8-87B8	3R:8191902..8193286	glutathione transferase activity	Unknown
GstD1	87B8-87B8	3R:8193269..8194987	DDT-dehydrochlorinase activity; glutathione transferase activity	Unknown
GstD2	87B8-87B8	3R:8197693..8198426	glutathione transferase activity	Unknown
GstD3	87B8-87B8	3R:8198790..8199535	glutathione transferase activity	Unknown
GstD4	87B8-87B8	3R:8199824..8200546	glutathione transferase activity	Unknown
GstD5	87B8-87B8	3R:8201486..8202136	glutathione transferase activity	Unknown
GstD6	87B8-87B8	3R:8202894..8203629	glutathione transferase activity	Unknown
GstD7	87B8-87B8	3R:8204261..8204977	glutathione transferase activity	Unknown
GstD8	87B8-87B8	3R:8205745..8206537	glutathione transferase activity	Unknown
CG10035	87B9-87B9	3R:8207373..8208412	Unknown	Unknown
CG17639	87B9-87B9	3R:8209058..8211346	glutathione transferase activity	Unknown
CG34402	87B9-87B9	3R:8211971..8228627	Unknown	Unknown

CG33098	87B9-87B9	3R:8212910..8214517	calcium ion binding	Unknown
CG10097	87B9-87B9	3R:8216405..8221702	oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor; nucleotide binding	oxidation-reduction process
CG10096	87B9-87B9	3R:8216405..8221702	oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor; nucleotide binding	oxidation-reduction process
Cyp9f3Psi	87B9-87B9	3R:8221729..8224651	monooxygenase activity; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen; heme binding; electron carrier activity	oxidation-reduction process
lig3	87B9-87B9	3R:8224733..8227561	DNA ligase (ATP) activity	DNA ligation involved in DNA repair; DNA recombination; DNA replication
Cyp9f2	87B9-87B9	3R:8229062..8231750	electron carrier activity	wing disc development
CG5167	87B9-87B9	3R:8232254..8233824	nucleotide binding; oxidoreductase activity	oxidation-reduction process

CG5196	87B9-87B9	3R:8234044..8237380	protein-cysteine S-palmitoleyltransferase activity	Golgi organization
Dip-C	87B9-87B9	3R:8237531..8239460	dipeptidyl-peptidase activity	Proteolysis
snmRNA:649	87B9-87B9	3R:8239110..8239168	Unknown	Unknown
pps	87B9-87B9	3R:8239550..8246973	protein binding	RNA splicing
Scgbeta	87B9-87B9	3R:8246958..8248151	structural constituent of muscle	cytoskeleton organization
CG17202	87B9-87B9	3R:8248490..8249328	cAMP-dependent protein kinase regulator activity	signal transduction
Pp1-87B	87B9-87B10	3R:8249343..8251639	myosin phosphatase activity; protein serine/threonine phosphatase activity	terms, many of which group under: cellular component organization or biogenesis; cell cycle; cell cycle process; associative learning; anatomical structure development; localization; learning; organelle organization; multicellular organismal process; cellular macromolecule metabolic process
Sas	87B10-87B10	3R:8252300..8253599	N-acylneuraminate-9-phosphate synthase activity	Glycosylation

CG5641	87B10-87B10	3R:8253515..8255002	DNA binding; double-stranded RNA binding	positive regulation of transcription, DNA-dependent
CG5245	87B10-87B10	3R:8255307..8256960	zinc ion binding; nucleic acid binding	Unknown
Pros25	87B10-87B10	3R:8257047..8258279	endopeptidase activity	mitotic spindle elongation; cell proliferation; cellular process; mitotic spindle organization
Aos1	87B10-87B10	3R:8258255..8259626	protein binding	positive regulation of NF-kappaB transcription factor activity; neurogenesis
CG5844	87B10-87B10	3R:8259824..8261365	dodecenoyl-CoA delta-isomerase activity	cellular response to hypoxia; phagocytosis, engulfment
desat2	87B10-87B10	3R:8261970..8263582	stearoyl-CoA 9-desaturase activity	fatty acid biosynthetic process; cuticle hydrocarbon biosynthetic process
CG17207	87B10-87B10	3R:8263536..8264861	Unknown	Unknown
desat1	87B10-87B11	3R:8265322..8273338	stearoyl-CoA 9-desaturase activity	cuticle hydrocarbon biosynthetic process; regulation of lipid metabolic process; pheromone biosynthetic process; male mating behavior; mating behavior, sex discrimination;

				regulation of autophagy
CG5538	87B11-87B11	3R:8273375..8274768	Unknown	Unknown
CG18549	87B11-87B11	3R:8275018..8282084	Unknown	Unknown
CG5509	87B11-87B11	3R:8278186..8278918	Unknown	Unknown
trus	87B11-87B11	3R:8282256..8284166	Unknown	Unknown
CG5961	87B11-87B11	3R:8284143..8286016	Unknown	Unknown
CG12267	87B11-87B11	3R:8286136..8288140	DNA-directed RNA polymerase activity	transcription from RNA polymerase III promoter
CG5608	87B11-87B12	3R:8288284..8291020	binding	lateral inhibition
Hsp70Ba	87B12-87B12	3R:8291026..8293500	ATP binding	response to hypoxia; heat shock-mediated polytene chromosome puffing; response to heat
alphagamma-element:CR32865	87B13-87B13	3R:8301058..8303989	Unknown	Unknown

Hsp70Bbb	87B14-87B14	3R:8328232..8330822	ATP binding	response to hypoxia; heat shock-mediated polytene chromosome puffing; response to heat
Hsp70Bb	87B14-87B14	3R:8331515..8334105	ATP binding	response to hypoxia; heat shock-mediated polytene chromosome puffing; response to heat
Hsp70Bc	87B14-87B15	3R:8334798..8337183	ATP binding	response to hypoxia; heat shock-mediated polytene chromosome puffing; response to methotrexate; response to heat
* ***Octbeta3R***	87B15-87C1	3R:8337292..8373951	octopamine receptor activity	G-protein coupled receptor signaling pathway
****hug***	87B15-87B15	3R:8351910..8354798	myostimulatory hormone activity	larval feeding behavior; ecdysis, chitin-based cuticle
****mir-284***	87C1-87C1	3R:8377237..8377336	Unknown	Unknown
****Octbeta2R***	87C1-87C2	3R:8378211..8421447	octopamine receptor activity	positive regulation of synaptic growth at neuromuscular junction
****Vha55***	87C2-87C3	3R:8449484..8453551	proton-transporting ATPase activity, rotational mechanism	ATP hydrolysis coupled proton transport; vacuolar

				acidification
Snx3	87C3-87C3	3R:8453763..8455783	phosphatidylinositol binding	Wnt protein secretion; wing disc development; positive regulation of canonical Wnt receptor signaling pathway; protein stabilization; canonical Wnt receptor signaling pathway; regulation of Wnt receptor signaling pathway by Wnt protein secretion; positive regulation of Wnt protein secretion; phagocytosis, engulfment
CG18616	87C3-87C3	3R:8456364..8458747	sequence-specific DNA binding transcription factor activity; sequence-specific DNA binding; zinc ion binding	regulation of transcription, DNA-dependent
CG18530	87C3-87C3	3R:8459081..8460374	triglyceride lipase activity	lipid metabolic process
CG11598	87C3-87C3	3R:8460639..8462005	lipase activity; triglyceride lipase activity	multicellular organism reproduction
CG11600	87C3-87C3	3R:8462275..8463602	triglyceride lipase activity	lipid metabolic process
CG11608	87C3-87C3	3R:8464203..8465669	triglyceride lipase activity	multicellular organism reproduction

***CG6753**	87C3-87C3	3R:8465885..8468295	triglyceride lipase activity	lipid metabolic process
***CG6234**	87C3-87C3	3R:8473782..8478635	Unknown	Gastrulation
***CG6225**	87C3-87C3	3R:8480299..8486588	aminopeptidase activity	Neurogenesis
***CG14395**	87C4-87C5	3R:8488553..8499681	Unknown	Unknown
***CG6188**	87C5-87C5	3R:8500332..8501806	glycine N-methyltransferase activity	methionine metabolic process
***CG43630**	87C5-87C5	3R:8501732..8501982	Unknown	Unknown
***mbo**	87C5-87C5	3R:8502209..8504766	protein binding	negative regulation of protein export from nucleus; antimicrobial humoral response; protein import into nucleus; neurogenesis
***Cyp313a4**	87C5-87C5	3R:8505001..8507097	electron carrier activity	oxidation-reduction process
***kar**	87C5-87C5	3R:8507908..8515089	monocarboxylic acid transmembrane transporter activity	ommochrome biosynthetic process
***Su(fu)**	87C5-87C5	3R:8515595..8517434	transcription factor binding	negative regulation of sequence-specific DNA binding transcription factor activity; cytoplasmic

				sequestering of transcription factor; negative regulation of smoothened signaling pathway
CG31347	87C5-87C5	3R:8517455..8518319	Unknown	Unknown
Arp87C	87C5-87C5	3R:8517458..8519954	structural constituent of cytoskeleton; actin binding	axon transport of mitochondrion; retrograde axon cargo transport; mitosis; anterograde axon cargo transport
CG14391	87C5-87C5	3R:8519950..8520564	Unknown	Unknown
CG14394	87C5-87C6	3R:8520868..8523265	Unknown	tissue regeneration; cell adhesion
Past1	87C6-87C6	3R:8523044..8530597	calcium ion binding	endocytosis; imaginal disc-derived wing margin morphogenesis; sperm individualization; imaginal disc-derived wing vein specification; oogenesis
CG12279	87C6-87C6	3R:8530816..8531540	unfolded protein binding	protein folding
mus308	87C6-87C6	3R:8531245..8538021	DNA-directed DNA polymerase activity; helicase activity	double-strand break repair via nonhomologous end joining; nucleotide-excision repair

Men	87C6-87C7	3R:8538825..8548267	malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) activity	regulation of cell death
CG5724	87C8-87C8	3R:8565384..8567139	glucuronosyltransferase activity	metabolic process
CG5999	87C8-87C8	3R:8567680..8569402	glucuronosyltransferase activity	metabolic process
beat-Vc	87C8-87D1	3R:8571758..8603874	Unknown	Unknown
CG31345	87D1-87D1	3R:8636867..8642751	calcium ion binding	phagocytosis, engulfment
beat-Va	87D2-87D2	3R:8667040..8674141	Unknown	Unknown
CG10126	87D2-87D2	3R:8681260..8684939	calcium ion binding	Neurogenesis
d-cup	87D3-87D3	3R:8717717..8718880	calcium ion binding	Unknown
CR33929	87D3-87D3	3R:8719694..8720251	Unknown	Unknown
CG10909	87D4-87D4	3R:8735842..8737014	RNA binding; methyltransferase activity	rRNA processing
beat-Vb	87D4-87D4	3R:8741844..8760535	Unknown	Unknown
grsm	87D5-87D5	3R:8775764..8793434	aminopeptidase activity	Proteolysis

Spc25	87D5-87D5	3R:8787474..8788975	Unknown	mitotic metaphase plate congression; chromosome segregation; mitotic spindle organization
Cyp304a1	87D5-87D5	3R:8789519..8791652	electron carrier activity	insecticide metabolic process
CG14384	87D5-87D5	3R:8791660..8792442	Unknown	Unknown
CG7381	87D5-87D6	3R:8793497..8803193	Unknown	Unknown
CG7091	87D6-87D6	3R:8803722..8806257	high affinity inorganic phosphate:sodium symporter activity	transmembrane transport
Paip2	87D6-87D7	3R:8806768..8811210	protein binding	regulation of cell growth; negative regulation of translation
CG31342	87D7-87D7	3R:8811763..8817265	Unknown	Unknown
CG14383	87D7-87D7	3R:8813098..8814233	Unknown	Unknown
yellow-f	87D7-87D7	3R:8817171..8818923	dopachrome isomerase activity	indole-containing compound biosynthetic process
yellow-f2	87D7-87D7	3R:8819117..8820869	dopachrome isomerase activity	indole-containing compound biosynthetic process

CG7488	87D7-87D7	3R:8821342..8822647	GTP binding	Unknown
CG17327	87D7-87D7	3R:8822590..8823404	aminoacyl-tRNA hydrolase activity	Unknown
CG7518	87D7-87D8	3R:8823811..8834126	DNA binding	Unknown
CG8031	87D8-87D8	3R:8834106..8837010	Unknown	Unknown
CG11656	87D8-87D8	3R:8835617..8836808	Unknown	Unknown
CtBP	87D8-87D9	3R:8837388..8851905	protein binding; transcription factor binding; protein homodimerization activity; repressing transcription factor binding; transcription corepressor activity; transcription coactivator activity	chaeta development; wing disc development; regulation of Wnt receptor signaling pathway; regulation of transcription, DNA-dependent; embryonic development via the syncytial blastoderm; regulation of transcription from RNA polymerase II promoter; Wnt receptor signaling pathway; positive regulation of JAK-STAT cascade
CG12360	87D9-87D9	3R:8852747..8856372	Unknown	Unknown
l(3)87Df	87D9-87D9	3R:8856664..8857317	Unknown	Unknown

ry	87D9-87D9	3R:8858259..8863748	pyrimidine base metabolic process; determination of adult lifespan; arginine metabolic process; tryptophan metabolic process; glycerophospholipid metabolic process; purine base metabolic process	
CG11668	87D9-87D9	3R:8864135..8865906	serine-type endopeptidase activity	Proteolysis
snk	87D9-87D9	3R:8865887..8868492	serine-type endopeptidase activity	Toll signaling pathway; protein processing
CG11670	87D9-87D10	3R:8867790..8870123	serine-type endopeptidase activity	Proteolysis
Hsc70-2	87D10-87D10	3R:8870481..8873112	unfolded protein binding	protein folding
CG31157	87D10-87D10	3R:8873234..8875018	Unknown	Unknown
CG7966	87D10-87D10	3R:8875120..8877172	selenium binding	Unknown
pic	87D11-87D11	3R:8877978..8882454	protein binding	mitotic cell cycle G2/M transition DNA damage checkpoint; eggshell chorion gene amplification

sim	87D11-87D11	3R:8883481..8903950	RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity; sequence-specific DNA binding transcription factor activity; RNA polymerase II core promoter proximal region sequence-specific DNA binding; sequence-specific DNA binding; RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription; protein heterodimerization activity	adult walking behavior; positive regulation of transcription from RNA polymerase II promoter; axon guidance; determination of genital disc primordium; ventral midline development; brain development; axonogenesis; ventral cord development
CG43063	87D12-87D12	3R:8911946..8912483	Unknown	Unknown
timeout	87D12-87D14	3R:8914373..8989598	Unknown	entrainment of circadian clock; DNA damage checkpoint
CG34308	87D12-87D12	3R:8915021..8915805	zinc ion binding	Unknown
2mit	87D13-87D14	3R:8947673..8981386	Unknown	Unknown

CG8138	87E1-87E1	3R:9007026..9007876	Unknown	Unknown
CG8508	87E1-87E1	3R:9011248..9012232	Unknown	Unknown
CG14380	87E1-87E1	3R:9014287..9015290	Unknown	Unknown
CG8141	87E1-87E1	3R:9017423..9018231	zinc ion binding	Unknown
CG8483	87E2-87E2	3R:9034470..9040187	Unknown	Unknown
CG8476	87E2-87E2	3R:9045362..9046300	heat shock protein binding; unfolded protein binding	protein folding
Ace	87E2-87E3	3R:9053964..9085240	cholinesterase activity; protein homodimerization activity; acetylcholinesterase activity	phototaxis; synaptic transmission; choline catabolic process; acetylcholine catabolic process
CG11686	87E3-87E3	3R:9086079..9087949	Unknown	Unknown
Ravus	87E3-87E3	3R:9088454..9089755	DNA binding	Unknown
Su(var)3-7	87E3-87E3	3R:9090077..9095760	protein binding; chromatin binding	dosage compensation by inactivation of X chromosome; positive regulation of chromatin silencing at centromere

CG8449	87E3-87E3	3R:9094859..9097748	Rab GTPase activator activity	regulation of Rab GTPase activity
CG8630	87E3-87E4	3R:9105445..9110316	stearoyl-CoA 9-desaturase activity	oxidation-reduction process; lipid metabolic process
CG15888	87E4-87E4	3R:9110671..9112251	Unknown	Unknown
CG15887	87E4-87E4	3R:9113371..9114532	Unknown	Unknown
Osi22	87E4-87E4	3R:9116438..9117568	Unknown	Unknown
wntD	87E4-87E4	3R:9117774..9118920	frizzled binding	ventral furrow formation; defense response to Gram-positive bacterium; pole cell migration; regulation of embryonic development
CG8773	87E4-87E4	3R:9120472..9124010	aminopeptidase activity	dsRNA transport
CG8774	87E4-87E5	3R:9124569..9128056	aminopeptidase activity	Proteolysis
CG32473	87E5-87E5	3R:9128375..9138507	aminopeptidase activity	Proteolysis
CG43208	87E5-87E5	3R:9129148..9129578	Unknown	Unknown
CG8795	87E6-87E6	3R:9154787..9159885	peptide receptor activity; G-protein coupled receptor	G-protein coupled receptor signaling pathway

			activity; neuropeptide receptor activity	
CG8784	87E6-87E6	3R:9165685..9170828	G-protein coupled receptor activity; neuropeptide receptor activity	G-protein coupled receptor signaling pathway
mthl12	87E7-87E7	3R:9179395..9181135	G-protein coupled receptor activity	G-protein coupled receptor signaling pathway; determination of adult lifespan; response to stress
poly	87E7-87E8	3R:9187538..9192629	Unknown	oocyte microtubule cytoskeleton polarization; melanotic encapsulation of foreign target
Dic1	87E8-87E8	3R:9190661..9194813	inorganic phosphate transmembrane transporter activity; sulfate transmembrane transporter activity; malate transmembrane transporter activity; thiosulfate transmembrane transporter activity; oxaloacetate transmembrane transporter activity; succinate transmembrane transporter activity	oxaloacetate transport; thiosulfate transport; malate transmembrane transport; sulfate transport; succinate transmembrane transport; phosphate ion transmembrane transport
CheA87a	87E8-87E8	3R:9195391..9196068	Unknown	sensory perception of chemical

				stimulus
Lip3	87E8-87E8	3R:9195962..9197626	triglyceride lipase activity	lipid metabolic process
CG34309	87E8-87E8	3R:9198352..9199048	Unknown	Unknown
CG9813	87E8-87E8	3R:9198821..9205494	ATP binding	Unknown
CG8870	87E8-87E8	3R:9205862..9207065	serine-type endopeptidase activity	Proteolysis
mRpS21	87E8-87E8	3R:9207051..9207514	structural constituent of ribosome	Translation
Droj2	87E8-87E8	3R:9207799..9211013	heat shock protein binding; unfolded protein binding; ATP binding	Neurogenesis
CG9799	87E8-87E8	3R:9211370..9214436	Unknown	rRNA processing
CCHa2	87E8-87E8	3R:9214781..9220912	neuropeptide hormone activity	neuropeptide signaling pathway
CG14374	87E9-87E9	3R:9222416..9222855	Unknown	Unknown
CG14377	87E9-87E9	3R:9223557..9224305	Unknown	Unknown
CG9796	87E9-87E9	3R:9224248..9227580	Unknown	Unknown

CG33977	87E9-87E10	3R:9228281..9228771	Unknown	Unknown
yellow-e3	87E10-87E10	3R:9229145..9230590	Unknown	Unknown
yellow-e2	87E10-87E10	3R:9230831..9232689	Unknown	Unknown
yellow-e	87E10-87E10	3R:9235264..9241006	Unknown	Unknown
Ir87a	87E10-87E11	3R:9245995..9248862	ligand-gated ion channel activity	detection of chemical stimulus
Act87E	87E11-87E11	3R:9251707..9253811	structural constituent of cytoskeleton	cytoskeleton organization
yrt	87E11-87E11	3R:9254589..9260704	cytoskeletal protein binding	dorsal closure; amnioserosa maintenance; establishment or maintenance of apical/basal cell polarity; germ-band shortening; eye photoreceptor cell development; head involution
CR42756	87E11-87E11	3R:9264309..9264931	Unknown	Unknown
CG14372	87E12-87E12	3R:9276312..9289906	Unknown	Unknown

CR17025	87E12-87E12	3R:9281233..9291828	Unknown	Unknown
mir-252	87E12-87E12	3R:9289941..9290033	Unknown	Unknown
CG12538	87F1-87F1	3R:9348673..9349257	acid phosphatase activity	Unknown
CG42778	87F2-87F2	3R:9354319..9355078	Unknown	Unknown
CG31337	87F2-87F2	3R:9369688..9371106	Unknown	Unknown
CR43848	87F3-87F3	3R:9400547..9401201	Unknown	Unknown
CG14370	87F3-87F3	3R:9413719..9414447	Unknown	Unknown
CG14369	87F3-87F3	3R:9417998..9418336	Unknown	Unknown
CG42760	87F4-87F4	3R:9433304..9433543	Unknown	Unknown
CG42761	87F4-87F4	3R:9433771..9434112	Unknown	Unknown
CG9759	87F4-87F4	3R:9434475..9434968	Unknown	Unknown
CG9757	87F4-87F4	3R:9437588..9438151	Unknown	Unknown
CG9269	87F5-87F5	3R:9441726..9442166	Unknown	Unknown

CG10841	87F5-87F5	3R:9457664..9460583	calcium ion binding	Unknown
sqd	87F5-87F6	3R:9460679..9472026	mRNA binding	terms, many of which group under: localization; cellular component organization or biogenesis; multicellular organism reproduction; anterior/posterior axis specification; biological regulation; regulation of RNA splicing; ovarian follicle cell development; regulation of RNA metabolic process; establishment or maintenance of polarity of follicular epithelium; oocyte anterior/posterior axis specification
rin	87F6-87F7	3R:9472726..9480285	mRNA binding; SH3 domain binding	Ras protein signal transduction; compound eye photoreceptor fate commitment; ommatidial rotation
Rbp4	87F7-87F7	3R:9480278..9482116	single-stranded RNA binding	mRNA processing
Hrb87F	87F7-87F7	3R:9482568..9486241	sequence-specific DNA binding	mitosis; regulation of alternative nuclear mRNA splicing, via spliceosome;

				neurogenesis
B52	87F7-87F7	3R:9487022..9492613	protein binding	nuclear mRNA splicing, via spliceosome; regulation of nuclear mRNA splicing, via spliceosome; mitosis; regulation of alternative nuclear mRNA splicing, via spliceosome
Task6	87F7-87F7	3R:9494313..9497823	potassium channel activity	potassium ion transmembrane transport
CG9588	87F7-87F7	3R:9500814..9502398	Unknown	response to DNA damage stimulus
lkb1	87F7-87F9	3R:9502271..9505152	protein kinase activity	border follicle cell delamination; dorsal closure; regulation of JNK cascade; oocyte microtubule cytoskeleton organization; determination of adult lifespan; pole plasm oskar mRNA localization; mitotic spindle organization; asymmetric neuroblast division; positive regulation of apoptotic process
omd	87F9-87F11	3R:9505151..9512932	Unknown	snRNA 3'-end processing;

				neurogenesis
flfl	87F10-87F11	3R:9509680..9519629	protein binding	Rac protein signal transduction; dorsal closure; neuroblast proliferation; asymmetric neuroblast division; asymmetric protein localization; phagocytosis, engulfment
CG14367	87F11-87F11	3R:9519775..9521484	Unknown	cilium assembly
f-cup	87F11-87F11	3R:9520979..9526560	Unknown	Ras protein signal transduction
ninaB	87F11-87F11	3R:9526308..9528844	retinal isomerase activity; beta-carotene 15,15'-monooxygenase activity; carotenoid dioxygenase activity	retinal metabolic process; carotene metabolic process; rhodopsin biosynthetic process; phototransduction, UV; vitamin biosynthetic process; phototransduction, visible light; vitamin A biosynthetic process
Adgf-D	87F11-87F11	3R:9528806..9531180	growth factor activity; adenosine deaminase activity	cell proliferation
Adgf-C	87F12-87F12	3R:9532479..9534851	growth factor activity	purine ribonucleoside monophosphate biosynthetic

				process
CG31469	87F12-87F12	3R:9534881..9535661	protein tyrosine phosphatase activity	protein dephosphorylation
primo-2	87F12-87F12	3R:9535748..9537557	protein tyrosine phosphatase activity; acid phosphatase activity	protein dephosphorylation
primo-1	87F12-87F12	3R:9535748..9537557	protein tyrosine phosphatase activity; acid phosphatase activity	protein dephosphorylation
CR34044	87F12-87F12	3R:9538777..9539926	Unknown	Unknown
CG34383	87F12-87F13	3R:9540931..9590389	phospholipid binding	Unknown
CG9602	87F12-87F12	3R:9543176..9543898	ubiquitin-protein ligase activity	Unknown
CG9312	87F13-87F13	3R:9591497..9592829	Unknown	Unknown
Cht5	87F13-87F13	3R:9593271..9596454	chitin binding; chitinase activity	chitin catabolic process
CG9297	87F13-87F13	3R:9597217..9605039	calcium ion binding	calcium ion transport

CG9288	87F13-87F13	3R:9605346..9606517	Unknown	glycine catabolic process
CG42375	87F13-87F13	3R:9605346..9606517	Unknown	Unknown
CG9286	87F13-87F13	3R:9606686..9607804	Unknown	Unknown
Dip-B	87F13-87F13	3R:9608012..9611401	tripeptidyl-peptidase activity; dipeptidyl-peptidase activity	Proteolysis
tRNA:CR31588	87F14-87F14	3R:9623675..9623747	UUC codon-amino acid adaptor activity	Translation
tRNA:CR31331	87F14-87F14	3R:9623920..9623992	UUC codon-amino acid adaptor activity	Translation
tal-AA	87F14-87F14	3R:9638831..9640370	Unknown	morphogenesis of an epithelium; actin filament organization
tal-3A	87F14-87F14	3R:9638831..9640370	Unknown	morphogenesis of an epithelium; actin filament organization
tal-2A	87F14-87F14	3R:9638831..9640370	Unknown	morphogenesis of an epithelium; actin filament organization
tal-1A	87F14-	3R:9638831..9640370	Unknown	morphogenesis of an

	87F14			epithelium; imaginal disc-derived wing morphogenesis; actin filament organization
CR43641	87F14-87F14	3R:9641001..9641500	Unknown	Unknown
CR43642	87F14-87F14	3R:9641953..9642849	Unknown	Unknown
Mst87F	87F14-87F14	3R:9652873..9653432	structural molecule activity	Spermatogenesis
CR43300	87F14-87F14	3R:9653690..9654149	Unknown	Unknown
Nsf2	87F15-87F15	3R:9660195..9663708	nucleoside-triphosphatase activity; ATP binding	neuromuscular synaptic transmission; regulation of synaptic growth at neuromuscular junction
CG31495	87F15-87F15	3R:9663960..9665652	ATP binding	Unknown
CG14362	88A1-88A1	3R:9680321..9681176	calcium ion binding	Unknown
E5	88A1-88A1	3R:9693067..9700638	sequence-specific DNA binding transcription factor activity	regulation of transcription, DNA-dependent

Appendix C: Candidate genes within region 93A1-93D5

Gene Name	Cytological Location	Base Position	Molecular Function	Biological Process
Fancd2	93A1-93A1	3R:16636525..16641923	protein kinase activity; ATP binding	DNA repair; intra-S DNA damage checkpoint
CG17270	93A1-93A1	3R:16642167..16644646	Unknown	Unknown
CG17271	93A1-93A1	3R:16644849..16647587	Calcium ion binding	Unknown
RpS20	93A1-93A1	3R:16647901..16649078	structural constituent of ribosome	Translation
snoRNA:Psi18S-1295	93A1-93A1	3R:16648332..16648471	Unknown	Unknown
snoRNA:Psi28S-2562	93A1-93A1	3R:16648648..16648782	Unknown	Unknown
CG17272	93A1-93A1	3R:16649035..16649951	Calcium ion binding	Unknown
CG17273	93A1-93A1	3R:16650389..16656337	adenylosuccinate synthase	Neurogenesis

			activity	
CG31223	93A1-93A1	3R:16656914..16658177	Unknown	Neurogenesis
Synd	93A1-93A2	3R:16658507..16665840	Unknown	neurotransmitter secretion; synaptic vesicle endocytosis
CG15695	93A2-93A2	3R:16667864..16670709	catalytic activity	metabolic process
CG15696	93A2-93A2	3R:16672833..16673372	sequence-specific DNA binding transcription factor activity	regulation of transcription, DNA-dependent
RpS30	93A2-93A2	3R:16676271..16677156	structural constituent of ribosome	mitotic spindle elongation; mitotic spindle organization
Ir93a	93A2-93A2	3R:16677428..16685667	extracellular-glutamate-gated ion channel activity; ligand-gated ion channel activity	detection of chemical stimulus involved in sensory perception
mir-1011	93A2-93A2	3R:16679027..16679080	Unknown	Unknown
CG3822	93A2-93A2	3R:16685904..16691951	kainate selective glutamate receptor activity	ion transport

TotA	93A2-93A2	3R:16696758..16697427	Unknown	response to bacterium; response to water deprivation; cellular response to heat; cellular response to UV; cellular response to oxidative stress; response to methylmercury; cellular response to mechanical stimulus
TotC	93A2-93A2	3R:16698710..16699310	Unknown	response to bacterium; cellular response to heat; cellular response to UV
TotB	93A2-93A2	3R:16699672..16700259	Unknown	response to bacterium; cellular response to heat; cellular response to UV
TotZ	93A2-93A2	3R:16703458..16704164	Unknown	response to bacterium; cellular response to heat; cellular response to UV; cellular

				response to oxidative stress
tRNA:CR31480	93A2-93A2	3R:16716420..16716491	ACG codon-amino acid adaptor activity	Translation
tRNA:CR31334	93A2-93A2	3R:16716844..16716915	ACG codon-amino acid adaptor activity	Translation
tRNA:CR31333	93A2-93A2	3R:16717380..16717451	ACG codon-amino acid adaptor activity	Translation
CG10830	93A2-93A2	3R:16720174..16723042	Unknown	protein homooligomerization
CG5621	93A2-93A3	3R:16727716..16733987	kainate selective glutamate receptor activity	ion transport
TotX	93A3-93A3	3R:16730638..16731240	Unknown	response to bacterium; cellular response to heat; cellular response to oxidative stress
CG31191	93A3-93A4	3R:16734935..16771252	Unknown	signal transduction
snoRNA:CG31191-a	93A3-93A3	3R:16738455..16738601	Unknown	Unknown

CG5630	93A3-93A4	3R:16740023..16754935	Unknown	Unknown
Atpalpha	93A4-93A4	3R:16774436..16801961	sodium:potassium-exchanging ATPase activity; cation transmembrane transporter activity	biological regulation; anatomical structure development; locomotory behavior; cellular component organization or biogenesis; response to stimulus; multicellular organismal process; determination of adult lifespan; synaptic transmission; response to mechanical stimulus; behavior; localization; neuromuscular process; response to temperature stimulus; regulation of anatomical structure size
Calx	93A4-93B3	3R:16803907..16841335	calcium ion binding; calcium:sodium antiporter	Phototransduction

			activity	
CG43446	93B3-93B3	3R:16812226..16813658	Unknown	Unknown
CG10827	93B3-93B3	3R:16830882..16832709	alkaline phosphatase activity	metabolic process
CG5697	93B4-93B4	3R:16841671..16842842	Unknown	Unknown
Rlip	93B4-93B4	3R:16843423..16846475	protein binding; Ral GTPase binding	receptor-mediated endocytosis
CG17278	93B4-93B4	3R:16846756..16849885	Unknown	negative regulation of Wnt receptor signaling pathway
Dhc93AB	93B5-93B7	3R:16850741..16868141	ATPase activity, coupled; motor activity	microtubule-based movement
****CG12278***	93B7-93B7	3R:16869815..16870627	Unknown	Unknown
****CG31189***	93B7-93B7	3R:16870620..16871533	Unknown	Unknown
****CG31207***	93B7-93B7	3R:16871754..16872749	Unknown	Unknown
****CG7079***	93B7-93B7	3R:16873487..16874543	Unknown	Unknown

***CG17279**	93B7-93B7	3R:16875736..16876637	Unknown	Unknown
***Mvl**	93B7-93B8	3R:16877105..16886517	manganese ion transmembrane transporter activity; iron ion transmembrane transporter activity; copper ion transmembrane transporter activity; symporter activity	iron assimilation; multicellular organismal iron ion homeostasis; copper ion transport; divalent metal ion transport; copper ion import; sensory perception of sweet taste; viral entry into host cell; transition metal ion transport; copper ion homeostasis; transition metal ion homeostasis
***Cortactin**	93B8-93B9	3R:16887402..16890193	proline-rich region binding	regulation of cell shape; positive regulation of receptor-mediated endocytosis; border follicle cell migration; female germline ring canal formation
***AnnIX**	93B9-	3R:16890963..16896639	calcium ion binding; calcium-	wing disc dorsal/ventral pattern

	93B10		dependent phospholipid binding; actin binding	formation
r-l	93B10-93B10	3R:16896279..16899041	orotidine-5'-phosphate decarboxylase activity; orotate phosphoribosyltransferase activity	'de novo' pyrimidine base biosynthetic process
dmrt93B	93B10-93B10	3R:16899729..16902520	sequence-specific DNA binding transcription factor activity	sex differentiation; regulation of transcription, DNA-dependent
CG7056	93B10-93B10	3R:16902635..16906405	sequence-specific DNA binding transcription factor activity	dendrite morphogenesis; neurogenesis
RhoGAP93B	93B10-93B11	3R:16908758..16923527	Unknown	axon guidance
CG7044	93B11-93B12	3R:16923951..16927440	Binding	Unknown
CG5745	93B12-	3R:16927921..16930835	GTPase activator activity	phagocytosis, engulfment

	93B12			
**** sec15***	93B12-93B12	3R:16930768..16933694	Unknown	chaeta development; phototaxis; border follicle cell migration; axon guidance; endocytic recycling
****Rtet***	93B12-93B12	3R:16934168..16936556	sugar transmembrane transporter activity	Oogenesis
****Rab11***	93B12-93B13	3R:16937130..16941868	GTPase activity	cellular component organization or biogenesis; sensory organ development; multicellular organism reproduction; biological regulation; localization; gamete generation; organelle organization; cell cycle; regulation of developmental process; cell cycle process; fusome organization

***Ppan**	93B13-93B13	3R:16941976..16943621	Unknown	imaginal disc development; larval development; oogenesis; neurogenesis
***CG17282**	93B13-93B13	3R:16943579..16944578	Binding	Unknown
***Slmb**	93B13-93C1	3R:16944974..16953207	phosphoprotein binding	biological regulation; multicellular organism reproduction; ovarian follicle cell development; gamete generation; cellular component organization or biogenesis; localization; learning or memory; rhythmic process; regulation of cellular component organization; regulation of Wnt receptor signaling pathway
***CG5793**	93C1-93C1	3R:16953295..16955323	catalytic activity	metabolic process

***Obp93a**	93C1-93C1	3R:16954672..16955439	odorant binding	sensory perception of chemical stimulus
***Ice2**	93C1-93C1	3R:16955941..16958442	Unknown	Unknown
***CG7009**	93C1-93C1	3R:16958456..16959599	rRNA (uridine-2'-O-)-methyltransferase activity	rRNA modification
***Ubpy**	93C1-93C1	3R:16959984..16963936	ubiquitin thiolesterase activity	positive regulation of canonical Wnt receptor signaling pathway; imaginal disc-derived wing margin morphogenesis
***CG5802**	93C1-93C1	3R:16963992..16966087	UDP-galactose transmembrane transporter activity	transmembrane transport
***SNF4Agamma**	93C1-93C5	3R:16966461..17039991	AMP-activated protein kinase activity	lipid metabolic process; positive regulation of cell cycle; behavioral response to starvation; sequestering of triglyceride; cellular response

				to starvation; autophagy; cholesterol homeostasis
CG10824	93C2-93C2	3R:16984960..16986814	Unknown	Unknown
CG5810	93C2-93C2	3R:16989500..16990883	Unknown	Unknown
Snmp1	93C2-93C2	3R:16990889..16993927	scavenger receptor activity	response to pheromone; cell surface receptor signaling pathway

Appendix D: Candidate genes within region 96A20-96C3

Gene Name	Cytological Location	Base Position	Molecular Function	Biological Process
CG42331	96A20-96A22	3R:20597903..20610284	peroxidase activity	oxidation-reduction process; response to oxidative stress
CG5805	96A22-96A22	3R:20610459..20613152	transmembrane transporter activity	transmembrane transport
CG13634	96A22-96A22	3R:20614072..20615858	Unknown	Unknown
Ets96B	96A22-96A22	3R:20617465..20623678	sequence-specific DNA binding transcription factor activity; DNA binding	regulation of transcription, DNA-dependent
Ude	96A22-96A22	3R:20623317..20625390	RNA binding; DNA binding	pupation; DNA catabolic process
Polybromo	96A22-96A23	3R:20625689..20630981	DNA binding; zinc ion binding	eggshell chorion assembly; negative regulation of

				chromatin silencing; vitelline membrane formation involved in chorion-containing eggshell formation; imaginal disc-derived leg morphogenesis
CG5807	96A23-96A23	3R:20631167..20639734	Unknown	Unknown
CG6980	96A23-96A23	3R:20633343..20634399	binding	Unknown
CG34150	96A23-96A23	3R:20639984..20640652	Unknown	Unknown
CG5808	96A23-96A23	3R:20640625..20642729	mRNA binding	Neurogenesis
Saf-B	96A23-96A23	3R:20643186..20648072	mRNA binding	regulation of alternative nuclear mRNA splicing, via spliceosome

Niki	96A23-96A24	3R:20648123..20651105	receptor signaling protein serine/threonine kinase activity	protein phosphorylation
CG43166	96A24-96A24	3R:20651513..20652193	Unknown	Unknown
RabX4	96A24-96A24	3R:20652387..20653691	GTPase activity	protein transport; small GTPase mediated signal transduction
CG31357	96A24-96A24	3R:20654001..20655645	Unknown	Unknown
CG43273	96A24-96A24	3R:20656023..20656695	Unknown	Unknown
CG13636	96A24-96A25	3R:20659676..20666217	Unknown	Unknown
CG33658	96A25-96B1	3R:20666231..20666885	Unknown	Unknown
Esp	96B1-96B1	3R:20667765..20676134	secondary active sulfate	sulfate transport

			transmembrane transporter activity	
CG7006	96B1-96B1	3R:20676341..20677107	RNA binding	ribosome assembly
Nct	96B1-96B1	3R:20677294..20680730	Unknown	Notch receptor processing; regulation of compound eye photoreceptor development; lateral inhibition; membrane protein ectodomain proteolysis; Notch signaling pathway; R8 cell development; photoreceptor cell morphogenesis; cytoskeleton organization
HdacX	96B1-96B1	3R:20680218..20681965	histone deacetylase activity	histone deacetylation
CG10899	96B1-96B1	3R:20682105..20683281	carbonate dehydratase activity	one-carbon metabolic process

CG31105	96B1-96B1	3R:20683344..20685475	sodium channel activity	sodium ion transport
CG13639	96B1-96B1	3R:20685622..20686088	Unknown	Unknown
CG13640	96B1-96B1	3R:20687381..20687884	Unknown	Unknown
CG7016	96B1-96B1	3R:20688654..20689938	Unknown	Unknown
CG13641	96B1-96B1	3R:20689816..20690502	Unknown	Unknown
Ela	96B1-96B1	3R:20691073..20692425	Unknown	response to nicotine
CycB3	96B1-96B2	3R:20694075..20696617	protein binding	cytokinesis after mitosis; regulation of chromatin binding; mitosis; mitotic spindle organization;

				syncytial blastoderm mitotic cell cycle
CG3744	96B2-96B2	3R:20697503..20702366	dipeptidyl-peptidase activity	Proteolysis
CG11089	96B2-96B2	3R:20702790..20707202	phosphoribosylaminoimidazole carboxamide formyltransferase activity; IMP cyclohydrolase activity	wound healing
CG31381	96B2-96B2	3R:20702790..20707202	zinc ion binding; ATP binding	tRNA modification
CG31121	96B2-96B4	3R:20707180..20720327	ATPase activity, coupled to transmembrane movement of substances; transporter activity	Transport
CG11069	96B4-96B4	3R:20720466..20723335	ATPase activity, coupled to transmembrane movement of substances; transporter activity	Unknown
CG13643	96B4-96B5	3R:20723407..20731723	chitin binding	chitin metabolic process

CG10845	96B4-96B5	3R:20726838..20728770	motor activity	microtubule-based movement
CG31120	96B6-96B6	3R:20738938..20743582	iron ion binding; L-ascorbic acid binding; oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, 2-oxoglutarate as one donor, and incorporation of one atom each of oxygen into both donors	oxidation-reduction process
tRNA:CR31382	96B6-96B6	3R:20740556..20740627	AAC codon-amino acid adaptor activity	Translation
tRNA:CR31416	96B6-96B6	3R:20740782..20740853	AAC codon-amino acid adaptor activity	Translation
CG11168	96B6-96B8	3R:20743868..20748111	Unknown	Unknown

CG11120	96B8-96B9	3R:20748168..20750882	Unknown	Unknown
Trf4-2	96B9-96B9	3R:20750938..20752576	DNA-directed DNA polymerase activity	sister chromatid cohesion
CG11771	96B10-96B10	3R:20757739..20760637	metalloendopeptidase activity	Proteolysis
Osbp	96B10-96B10	3R:20760591..20763959	oxysterol binding	Golgi organization; sperm individualization; intracellular protein transport
Ssh	96B10-96B11	3R:20764725..20770678	protein tyrosine/serine/threonine phosphatase activity	mitotic cell cycle; regulation of axonogenesis; regulation of lamellipodium assembly; compound eye development; mushroom body development; regulation of actin polymerization or

				depolymerisation
Nmnat	96B11-96B11	3R:20770959..20773122	nicotinamide-nucleotide adenylyltransferase activity	dendritic spine maintenance; photoreceptor cell maintenance
CG13646	96B11-96B11	3R:20773354..20775261	amino acid transmembrane transporter activity	amino acid transmembrane transport
Tnc	96B13-96B15	3R:20810106..20848589	integrin binding	wing disc morphogenesis; imaginal disc-derived male genitalia morphogenesis; imaginal disc-derived wing morphogenesis
beta4GalT7	96B16-96B16	3R:20848995..20850205	xylosylprotein 4-beta-galactosyltransferase activity; galactosyltransferase activity	proteoglycan biosynthetic process
CG11781	96B16-96B16	3R:20850193..20850727	Unknown	Unknown
Hr96	96B16-96B17	3R:20850961..20855075	RNA polymerase II regulatory	response to starvation;

			region sequence-specific DNA binding	triglyceride homeostasis; regulation of glycogen metabolic process; cholesterol homeostasis
Smg6	96B17-96B17	3R:20855145..20859177	ribonuclease activity	neuromuscular synaptic transmission; nuclear-transcribed mRNA catabolic process, nonsense-mediated decay; synapse organization
CG6422	96B17-96B17	3R:20859866..20864801	Unknown	Unknown
CG31115	96B17-96B17	3R:20862666..20863708	S-methyl-5-thioadenosine phosphorylase activity	nucleoside metabolic process
Bai	96B17-96B17	3R:20865097..20866172	Unknown	maternal specification of dorsal/ventral axis, oocyte, germ-line encoded
5PtaseI	96B17-96B18	3R:20866149..20870857	inositol-polyphosphate 5-	inositol phosphate

			phosphatase activity	dephosphorylation
CG11786	96B18-96B18	3R:20872047..20873131	Unknown	Unknown
CG11790	96B18-96B19	3R:20873422..20875177	Unknown	cell redox homeostasis
CG11791	96B19-96B19	3R:20875514..20878799	Unknown	Unknown
CG31109	96B19-96B19	3R:20879199..20880356	zinc ion binding	Unknown
CG31111	96B19-96B19	3R:20880798..20881899	Unknown	Neurogenesis
CG34349	96B19-96B19	3R:20882812..20890070	Unknown	Unknown
Veli	96B19-96B19	3R:20890140..20891317	Unknown	regulation of synaptic growth at neuromuscular junction
PQBP1	96B19-96B19	3R:20891527..20892440	Unknown	olfactory learning

OstStt3	96B19-96B20	3R:20892434..20895372	oligosaccharyl transferase activity	protein glycosylation
CG11839	96B20-96B20	3R:20895649..20896636	Unknown	Unknown
CG11836	96B20-96B20	3R:20896853..20898434	serine-type endopeptidase activity	Proteolysis
CG9996	96B20-96B20	3R:20898451..20900329	transferase activity, transferring glycosyl groups	Unknown
CG11920	96B20-96B20	3R:20900604..20902104	Unknown	Unknown
fd96Ca	96B20-96B20	3R:20909274..20910392	sequence-specific DNA binding transcription factor activity	embryo development
fd96Cb	96B20-96B21	3R:20920831..20921655	sequence-specific DNA binding transcription factor	embryo development

			activity	
CG33096	96B21-96B21	3R:20923364..20925507	hydrolase activity	Unknown
CG33095	96B21-96C1	3R:20924610..20927868	Unknown	Unknown
CG34027	96C1-96C1	3R:20929392..20934754	Unknown	Unknown
CG13650	96C1-96C1	3R:20934948..20938238	metalloendopeptidase activity	Proteolysis
CG31108	96C1-96C1	3R:20938587..20948631	tubulin-tyrosine ligase activity	cellular protein modification process
CG31510	96C1-96C1	3R:20943147..20948631	zinc ion binding	Unknown
vig2	96C1-96C1	3R:20949095..20951341	Unknown	heterochromatin organization; histone H3-K9 methylation; regulation of chromatin

				silencing
CG42503	96C1-96C1	3R:20951354..20953081	Unknown	Mo-molybdopterin cofactor biosynthetic process
Mocs2	96C1-96C1	3R:20951354..20953081	Unknown	Mo-molybdopterin cofactor biosynthetic process
Clbn	96C1-96C1	3R:20953224..20956877	Unknown	protein export from nucleus
Bili	96C1-96C1	3R:20957012..20962431	Unknown	negative regulation of Wnt receptor signaling pathway
Danr	96C2-96C2	3R:20963799..20965625	sequence-specific DNA binding transcription factor activity; protein binding	segment specification; regulation of transcription, DNA-dependent; compound eye development
Lobo	96C2-96C4	3R:20995286..21019498	Unknown	sperm motility; sperm storage
Dan	96C3-96C4	3R:21010105..21018528	sequence-specific DNA	segment specification;

binding transcription factor activity; protein binding	regulation of transcription, DNA-dependent; compound eye development

Appendix E: Candidate genes within region 98E5-99A5

Gene Name	Cytological Location	Base Position	Molecular Function	Biological Process
Apc	98E5-98E6	3R:24658022..24670372	beta-catenin binding	system development; biological regulation; multicellular organismal development; cellular process; cellular component organization or biogenesis; localization; regulation of Wnt receptor signaling pathway; cell proliferation; larval chitin-based cuticle development; regionalization; embryonic development via the syncytial blastoderm; regulation of signal transduction
Spg	98E6-98F1	3R:24670992..24695340	GTP binding; GTPase binding;	central nervous system

			guanyl-nucleotide exchange factor activity	development
inx3	98E6-98F1	3R:24679328..24684915	gap junction channel activity	intercellular transport
CG33203	98F1-98F1	3R:24696328..24714723	Unknown	Lateral inhibition
CG14529	98F1-98F1	3R:24698627..24700753	metalloendopeptidase activity	Proteolysis
CG14528	98F1-98F1	3R:24701345..24703529	metalloendopeptidase activity	Proteolysis
CG14523	98F1-98F1	3R:24703746..24705963	metalloendopeptidase activity	Proteolysis
CG14527	98F1-98F1	3R:24706525..24708627	metalloendopeptidase activity	Proteolysis
CG14526	98F1-98F1	3R:24709415..24711737	metalloendopeptidase activity	Proteolysis

Doa	98F1-98F2	3R:24713799..24748494	protein serine/threonine kinase activity; protein kinase activity	biological regulation; programmed cell death; sensory organ development; system development; response to stimulus; multicellular organism reproduction; macromolecule modification; cellular component organization or biogenesis; sex differentiation; localization; neuromuscular process; embryonic pattern specification; gland morphogenesis; locomotory behavior; compound eye photoreceptor development
CG11828	98F2-98F2	3R:24748766..24750808	procollagen-proline 4-dioxygenase activity	peptidyl-proline hydroxylation to 4-hydroxy-

				L-proline
CG14521	98F2-98F4	3R:24750717..24796076	Unknown	Unknown
CG1443	98F5-98F5	3R:24818699..24846128	oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor; nucleotide binding	oxidation-reduction process
mir-4947	98F5-98F5	3R:24834475..24834574	Unknown	Unknown
CG14518	98F5-98F5	3R:24850693..24851881	Unknown	Unknown
eIF4E-6	98F5-98F5	3R:24852161..24852896	RNA 7-methylguanosine cap binding	negative regulation of translation
CG1951	98F5-98F5	3R:24852969..24856660	protein kinase activity	protein phosphorylation
Ssl2	98F5-98F5	3R:24856580..24858255	strictosidine synthase activity	biosynthetic process

beta4GalNAcTB	98F5-98F5	3R:24858382..24859730	N-acetyl-beta-glucosaminyl-glycoprotein 4-beta-N-acetylgalactosaminyltransferase activity; acetylgalactosaminyltransferase activity	N-acetylglucosamine metabolic process; glycolipid biosynthetic process
Cpsf100	98F5-98F5	3R:24860325..24862784	mRNA 3'-UTR binding	mRNA polyadenylation; histone mRNA 3'-end processing; neurogenesis
Slu7	98F5-98F6	3R:24862780..24864788	zinc ion binding; nucleic acid binding	mitotic spindle organization
Pkc98E	98F6-98F6	3R:24864896..24872756	protein kinase C activity; diacylglycerol binding	Golgi organization; response to ethanol; negative regulation of cell proliferation; lipid particle organization
CG11837	98F6-98F6	3R:24875217..24876443	rRNA (adenine-N6,N6-)-	Neurogenesis

			dimethyltransferase activity	
CG11841	98F6-98F6	3R:24876778..24877989	serine-type endopeptidase activity	Proteolysis
CG11842	98F6-98F6	3R:24878402..24879635	serine-type endopeptidase activity	Proteolysis
CG11843	98F6-98F6	3R:24880244..24881559	serine-type endopeptidase activity	Proteolysis
Sirt7	98F6-98F6	3R:24881654..24884926	zinc ion binding; NAD+ binding	protein deacetylation
Cul-5	98F6-98F6	3R:24884992..24888881	ubiquitin protein ligase binding	sensory organ precursor cell fate determination; growth of a germarium-derived egg chamber; neuromuscular junction development; negative regulation of cell proliferation; germarium-derived egg chamber

				formation; regulation of synaptic growth at neuromuscular junction
CG11873	98F6-98F10	3R:24888762..24934670	Unknown	Unknown
CG11874	98F10-98F10	3R:24934775..24938542	mannosyl-oligosaccharide 1,2-alpha-mannosidase activity	Unknown
CG11876	98F10-98F10	3R:24938793..24941919	pyruvate dehydrogenase (acetyl-transferring) activity	cytoplasmic microtubule organization
CG11877	98F10-98F10	3R:24942113..24944112	Unknown	positive regulation of autophagy
yemalpha	98F10-98F10	3R:24944052..24948601	DNA binding	female meiosis
CG11880	98F10-98F12	3R:24949104..24953972	Unknown	Unknown
dgt6	98F12-	3R:24954306..24956651	Unknown	regulation of mitosis; mitotic

	98F12			spindle organization
Vha100-1	98F12-98F12	3R:24956772..24963702	calmodulin binding	intracellular pH reduction
CG14512	98F12-98F12	3R:24963692..24964295	carbohydrate binding; transferase activity, transferring hexosyl groups	lipid glycosylation
CG14516	98F12-98F12	3R:24964388..24971515	aminopeptidase activity	Proteolysis
CG14511	98F12-98F12	3R:24971400..24972615	UDP-N-acetylglucosamine transmembrane transporter activity	transmembrane transport
CG11882	98F12-98F12	3R:24972738..24973894	Unknown	Unknown
Pglym78	98F12-98F13	3R:24973970..24975623	phosphoglycerate mutase activity	Glycolysis
ligatin	98F13-	3R:24975618..24977512	translation initiation factor	translational initiation

	98F13		activity	
Slbp	98F13-98F13	3R:24977793..24978969	mRNA binding; RNA stem-loop binding	histone mRNA metabolic process; histone mRNA 3'-end processing; cell cycle
Rpn2	98F13-98F13	3R:24979185..24982981	endopeptidase activity	response to DNA damage stimulus; proteolysis
CG11897	98F13-98F13	3R:24983847..24990694	xenobiotic-transporting ATPase activity; drug transmembrane transporter activity	transmembrane transport
CG11898	98F13-98F13	3R:24991515..24996871	xenobiotic-transporting ATPase activity; drug transmembrane transporter activity	transmembrane transport
CG14509	98F13-99A1	3R:24998986..25020716	Unknown	olfactory behavior
CG14515	99A1-99A1	3R:25023305..25024933	Unknown	Unknown

CG11899	99A1-99A1	3R:25026174..25027492	O-phospho-L-serine:2-oxoglutarate aminotransferase activity	pyridoxine biosynthetic process; L-serine biosynthetic process
Mesh1	99A1-99A1	3R:25027478..25028247	guanosine-3',5'-bis(diphosphate) 3'-diphosphatase activity	response to starvation; guanosine tetraphosphate catabolic process
CG14508	99A1-99A1	3R:25028387..25029855	electron transporter, transferring electrons within CoQH2-cytochrome c reductase complex activity	mitochondrial electron transport, ubiquinol to cytochrome c; oxidative phosphorylation
CR31044	99A1-99A1	3R:25040284..25045389	Unknown	Unknown
mir-279	99A1-99A1	3R:25041307..25041406	Unknown	startle response; brain morphogenesis; wing disc dorsal/ventral pattern formation; locomotion involved in locomotory

				behavior
mir-996	99A1-99A1	3R:25042906..25043002	Unknown	Unknown
Ef1gamma	99A1-99A1	3R:25045888..25047831	translation elongation factor activity	autophagic cell death; salivary gland cell autophagic cell death
CG1458	99A1-99A1	3R:25047939..25049149	2 iron, 2 sulfur cluster binding	Unknown
Brd8	99A1-99A1	3R:25049213..25051998	Unknown	negative regulation of gene expression
CG14507	99A1-99A1	3R:25051926..25053447	phospholipase A2 activity	lipid catabolic process; phospholipid metabolic process
CG15817	99A1-99A1	3R:25054883..25061024	ubiquitin thiolesterase activity	ubiquitin-dependent protein catabolic process
CG11951	99A1-99A1	3R:25061039..25064426	aminopeptidase activity	proteolysis

CG31427	99A1-99A1	3R:25064552..25066085	metallopeptidase activity; zinc ion binding	proteolysis
CG31445	99A1-99A4	3R:25066302..25070549	aminopeptidase activity	proteolysis
SP1029	99A4-99A5	3R:25070680..25076167	aminopeptidase activity	lateral inhibition

Curriculum Vitae

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