

# Evaluating proximate causes of longevity in ant queens by RNA-sequencing



DISSERTATION ZUR ERLANGUNG DES  
DOKTORGRADES DER NATURWISSENSCHAFTEN (DR. RER. NAT.)  
DER FAKULTÄT FÜR BIOLOGIE UND VORKLINISCHE MEDIZIN  
DER UNIVERSITÄT REGENSBURG

vorgelegt von  
Katharina von Wyschetzki

aus  
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# Chapter 1

## General introduction

### 1.1 What is aging?

The diversity on Earth is reflected in an enormous variation of life forms that range from primitive cells via multicellular organisms with specialized organs to complex societies, which are based on the division of labor. Evolutionary trends towards an increase in hierarchy (Bourke 2011) and morphological complexity (Carroll 2001) of biological systems are generally observed. Basically, such directional changes determine phylogenesis, which is the historical evolution of traits. However, these developments do not always provide a satisfactory explanation for differences and similarities between taxa. An important trait that appears to be relatively plastic across the animal kingdom is lifespan. Even considering exclusively sexually reproducing animals with determinate growth, the length of an organism's life can differ by a factor of 10–100 among species with similar bodyplans. For example, some parasitic nematodes live up to 15 years, whereas soil nematodes live for only a few months; lifespans of fish vary from several months (turquoise killifish) to more than 150 years (lake sturgeon); rodents are usually short-lived with the exception of the naked mole-rat that survives several decades in captivity (Finch 1990). This suggests that other principles than phylogenetic relatedness underlie the pattern of variation.

Why some species live longer than others, and why some people age faster than their companions, has been on mankind's mind long before the emergence of the modern life sciences. Aristotle already pointed out in his work 'On longevity and the shortness of life' (350BC) that "the larger live longer than the smaller", "salacious animals and those abounding in seed age quickly" and "males subject to great toil are short-lived and age more quickly owing to the labour". His observations on the relationships between lifespan and body size, energy expenditure and sexual activity may sound too simplistic, but exactly these correlations serve nowadays as a basis for the most comprehensive, broadly accepted and empirically supported theories on aging.

Comparing individuals of different ages illustrates that aging is a gradual process, which is characterized by an increase in the probability to die and a decrease in vitality (Medawar 1952), measurable on the physiological level through a progressive functional decline. Therefore, aging is often synonymous with 'senescence', although the term aging can also be used in a neutral sense. The general concept of senescence is that damage accumulates with age causing an impairment of body functions and an enhanced susceptibility to certain diseases (see Kirkwood 2005; López-Otín *et al.* 2013). The initial notion that the deterioration of the body is caused by its sole 'wear and tear', which presumably originated in the time of industrial revolution (see Speakman *et al.* 2002; Speakman 2005), has been increasingly extended in the last

decades by experimental findings about regulatory mechanisms involving gene networks.

The free radical theory of aging provided a first mechanistic, damage-based explanation for senescence (Harman 1956). Free oxygen radicals and oxidants, known as ROS (= reactive oxygen species), are toxic by-products of basic reactions in cells, mainly of aerobic respiration in mitochondria. ROS are thought to cause random damage to macromolecules, in particular proteins, lipids and DNA, hence linking energy metabolism with aging (e. g. Finkel & Holbrook 2000). However, it remains controversial if the pace of aging is correlated with the rate of energy metabolism (Austad 2010). It has been argued that the reason for the positive correlation between longevity and body size, first described by Aristotle, might be a lower mass-specific metabolic rate of larger mammals (Speakman 2005; Hulbert *et al.* 2007). Conflicting results were produced by an experimental intervention that extends lifespan in nearly all model organisms: caloric restriction does not decrease metabolic rate (Hulbert *et al.* 2004; Faulks *et al.* 2006), but reduces oxidative damage (Masoro 2000; Gredilla & Barja 2005). What further complicates the understanding of the connection between aging and metabolic activity is the presence of endogenous antioxidant defenses and mechanisms to repair or remove damaged macromolecules (reviewed by Beckman & Ames 1998; Finkel & Holbrook 2000). The oxidative stress theory predicts that longer-lived species either produce less ROS than shorter-lived ones, or possess a better protection against damage through antioxidant enzymes (e. g. superoxide dismutase, catalase, glutathione peroxidase), free radical scavengers, repair mechanisms or resistant cell membranes (Hulbert 2005).

If organisms are equipped with machineries to counteract damage accumulation, why do some species age faster than others? Purely mechanistic theories do not provide a satisfying answer to this question, but an explanation can be found in the framework of life history theory. Its central theme is that life history traits, such as body size, reproductive effort or parental survival, are costly due to the scarcity of resources. For this reason, fitness traits cannot be optimized all at once, leading to trade-offs between them (Reznick 1985; Stearns 1989). The model of 'r- and K-selection' distinguishes two types of life history tactics (Pianka 1970; Stearns 1976). Fast development, small body size, early reproduction, semelparity, numerous offspring and short lifespan are favored by r-selection, corresponding to the maximization of reproductive rate. Slow maturation, larger body size, delayed reproduction, iteroparity, few offspring and long lifespan are favored by K-selection, which is equivalent to the maximization of competitive ability (Stearns 1976). Insects are relatively r-selected and vertebrates relatively K-selected (with several exceptions, as for example rodents and amphibians; see Pianka 1970). Kirkwood (1977) combined the concept of life-history trade-offs with the idea of age-dependent damage accumulation to one evolutionary explanation for aging. The disposable soma theory is based on an optimal resource allocation of metabolic resource between somatic maintenance and reproduction (Kirkwood & Austad 2000). Somatic maintenance, which comprises DNA repair, antioxidants, stress response, the immune system and tumor suppression (Kirkwood 1996), is costly and



can only be optimized at a disadvantage to other energy-demanding processes, as for example the production of offspring. Thus, evolution might favor genes that increase early reproductive success, but reduce later survival. This idea that genes which have beneficial effects on fitness early in life would negatively affect fitness at old ages, had already been taken up by Williams (1957) before genes with such pleiotropic functions were discovered.

Since the discovery of the *Caenorhabditis elegans* mutant *age-1* in the late 1980s (Friedman & Johnson 1988), there is growing evidence that the evolutionary conserved insulin/IGF signaling (IIS) pathway has antagonistic effects on fecundity and longevity (Partridge *et al.* 2005; Flatt 2011; Partridge *et al.* 2011, and references therein). IIS mediates the adjustment of energy-demanding processes, such as growth (Brogiolo *et al.* 2001), reproduction (Burks *et al.* 2000) and immune response (DiAngelo *et al.* 2009), to nutrient status. Apart from nutrient availability, also stress signals are communicated to the IIS network, allowing the animal to adapt to environmental challenges. The Toll and JNK pathways, which are both involved in innate immunity, have been shown to antagonize IIS by direct molecular interactions, leading to an impairment of growth and nutrient storage (Wang *et al.* 2005; DiAngelo *et al.* 2009). The longevity-promoting effects of these signaling pathways are thought to be caused by the enhanced expression of protective genes (Wang *et al.* 2003). This molecular antagonism between growth and stress tolerance/longevity agrees with the traditional Y model of resource allocation (Zera & Harshman 2001; Figure 1.1). Similar signaling cascades involving IIS and downstream hormones, as for example juvenile hormone in insects, might also be the underlying mechanism of the fecundity/longevity trade-off (Harshman & Zera 2007). However, the Y model is more difficult to apply in this context, because endocrine signals from gonadal tissue can directly repress longevity without altering resource allocation (Tatar *et al.* 2003; Harshman & Zera 2007; Kenyon 2010).

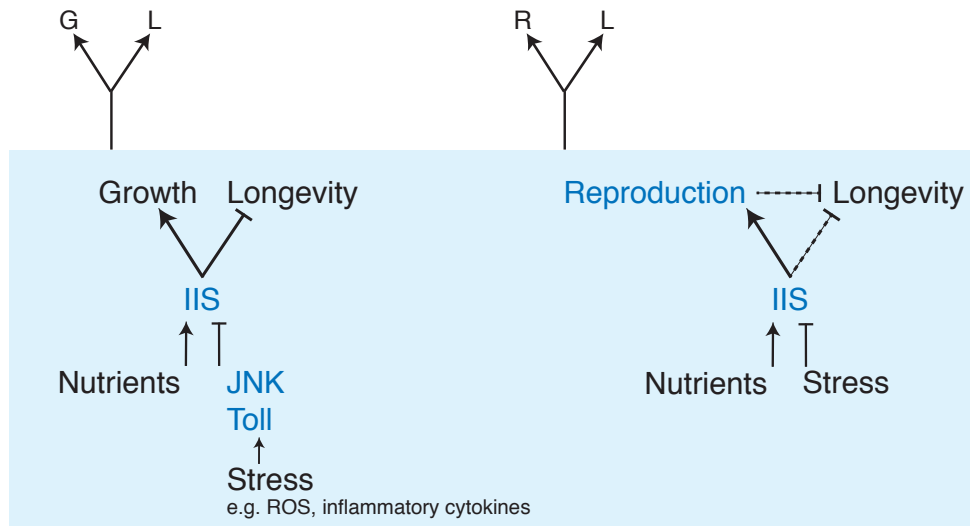


Figure 1.1: Models for the mechanistic basis of the growth/longevity (left) and fecundity/longevity (right) trade-offs integrating Y models for resource allocation (top), and activating and inhibitory links of insulin/IGF signaling. G, growth; L, longevity; R, reproduction; IIS, insulin/IGF signaling; ROS, reactive oxygen species.

The discovery of the role of endocrine systems in the regulation of lifespan has given rise to the notion that aging is a genetically controlled process that could be programmed. The term 'programed' can be misleading and is probably not appropriate to use with respect to aging. Senescence is not understood as a product of evolution in the sense of serving a purpose, even if some authors tend to draw such conclusions (Longo *et al.* 2005). The majority of evolutionary biologists consider senescence not as a trait favored by selection, but rather as a byproduct of the selection for reproduction (Austad 2004).

Even though many candidate genes have now been identified, it is still puzzling which molecular changes induce the gradual decay during the natural course of aging in most mammals, and which lead to the rapid senescence, for example in short-lived insects and semelparous species. Recent studies that measured genome-wide gene expression and DNA methylation at different time points throughout life revealed parallels between development and aging (de Magalhães 2012). Therefore, the search for a 'pacemaker' remains appealing.

## 1.2 (Co-) Evolution of eusociality and longevity

Studies addressing putative universal mechanisms of aging are traditionally performed with rapidly aging, short-lived animals. Gradual (e. g. in humans and rodents) or rapid senescence (e. g. in flies and nematodes; classification according to Finch 1990) might be common, but it is important to bear in mind that certain organisms do not show an age-related increase in mortality (Vaupel *et al.* 2004; Jones *et al.* 2014). The phenomenon of negligible senescence is not restricted to long-lived species with

indeterminate growth, as for example sea anemones and trees; it can also be found among colony-forming eusocial animals.

Species which are classified as 'eusocial' show a reproductive division of labor, cooperative brood care, and an overlap of parental and off-spring generations (Wilson 1971). Hence, a prominent feature of eusociality is the presence of altruistic individuals that gain indirect fitness by raising the offspring of close relatives (Hamilton 1964). In facultative eusocial animals (Crespi & Yanega 1995), individuals are not irreversibly confined to the reproductive or the non-reproductive helper caste, which makes it difficult to distinguish them by definition from animals regarded as 'cooperative breeders'. According to the broad definition after Bourke (2011), eusociality evolved several times independently in insects (termites, ambrosia beetle, aphids, wasps, bees and ants), crustaceans (social shrimps) and rodents (naked and Damaraland mole-rat). Other social animals, which are not strictly classified as eusocial, are cooperatively breeding vertebrates and spiders, and colonial marine invertebrates (e. g. corals and sea anemones).

A thorough investigation of the association between sociality and lifespan throughout all metazoans has possibly been hampered by the lack of detailed data on most species, but there is now profound empirical evidence that social lifestyle promotes longevity (Keller & Genoud 1997; Carey & Judge 2001; Healy 2015). Both low extrinsic mortality risk and help by workers are factors that favored the extension of lifespan in at least three clades of eusocial insects: the female reproductives, which are often referred to as queens, of honeybees, ants and termites, have extraordinary lifespans in comparison to solitary insects (Keller & Genoud 1997; Keller 1998; Carey 2001a; b). The degree of correlation between social behavior and longevity in mammals depends on the definition of sociality in this taxon (Lukas & Clutton-Brock 2012; Healy 2015), but the long lifespans of African mole-rats in general, and of the eusocial naked mole-rat *Heterocephalus glaber* in particular, indicates a strong association of these two traits.

The queens of eusocial insects and mammals belong to the category of organisms in which dysfunctional changes have so far eluded from detection (Finch 1990; Buffenstein 2008). Their long life expectancy, which can be up to 30 years (Keller 1998; Buffenstein & Jarvis 2002), makes it difficult to collect demographic data, as for example age-specific survival. For most species, only records for maximum lifespan exist. Exceptions are queens of the ant genus *Cardiocondyla*. Several studies conducted in the last years showed that they rarely survive more than one year even under optimal laboratory conditions (Schrempf *et al.* 2005; Heinze *et al.* 2013; Fuessl *et al.* 2015). The linear survival curves of *Cardiocondyla* queens from reproductive maturity onwards (Schrempf *et al.* 2005; Schrempf & Heinze 2008; Schrempf *et al.* 2011; Fuessl *et al.* 2015) are an indication for the lack of an acceleration of age-specific mortality and consequently for the lack of senescence as it is currently defined (Finch 1990; Jones *et al.* 2014). The absence of a decline in fecundity also points to negligible senescence in queens of eusocial animals (Buffenstein 2008; Heinze & Schrempf 2012). The well-studied naked mole-rat generally shows no typical signs of age-related

deteriorations in physiological or biochemical function (Buffenstein 2008). Comparable data on age-related changes is not available for insect queens. It is known that their behavior and physiology change significantly during the transition from a virgin to a mature, egg-laying queen (Fahrenholz *et al.* 1992; Fahrbach *et al.* 1995a; Julian & Gronenberg 2002; Groh *et al.* 2006; Bernadou & Heinze 2013) and these adaptive processes might persist throughout life.

### 1.3 Eusociality and the reversal of the fecundity/longevity trade-off

Social insect queens are difficult to classify with regard to the r- and K-selection continuum. They appear to be selected for high reproductive output (some ant species lay up to a million of eggs per year), long lifespan and relatively late onset of sexual reproduction, as the first sexuals are only produced after an ergonomic stage in which a stabile workforce has been build up (Hölldobler & Wilson 1990). Furthermore, the disposable soma theory predicts that the female workers, which do not have to invest resources into the production of eggs, should live longer than the reproductive females. This seems not to be the case because in various ants, termites, bees and *Fukomys* mole-rats the worker caste lives considerably shorter than the queen caste (Hölldobler & Wilson 1990; Carey 2001a; Dammann & Burda 2006; Dammann *et al.* 2011; Schmidt *et al.* 2013). This observation contradicts the fecundity/longevity trade-off on the level of the population or colony, but it makes evolutionary sense regarding the workers as the disposable soma which can be regenerated and the queen as the germ line of one colonial superorganism. Fascinatingly, the release of the reproductive female from the costs of reproduction on the colony level seems to have been translated into a reversal of the negative association between fecundity and longevity on the individual level. Experiments in the three ant genera *Cardiocondyla*, *Platythyrea* and *Diacamma* revealed a positive association between lifespan and reproductive success (Tsuji *et al.* 1996; Hartmann & Heinze 2003; Heinze *et al.* 2013; Kramer *et al.* 2015). The reversal was also detected in annual bumblebee queens (Lopez-Vaamonde *et al.* 2009) and might be a universal feature of social Hymenoptera and termites. The reason for this exceptional relationship is unknown, but the fact that isolated *Cardiocondyla obscurior* queens only live longer than workers if they start to lay eggs (Rueppell *et al.* 2015) strongly supports a causal link. Finding the mechanistic basis for this phenomenon is a challenge because the molecular interactions that cause the trade-off in solitary species are not sufficiently resolved (see Figure 1.1).

## 1.4 Eusociality and female-male coevolution

The assumed positive effect of reproduction in eusocial animals is not the only determinant of queen lifespan. With the exception of some clonal species, as for example the ant *Platythyrea punctata* (Bernadou *et al.* 2015), females of most eusocial animals mate with one or several males to reach their full reproductive potential. Since the early experiments by Partridge *et al.* (1986; 1987), it is known that insect males have an effect on female survival that cannot be explained by the mating-induced boost in fecundity. In the model organism *Drosophila melanogaster*, survival costs of mating are attributable to various manipulative accessory gland proteins, which are transferred in the seminal fluid during copulation. These substances have a significant impact on female physiology by preventing remating, eliminating competitive sperm, and increasing short-term fecundity (Chen *et al.* 1988; Herndon & Wolfner 1995; Lung *et al.* 2002).

To disentangle the effects of reproduction and mating on survival in queens of the species *C. obscurior*, the lifespans of three types of queens with varying mating and reproductive status were compared: 1) mated queens with high egg-laying rate; 2) sham-mated queens with low egg-laying rate, which were mated with a sterilized male to prevent fertilization of eggs, and 3) virgin queens with low egg-laying rate (Schrempf *et al.* 2005). As sham-mated queens lived as long as mated queens, despite having a reduced fecundity as the shorter-lived virgin queens, the conclusion could be drawn that mating alone prolongs queen lifespan. More recent data which shows a positive linear correlation of individual lifespan and egg-laying rate (Heinze *et al.* 2013; Kramer *et al.* 2015) suggests that a combination of mating- and fecundity-driven factors extends lifespan in *Cardiocondyla* queens. The sample size of sham-mated queens in the early experiment by Schrempf *et al.* (2005) was comparably low ( $n = 18$ ) and might have not revealed the full spectrum of fitness differences.

A positive effect of mating is rare, but not surprising in eusocial organisms because males are expected to benefit from a long lifespan of their mate. In contrast to females of solitary insects, virgin queens usually mate with one or few males early in life and never remate (Hughes *et al.* 2008). This stable pair bond and the late onset of reproduction should generally prevent the evolution of manipulative strategies that cause harmful side effects to the female. It has been suggested that analogous to the accessory gland proteins in solitary insects, seminal substances of eusocial insects mediate beneficial effects (Schrempf & Heinze 2008).

Nevertheless, male-male competition also exists in eusocial insects, especially in species in which queens mate multiply (Baer 2014). The accessory gland secretions of the highly polyandrous leafcutter ants and honeybees enhance the survival of their own (Boer *et al.* 2009; 2010), but reduce the survival of alien sperm. The fluids of the sperm-storage organ in *Atta* queens counteract this incapacitation indicating negative side effects on female fitness (Boer *et al.* 2010).

The degree of cooperation and conflict between the sexes might strongly depend on the species' mating system. Sexual cooperation is expected to be maximal when

females and males coevolve and fully agree with each other on their reproductive interests. Such a monogamous relationship can be found in the ant genera *Hypoponera* (Yamauchi *et al.* 1996) and *Cardiocondyla* (Kinomura & Yamauchi 1987; Stuart *et al.* 1987; Heinze *et al.* 1998), in which wingless males stay in the mother colony and fight for the control over all virgin queens. In this situation, a newly emerged queen only mates once with a closely related male, which has killed all his rivals before. Coadaptation may be less pronounced if males disperse, as in most eusocial insects including the winged males of some *Cardiocondyla* ants. The species *C. obscurior* possesses both wingless fighter males and winged disperser males and therefore provides a unique opportunity to test this hypothesis.

### 1.5 A tiny ant and computational biology offer new opportunities to study the regulation of longevity

Eusocial animals are still exotic, but attractive model organisms, and more and more used in the field of biogerontological research. An exploration of the proximate causes of slowed aging in eusocial insect queens has possibly been initiated by a study that demonstrated the enormous quantitative lifespan difference between solitary and social insects nearly 20 years ago (Keller & Genoud 1997). Since then, potential mechanisms have often been discussed (Jemielity *et al.* 2005; Keller & Jemielity 2006; Heinze & Schrempf 2008; Remolina & Hughes 2008; Lucas & Keller 2014), but mostly remained hypothetical because unequivocal evidence has not been found yet. A pitfall of previous studies is the assumption that a physiological factor that differs between queens and workers, or between reproductives and non-reproductives of the same species, is involved in the regulation of lifespan. Unfortunately, it is difficult to draw conclusions from the results of those former studies. For example, it is not clear if the expression of antioxidant enzymes is lower in queens than in workers (Parker *et al.* 2004; Corona *et al.* 2005; Schneider *et al.* 2011) because queens produce less oxidative damage, need less protection against oxidative damage, use other radical scavengers or because oxidative damage is not associated with senescence. More progress has been achieved in understanding senescence in the worker caste of the honeybee *Apis mellifera* by studying individuals of different age and social task (Seehuus *et al.* 2006; Rascón *et al.* 2012; Seehuus *et al.* 2013; Münch *et al.* 2013; Paoli *et al.* 2014). Such an approach has so far been hampered in queens by the difficulty to monitor their survival and their lifetime reproductive behavior, and to perform experiments under standardized conditions on a reasonable number of replicates. A species that is relatively short-lived in comparison to other eusocial insects in the field of biogerontology (Table 1.1), and therefore offers more opportunities to monitor and manipulate aged individuals, is the ant species *Cardiocondyla obscurior* (Wheeler, 1929).

Table 1.1: Overview of eusocial insects which are used for laboratory studies addressing the mechanistic basis of queen longevity. Maximum-recorded lifespan of queens and corresponding publications are listed.

Species	Lifespan (max.)	Studies Topic	References
<i>Apis mellifera</i>	8 yrs	Oxidative stress	Corona <i>et al.</i> 2005 Haddad <i>et al.</i> 2007
<i>Lasius niger</i>	29 yrs	Endocrine control Oxidative stress Telomere length	Corona <i>et al.</i> 2007 Parker <i>et al.</i> 2004 Jemielity <i>et al.</i> 2007
<i>Harpegnathos saltator</i>	> 3 yrs	Oxidative stress	Schneider <i>et al.</i> 2011
<i>Cardiocondyla obscurior</i>	1 yr	-	

Besides the relatively low life expectancy and the short generation time (around 4 weeks), this species fulfills additional requirements important for controlled laboratory studies. It forms small colonies in the field consisting of 20 to 30 workers on average, several reproductive queens, and a single wingless male (Kinomura & Yamauchi 1987; Heinze & Delabie 2005). Its original natural habitat is unknown. Single nests have presumably been transferred from Southeast Asia to other subtropical regions by human activities (Heinze *et al.* 2006). Nowadays it can be found in anthropogenic habitats around the globe, as for example in park trees in East Asia (Figure 1.2) and in coconut and lemon plantations in South America. Because the small colonies inhabit cavities in bark, dead twigs, and enrolled leaves, whole nests do not have to be excavated and can easily be collected. Like all other *Cardiocondyla* species, *C. obscurior* is a tiny little ant (1–2 mm), which can be kept in Petri dishes or plastic boxes with a plaster floor.

A tremendous advantage over other eusocial insects is that sexuals are produced year-round and mate in the nest (Figure 1.2). Its general flexibility regarding colony characteristics facilitates the creation of experimental colonies from larger stock colonies with varying numbers of queens and workers. This opens the possibility to investigate the effect of single mating (Schrempf *et al.* 2005), polygyny (Schrempf *et al.* 2011), mating with alternative male morphs (Heinze & Schrempf 2008), and other factors on queen lifespan and lifetime fecundity. As mentioned above, colonies usually contain one wingless male, also referred to as ergatoid (worker-like) male, which monopolizes mating with all virgin queens by killing his rivals (Kinomura & Yamauchi 1987; Stuart *et al.* 1987). Winged disperser males, which are produced in most other eusocial insects and in some *Cardiocondyla* species including *C. obscurior*, have never been observed in the field, but develop from unfertilized eggs under specific conditions in the lab (Cremer & Heinze 2003).

Because of its short life cycle, this species is well suited to study the interplay among aging, reproduction and mating using molecular and in-silico techniques. Recent advances in the field of DNA sequencing are now enabling the application of functional genomic studies to non-model organisms. One of the latest high throughput methods is RNA-Seq, which allows the determination and quantification of all mRNA molecules of a given organism, a specific tissue or even a single cell (Wang *et al.* 2009; Wilhelm &

Landry 2009). Transcriptional profiling is commonly used to monitor the activity of genes and to generate hypotheses about physiological differences between varying natural or experimental conditions. Genome-wide gene expression analysis is extensively applied to investigations of aging, for example to identify general age-related changes, biomarkers for aging and modifications caused by life-prolonging interventions like caloric restriction (reviewed in de Magalhães *et al.* 2010; Wieser *et al.* 2011).

Whereas the preparation of the sequencing library and the sequencing itself is relatively straightforward and basically the same for model and non-model organisms, the strategy for the subsequent bioinformatic analysis depends on the species' available resources. Because the genome and transcriptome of *C. obscurior* was recently sequenced and electronically annotated (Schrader *et al.* 2014), next-generation sequencing reads can be mapped and counted against the 17552 predicted gene models. Further computational methods can then be used to predict the functions of genes and to test for a functional enrichment in a specific set of genes.



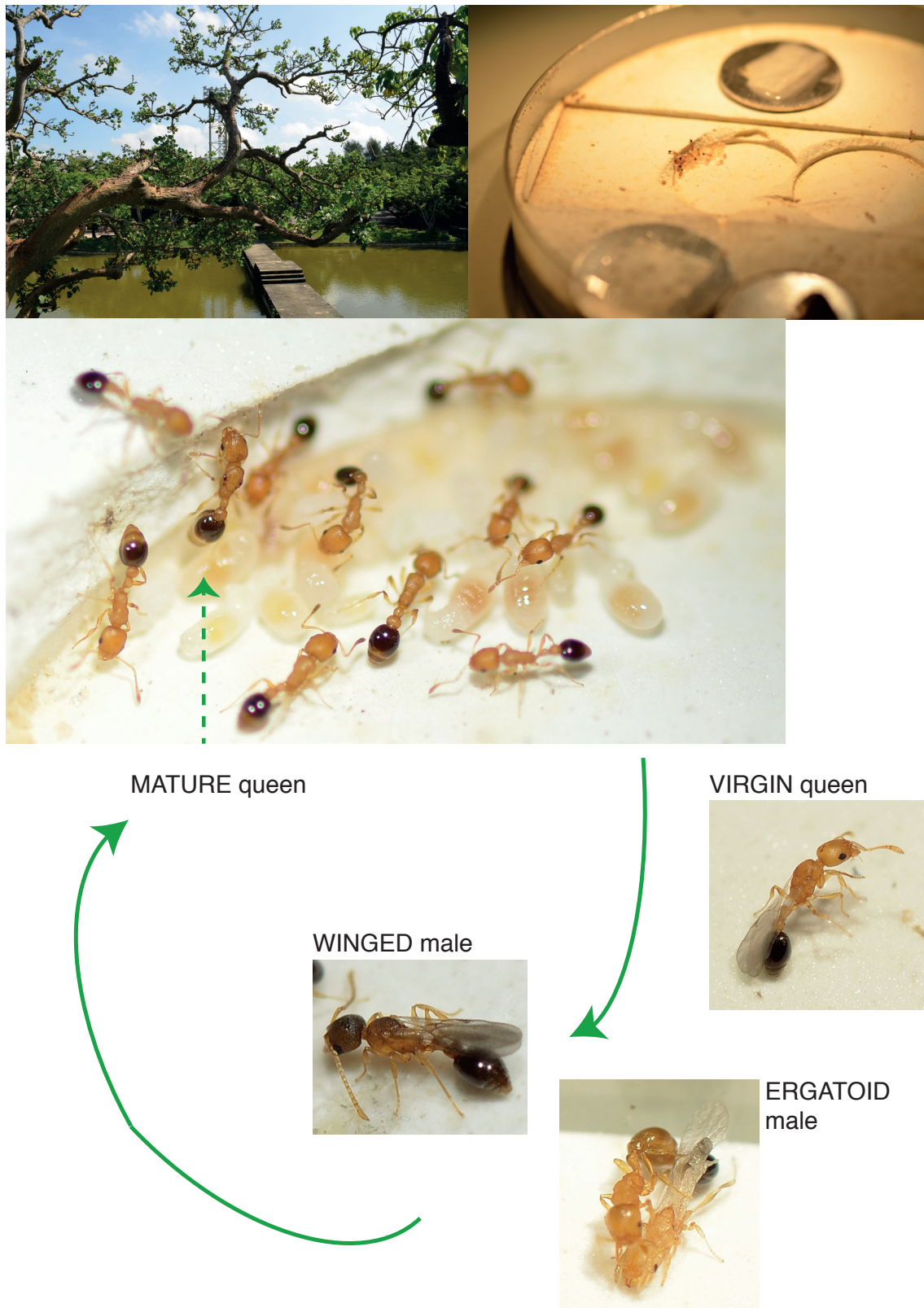


Figure 1.2: Photographs of the natural habitat (top left), a laboratory colony (top right and middle) and the two male phenotypes of the ant *Cardiocondyla obscurior* (bottom). On the Japanese island Okinawa, colonies can typically be found in coral trees in the Oonoyama-park in Naha. The small colonies are kept in Petri dishes and fed ad libitum with chopped cockroaches and diluted honey 2–3 times per week. Young queens lose their wings after they mated with a territorial ergatoid or a winged disperser male, and start to lay eggs.

## 1.6 Aims of this thesis

The four different experiments carried out in the framework of this thesis were initiated to improve the understanding of the molecular causes for two phenomena: 1) the reversal of the usually observed negative association between reproduction and lifespan, and 2) the positive effect of mating on lifespan.

Previous comparisons of reproductives and non-reproductives regarding antioxidant enzyme levels, membrane composition, vitellogenin expression, hormone titres, and telomere length, yielded interesting results. For an evaluation of the factors that really play a role in the regulation of queen longevity, it is important to determine the progression of age-related changes within this caste. Transcriptomic comparisons of young and old individuals have been carried out multiple times in the model organism *Drosophila melanogaster* (Zou *et al.* 2000; Pletcher *et al.* 2002; Landis *et al.* 2004; Girardot *et al.* 2006; Zhan *et al.* 2007; Doroszuk *et al.* 2012; Zhou *et al.* 2014), but not in eusocial insects before. For this reason, the whole body transcriptomes of 4-week-old and 18-week-old *C. obscurior* queens, which had mated with a single ergatoid male shortly after eclosing, were contrasted (chapter 2). Analogous to cross-species comparisons of genome-wide data among model organisms (McCarroll *et al.* 2004; de Magalhães *et al.* 2009), the study aimed to identify commonalities and differences between solitary and social insect females.

As the trade-off between fecundity and longevity seems to be reversed in ants and other eusocial animals, the question arises if queens do not have to compensate for the costs of reproduction. The second study dealt with this topic by challenging *C. obscurior* queens through amputating both middle legs and by measuring the effect of this injury on egg-laying rate and the expression of immune, reproductive, and metabolic genes (chapter 3).

Building on the knowledge that mating is beneficial in *C. obscurior* queens (Schrempf *et al.* 2005) and that the extent of this effect depends on the type of male (Schrempf & Heinze 2008), three experiments were performed to better understand the physiological link between mating and longevity, and the impact of female-male coevolution. The idea was to disentangle the effects of mating and reproduction on lifespan by comparing the transcriptomes of 18-weeks-old queens, which had either mated with a fertile male, a sterile male, or no male (chapter 2). Furthermore, it was tested if differences in the degree of cooperation between the sexes through varying male phenotype (chapter 4) and varying male origin (chapter 5) are reflected in the transcriptome.

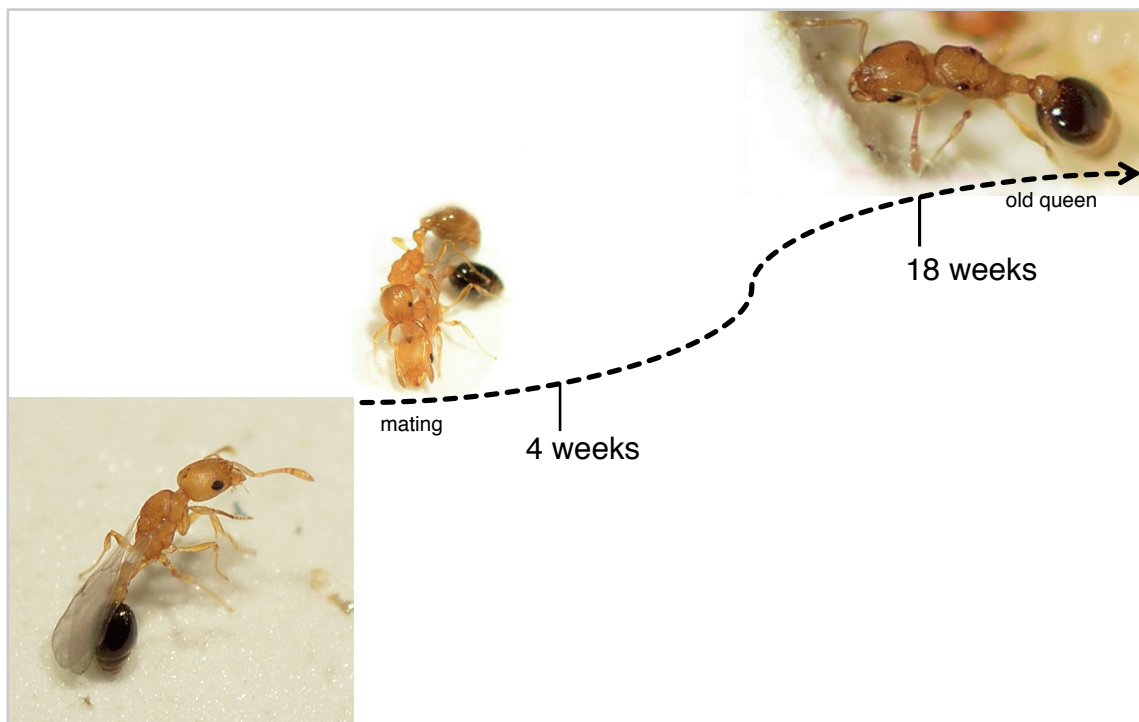
## Chapter 2

# Transcriptomic signatures mirror the lack of the fecundity/longevity trade-off in ant queens\*

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Transition from a virgin (left) to a mated, egg-laying *Cardiocondyla obscurior* queen (right).

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## Abstract

Life history theory predicts a trade-off between reproductive investment and self-maintenance. The negative association between fertility and longevity found throughout multicellular organisms supports this prediction. As an important exception, the reproductives of many eusocial insects (ants, bees, termites) are simultaneously very long-lived and highly fertile. Here, we examine the proximate basis for this exceptional relationship by comparing whole body transcriptomes of differently aged queens of the ant *Cardiocondyla obscurior*. We show that the sets of genes differentially expressed with age significantly overlap with age-related expression changes previously found in female *Drosophila melanogaster*. We identified several developmental processes, such as the generation of neurons, as common signatures of aging. More generally, however, gene expression in ant queens and flies changes with age mainly in opposite directions. In contrast to flies, reproduction-associated genes were upregulated and genes associated with metabolic processes and muscle contraction were downregulated in old relative to young ant queens. Furthermore, we searched for putative *C. obscurior* longevity candidates associated with the previously reported lifespan-prolonging effect of mating by comparing the transcriptomes of queens that differed in mating and reproductive status. We found 21 genes, including the putative aging candidate *NLaz* (an insect homolog of *APOD*), which were consistently more highly expressed in short-lived, unmated queens than in long-lived, mated queens. Our study provides clear evidence that the alternative regulation of conserved molecular pathways that mediate the interplay among mating, egg laying, and aging underlies the lack of the fecundity/longevity trade-off in ant queens.

**Keywords:** fecundity/longevity trade-off, transcriptome, aging, mating, social insect, RNA-Seq

## 2.1 Introduction

Why organisms age and die and why they do so at different rates are among the most fundamental and least understood phenomena in biology. Of the various mechanistic and evolutionary explanations for aging and death (e.g., Rose 1990; Hughes & Reynolds 2005), those that involve a trade-off between fecundity and longevity have gained considerable empirical support. Throughout multicellular organisms, including volvocine algae, *Drosophila*, *Caenorhabditis* and human beings, increased investment in early and current reproduction negatively affects longevity (Stearns 1989; Westendorp & Kirkwood 1998; Michod *et al.* 2006; Flatt 2011; Tabatabaie *et al.* 2011). On the molecular level, there is evidence that this trade-off might be mediated by Insulin/insulin-like growth factor signaling (IIS) and downstream endocrine signals, for example, juvenile hormone (JH) in insects (Flatt *et al.* 2005; Flatt & Kawecki 2007). Mutations in the IIS pathway were shown to have antagonistic pleiotropic effects on lifespan and reproduction in *Drosophila melanogaster*, *Caenorhabditis elegans*, and mice (Tatar *et al.* 2001; Partridge *et al.* 2005).

Perennial eusocial insects, such as termites, ants, and many bees, and eusocial *Fukomys* mole-rats, are a striking exception: dependent on their environment, individuals may grow into long-lived reproductives or short-lived, non-reproductive workers (Keller & Genoud 1997; Keller 1998; Jemielity *et al.* 2005; Heinze & Schrempf 2008; Schmidt *et al.* 2013). This suggests that, on the population level, the trade-off between fecundity and longevity is reversed in these organisms. In addition, mating might not be that detrimental for the female reproductives (queens) of social insects as for solitary insects (Partridge *et al.* 1987; Trevitt & Partridge 1991). The short mating period early in life and the storage of sperm by queens result in a lifelong pair bond of males and females. This predicts that males benefit from increasing the lifespan of their female partners, as was already shown in the ant *Cardiocondyla obscurior* (Schrempf *et al.* 2005).

Understanding how reproductives of eusocial animals evade the fecundity/longevity trade-off not only serves to identify idiosyncratic pathways that link mating, fecundity, and lifespan, but might also provide fundamental insight into the evolution of aging in general. Hence, considerable efforts have been made to reveal the physiological, endocrine, and transcriptomic correlates of the different life expectancies of reproductives and non-reproductives (Parker *et al.* 2004; Corona *et al.* 2005; Jemielity *et al.* 2007; Corona *et al.* 2007; Haddad *et al.* 2007; Grozinger *et al.* 2007; Schneider *et al.* 2011). For example, experiments addressing the oxidative stress theory of aging (which considers reactive oxygen species as a cause of aging; e.g., Finkel & Holbrook 2000) consistently showed that antioxidant enzyme gene expression and activity are lower in queens than in workers (Parker *et al.* 2004; Corona *et al.* 2005; Schneider *et al.* 2011). This might be explained either by a lower generation of reactive oxygen species in queens or by the mediation of oxidative stress resistance through other molecules, such as vitellogenin (Vg; Seehuus *et al.* 2006; Havukainen *et al.* 2013). Honeybee queens indeed have a higher titer of vitellogenin, associated with

lower JH titers and lower expression of insulin-like peptide and receptor genes compared with workers (Corona *et al.* 2007). As this observation disagrees with the opposing effects of IIS and JH on lifespan and reproduction in *Drosophila melanogaster* (Flatt *et al.* 2005), it has been suggested that the traditional positive relationships between nutrition and IIS, and between JH and Vg, are reversed in honeybee queens (Corona *et al.* 2007; Remolina & Hughes 2008).

However, comparisons between queens and workers are often confounded because the two female castes typically differ not only in fecundity, but also in developmental, morphological, physiological, and behavioral traits. All of these might affect the tempo of aging and senescence. To disentangle the effects of variation in phenotype, mating status, fecundity, and resource availability on lifespan requires alternative approaches, for example, a comparison among reproductives of different fecundity and longevity.

Here, we used the ant *Cardiocondyla obscurior* as a social insect model to investigate the proximate mechanisms underlying variation in lifespan independent of variation in genotype, development, and morphology. Its colonies are typically inbred because young queens mate in the nest with wingless males and stay there to reproduce (e.g., Heinze & Hölldobler 1993). Queens are relatively short-lived (approximately 6 months), which allows monitoring their total lifespan and lifetime reproductive success (Schrempf *et al.* 2005; Heinze & Schrempf 2012). We used two approaches to investigate the effects of age and mating on gene expression.

First, we compared the transcriptomes of young mated (4-week-old) and old mated (18-week-old) *C. obscurior* queens to identify general signatures of aging. We then compared these data with transcriptomes of female *D. melanogaster* of different age (Pletcher *et al.* 2002; Doroszuk *et al.* 2012).

Second, we contrasted transcriptome data among three different types of 18-week-old queens, which were subjected to different mating regimes known to affect future life expectancy and fecundity: 1) Virgin queens (short average lifespan and low average fecundity, 18.2 weeks and 6.8 eggs per week); 2) mated queens (long lifespan and high fecundity, 26.0 weeks and 20.5 eggs per week), and 3) queens mated to sterilized males (Schrempf *et al.* 2005).

Finally, we investigated whether mating-induced gene expression changes in *C. obscurior* match those previously found in female *D. melanogaster* and honeybees.

Our results reveal for the first time a comprehensive picture of gene expression patterns associated with age, mating, and fecundity in a social insect and indicate that conserved pathways involved with senescence in solitary species may experience a reversal in gene expression patterns. The commonality of aging found between two species with opposite life histories indicates a persistent action of developmental genes later in life.

## 2.2 Materials and methods

### 2.2.1 The study organism

*Cardiocondyla obscurior* is a tropical tramp species (Heinze *et al.* 2006), which nests in cavities of dead twigs and leaves (Seifert 2002). Its successful establishment around the globe through human activities is possible because of several specific life history traits, such as the continuous production of sexuals, the presence of multiple queens per nest, mating in the nest, and colony propagation by budding. These traits also facilitate rearing and maintenance of *Cardiocondyla* colonies in the lab. Its small colonies contain on average 20 female workers, several reproductive queens, and a single wingless male, which monopolizes mating with any newly produced queen by killing younger rival males reared in the colony (Kinomura & Yamauchi 1987; Stuart *et al.* 1987; Heinze & Delabie 2005).

### 2.2.2 Experimental design and sampling

We established 73 experimental colonies from laboratory stock colonies derived from the genome reference population in Bahia, Brazil (Schrader *et al.* 2014). Each nest contained 20 workers, 10 larvae, and a single queen pupa, which was assigned to one of three treatments: Mated (MQ), sham-mated (SQ) and virgin (VQ). MQ colonies were set up with an additional male pupa about to eclose simultaneously with the queen, whereas VQ did not have contact to males. For the SQ treatments, we sterilized the added male prior to its introduction to the nest by exposure to X-rays (120 G;  $2.95 \pm 0.12$  G/min; Schrempf *et al.* 2005). Colonies with males that died within 1 week after irradiation were excluded from further analysis. Sterilized males transfer only unviable sperm and consequently SQ can only produce male offspring from unfertilized eggs (Schrempf *et al.* 2005). From these three treatments (MQ, SQ, and VQ), individuals were sampled after 18 weeks (MQ18, SQ18, and VQ18), corresponding to the age when 50 % of virgin queens had died in a previous experiment (Schrempf *et al.* 2005). In addition, mated queens which were set up as the mated queens described above were sampled after 4 weeks (= MQ4; Schrempf *et al.* 2015) to assess age-related changes under normal circumstances. These young queens started to lay eggs 1 week after emergence and consistently increased their egg-laying rate within the 3 weeks before sampling.

The colonies were reared in Petri dishes with plaster and fed three times per week with chopped cockroaches and diluted honey according to standard protocols *ad libitum*. All eggs were counted and removed twice per week in the first month and subsequently once per week. The number of workers and larvae was standardized by adding or removing individuals to 20 workers and 10 larvae per colony. Developing male and queen pupae were removed to avoid replacement or (additional) mating of the

focal queens. All queens were individually snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until further processing.

We monitored survival and reproductive output of queens. Mating type had no significant effect on the survival of queens until 18 weeks, but mean egg-laying rates differed significantly between all three queen types (details in Appendix 7.1.1).

### 2.2.3 Library preparation and sequencing

Individual queens were transferred into RLT Plus buffer (QIAGEN) and homogenized by using Lysing Matrix Tubes and a FastPrep bead shaker (MP Biomedicals). Subsequently, total RNA was extracted following the RNeasy Plus Micro Kit protocol (QIAGEN). We measured RNA content and quality with an Agilent 2100 Bioanalyzer, which indicated yields of 20–200 ng of total RNA per queen. To obtain sufficient RNA to prepare a sequencing library, whole RNA was amplified after conversion into cDNA (NuGEN Ovation RNA-Seq System V2). After sonic fragmentation, and adapter ligation and incorporation of multiplex barcodes (NuGEN Encore Rapid Library Systems), the 28 samples were randomly distributed across different lanes of a flow cell and sequenced on an Illumina HiSeq1000 platform.

### 2.2.4 RNA-Seq analysis

On average, 24 million 100-bp single reads were generated per sample. Quality of raw reads (phred scores  $> 30$ ) was assessed by FastQC version 0.10.1 (Andrews 2010). Adapter residuals were trimmed with Cutadapt version 1.2.1 (Martin 2011). Using Bowtie2 version 2.1.0 (Langmead & Salzberg 2012) in combination with the splice junction mapper TopHat version 2.0.8 (Trapnell *et al.* 2009), the sequences were mapped with default settings against the *C. obscurior* reference genome Cobs1.4 (Schrader *et al.* 2014; mapping statistics in Appendix 7.1.2). Subsequently, HTSeq-count version 0.5.4 (Anders *et al.* 2015) was used for counting reads. Normalization of raw counts and the tests for differential gene expression were performed with DESeq2 version 1.6.2 (Love *et al.* 2014) in R version 3.1.2 (R Core Team 2014). We tested MQ18 against MQ4 and then contrasted the three treatments MQ18, SQ18 and VQ18. Raw *P* values were adjusted for multiple testing (Benjamini & Hochberg 1995). A principal component analysis was conducted on expression values of the top 500 genes with the highest variance across all samples after variance stabilization in DESeq2. Centroids were added by means of the package Vegan version 2.2-1 (Oksanen *et al.* 2015). Area-proportional Venn diagrams were generated with EulerAPE (Micallef & Rodgers 2014).



### 2.2.5 Functional annotation

We inferred orthology by applying a reciprocal BLASTp [Basic Local Alignment Search Tool] between all 17,552 predicted *C. obscurior* genes and the *D. melanogaster* (dmel-all-translation-r5.56.fasta) and *A. mellifera* (amel\_OGSv1.1\_pep.fa) genomes by means of the BLAST+ toolkit (Camacho *et al.* 2009). This resulted in 6,959 fruit fly and 7,948 honeybee orthologs, corresponding to 68 % and 72 % of the annotated fly or bee genes, respectively. For the remaining genes, the most similar homolog was defined as the best hit of the one-way protein BLAST against the fruit fly or honeybee genome on condition that the *e* value was smaller than  $10^{-05}$ . Functional annotation of genes was obtained by loading all 6,959 genes with reciprocal orthologous relationships to fly genes as background into DAVID (Huang *et al.* 2009). A modified Fisher's exact test was used for testing of enrichment (EASE < 0.05) for Gene Ontology (GO) terms (Ashburner *et al.* 2000) and KEGG pathways (Kanehisa & Goto 2000) in the sets of differentially expressed genes (DEGs) relative to the background. As the lists of DEGs between VQ18 and MQ18 and of the common genes between the contrasts VQ18–MQ18 and VQ18–SQ18 did not reveal a functional enrichment considering orthologs, we repeated the test by including homologs.

### 2.2.6 Cross-species comparisons

We compared our results with similar studies in insects that addressed mating- and aging-related transcriptome changes. We compared our data with two independent studies to determine overlap in age-related changes between *C. obscurior* queens and *D. melanogaster* females: A study of young and old virgin female *D. melanogaster* (90 % vs. 10 % survival) by Doroszuk *et al.* (2012) and a study that compared the transcriptomes of 7- and 23-day-old mated fly females (= aged females with about 65 % survival) from Pletcher *et al.* (2002). We did not identify any other data sets that were suitable to compare age-related transcriptome changes in insect females.

We assessed six further data sets for similarities to our transcriptomic comparisons among mated, sham-mated, and virgin *C. obscurior* queens. Three of these data sets focused on mating-induced changes in gene expression of *D. melanogaster* females (McGraw *et al.* 2004) and *A. mellifera* queens (Kocher *et al.* 2008; 2010). The other three comparative data sets were derived from studies of reproduction- or ovary-status-associated gene expression changes in *A. mellifera* workers (Grozinger *et al.* 2007; Cardoen *et al.* 2011; Wang *et al.* 2012). We excluded another study (Zhou *et al.* 2014) because it did not report on a sufficient number of orthologs of our genes (< 50 %) to allow for a meaningful comparative analysis (see Appendix 7.1.3). We also screened our lists of DEGs for the presence of putative *D. melanogaster* aging candidates retrieved from the GenAge database (Tacutu *et al.* 2013).

The given identifiers were converted to the current gene annotations that we used for determining orthologs (as described above). Normalized log-transformed

expression values from microarrays of two studies on flies (Pletcher *et al.* 2002; Doroszuk *et al.* 2012) were analyzed with limma version 3.22.4 (Ritchie *et al.* 2015). The definition of DEGs was based on a false discovery rate (FDR)  $< 0.05$  for all data sets, only Grozinger *et al.* (2007) applied a 97.5 % confidence level cutoff. As for the functional enrichment analyses, we restricted the comparison to the set of unambiguous orthologs. Up- and downregulated genes were analyzed separately. To perform quantitative comparisons we generated contingency tables containing the number of DEGs found in both studies, the number of DEGs not found in the other study in each case and the number of all remaining genes. A one-sided Fisher's exact test then revealed whether more genes overlapped than expected by chance.

## 2.3 Results

### 2.3.1 Gene expression patterns of all four types of queens

To reveal the effects of age and mating on gene expression, we analyzed transcriptomic data of four types of queens: MQ4 = 4-week-old mated queens, MQ18 = 18-week-old mated queens, SQ18 = 18-week-old sham-mated queens, VQ18 = 18-week-old virgin queens. We sequenced individual queens to account for biological variation across samples and achieved a final sample size of seven (MQ4, MQ18, SQ18) and six (VQ18) replicates. The principal component plot of all samples indicated a separation of MQ4 and VQ18 from all other groups and considerable overlap of SQ18 and MQ18 (Figure 2.1). To analyze age-specific expression, we first compared the transcriptomes of MQ4 and MQ18 and found 783 DEGs (adjusted  $P$  value  $< 0.05$ , Appendix 7.1.4).

To disentangle the effects of mating and reproduction, we contrasted the gene expression profiles of MQ18, SQ18, and VQ18. VQ18 differed from MQ18 in 37 genes and from SQ18 in 350 genes, whereas SQ18 and MQ18 differed in five genes (adjusted  $P$  value  $< 0.05$ , Appendix 7.1.4).

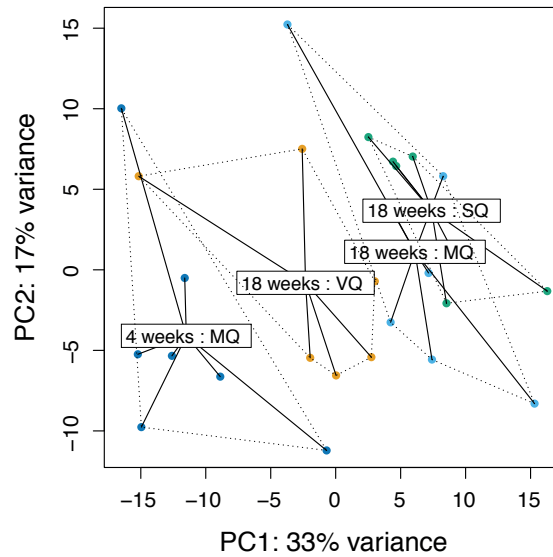


Figure 2.1: Principal component analysis plot of the top 500 genes with highest variance across all samples illustrating variation within and between treatments. Variance stabilizing transformation of expression values was performed prior to analysis. The labels represent the center of mass of each group.

### 2.3.2 Functional annotation of genes differentially expressed with age and reproduction (MQ4 vs. MQ18)

We used the corresponding *D. melanogaster* orthologs of all genes (determined by a reciprocal BLAST) in DAVID to test for a functional enrichment in all lists of DEGs. The 242 genes more highly expressed in MQ18 compared with MQ4 (160 orthologs) revealed an enrichment for GO terms associated with reproduction, which reflects the higher rate of reproduction in older queens (Appendix 7.1.5). Clustering of these 102 GO annotations for biological processes (BP) resulted in five groups, which are best described by the terms cell cycle, cellular component assembly, female germ-line cyst encapsulation, germ cell development, and establishment or maintenance of cell polarity (Table 2.1).

KEGG pathway analysis of the 541 genes more highly expressed in MQ4 (336 orthologs) revealed a significant enrichment in fructose and mannose metabolism ( $P = 0.01$ ), purine metabolism, pentose phosphate pathway, and metabolism of xenobiotics by cytochrome P450 ( $P < 0.05$ ). The 37 significant GO terms also pointed to an association with mainly catabolic and biosynthetic processes. The most significant terms of the three generated clusters were cellular carbohydrate catabolic, organic acid metabolic, and nucleoside monophosphate biosynthetic process. Besides, genes involved in muscle contraction were overrepresented in the set of downregulated genes ( $P = 0.0009$ ).

Table 2.1: Enriched GO terms for BP in aged (MQ18) vs. young mated queens (MQ4). Annotation clusters are listed with their most significant GO term representing the biological meaning of the group. ES, Enrichment Score.

	ES	Representative GO Term	Count	%	P value
Upregulated in MQ18					
Annotation Cluster 1	2.3	Cell cycle	22	15	0.0001
Annotation Cluster 2	2.0	Cellular component assembly	20	13	0.0013
Annotation Cluster 3	2.0	Female germ-line cyst encapsulation	4	3	0.0005
Annotation Cluster 4	1.8	Germ cell development	10	7	0.0042
Annotation Cluster 5	1.7	Establishment or maintenance of cell polarity	7	5	0.0161
Downregulated in MQ18					
Annotation Cluster 1	3.3	Cellular carbohydrate catabolic process	9	3	< 0.0001
Annotation Cluster 2	2.4	Organic acid metabolic process	19	6	0.0025
Annotation Cluster 3	2.3	Nucleoside monophosphate biosynthetic process	7	2	0.0026

### 2.3.3 Functional annotation of genes associated with mating (VQ18 vs. MQ18 and SQ18, SQ18 vs. MQ18)

Neither the 33 genes with higher expression (19 orthologs), nor the 4 genes with lower expression (one ortholog) in VQ18 compared with MQ18 showed a significant functional enrichment. However, when we included eight genes for which homology was established by simple BLAST to *D. melanogaster* ( $e$  value <  $10^{-05}$ ), GO analysis suggested an elevation in carbohydrate metabolic process in VQ18 ( $P = 0.008$ ).

The 93 genes with lower expression in VQ18 compared with SQ18 (74 orthologs) were enriched for the GO terms protein localization in organelle ( $P = 0.004$ ), organelle fission ( $P = 0.022$ ), and 14 related BP. The 257 genes with lower expression in SQ18 (186 orthologs) were enriched for more diverse categories, which were summarized by the following terms: neurological system process, muscle cell development, phototransduction, cyclic nucleotide metabolic process ( $P < 0.001$ ), alcohol catabolic process, and homeostatic process ( $P < 0.01$ , a complete list of enriched terms is provided in Appendix 7.1.5).

An enrichment in nucleobase, nucleoside, nucleotide, and nucleic acid metabolism ( $P = 0.045$ ) was found in the four genes more highly expressed in SQ18 compared with MQ18 (four orthologs).

### 2.3.4 Overlap of age- and mating-associated expression patterns

We determined the overlap of age- and mating-associated expression differences by comparing all four lists of DEGs (Figure 2.2). We found the highest overlap between genes with higher expression in MQ4 relative to MQ18 and genes with higher expression in VQ18 than in MQ18 or SQ18 (Figure 2.2, bottom right). Fewer genes showed the opposite pattern, that is, had lower expression in MQ4 and VQ18 than in MQ18 (top right).

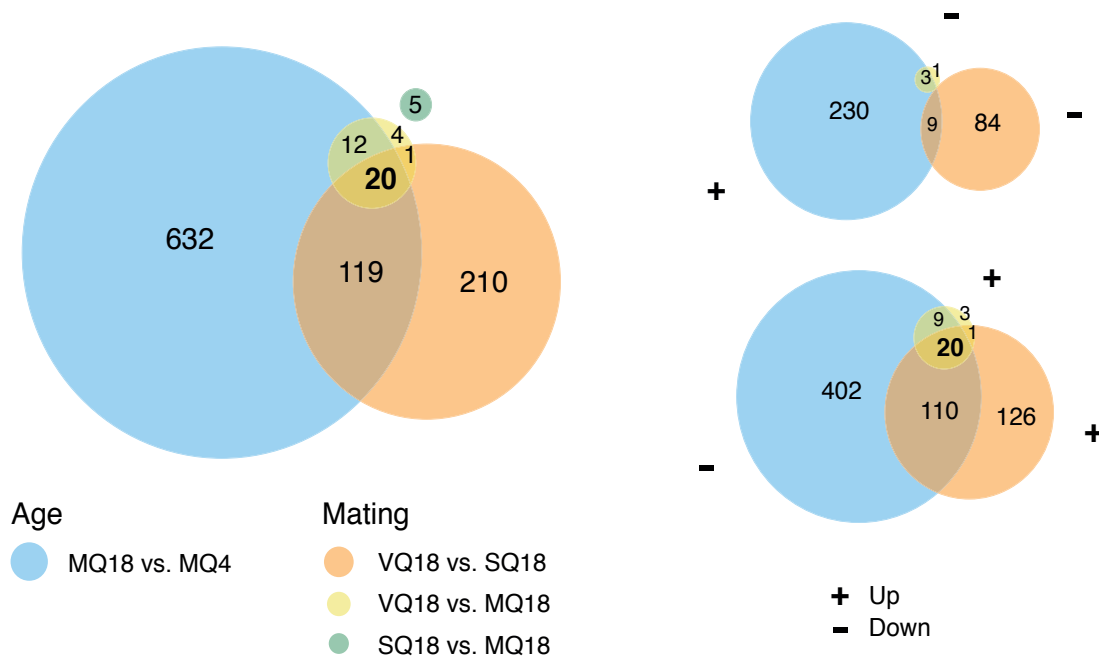


Figure 2.2: Overlap of age- and mating-associated expression patterns. Venn diagrams showing the general overlap between all four comparisons (left) and specifically the overlap of genes upregulated with age in the mated queen type and downregulated in virgin compared to mated or sham-mated queens (top right) as well as vice versa (bottom right).

Twenty-one genes were differentially expressed in both the comparison of VQ18–MQ18 and VQ18–SQ18. They presumably reflect physiological changes as consequence of mating rather than of fertilization. All of them had higher transcript abundances in the shorter-lived phenotype, and all except *PRL-1* were more highly expressed in MQ4 than in MQ18 (Figure 2.3). Thirteen genes have orthologs in *D. melanogaster* (Table 2.2). Including five genes with putative homologs in *D. melanogaster* yielded a significant enrichment in carbohydrate metabolic process ( $P = 0.024$ ). Across all three treatments, expression of these 21 genes showed a minimum at intermediate egg-laying rates instead of a linear relationship (Appendix 7.1.6).

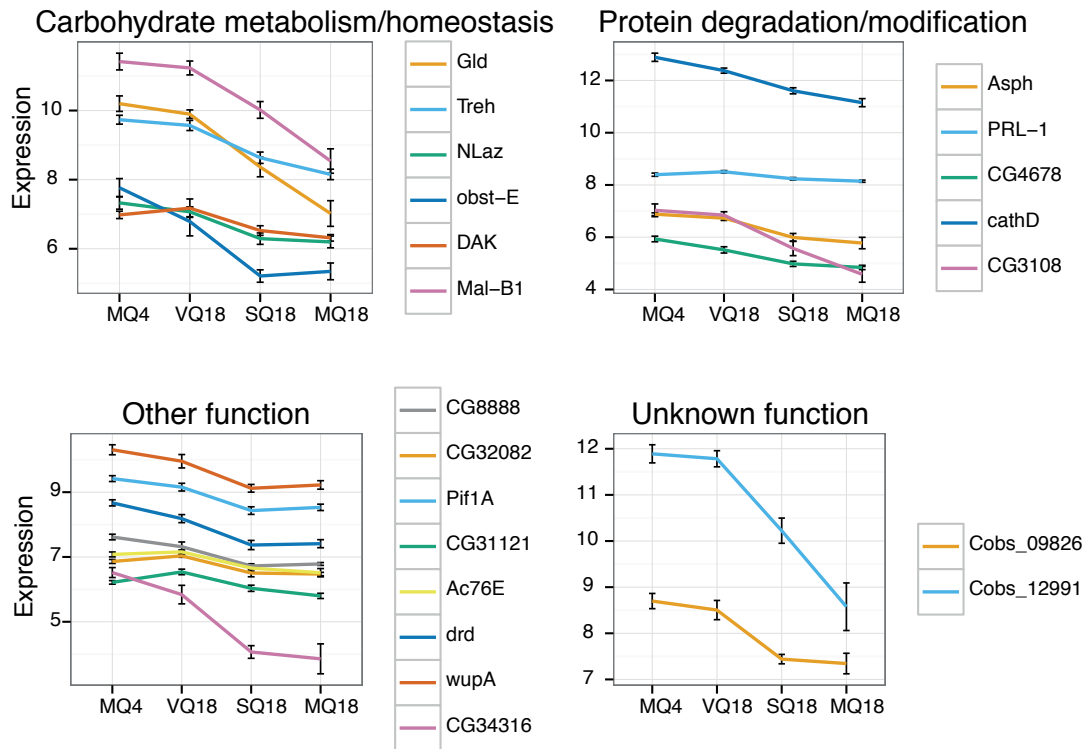


Figure 2.3: Expression of all 21 mating-associated genes across all four conditions. Illustrated are mean and standard errors of  $\log_2$ -values of normalized counts. Genes are named according to *D. melanogaster* orthologs/homologs if present and grouped by functions.

Table 2.2: List of genes downregulated by mating. Corresponding *D. melanogaster* orthologs, human homologs and fold changes of both VQ18-MQ18 and VQ18-SQ18 comparisons are indicated.

Gene ID	Fold change		<i>D. melanogaster</i> (o, ortholog; h, homolog)		
	VQ18 vs. MQ18	VQ18 vs. SQ18	Name	Human homologs	Function
Cobs_05812	1.5	1.5	<i>Neural Lazarillo</i>	o <i>APOD</i>	carbohydrate and triglyceride homeostasis
Cobs_09609	1.5	1.3	<i>Adenylyl cyclase 76E</i>	o <i>ADCY2, ADCY4, ADCY7</i>	negative regulation of growth
Cobs_03273	2.1	1.6	<i>Trehalase</i>	h <i>TREA</i>	carbohydrate metabolism
Cobs_15800	2.2	1.5	<i>Maltase B1</i>	h <i>RBAT</i>	carbohydrate metabolism
Cobs_02493	2.1	1.5	<i>Glucose dehydrogenase</i>	h -	carbohydrate metabolism
Cobs_06870	1.7	1.8	<i>obstructor-E</i>	o -	carbohydrate metabolism
Cobs_08335	1.6	1.4	-	- <i>DAK</i>	carbohydrate metabolism
Cobs_10061	2.0	1.5	<i>cathD</i>	o <i>CATD and others</i>	lysosomal proteolysis
Cobs_08331	1.5	1.4	CG4678	o <i>CBPD, CBPM</i>	proteolysis
Cobs_15592	2.5	1.7	CG3108	o <i>CBPA4 and other CBPs</i>	proteolysis
Cobs_16232	2.0	2.0	CG34316	h -	haemolymph juvenile hormone binding
Cobs_01124	1.4	1.5	CG8888	o <i>BDH, DHI2</i>	metabolism
Cobs_15266	1.5	1.6	<i>wings up A</i>	o -	muscle development and contraction
Cobs_05213	1.4	1.5	<i>PFTAIRE-interacting factor 1A</i>	o -	regulation of transcription
Cobs_01240	1.3	1.2	<i>PRL-1</i>	o <i>PRL1, PRL2, PRL3</i>	protein dephosphorylation
Cobs_03852	1.4	1.4	CG32082	o <i>BAIAP2, BI2L1, BI2L2</i>	membrane organization
Cobs_05552	1.6	1.3	CG31121	o -	transport
Cobs_01171	1.6	1.4	<i>Aspartyl <math>\beta</math>-hydroxylase</i>	o <i>ASPH</i>	protein modification
Cobs_14086	1.5	1.6	<i>drop dead</i>	h -	digestion, oogenesis
Cobs_09826	1.7	1.7	-	- -	-
Cobs_12991	2.1	1.5	-	- -	-

### 2.3.5 Comparison of genes differentially expressed with age in mated queens and fruit fly females

We performed a quantitative comparison of age-related gene expression changes in *C. obscurior* and *D. melanogaster* females by including expression differences between young and aged females (about 65 % survival; Pletcher *et al.* 2002) as well as between young and extremely old females (10 % survival; Doroszuk *et al.* 2012). The *D. melanogaster* studies resembled each other regarding the lists of genes upregulated in older individuals (Table 2.3). A significant number of these upregulated genes were downregulated in aged *C. obscurior* queens. Ten genes were consistently upregulated in flies and downregulated in ant queens (Figure 2.4). Analysis of GO terms suggested that cellular ketone, carbohydrate and organic acid metabolic processes are oppositely regulated in aging ant and fly females (Table 2.4). Furthermore, transcripts, which contribute to the development and contraction of muscles, were less abundant in MQ18, but more highly abundant in extreme old flies. Genes that were increasingly expressed with age in *C. obscurior*, but downregulated in very old *D. melanogaster*, play a role in cell division and reproduction.

Genes expressed in the same direction did not overlap significantly, but showed a significant enrichment in cell differentiation ( $P = 0.004$ ). In addition, cell fate determination, neurogenesis, and anatomical structure homeostasis were identified as processes upregulated with age in both species.

Table 2.3: Overlap of genes found to be upregulated (+) or downregulated (-) with age in *Cardiocondyla obscurior* and *Drosophila melanogaster* females (Pletcher *et al.* 2002; Doroszuk *et al.* 2012). Number of common genes are shown with statistical significance as extracted from Fisher's exact tests and FDR-correction in parentheses. Significant overlaps are italicized.

		MQ18 vs. MQ4		Pletcher <i>et al.</i>	
		+	-	+	-
		DEGs			
Pletcher <i>et al.</i> (65 % survival)	+	176	2 (1)	<i>16 (0.026)</i>	
	-	134	3 (1)	10 (0.2)	
Doroszuk <i>et al.</i> (10 % survival)	+	648	12 (1)	<i>84 (2.6e-15)</i>	<i>50 (1.0e-12)</i>
	-	1,233	<i>43 (0.009)</i>	16 (1)	7 (1)



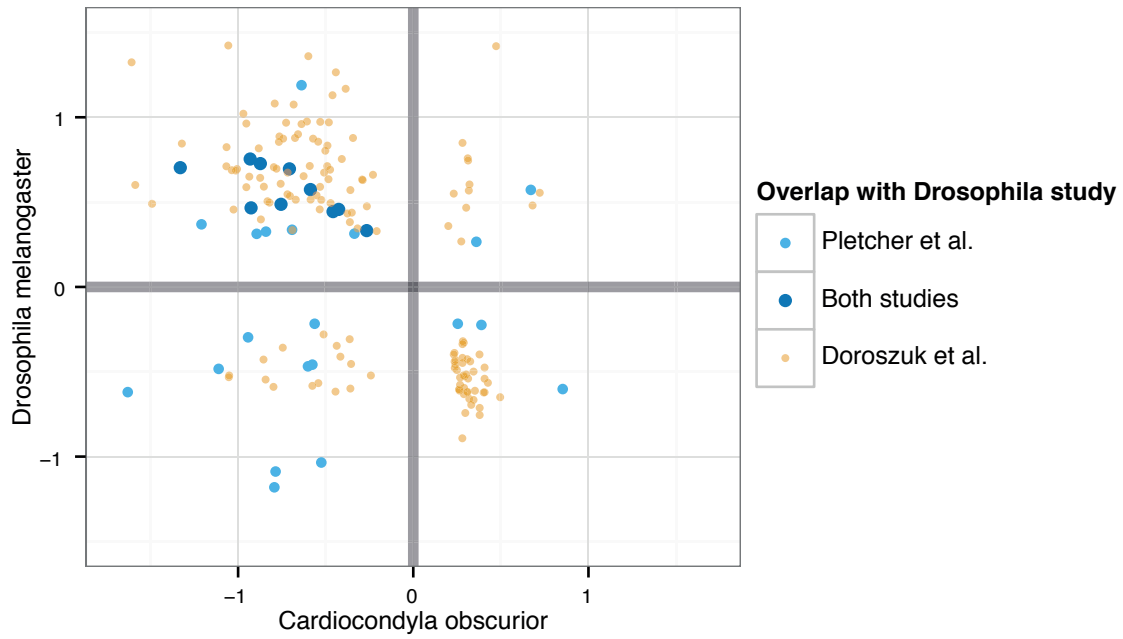


Figure 2.4: Relation of expression changes ( $\log_2$  fold changes = lfc) of genes showing age-regulated transcription in *C. obscurior* and *D. melanogaster*. Ten genes were consistently upregulated in aged (Pletcher *et al.* 2002) and very old flies (Doroszuk *et al.* 2012) and downregulated in ant queens: *ref(2)P*, *emp*, *IscU*, *P5cr-2*, *CAP*, *CCHa2*, CG3168, CG13124, CG9701, CG11796. For these genes, the mean lfc of both analyses is given.

Table 2.4: Overlap of significant GO terms in genes upregulated (+) or downregulated (-) with age in *Cardiocondyla obscurior* (CO) and *Drosophila melanogaster* (DM). The number of annotated genes for each GO category contained in the lists of DEGs is given in the last two columns together with whole-genome annotations for both organisms in parentheses. Underlined, both *D. melanogaster* studies (first number corresponds to Pletcher *et al.*, second to Doroszuk *et al.*); italic, Doroszuk *et al.* only.

Biological process	GO Code	CO	DM	CO	DM
<u>Gene expression changes in opposite directions</u>					
<u>Metabolism</u>					
<u>Oxidation reduction</u>	GO:0055114	-	+	39 (306)	42/115 (620)
<u>Cellular ketone metabolism</u>	GO:0042180	-	+	19 (195)	21/51 (290)
<u>Carbohydrate metabolism</u>	GO:0005975	-	+	16 (193)	24/73 (393)
<i>Carbohydrate catabolism</i>	GO:0016052	-	+	10 (39)	21 (83)
Cellular carbohydrate metabolism	GO:0044262	-	+	11 (109)	12 (182)
Monosaccharide metabolism	GO:0005996	-	+	8 (55)	10 (97)
Hexose metabolism	GO:0019318	-	+	8 (46)	10 (85)
Glucose metabolism	GO:0006006	-	+	8 (33)	9 (61)
<u>Organic acid metabolism</u>	GO:0006082				
<u>Oxoacid metabolism</u>	GO:0043436	-	+	19 (181)	21/50 (270)
<u>Carboxylic acid metabolism</u>	GO:0019752				
<i>Cellular amino acid metabolism</i>	GO:0006520	-	+	16 (145)	32 (199)
<i>Amine metabolism</i>	GO:0009308	-	+	13 (117)	28 (151)
<i>Cellular amine metabolism</i>	GO:0044106	-	+	18 (188)	60 (332)
<i>Aromatic amino acid family metabolism</i>	GO:0009072	-	+	5 (14)	8 (19)
Alcohol metabolism	GO:0006066	-	+	16 (103)	16 (173)
Alcohol catabolism	GO:0046164	-	+	9 (26)	5 (51)
<i>DNA metabolism</i>	GO:0006259	+	-	9 (159)	110 (221)
<i>DNA packaging</i>	GO:0006323	+	-	5 (42)	28 (65)
<u>Development/Reproduction</u>					
<u>Anatomical structure formation</u>	GO:0048646	+	-	12 (203)	16/63 (369)
Myofibril assembly	GO:0030239	-	-/+	4 (11)	3/8 (14)
<i>Actomyosin structure organization</i>	GO:0031032	-	+	5 (23)	10 (38)
<i>Mesoderm development</i>	GO:0007498	-	+	8 (59)	21 (91)
<i>Cell differentiation</i>	GO:0030154	+	-	26 (636)	158 (1060)
<i>Anterior/posterior axis specification</i>	GO:0009948	+	-	8 (84)	28 (147)
<i>Reproduction</i>	GO:0000003	+	-	21 (408)	126 (848)
<i>Reproductive process</i>	GO:0022414	+	-	21 (408)	126 (848)
<i>Female gamete generation</i>	GO:0007292	+	-	15 (286)	96 (546)
<i>Cell cycle</i>	GO:0007049	+	-	22 (349)	173 (616)
<i>Cell cycle checkpoint</i>	GO:0000075	+	-	5 (20)	17 (24)
<i>Cell cycle phase</i>	GO:0022403	+	-	16 (270)	136 (496)
<i>M phase</i>	GO:0000279	+	-	15 (255)	126 (478)
<i>Meiotic M phase</i>	GO:0051327	+	-	7 (72)	49 (200)
<i>Mitotic M phase</i>	GO:0000087	+	-	8 (92)	49 (148)
<i>Cell division</i>	GO:0051301	+	-	13 (136)	61 (205)
<i>Asymmetric cell division</i>	GO:0008356	+	-	6 (47)	15 (58)
<i>Chromosome segregation</i>	GO:0007059	+	-	9 (73)	54 (117)
<i>Organelle fission</i>	GO:0048285	+	-	8 (97)	59 (153)
<u>Other processes</u>					
<i>Muscle system process</i>	GO:0003012	-	+	5 (10)	7 (13)
<i>Muscle contraction</i>	GO:0006936	-	+	5 (10)	7 (13)

Biological process	GO Code	CO	DM	CO	DM
<i>RNA localization</i>	GO:0006403	-	+	6 (80)	39 (125)
<i>Macromolecular complex assembly</i>	GO:0065003	+	-	10 (177)	75 (246)
<i>Regulation of programmed cell death</i>	GO:0043067	+	-	6 (75)	22 (111)
<i>Negative regulation of biological process</i>	GO:0048519	+	-	17 (369)	116 (604)
<i>Negative regulation of cellular process</i>	GO:0048523	+	-	15 (323)	97 (538)
<b>Gene expression changes in same direction</b>					
<i>Cell fate determination</i>	GO:0001709	+	+	8 (89)	22 (129)
<i>Neurogenesis</i>	GO:0022008	+	+	16 (347)	76 (499)
<i>Generation of neurons</i>	GO:0048699	+	+	15 (332)	69 (477)
<i>Anatomical structure homeostasis</i>	GO:0060249	+	+	4 (31)	10 (43)

### 2.3.6 Cross-species comparison of mating- and reproduction-associated transcriptomic changes

We compared our data with several previous studies in *D. melanogaster* and honeybees, *Apis mellifera*, which focused on short-term gene expression changes linked to mating or reproduction (Appendix 7.1.3). Though we examined queens only several weeks after mating, we found significant overlap of the DEGs in these studies with our DEGs in VQ18 versus SQ18, but not with the other two contrasts (Table 2.5). Genes with higher expression in VQ18 were significantly enriched for genes downregulated by sperm, but surprisingly also for genes upregulated by accessory gland proteins in female fruit flies. Likewise, genes downregulated in the brains of mated honeybee queens and genes downregulated in reproductive honeybee workers were overrepresented in the list of genes with higher expression in VQ18. A GO term enrichment analysis revealed that the expression of genes involved in muscle development and contraction is consistently reduced by mating in *C. obscurior* queens and *D. melanogaster* females and by the onset of reproduction in *A. mellifera* workers. Furthermore, significantly more genes were found to be upregulated in SQ18 and brains of incompletely mated honeybee queens ("intermediate") compared to virgin individuals than expected by chance.

Table 2.5: Overlap of genes found to be differentially expressed due to mating and/or the onset of reproduction in *Cardiocondyla obscurior*, *Drosophila melanogaster* and *Apis mellifera* (-, downregulated; +, upregulated). Fisher's exact tests were performed on comparisons revealing at least two common genes, resulting *P* values after FDR-correction are shown in parenthesis. Significant overlaps are italicized.

				VQ18 versus SQ18		VQ18 versus MQ18		SQ18 versus MQ18		
				+	-	+	-	+	-	
<i>Drosophila melanogaster</i> orthologs (6,959)				DEGs	186	74	19	1	4	1
Whole Female (McGraw <i>et al.</i> 2004)	Sperm	+	139	3 (0.9)	1	0	0	0	0	
		-	152	<i>16 (3.8e-5)</i>	2 (0.8)	2 (0.2)	0	0	0	
	Acps	+	41	5 <i>(0.027)</i>	1	0	0	0	0	
		-	29	0	0	0	0	0	0	
	Non-sperm/-Acps	+	317	13 (0.2)	2 (0.3)	0	0	0	0	
		-	196	11 (0.4)	0	0	0	0	0	
<i>Apis mellifera</i> othologs (7,948)				DEGs	215	80	27	3	4	1
Queen Brain (Kocher <i>et al.</i> 2008)	Virgin vs. Mated	+	42	1	0	0	0	0	0	
		-	30	1	2 (0.1)	0	0	0	0	
	Virgin vs. Intermediate	+	66	3 (0.4)	1	0	0	0	0	
		-	336	2 (1)	<i>12 (0.001)</i>	0	0	0	0	
	Intermediate vs. Mated	+	261	1	4 (0.4)	0	0	0	0	
		-	100	3 (0.7)	1	0	0	0	0	
Queen Ovary (Kocher <i>et al.</i> 2008)	Virgin vs. Mated	+	74	2 (0.8)	0	1	0	0	0	
		-	11	0	1	0	0	0	0	
	Virgin vs. Intermediate	+	119	2 (1)	2 (0.5)	1	0	0	0	
		-	26	1	0	0	0	0	0	
Intermediate vs. Mated	+	18	0	0	0	0	0	0		
	-	19	0	0	0	0	0	0		
Queen Brain (Kocher <i>et al.</i> 2010)	Virgin vs. Mated	+	84	9 <i>(0.003)</i>	1	1	0	0	0	
		-	45	0	0	0	0	0	0	
Worker Brain (Grozinger <i>et al.</i> 2007)	Sterile vs. Reproductive	+	21	1	0	0	0	0	0	
		-	59	4 (0.2)	0	0	0	0	0	
Whole Worker (Cardoen <i>et al.</i> 2011)	Sterile vs. Reproductive	+	410	<i>39 (8.9e-11)</i>	2 (1)	3 (0.3)	0	0	0	
		-	632	5 (1)	12 (0.1)	1	0	1	0	
Worker Ovary (Wang <i>et al.</i> 2012)	Low pollen vs. high pollen strain	+	616	25 (0.1)	2 (1)	0	0	0	0	
		-	1,140	21 (1)	18 (0.1)	3 (1)	0	2 (0.2)	0	

### 2.3.7 Genes related to aging in *D. melanogaster*

We screened the *C. obscurior* genome for 136 genes with documented effects on longevity in *D. melanogaster* and found 95 orthologs. Eight of them showed differential expression with age or mating status or both (Table 2.6). *Neural Lazarillo* (*NLaz*) was identified to be less expressed in both SQ18 and MQ18 types. *Nlaz* and *Sirt6* were oppositely expressed in ant queens and fly females with regard to age, whereas *rutabaga* and *Muscle LIM protein at 84B* were regulated in the same direction.

Table 2.6: *Drosophila melanogaster* aging candidate genes showing differential expression (-, downregulated; +, upregulated) with age and/or mating in *Cardiocondyla obscurior* (CO). The following references provided information about age- and mating-related expression in *D. melanogaster* (DM) and *Apis mellifera* (AM): (1) Pletcher *et al.* (2002), (2) Kocher *et al.* (2008), (3) Kocher *et al.* (2010), (4) Doroszuk *et al.* (2012), (5) Zhou *et al.* (2014).

Gene ID	Name	Longevity effect	Age		Mating	
			CO	DM	CO	AM
<b>Age-specific changes in opposite directions</b>						
Cobs_05812	<i>Neural Lazarillo</i>	Pro	-	+	VQ18 > SQ18 and MQ18	Virgin > Mated (3)
Cobs_12553	<i>Sirt6</i>	Pro	+	-		Intermediate < Mated (2)
<b>Age-specific changes in same direction</b>						
Cobs_05934	<i>Muscle LIM protein at 84B</i>	Pro	-	-	VQ18 > SQ18	
Cobs_00579	<i>rutabaga</i>	Pro	-	-		
<b>Other pattern</b>						
Cobs_15768	<i>Insulin-like receptor</i>	Anti	+			
Cobs_03800	<i>myospheroid</i>	Anti		+	VQ18 > SQ18	
Cobs_01798	<i>parkin</i>	Pro			VQ18 < SQ18	Virgin < Intermediate (2, brain)
Cobs_04768	CG3776	Anti+pro			VQ18 < SQ18	Virgin > Intermediate and Mated (2, ovary)

## 2.4 Discussion

### 2.4.1 Opposite gene expression changes in aging queens and female flies

Female reproductives of social insects appear to suffer less “mortality costs” from mating and reproduction than females of solitary insects (Partridge *et al.* 1987; Trevitt & Partridge 1991; Eady *et al.* 2007). On the contrary, mating extends the lifespan of *Cardiocondyla* ant queens (Schrempf *et al.* 2005) and their longevity increases with egg-laying rate (Heinze & Schrempf 2012; Heinze *et al.* 2013). Evolutionary theories of

aging explain the long lifespan of social insect queens and the absence of the fecundity/longevity trade-off from their low extrinsic mortality, as queens live in the relative safety of well-protected, often subterranean, nests (Keller & Genoud 1997; Carey 2001b). Proximally, this suggests an alternative regulation of the conserved pathways that mediate the interplay among mating, egg-laying, and aging. Our study provides support for this hypothesis at the transcriptome level: age-related changes in gene expression had opposite directions in two taxa with opposite life histories.

Transcriptional changes of aged female (Pletcher *et al.* 2002; Doroszuk *et al.* 2012) and male (Zou *et al.* 2000; Girardot *et al.* 2006) flies reflect their decline in reproductive capacity (e.g., Tatar *et al.* 1996). In contrast, the observation that *C. obscurior* queens increase their reproductive efforts with age and show reproductive senescence only immediately before they die, if at all (Heinze & Schrempf 2012), is consistent with the differences between the transcriptomes of young and older *C. obscurior* queens found in this study.

Furthermore, aged and extremely old female *D. melanogaster* exhibit a higher expression of genes involved in cellular ketone, carbohydrate, and organic acid metabolism than young female flies, whereas these genes were downregulated in older relative to young *C. obscurior* queens. The decline of muscle formation and contraction in aging *C. obscurior* queens is consistent with the adaptation to a stationary mode of life and might contribute to save energy. Together with the downregulation of metabolism genes, it might also delay the accumulation of physiological damage. Reproductives of several social insects have lower levels and activities of oxidant enzymes than non-reproductives, which might indicate a reduced generation of oxygen radicals (Parker *et al.* 2004; Corona *et al.* 2005; Schneider *et al.* 2011), perhaps because of reduced metabolism.

#### 2.4.2 Common gene expression changes in aging queens and female flies

Aging is largely regarded as the result of wear and tear. At the same time, the overlap of gene expression changes found during aging and during development in mammals suggests that aging is a regulated process under genetic control (de Magalhães 2012). From this point of view, genes showing age-specific expression changes in the same direction across taxa might be universal regulators of aging. Here, we identified the downregulation of *rut* and *Mlp84B* and the upregulation of genes involved in cell fate determination, neurogenesis, and anatomical structure homeostasis as common signatures of aging. This result supports the idea that developmental processes might continue beyond maturity and become detrimental later in life when selection is relaxed (de Magalhães & Church 2005). In *Caenorhabditis elegans*, age-related expression changes are controlled by three transcription factors, which are not affected by the accumulation of damage (Budovskaya *et al.* 2008). Extending this theory to our study, selection for late reproduction in social insect queens might specifically prevent the drift

or cessation of developmental programs, which optimize reproduction, such as insulin signaling.

### 2.4.3 Gene expression changes associated with the lifespan-prolonging effect of mating

A comparison of the transcriptomes among 18-week-old egg-laying virgin queens, mated queens, and queens mated with sterilized males yielded additional insight into the effects of mating and reproduction. *Cardiocondyla obscurior* queens that are unmated (or sham-mated) and lay only few eggs are tolerated in the colony and receive the same treatment from workers as more fecund, mated queens (Schrempf *et al.* 2005; 2011). Nevertheless, both mated and sham-mated queens live significantly longer than virgin queens do. Consistent with this phenotypic similarity, MQ18 and SQ18 differed in their transcriptomic profiles in only five genes. Interestingly, the transcriptomic profile of VQ18 was more similar to the profile of MQ18 than to the profile of SQ18 considering the number of gene expression differences. Similar to our results, Kocher *et al.* (2008) found more DEGs between unmated and "intermediate" queens than between unmated and mated, egg-laying queens. Furthermore, significant overlap of genes downregulated in SQ18 compared with VQ18 with genes downregulated by sperm in *D. melanogaster* (McGraw *et al.* 2004) and by mating in brains of honey bees (Kocher *et al.* 2008; 2010) indicates that short- and long-term consequences of the mating event are similar, even across taxa. However, our analysis revealed that genes downregulated by sham-mating in ant queens contained a significant part of genes upregulated by the transfer of accessory gland proteins during mating in flies (McGraw *et al.* 2004).

The differential regulation of conserved, public mechanisms may relate to lifespan regulation in these different biological contexts, although presumably with opposing consequences. This corresponds to the contrasting effect of mating on longevity in these taxa. Out of the 257 genes with higher expression in VQ18 compared with SQ18, 21 genes had also significant higher expression than in MQ18. Given that sham-mated queens live as long as mated queens and at the same time display low fecundity similar to that of virgin queens, these genes are particularly interesting because they might be correlated with the different speed of aging. The five carbohydrate-degrading and proteolytic enzymes *Trehalase*, *Maltase B1*, *cathD*, CG4678, and CG3108 point to a reduced need of these energy resources in mated queens. The differential expression of *Trehalase*, *Maltase B1*, *NLaz*, *obstructor-E*, *Glucose dehydrogenase* and a homolog of human *DAK* give further support that mating has an effect on carbohydrate metabolism and homeostasis. In addition, *Neural Lazarillo*, *Adenylyl cyclase 76E (Ac76E)* and a JH binding protein (homolog to CG34316) indicate an involvement of IIS and JH. Noticeably, our data does not hint at a major role of vitellogenin in regulating fecundity or longevity in *C. obscurior*. So far, we identified Cobs\_01486 as the only gene in the genome of this species possessing the vitellogenin domain (pfam01347). This gene is orthologous to the honeybee

"vitellogenin-like" GB52464 and was moderately downregulated in MQ18 compared with MQ4 (fold change = 0.7).

#### 2.4.4 Candidate genes

The lipocalin *NLaz*, which is homologous to vertebrate *Apolipoprotein D (APOD)*, was shown to promote metabolic homeostasis and tolerance to certain types of stress by repressing IIS in the fly model (Hull-Thompson *et al.* 2009). Consequently, flies overexpressing *NLaz* have an extended lifespan at the expense of reduced growth (Hull-Thompson *et al.* 2009; Ruiz *et al.* 2011). Experiments with female flies predict that *NLaz* decreases food intake, decreases fat storage with age, increases locomotor activity, and enhances mating behavior. Ant queens depend on extensive energy intake for the continuous production of eggs. It is therefore not surprising that this gene is less expressed in older, more fertile queens than in younger queens. Furthermore, *NLaz* expression was reduced in mated *C. obscurior* and *A. mellifera* queens (Kocher *et al.* 2010) relative to virgin queens, indicating a regulatory function of post-mating behavior and metabolism. Consistent with the expression pattern of *NLaz*, the differential regulation of *Ac76E* – a direct transcriptional target of *foxo* (Mattila *et al.* 2009) – indicates that IIS activity is lower in short-lived virgin queens. Corona *et al.* (2007) hypothesized that a reduction of IIS in the head of bee queens contributes to their longer lifespan compared with workers. In contrast, *Insulin-like receptor (InR)* was shown to be important for ovary development and reproduction in dipterans and ants (Tatar *et al.* 2001; Okada *et al.* 2010; Lu & Pietrantonio 2011). Our results, including the upregulation of *InR* in older, more fertile queens, point to the involvement of IIS but do not suggest a general reversal of the traditional relationship between nutrition and IIS as proposed for the honeybee (Corona *et al.* 2007). Instead, we found that lifespan differences are accompanied by the differential expression of carbohydrate-metabolizing enzymes. This suggests that mating triggers a change in metabolism to allow a long life and maximize the reproductive output at the same time.

## Conclusions

Our study reveals a number of genes that change expression with age and as a function of reproductive status. The important commonalities and differences in age-related expression changes between *Cardiocondyla obscurior* queens and *Drosophila melanogaster* females may be of broad interest in the community of aging researchers working on diverse organisms.

The comparison among virgin, sham-mated, and mated queens shows how the effects of mating and fecundity on queen longevity can be separated and suggest a number of promising candidates for further in depth studies on the complex regulation of fundamental life history traits in social insects.



## Data accessibility

Raw sequencing data have been deposited in SRA under the BioProject accession numbers PRJNA293450 (MQ18, SQ18, VQ18: SRR2177525–SRR2177544) and PRJNA284224 (MQ4: SRR2033894–SRR2033897, SRR2033903–SRR2033905).

## Supplementary material

Supplementary files S1–S5 are available at Molecular Biology and Evolution online (<http://www.mbe.oxfordjournals.org/>).

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# Chapter 3

## Transcriptomic response to injury sheds light on the physiological costs of reproduction in ant queens\*

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## Abstract

The trade-off between reproduction and longevity is widespread among multicellular organisms. As an important exception, the reproductive females of perennial social insects (ants, honeybees, termites) are simultaneously highly fertile and very long-lived relative to their non-reproductive nestmates. The observation that increased fecundity is not coupled with decreased lifespan suggests that social insect queens do not have to reallocate resources between reproduction and self-maintenance. If queens have to compensate for the costs of reproduction on the level of the individual, the activation of other energy-demanding physiological processes might force them to reduce the production of eggs. To test this hypothesis in ant queens, we increased immunity costs by injury and measured the effect of this treatment on egg-laying rates and genome-wide gene expression. Amputation of both middle legs led to a temporary decrease of egg-laying rates and affected the expression of 947 genes corresponding to 9 % of the transcriptome. The changes comprised the upregulation of the immune and wound healing response on one hand, and the downregulation of germ cell development, central nervous system development and learning ability on the other hand. Injury strongly influenced metabolism by inducing catabolism and repressing amino acid and nitrogen compound metabolism. By comparing our results to similar transcriptomic studies in insects, we found a highly consistent upregulation of immune genes due to sterile and septic wounding. The gene expression changes, complemented by the temporary decline of egg-laying rates, clearly reveal a trade-off between reproduction and the immune response in social insect queens.

*Keywords:* reproduction, trade-off, immunity, social insect, transcriptome, RNA-Seq

### 3.1 Introduction

Life history theory explains how evolution shapes fitness-relevant traits to maximize reproductive success of organisms (Stearns 1976; Bell 1980; Stearns 2000). Individual fitness traits are typically costly and can only be optimized at a disadvantage to other fitness traits (Reznick 1985; Stearns 1989). The constraints intrinsic to organisms result in compromises, such as a trade-off between reproduction and longevity. Investment into reproduction reduces metabolic resources, which otherwise could be used for the maintenance and repair of the soma, and therefore indirectly shortens lifespan (Kirkwood 1977; Kirkwood & Austad 2000).

While numerous studies have revealed this trade-off (Westendorp & Kirkwood 1998; Michod *et al.* 2006; Tabatabaie *et al.* 2011), the female reproductives of perennial eusocial insects (often referred to as queens in ants, honeybees, termites) appear to be an exception: they are simultaneously highly fertile and extraordinarily long-lived (Keller & Genoud 1997; Keller 1998). The observation that queens outlive their non-reproductive nestmates, the workers (Hölldobler & Wilson 1990; Carey 2001b; Heinze & Schrempf 2008), suggests that an increase in fecundity is not coupled with a decrease in survival. Clear evidence that the fecundity/longevity trade-off is absent on the level of the individual was found in several ant species (Tsuji *et al.* 1996; Hartmann & Heinze 2003; Schrempf *et al.* 2005). Furthermore, lifespan and weekly egg-laying rate were shown to be positively correlated in the ant genus *Cardiocondyla* (Heinze & Schrempf 2012; Heinze *et al.* 2013; Kramer *et al.* 2015; Rueppell *et al.* 2015).

However, the lack of a trade-off under certain environmental conditions does not necessarily imply the general absence of costs (Flatt 2011). In social species, the costs of reproduction are thought to be compensated on the level of the whole colony, i.e., by the help of workers. Through the release from other energy-demanding tasks, as for example resource acquisition, the queen might be able to reduce its investment in non-essential somatic repair systems to a minimum, allowing most resources to be used for the production of eggs. Especially defense systems, such as the stress and immune response, are not expected to be continuously activated at high levels, as mature ant queens live in protected nests under predictable conditions.

To understand the mechanisms of longevity in social insect queens, it is important to clarify if queen physiology is affected by the costs of reproduction. We predicted that the costs associated with a challenge of the immune system can not be compensated by the workers and forces the queen to balance them by the reduction of other nutrient-demanding processes, in particular growth and reproduction (Sheldon & Verhulst 1996; Siva-Jothy *et al.* 1998; Lochmiller & Deerenberg 2000; Schmid-Hempel 2003).

To test this hypothesis, we challenged the somatic maintenance and repair systems of *C. obscurior* queens by mimicking a physical injury, which is observed to occur in the natural environment but does not lead to the queen's death. Insects generally accumulate injuries, such as the loss of legs, antennae and parts of wings, with age (Burkhard *et al.* 2002; Sepulveda *et al.* 2008). Queens in our lab colonies

occasionally lack one or several legs, but whether this impairment affects fitness has not been studied. Female *Drosophila melanogaster* were shown to cope with artificial leg amputations by decreasing fecundity (Carey *et al.* 2007; Sepulveda *et al.* 2008). Since the loss of the middle leg is the least harmful among the interventions tested in Carey *et al.* (2007), we decided to amputate both middle legs, and compared egg-laying rate before and immediately after the manipulation.

The immune response is known to deprive resources from growth and reproduction by molecular interactions between inflammatory signals and the insulin-like/IGF signaling (IIS) pathway in the insect fat body (Dionne *et al.* 2006; DiAngelo *et al.* 2009). We expected to find signatures of altered resource allocation after injury in the whole body transcriptome and measured genome-wide gene expression differences by RNA-Seq. For a detailed analysis of the treatment's effect on immunity, reproduction and metabolism, we screened the *C. obscurior* genome for genes known to be involved in these physiological systems. By comparing our result with similar insect studies on the level of shared differently expressed genes, we aimed to determine the degree of conservation of the transcriptomic response to different treatments (sterile and septic wounding) and across different species (*C. obscurior*, carpenter ant *Camponotus floridanus*, and fruit fly *D. melanogaster*).

Our comparison of injured and uninjured queens demonstrates that a considerable part of the transcriptome is affected by the treatment. The gene expression changes clearly reflect a trade-off between the immune response and reproduction, which is also reflected in a temporary decline of egg-laying rates.

## 3.2 Materials and methods

### 3.2.1 The organism and stock colonies

The cosmopolitan tramp ant *Cardiocondyla obscurior* occurs in anthropogenic habitats of subtropical regions (Heinze *et al.* 2006). Colonies contain one or several reproductive queens, which mate in their natal nest after eclosing, and continuously lay eggs until the end of their life (Heinze & Schrempf 2012). Different from solitary insects, the fertile queens never leave the sheltered nest and are cared for by the sterile workers.

The genome reference stock colony was collected in Una (Bahia, Brazil) in 2009 (Schrader *et al.* 2014). All laboratory colonies, which were used for RNA-Seq, were derived from this stock colony. Queens whose egg-laying rates were studied after injury also came from Bahia, Brazil, but have been kept in the lab since being collected in 2004. All colonies were kept under standard conditions in incubators (12h 28°C light/12h 24°C dark) and fed ad libitum with chopped cockroaches and honey.

### 3.2.2 Injury treatment

For the gene expression analysis, queen pupae were reared individually with 1 male pupa, 20 workers and 10 larvae in a Petri dish with plaster. Young queens and males usually mate a few days after eclosion (Schrempf *et al.* 2005). Once per week we counted the number of eggs in the nest. Queens laying only a low amount of eggs, which developed into haploid males due to the failure of mating, were excluded. Additionally developing queen and male pupae were removed from the nest as well as surplus worker pupae and larvae to maintain the initial size of the colony. At the age of 6 months, which is equivalent to the average life span of mated *C. obscurior* queens (Schrempf & Heinze 2008), the 50 surviving queens were divided equally among the two treatments: "injured" and "control". Injured individuals were artificially wounded by amputating both middle legs at the level of the tibia with fine clippers as shown in Figure 3.1. Before freezing, injured queens were kept in vials in the incubator for six hours. Injured and control queens were snap-frozen and stored at  $-80^{\circ}\text{C}$  until further processing.

To determine the impact of the injury on the reproductive performance of queens, we set up 30 colonies consisting each of a dealate queen of unknown age, 20 workers, and 5 large larvae from two large stock colonies. Because egg-laying rates differed tremendously among queens we decided to compare individual egg-laying rates before and after injury rather than comparing injured queens with uninjured controls. Egg-laying rates were monitored during two weeks by counting all eggs and small larvae, then the middle legs of all queens were cut and brood items were counted again during the next two weeks. Nine queens died during the first weeks of the experiment. Among the 21 surviving queens, the increase of the number of brood items during 48 hours one week before, immediately after injury, and one week after injury was compared by Friedman ANOVA with subsequent Bonferroni-corrected Wilcoxon signed rank tests using the software PAST 3.0.7 (Hammer *et al.* 2001). The change in the number of brood items reflects both the number of newly laid eggs but takes the development of eggs to larvae into account. Twelve of the 21 queens produced only male offspring and might therefore not have been inseminated.

### 3.2.3 RNA sequencing and analysis

We extracted total RNA of queens by homogenizing whole bodies with a FastPrep bead shaker (MP Biomedicals) and subsequent application of the RNeasy Plus Micro Kit protocol (QIAGEN) as described before (Wyschetzki *et al.* 2015). We performed an additional DNase treatment step before the RNA was converted into cDNA and amplified (NuGEN Ovation RNA-Seq System V2). Following fragmentation and ligation of adapters with barcodes (NuGEN Rapid DR Multiplex System), 14 individual queens per treatment were sequenced on an Illumina HiSeq1000 platform.

Twenty-five to 58 million 75-bp single reads were generated per sample.

Adapter sequences were removed with Cutadapt version 1.7.1 (Martin 2011). The trimmed reads were mapped against the *C. obscurior* reference genome Cobs1.4 (Schrader *et al.* 2014) using Bowtie2 version 2.2.3 (Langmead & Salzberg 2012) and TopHat version 2.0.13 (Trapnell *et al.* 2009; see mapping statistics in Appendix 7.2.1). Counting of reads, which mapped to gene models, was performed with HTSeq-count version 0.6.1 (Anders *et al.* 2015). We analyzed the quality of the data by means of the clustering functions implemented in the packages 'DESeq2' version 1.6.3 (Love *et al.* 2014) and 'Vegan' version 2.2-1 (Oksanen *et al.* 2015) in R (R Core Team 2014). The PCA indicated a batch effect caused by the distribution of biological samples on eight different lanes of the flow cell (Appendix 7.2.2). To account for this lane effect, we included "lane" as a second variable in the generalized linear model implemented in DESeq2 (design = ~ lane + treatment; see Leek *et al.* 2010). *P* values were adjusted for multiple testing by the FDR-procedure (Benjamini & Hochberg 1995). To enable a PCA with expression values corrected for the batch effect, the ComBat function of the package 'SVA' version 3.12.0 (Leek *et al.* 2012) was applied on the normalized and transformed (VST=variance-stabilizing transformation) gene counts. Furthermore, the 20 genes with the highest variance were chosen to conduct a hierarchical clustering in 'pheatmap' version 1.0.2 (Kolde 2015).

### 3.2.4 General gene annotation and functional enrichment

In order to determine orthologs, we performed a reciprocal protein blast between all 17552 predicted *C. obscurior* genes and the proteomes of *Drosophila melanogaster* (dmel-all-translation-r5.56.fasta) and the ant *Camponotus floridanus* (protein\_sequences.fa retrieved from <http://www.bioinfo.biozentrum.uniwuerzburg.de/computing/Camponotus>) by means of the Blast+ toolkit (Camacho *et al.* 2009). The closest homolog of the genes remaining without ortholog was defined as the best hit of the one-way protein blast on condition that the *e* value was smaller than  $10^{-5}$ . Gene names were adopted from *D. melanogaster*.

Functions of *C. obscurior* genes were predicted from annotations of the corresponding *D. melanogaster* orthologs. All genes with reciprocal orthologous relationships to fly genes were uploaded as background into DAVID (Huang *et al.* 2009). The sets of up- and downregulated genes (*P* adjust < 0.05) were tested for enrichment in Gene Ontology (GO) terms (Ashburner *et al.* 2000) and KEGG pathways (Kanehisa & Goto 2000) relative to the background by the implemented modified Fisher's exact test. Significant GO terms of all levels in the category biological process (EASE < 0.05) were clustered to reduce redundancy.

### 3.2.5 Screening of immune, developmental and metabolic genes

To identify putative immune genes in the *C. obscurior* transcriptome, we retrieved the set of *D. melanogaster* immune genes from Insect Innate Immunity Database (De

Gregorio *et al.* 2001; Bordenstein Lab 2011) and the set of recently classified immune genes of the carpenter ant *C. floridanus* from Gupta *et al.* (2015).

In addition, we were interested in the regulation of genes involved in metabolic and developmental processes. The classification in energy storage (glycogen synthesis or degradation, triglyceride synthesis) and energy generation genes (glycolysis or gluconeogenesis, mitochondrial and peroxisomal fatty acid beta-oxidation, pyruvate dehydrogenase or citric acid cycle, pentose-phosphate pathway, lactate production or degradation) was adopted from the *D. melanogaster* study by Dionne *et al.* (2006). Finally, we screened our data and the above-mentioned publications for DEGs represented in the GO categories amino acid metabolism (GO:0006520), nucleobase, nucleoside and nucleotide metabolism (GO:0055086), germ cell development (GO:0007281), central nervous system development (GO:0007417), learning or memory (GO:0007611) and programmed cell death (GO:0012501). We exclusively considered genes with reciprocal orthologous relationships. Figures were mainly generated with ggplot 2 in R (Wickham 2009).

### 3.2.6 Quantitative comparisons with other studies on sterile or septic wounding

Previous studies investigated genome-wide gene expression changes due to puncture wounding in larvae (Patterson *et al.* 2013) and bacterial injection in mated females and males of *D. melanogaster* (Dionne *et al.* 2006; Short & Lazzaro 2013) and *C. floridanus* pooled workers and larvae (Gupta *et al.* 2015). We determined the overlap with these studies on the level of shared differently expressed genes (DEGs). First, we collected the reported gene identifiers with significant expression changes (corrected  $P < 0.01$  in Patterson *et al.* (2013)/  $< 0.05$  in others) and converted them to the current gene annotations in FlyBase if needed. We tested by a one-sided Fisher's exact test in R if more genes overlapped than expected by random sampling the set of 6959 (*D. melanogaster*) and 8824 (*C. floridanus*) orthologs. GO term enrichment analysis of the shared DEGs was done in DAVID as described above.

## 3.3 Results

### 3.3.1 Fecundity of injured queens

Three of 50 queens of this experiment lost one leg due to unknown causes. All three queens were still alive at the age of 6 months; survival seemed not to be affected by this impairment. Weekly egg-laying rates of uninjured and naturally injured queens strongly varied within individuals and between individuals in the 3 months before sampling (median 17, range 0–35; Figure 3.1). We did not detect a significant difference in the number of eggs laid in the week before and the week after the loss of one leg occurred ( $n = 3$ , Wilcoxon signed rank test,  $P = 0.17$ ).



For a more detailed analysis of the effect of injury on reproduction we cut off the two middle legs of queens in small experimental colonies. On average, queens produced 2.6 eggs in 48 hours 1 week before leg amputation ( $n = 21$ , median, quartiles: 2, 1, 3, range 0–8), 0.9 eggs in the 48 hours immediately after leg amputation (median, quartiles: 0, 0, 1.5, range 0–4), and 1.9 eggs (median, quartiles: 1, 1, 3; range 0–8) in 48 hours 1 week after leg amputation (Friedman test, tie-corrected  $\chi^2 = 12.8$ ,  $P = 0.0017$ ; Bonferroni-corrected Wilcoxon tests, 1 week before vs. immediately after injury,  $P = 0.0022$ , 1 week before vs. 1 week after injury:  $P = 1$ , immediately after vs. 1 week after injury:  $P = 0.012$ ; Figure 3.1).

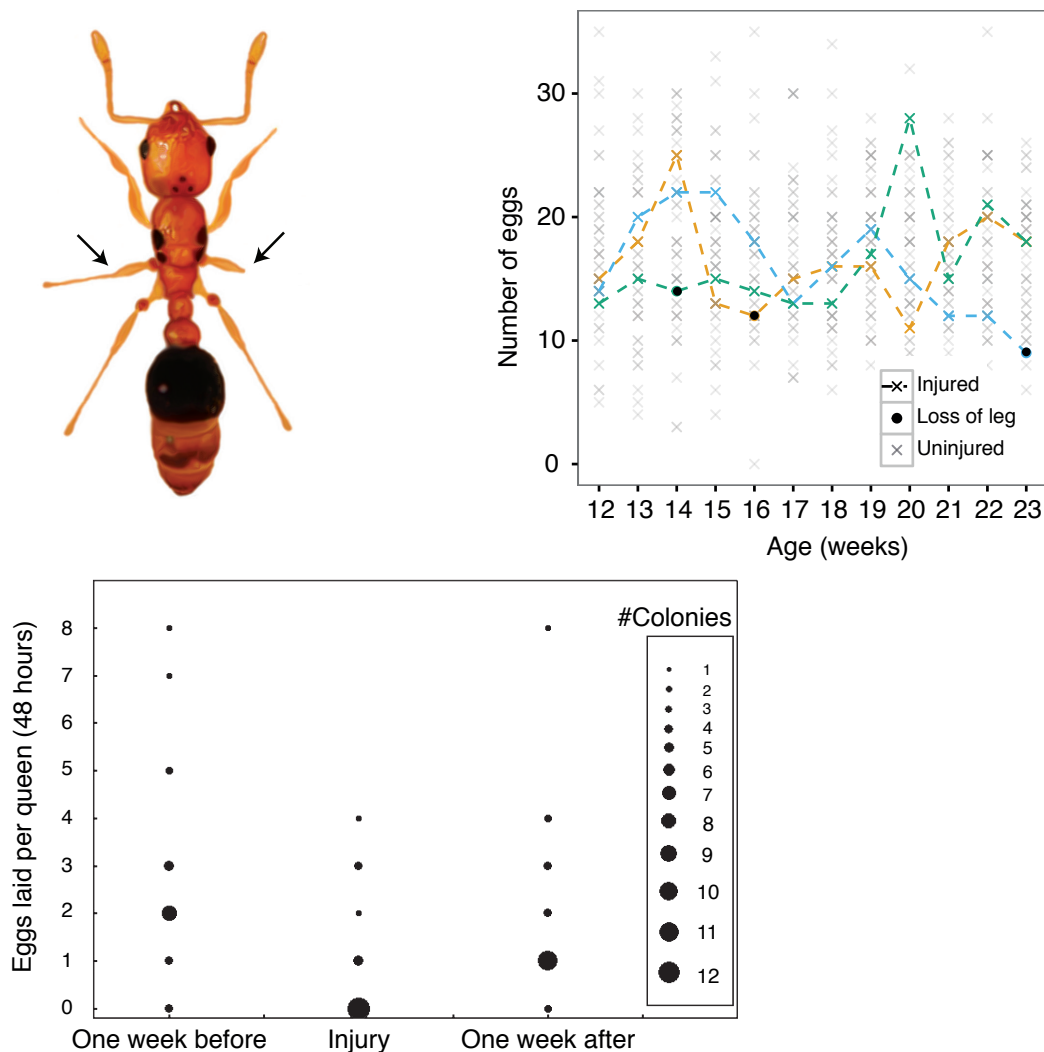


Figure 3.1: Leg amputation and fecundity in *Cardiocondyla obscurior* queens. Schematic view of the surgical treatment resulting in the loss of both middle legs from the tibia (top left). Reproductive investment of the three queens that naturally lost one leg in the course of the experiment (top right). The number of eggs produced by individual queens over 48 hours one week before, in the 48 hours immediately after and one week after artificial leg amputation is given (bottom).

### 3.3.2 Overview of gene expression differences 6 hours after leg amputation

We analyzed the early response to experimental leg amputation by comparing the transcriptomes of 14 injured and 14 control animals. As illustrated by the first principal components of the PCA with the 500 most variable genes, samples clustered in accordance with the treatment (Figure 3.2). We found 947 differently expressed genes (DEGs; adjusted  $P$  value  $< 0.05$ ; Appendix 7.2.3) corresponding to 5 % of all annotated genes and 9 % of all genes whose transcription could be confirmed in this study (average gene count  $\geq 10$ ). Plotting the number of DEGs for several FDR cutoffs showed that the treatment had caused considerable differences in transcript abundances even at more stringent significance levels (Figure 3.2, center).

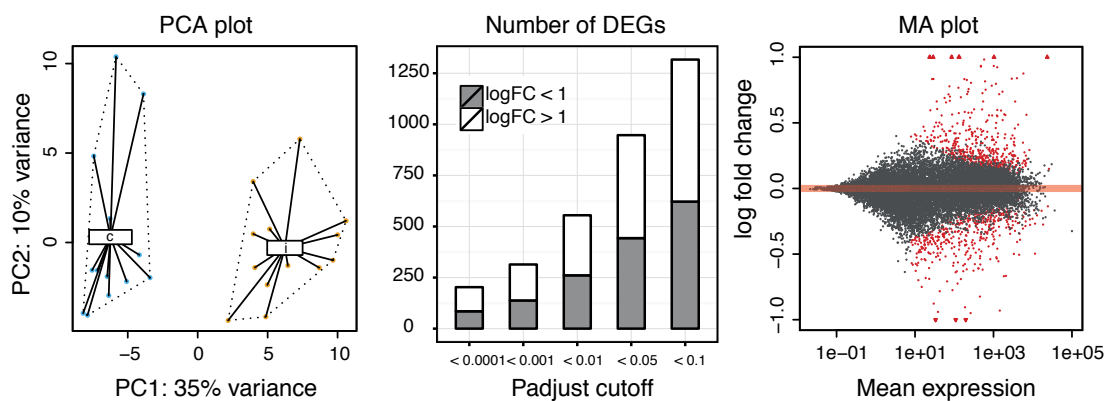


Figure 3.2: PCA, DEG and MA plots of the RNA-Seq data. The PCA plot was generated with transformed (VST) and batch effect-corrected expression values of the top 500 genes with the highest variance; labels represent the center of masses of each treatment (c, control; i, injury). The bar graph in the middle shows the number of differently expressed genes at different  $P$  value (FDR) cutoffs; positive/negative  $\log_2$  fold changes refer to genes upregulated/downregulated by injury. Red dots in the MA plot represent differentially expressed genes with adjusted  $P$  value  $< 0.05$ .

*Hymenoptaecin*, an antimicrobial peptide specific to Hymenoptera, was identified to be the most variable gene across all samples (Table 3.1). It was consistently more highly expressed in all injured queens (Figure 3.3). Hierarchical clustering of the 20 top-ranked variable genes resulted in an almost complete separation of injured and control queens into two distinct clades. Five further genes with significant expression changes separated injured and control queens. Two immune genes (Cobs\_11839, homologous to *Tep2*, and *pale*) and *Dopamine transporter (DAT)* were upregulated, and two metabolic genes (Cobs\_17854, homologous to *Ugt86De*, and CG5618) were downregulated due to the injury.

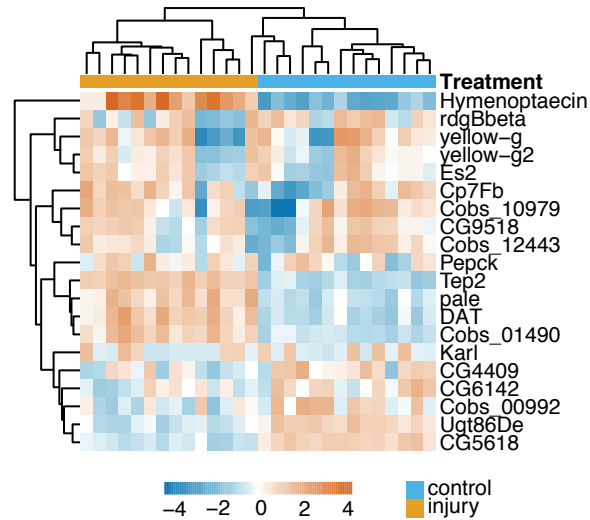


Figure 3.3: Heatmap based on hierarchical clustering of the 20 most variable genes. Expression values were transformed (VST), and corrected for the batch effect prior to the analysis. The color-coding in each cell reflects the deviation from the gene's average across all samples.

Table 3.1: List of top 20 genes with the highest expression variance across all samples. The order of genes corresponds to the ranking according to row variance.

Gene ID	Injury versus control		<i>D. melanogaster</i> (o=ortholog, h=homolog, bo=ortholog in <i>A. mellifera</i> )	
	BaseMean	Fold Change	Name	Function
Cobs_04663	3954	1.8	<i>hymenoptaecin</i>	bo Antimicrobial peptide
Cobs_10979	20776	n.s.	GB55450	bo
Cobs_16707	13705	n.s.	<i>yellow-g</i>	o Eggshell matrix
Cobs_11017	2300	n.s.	<i>Cp7Fb</i>	o Eggshell matrix
Cobs_04675	1950	n.s.	<i>rdgBbeta</i>	o Lipid transport
Cobs_11839	23439	2.9	<i>Tep2</i>	h JAK/STAT pathway
Cobs_12361	479	1.9	<i>DAT</i>	o Circadian sleep/wake cycle
Cobs_03171	513	n.s.	CG9518	h Response to ER stress
Cobs_12443	530	n.s.	GB47943	bo
Cobs_14710	578	2.0	<i>pale</i>	o Humoral response
Cobs_16713	1087	n.s.	<i>yellow-g2</i>	o Eggshell matrix
Cobs_00992	960	n.s.	-	
Cobs_11014	5657	n.s.	<i>Es2</i>	o Splicing
Cobs_01490	133	2.1	-	
Cobs_08980	4237	n.s.	<i>Pepck</i>	o Gluconeogenesis
Cobs_18066	855	n.s.	CG4409	o Insect-allergen-related
Cobs_17854	261	0.5	<i>Ugt86De</i>	h Metabolism
Cobs_17036	196	0.4	CG5618	o Metabolism
Cobs_10405	791	n.s.	CG6142	h Oxidation-reduction
Cobs_01807	11	n.s.	<i>Karl</i>	h Lipocalin

### 3.3.3 Functional enrichment of DEGs

Two thirds of all DEGs had reciprocal orthologous relationships to genes from *Drosophila melanogaster* (Figure 3.4 A). To obtain an overview of gene functions, we extracted the ortholog's annotations for the first Gene Ontology (GO) term level of the category biological process from DAVID. An essential part of the genes were involved in metabolism, development and the response to stimulus (Figure 3.4 B). The proportion of genes annotated for the term reproduction was twice as high in the set of downregulated (11.7 %) than in the set of upregulated DEGs (5.7 %).

Enrichment analysis of genes more highly expressed in injured queens yielded 78 significant GO terms which could be grouped to six annotation clusters and described by the following processes: immune and stress response, growth, response to external stimulus, programmed cell death/ gland development, aging and protein modification (Figure 3.4 C, Table 3.2). Furthermore, the number of genes associated with catabolism, proteolysis, alcohol metabolism, nicotinate and nicotinamide metabolism as well as the mTOR pathway in the list of upregulated genes was higher than expected by chance. Decreased gene expression due to wounding affected developmental processes, in particular the formation of germ cells, the nervous system and memory (Figure 3.4 C, Table 3.2). In addition, the treatment repressed metabolic processes involving carboxylic acids, amino acids, and other nitrogenous compounds, as for example nucleotides, as well as the biosynthesis of proteins. All significant categories can be found in Appendix 7.2.4.

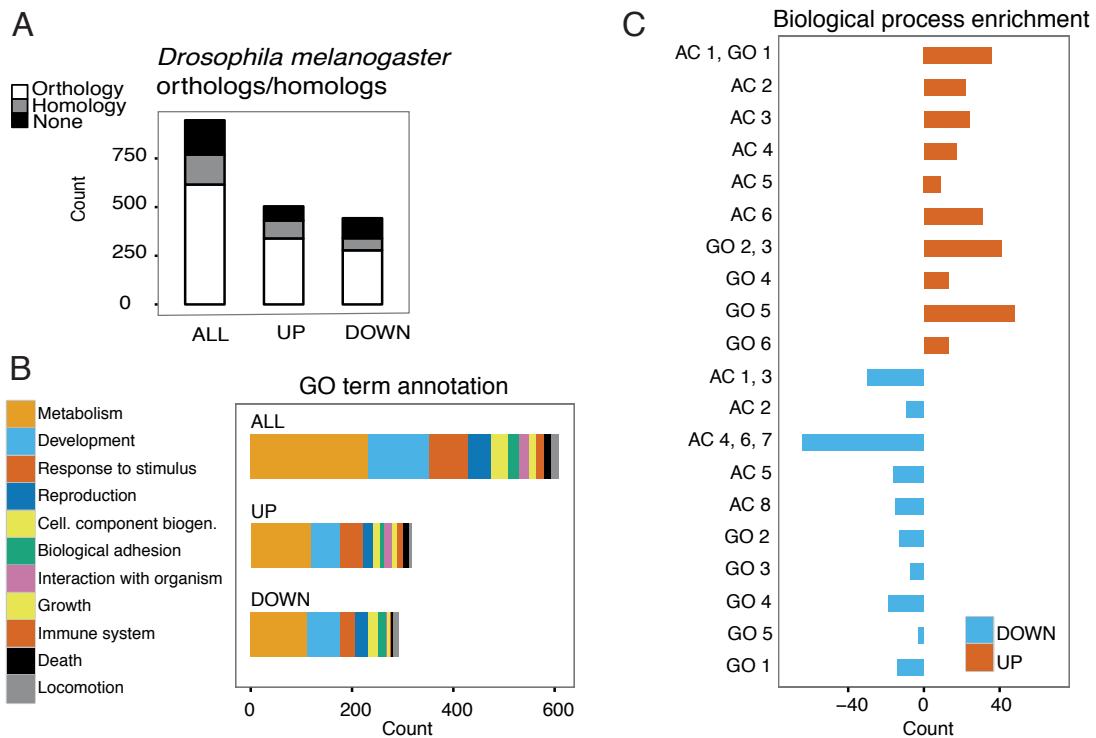


Figure 3.4: Functional annotation of DEGs. A) Proportion of DEGs possessing an ortholog (reciprocal relationship) or a homolog (unidirectional relationship) in *D. melanogaster*. B) GO term annotation for the category biological process (first level). C) Number of DEGs in enriched clusters (AC) and single GO terms (GO) in all levels of the category biological process which can be found in Table 3.2. Count, number of DEGs in each category; Up, upregulated by injury; Down, downregulated by injury.

Table 3.2: Enriched annotation terms. Significant GO term annotation clusters (AC), single GO terms (GO) and KEGG pathways are listed with corresponding enrichment scores (ES) and *P* values respectively.

	Category	Name	ES/ <i>P</i> value
Upregulated	AC 1	Immune response	3.2
	GO 1	Response to stress	1.6e-04
	AC 2	Growth	2.4
	AC 3	Response to external stimulus	2.0
	AC 4	Programmed cell death/ gland development	1.8
	AC 5	Aging	1.8
	AC 6	Protein modification	1.5
	GO 2	Catabolism	3.1e-02
	GO 3	Proteolysis	2.5e-03
	GO 4	Alcohol metabolism	4.9e-03
	GO 5	Signal transduction	9.1e-04
	GO 6	Homeostasis	7.8e-03
	KEGG pathway	Nicotinate & nicotinamide metabolism	3.5e-02
	KEGG pathway	mTOR signaling pathway	4.9e-02
Downregulated	AC 1	Metabolism of carboxylic acids/ amino acids 1	4.1
	AC 2	Maintenance of location	3.6
	AC 3	Metabolism of carboxylic acids/ amino acids 2	2.7
	AC 4	Development 1	1.6
	AC 5	Metabolism of nitrogenous compounds	1.5
	AC 6	Development 2	1.5
	AC 7	Development 3	1.5
	AC 8	Germ cell development	1.4
	GO 1	Cell adhesion	2.3e-03
	GO 2	Central nervous system development	1.5e-02
	GO 3	Learning or memory	2.7e-02
	GO 4	Protein polymerization	9.2e-03
	GO 5	Carbohydrate transport	5.0e-02
	KEGG pathway	ECM-receptor interaction	1.4e-04
	KEGG pathway	Alanine, aspartate & glutamate metabolism	1.8e-03
	KEGG pathway	Nitrogen metabolism	9.6e-03
KEGG pathway	Cysteine and methionine metabolism	4.99e-02	

### 3.3.4 Differential expression of immune, developmental and metabolic genes

The functional enrichment and comparative analyses indicated that the injury led to an activation of the innate immune system. By means of our orthology search, we found 157 orthologs of *D. melanogaster* immune genes (De Gregorio *et al.* 2001; Bordenstein Lab 2011) and 343 orthologs of putative immune genes recently classified in *C. floridanus* (Gupta *et al.* 2015). The early response to injury comprised the elevated expression of genes associated with the Toll (*GNB1*, *PGRP-SA*, *Pellino*, *spirit*, *Serpina27A*, *Serpina42Da*, *Serpina88Ea*, *Toll*, *Traf6*), IMD (*immune deficiency*, *croquemort*, *Nos*, *pirk*, *Relish*, *santa-maria*, homolog of *PGRP-LC*), Jak/Stat (*eater*, *Cdk4*, *Pdk1*, *Pi3K92E*, *Socs44A*, *Tep3*) and, to a lesser extent, the JNK pathway (*Diap1*, *kayak*; Figure 3.5 A.1). In total, 55 putative immune genes were upregulated and 22 downregulated, corresponding to 13 and 6 % respectively of all DEGs with orthologs in either *D. melanogaster*, *C. floridanus*, or both species (Figure 3.5 A.2).

Expression of many genes known to play a role in insect development (122 of 1218 annotated orthologs) was significantly changed after leg amputation. First, the enrichment test pointed to a reduced development of germ cells (*Actin 5C*, *Dystroglycan*, *boule*, *Hsp83*, *nudel*, *windbeutel*) and structures of the central nervous system (*brain tumor*, *Calreticulin*, *disembodied*, *Drop*, *Laminin A*, *Dsam1*, *Semaphorin-5c*, *short stop*, *twinstar*), as well as a decline in learning or memory formation (for example *foraging*, *period*, *nord*; Figure 3.5 B). A part of the respective genes are pleiotropic and cannot be conclusively classified in one of the mentioned processes (*14-3-3ε*, *alpha Spectrin*, *chickadee*, *hedgehog*, *minibrain*, *pumilio*, *staufen*). The few representatives that we identified among the upregulated genes were linked to immunity (*Pvr*, *zfh1*, *kayak*, *gastrulation-defective*) and cell growth (*InR*, *chico*, *Dopa decarboxylase*). Second, genes assigned to the category programmed cell death (*ALiX*, *bigmax*, *Cysteine proteinase-1*, *croquemort*, *Diap1*, *Drep4*, *Drice*, *Pdk1*, *Rab7*, *raw*, *santa-maria*) were overrepresented in the set of upregulated genes.

The significant enrichment for catabolic and anabolic processes, as for example nucleotide biosynthesis, suggested that metabolism was considerably affected already six hours after the amputation. Following Dionne *et al.* (2006), we specifically searched our data for gene expression changes in energy metabolism. We noticed a slight tendency towards downregulation of genes involved with the storage of energy in the form of glycogen and fat or triglycerides (*fabp*, *miday*, *scheggia*; Figure 3.5 C.1). However, the proportion of genes associated with the generation of energy, for example through glycolysis and beta-oxidation, was higher in the set of induced genes (*brummer*, CG3902, CG44252, *Hexokinase A*, *Hsl*, *l(1)G0156*, *Nc73EF*, *Rim2*, *whd*) matching the enrichment for catabolism.

On the contrary, we identified a repression of amino acid and nucleobase, nucleoside and nucleotide metabolism. In total, we found 23 genes associated with either one or both metabolic categories to have lower mRNA levels in injured *C. obscurior* queens (*Adenylosuccinate Lyase*, CG1315, CG33298, CG3714, CG3999, CG5421, CG6415, CG8745, CG9510, CG9674, *Eip55E*, *Glutamate dehydrogenase*, *Mtap*, *nahoda*, *Phosphogluconate dehydrogenase*, *PMCA*, *α-PheRS*, *pugilist*, *rudimentary*, *rudimentary-like*, *S-adenosylmethionine Synthetase (Sam-S)*, *Sarcosine dehydrogenase (Sardh)*, *vermilion*).

Recent work in *D. melanogaster* suggests associations between methionine metabolism and the regulation of metabolic homeostasis during inflammation and aging (Obata *et al.* 2014; Obata & Miura 2015). S-adenosyl-methionine (SAM) is an important methyl-group donor and enhanced SAM catabolism mediated by the enzyme *glycine N-methyltransferase (Gnmt)* has beneficial effects on lifespan. Interestingly, expression of *Gnmt* was not changed in *C. obscurior* queens ( $P = 0.7$ ), which contrasts with the widespread upregulation in immune challenged flies (Patterson *et al.* 2013; Short & Lazzaro 2013; Obata *et al.* 2014). Instead, we detected the downregulation of three other enzymes of the methionine and sarcosine metabolism (*Sam-S*, *Sardh*, *Ahcy13*; Figure 3.5 C.2).

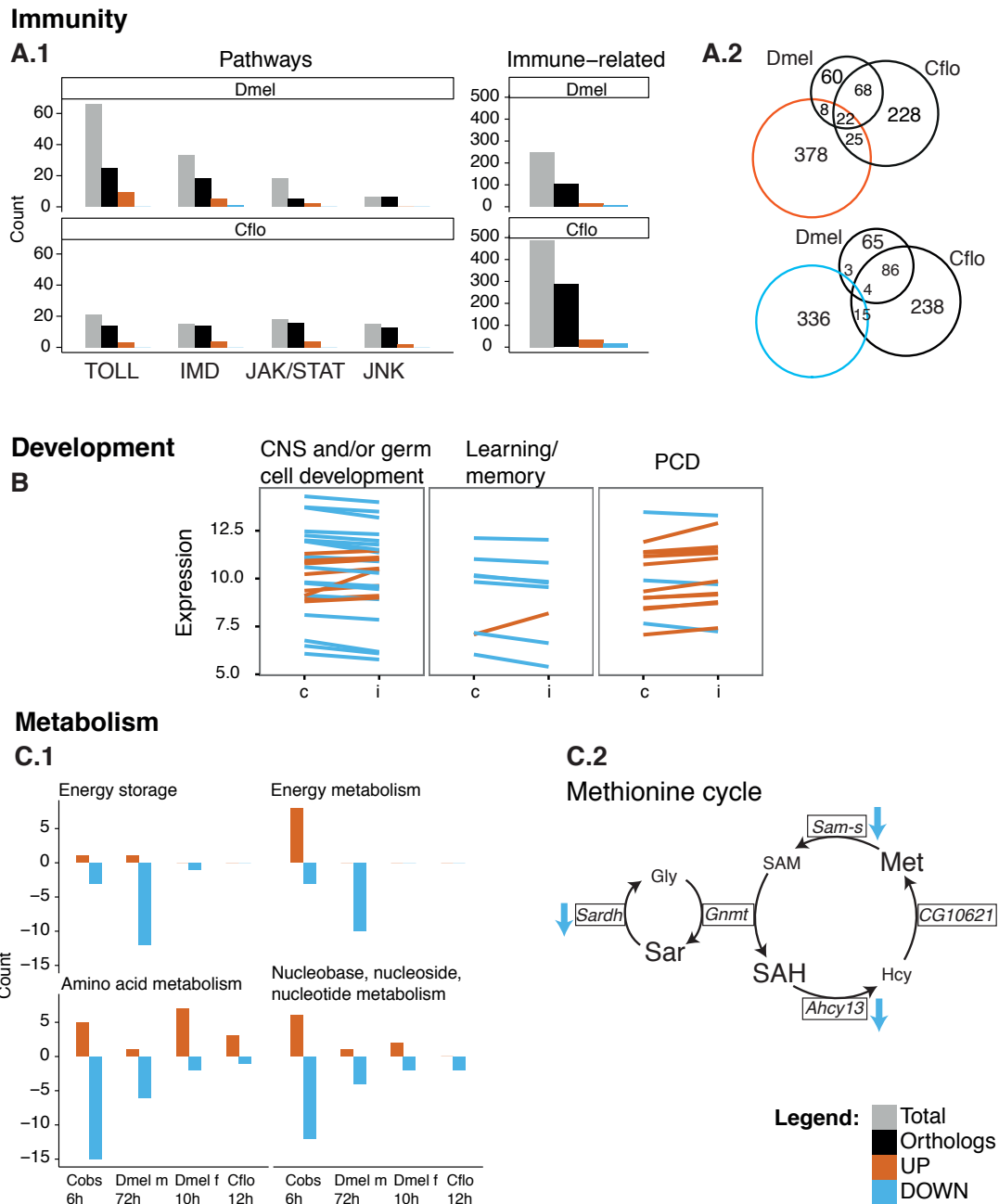


Figure 3.5: Differential expression of immune, developmental and metabolic genes. A.1) Number of immune genes in *D. melanogaster* (Dmel) and the ant *C. floridanus* (Cflo) according to classifications in previous studies (Total) and orthologous genes found in *C. obscurior* (Orthologs). A.2) Overlap of immune gene sets and DEGs. B) Means of transformed (VST) and batch effect-corrected expression values of all DEGs involved in central nervous system (CNS) development, germ cell development, learning or memory, and programmed cell death (PCD). Several genes are annotated for multiple of these processes. C.1) Up- and downregulated metabolic genes in injured *C. obscurior* queens (Cobs), infected *D. melanogaster* males (Dmel m, Dionne *et al.* 2006), females (Dmel f, Short & Lazzaro 2013) and infected *C. floridanus* workers/larvae (Cflo, Gupta *et al.* 2015). C.2) Schematic overview of the methionine cycle with arrows indicating downregulated enzymes. Gly, glycine; Sar, sarcosine; SAM, S-adenosyl-methionine; SAH, S-adenosyl-homocysteine; Hcy, homocysteine; Met, methionine. Up, upregulated by injury; Down, downregulated by injury.



### 3.3.5 Comparison with previous studies on sterile and septic wounding in insects

We found one suitable *D. melanogaster* study for a quantitative comparison of expression changes induced by sterile wounding (Patterson *et al.* 2013). Experiments on septic wounding are more common in insects and usually performed by the injection of bacteria (*Drosophila*: Short & Lazzaro 2013; Dionne *et al.* 2006; the carpenter ant *Camponotus floridanus*: Gupta *et al.* 2015).

Genes upregulated in injured *C. obscurior* queens significantly overlapped with the sets of upregulated genes in all four studies, with the highest conformity to the study in *C. floridanus* (Gupta *et al.* 2015; Table 3.3). GO term enrichment of shared genes showed that wounding and infection in ants and fruit flies consistently induced an immune response. We found much less congruence among the repressed genes. Expression of several genes contributing to protein targeting or amine metabolism was reduced in ant queens, but elevated in fruit flies. Only the data obtained from fly males 3 days after the treatment (Dionne *et al.* 2006) indicated a repression of specific metabolic processes involving carboxylic acids and other amines, matching our results. The complete comparative gene matrices can be accessed in Dryad (doi:10.5061/dryad.d6bc7).

Table 3.3: Overlap of genes significantly upregulated (+) or downregulated (-) due to sterile or septic wounding in the social insect species *Cardiocondyla obscurior* and *Camponotus floridanus* as well as in the solitary model *Drosophila melanogaster*. Sampling time after treatment in brackets.

	DEGs		DEGs	DEG overlap	P value (Fisher)	Adjusted (BH)	GO enrichment	
<i>C. obscurior</i> females (queens) sterile (6h)	+ 414	<i>C. floridanus</i> workers and larvae	+ 67	21	1.3e-12	1.9e-11	Immune response, Amine metabolism	
	414		- 83	3	7.5e-01	7.5e-01		
	-	347	septic (12h) Gupta <i>et al.</i> 2015	+ 67	4	2.7e-01	3.1e-01	
				- 83	5	2.3e-01	3.1e-01	
	+	339	<i>D. melanogaster</i> males septic (72h)	+ 87	9	2.5e-02	5.0e-02	Immune response, Amine metabolism
				- 144	9	2.7e-01	3.1e-01	
	-	278	Dionne <i>et al.</i> 2006	+ 87	11	6.4e-04	2.2e-03	Protein targeting
				- 144	18	1.5e-05	1.1e-04	
	+	339	<i>D. melanogaster</i> females (mated) septic (10h)	+ 68	9	5.4e-03	1.3e-02	Immune response
				- 56	3	5.2e-01	5.6e-01	
	-	278	Short & Lazzaro 2013	+ 68	9	1.4e-03	4.0e-03	Amine metabolism
				- 56	5	7.2e-02	1.3e-01	
+	339	<i>D. melanogaster</i> larvae sterile (2h)	+ 52	10	1.7e-04	7.8e-04	Immune response	
-	278	Patterson <i>et al.</i> 2013	+ 52	4	1.5e-01	2.4e-01		

### 3.4 Discussion

Lifespan and offspring number are positively associated in *Cardiocondyla* ant queens (Heinze & Schrempf 2012; Heinze *et al.* 2013). This challenges the concept of an optimal resource allocation between different energy-demanding processes, in particular reproduction and self-maintenance (Reznick 1985). Though the fecundity/longevity trade-off is a central tenet of life history theory, several recent studies show that the costs of reproduction may be difficult to document when individuals live in a super-optimal environment or differ in the availability or acquisition rate of resources (e. g., Reznick *et al.* 2000). To reveal the costs of reproduction in *C. obscurior* queens, we increased immunity costs by injury and measured the early effect of injury on egg-laying rate and genome-wide gene expression. Our results clearly show that the activation of immune and wound healing mechanisms is accompanied by the suppression of competing processes including reproduction and changes in metabolism, which on the organismic level are reflected in a transient decreased reproductive performance. We outline the transcriptomic signatures of a fecundity/immunity trade-

off in the following sections. In addition, we discuss indications for a repression of learning ability and metabolism.

#### 3.4.1 Upregulation of the wound healing and immune response

Insects have open circulatory systems and therefore must quickly stop the loss of hemolymph and the invasion of pathogens caused by epithelial injuries (Theopold *et al.* 2004). The response to tissue damage comprises the sealing of wounds by clotting, regeneration of the epithelium and activation of the immune system (Razzell *et al.* 2011). Six hours after leg amputation, we discovered that many genes involved in the latter two events were upregulated.

Epithelial tissue repair in adult *Drosophila* is characterized by the polyploidization and growth of healthy epithelial cells, and the subsequent fusion of these cells to giant syncytia to close the wound (Rämet *et al.* 2002; Razzell *et al.* 2011; Losick *et al.* 2013). The JNK pathway activator *kayak* controls the initiation of the cell shape change in adult *D. melanogaster* (Rämet *et al.* 2002) and had significant higher expression in injured than in control queens. The significant enrichment for processes contributing to cell growth, including insulin receptor and mTOR signaling, in the set of upregulated DEGs, is consistent with an early stage of re-epithelialization. Furthermore, the migration of cells or cell protrusions might have been facilitated by the downregulation of genes involved in cell adhesion and the maintenance of location.

By screening the *C. obscurior* genome for genes with immune-related functions in other insects, we detected the upregulation of the conserved key immune pathways Toll, Imd, JAK/STAT, and JNK (Viljakainen 2015) upon injury. Sterile laser wounding of *D. melanogaster* embryos, which does not breach the vitelline membrane, demonstrated that a part of the insect innate immune repertoire is activated in the absence of microbes (Stramer *et al.* 2008). Bacteria from the surface of the animal might have entered the wound in our experiment, as well as in similar manipulations (Patterson *et al.* 2013; Johnston & Rolff 2013), but it is possible that the pathogen response was partially induced by endogenous signals to reduce the risk of future infection (Razzell *et al.* 2011).

Among upregulated genes, we found a significant enrichment in the category programmed cell death (PCD). Corresponding genes with elevated transcript levels might have mediated PCD or apoptosis of damaged cells and promoted regeneration (Fuchs & Steller 2011).

#### 3.4.2 Competing processes: reproduction and learning

Developmental processes apart from the above-mentioned, in particular germ cell development, were exclusively overrepresented in the set of downregulated genes. This matches the observation that experimental leg amputation led to a transient decline of egg-laying rates. *C. obscurior* queens lay eggs until the end of their lives (Heinze &

Schrempf 2012) and the expression of genes associated with reproduction increases with age (Wyschetzki *et al.* 2015). The downregulation of genes involved in oocyte development including axis specification clearly demonstrates that nutrients and energy are redistributed away from reproductive systems to vitally important metabolic processes, indicating the cost of reproduction (Lochmiller & Deerenberg 2000).

In addition, several genes associated with central nervous system and brain development were downregulated in injured queens. Whereas the formation of tissues and organs usually terminates at the pupal stage in insects, adult neurogenesis has been found in the mushroom body of several insect species (Corley & Lavine 2006) and only recently in *D. melanogaster* optic lobes (Fernández-Hernández *et al.* 2013). Plasticity in the visual and olfactory systems and higher brain regions is documented for adult insects and correlates with caste-dependent behavioral changes in Hymenoptera (Julian & Gronenberg 2002; Groh & Meinertzhagen 2010). The downregulation of several genes known to be involved in memory foundation in *Drosophila* (e. g., Figure 3.5 B) suggests a diminished learning ability of ant queens in response to injury. Challenging the immune system of honeybees or bumblebees indeed reduces the ability for associative learning (Mallon *et al.* 2003; Riddell & Mallon 2006; Alghamdi *et al.* 2008), but data for the learning capability of ant queens are not available.

### 3.4.3 Metabolic changes

Our data shows that metabolism was strongly affected by the treatment. Which changes are expected? Infection (Dionne *et al.* 2006; Chambers *et al.* 2012) and sterile inflammation (Obata *et al.* 2014) may lead to an increase in metabolic rate (Martin *et al.* 2003; Ardia *et al.* 2012) followed by a lasting loss of fat and glycogen, generally referred to as wasting of body tissue (Beisel 1977).

The transcriptome data of queens shows signs of elevated catabolic and energy-generating activities. The accelerated breakdown of lipids, carbohydrates and proteins might be necessary to supply the fuel for macrophages and the synthesis of acute-phase inflammatory proteins like antimicrobial peptides (Lochmiller & Deerenberg 2000). The closure of the relatively large wounds caused by leg amputation probably claims additional resources.

We did not find indications for an excessive depletion of fat and carbohydrate stores that would be comparable to the long lasting changes observed in male fruit flies (Dionne *et al.* 2006) and female *Tenebrio molitor* beetles (Johnston *et al.* 2014). Instead, the repression of amino acid and nitrogen compound metabolism in injured queens points to a lack of certain amino acids and a negative nitrogen balance, which are commonly observed consequences of infection in vertebrates (Beisel 1977; Le Floc'h *et al.* 2004). In contrast, amino acid metabolism was upregulated in immune challenged female flies (Patterson *et al.* 2013). This matches the general inconsistency of repressed processes we found across different species and treatments with possibly varying impacts (see Table 3.3).

## Conclusion

Our study gives molecular evidence that the deployment of immune and wound healing mechanisms in ant queens requires the reduction of reproductive and other somatic systems. This demonstrates that eusocial organisms have to compensate the elevated need for energy and nutrients on the level of the individual and strongly suggests that queens are not exempted from the physiological costs of reproduction. How the exceptional longevity of queens is facilitated despite these costs is an open question. This experiment, as well as our previous genome-wide gene expression analysis of young and aged *C. obscurior* queens (Wyschetzki *et al.* 2015), indicates a repression of metabolism that is undetectable in comparable data sets of female reproductives of the solitary model *D. melanogaster*. This could be an adaptive strategy to cope with the burden of continuous and extensive egg production and other highly energy-demanding challenges, as inflammation.

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## Data accessibility

Raw sequencing data have been deposited in SRA under the BioProject accession number PRJNA309926 (SRP069794). Fecundity data of queens from both experiments, RNA-Seq counts and results, a script for the RNA-Seq analysis in R and the cross-species comparison matrix have been uploaded to Dryad doi:10.5061/dryad.d6bc7.

## Author contributions

K.v.W. and J.H. designed the study; K.v.W prepared the RNA-Seq experiment, analyzed the sequencing data and drafted the manuscript; H.L. conducted RNA extractions and library preparation; J.H. performed the experiment on egg-laying rates

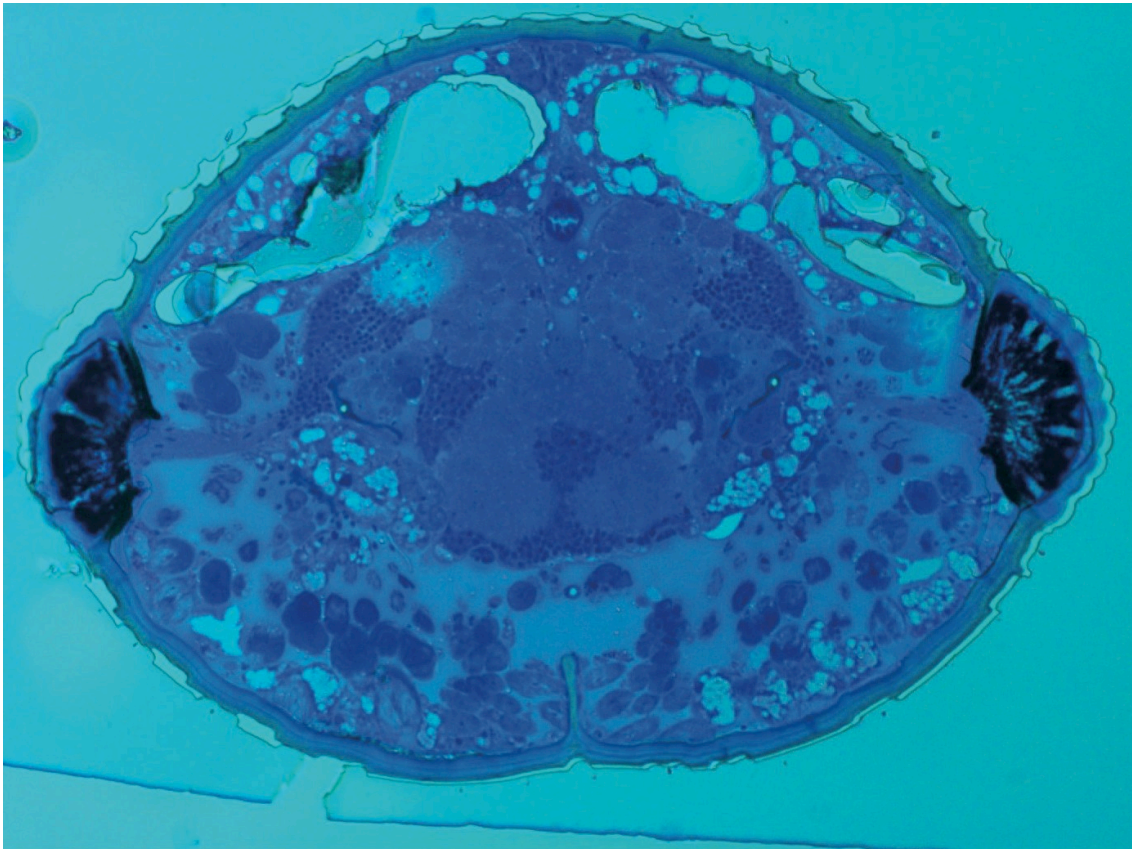
and drafted the corresponding part of the manuscript; all authors carefully revised the manuscript.

# Chapter 4

## Increased fitness in mated ant queens depends on the expression of genes associated with neural and reproductive activity\*

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Section of the head of a 6-month-old mated queen. Photograph provided by Jürgen Heinze.

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## Abstract

Mating can be harmful to female insects and shorten their lifespans. This is a result of the male's attempt to prevent the female from remating and maximize its short-term fecundity. However, if females and males form stable pairs over their whole lifetime, as it is the case in eusocial insects (ants, bees, wasps, termites), both partners benefit from female longevity and are expected to cooperate. Consistent with this prediction, mating prolongs the life of *Cardiocondyla obscurior* queens, and this effect is influenced by the magnitude of female-male coevolution and the phenotype of the male. In this study, we find that queens paired with a wingless fighter male from the same colony have a higher survival advantage over virgin queens than queens paired with a nest-mate winged male. Our transcriptome analysis shows that the higher fitness of queens mated with the fighter male morph is associated with enhanced oogenesis, neurogenesis and neurotransmitter transport revealing reproductive and neural activity as important determinants of queen longevity.

*Keywords:* mating, sexual cooperation, queen, fecundity, transcriptome, RNA-Seq



## 4.1 Introduction

Females and males of sexually reproducing organisms have evolved elaborate strategies to manipulate the behavior and physiology of their mates for their own benefit. A well-known example are the seminal fluid proteins of insect males, which are produced in the accessory glands and may have a striking impact on female biology (Chen *et al.* 1988; Herndon & Wolfner 1995; Wolfner 1997). Surprisingly, seminal fluid can be harmful to the female and shorten her lifespan (Chapman *et al.* 1995). What seems to be a paradox is the outcome of the male's attempts to prevent the female from remating, eliminate competitive sperm, and boost the female's investment into production of the male's progeny. The costs of mating in *Drosophila melanogaster* females were shown to be attributed to the transfer of toxic substances (Lung *et al.* 2002) and pheromones, which interfere with the endocrine system (Rice 2000; Wigby & Chapman 2005). Thus, male-male competition indirectly gives rise to a conflict between the sexes when females repeatedly mate with different males (Arnqvist & Nilsson 2000; Johnstone & Keller 2000; Rice 2000; Snook 2001).

In contrast, when females form a lifelong association with only a single male, both partners benefit from maximizing female longevity. Stable pair formation seems to be rare in solitary species, but is distinctive of eusocial insects, such as ants, bees and termites. The queens of social Hymenoptera mate with one or few males in a short period of time early in their lives (Hughes *et al.* 2008). The rearing of sexual offspring begins only after an ergonomic phase during which sterile workers are produced. Therefore, males do not benefit from trying to increase the queen's short-term reproductive efforts at a cost to its longevity and lifetime reproductive success (Boomsma *et al.* 2005). In the ant species *Cardiocondyla obscurior*, mating with both fertile and sterile males even increased the lifespan of queens (Schrempf *et al.* 2005). Nevertheless, sperm competition, as in honeybees and leafcutter ants (Boer *et al.* 2008; 2009; 2010; Baer 2014), may lead to collateral damage in the queen.

The utilization of harmful substances in eusocial insects might depend on the male's strategy to monopolize paternity. A male morph, which is relatively long-lived among social Hymenoptera (Heinze & Schrempf 2008), occurs in the genus *Cardiocondyla* (Kugler 1983). The wingless males are territorial and possess sabre-shaped mandibles in some species in order to fight to death. In *C. obscurior*, one ergatoid (worker-like) male eliminates all his rivals and thus acquires absolute control over all virgin queens in the colony (Kinomura & Yamauchi 1987; Stuart *et al.* 1987; Schrempf *et al.* 2007). Even though *C. obscurior* queens have been observed to mate multiply (Kinomura & Yamauchi 1987), the likelihood of encountering another wingless male is very low. Occasionally, colonies also produce peaceful winged males, which in morphology and behavior resemble males from other ants and which cannot rely on their predominance in the nest (Cremer & Heinze 2003; Schrempf *et al.* 2007). They possibly increase their fitness by investing more into postcopulatory reproductive tactics, such as seminal fluids, consistent with their larger accessory glands (Schrempf & Heinze 2008). More than 50 accessory gland proteins, which are not yet completely

identified, differ in their relative quantities between winged and ergatoid males (Fuessl *et al.* 2014). The transfer of semen with similar amounts of sperm (Schrempf *et al.* 2007), but varying compositions of accompanying substances, might explain why mating with a winged male has a different effect on queen survival than mating with a wingless male (Schrempf & Heinze 2008).

To extend our knowledge about proximate causes leading to longevity differences between queens mated with an ergatoid male, queens mated with a winged male, and virgin queens, we monitored survival and egg-laying rates of all three groups and compared their whole body transcriptomes at the age of 6 months. To avoid any confounding effects of varying genetic relatedness among mates (Schrempf *et al.* 2015), we exclusively used queens produced in one stock colony and paired them with a male from the same colony. Our results show that the survival advantage of queens, which were mated with an ergatoid male from the same nest, over queens of the other two treatments, is reflected in profound gene expression differences.

## 4.2 Materials and methods

### 4.2.1 The study organism and experimental design

*Cardiocondyla obscurior* is a tramp ant, which was globally transferred to subtropical regions through human activities (Heinze *et al.* 2006). Colonies found in the field in Japan and Brazil are usually small and contain on average 20 to 30 workers, several reproductive queens, and a single wingless male (Kinomura & Yamauchi 1987; Heinze & Delabie 2005). Wingless fighter males, also referred to as ergatoid males, monopolize mating with any newly produced queen by killing younger rival males reared in the colony (Kinomura & Yamauchi 1987; Stuart *et al.* 1987). In the lab, queens and ergatoid males are produced year-round, whereas winged disperser males only develop under specific stressful conditions, for example the decrease of colony size and temperature (Cremer & Heinze 2003).

We used laboratory colonies, which were derived from the genome reference stock colony, collected in Bahia, Brazil in 2009 (Schrader *et al.* 2014) and kept under controlled conditions in incubators (12h 28°C light/12h 24°C dark). Few weeks after splitting half of the colonies into smaller fragments and moving them to room temperature in summer 2013, we found emerging winged disperser males besides pupae of ergatoid males in the nests. We established 200 experimental colonies from these stocks by transferring one queen pupae, 20 workers and ten larvae into a Petri dish with plaster. We assigned the queens to one of the three treatments: mated with an ergatoid male (MQE), mated with a winged male (MQW), virgin (VQ). One ergatoid male (EM) pupa was added to the MQE colonies and one winged male (WM) pupa to the MQW colonies, whereas VQ were reared without male.

The experimental colonies were kept under standard conditions in incubators (as described above) and fed three times per week with chopped cockroaches and diluted

honey ad libitum. All colonies were scanned once per week and additionally developing male and queen pupae were removed. Furthermore, the number of workers and larvae was standardized by adding or removing individuals to 20 workers and 10 larvae per colony. From three months after eclosing, all eggs in the nest were counted as part of the weekly scan. Mated queens, which did not produce diploid offspring (worker or queen pupae) as well as virgin queens, which produced diploid offspring were excluded from subsequent analyses. Individuals of all treatments were sampled after 24 weeks, corresponding to the age when 50% of queens mated with a wingless male had died in a previous experiment (Schrempf & Heinze 2008). All queens were individually snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until further processing.

#### 4.2.2 Analysis of phenotypic data

We analyzed survival of queens beyond the age of four weeks by fitting a Cox proportional hazards regression model with the package 'survival' version 2.38-1 (Therneau 2015) in R version 3.2.1 (R Core Team 2014). We excluded queens, which had died in the first four weeks, as it was done in all previous studies on *C. obscurior* queen survival (MQE:  $n = 47$ , MQW:  $n = 52$ , VQ:  $n = 40$ ). Survival of corresponding male mating partners was similarly evaluated by including individuals, which outlived the queen, as censored (EM:  $n = 44$ , WM:  $n = 52$ ). Mortality curves were fitted by means of the Gompertz model in 'flexsurv' version 0.6 (Royston & Parmar 2002). The y-intercept in the mortality curve represents the background (age-independent) mortality and the slope represents the age-dependent acceleration of the mortality rate indicating senescence.

Furthermore, we compared survival of the three queen types to data obtained in previous *C. obscurior* longevity studies (Schrempf *et al.* 2005; Schrempf & Heinze 2008; Heinze & Schrempf 2012; Schrempf *et al.* 2015) and to lifespans of queens from a former RNA-Seq experiment (Wyschetzki *et al.* 2015). Since the origin of the mate can have profound effects on longevity in *C. obscurior* queens (Schrempf *et al.* 2015), we only considered lifespans of queens, which were collected in Brazil and mated with a male from the same sample site. Individuals, which lived longer than 6 months, were included as censored data.

The number of eggs laid per week represents the queen's reproductive activity. As most queens were sampled before their natural death, we could not determine their lifetime reproductive investment. Instead, we calculated the mean number of eggs laid in each week from the age of 12 to 23 weeks (MQE:  $n = 30$ , MQW:  $n = 19$ , VQ:  $n = 15$ ). To reveal putative differences in fecundity close before natural death, we determined age-specific egg-laying rates of queens, which had died during the experiment and calculated the means over week 12 to week 1 before death (MQE:  $n = 7-12$ , MQW:  $n = 10-22$ , VQ:  $n = 18-25$ ). We compared these values among the three treatments by non-parametric tests with Bonferroni correction in R.

### 4.2.3 Sequencing and RNA-Seq analysis

Total RNA of individual queens was extracted following the RNeasy Plus Micro Kit protocol (QIAGEN) as described before (Wyschetzki *et al.* 2015). Following DNase treatment, the pure RNA was amplified and converted into cDNA (NuGEN Ovation RNA-Seq System V2). We sequenced seven samples per treatment on an Illumina HiSeq1000 platform. Eight barcodes were used for multiplexing (NuGEN Rapid DR Multiplex System).

We generated on average 44 million 75 bp single reads per sample. After trimming adapter sequences with Cutadapt version 1.7.1 (Martin 2011), the reads were mapped against the *C. obscurior* reference genome Cobs1.4 (Schrader *et al.* 2014) using Bowtie2 version 2.2.3 (Langmead & Salzberg 2012) and TopHat version 2.0.13 (Trapnell *et al.* 2009; see mapping statistics in Appendix 7.3.1). Subsequently, reads mapping to each gene model were counted with HTSeq-count version 0.6.1 (Anders *et al.* 2015). We performed normalization of raw counts, variance-stabilizing transformation (VST) and subsequent sample clustering by means of the packages 'DESeq2' version 1.6.3 (Love *et al.* 2014) and Vegan version 2.2-1 (Oksanen *et al.* 2015). By means of the plot of the first two principal components, we identified a batch effect caused by the distribution of biological samples on eight different lanes of the flow cell (Appendix 7.3.2). As recommended for linear models (Leek *et al.* 2010), we could account for this batch effect by incorporating lane as a second variable in addition to treatment in the generalized linear model implemented in DESeq2. Then we contrasted all three treatments and adjusted the *P* values for multiple testing (Benjamini & Hochberg 1995). We used Gene Set Enrichment Analysis (GSEA; Subramanian *et al.* 2005) to test if the genes significantly up- or downregulated in VQ relative to MQE were similarly rank-ordered by fold change in the contrasts VQ vs. MQW and MQW vs. MQE. The area-proportional Venn diagram was drawn with EulerAPE (Micallef & Rodgers 2014). For downstream visualization, we corrected the counts for the lane effect by applying the ComBat function of the package 'SVA' version 3.12.0 (Leek *et al.* 2012).

### 4.2.4 Gene annotation and functional enrichment

We determined *Drosophila melanogaster* and *Apis mellifera* orthologs by a reciprocal protein blast as described before (Wyschetzki *et al.* 2015). The closest homolog of the genes remaining without ortholog was defined as the best hit of the one-way protein blast on condition that the *e* value was smaller than  $10^{-5}$ . Gene names were adopted from *D. melanogaster*. We uploaded all genes with reciprocal orthologous relationships to fly genes as background into DAVID (Huang *et al.* 2009) and tested for a functional enrichment for Gene Ontology terms (Ashburner *et al.* 2000) in the sets of differentially expressed genes (DEGs) relative to the background.

To find all putative neurotransmitter transporters of the SLC6 subfamily

encoded in the *C. obscurior* genome, we screened the list of *D. melanogaster* orthologs and homologs for the 21 members identified by Thimgan *et al.* (2006). Furthermore, we were interested in the most variable genes in the whole data set and calculated expression variances across all samples in R. Hierarchical clustering of 500 most variable genes was performed with 'pheatmap' version 1.0.2 (Kolde 2015). Finally, we determined the overlap with previous transcriptomic comparisons of 18-week-old mated and virgin *C. obscurior* queens (Wyschetzki *et al.* 2015) and young mated and virgin *A. mellifera* queens (Kocher *et al.* 2008; 2010; Manfredini *et al.* 2015) on the level of shared orthologous DEGs.

## 4.3 Results

### 4.3.1 Survival of queens and males

Mating status had a significant effect on survival of queens until they were killed at the age of 24 weeks (Cox proportional hazards model, logrank-test:  $P = 0.007$ ; Kaplan Meier estimates: MQE: 0.7, MQW: 0.5, VQ: 0.4; Figure 4.1). Pairwise comparisons revealed that MQE had a significant lower risk to die than VQ, whereas the difference between MQE and MQW was only marginally significant (Cox proportional hazards model, pairwise logrank-tests with FDR-correction: MQE-VQ:  $P = 0.005$ , MQE-MQW:  $P = 0.08$ , MQW-VQ:  $P = 0.2$ ). Fitted Gompertz curves confirmed that age-independent mortality was highest in VQ, whereas MQW showed the highest age-dependent parameter (Gompertz intercept: MQE: 0.002, MQW: 0.004, VQ: 0.008; slope: MQE: 0.12, MQW: 0.13, VQ: 0.11).

Singly mated ergatoid males lived longer than singly mated winged males (Cox proportional hazards model, logrank-test:  $P = 2.2e-16$ ; Kaplan Meier estimates: EM: 0.1, WM: 0.0; median lifespans: EM: 19 weeks (estimated from censored data), WM: 4 weeks). Background mortality of both male morphs exceeded the values of queens, whereas the age-dependent parameter was only higher in the winged phenotype (Gompertz intercept: EM: 0.01, WM: 0.1; slope: EM: 0.13, WM: 0.17).

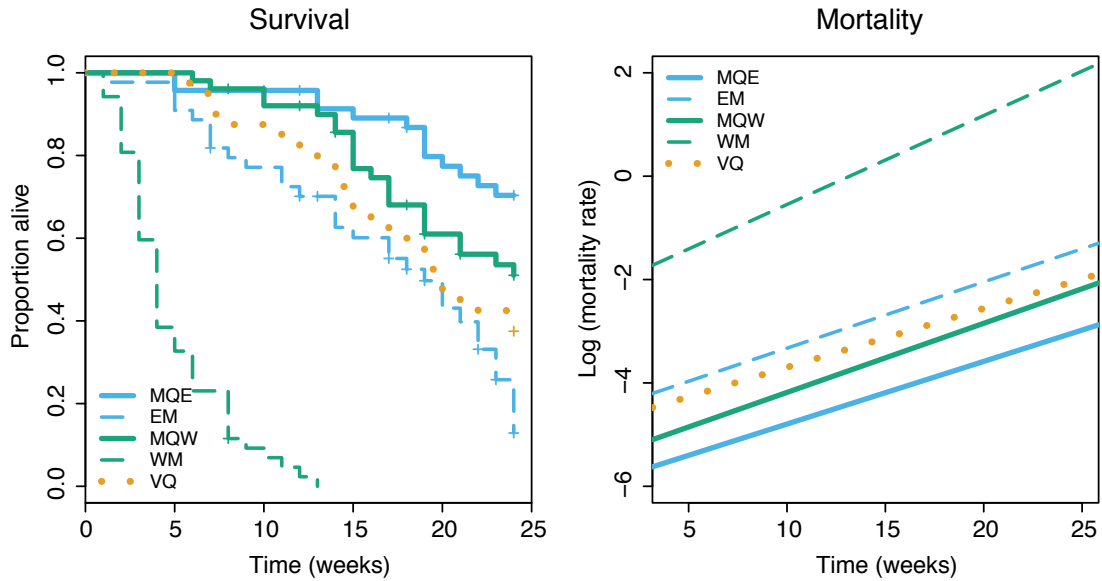


Figure 4.1: Survival and mortality of all queen and male types until the age of 24 weeks. Markings represent censored individuals. MQE, queens mated with ergatoid (wingless) male; EM, ergatoid male; MQW, queens mated with winged male; WM, winged male; VQ, virgin queen.

#### 4.3.2 Fecundity of sampled and dead queens

Egg-laying rates of surviving queens differed significantly among groups (Kruskal-Wallis test:  $P = 8.166e-06$ ; Figure 4.2). Pairwise tests revealed differences between virgin and both types of mated queens, but not between the two mating types (pairwise Wilcoxon rank sum tests with Bonferroni correction: MQE-VQ:  $P = 0.0001$ , MQW-VQ:  $P = 0.0001$ , MQE-MQW:  $P = 1$ ; median, quartiles, range: MQE: 16.9, 16.3, 17.5, 16.2–19.2, MQW: 17.6, 16.0, 18.3, 15.1–19.7, VQ: 4.5, 4.2, 5.6, 3.4–6.3). The same held true when only sequenced queens were taken into account ( $n = 21$ ).

In contrast to the sampled queens, we could compare the queens, which had died before sampling with respect to their lifetime reproductive investment (Figure 4.2). This approach revealed that MQW laid significantly fewer eggs in the twelve weeks before death than MQE (pairwise Wilcoxon rank sum tests with Bonferroni correction: MQE-VQ:  $P = 0.0001$ , MQW-VQ:  $P = 0.0001$ , MQE-MQW:  $P = 0.001$ ; median, quartiles, range: MQE: 15.6, 14.8, 16.2, 13.4–14.8, MQW: 12.7, 11.7, 13.8, 9.9–14.5, VQ: 3.9, 3.6, 4.4, 3.0–5.5).

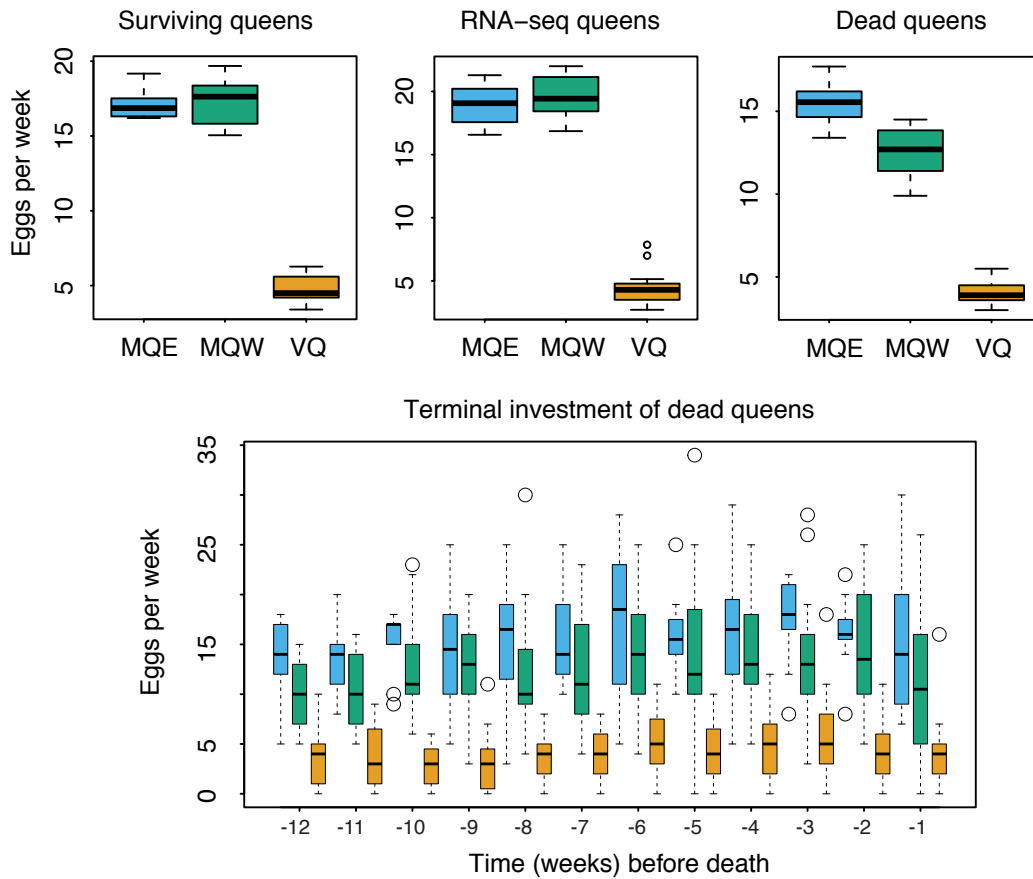


Figure 4.2: Fecundity of queens. Distribution of weekly egg counts for queens which survived until the age of 24 weeks (top left), queens which were sequenced (top middle) and queens which died before the age of 24 weeks (top right); egg counts refer to means per week from week 12 to week 23 after eclosing (across all replicates per treatment). Egg counts of dead queens for each of the twelve weeks before death illustrate the terminal investment phase (bottom); treatments appear in the following order at each time point: MQE, MQW, VQ.

### 4.3.3 Overview of gene expression differences between MQE, MQW and VQ

We analyzed the effect of mating with alternative male morphs on gene expression by contrasting the whole body transcriptomes of MQE, MQW and VQ ( $n = 7, 7, 7$ ). Multivariate analyses of gene counts resulted in a separation of all samples into three clusters, which were in accordance with the three treatments (hierarchical clustering; Figure 4.3, top), but also indicated a considerable overlap of MQW with MQE and VQ (principal component analysis; Figure 4.3, middle).

We found 293 differentially expressed genes (DEGs) between VQ and MQE (adjusted  $P$ -value  $< 0.05$ ; Appendix 7.3.3). MQW showed few DEGs to both other queen types at a FDR of 5%. MQW and VQ differed in eleven, whereas MQW and MQE differed in only two genes. We applied Gene Set Enrichment Analysis (GSEA) to test if all genes up- or downregulated in VQ compared to MQE were similarly rank-ordered by fold change in both other contrasts VQ vs. MQW and MQW vs. MQE. Indeed, the 136 genes with higher expression in VQ than in MQE were significantly overrepresented at the top of both pre-ranked gene lists implying that they had mainly lower expression values in VQ than in MQW and higher values in MQW than in MQE (Normalized Enrichment Score, NES, and FDR: VQ vs. MQW: 8.7,  $P < 0.001$ , MQW vs. MQE: 9.8,  $P < 0.001$ ; Appendix 7.3.4). Similarly, 157 genes with higher expression in MQE were significantly overrepresented at the bottom of both lists (NES and FDR: VQ vs. MQW: -11.2,  $P < 0.001$ , MQW vs. MQE: -9.2,  $P < 0.001$ ).



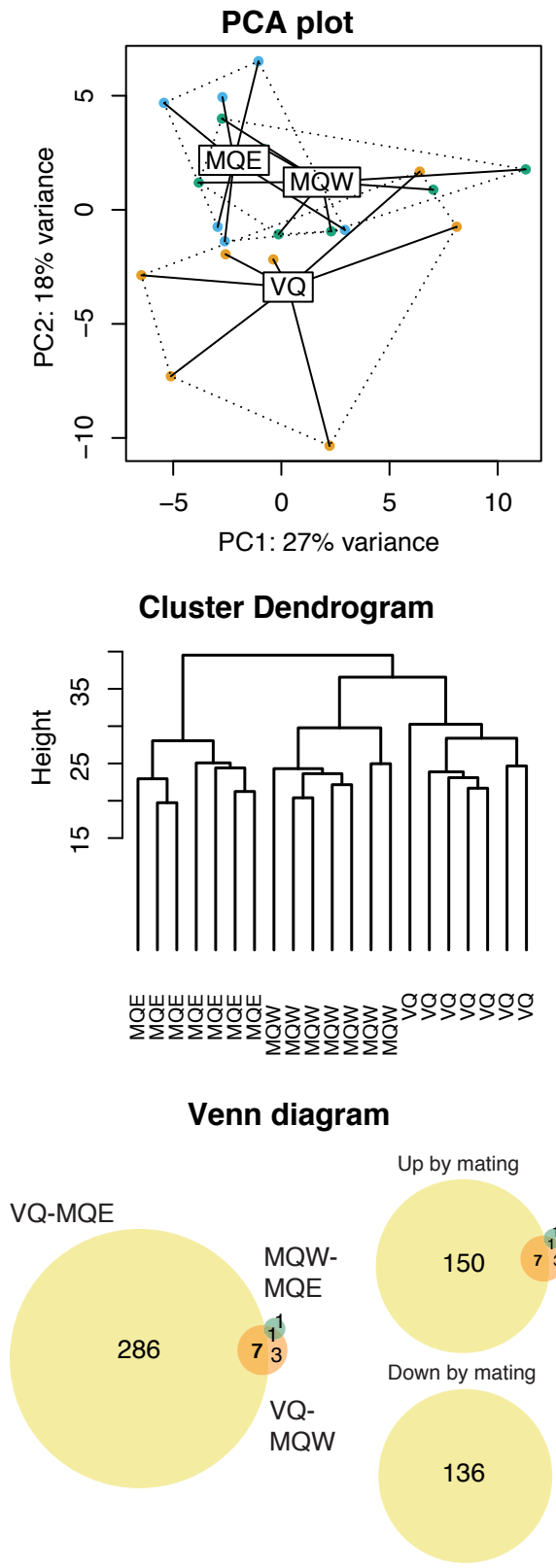


Figure 4.3: Clustering of all samples and Venn diagram of DEGs. Normalized counts were transformed (VST) and corrected for the batch effect prior to principal component analysis (top) and hierarchical clustering (middle); the top 500 genes with the highest variance were analyzed. The Venn diagram illustrates the overlap of all three pairwise comparisons; upregulated by mating refers to a higher expression in MQE and MQW than VQ, as well as upregulation in MQW compared to MQE.

#### 4.3.4 Expression changes specific to treatments and single contrasts

By determining the overlap of all three pairwise comparisons, we could isolate gene expression changes associated with a certain queen type (MQE, MQW, VQ) from those specific to a single contrast (Figure 4.3, bottom).

Seven genes were upregulated in both MQE and MQW compared to VQ (Table 4.1). These mating-associated genes were enriched for 42 GO terms related to cell and neuron development (Table 4.2, Appendix 7.3.5). The MQW treatment was associated with the upregulation of the neurotransmitter transporter CG5549, whereas no gene was consistently associated with the MQE treatment.

The 150 genes exclusively upregulated in MQE compared to VQ were enriched for 90 GO categories, which could be grouped to five annotation clusters (Table 4.2, Appendix 7.3.5). A considerable number of DEGs that contributed to the enrichment for neurogenesis, neurotransmitter transport and/or oogenesis (*alpha-Spec*, *AP-1-2beta*, *Apc*, *ash1*, *asteroid*, *bazooka*, *bruchpilot*, *Chc*, *Chd1*, *coracle*, *Dap160*, *Disabled*, *gbb*, *Hsp83*, *Khc*, *klarsicht*, *l(2)gl*, *Liprin-alpha*, *Moesin*, *nudC*, *off-track*, *Rab3-GEF*, *still life*, *trio*) had maximum expression levels in MQE, minimum levels in VQ, and intermediate levels in MQW (Figure 4.4). The neurotransmitter transporter CG5549 and  *fend*, which is a gene involved in axon growth of motor neurons, were exceptionally most highly expressed in MQW.

The 136 genes downregulated in MQE showed a functional enrichment in only 8 GO categories, which were related to the biosynthesis of macromolecules and apoptosis (Table 4.2, Appendix 7.3.5).

Table 4.1: List of the number of expression changes associated with the three queen treatments (MQE, MQW, VQ) and those that are specific to each of the three comparisons (VQ-MQE, VQ-MQW, MQW-MQE). o, ortholog; h, homolog.

	N	Gene ID	<i>Drosophila</i> (o/h)	Function	
Treatment-associated genes					
VQ	MQE-MQW > VQ	7	Cobs_00112	<i>split ends (spen)</i>	o Neurogenesis
			Cobs_10941	-	-
			Cobs_12564	<i>Maltase B1 (Mal-B1)</i>	h Carbohydrate metabolism
			Cobs_00193	<i>karst (kst)</i>	o Oogenesis
			Cobs_05260	CG43340	o unknown
			Cobs_04423	<i>short stop (shot)</i>	o Neurogenesis
			Cobs_05750	<i>β Spectrin (beta-Spec)</i>	o Neurogenesis, Neurotransmitter transport
MQW	MQW > MQE-VQ	1	Cobs_05219	CG5549	o Neurotransmitter transport
MQE	MQE vs. MQW-VQ	0	-		
Private genes					
	MQE </> VQ	286	(Appendix)		
	MQW > VQ	3	Cobs_00659	CG14516	h Proteolysis
			Cobs_03549	CG13907	o Transmembrane transport
			Cobs_10116	CG15279	h Neurotransmitter transport
	MQW > MQE	1	Cobs_00566	<i>forked end (fend)</i>	o Neurogenesis

Table 4.2: Functional enrichment of treatment- and contrast-specific DEGs. Significant GO terms for biological processes were clustered; one representative term of each cluster is shown including statistics. dev., development.

Genes	Cluster	Representative GO term	Count	%	P value
Treatment: mating					
MQE-MQW > VQ	7	Cell/Neuron dev.	Axonogenesis	3	75 0.0035
Comparison: VQ vs. MQE					
MQE < VQ	136	Biosynthesis	Macromolecule biosynthesis	17	17 0.0146
MQE > VQ	150	Cell communication	Neurotransmitter secretion	8	8 0.0003
		Localization	Protein localization	12	12 0.0101
		Cell/Neuron dev.	Neurogenesis	16	16 0.0007
		Regulation	Reg. of synaptic transmission	3	3 0.0173
		Oocyte/neuron dev.	Oogenesis	11	11 0.0222

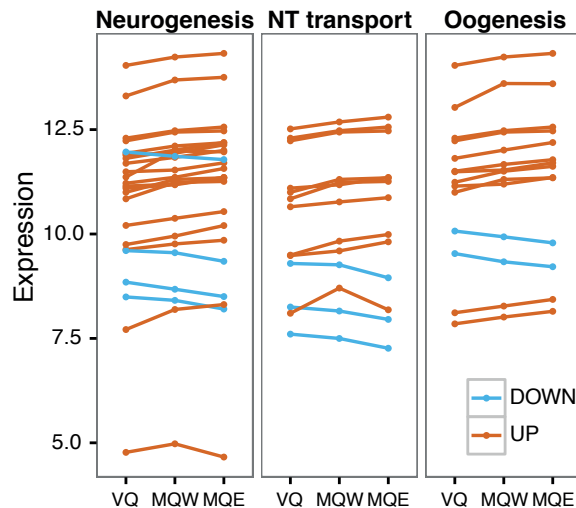


Figure 4.4: Mean expression of all DEGs, which are involved in neurogenesis, the transport of neurotransmitters (NT) and oogenesis, across all three treatments. VST and batch-effect correction were applied to normalized counts prior to plotting. A part of the DEGs are involved in several of the three processes.

#### 4.3.5 Differential expression of SLC6 transporters

Two genes with elevated transcript levels in MQW belong to the SLC6 family of neurotransmitter transporters (Figure 4.5). CG5549 is a glycine transporter and the gene homologous to CG15279 is a putative member of a novel subfamily described as insect amino acid transporters (Thimgan *et al.* 2006) or nutrient amino acid transporters (NATs; Boudko *et al.* 2005). We detected nine further expressed homologs of the 21 *Drosophila melanogaster* SLC6 transporters in the *Cardiocondyla obscurior* genome (mean number of counts > 10, Table 4.3). Five of all eleven representatives of this group differed in expression between mated and virgin insect females in previous transcriptomic studies.

Table 4.3: Homologs of SLC6 transporters and their mating-associated expression patterns. Classification and characterization of *Drosophila melanogaster* homologs refers to (2006). Information about functions was provided in three references: (1) (2006), (2) (2015), (3) (2014). The following transcriptomic data sets were screened for mating-associated expression changes in *Cardiocondyla obscurior* queens (4), and corresponding orthologs in *Apis mellifera* queens (5,7) and *D. melanogaster* females (6): (4) (2015), (5) (2015), (6) (2004), (7) (2010). o, ortholog; h, homolog, AA, amino acid; NT, neurotransmitter; MT, malpighian tubules; M, mated; S, sham-mated; V, virgin.

Gene ID	<i>Drosophila</i> (o/h)	Subfamily	Substrate	Localization	Putative role	Expression	Other studies
Cobs_05219	CG5549	o AA	Glycine	Broad CNS	Nutrient uptake (1)	MQW > MQE, VQ	
Cobs_10116	CG15279	h Insect AA	-	Gut, MT	-	MQW > VQ	M > V (5)
Cobs_00503	ine	o -	-	Hindgut	Water homeostasis (2)	-	-
Cobs_16363	Gat	h				-	M < V (5)
Cobs_00124	Gat	o GABA	GABA	Glia	GABA transport	-	-
Cobs_01306	Gat	h				-	-
Cobs_12361	DAT	o Mono-amine	Dopa-mine	CNS cells	Sleep and memory (3)	-	S < V (4), M > V (6)
Cobs_16158	CG43066	o Orphan NT	-	Broad CNS	-	-	-
Cobs_04734	CG10804	o Orphan NT	-	Broad CNS	-	-	M < V (7)
Cobs_10107	CG15279	o Insect AA	-	Gut, MT	-	-	M > V (5), M > V (6)
Cobs_07471	bdg	o -	-	Reprod. cells	-	-	-

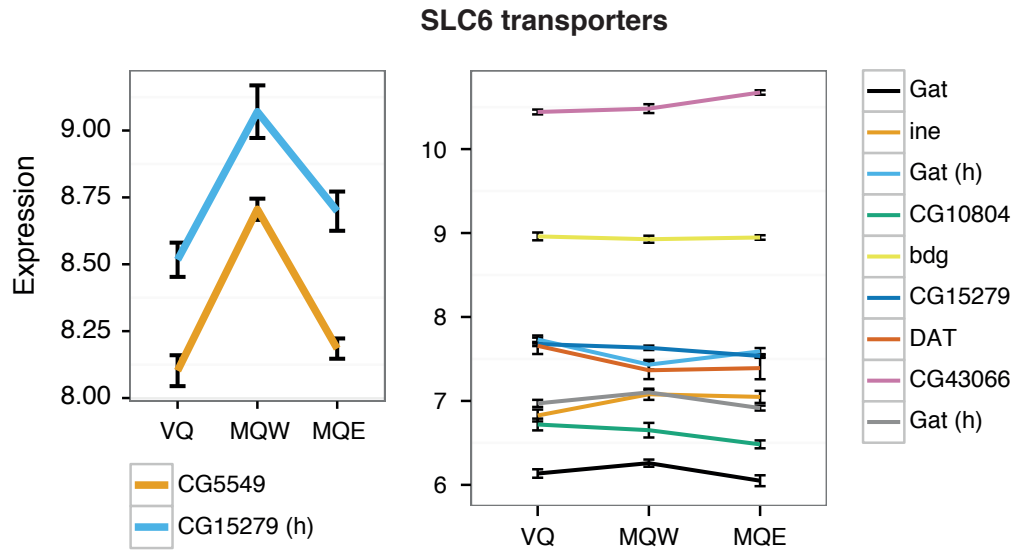


Figure 4.5: Expression of neurotransmitter transporters of the SLC6 family across all treatments. Mean transcript levels of two genes significantly upregulated in MQW (FDR < 0.05) are shown in the left panel; all other genes in the right panel. Error bars represent standard errors of VST- and batch-corrected counts. Gene names were adopted from *D. melanogaster* orthologs and homologs (h).

#### 4.3.6 Gene expression variability among queens independent of mating status

The gene *Cobs\_10979*, which is orthologous to *GB55450* in *Apis mellifera*, but has no homolog in *D. melanogaster*, showed the highest variance across all 21 queens. Hierarchical clustering of the 500 most variable genes resulted in a clear separation of a clade consisting of *Cobs\_10979* and eight further covarying genes (Appendix 7.3.6). This group contained genes which are thought to be involved in the formation of the egg shell (*Cp7Fb*, *Es2*, *yellow-g* and *yellow-g2*). Their expression was not correlated with the mating or reproductive status (Table 4.4, Figure 4.6). Analysis of genome-wide counts from a previous comparison of mated and virgin *C. obscurior* queens (Wyschetzki *et al.* 2015) confirmed their high variability and coexpression (Appendix 7.3.7).

Table 4.4: List of candidate genes with highly varying expression that is independent of mating status. For genes, which do not have a homolog in *D. melanogaster*, the honeybee ortholog is indicated (bo). Gene counts were normalized for library size and arithmetically averaged. The variability rank V refers to the position in the list of all genes rank-ordered according to their variance. V (2015) presents the variability rank in a previous transcriptomic comparison of mated and virgin *C. obscurior* queens (Wyschetzki *et al.* 2015).

Gene ID	Ortholog/homolog	Function	Counts	V	V (2015)
Cobs_10979	GB55450	bo	25581	1	1
Cobs_11017	<i>Cp7Fb</i>	o Eggshell formation	2788	2	3
Cobs_16707	<i>yellow-g</i>	o Eggshell formation	15411	3	2
Cobs_11014	<i>Es2</i>	o Splicing	5660	4	8
Cobs_12443	GB47943	bo	675	5	7
Cobs_03171	CG9518	h Ecdysteroid metabolism	570	6	13
Cobs_16713	<i>yellow-g2</i>	o Eggshell formation	1157	13	4
Cobs_03773	-		364	16	18
Cobs_03766	CG14946	o Metabolism	1501	52	238

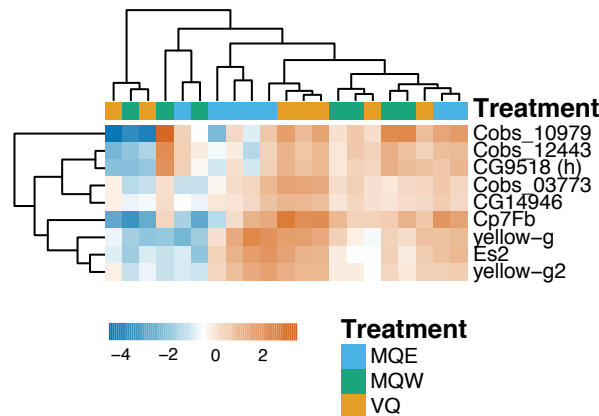


Figure 4.6: Hierarchical clustering of nine candidate genes showing a similar varying expression pattern across all queens independent of mating status. Normalized counts were transformed (VST), corrected for the batch effect and subtracted by the gene's mean expression value prior to clustering.

#### 4.3.7 Comparison with previous transcriptomic studies

We sequenced the whole body transcriptome of *C. obscurior* queens at a higher depth than in a former RNA-Seq study (Wyschetzki *et al.* 2015). As expected, an increase in the size of the sequencing library resulted in a considerable increase in the number of detected genes (count  $\geq 1$ ; Appendix 7.3.8). However, we noticed that the detection of new genes approximated a plateau in the larger, recent libraries suggesting saturation. The current study revealed eight times more DEGs between MQE and VQ than before. Out of the 37 previously discovered DEGs, only the transcription factor *Pif1A* exhibited altered expression levels. Finally, we compared the gene expression differences between MQE and VQ to the outcomes of transcriptomic comparisons of young mated

and virgin *A. mellifera* queens (Kocher *et al.* 2008; 2010; Manfredini *et al.* 2015). Consistent with the previous *C. obscurior* study, we did not find a significant overlap with these honeybee studies on the level of orthologous genes. The number of common genes can be found in Appendix 7.3.9.

#### 4.3.8 Cross-study comparison of queen survival

We compared the survival of queens of all three mating types (MQE, MQW, VQ) across this and four other longevity studies by fitting a Cox proportional hazards model with the variables 'mating type' and 'study'. Mating type had a significant effect on survival across studies ( $P = 0.0003$ ), whereas the factor 'study' did not significantly affect survival until the age of 24 weeks ( $P = 0.2$ ; Appendix 7.3.10 and 7.3.11). The reanalysis of a previous comparison of MQE and MQW lifespans confirmed that MQW had lived significantly longer than MQE as reported in the corresponding publication (Schrempf & Heinze 2008), but the difference was above the significance level when queens, which lived longer than 24 weeks, were censored (logrank-test:  $P = 0.09$ ).

We also analyzed survival until the age of 18 weeks to enable a comparison with a former RNA-Seq study in which queens were sampled at this time point (Wyschetzki *et al.* 2015). Differences between mating types turned out to be consistent with the analysis until 24 weeks (Cox proportional hazards model, pairwise logrank-tests with FDR-correction: MQE-VQ:  $P = 0.01$ , MQE-MQW:  $P = 0.06$ , MQW-VQ:  $P = 0.3$ ). In the former transcriptomic experiment, mated queens did not live significantly longer than virgin queens (logrank-test:  $P = 0.4$ ). Importantly, the sample size of mated queens was considerably lower than in this study (17 vs. 47, Appendix 7.3.10).

## 4.4 Discussion

### 4.4.1 Overview of survival and gene expression differences

By comparing the transcriptomic profiles of both mated queen types (MQE, MQW) with those of virgin queens (VQ) we could determine the overlap and dissimilarity of genes affected by mating with one of the two male morphs.

The overall pattern of gene expression differences matched the extent of survival differences between MQE, MQW and VQ. Mating with a long-lived ergatoid male prolonged the lifespan of queens and significantly influenced the expression of 293 genes. Mating with a short-lived winged male had a similar positive effect on survival until the age of three months, but then mortality increased drastically resulting in survival rates more similar to VQ, and a relatively low number of DEGs to both other treatments. Two findings indicate that mating with one or the other male type affects mainly the same genes. First, most of genes upregulated in MQW relative to VQ (seven out of eleven) were upregulated in both mated queen types. Second, gene set enrichment



analysis and the individual evaluation of genes involved in oogenesis and neurogenesis revealed that MQW had intermediate transcript levels of genes differently expressed between MQE and VQ. Significant changes in the majority of those genes might have not been detected on the level of the whole body transcriptome because differences in small tissues of a composite structure can be washed out by signals from larger tissues (Johnson *et al.* 2013).

#### 4.4.2 Survival advantage is associated with neurogenesis and oogenesis

Genes involved in axonogenesis were overrepresented in the set of genes with higher expression in both mating treatments. Furthermore, we found enrichments for oogenesis, neurogenesis and neurotransmitter secretion/transport in genes with significant higher expression in MQE and intermediate transcript levels in MQW.

MQE did not lay more eggs than MQW in the three months before sequencing, but the analysis of the terminal investment phase of dead queens revealed a higher fecundity of MQE. The absence of a difference in surviving queens possibly resulted from the advanced physiological age of MQW because egg-laying rates increase with age in *C. obscurior* (Heinze & Schrempf 2012). Higher reproductive activity in longer-lived queens contradicts the commonly observed fecundity/longevity trade-off, but is in agreement with recent studies indicating that these traits are positively correlated in *Cardiocondyla* ants (Heinze & Schrempf 2012; Heinze *et al.* 2013; Kramer *et al.* 2015; Rueppell *et al.* 2015).

Enhanced neuron development in mated queens is surprising, as ant queens were shown to lose brain volume after the mating flight and are generally expected to decrease nonessential costly neuronal tissue, for example of the visual system, to save energy (Julian & Gronenberg 2002). Adult neurogenesis in Hymenoptera is rare (Fahrbach *et al.* 1995b; Gronenberg *et al.* 1996). Therefore, age- and task-related volume changes, which have been found within the mushroom bodies of ants, bees and wasps (Fahrbach *et al.* 1995a; Gronenberg *et al.* 1996; Gronenberg & Liebig 1999; Molina & O'Donnell 2007), more likely arise through the growth of cell processes, i. e. axons and dendrites (Farris *et al.* 2001), and the plasticity of synaptic complexes (Groh *et al.* 2006). Mature honeybee queens - in contrast to virgin queens - rely more on olfactory than on visual cues, leading to a continuous increase of the corresponding olfactory-input region in the mushroom bodies with age (Groh *et al.* 2006). Environmental stimuli, especially those resulting from social interactions with conspecifics, may have an influence on the growth of neural structures in social insects, but empirical data on queens is not available.

A link between neurotransmitter activity and the drastic behavioral and physiological changes triggered through mating in social insect queens has been established by several studies (Harano *et al.* 2005; 2008; Aonuma & Watanabe 2012). Levels of dopamine, for example, permanently decrease in bee queens, probably associated with their reduced locomotor activity (Harano *et al.* 2008). The differential

expression of genes contributing to the transport or secretion of neurotransmitters predicts differences in neuronal signaling between mated and virgin queens (Gether *et al.* 2006; Kristensen *et al.* 2011). Interestingly, two of the five genes with exceptionally highest expression in queens mated with a winged male are transporters of the SLC6 family. Both CG5549 and CG15279 belong to subfamilies, which move compounds other than neuromodulators across membranes. The localization of these amino acid transporters in the broad CNS and the gut respectively point to a role in nutrient uptake (Thimman *et al.* 2006). By our comparative analysis, we found several other members of this family, including the dopamine transporter, to be differently regulated due to mating in solitary and social insect females. The classical SLC6 transporters (e.g. dopamine and serotonin transporters), as well as the less known representatives, provide promising candidates for future investigations on mating-induced physiological changes.

In summary, these findings suggest that queen longevity is associated with increased neural and reproductive activity. The high number of DEGs and the lack of an overlap with a former *C. obscurior* RNA-Seq dataset (Wyschetzki *et al.* 2015) could result from the higher sequencing depth and slight changes in the library preparation step, such as the additional DNase treatment and a larger size of cDNA fragments. Furthermore, the survival advantage of MQE over VQ seemed to be more strongly pronounced in the present study. Although the life-prolonging effect of mating is generally consistent across *C. obscurior* laboratory studies, individual survival curves vary considerably (Appendix 7.3.11). Among the factors, which could be responsible for this variation, is the degree of female-male co-evolution. We recently found out that queens mated with an ergatoid male from the same population live longer than queens mated with an ergatoid male from a population 50 km apart (Schrempf *et al.* 2015). Therefore, we standardized the evolutionary distance between females and males by crossing sexuals derived from the same collected colony, resulting in a higher survival of MQE than expected from the earlier 'out-crossing' experiments (Schrempf *et al.* 2005; Schrempf & Heinze 2008; Heinze & Schrempf 2012; Schrempf *et al.* 2015), but a lower survival of MQW (Schrempf & Heinze 2008). This confirms that queen physiology is optimally adapted to respond to coevolved ergatoid males (Schrempf *et al.* 2015), but also questions the degree of cooperation between queens and the rare winged phenotype. *Drosophila melanogaster* females, which are artificially prevented from coevolving with harmful males, evolve to be less resistant to male-induced harm (Rice 1996; Holland & Rice 1999). We analogously hypothesize that our examined queens, which had not been exposed to winged males at least since the year of sampling in 2009, are lacking mechanisms to counteract toxic seminal substances from winged males. This theory could be tested in future by experiments using artificial selection.

### 4.4.3 Candidate genes for egg development

In addition to the mentioned reproduction-related genes with higher expression in mated, more fecund queens, we identified nine candidates for egg development that are not associated with mating status. The chorion protein gene *Cp7Fb*, *Es2* and the two yellow genes *yellow-g* and *yellow-g2* are located on two different genomic regions in *D. melanogaster*. These are amplified in follicle cells at the late stages of oogenesis in order to rapidly increase the number of templates available for transcription (Claycomb *et al.* 2004; Fakhouri *et al.* 2006). Thus, the elevated expression of these genes in a part of the queens more likely arose from amplification of DNA rather than upregulation. Chorion proteins, such as *Cp7Fb*, and *yellow-g* are essential for the formation of the different eggshell layers in *D. melanogaster* (Claycomb *et al.* 2004). Several studies proposed a link between the expression of *yellow-g*, or its homologs, and the activation of the reproductive system in solitary and social insect females (Tian *et al.* 2004; Gräff *et al.* 2007; McGraw *et al.* 2008; Niu *et al.* 2014). In contrast to other insects, virgin reproductive females in *C. obscurior* regularly oviposit. This explains the large proportion of virgin queens in our dataset that had expression levels as high as mated queens.

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## Author contributions:

KW and JH designed the study; KW carried out the experiment, analyzed the data and drafted the manuscript; JH carefully revised the manuscript.

## Chapter 5

# Mating with an allopatric male triggers immune response and decreases longevity of ant queens\*

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Parasitized terminal shoots of *deigo* (coral tree), which are abundantly inhabited by Japanese *Cardiocondyla obscurior* colonies and other insects.

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## Abstract

In species with lifelong pair bonding, the reproductive interests of the mating partners are aligned, and males and females are expected to jointly maximize their reproductive success. Mating increases both longevity and fecundity of female reproductives (queens) of the ant *Cardiocondyla obscurior*, indicating a tight co-evolution of mating partners. Here, we show that mating with a male from their own population increases lifespan and reproductive success of queens more than mating with a male from a different population, with whom they could not co-evolve. A comparison of transcriptomes revealed an increased expression of genes involved in immunity processes in queens, which mated with males from a different population. Increased immune response might be proximately associated with decreased lifespan. Our study suggests a synergistic co-evolution between the sexes and sheds light on the proximate mechanisms underlying the decreased fitness of allopatrically mated queens.

*Keywords:* longevity, outbreeding depression, sexual cooperation, social insects

## 5.1 Introduction

Choosing the right mating partner(s) is one of the most important decisions in the life of a sexually reproducing organism. On the one hand, mating among close relatives may decrease progeny fitness due to homozygosity of recessive, deleterious mutations ('inbreeding depression'; Charlesworth & Charlesworth 1987). On the other hand, mating of distantly related partners may result in the disruption of co-adapted gene complexes ('outbreeding depression'; Templeton 1986; Waser & Price 1989; Edmands 2002). Many studies have documented the costs of mating with a 'wrong partner' by analysing the quantity and fitness of the offspring of the breeding pair. However, mating may also directly affect life history traits of the mating partners themselves, for example when males manipulate females via nutritious nuptial gifts and spermatophores, injurious genital spines or toxic seminal fluids (e.g. Merritt 1989; Chapman *et al.* 1995; Chapman & Partridge 1996; Schoofs *et al.* 1997; Yi & Gillott 1999; Lung *et al.* 2002; Bonduriansky *et al.* 2005). For example, in *Drosophila*, seminal fluids transferred during mating enhance the fecundity of females (Herndon & Wolfner 1995) and stimulate their innate immune system (Peng *et al.* 2005), but at the same time reduce their attractiveness to further mates, their probability of mating again (Chen *et al.* 1988) and their future lifespan (Chapman *et al.* 1995).

Manipulative sexual traits and mate preferences may diverge rapidly among local populations because of Fisherian sexual selection, co-evolutionary arms races driven by sexual conflict, and other causes (Rowe *et al.* 2003). Mating of individuals from different populations (allopatric crosses) therefore may have other consequences for the mating partners than mating of individuals from the same population (sympatric crosses). Several studies indicate that females are able to evolve resistance against manipulating males when they are able to co-evolve with them, but suffer from manipulations after mating with males from another population (e.g. Holland & Rice 1998; Parker & Partridge 1998; Holland & Rice 1999; Nilsson *et al.* 2002). However, the extent of male harm and female resistance might also depend on the local environment and condition of the interacting individuals (e.g. Fricke *et al.* 2009; Arbuthnott *et al.* 2014). It remains controversial whether this indeed reflects an ongoing arms race driven by sexual conflict or whether other processes of sexual selection are responsible (Chapman *et al.* 2003; Rowe *et al.* 2003; Arnqvist 2004; Long *et al.* 2006).

Ants are characterized by lifelong pair bonding, that is queens mate with one or a few males early in their adult life and use the obtained sperm to fertilize all female-destined eggs throughout their lives without ever mating again (Boomsma *et al.* 2005; Boomsma 2009). Hence, the interests of the mating partners are predicted to be aligned and males are not expected to harm their mate or to shorten its lifespan (even though immunity costs of sperm storage and competition among the ejaculates from different males can negatively affect a multiply mated queen; Baer *et al.* 2006; Boer *et al.* 2010). By comparing the lifespans of virgin queens, mated queens and queens mated with sterilized males, we could show that the act of mating and/or the transfer of seminal fluids significantly increase the lifespan and lifetime reproductive success of queens of

the ant *Cardiocondyla obscurior* (Schrempf *et al.* 2005), suggesting ‘sexual cooperation’.

Here, we investigate whether sympatric mating and allopatric mating differently affect the fitness of queens by comparing their longevity and reproductive output. We show that outcrossing drastically lowers the fitness of queens. To elucidate associated physiological mechanisms, we compared genome-wide gene expression of queens from sympatric and allopatric crosses. Upregulation of genes linked to immunity in outcrossed queens suggests that mating with alien males triggers physiological responses typical of infections.

## 5.2 Materials and methods

### 5.2.1 Study organism

Colonies of *Cardiocondyla obscurior* (Wheeler, 1929) were collected in two sites in Bahia, Brazil, *c.* 50 km apart (Ilheus and Una), in February 2004, and in Okinawa, Japan, in June 2005.

*Cardiocondyla obscurior* is a cosmopolitan tramp species, presumably of South-East Asian origin, which through trade with potted plants, fruits, etc., has been distributed across large parts of the tropics and subtropics (e.g. Heinze *et al.* 2006). Colonies are small (Heinze & Delabie 2005), so that our experimental set-up (see below) reflects natural colony size. Queens are short-lived with an average lifespan of 26 weeks (maximum 56 weeks; Schrempf *et al.* 2005), which makes it possible to record the complete reproductive output of a queen.

In our study populations in Brazil, colonies were found in aborted fruits of coconut trees and rolled leaves of lemon trees, while in Japan, colonies were found in bark cavities of coral trees. Since then, all colonies were reared under the same conditions in the laboratory, eliminating possible effects of different environment. Ants were kept in three-chambered nest boxes with a plaster floor in incubators under semi-natural temperature and light cycles (12 h light 30 °C/12 h dark 25 °C) and fed three times a week with honey and pieces of insects (cockroaches and fruit flies). *C. obscurior* is characterized by a male diphenism with wingless fighter males and winged disperser males (Kinomura & Yamauchi 1987; Stuart *et al.* 1987; Cremer *et al.* 2002). Winged males are only produced under extreme environmental conditions (Cremer & Heinze 2003), and colonies usually contain a single wingless male, which eliminates all rival males. Queens mate only during the first few days of their adulthood and queen monogamy appears to be the rule, probably because of the mating monopoly of wingless males. Genetic data from 18 experimentally double-mated queens suggest that queens use sperm only from a single male, in most cases the first one (A. Schrempf and J. Heinze, unpublished).

### 5.2.2 Experimental crosses

We determined longevity (day of eclosion till death) and lifetime reproductive success (total number of male and female sexuals produced) from (i) queens from the Ilheus and the Okinawa population, which had mated with males from their own population (sympatric crosses: Ilheus queen with Ilheus male: IxI,  $n = 12$ ; Okinawa queen with Okinawa male: OxO,  $n = 12$ ), (ii) Ilheus queens, which had mated with males from a different, but relatively close population (allopatric crosses: distance *c.* 50 km, Una: IxU,  $n = 10$ ), and (iii) queens from Ilheus and Okinawa, which had mated with a male from the other population (allopatric crosses: Ilheus queens mated with a male from Okinawa: IxO,  $n = 16$ ; Okinawa queens mated with males from Ilheus, OxI,  $n = 13$ ).

For the experimental crosses, we transferred a queen pupa and 20 workers from their natal nest into a new nest box and added a wingless male pupa from the same or a different population. Males usually mate with virgin queens within a few days after eclosion (Schrempf *et al.* 2005). We counted the number of eggs twice per week until the death of the queen. Workers of *C. obscurior* do not have ovaries; thus, all eggs were laid by the queens. To standardize between colonies, we kept the number of adult workers constant by removing surplus worker pupae. Similarly, all sexual pupae were removed and counted to obtain complete sex ratio data. All queens produced at least one (female) worker offspring, indicating that all queens had been inseminated as males are haploid and can emerge from unfertilized eggs (Schrempf *et al.* 2006).

Where data showed deviance from normality (Kolmogorov–Smirnov tests), we used appropriate nonparametric statistics. For each queen, we had a data point on its longevity and the number of sexuals produced, from which we calculated sex ratio (female sexuals/total sexuals). We were able to distinguish between the effects of queen origin (Ilheus or Okinawa) and mating combination (allopatric or sympatric) by conducting generalized linear models (GLMs) in R version 3.1.2 (R Core Team 2013).

Starting with the full models including queen origin, mating combination and the interaction of both, we successively excluded nonsignificant terms. We considered overdispersion by assuring that the residual deviance was smaller than the degrees of freedom using chi-squared tests. As data on longevity followed a right-skewed distribution, we used a GLM with gamma distributed error structure. For the number of sexuals produced, we used a negative binomial model (`glm.nb()` in the MASS package) as count data typically exhibits overdispersion and negative binomial models account for this. For modelling of sex ratio, we started with a GLM with binomial error distribution, as sex ratio data are proportions (females/total number of sexuals) and finally used a model of the quasibinomial family to deal with overdispersion.

For egg-laying rate, we had continuous data for each single queen over the complete lifespan available. We compared egg-laying rates with a generalized linear mixed model (GLMM, `lme4` package, R version 3.1.2), including colony as random factor and testing for the influence of queen origin, mating combination and the interaction of both. In addition, to indicate the differences between the distinct groups, we compared the means/medians of the colonies and conducted an ANOVA with



subsequent post hoc Tukey's HSD tests for egg-laying (Kolmogorov–Smirnov test n. s.) and median tests with subsequent Bonferroni-adjusted pairwise comparisons for the number of sexuals and sex ratio (Table 5.1 and Table 5.2).

Finally, we compared the lifespans of differently mated queens by a survival analysis. Two queens that were still alive at the end of the experiment and their lifespans were included as censored data.

### 5.2.3 Gene expression and *Wolbachia* strains

To detect underlying physiological causes for the different performance of allopatric and sympatric crosses, we sequenced the transcriptomes of 14 individual Ilheus queens mated with either an Ilheus ( $n = 7$ ) or an Okinawa male ( $n = 7$ ), 3 weeks after the queens had begun to lay eggs. Queen mating types were set up as described above. For each sample, total RNA (Qiagen RNeasy Plus) from whole bodies was converted into cDNA and amplified using oligo-dT and random 9-mer primers (Ovation RNA-Seq System V2, Nugen). Between 17 million and 25 million 100-bp-long reads per sample were generated on an Illumina HiSeq1000 (Encore Rapid Multiplex System, Nugen). Following adapter trimming (cutadapt, Martin 2011), reads were mapped against the reference genome of *C. obscurior* (Schrader *et al.* 2014) using the TOPHAT (v2.0.8b) /BOWTIE (2.1.0.0) package (Trapnell *et al.* 2009; Langmead & Salzberg 2012). Gene counts for all three data sets were obtained with HTSeq-count 0.5.4 (Anders *et al.* 2015) by counting reads that unambiguously mapped to a single gene model. Normalization of raw counts and statistical inference was performed in DESEQ2 (v1.2.9, Love *et al.* 2014) using a Benjamini–Hochberg correction for multiple testing. The implemented filtering function automatically excludes genes with low expression to optimize the number of adjusted *P* values. Raw sequencing reads have been deposited in the NCBI short read archive.

The common endosymbiotic bacterium *Wolbachia* could induce cytoplasmic incompatibilities when insects are infected with different strains, which may contribute to reproductive isolation among populations (Bordenstein *et al.* 2001). To test whether populations have different *Wolbachia* strains, we isolated DNA of individual workers of the different populations and conducted a PCR of the highly variable *wsp* gene using the primers *wsp81F* and *wsp691R* (Braig *et al.* 1998).

## 5.3 Results

### 5.3.1 Experimental crosses

Queens that mated with allopatric males had a reduced longevity ( $16.4 \pm \text{SD } 5.1$  vs.  $21.6 \pm 5.9$  weeks), laid fewer eggs per week ( $7.6 \pm 3.2$  vs.  $12.5 \pm 3.5$  eggs) and had a lower lifetime reproductive success ( $14.9 \pm 12.1$  vs.  $34.7 \pm 42.9$  sexuals) than queens

that mated with a male from their own population. The shortest-lived queen died after 6.5 weeks. Two of the queens were still alive at the end of the experiment (after 29 weeks), and their longevitys were included as censored data.

Longevity was significantly associated with mating combination (allopatric vs. sympatric), but not with queen origin (GLM with a ‘log’ link function and gamma errors: mating combination:  $F_{61} = 12.96$ ,  $P < 0.001$ ; queen origin  $F_{60} = 0.313$ ,  $P = 0.58$ ; the interaction mating combination x queen origin was removed because it was not significant). The survival analysis revealed that sympatrically mated queens lived longer than allopatrically mated queens (Cox F test allopatric vs. sympatric:  $F = 2.50$ ,  $P = 0.0002$ ); yet, the difference between allopatric and sympatric mating within the Brazilian populations was just marginally significant in a pairwise comparison (survival analysis over all five groups:  $\chi^2 = 11.53$ , d.f. = 4,  $P = 0.02$ , pairwise comparison Cox F test: sympatric vs. allopatric: all  $P < 0.04$ , IxI vs. IxU:  $P = 0.06$ ; all other comparisons  $P > 0.1$ ; mean lifespan in weeks  $\pm$  SD: IxI  $21.83 \pm 4.05$ ; OxO  $21.46 \pm 7.58$ ; IxU:  $17.05 \pm 6.21$ ; IxO  $16.5 \pm 5.48$ ; OxI  $15.69 \pm 4.06$ ; Figure 5.1)

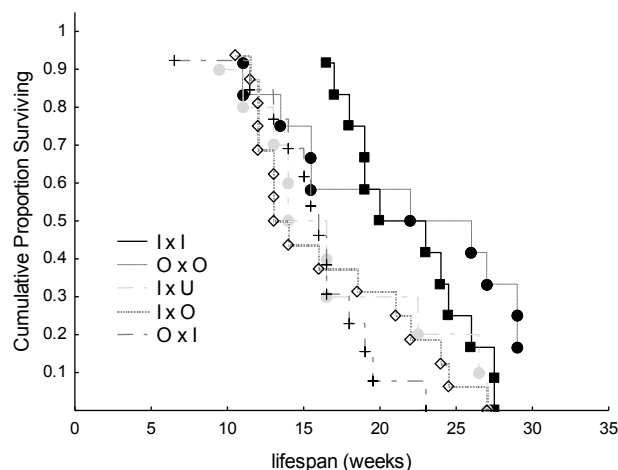


Figure 5.1: Lifespan (weeks) of queens of the ant *Cardiocondyla obscurior* dependent on mating partner (male from the own population: IxI (Ilheus, Brazil, black rectangles) and OxO (Okinawa, Japan, black circles); male from another Brazilian population: IxU (Ilheus x Una, Brazil; grey circles); cross between the Japanese and Brazilian population: IxO (Ilheus x Okinawa, Japan; white diamonds), OxI (Okinawa x Ilheus; crosses)). Censored data are indicated by a black star.

Likewise, the GLMM (Poisson errors with colony origin as random factor) shows that egg number is independent of queen origin ( $\chi^2 = 0.90$ ,  $P = 0.34$ ), but highly affected by mating combination ( $\chi^2 = 24.87$ ,  $P < 0.001$ ). The interaction term queen origin x mating combination was removed from the final model as it was not significant.

The ANOVA with post hoc pairwise comparisons shows that egg number was generally higher in Ilheus queens than in Okinawa queens (ANOVA:  $F_{4,58} = 20.27$ ,  $P < 0.00001$ ; post hoc Tukey’s HSD test; IxI vs. OxO:  $P = 0.005$ ). Sympatrically mated Ilheus queens had more eggs than allopatrically mated Ilheus queens (IxI vs. IxO,  $P <$

0.001; IxI vs. IxU,  $P < 0.02$ ; Figure 5.2). Okinawa queens showed a similar trend, but the difference between sympatrically and allopatrically mated queens was not significant at the 0.05 level (OxO vs. OxI:  $P = 0.068$ ; Figure 5.2).

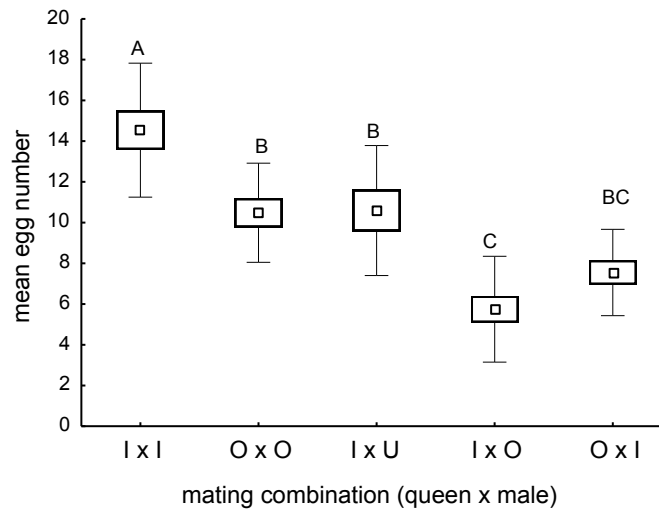


Figure 5.2: Mean ( $\pm$  SE, SD) number of eggs (left y-axis) observed per observation scan in nests with a single queen after mating. Queens mated with a male from their own population (Ilheus, Brazil: IxI, Okinawa, Japan: OxO), a male from a second Brazilian population (Ilheus x Una: IxU) or a reciprocal cross of a Brazilian and Japanese population (Ilheus x Okinawa: IxO, Okinawa x Ilheus: OxI). Significant differences are indicated by different letters.

A GLM (with a negative binomial error distribution and a log link function) shows that total number of sexual offspring was significantly correlated with queen origin but more so with mating combination (queen origin:  $\chi^2 = 6.88$ , d.f. = 60,  $P < 0.01$ ; mating combination:  $\chi^2 = 11.88$ , d.f. = 61,  $P < 0.001$ ; queen origin x mating combination: n. s.). Queens from Ilheus produced significantly more sexual offspring when mating with a male from their own population than in either type of allopatric cross. The number of sexual offspring of sympatrically mated Okinawa queens varied among colonies and overlapped with allopatric crosses (median test:  $\chi^2 = 10.93$ , d.f. = 4,  $P = 0.027$ , for pairwise comparisons see Table 5.1).

Table 5.1: Lifetime production of sexual offspring of queens mated with different males (median, upper and lower quartiles;  $P$  values and  $Z$  values (in parentheses) after post hoc comparison with Bonferroni-adjusted significance levels;  $P^*$  values indicate significant differences between the groups (I: individuals from Ilheus, Brazil; O: individuals from Okinawa, Japan; U: individuals from Una, Brazil)).

	Total reproductive output (median, quartiles)	I×O	I×U	O×O	O×I
I×I, $n = 12$	19.5 (17.5; 27.5)	< 0.03* ( $Z=3.01$ )	1 ( $Z=1.0$ )	1 ( $Z=0.44$ )	1 ( $Z=0.02$ )
O×O, $n = 12$	19.5 (4; 66)	1 ( $Z=2.54$ )	1 ( $Z=0.58$ )	–	1 ( $Z=0.43$ )
I×U, $n = 10$	19.0 (5; 25)	0.77 ( $Z=1.79$ )	–	1 ( $Z=0.58$ )	0.1 ( $Z=1.00$ )
I×O, $n = 16$	5.5 (3.5; 10.5)	–	0.77 ( $Z=1.79$ )	0.11 ( $Z=2.54$ )	0.02* ( $Z=3.06$ )
O×I, $n = 13$	24 (16; 32)	0.02* ( $Z=3.06$ )	1 ( $Z=1.01$ )	1 ( $Z=0.43$ )	–

Finally, sex ratio (number of female sexuals/total sexual offspring) also differed considerably among the five combinations (median test:  $\chi^2 = 25.39$ , d.f. = 4,  $P = 0.001$ ; for pairwise comparisons see Table 5.2). The GLM, in which we controlled for the number of sexuals produced, revealed that sex ratio is strongly associated with both queen origin and mating combination (GLM with quasi-binomial error and a logit link function: queen origin:  $\chi^2 = 123.7$ , d.f. = 56,  $P < 0.001$ ; mating combination:  $\chi^2 = 62.43$ , d.f. = 57,  $P < 0.001$ ; queen origin x mating combination: n. s.).

Table 5.2: Sex ratio of queens mated with different males (I: individuals from Ilheus, Brazil; O: individuals from Okinawa, Japan; U: individuals from Una, Brazil). Sex ratio is calculated as number of female sexuals/total sexual offspring (median, quartiles;  $P$  values and  $Z$  values (in parentheses) after post hoc comparison with Bonferroni-adjusted significance levels;  $P^*$  values differences between the groups).

	Sex ratio (median, quartiles)	I×O	I×U	O×O	O×I
I×I, $n = 12$	0.57 (0.46; 0.68)	0.06 ( $Z=2.73$ )	0.18 ( $Z=2.36$ )	1 ( $Z=1.41$ )	1 ( $Z=1.14$ )
O×O, $n = 12$	0.80 (0.56; 0.875)	0.0004* ( $Z=4.07$ )	0.003 ( $Z=3.61$ )	–	1 ( $Z=0.32$ )
I×U, $n = 10$	0.27 (0.22; 0.33)	1 ( $Z=0.11$ )	–	0.003* ( $Z=3.61$ )	0.006* ( $Z=3.45$ )
I×O, $n = 16$	0.14 (0; 0.5)	–	1 ( $Z=2.73$ )	0.0003* ( $Z=4.07$ )	< 0.001* ( $Z=3.93$ )
O×I, $n = 13$	0.67 (0.61; 0.72)	< 0.001* ( $Z=3.93$ )	0.006* ( $Z=3.45$ )	1 ( $Z=0.32$ )	–

### 5.3.2 Gene expression and *Wolbachia* strains

We analysed whole-body gene expression patterns of individual 4-week-old mature queens. At a false discovery rate of 10 %, we found 13 genes to be significantly differentially expressed between sympatrically and allopatrically mated queens (Table 5.3). A reciprocal blast between the corresponding protein sequences and the *Drosophila melanogaster* proteome (dmel-all-translation-r5.56.fasta) showed orthology for ten of these candidates. The *Drosophila* homolog for Cobs\_00625 was defined as the best hit of a one-way BLASTP against the fruit fly proteome, and the two remaining genes have orthologs in other sequenced ant genomes but not in other insects.

Three of the ten genes more highly expressed in allopatrically compared to sympatrically mated queens are associated with endoplasmic reticulum-associated processes (*Derlin-2*, *ergic53*, CG32276). Three further genes encode for lipid or sterol binding and transporting proteins (*Apolipoprotein lipid transfer particle*, *Niemann–Pick type C-2b*, CG3246). *Hayan* and *Derlin-2* were shown and CG3246 is predicted to be involved in innate immunity. Furthermore, RNA levels of an odorant-binding protein and the transketolase CG8036 were elevated in allopatrically mated queens. Only three genes were less expressed in allopatrically mated queens, including the isocitrate dehydrogenase *lethal (1) G0156* and midnolin-like CG32676.

The 577-bp-long *wsp* sequences from representative workers of each population were identical and matched the *Wolbachia* strain previously described for *C. obscurior* (Russell *et al.* 2012).

Table 5.3: Differentially expressed genes between Brazilian queens mated with a sympatric (IxI; male and female from Ilheus, Brazil) or allopatric male (IxO; Ilheus female mated with a male from Okinawa) with corresponding orthologs in Flybase when present or best blast result in NCBI (*E* values of protein blast are indicated). Adjusted *P* values and fold changes (FC) were generated in DESEQ2 by defining sympatric mating as base level.

Gene ID	BaseMean	<i>P</i> -adj	FC	Flybase ID	Name	<i>E</i> value
Cobs_06240	9479	0.085	1.8	FBgn0034769	<i>Odorant-binding protein 58c</i>	0.020
Cobs_00625	371	0.085	1.6	FBgn0030925	<i>Hayan</i>	1e-33
Cobs_06357	743	0.031	1.6	_	Hypothetical protein <i>SINV_15037</i> [ <i>S. invicta</i> ]	0.0
Cobs_06964	556	0.085	1.4	FBgn0031538	CG3246	2e-132
Cobs_02469	1361	0.085	1.3	FBgn0038198	<i>Niemann-Pick type C-2b</i>	2e-28
Cobs_01349	4423	0.085	1.3	FBgn0032136	<i>Apolipoprotein lipid transfer particle</i>	0.0
Cobs_08680	557	0.085	1.3	FBgn0047135	CG32276	9e-23
Cobs_04691	437	0.031	1.2	FBgn0038438	<i>Derlin-2</i>	6e-113
Cobs_05595	623	0.085	1.2	FBgn0035909	<i>ergic53</i>	0.0
Cobs_11650	3887	0.085	1.2	FBgn0037607	CG8036	0.0
Cobs_08293	661	0.085	0.8	FBgn0027291	<i>lethal (1) G0156</i>	0.0
Cobs_09323	604	0.085	0.8	FBgn0052676	CG32676	2e-34
Cobs_09776	387	0.085	0.8	_	PREDICTED: uncharacterized protein LOC101046512 [ <i>S. boliviensis</i> ]	8e-27

## 5.4 Discussion

Our results show that the origin of their mating partners directly affects the fitness of *Cardiocondyla obscurior* ant queens. Allopatric mating reduced the lifespan of *C. obscurior* queens to about 75 % of the lifespan of sympatrically mated queens and also negatively affected the number of eggs they produced. Surprisingly, matings between sexuals from two neighbouring populations just 50 km apart, which both were presumably introduced to Brazil less than a few hundred years ago, had a similar negative effect on queen longevity as mating with a male from a very distant population in Japan.

#### 5.4.1 Consequences of mating on queen fecundity

Outbreeding depression is commonly associated with decreased reproductive success due to the disruption of co-adapted gene complexes (Lynch 1991; Edmands 2002), maternal effects (Wolf 2000; Kawasaki *et al.* 2010), or through endosymbiotic bacteria, in particular *Wolbachia* (Hoffmann & Turelli 1997; Kawasaki *et al.* 2010; Cordaux *et al.* 2011; Brucker & Bordenstein 2012; 2013). According to the sequence similarity of the *wsp* gene, all studied populations had the same *Wolbachia* strain, speaking against an involvement of this endosymbiont. However, we cannot completely rule out possible double infections or differences in strains, which might have contributed to the observed outbreeding depression.

Alternatively, disruption of co-adapted gene complexes or maternal effects could in principle explain the lower productivity of outcrossed *C. obscurior* queens in our study. Cytoplasmic incompatibility through a mismatch of mother–offspring genes might explain variation in queen bias, that is the propensity of fertilized eggs to develop into queens rather than workers, and consequently also sex ratio. Such a phenomenon has been suggested to cause the variation in queen bias associated with the origin of males and queens in *Pogonomyrmex* harvester ants (Cahan *et al.* 2002; Volny & Gordon 2002; Schwander & Keller 2008), although maternal effects may also play an important role (Schwander *et al.* 2008). As an alternative nongenetic explanation, different treatment by workers might have contributed to the observed differences in our study. Social insect workers are involved in brood care and have the power to manipulate sex ratios (Trivers & Hare 1976). In our study, queens were initially kept with workers from their maternal nests, which were gradually replaced by the queens' own offspring. Assuming that workers are capable of recognizing their relatedness to the queen's offspring, original workers in colonies with allopatrically mated queens might have preferred the queen's sons over 'hybrid' daughters. However, this preference should quickly have vanished with the eclosion of F1 workers. Furthermore, *C. obscurior* queens appear to be capable of predetermining the caste fate of their eggs and workers have only a limited influence on sex ratio if at all (Cremer & Heinze 2002).

#### 5.4.2 Consequences of mating on queen longevity

Our study shows that queens suffer reduced longevity after mating with a male from a different population. This matches previous observations in *Drosophila*, where the life-shortening effects of allopatric mating have originally been interpreted as evidence for sexually antagonistic co-evolution (Rice 1996; Holland & Rice 1998; Parker & Partridge 1998; Holland & Rice 1999). While this phenomenon may also arise through other factors (Rowe *et al.* 2003; Long *et al.* 2006), the original idea of antagonistic evolution remains appealing (Fricke & Arnqvist 2004; Geuverink *et al.* 2009; Matute & Coyne 2010).

By analogy, we propose two mutually nonexclusive mechanisms of sexual selection to explain the decreased life expectancy of outcrossed queens of *C. obscurior*. First, we have previously shown that mating with a sterilized male prolongs the lifespan of queens relative to that of virgin queens even though both sterile-mated and virgin queens laid only few haploid, male-destined eggs (Schrempf *et al.* 2005). This suggests a positive effect of seminal fluid or tactile stimulation during mating on queen physiology. We suggest that sexuals of *C. obscurior* are co-adapted so that queen physiology responds optimally to sympatric males. This ‘sexual cooperation’ (Schrempf *et al.* 2005) might be impaired when queens mate with males from another population.

Second, co-evolution might allow queens mated to males from their own population to avoid costs resulting from ejaculate competition after multiple mating (Boer *et al.* 2010). Traits evolved by males to promote their own sperm at the cost of other males might accidentally harm queens despite sexual cooperation, and queens might be better adapted to neutralize ‘collateral damage’ from copulations with local males. Under laboratory conditions, *C. obscurior* queens have been observed to mate with multiple males, but at present there is no evidence that seminal fluid and sperm are transferred during all copulations. In contrast, genetic maternity analyses show that queens use only sperm from a single male to fertilize their eggs, indicating that multiple mating does not lead to the storage of several ejaculates (A. Schrempf, unpublished). However, a higher sample size is necessary to confirm that sperm mixing never occurs. Moreover, to distinguish between strict sexual cooperation and adaptation to harmful by-products of male–male conflict will require more detailed investigations into the mechanisms of sperm transfer and the frequency of multiple copulations. In both scenarios, divergence of male and female sexual traits among the three populations parsimoniously explains all the results of our study: the shorter lifespan of outbred queens, their lower productivity and also the lower queen bias and the more male-biased sex ratio of their broods.

The alternative nongenetic explanation that workers differentially provision or groom the queen depending on queen fecundity and in this way affect lifespan is unlikely. Previous studies did not reveal worker discrimination among differently fecund queens (Schrempf *et al.* 2005; 2011), and although productivity differences among differently mated Okinawa queens were small, lifespan differences matched those of Ilheus queens, indicating that queen physiology is affected by mating combination and not by workers.

### 5.4.3 Gene expression

In general, seminal proteins evolve rapidly and have been shown to evoke immune responses in females (Peng *et al.* 2005; Rodríguez-Martínez *et al.* 2011). In Ilheus queens, the origin of the mating partner affected the expression of 13 genes (at a FDR < 0.1). While this cut-off allows for 1.3 false-positive genes, the redundancy in gene functions and the analogy to previous studies suggest that our results are reliable. Of the



nine characterized genes overexpressed in allopatrically mated *Ilheus* queens, five have also been found to be overexpressed after single and/or double mating in *Drosophila* (Innocenti & Morrow 2009). Four of these five genes code for proteins suggested to be involved in the innate immune response: *Hayan* is a serine protease activated following injury (Nam *et al.* 2012), CG3246 codes for an antimicrobial protein domain (IPR017943), *Niemann–Pick type C-2b* is one of eight *npc2* genes assumed to have a function in immune signalling pathways (Shi *et al.* 2012), and *Apolipoprotein lipid transfer particle* is overexpressed in immune challenged haemocytes (Johansson *et al.* 2005). Furthermore, *ergic53* is associated with endoplasmic reticulum stress (Chow *et al.* 2013) as are two other overexpressed candidates *Derlin-2* (IPR007599) and CG32276 (IPR010580).

It has been hypothesized that the exclusive overexpression of *Hayan*, *Niemann–Pick type C-2b* and 30 other immune response genes after the second but not the first mating in *Drosophila* females is a counter-reaction to seminal fluids (Innocenti & Morrow 2009). The immune response in our study therefore might similarly be caused by incompatible seminal fluid proteins (comparable to sex peptide influencing the TOLL pathway in *Drosophila* (Peng *et al.* 2005)). Whether undetected variability of *Wolbachia* strains or immune responses resulting from a mismatch of genitalia in allopatric mating (Yassin & Orgogozo 2013) also contribute to the observed differences remains to be studied.

## Conclusion

Male–female co-evolution in species with lifelong pair bonding appears to be an important determinant of reproductive success. In our study, the disruption of co-evolved sexual traits may be an explanation for the overall reduction in fitness in outcrossed queens. Incompatible seminal fluid proteins potentially cause an immune response in females, which in turn reduces their lifespan. Given the interest in the interdependencies among population structure, genetic relatedness and reproductive tactics (Boomsma *et al.* 2005; Boomsma 2007; Bourke 2009; Rankin 2011), we believe that our finding will be of considerable interest for disentangling the complex interrelations between reproductive isolation and sexual selection.

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## Author contributions

A.S., K.v.W. and A.K. carried out the practical work; A.K., A.S., K.v.W., L.S. and J.O. analysed the data and participated in the design of the study; A.S., J.H., J.O. and K.v.W. drafted the manuscript; A.S. and J.H. designed the study. All authors gave final approval for publication.

## Data accessibility

Raw sequencing data have been deposited in SRA under the BioProject accession PRJNA284224 (SRR2033892-SRR2033905).

HTseq-count data, Wolbachia sequences (363 bp alignment) and fitness data: Dryad doi:10.5061/dryad.846qk

# Chapter 6

## General discussion

### 6.1 General considerations

The four studies conducted in the framework of this thesis provide new and important insights into the proximate regulation of longevity in eusocial insect queens. The myrmicine ant *Cardiocondyla obscurior* served as a model for investigations concerning the reversal of the fecundity/longevity trade-off (chapter 2 and 3). This phenomenon is widespread among eusocial insects and seems to be a universal principle of eusocial organisms. The possibility to carry out controlled crossings, as it is routinely done in model organisms, for example in the fruit fly *Drosophila melanogaster*, and the relative short lifespan of the study organism were essential prerequisites for experiments related to the effect of mating (chapter 2) and the effect of mating with varying partners (chapter 4 and 5).

On one side, the state-of-the-art technique RNA-Seq allowed to test concrete hypotheses, whereas on the other side, this approach was to a certain extent exploratory. For example, the differential expression of genes, which are involved in enhanced reproduction, in older (see chapter 2) and injured (see chapter 3) queens gave support for theoretical considerations and was consistent with phenotypic data. For genes with other functions there were no precise expectations. A great advantage of RNA-Seq is that the whole mRNA content of organisms can be quantified. This inspection of the full genetic spectrum can yield interesting and surprising results, which would otherwise remain concealed.

The conclusions drawn from the experiments of this thesis relied basically on the knowledge achieved from the model organism *D. melanogaster*. Even if the functional characterization of genes is by far not complete in the fruit fly, the determination of orthologs contributed substantially to the realization of this work. Barely 7000 out of 17552 genes are reciprocal orthologs and presumably have the same functions in *D. melanogaster* and *C. obscurior* (see Koonin 2005). Although these genes make up only 40 % of the whole *C. obscurior* gene repertoire, the proportion of differently expressed genes (DEGs) with *D. melanogaster* orthologs was generally higher (at least 60 %; see Figure 3.4 and Appendix 7.1.3).

For a comparison with previous transcriptomic studies, the protein coding sequences of two further pioneer species in field of genomics were screened for homologs. The honeybee *Apis mellifera* and the carpenter ant *Camponotus floridanus* were the first eusocial insects for which genome assemblies and electronic gene annotations were obtained (Honeybee Genome Sequencing Consortium 2006; Bonasio *et al.* 2010). These early annotations were incomplete and recently re-evaluated (Elsik *et al.* 2014; Gupta *et al.* 2015). Considering the latest genome versions, *C. obscurior*

shares 8824 reciprocal orthologs with *C. floridanus* and 7948 with *A. mellifera*. The low number of 1:1 orthologs with other eusocial insect genomes is surprising. If the remaining 50 % of genes are orphan genes or *Cardiocondyla*-specific homologs has not been analyzed in detail. As in the comparison with *D. melanogaster*, the proportion of DEGs with orthologs in the carpenter ant and the honeybee was considerably higher (up to 80 %; see Appendix 7.1.3). Therefore, the downstream analysis of the genes with orthologous relationships should be representative for the whole set of DEGs. The remaining DEGs were excluded from the subsequent enrichment tests and cross-study comparisons.

Because comprehensive functional information on genes, for example in the form of Gene Ontology (GO) terms, cannot be derived from other insects than the fruit fly, a transcriptome- or proteome-wide BLAST search is an obligatory step for all eusocial insect microarray and RNA-seq studies. However, previous studies defined different criteria for the adoption of GO terms. Many authors applied a unidirectional BLAST against the *D. melanogaster* annotation, for example by means of the tool Blast2GO, without verifying orthology reciprocally (Yek *et al.* 2013; e. g. Nipitwattanaphon *et al.* 2013; Feldmeyer *et al.* 2013). So far, there is no general agreement whether to use all homologs, which possibly contain paralogs, or exclusively 1:1 orthologs. The stringent strategy used in the experiments of this thesis reduced the risk of comparing genes, which are closely related but have not kept the same functions.

Another unclear point in transcriptomics is the definition of false discovery rate (FDR)- and fold change-cutoffs for DEGs. In principle, the investigator is free to adjust the application of such thresholds to the purpose of the study. Downstream analyses usually profit from larger input lists. Thus, overly stringent values might only be helpful if the focus is on a specific set of genes. As it is common in this field, an FDR of 0.05 was applied in the majority of tests for differential expression (chapter 2–4). Because the number of DEGs was comparably low in one study (chapter 5), the proportion of false positives was increased to 10 %. Additional filtering criteria were not used. The various physiological insights revealed by the corresponding gene lists are discussed in the following paragraphs.

## 6.2 Results concerning the reversal of the fecundity/longevity trade-off

The DEGs between 4-week-old and 18-week-old queens (chapter 2) present the first report of genome-wide age-related expression changes in eusocial insect queens. Because of their long lifespan, high vitality at old ages and general low mortality, it is assumed that queens do not suffer from senescence. The idea of negligible senescence in female reproductives of eusocial animals (Finch 1990; Buffenstein 2008) is now supported by our comparison of young and aged individuals. First, the higher expression of reproduction-associated genes in older queens is consistent with an increase in egg-laying rate with age (Heinze & Schrempf 2012; Kramer *et al.* 2015). Second, no other senescence-related changes, as for example an elevation of the

immune response as in *D. melanogaster* (Pletcher *et al.* 2002; Landis *et al.* 2004; Girardot *et al.* 2006; Curtis *et al.* 2007; Doroszuk *et al.* 2012), could be identified. The most important finding of this study was that gene expression changes are reversed in *C. obscurior* queens in comparison to *D. melanogaster* females reflecting the opposite life histories in ants and fruit flies. Oppositely regulated processes are mainly reproduction and metabolic processes involving carbohydrates, amino acids and alcohol. This indicates that an increase in reproductive effort is accompanied by a slowed metabolism.

The connection between aging and metabolism seems to be more complex than initially postulated (see rate of living theory; reviewed in Speakman 2005), but the generation of toxic by-products by metabolic reactions provides mechanistic support for an association (Harman 1956). According to the oxidative stress theory, either the production of less damage or the better protection against damage in queens than in workers or females of solitary insects could be responsible for the longevity of queens. Previous experiments gave evidence for the first possibility because antioxidant enzymes were shown to have lower expression and activity levels in reproductive than in non-reproductive females in the three Hymenopteran species *Apis mellifera*, *Lasius niger* and *Harpegnathos saltator* (Parker *et al.* 2004; Corona *et al.* 2005; Schneider *et al.* 2011). Moreover, the expression of antioxidant enzymes and mitochondrial genes involved in respiration showed a significant age-related decline in honeybee queens (Corona *et al.* 2005). The RNA-seq data of *C. obscurior* queens reveals that *Superoxide dismutase*, a homolog of *Glutathione peroxidase* and *Glutathione S transferase D1* are downregulated in older queens (Table 6.1). None of the antioxidant enzyme genes tested in honeybees is upregulated. Unfortunately, mitochondrial genes could not be screened because a mitochondrial genome has not been assembled yet for *C. obscurior*.

Table 6.1: Differential expression of antioxidant enzymes between 18-week-old and 4-week-old *C. obscurior* queens. If a gene is duplicated, it is indicated which gene is the ortholog (o) or a non-orthologous homolog (h) to the *D. melanogaster* gene.

Name	Gene ID	BaseMean	P-adj	Fold change
Superoxide dismutase	Cobs_08876	35	5.5e-06	0.5
Superoxide dismutase 2 (Mn)	Cobs_13627	455	0.4	1.1
Superoxide dismutase 3	Cobs_08867	1356	0.9	1.0
Catalase	Cobs_01787	1661	0.4	0.9
Glutathione peroxidase (PHGPx)	Cobs_09272 (h) Cobs_18028 (o)	1098 460	6.4e-03 0.1	0.6 0.8
Thioredoxin reductase-1	Cobs_10638	888	0.7	1.0
Glutathione S transferase D1	Cobs_05225 (o) Cobs_00703 (h)	668 0	1.2e-07 NA	0.6 NA

Calorimetric measurements in honeybees demonstrated that metabolic rates decrease with age in queens, but increase in workers (Fahrenholz *et al.* 1992). The authors examined virgin and relatively young egg-laying queens. Thus, the difference between

workers and queens might be even more pronounced at older ages. Building on the knowledge gained so far, it could be the next step to test if *C. obscurior* queens accumulate less damage than workers, and if so because of a slowed metabolism. In the Damaraland mole-rat, protein carbonyls and malondialdehyde, which are biomarkers for oxidative damage, are less abundant in specific tissues of reproductive than of non-reproductive females (Schmidt *et al.* 2014).

In the light of the disposable soma theory (Kirkwood & Austad 2000), the idea of 'metabolic quiescence' only makes sense if queens invest less resources into self-maintenance than workers and solitary insects. In social insects, resources might not have to be allocated between reproduction and self-maintenance because each individual of the colony is responsible for just one of these two tasks. The workers of a colony can be regarded as the disposable soma that protects the germ line, i. e. the queen. The importance of work load was highlighted by previous studies on *Diacamma* subordinate workers, which live shorter when their selfish dominant nest-mates start to reproduce (Tsuji *et al.* 2012). Reproductive behavior might not be less metabolically intense than the tasks carried out by the workers, as for example foraging, nest guarding, cleaning and nursing, but the execution of the latter may require the additional activation of energy-demanding maintenance systems, for example the immune response. The above described results regarding lower antioxidant enzyme levels, and the repression of 15 immune genes in older, more fertile *C. obscurior* queens (Table 6.2) indicate that reproductives invest less into stress resistance and are consequently to some degree released from the costs associated with these expensive defense systems.

Table 6.2: Upregulated (fold change > 1) and downregulated (fold change < 1) immune genes in 18-week-old compared with 4-week-old queens. Immune genes were identified by means of the *D. melanogaster* annotation (see chapter 3).

Symbol	Category	Gene ID	BaseMean	<i>P</i> -adj	Fold change
CG13618	undesignated	Cobs_10873	o 111	3.8e-10	0.4
CG14661	undesignated	Cobs_04248	h 18	8.5e-9	0.4
CG10345	IMD	Cobs_10252	o 104	1.8e-9	0.4
Snmp1	IMD	Cobs_00383	o 32	6.9e-6	0.5
CG1358	undesignated	Cobs_09767	h 116	9.0e-6	0.5
Toll-6	TOLL	Cobs_01322	h 10	5.5e-4	0.5
CG9701	undesignated	Cobs_17851	o 36	8.5e-5	0.5
CG14661	undesignated	Cobs_04247	h 330	5.2e-4	0.6
PebIII	Antimicrobial peptide	Cobs_03389	h 3472	8.2e-3	0.6
emp	IMD	Cobs_00088	o 166	4.5e-5	0.6
CG14661	undesignated	Cobs_04238	o 384	2.0e-2	0.6
DNaseII	Cell cycle regulation	Cobs_01796	o 62	3.4e-3	0.7
Pu	Humoral response	Cobs_18022	o 282	7.9e-5	0.7
Nos	IMD	Cobs_06158	o 176	9.0e-3	0.8
ref(2)P	Antimicrobial peptide	Cobs_14221	o 1547	3.7e-2	0.8
Tsf3	Cell cycle regulation	Cobs_01059	o 548	5.4e-3	1.2
N	Humoral response	Cobs_08231	o 451	4.2e-2	1.3
AGO2	Antimicrobial peptide	Cobs_05737	h 102	3.4e-2	1.4

Based on the trade-off between reproduction and stress response, two life history modes have been proposed for solitary animals by Tatar *et al.* (2003). Quiescence, in which reproduction and metabolism is reduced to ensure survival under adverse conditions, corresponds to diapause and hibernation. Analogous to this model, a new mode termed 'social reproduction' is suggested for eusocial insect queens, in which the investment of resources into self-maintenance is low to facilitate a continuous high rate of egg production (Figure 6.1).

#### Life history modes

	Reproduction	Metabolism	Stress response	Solitary	Social
Reproduction	+	+	–	favorable conditions	
Quiescence	–	–	+	adverse conditions e.g. diapause, stress/immune response	
Social reproduction	+	–	–		favorable conditions

Figure 6.1: Life history modes in reproductive females of solitary and social animals. The reproductive and quiescent modes are based on the model by Tatar *et al.* (2003). Survival of social insect queens under favorable conditions might not require an elevation of the stress response. The additional deployment of maintenance mechanisms under adverse conditions necessitates the decrease in reproduction.

According to this model, reproduction and stress response are negatively associated in social insects despite the reversal of the fecundity/longevity trade-off. As in solitary insects, this interaction might be mediated by juvenile hormone (JH), which was shown to promote egg production and to suppress stress resistance and immunity leading to reduced survival in a solitary (Flatt *et al.* 2005, and references therein), but possibly not in a social environment. JH titers were not directly measured in *C. obscurior* queens, but the age-related expression changes of genes involved in JH biosynthesis and degradation suggest higher levels in older, more fertile queens (Table 6.3). Therefore, it is unlikely that JH has reversed its positive gonadotropic function as proposed by some authors for other eusocial insect species (Corona *et al.* 2007; Pamminer *et al.* 2016).

Table 6.3: Age-related expression changes of enzymes involved in the biosynthesis and degradation of juvenile hormone in *C. obscurior*. Fold change > 1 refers to higher expression in 18-week-old compared with 4-week-old queens. No other homologs were found in the *C. obscurior* genome.

Name	Function	Gene ID	BaseMean	<i>P</i> -adj	Fold change
JH acid methyltransferase	JH biosynthesis	Cobs_04150	387	1.0e-4	1.5
JH epoxide hydrolase 2	JH catabolism	Cobs_05866	o 2060	0.04	0.8
	JH catabolism	Cobs_05631	h 463	0.2	0.8

The model also predicts that defense systems can only be activated at the expense of a reduced fecundity, similar to the quiescent stage in solitary insects. That the costs of reproduction can be detected indirectly by increasing immunity costs was clearly demonstrated by the amputation of both middle legs (chapter 3). Both egg-laying rates and the expression of genes involved in germ cell development showed a significant decline after injury. Notably, *C. obscurior* queens are extremely robust and survive the loss of one or several legs, which was observed to occur in laboratory colonies. The consequences of this injury on fitness seem to be temporary.

### 6.3 Results concerning the effect of mating

The positive effect of single-mating on queen survival was revealed by Schrempf *et al.* (2005). This result was pioneering, but it did not allow conclusions on whether virgin queens have a higher age-independent background mortality or whether they age faster. Considering new data which was collected in the framework of this thesis (see 4.3.1), the former case may be more likely because background mortality was higher in virgin queens, whereas age-specific mortality rate was similar for both virgin and mated queens. However, as usually much larger sample sizes are required to accurately estimate Gompertz parameters (Promislow *et al.* 1999), this finding should be treated with care for the moment.

Importantly, survival of queens that had mated with a single ergatoid male (chapter 2 and 4) was higher than expected from average lifespan estimates of previous experiments (Schrempf *et al.* 2005; Schrempf & Heinze 2008; Heinze & Schrempf 2012; see Appendix 7.3.11). Even more surprising, the survival advantage of queens mated with a winged disperser male over queens mated with an ergatoid male was reversed (Schrempf & Heinze 2008; chapter 4). In contrast, median lifespans of virgin queens were consistent across studies (Schrempf *et al.* 2005 and chapter 4). Therefore, this discrepancy cannot be explained by differences between *C. obscurior* populations or experimental conditions. A factor that likely had an impact is the degree of female-male coevolution. Queens which were mated with a male from the same collection site lived longer, laid more eggs and showed a decreased expression of genes involved in immune and stress response than queens which were mated with an allopatric male (chapter 5). All queens examined in the experiments of chapter 2 and 4 originated from



one single colony collected in Una (Brazil) in 2009. Since this colony was brought to the laboratory, queens have been regularly exposed to closely related ergatoid males, but never to males from other colonies and presumably not to winged males. Inbreeding and monogamy are predicted to reduce sexual conflict (Holland & Rice 1999; Hosken *et al.* 2001; Chapman *et al.* 2003) and promote sexual cooperation (Schrempf *et al.* 2005). To what extent the effect of mating in *C. obscurior* and other eusocial insects is shaped by the adaptation to male-induced benefits, male-induced harm, or both, and if accessory gland proteins are proximately involved, are open questions.

Fitness differences between shorter-lived virgin and longer-lived mated queens are reflected in the whole body transcriptomes of mature queens. A deeper sequencing in the second experiment (chapter 4) enabled the discovery of vastly more differently expressed genes than in the first study (chapter 2) at the same significance cutoff. Combining the results of both sequencing runs, longer-lived queens possibly have a lower carbohydrate metabolism, which is consistent with a downregulation of specific metabolic processes with increasing age and fecundity, and produce more eggs as it is predicted from the positive correlation between egg-laying rate and lifespan. Enhanced neuron development is an unexpected finding because Hymenoptera do not seem to have adult neurogenesis (Fahrbach *et al.* 1995b; Gronenberg *et al.* 1996). A possibility is that these genes induce structural changes, as for example axon growth and the formation of synapses, in already existing cells (Farris *et al.* 2001; Groh *et al.* 2006). Environmental stimuli, in particular social interactions, could be responsible for an increase in brain volume (Scotto Lomassese *et al.* 2000; Scotto-Lomassese *et al.* 2002; Molina *et al.* 2009; Smith *et al.* 2010).

The DEGs between mated and virgin queens from both experiments did not overlap with gene lists from former studies which focused on the short-term effect of mating in *D. melanogaster* and *A. mellifera* (McGraw *et al.* 2004; Kocher *et al.* 2008; 2010; Manfredini *et al.* 2015; see Table 2.5 and Appendix 7.3.9). Interestingly, more genes were shared between these previous transcriptomic investigations and the DEGs resulting from the comparison between sham-mated and virgin queens. Time course data shows that the majority of gene expression changes after mating are transient (McGraw *et al.* 2008; Zhou *et al.* 2014). Due to the lack of fertilization, sham-mated queens might not complete the transitions that normal mated queens undergo.

## 6.4 Conclusion

Eusocial insect queens do not seem to suffer 'mortality costs' from reproduction or mating. Transcriptomic experiments reveal that proximate mechanisms of *Cardiocondyla obscurior* queen longevity involve oogenesis, metabolism, immunity and neural activity. Furthermore, comparisons with reproductive females of the fruit fly *Drosophila melanogaster* show that conserved genes which are associated with senescence in solitary species experience a reversal in gene expression patterns. The alternative regulation of genes that mediate the interplay among aging, egg-laying and

mating might be responsible for the exceptional positive association of fecundity and longevity in eusocial species. An experimental increase of immunity costs demonstrates that the stress response can only be upregulated at the expense of a reduced production of eggs as predicted by life history theory. Consequently, queens might not avoid the costs of reproduction, but the costs of self-maintenance which can be borne by the workers under favorable conditions.

# Chapter 7

## Appendix

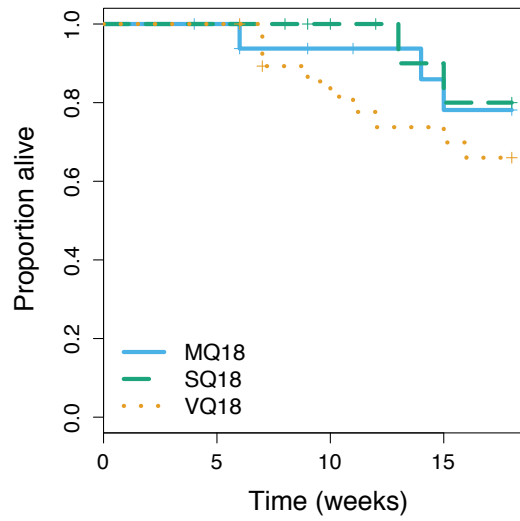
### 7.1 Appendix for Chapter 2

#### 7.1.1 Survival and fecundity of queens

We analyzed survival of queens beyond the age of four weeks with the package *Survival* version 2.37-7 (Therneau 2015) in R. Data on queens, which died from other causes, as for example squeezed by the glass cover of the nest, or in whose colonies males or additional queens had eclosed, were included as censored data. One-way ANOVA was applied to ln-transformed data on fecundity of the experimental 18-week-old queens in SPSS (22.0).

Log-rank test did not reveal a significant difference in survival between the mated, sham-mated, and virgin queens until the age of 18 weeks when they were censored (Kaplan-Meier estimates: MQ18: 0.78, SQ18: 0.8, VQ18: 0.66,  $P = 0.4$ ). Similarly, a re-analysis of data from the previous study of (Schrempf *et al.* 2005) revealed that up to week 18 the MQ, SQ, and VQ survival did not differ ( $P = 0.09$ ).

MQ18 produced more eggs per week than the other two types of queens (mean  $\pm$  standard deviation: MQ: 18.8  $\pm$  2.9, SQ: 7.5  $\pm$  0.8, VQ: 4.7  $\pm$  0.5). Egg-laying rates were also significantly different between sham-mated and virgin queens (ANOVA on ln-transformed number of eggs laid per week with post hoc Bonferroni t-test, all pairwise: VQ18-MQ18, SQ18-MQ18, VQ18-SQ18:  $P < 0.001$ ). MQ18 started to lay eggs earlier than virgins did, but the onset of reproduction was not different between SQ18 and VQ18 (mean  $\pm$  standard deviation of queen age at first egg-laying MQ18: 6.9  $\pm$  2.0, SQ18: 8.9  $\pm$  2.7, VQ18: 14.0  $\pm$  7.1; ANOVA on ln-transformed values with post hoc Bonferroni t-test, all pairwise: VQ18-MQ18:  $P = 0.023$ , SQ18-MQ18:  $P = 0.7$ , VQ18-SQ18:  $P = 0.3$ ).



Plot of the survival of queens destined for sequencing at the age of 18 weeks. MQ18, mated queens; SQ18, sham-mated queens; VQ18, virgin queens. Markings illustrate censored individuals.

## 7.1.2 Mapping statistics of reads

Sample-wise number of raw, adapter-trimmed and mapped reads; the proportion of mapped reads is given in the last column.

Sample	Treatment	Raw	Trimmed	Mapped	%
BB1	MQ4	18222123	18222118	6439873	35
BB14	MQ4	22467486	22467483	6524822	29
BB54	MQ4	18630022	18630020	5950027	32
BB51	MQ4	23378463	23378452	8288877	35
BB16	MQ4	17817900	17817897	6477525	36
BB17	MQ4	17205236	17205230	5899270	34
BB15	MQ4	24895029	24895016	8903601	36
M12	MQ18	26731190	26731179	8403595	31
M14	MQ18	24393533	24393524	8226756	34
M15	MQ18	28309885	28309879	8298313	29
M16	MQ18	22568062	22568053	4697763	21
M6	MQ18	22373637	22373636	7097131	32
M8	MQ18	16144761	16144756	4439243	27
M9	MQ18	24219045	24219033	8120858	34
SM12	SQ18	21103588	21103578	6562783	31
SM13	SQ18	27473219	27473215	8452637	31
SM14	SQ18	21976024	21976013	7042177	32
SM20	SQ18	22430021	22430017	6299139	28
SM29	SQ18	29800830	29800823	9239136	31
SM30	SQ18	26877685	26877672	8620130	32
SM7	SQ18	23076134	23076129	6072563	26
V11	VQ18	21100665	21100659	5845971	28
V12	VQ18	23732612	23732606	7822134	33
V3	VQ18	20325873	20325866	5258076	26
V7	VQ18	21429548	21429542	7334315	34
V8	VQ18	21134934	21134925	7511966	36
V9	VQ18	21462076	21462068	5398643	25
Mean		22565910	22565903	7008419	31

### 7.1.3 List of similar experiments

The total number of reported DEGs is given after conversion to new IDs. In accordance with the studied organism, either the set of *Drosophila* or the set of *Apis* orthologs was used to determine the number and proportion of comparable DEGs.

Dataset	Author	Year	Set of orthologs	Body part	DEGs	DEGs in set of orthologs	Proportion
Young vs. aged queens; mated vs. sham-mated vs. virgin queens	this study		<i>Drosophila</i>	Whole body	1003	665	0.7
Mated vs. sham-mated vs. virgin queens	this study		<i>Apis</i>	Whole body	371	312	0.8
Young vs. aged mated females flies (7 days vs. 23 days)	Pletcher <i>et al.</i>	2002	<i>Drosophila</i>	Whole body	648	310	0.5
Mated vs. virgin female flies (1-3h postmating)	McGraw <i>et al.</i>	2004	<i>Drosophila</i>	Whole body	1790	873	0.5
Reproductive vs. sterile workers (10-day-old)	Grozinger <i>et al.</i>	2007	<i>Apis</i>	Brain	99	80	0.8
Mated vs. intermediate-mated vs. virgin queens (5 days postmating)	Kocher <i>et al.</i>	2008	<i>Apis</i>	Brain, ovary	1076	837	0.8
Mated vs. virgin queens (2 days postmating)	Kocher <i>et al.</i>	2010	<i>Apis</i>	Brain	175	129	0.7
Reproductive vs. sterile workers (18-day-old)	Cardoen <i>et al.</i>	2011	<i>Apis</i>	Whole body	1261	1041	0.8
High pollen vs. low pollen-hoarding workers (< 1-day-old)	Wang <i>et al.</i>	2012	<i>Apis</i>	Ovary	2151	1756	0.8
Young vs. old virgin females (10 % vs. 90 % survival)	Doroszuk <i>et al.</i>	2012	<i>Drosophila</i>	Whole body	3239	1870	0.6
Young vs. middle-aged virgin and mated female flies (3 to 5-day-old and 4-week-old)	Zhou <i>et al.</i>	2014	<i>Drosophila</i>	Whole body	649	162	0.2

## 7.1.4 List of DEGs

All genes differently expressed in at least one of the four pairwise tests. Mean, mean of counts per gene across replicates; logFC, log<sub>2</sub>-transformed fold change; *P*-adj, BH corrected *P* value.

	MQ18 versus MQ4			VQ18 versus MQ18			VQ18 versus SQ18			SQ18 versus MQ18		
	Mean	logFC	<i>P</i> -adj	Mean	logFC	<i>P</i> -adj	Mean	logFC	<i>P</i> -adj	Mean	logFC	<i>P</i> -adj
Cobs_00021							106	0.62	2.0e-02			
Cobs_00067	556	-0.2	3.3e-02	560	0.27	2.8e-02						
Cobs_00070	835	-0.2	4.1e-02									
Cobs_00088	166	-0.8	4.5e-05									
Cobs_00096							140	0.47	1.1e-02			
Cobs_00102							35	0.52	3.5e-02			
Cobs_00114	628	-0.4	1.6e-02									
Cobs_00115	737	0.4	1.9e-04				821	-0.35	4.0e-02			
Cobs_00129	60	-0.4	4.4e-02									
Cobs_00145	20	0.6	4.2e-02									
Cobs_00163							513	-0.34	2.0e-02			
Cobs_00180				41	0.51	4.0e-02						
Cobs_00193	4427	0.3	4.4e-02									
Cobs_00202	391	0.3	1.9e-02									
Cobs_00218	341	0.4	6.4e-03									
Cobs_00231	348	0.3	1.0e-02									
Cobs_00239	489	-0.6	1.6e-02									
Cobs_00252	11	-1.3	4.0e-06									
Cobs_00346	350	-0.3	2.3e-02									
Cobs_00347	36	-1.0	9.2e-07									
Cobs_00356	109	0.4	1.6e-02									
Cobs_00376	334	0.4	2.3e-02									
Cobs_00378							223	-0.39	2.6e-02			
Cobs_00383	32	-1.1	6.9e-06									
Cobs_00397							89	-0.52	4.2e-02			
Cobs_00442	174	-0.8	3.4e-07									
Cobs_00506	37	-1.0	1.0e-04				24	0.6	3.4e-02			
Cobs_00579	33	-0.6	3.2e-02									
Cobs_00582	23	-1.1	8.5e-06									
Cobs_00587	17	-0.7	9.7e-03									
Cobs_00591	267	-0.8	1.8e-02									
Cobs_00621	95	-0.5	3.8e-03									
Cobs_00641							577	0.51	3.8e-02			
Cobs_00670	1465	-1.2	1.2e-08									
Cobs_00710							164	-0.55	1.6e-02			
Cobs_00713	13	0.6	3.4e-02									
Cobs_00767	282	0.2	4.9e-02									
Cobs_00768							157	0.48	1.2e-02			
Cobs_00805	268	-0.8	1.7e-02									
Cobs_00868							86	0.72	3.5e-03			
Cobs_00875							124	-0.42	1.6e-02			
Cobs_00879	452	0.3	2.8e-02									
Cobs_00885							50	0.54	1.1e-02			
Cobs_00906	742	0.3	3.0e-02									
Cobs_00907	63	-0.5	6.5e-03									
Cobs_00916							111	0.43	1.7e-02			
Cobs_00923	6291	1.1	2.9e-10									
Cobs_00927	54	0.9	1.5e-05									
Cobs_00933	112	-0.5	5.2e-03									
Cobs_00934							234	0.46	2.6e-02			
Cobs_00950							46	-0.47	4.2e-02			
Cobs_00964							57	0.67	4.4e-03			
Cobs_01014	288	0.4	9.4e-03				302	-0.39	3.0e-02			
Cobs_01018	81	-1.1	8.9e-05									
Cobs_01020	999	-0.7	1.7e-04									
Cobs_01025	549	-0.6	2.3e-02									
Cobs_01045	61	-0.7	5.3e-04				49	0.44	4.8e-02			
Cobs_01047										704	0.35	3.6e-03
Cobs_01053	380	0.3	2.9e-02									
Cobs_01059	548	0.3	5.4e-03									
Cobs_01070	187	-1.7	8.3e-11									
Cobs_01100	99	0.3	4.6e-02				104	-0.54	4.8e-03			
Cobs_01116	188	-0.3	3.7e-02									
Cobs_01117							142	-0.39	1.5e-02			
Cobs_01124	154	-0.8	2.4e-10	125	0.49	2.4e-02	125	0.54	4.1e-03			
Cobs_01143	30	-0.6	1.8e-02									
Cobs_01147							75	-0.42	4.2e-02			

	MQ18 versus MQ4			VQ18 versus MQ18			VQ18 versus SQ18			SQ18 versus MQ18		
	Mean	logFC	P-adj	Mean	logFC	P-adj	Mean	logFC	P-adj	Mean	logFC	P-adj
Cobs_01162	351	0.4	4.0e-02									
Cobs_01166	63	0.5	2.1e-02									
Cobs_01171	88	-0.9	1.2e-05	76	0.64	2.4e-02	76	0.53	3.6e-02			
Cobs_01190	429	-0.2	3.8e-02									
Cobs_01193							181	0.65	2.4e-02			
Cobs_01195	408	-1.7	7.1e-18	250	0.79	2.1e-04						
Cobs_01203	225	0.3	2.6e-02									
Cobs_01221	171	0.5	8.8e-04									
Cobs_01240				314	0.34	7.4e-03	314	0.25	3.8e-02			
Cobs_01277	68	-0.6	1.4e-02									
Cobs_01279	107	-1.6	1.4e-12									
Cobs_01306	85	-0.6	2.0e-03									
Cobs_01309	2256	-0.6	8.1e-12				1936	0.41	5.0e-03			
Cobs_01311	72	-0.7	4.5e-04				57	0.54	2.6e-02			
Cobs_01312	54	-0.7	1.4e-05				40	0.59	2.9e-02			
Cobs_01322	10	-1.0	5.5e-04									
Cobs_01359										357	0.47	4.9e-02
Cobs_01382	73	-1.0	7.5e-11									
Cobs_01441	14	-0.8	4.8e-03									
Cobs_01442	315	0.3	2.3e-02									
Cobs_01474	82	-0.4	3.4e-02									
Cobs_01486	50	-0.5	2.2e-02									
Cobs_01567	104	-0.6	5.0e-03									
Cobs_01583	154	-1.0	4.5e-04									
Cobs_01594	162	0.4	1.0e-02									
Cobs_01637	42	-1.0	1.1e-06									
Cobs_01644	437	-0.6	7.8e-03									
Cobs_01682	2146	-1.1	4.3e-09									
Cobs_01757	119	-0.6	3.2e-02									
Cobs_01768	121	-0.6	1.3e-03	127	0.58	4.6e-02						
Cobs_01771	372	0.2	4.3e-02									
Cobs_01779	109	0.9	9.6e-07									
Cobs_01782	79	-0.7	4.1e-04									
Cobs_01783	598	0.3	3.9e-02									
Cobs_01796	62	-0.6	3.4e-03									
Cobs_01798							96	-0.38	4.8e-02			
Cobs_01820	446	-0.7	2.7e-07									
Cobs_01828							74	-0.42	4.7e-02			
Cobs_01899	28	-0.9	2.0e-03									
Cobs_01901	640	0.4	1.2e-02									
Cobs_01907							589	-0.25	1.2e-02			
Cobs_01908							842	-0.29	2.9e-02			
Cobs_01914	774	-0.5	2.8e-03									
Cobs_02011	35	-0.5	3.8e-02									
Cobs_02020	1100	0.2	3.2e-02									
Cobs_02075	50	-0.5	1.2e-02									
Cobs_02145	56	-0.4	4.9e-02									
Cobs_02152	525	0.3	1.8e-03									
Cobs_02166							561	0.72	2.9e-03			
Cobs_02168	1127	0.2	4.7e-02									
Cobs_02174	504	-0.8	8.3e-04				358	0.51	4.5e-02			
Cobs_02185	117	-0.8	1.4e-03				90	0.82	3.5e-03			
Cobs_02194	42	-1.2	1.3e-09									
Cobs_02231	22	1.0	1.4e-03									
Cobs_02246	360	0.3	2.4e-02									
Cobs_02247							276	0.37	3.3e-02			
Cobs_02251	13	-0.7	3.4e-02									
Cobs_02253							594	0.39	3.8e-02			
Cobs_02268	122	-0.4	1.0e-02									
Cobs_02283	894	0.3	2.2e-02									
Cobs_02360	437	-1.4	8.0e-09									
Cobs_02362							312	-0.27	3.1e-02			
Cobs_02373	190	0.3	4.7e-02									
Cobs_02406	200	-0.5	1.3e-03									
Cobs_02459	600	0.2	1.8e-02									
Cobs_02469	891	-0.5	9.0e-04									
Cobs_02475							410	-0.29	3.9e-02			
Cobs_02490							86	-0.67	9.1e-03			
Cobs_02493	715	-1.5	1.1e-07	479	1.05	1.4e-05	479	0.63	2.9e-02			
Cobs_02515							227	-0.54	2.3e-03			
Cobs_02525	861	-0.4	3.4e-03				798	0.31	3.6e-02			
Cobs_02537	194	-0.4	2.3e-03									
Cobs_02551	412	0.2	3.4e-02									
Cobs_02574	183	-0.5	3.4e-03				137	0.55	2.0e-02			
Cobs_02624							58	0.45	3.5e-02			











	MQ18 versus MQ4			VQ18 versus MQ18			VQ18 versus SQ18			SQ18 versus MQ18		
	Mean	logFC	P-adj	Mean	logFC	P-adj	Mean	logFC	P-adj	Mean	logFC	P-adj
Cobs_07714	576	0.3	2.4e-02									
Cobs_07719	726	-0.7	3.8e-10				554	0.51	7.4e-03			
Cobs_07725	1192	-0.5	7.5e-03									
Cobs_07808	347	0.2	2.9e-02									
Cobs_07831							457	-0.38	1.1e-02			
Cobs_07835							52	0.61	1.1e-02			
Cobs_07839							411	0.62	1.2e-02			
Cobs_07842							167	-0.43	1.1e-02			
Cobs_07843	33	-0.6	2.1e-02									
Cobs_07846	5959	-0.4	2.9e-02									
Cobs_07848	30	-1.0	3.1e-07									
Cobs_07854	7	-0.8	9.7e-03									
Cobs_07880	136	-0.6	9.7e-04				112	0.43	3.9e-02			
Cobs_07881	21	-0.8	5.8e-03									
Cobs_07891	43	0.5	1.8e-02									
Cobs_07893	61	-0.5	3.4e-02									
Cobs_07903	77	-0.7	6.1e-05									
Cobs_07931	340	-0.6	2.2e-03									
Cobs_07937							328	-0.36	4.2e-02			
Cobs_07940	215	-0.8	1.5e-04									
Cobs_07963							92	0.41	4.8e-02			
Cobs_08107	33	-0.7	1.3e-03									
Cobs_08123	213	-0.8	2.5e-06				172	0.69	5.1e-03			
Cobs_08125	394	0.3	3.8e-04									
Cobs_08142							149	0.43	3.0e-02			
Cobs_08162	104	-0.5	7.2e-03									
Cobs_08169	195	-0.9	4.7e-03				125	0.51	4.4e-02			
Cobs_08175							334	0.78	3.5e-03			
Cobs_08184	52	-0.6	4.4e-03									
Cobs_08196	2773	-0.9	1.7e-10									
Cobs_08211	227	-0.5	2.2e-02									
Cobs_08231	451	0.4	4.2e-02									
Cobs_08253							331	-0.26	2.7e-02			
Cobs_08259	900	0.4	4.1e-02									
Cobs_08302	613	0.2	3.4e-02									
Cobs_08304	17	-1.0	1.8e-04									
Cobs_08305	185	-0.4	8.3e-03									
Cobs_08313							135	-0.46	2.1e-02			
Cobs_08321	33	-1.1	1.3e-04				18	0.81	3.5e-03			
Cobs_08331	45	-1.0	2.6e-08	35	0.54	4.9e-02	35	0.45	4.9e-02			
Cobs_08335	104	-0.6	1.1e-04	109	0.7	1.1e-02	109	0.53	4.2e-02			
Cobs_08336	55	-0.5	1.4e-02									
Cobs_08344	376	0.3	4.4e-02									
Cobs_08354							343	-0.46	1.1e-02			
Cobs_08355							29	0.52	3.6e-02			
Cobs_08365	193	0.4	3.7e-02									
Cobs_08367	188	-0.7	1.8e-04									
Cobs_08379	693	-1.0	3.0e-04									
Cobs_08387	277	-1.0	1.8e-08				186	0.5	3.5e-02			
Cobs_08390	241	-0.3	5.3e-03									
Cobs_08414	54	-1.0	6.7e-07									
Cobs_08419	46	0.7	2.2e-02									
Cobs_08426	612	0.4	2.2e-02									
Cobs_08432	16	-0.8	3.8e-03				12	0.82	3.5e-03			
Cobs_08458							1184	0.23	4.9e-02			
Cobs_08477	37	-0.5	1.6e-02									
Cobs_08501	63	-0.6	3.0e-03									
Cobs_08510	257	-0.3	2.7e-02				210	0.43	8.0e-03			
Cobs_08513							655	0.24	3.6e-02			
Cobs_08560	59	0.8	3.1e-06									
Cobs_08605	695	-0.2	2.3e-02									
Cobs_08610	139	-1.6	4.4e-21									
Cobs_08620	202	0.3	2.7e-02									
Cobs_08653	17	1.1	3.4e-05									
Cobs_08669	1124	-0.6	4.1e-04									
Cobs_08672	122	-0.8	1.8e-04				98	0.56	2.7e-02			
Cobs_08675	133	0.5	1.6e-02				156	-0.35	4.8e-02			
Cobs_08706										1444	0.31	1.5e-02
Cobs_08721	229	-0.5	3.7e-02									
Cobs_08731	42	-1.1	4.1e-07									
Cobs_08740	264	-0.5	9.9e-06									
Cobs_08750							531	-0.36	2.9e-02			
Cobs_08751	273	-0.3	4.8e-02									
Cobs_08768							108	-0.39	3.5e-02			
Cobs_08776										535	0.38	2.5e-02

















### 7.1.5 Lists of significant GO terms of the category biological process

Functional enrichments in genes differently expressed with age (MQ18 versus MQ4) or due to sham-mating (VQ18 versus SQ18). Count, number of genes in list annotated for the corresponding term; Pop Hits, total number of annotated genes; FE, fold enrichment.

Genes upregulated in MQ18 compared with MQ4.

Term	Count	%	P value	Pop Hits	FE
GO:0000003~reproduction	21	14.1	2.3e-03	408	2.0
GO:0000075~cell cycle checkpoint	5	3.4	1.3e-03	20	9.9
GO:0000087~M phase of mitotic cell cycle	8	5.4	7.8e-03	92	3.4
GO:0000278~mitotic cell cycle	12	8.1	4.5e-02	248	1.9
GO:0000279~M phase	15	10.1	4.1e-03	255	2.3
GO:0000280~nuclear division	8	5.4	8.2e-03	93	3.4
GO:0000578~embryonic axis specification	7	4.7	1.6e-02	82	3.4
GO:0001709~cell fate determination	8	5.4	6.5e-03	89	3.6
GO:0001738~morphogenesis of a polarized epithelium	5	3.4	4.8e-02	55	3.6
GO:0002009~morphogenesis of an epithelium	10	6.7	1.8e-02	160	2.5
GO:0003006~reproductive developmental process	16	10.7	2.6e-03	269	2.4
GO:0006259~DNA metabolic process	9	6.0	4.5e-02	159	2.2
GO:0006267~pre-replicative complex assembly	3	2.0	1.2e-02	7	16.9
GO:0006323~DNA packaging	5	3.4	2.0e-02	42	4.7
GO:0006403~RNA localization	6	4.0	4.9e-02	80	3.0
GO:0006996~organelle organization	25	16.8	2.9e-02	651	1.5
GO:0007018~microtubule-based movement	6	4.0	1.2e-02	55	4.3
GO:0007043~cell-cell junction assembly	4	2.7	1.9e-02	23	6.9
GO:0007049~cell cycle	22	14.8	1.1e-04	349	2.5
GO:0007059~chromosome segregation	9	6.0	4.1e-04	73	4.9
GO:0007067~mitosis	8	5.4	7.8e-03	92	3.4
GO:0007126~meiosis	7	4.7	8.8e-03	72	3.8
GO:0007143~female meiosis	5	3.4	1.4e-02	38	5.2
GO:0007163~establishment or maintenance of cell polarity	7	4.7	1.6e-02	82	3.4
GO:0007276~gamete generation	19	12.8	2.6e-03	354	2.1
GO:0007280~pole cell migration	3	2.0	4.1e-02	13	9.1
GO:0007281~germ cell development	10	6.7	4.2e-03	127	3.1
GO:0007283~spermatogenesis	6	4.0	3.2e-02	71	3.3
GO:0007292~female gamete generation	15	10.1	1.1e-02	286	2.1
GO:0007293~germarium-derived egg chamber formation	5	3.4	1.6e-02	39	5.1
GO:0007308~oocyte construction	6	4.0	2.7e-02	68	3.5
GO:0007309~oocyte axis specification	6	4.0	2.3e-02	65	3.7
GO:0007314~oocyte anterior/posterior axis specification	6	4.0	3.3e-03	41	5.8
GO:0007350~blastoderm segmentation	8	5.4	4.8e-02	133	2.4
GO:0007351~tripartite regional subdivision	7	4.7	1.3e-02	78	3.5
GO:0007419~ventral cord development	4	2.7	1.7e-02	22	7.2
GO:0007446~imaginal disc growth	3	2.0	4.1e-02	13	9.1
GO:0008283~cell proliferation	7	4.7	2.7e-02	92	3.0
GO:0008356~asymmetric cell division	6	4.0	6.0e-03	47	5.0
GO:0008358~maternal determination of anterior/posterior axis. embryo	6	4.0	3.7e-03	42	5.6
GO:0008595~determination of anterior/posterior axis. embryo	7	4.7	1.3e-02	78	3.5
GO:0009798~axis specification	8	5.4	2.8e-02	118	2.7
GO:0009880~embryonic pattern specification	9	6.0	2.6e-02	143	2.5
GO:0009948~anterior/posterior axis specification	8	5.4	4.7e-03	84	3.8
GO:0009952~anterior/posterior pattern formation	10	6.7	7.9e-04	100	4.0
GO:0009994~oocyte differentiation	7	4.7	1.3e-02	78	3.5
GO:0010941~regulation of cell death	6	4.0	3.9e-02	75	3.2
GO:0016043~cellular component organization	43	28.9	8.1e-03	1205	1.4
GO:0016333~morphogenesis of follicular epithelium	5	3.4	1.6e-03	21	9.4
GO:0017145~stem cell division	5	3.4	1.3e-02	37	5.3
GO:0019953~sexual reproduction	20	13.4	1.3e-03	360	2.2
GO:0021700~developmental maturation	6	4.0	4.7e-02	79	3.0
GO:0022008~neurogenesis	16	10.7	2.5e-02	347	1.8
GO:0022402~cell cycle process	19	12.8	3.5e-04	298	2.5
GO:0022403~cell cycle phase	16	10.7	2.7e-03	270	2.3
GO:0022414~reproductive process	21	14.1	2.3e-03	408	2.0
GO:0022607~cellular component assembly	20	13.4	1.3e-03	360	2.2
GO:0030010~establishment of cell polarity	3	2.0	4.7e-02	14	8.5
GO:0030154~cell differentiation	26	17.4	1.2e-02	636	1.6
GO:0030708~germarium-derived female germ-line cyst encapsulation	3	2.0	8.7e-03	6	19.8
GO:0030720~oocyte localization during gemmarium-derived egg chamber formation	3	2.0	2.5e-02	10	11.9
GO:0032504~multicellular organism reproduction	20	13.4	1.9e-03	373	2.1
GO:0034329~cell junction assembly	4	2.7	1.9e-02	23	6.9

Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0034330~cell junction organization	4	2.7	2.9e-02	27	5.9
GO:0034622~cellular macromolecular complex assembly	8	5.4	2.5e-02	116	2.7
GO:0035265~organ growth	3	2.0	4.1e-02	13	9.1
GO:0040029~regulation of gene expression, epigenetic	6	4.0	3.7e-02	74	3.2
GO:0042078~germ-line stem cell division	4	2.7	1.7e-02	22	7.2
GO:0043067~regulation of programmed cell death	6	4.0	3.9e-02	75	3.2
GO:0043297~apical junction assembly	4	2.7	1.7e-02	22	7.2
GO:0044085~cellular component biogenesis	20	13.4	5.7e-03	410	1.9
GO:0045132~meiotic chromosome segregation	5	3.4	6.2e-03	30	6.6
GO:0045165~cell fate commitment	9	6.0	4.6e-02	160	2.2
GO:0045186~zonula adherens assembly	3	2.0	2.0e-02	9	13.2
GO:0045216~cell-cell junction organization	4	2.7	2.9e-02	27	5.9
GO:0048138~germ-line cyst encapsulation	4	2.7	5.0e-04	7	22.6
GO:0048139~female germ-line cyst encapsulation	4	2.7	5.0e-04	7	22.6
GO:0048232~male gamete generation	6	4.0	3.2e-02	71	3.3
GO:0048285~organelle fission	8	5.4	1.0e-02	97	3.3
GO:0048468~cell development	19	12.8	4.6e-02	475	1.6
GO:0048469~cell maturation	6	4.0	3.7e-02	74	3.2
GO:0048477~oogenesis	14	9.4	2.3e-02	283	2.0
GO:0048519~negative regulation of biological process	17	11.4	2.0e-02	369	1.8
GO:0048523~negative regulation of cellular process	15	10.1	2.9e-02	323	1.8
GO:0048599~oocyte development	6	4.0	3.0e-02	70	3.4
GO:0048609~reproductive process in a multicellular organism	20	13.4	1.9e-03	373	2.1
GO:0048610~reproductive cellular process	16	10.7	1.8e-03	260	2.4
GO:0048646~anatomical structure formation involved in morphogenesis	12	8.1	1.2e-02	203	2.3
GO:0048699~generation of neurons	15	10.1	3.6e-02	332	1.8
GO:0048729~tissue morphogenesis	10	6.7	2.9e-02	174	2.3
GO:0048869~cellular developmental process	27	18.1	1.8e-02	689	1.5
GO:0050768~negative regulation of neurogenesis	3	2.0	4.7e-02	14	8.5
GO:0051093~negative regulation of developmental process	6	4.0	3.2e-02	71	3.3
GO:0051276~chromosome organization	13	8.7	1.8e-03	182	2.8
GO:0051301~cell division	13	8.7	1.2e-04	136	3.8
GO:0051321~meiotic cell cycle	7	4.7	1.1e-02	75	3.7
GO:0051327~M phase of meiotic cell cycle	7	4.7	8.8e-03	72	3.8
GO:0051726~regulation of cell cycle	9	6.0	7.3e-03	114	3.1
GO:0060249~anatomical structure homeostasis	4	2.7	4.1e-02	31	5.1
GO:0060429~epithelium development	10	6.7	2.2e-02	166	2.4
GO:0065003~macromolecular complex assembly	10	6.7	3.2e-02	177	2.2
GO:0065004~protein-DNA complex assembly	5	3.4	7.8e-03	32	6.2

## Genes downregulated in MQ18 compared with MQ4.

Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0003012~muscle system process	5	1.6	8.8e-04	10	10.3
GO:0005975~carbohydrate metabolic process	16	5.2	4.4e-02	193	1.7
GO:0005996~monosaccharide metabolic process	8	2.6	1.5e-02	55	3.0
GO:0006006~glucose metabolic process	8	2.6	8.0e-04	33	5.0
GO:0006007~glucose catabolic process	8	2.6	3.6e-05	21	7.9
GO:0006066~alcohol metabolic process	16	5.2	1.0e-04	103	3.2
GO:0006082~organic acid metabolic process	19	6.2	2.5e-03	181	2.2
GO:0006096~glycolysis	6	1.9	3.5e-04	14	8.8
GO:0006519~cellular amino acid and derivative metabolic process	16	5.2	3.8e-03	145	2.3
GO:0006520~cellular amino acid metabolic process	13	4.2	1.0e-02	117	2.3
GO:0006575~cellular amino acid derivative metabolic process	6	1.9	4.1e-02	40	3.1
GO:0006576~biogenic amine metabolic process	5	1.6	4.8e-02	29	3.6
GO:0006936~muscle contraction	5	1.6	8.8e-04	10	10.3
GO:0007498~mesoderm development	8	2.6	2.2e-02	59	2.8
GO:0009056~catabolic process	25	8.1	2.1e-02	323	1.6
GO:0009072~aromatic amino acid family metabolic process	5	1.6	3.6e-03	14	7.4
GO:0009123~nucleoside monophosphate metabolic process	7	2.3	4.3e-03	33	4.4
GO:0009124~nucleoside monophosphate biosynthetic process	7	2.3	2.6e-03	30	4.8
GO:0009187~cyclic nucleotide metabolic process	5	1.6	7.6e-03	17	6.1
GO:0009190~cyclic nucleotide biosynthetic process	5	1.6	3.6e-03	14	7.4
GO:0009308~amine metabolic process	18	5.8	8.4e-03	188	2.0
GO:0016052~carbohydrate catabolic process	10	3.2	7.1e-05	39	5.3
GO:0019318~hexose metabolic process	8	2.6	5.9e-03	46	3.6
GO:0019320~hexose catabolic process	8	2.6	3.6e-05	21	7.9
GO:0019752~carboxylic acid metabolic process	19	6.2	2.5e-03	181	2.2
GO:0030239~myofibril assembly	4	1.3	1.4e-02	11	7.5
GO:0031032~actomyosin structure organization	5	1.6	2.3e-02	23	4.5
GO:0042180~cellular ketone metabolic process	19	6.2	5.5e-03	195	2.0
GO:0043436~oxoacid metabolic process	19	6.2	2.5e-03	181	2.2
GO:0044106~cellular amine metabolic process	15	4.9	4.7e-03	134	2.3
GO:0044262~cellular carbohydrate metabolic process	11	3.6	3.6e-02	109	2.1
GO:0044271~nitrogen compound biosynthetic process	15	4.9	1.3e-02	150	2.1
GO:0044275~cellular carbohydrate catabolic process	9	2.9	1.9e-05	26	7.1
GO:0046164~alcohol catabolic process	9	2.9	1.9e-05	26	7.1
GO:0046365~monosaccharide catabolic process	8	2.6	5.0e-05	22	7.5
GO:0051186~cofactor metabolic process	9	2.9	3.1e-02	77	2.4
GO:0055114~oxidation reduction	39	12.7	2.7e-08	306	2.6

## Genes upregulated in VQ18 compared with SQ18.

Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0001505~regulation of neurotransmitter levels	10	6.2	5.4e-04	82	4.2
GO:0003001~generation of a signal involved in cell-cell signaling	7	4.3	2.2e-02	76	3.1
GO:0003008~system process	23	14.3	1.1e-05	278	2.8
GO:0006007~glucose catabolic process	4	2.5	2.2e-02	21	6.5
GO:0006096~glycolysis	4	2.5	6.9e-03	14	9.7
GO:0006163~purine nucleotide metabolic process	6	3.7	4.7e-02	68	3.0
GO:0006182~cGMP biosynthetic process	3	1.9	1.2e-02	6	17.1
GO:0006753~nucleoside phosphate metabolic process	9	5.6	1.1e-02	105	2.9
GO:0006810~transport	35	21.7	2.1e-02	838	1.4
GO:0006814~sodium ion transport	5	3.1	4.6e-03	24	7.1
GO:0006836~neurotransmitter transport	8	5.0	9.9e-03	83	3.3
GO:0006873~cellular ion homeostasis	4	2.5	4.6e-02	28	4.9
GO:0006887~exocytosis	6	3.7	1.3e-02	49	4.2
GO:0007154~cell communication	13	8.1	5.9e-03	181	2.5
GO:0007165~signal transduction	23	14.3	1.8e-03	393	2.0
GO:0007186~G-protein coupled receptor protein signaling pathway	11	6.8	2.6e-03	122	3.1
GO:0007242~intracellular signaling cascade	13	8.1	1.5e-02	205	2.2
GO:0007267~cell-cell signaling	13	8.1	1.1e-03	148	3.0
GO:0007268~synaptic transmission	13	8.1	4.1e-04	133	3.3
GO:0007269~neurotransmitter secretion	7	4.3	2.1e-02	75	3.2
GO:0007416~synaptogenesis	4	2.5	8.4e-03	15	9.1
GO:0007517~muscle organ development	9	5.6	7.6e-03	99	3.1
GO:0007519~skeletal muscle tissue development	6	3.7	9.2e-04	27	7.6
GO:0007528~neuromuscular junction development	4	2.5	1.4e-02	18	7.6
GO:0007600~sensory perception	8	5.0	1.0e-02	84	3.2
GO:0007601~visual perception	7	4.3	2.7e-03	49	4.9
GO:0007602~phototransduction	6	3.7	1.3e-03	29	7.1
GO:0007603~phototransduction, visible light	5	3.1	5.4e-04	14	12.2
GO:0008088~axon cargo transport	3	1.9	3.9e-02	11	9.3
GO:0008277~regulation of G-protein coupled receptor protein signaling pathway	4	2.5	1.0e-02	16	8.5
GO:0009117~nucleotide metabolic process	9	5.6	1.1e-02	105	2.9
GO:0009123~nucleoside monophosphate metabolic process	7	4.3	3.1e-04	33	7.2
GO:0009124~nucleoside monophosphate biosynthetic process	6	3.7	1.5e-03	30	6.8
GO:0009165~nucleotide biosynthetic process	7	4.3	3.1e-02	82	2.9
GO:0009187~cyclic nucleotide metabolic process	7	4.3	5.1e-06	17	14.0
GO:0009190~cyclic nucleotide biosynthetic process	6	3.7	3.1e-05	14	14.6
GO:0009314~response to radiation	7	4.3	1.3e-02	68	3.5
GO:0009416~response to light stimulus	7	4.3	5.8e-03	57	4.2
GO:0009581~detection of external stimulus	6	3.7	6.9e-03	42	4.9
GO:0009582~detection of abiotic stimulus	6	3.7	5.6e-03	40	5.1
GO:0009583~detection of light stimulus	6	3.7	3.1e-03	35	5.8
GO:0009584~detection of visible light	5	3.1	2.3e-03	20	8.5
GO:0009586~rhodopsin mediated phototransduction	5	3.1	2.8e-04	12	14.2
GO:0009605~response to external stimulus	11	6.8	1.7e-03	115	3.3
GO:0009628~response to abiotic stimulus	9	5.6	1.4e-02	110	2.8
GO:0009888~tissue development	18	11.2	5.5e-03	300	2.0
GO:0014706~striated muscle tissue development	6	3.7	1.5e-03	30	6.8
GO:0016056~rhodopsin mediated signaling pathway	4	2.5	5.5e-03	13	10.5
GO:0016059~deactivation of rhodopsin mediated signaling	4	2.5	2.5e-03	10	13.6
GO:0016079~synaptic vesicle exocytosis	6	3.7	4.0e-03	37	5.5
GO:0019226~transmission of nerve impulse	13	8.1	5.8e-04	138	3.2
GO:0019320~hexose catabolic process	4	2.5	2.2e-02	21	6.5
GO:0019725~cellular homeostasis	7	4.3	1.3e-02	68	3.5
GO:0022400~regulation of rhodopsin mediated signaling pathway	4	2.5	2.5e-03	10	13.6
GO:0030001~metal ion transport	7	4.3	3.6e-02	85	2.8
GO:0030239~myofibril assembly	4	2.5	3.3e-03	11	12.4
GO:0031032~actomyosin structure organization	4	2.5	2.8e-02	23	5.9
GO:0032501~multicellular organismal process	50	31.1	3.7e-02	1352	1.3
GO:0032940~secretion by cell	7	4.3	4.4e-02	89	2.7
GO:0034404~nucleobase, nucleoside and nucleotide biosynthetic process	7	4.3	4.0e-02	87	2.7
GO:0034654~nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process	7	4.3	4.0e-02	87	2.7
GO:0042133~neurotransmitter metabolic process	3	1.9	1.6e-02	7	14.6
GO:0042592~homeostatic process	10	6.2	4.1e-03	109	3.1
GO:0042692~muscle cell differentiation	8	5.0	7.5e-04	53	5.2
GO:0043062~extracellular structure organization	6	3.7	9.2e-03	45	4.5
GO:0043954~cellular component maintenance	3	1.9	2.6e-02	9	11.4
GO:0044271~nitrogen compound biosynthetic process	11	6.8	1.1e-02	150	2.5
GO:0044275~cellular carbohydrate catabolic process	4	2.5	3.8e-02	26	5.2
GO:0046068~cGMP metabolic process	4	2.5	1.2e-03	8	17.1
GO:0046164~alcohol catabolic process	5	3.1	6.2e-03	26	6.6
GO:0046365~monosaccharide catabolic process	4	2.5	2.5e-02	22	6.2
GO:0048489~synaptic vesicle transport	8	5.0	2.8e-03	66	4.1
GO:0048741~skeletal muscle fiber development	4	2.5	1.4e-02	18	7.6

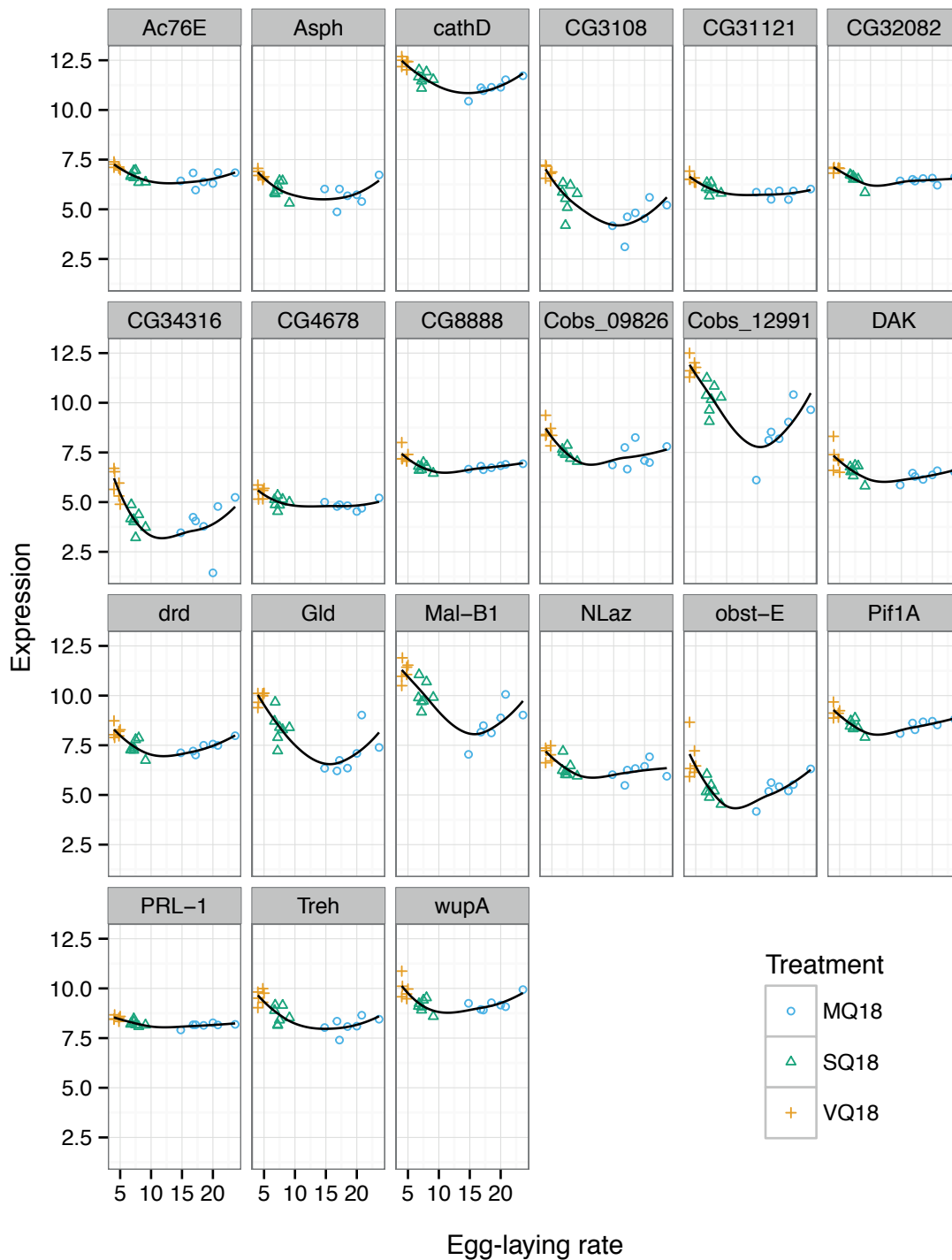


Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0048747~muscle fiber development	4	2.5	3.5e-02	25	5.5
GO:0048878~chemical homeostasis	6	3.7	9.2e-03	45	4.5
GO:0050801~ion homeostasis	5	3.1	1.6e-02	34	5.0
GO:0050808~synapse organization	6	3.7	1.3e-03	29	7.1
GO:0050877~neurological system process	21	13.0	7.1e-05	268	2.7
GO:0050890~cognition	10	6.2	1.3e-02	131	2.6
GO:0050896~response to stimulus	26	16.1	2.2e-02	572	1.6
GO:0050906~detection of stimulus involved in sensory perception	5	3.1	4.6e-03	24	7.1
GO:0050908~detection of light stimulus involved in visual perception	5	3.1	1.2e-03	17	10.0
GO:0050953~sensory perception of light stimulus	7	4.3	2.7e-03	49	4.9
GO:0050962~detection of light stimulus involved in sensory perception	5	3.1	1.2e-03	17	10.0
GO:0051146~striated muscle cell differentiation	8	5.0	3.5e-04	47	5.8
GO:0051179~localization	39	24.2	4.9e-02	1020	1.3
GO:0051234~establishment of localization	35	21.7	3.4e-02	868	1.4
GO:0051606~detection of stimulus	6	3.7	1.1e-02	47	4.4
GO:0055001~muscle cell development	8	5.0	6.1e-05	36	7.6
GO:0055002~striated muscle cell development	8	5.0	6.1e-05	36	7.6
GO:0055065~metal ion homeostasis	3	1.9	3.9e-02	11	9.3
GO:0055074~calcium ion homeostasis	3	1.9	2.1e-02	8	12.8
GO:0055080~cation homeostasis	4	2.5	3.1e-02	24	5.7
GO:0055086~nucleobase, nucleoside and nucleotide metabolic process	9	5.6	2.2e-02	119	2.6
GO:0060537~muscle tissue development	6	3.7	1.8e-03	31	6.6
GO:0060538~skeletal muscle organ development	7	4.3	1.2e-03	42	5.7
GO:0065008~regulation of biological quality	24	14.9	1.8e-03	419	2.0

Genes downregulated in VQ18 compared with SQ18.

Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0000070~mitotic sister chromatid segregation	3	4.1	4.6e-02	31	8.5
GO:0000819~sister chromatid segregation	3	4.1	4.9e-02	32	8.3
GO:0006605~protein targeting	5	6.8	1.0e-02	77	5.7
GO:0006886~intracellular protein transport	6	8.2	1.3e-02	128	4.1
GO:0008104~protein localization	8	11.0	3.9e-02	290	2.4
GO:0010948~negative regulation of cell cycle process	3	4.1	1.2e-02	15	17.6
GO:0015031~protein transport	7	9.6	2.4e-02	204	3.0
GO:0033365~protein localization in organelle	5	6.8	4.4e-03	61	7.2
GO:0034613~cellular protein localization	6	8.2	1.4e-02	131	4.0
GO:0045184~establishment of protein localization	7	9.6	2.7e-02	209	3.0
GO:0045786~negative regulation of cell cycle	3	4.1	1.8e-02	19	13.9
GO:0046907~intracellular transport	7	9.6	3.8e-02	227	2.7
GO:0048285~organelle fission	5	6.8	2.2e-02	97	4.5
GO:0051641~cellular localization	9	12.3	4.7e-02	368	2.2
GO:0051649~establishment of localization in cell	9	12.3	2.1e-02	315	2.5
GO:0070727~cellular macromolecule localization	6	8.2	3.4e-02	164	3.2

### 7.1.6 Relation between expression of mating-associated genes and rate of reproduction



Expression corresponds to means of log<sub>2</sub>-transformed normalized counts; egg-laying rate is equivalent to the mean of laid eggs per week until 18 weeks; the line is a fitted loess regression.

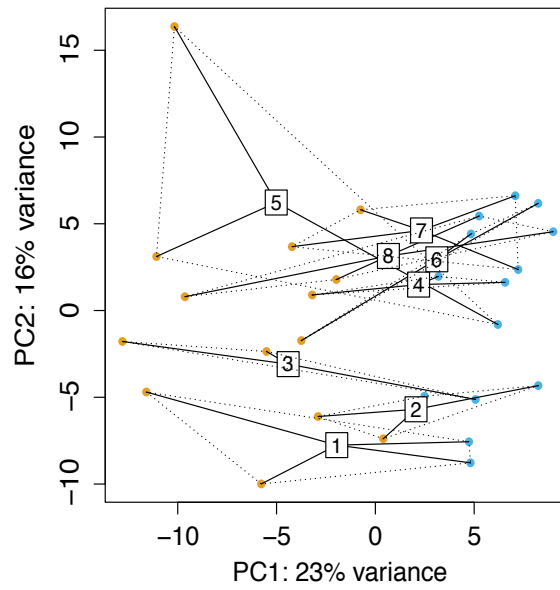
## 7.2 Appendix for Chapter 3

### 7.2.1 Mapping statistics of reads

Sample-wise numbers of raw and mapped reads are shown.

Sample	Treatment	Raw reads	Mapped reads	Mapped (%)
qe_5	control	36546316	11733378	32
qe_6	control	57677818	16318460	28
qe_9	control	36866997	11589379	31
qe_10	control	54635027	19088587	35
qe_13	control	57615421	23442385	41
qe_18	control	40524500	16896124	42
qe_22	control	37136707	8526999	23
qw_3	control	35986383	16204019	45
qw_5	control	46167534	17645426	38
qw_14	control	49572777	11286707	23
qw_22	control	46242996	15592877	34
qw_29	control	46247689	15697349	34
qw_34	control	41525145	16728049	40
qw_42	control	36646025	10669756	29
qes_20	injured	45059716	14853645	33
qes_24	injured	44506516	16157308	36
qes_25	injured	35043559	11507153	33
qes_26	injured	50533126	13567972	27
qes_27	injured	52869226	14425312	27
qes_36	injured	32313937	13950490	43
qes_41	injured	38704104	11619088	30
qws_4	injured	25230058	6475851	26
qws_11	injured	51968121	12385739	24
qws_27	injured	44653102	15429399	35
qws_33	injured	50698369	10276173	20
qws_38	injured	45326647	15673868	35
qws_43	injured	44689570	17934755	40
qws_15	injured	50406208	14897037	30
	Mean	44121200	14306189	33

## 7.2.2 Principal component plot of all samples



The graph was generated with normalized and transformed data (VST), and shows the grouping according to lane; orange = injured, blue = control.

## 7.2.3 List of DEGs

List of DEGs with corresponding homologs in *Drosophila* and *Camponotus*. BaseMean, mean expression; logFC, log2FoldChange; padj, BH corrected *P*-value; o, ortholog; h, homolog.

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56			<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h
Cobs_00035	1141	0.5	6.80e-08	FBgn0033584	CG7737	2.0e-109	o	Cflo_N_g7946t1	0.0e+00	o
Cobs_00037	243	0.5	4.73e-06	FBgn0039489	CG5880	4.0e-119	o	Cflo_N_g7944t1	0.0e+00	o
Cobs_00065	187	0.3	2.47e-02	FBgn0025186	ari-2	1.0e-14	h	Cflo_N_g13443t1	5.0e-95	h
Cobs_00073	470	0.2	1.05e-02	FBgn0062412	CtrlB	3.0e-09	h	Cflo_N_g14401t1	2.0e-42	o
Cobs_00088	153	-0.4	2.84e-03	FBgn0010435	emp	0.0e+00	o	Cflo_N_g9951t1	0.0e+00	o
Cobs_00091	427	-0.3	1.75e-02	FBgn0000566	eip55e	5.0e-178	o	Cflo_N_g9958t1	0.0e+00	o
Cobs_00093	103	0.2	3.20e-02	FBgn0033578	BBS4	2.0e-67	o	Cflo_N_g13545t1	0.0e+00	o
Cobs_00107	30	-0.4	8.22e-03	FBgn0033869	Cpr50Cb	4.0e-27	o	Cflo_N_g11707t1	7.0e-99	o
Cobs_00114	1991	0.4	4.17e-06	FBgn0032943	Tsp39D	1.0e-53	o	Cflo_N_g8522t1	1.0e-128	o
Cobs_00118	106	0.3	9.03e-03	FBgn0032149	CG4036	5.0e-115	o	Cflo_N_g8525t1	1.0e-158	o
Cobs_00119	385	-0.1	1.92e-02	FBgn0032434	CG5421	1.7e+00	o	Cflo_N_g7073t1	0.0e+00	o
Cobs_00125	354	0.4	3.47e-03	FBgn0030310	PGRP-SA	1.0e-51	o	Cflo_N_g8526t1	2.0e-97	o
Cobs_00129	88	0.3	1.72e-02	FBgn0003720	till	6.0e-01	h	Cflo_N_g6945t1	2.7e+00	h
Cobs_00182	186	-0.3	1.33e-02	FBgn0029881	pigs	3.0e-35	h	Cflo_N_g13892t1	2.0e-172	o
Cobs_00187	6380	0.2	1.28e-03	FBgn0024320	Npc1a	0.0e+00	o	Cflo_N_g5723t1	0.0e+00	o
Cobs_00207	111	-0.2	4.63e-02	FBgn0035206	CG9186	4.0e-77	o	Cflo_N_g3968t2	0.0e+00	o
Cobs_00226	4190	0.2	3.54e-03	FBgn0037580	DppIII	0.0e+00	o	Cflo_N_g14218t1	0.0e+00	o
Cobs_00229	1638	-0.1	4.83e-02	FBgn0028473	CG8801	0.0e+00	o	Cflo_N_g4759t1	0.0e+00	o
Cobs_00245	34	-0.5	6.49e-05	FBgn0024236	foi	4.7e+00	h	Cflo_N_g14221t1	1.0e-76	o
Cobs_00262	83	-0.3	1.43e-02	FBgn0027554	CG8042	1.3e-01	h	Cflo_N_g1195t1	0.0e+00	o
Cobs_00346	648	0.2	5.42e-03	FBgn0028424	JhI-26	5.0e-23	h	Cflo_N_g10570t1	3.0e-150	h
Cobs_00371	1243	0.2	9.01e-05	FBgn0265935	coro	4.0e-158	h	Cflo_N_g12001t1	0.0e+00	o
Cobs_00373	1363	0.2	2.86e-02	FBgn0037074	CG7324	0.0e+00	o	Cflo_N_g7986t1	7.0e-156	o
Cobs_00505	1557	0.6	2.03e-04	FBgn0037069	Cpr78Cc	9.0e-21	o	Cflo_N_g6024t1	8.0e-86	o
Cobs_00580	124	-0.3	2.90e-03	FBgn0032785	CG10026	2.0e-81	o	Cflo_N_g7426t1	0.0e+00	o
Cobs_00581	968	-0.4	2.81e-08	FBgn0260653	serp	0.0e+00	o	Cflo_N_g7421t1	0.0e+00	o
Cobs_00582	57	0.4	1.82e-02	FBgn0039620	wat	6.0e-127	h	Cflo_N_g1327t1	0.0e+00	o
Cobs_00625	544	0.7	9.84e-07	FBgn0263397	lh	4.0e-77	h	Cflo_N_g8446t1	3.0e-125	o
Cobs_00629	71	0.7	2.67e-06	FBgn0039559	Mes-4	7.8e-01	h	Cflo_N_g5613t1	7.1e-01	h
Cobs_00644	445	-0.1	4.81e-02	FBgn0004435	Galphaq	5.0e-162	o	Cflo_N_g517t3	0.0e+00	o
Cobs_00668	825	0.1	3.91e-02	FBgn0029736	CG4041	0.0e+00	o	Cflo_N_g11117t1	0.0e+00	o
Cobs_00740	13	-0.4	4.83e-02	FBgn0037657	hyx	8.6e-01	h	Cflo_N_g8081t1	1.6e+00	h
Cobs_00759	874	0.2	1.46e-02	FBgn0029737	CG6903	1.0e-84	h	Cflo_N_g8196t1	7.0e-143	h
Cobs_00766	703	0.2	4.45e-03	FBgn0005695	gcl	1.0e-116	o	Cflo_N_g4950t1	0.0e+00	o
Cobs_00768	191	-0.2	1.44e-02	FBgn0038641	CG7708	5.0e-167	h	Cflo_N_g4945t1	0.0e+00	o
Cobs_00777	10813	-0.2	1.45e-02	FBgn0029176	eflγ	6.0e-167	o	Cflo_N_g11160t1	0.0e+00	o
Cobs_00795	194	0.2	2.01e-03	FBgn0087041	CG42231	1.2e-01	h	Cflo_N_g6367t1	7.7e-01	h
Cobs_00796	138	0.3	1.39e-02	FBgn0023526	CG2865	1.0e-10	o	Cflo_N_g11159t1	5.0e-158	o
Cobs_00814	189	-0.4	1.30e-04	FBgn0027364	Six4	4.0e-93	o	Cflo_N_g8374t1	0.0e+00	o
Cobs_00856	872	0.2	1.23e-02	FBgn0038966	pinta	9.0e-44	o	Cflo_N_g6260t1	9.0e-90	h
Cobs_00860	14	0.4	2.59e-02	-	-	-	-	Cflo_N_g15127t1	7.9e-01	o
Cobs_00878	1305	-0.2	4.74e-04	FBgn0015240	Hr96	8.0e-106	o	Cflo_N_g8207t1	0.0e+00	o
Cobs_00883	480	-0.4	1.42e-05	-	-	-	-	Cflo_N_g4575t1	0.0e+00	o
Cobs_00886	11	-0.5	3.44e-03	FBgn0034660	lox2	0.0e+00	o	Cflo_N_g1972t1	0.0e+00	o
Cobs_00930	192	0.2	4.46e-02	FBgn0020377	Sr-CII	2.0e-45	o	Cflo_N_g79t2	0.0e+00	o
Cobs_00973	363	-0.3	4.00e-02	FBgn0025625	Sik2	1.0e-160	o	Cflo_N_g12525t1	0.0e+00	o
Cobs_00979	403	0.2	1.49e-02	FBgn0035498	Fit1	0.0e+00	o	Cflo_N_g4939t1	0.0e+00	o
Cobs_00993	770	-0.2	4.36e-02	FBgn0029715	CG11444	4.0e-35	o	Cflo_N_g3561t1	0.0e+00	o
Cobs_00994	280	-0.4	4.63e-09	FBgn0264711	CG43980	0.0e+00	o	Cflo_N_g8849t1	0.0e+00	o
Cobs_01007	61	0.4	8.03e-04	FBgn0030395	Mks1	7.0e-12	o	Cflo_N_g8855t1	0.0e+00	o
Cobs_01021	2399	-0.2	4.77e-02	FBgn0038108	CG7518	7.0e-05	o	Cflo_N_g7615t1	3.0e-153	h
Cobs_01040	154	0.2	3.40e-02	FBgn0032850	Kua	1.0e-131	o	Cflo_N_g4934t1	0.0e+00	o
Cobs_01042	686	-0.2	3.52e-03	FBgn0030329	prtp	3.0e-130	o	Cflo_N_g6609t1	9.0e-159	o
Cobs_01058	1390	-0.2	2.14e-03	FBgn0001250	if	0.0e+00	o	Cflo_N_g2526t2	0.0e+00	o

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015		
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h
Cobs_01059	2028	0.2	2.64e-02	FBgn0034094	Tsf3	2.0e-115	o	Cflo_N_g8219t1	3.0e-156	o
Cobs_01111	5998	0.2	5.12e-03	FBgn0030809	CG9086	0.0e+00	o	Cflo_N_g3741t1	0.0e+00	o
Cobs_01113	2108	-0.3	7.82e-03	FBgn0086357	Sec61 $\alpha$	0.0e+00	o	Cflo_N_g9808t1	0.0e+00	o
Cobs_01122	389	-0.2	7.78e-04	FBgn0010741	l(3)01239	4.0e-39	o	Cflo_N_g3742t1	4.0e-88	o
Cobs_01123	222	-0.2	2.39e-02	FBgn0030093	dalao	3.0e-118	o	Cflo_N_g3739t1	0.0e+00	o
Cobs_01142	864	-0.2	1.06e-02	FBgn0262737	mub	1.0e-156	o	Cflo_N_g2597t1	0.0e+00	o
Cobs_01163	4169	-0.3	4.25e-03	FBgn0037137	Nopp140	1.0e-12	o	Cflo_N_g5058t1	8.0e-59	o
Cobs_01193	491	-0.5	5.09e-03	FBgn0037801	CG3999	0.0e+00	o	Cflo_N_g2113t1	0.0e+00	o
Cobs_01201	360	0.2	6.00e-04	FBgn0039509	bigmax	9.0e-81	o	Cflo_N_g2609t1	2.0e-72	o
Cobs_01208	696	0.4	1.21e-05	FBgn0038056	CG5961	1.0e-87	o	Cflo_N_g2604t1	0.0e+00	o
Cobs_01209	37	-0.4	5.84e-03	FBgn0004644	hh	5.0e-90	o	Cflo_N_g5625t2	3.0e-64	o
Cobs_01214	534	0.2	7.34e-04	FBgn0040395	Unc-76	3.0e-123	o	Cflo_N_g14864t1	0.0e+00	o
Cobs_01257	780	0.2	9.61e-03	FBgn0003118	pnt	1.0e-65	o	Cflo_N_g12809t1	0.0e+00	o
Cobs_01259	819	0.2	1.46e-02	FBgn0003118	pnt	1.0e-40	h	Cflo_N_g4947t2	0.0e+00	o
Cobs_01268	1319	0.2	1.47e-02	FBgn0029976	snz	7.0e-22	h	Cflo_N_g13138t1	0.0e+00	o
Cobs_01278	2653	-0.3	5.79e-06	FBgn0033250	CG14762	0.0e+00	o	Cflo_N_g8133t1	0.0e+00	o
Cobs_01315	291	-0.4	1.46e-04	FBgn0261928	CG42795	1.0e-138	o	Cflo_N_g618t1	0.0e+00	o
Cobs_01322	15	-0.4	1.16e-02	FBgn0036494	Toll-6	5.0e-25	h	Cflo_N_g620t1	0.0e+00	o
Cobs_01323	133	-0.4	1.36e-04	FBgn0025879	Timp	2.0e-18	o	Cflo_N_g7650t1	0.0e+00	o
Cobs_01351	165	0.2	2.56e-02	FBgn0038763	CG4433	2.0e-68	o	Cflo_N_g13976t1	0.0e+00	o
Cobs_01356	463	-0.4	2.04e-04	FBgn0033062	Ars2	4.0e+00	h	Cflo_N_g9426t1	0.0e+00	o
Cobs_01375	94	-0.6	6.35e-09	FBgn0034275	CG5002	2.0e-120	h	Cflo_N_g9421t1	0.0e+00	o
Cobs_01404	6159	0.3	2.93e-02	FBgn0031143	CG1532	1.0e-103	o	Cflo_N_g8193t1	0.0e+00	o
Cobs_01417	1349	0.2	9.76e-03	FBgn0034110	Atg9	0.0e+00	o	Cflo_N_g5343t3	4.0e-156	o
Cobs_01419	217	0.2	6.85e-03	FBgn0051720	mthl15	4.0e-67	o	Cflo_N_g14191t1	0.0e+00	o
Cobs_01434	1171	0.5	1.44e-03	FBgn0054034	CG34034	1.7e-02	o	Cflo_N_g8099t1	1.3e+00	h
Cobs_01446	743	-0.2	6.12e-03	FBgn0005278	Sam-S	5.0e-168	o	Cflo_N_g535t3	0.0e+00	o
Cobs_01447	1503	0.1	1.09e-02	FBgn0264357	SNF4A $\gamma$	0.0e+00	o	Cflo_N_g9404t1	0.0e+00	o
Cobs_01449	66	-0.3	1.81e-02	FBgn0051151	wge	3.0e-47	o	Cflo_N_g7196t1	2.0e-137	h
Cobs_01485	213	0.7	2.76e-06	FBgn0000422	Ddc	0.0e+00	o	Cflo_N_g11755t1	0.0e+00	o
Cobs_01490	133	1.0	2.86e-13	FBgn0030455	CG4318	2.6e-01	h	Cflo_N_g7616t1	1.4e+00	h
Cobs_01493	25	0.6	5.28e-05	FBgn0037126	CG14567	2.0e-12	o	Cflo_N_g10198t1	9.0e-40	o
Cobs_01505	2081	-0.7	5.40e-18	FBgn0020385	pug	0.0e+00	o	Cflo_N_g10104t1	0.0e+00	o
Cobs_01516	63	-0.3	1.21e-02	FBgn0038629	CG14304	2.0e-69	o	Cflo_N_g4894t1	3.0e-124	o
Cobs_01557	410	-0.6	6.46e-13	FBgn0016075	vkq	0.0e+00	o	Cflo_N_g1734t1	0.0e+00	o
Cobs_01566	528	-0.6	3.06e-14	FBgn0000299	Cg25C	0.0e+00	o	Cflo_N_g1735t1	0.0e+00	o
Cobs_01612	44	-0.3	4.62e-02	FBgn0032713	CG17323	4.0e-98	o	Cflo_N_g8955t1	0.0e+00	o
Cobs_01614	3895	0.2	2.50e-02	FBgn0262124	uex	0.0e+00	o	Cflo_N_g1768t1	0.0e+00	o
Cobs_01616	1305	0.2	1.77e-02	FBgn0041191	Rheb	1.0e-98	o	Cflo_N_g11394t1	0.0e+00	o
Cobs_01668	871	0.2	9.61e-03	FBgn0035763	CG8602	0.0e+00	o	Cflo_N_g12696t1	5.0e-69	o
Cobs_01785	390	0.2	1.19e-02	FBgn0002774	mle	0.0e+00	o	Cflo_N_g7517t1	0.0e+00	o
Cobs_01786	322	-0.3	1.71e-04	FBgn0031717	Oscillin	8.0e-162	o	Cflo_N_g7522t1	0.0e+00	o
Cobs_01806	254	-0.5	3.19e-12	FBgn0030574	CG9413	0.0e+00	o	Cflo_N_g7960t1	0.0e+00	o
Cobs_01826	405	-0.3	4.70e-04	FBgn0004370	Ptp10D	0.0e+00	o	Cflo_N_g7140t1	0.0e+00	o
Cobs_01921	1313	-0.2	1.94e-02	FBgn0028686	Rpt3	0.0e+00	o	Cflo_N_g13801t1	0.0e+00	o
Cobs_01972	48	-0.3	4.41e-02	FBgn0265487	mbl	9.0e-14	h	Cflo_N_g13885t1	0.0e+00	o
Cobs_01975	22	-0.3	4.01e-02	FBgn0264672	eogt	4.9e+00	h	Cflo_N_g13885t1	5.0e-24	h
Cobs_02032	100	0.4	1.99e-02	FBgn0036316	CG10960	1.0e-94	h	Cflo_N_g5632t1	0.0e+00	o
Cobs_02036	15	-0.5	8.03e-03	-	-	-	-	Cflo_N_g6013t1	7.6e-01	h
Cobs_02039	365	0.2	2.69e-04	FBgn0039465	Tsp97e	7.0e-82	o	Cflo_N_g5633t1	2.0e-106	o
Cobs_02040	933	-0.2	3.58e-02	FBgn0025815	Mcm6	0.0e+00	o	Cflo_N_g10881t1	0.0e+00	o
Cobs_02046	3569	0.7	1.30e-14	FBgn0033017	CG10465	1.0e-132	o	Cflo_N_g4257t1	0.0e+00	o
Cobs_02114	997	-0.2	1.12e-02	FBgn0029155	Men-b	0.0e+00	o	Cflo_N_g2422t1	0.0e+00	o
Cobs_02129	120	0.4	5.67e-03	FBgn0032871	CG2611	2.0e-09	o	Cflo_N_g2214t1	3.0e-57	o
Cobs_02161	1013	0.4	7.39e-07	FBgn0032864	CG2493	6.0e-148	o	Cflo_N_g1923t1	0.0e+00	o
Cobs_02162	1491	0.1	3.87e-02	FBgn0014001	Pak	5.0e-101	h	Cflo_N_g2159t1	0.0e+00	o
Cobs_02166	1154	0.8	5.23e-10	FBgn0033644	Tret1-2	0.0e+00	o	Cflo_N_g2162t1	0.0e+00	o
Cobs_02172	575	0.3	2.89e-04	FBgn0029959	Rab39	4.0e-133	o	Cflo_N_g2147t1	9.0e-156	o
Cobs_02174	789	-0.3	2.02e-02	-	-	-	-	Cflo_N_g2150t1	4.0e-61	o
Cobs_02176	230	0.2	4.12e-02	FBgn0261451	trol	6.0e-27	h	Cflo_N_g2148t1	0.0e+00	o
Cobs_02179	747	0.2	3.48e-04	FBgn0031814	retm	0.0e+00	o	Cflo_N_g4714t1	0.0e+00	o

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_02181	877	-0.2	2.37e-02	FBgn0000542	ec	0.0e+00	o	Cflo_N_g4710t2	0.0e+00	o	
Cobs_02185	170	-0.6	5.17e-05	FBgn0003178	PyK	0.0e+00	o	Cflo_N_g5738t1	0.0e+00	o	
Cobs_02193	2721	0.2	1.58e-03	FBgn0052549	CG32549	0.0e+00	o	Cflo_N_g10944t3	0.0e+00	o	
Cobs_02278	251	-0.2	1.58e-02	FBgn0265298	SC35	5.0e-57	o	Cflo_N_g6751t1	5.0e-70	o	
Cobs_02341	484	-0.2	1.82e-02	-	-	-	-	Cflo_N_g2522t2	0.0e+00	o	
Cobs_02345	14	-0.5	3.33e-03	FBgn0259244	CG42342	1.0e-22	h	Cflo_N_g5625t2	1.0e-39	h	
Cobs_02471	147	-0.7	6.56e-11	FBgn0025837	CG17636	1.0e-152	o	Cflo_N_g1598t1	0.0e+00	o	
Cobs_02508	2095	0.2	4.85e-03	FBgn0000723	FeR	6.0e-17	h	Cflo_N_g13569t1	0.0e+00	o	
Cobs_02524	3582	-0.1	4.60e-02	FBgn0004907	14-3-3C	4.0e-86	h	Cflo_N_g7897t3	6.0e-97	h	
Cobs_02526	1019	-0.2	2.99e-02	FBgn0040340	TRAM	2.0e-94	o	Cflo_N_g5049t1	0.0e+00	o	
Cobs_02527	46	-0.4	6.76e-04	FBgn0016054	phr6-4	3.0e-172	o	Cflo_N_g2697t1	0.0e+00	o	
Cobs_02533	8226	0.3	3.44e-06	FBgn0263593	Lpin	0.0e+00	o	Cflo_N_g7899t1	0.0e+00	o	
Cobs_02539	171	0.3	4.24e-03	FBgn0035086	CG12851	1.0e-37	o	Cflo_N_g13620t1	0.0e+00	o	
Cobs_02568	376	-0.2	2.23e-02	FBgn0033741	CG8545	0.0e+00	o	Cflo_N_g5764t1	2.0e-151	o	
Cobs_02606	62	-0.4	4.67e-03	FBgn0051100	CG31100	2.0e-93	o	Cflo_N_g9601t1	0.0e+00	o	
Cobs_02643	73	0.3	3.18e-02	FBgn0051559	CG31559	3.0e-34	o	Cflo_N_g5129t1	0.0e+00	h	
Cobs_02816	2330	-0.2	3.58e-02	FBgn0263346	CG43427	3.0e-61	o	Cflo_N_g11106t1	0.0e+00	o	
Cobs_02822	30	-0.5	8.01e-04	FBgn0034476	Toll-7	4.0e-66	h	Cflo_N_g5858t1	3.0e-168	h	
Cobs_02966	55	-0.4	4.89e-03	FBgn0050503	CG30503	6.0e-21	h	Cflo_N_g9766t1	0.0e+00	o	
Cobs_03013	589	-0.2	2.70e-02	FBgn0035981	CG4452	9.0e-93	o	Cflo_N_g14507t2	5.0e-103	o	
Cobs_03047	24	-0.4	8.59e-03	FBgn0033108	CG15236	1.8e-01	h	Cflo_N_g10395t1	1.4e+00	h	
Cobs_03094	1002	-0.3	2.29e-06	FBgn0014455	Ahcy13	0.0e+00	o	Cflo_N_g12691t1	0.0e+00	o	
Cobs_03103	182	0.2	9.61e-03	FBgn0050010	CG30010	7.0e-59	o	Cflo_N_g8196t2	0.0e+00	o	
Cobs_03113	145	0.3	1.33e-02	FBgn0058196	Maf1	2.0e-104	o	Cflo_N_g5836t1	0.0e+00	o	
Cobs_03116	36	0.3	3.61e-02	-	-	-	-	-	-	-	
Cobs_03177	3273	0.2	1.32e-02	FBgn0260987	vtd	0.0e+00	o	Cflo_N_g11969t1	0.0e+00	o	
Cobs_03228	798	0.2	5.22e-03	FBgn0262937	Rabex-5	2.0e-111	o	Cflo_N_g14067t1	0.0e+00	o	
Cobs_03229	187	0.2	6.55e-03	FBgn0041147	ida	1.0e-53	o	Cflo_N_g6592t1	6.0e-41	h	
Cobs_03242	3641	-0.3	1.80e-03	-	-	-	-	Cflo_N_g15258t1	0.0e+00	o	
Cobs_03268	213	-0.2	2.31e-02	FBgn0038467	AdSL	0.0e+00	o	-	-	-	
Cobs_03271	1448	-0.9	1.21e-38	FBgn0034580	Cht8	1.0e-17	h	Cflo_N_g260t1	0.0e+00	o	
Cobs_03317	23	-0.5	2.06e-03	FBgn0036411	Sox21a	1.0e-47	o	Cflo_N_g15162t1	2.0e-157	o	
Cobs_03347	681	0.4	5.48e-05	FBgn0034501	CG13868	1.0e-100	o	Cflo_N_g3988t1	0.0e+00	o	
Cobs_03363	100	0.3	1.77e-02	FBgn0026602	Ady43A	5.0e-41	o	Cflo_N_g8952t1	1.0e-130	o	
Cobs_03364	27	-0.4	5.05e-03	FBgn0003884	$\alpha$ Tub84B	0.0e+00	o	Cflo_N_g13444t1	0.0e+00	o	
Cobs_03385	483	-0.3	7.50e-03	FBgn0030968	CG7322	8.0e-71	o	Cflo_N_g5978t2	0.0e+00	o	
Cobs_03388	561	0.2	6.04e-04	FBgn0015321	UbcD4	1.0e-84	o	Cflo_N_g8079t1	4.0e-103	o	
Cobs_03404	24	-0.4	1.46e-02	FBgn0038304	CG12241	6.6e+00	h	Cflo_N_g5940t1	2.1e+00	h	
Cobs_03406	52	-0.3	4.00e-02	FBgn0262160	CG9932	4.2e+00	h	Cflo_N_g588t1	2.0e-176	o	
Cobs_03411	17	-0.5	6.63e-04	FBgn0263706	CG43658	5.9e-01	h	Cflo_N_g13909t1	3.9e-01	h	
Cobs_03412	50	-0.5	1.61e-03	FBgn0001202	hk	1.5e+00	h	Cflo_N_g1759t1	6.6e-01	h	
Cobs_03423	239	0.2	4.47e-02	FBgn0085232	CG34203	3.0e-01	o	Cflo_N_g13463t1	3.0e-142	o	
Cobs_03424	26	0.4	1.33e-02	FBgn0034140	CG8317	1.5e-01	o	Cflo_N_g13461t1	4.0e-38	o	
Cobs_03430	1016	0.9	2.59e-13	FBgn0051217	modSP	2.0e-44	h	Cflo_N_g13922t1	0.0e+00	o	
Cobs_03500	3786	-0.2	3.30e-08	FBgn0034072	Dg	3.0e-136	o	Cflo_N_g14526t1	0.0e+00	o	
Cobs_03626	18	-0.4	9.68e-03	FBgn0264326	DNAPol-e	4.0e+00	h	Cflo_N_g14578t1	1.4e+00	h	
Cobs_03661	128	-0.2	2.34e-02	FBgn0034918	wibg	2.0e-37	o	Cflo_N_g11052t1	0.0e+00	o	
Cobs_03673	1406	0.1	1.70e-02	FBgn0035995	CG3529	2.0e-134	o	Cflo_N_g3494t1	0.0e+00	o	
Cobs_03732	1004	-0.3	1.28e-03	FBgn0015766	Msr-110	3.0e-34	o	Cflo_N_g8479t1	0.0e+00	o	
Cobs_03733	1581	-0.2	1.58e-03	FBgn0265434	zip	2.7e-01	h	Cflo_N_g14842t2	3.0e-136	o	
Cobs_03744	127	0.4	4.67e-05	FBgn0028406	Drep-4	2.0e-42	o	Cflo_N_g650t1	0.0e+00	o	
Cobs_03774	1918	0.4	5.07e-06	FBgn0037363	Atg17	4.0e-115	o	Cflo_N_g4875t1	0.0e+00	o	
Cobs_03786	264	0.3	2.85e-02	FBgn0037780	CG3925	4.0e-46	o	Cflo_N_g3284t1	0.0e+00	o	
Cobs_03806	473	0.5	3.62e-08	FBgn0031190	CG12576	2.7e-02	o	Cflo_N_g13820t1	2.0e-172	o	
Cobs_03831	1847	0.1	3.24e-02	FBgn0031384	CG4238	0.0e+00	o	Cflo_N_g3307t1	0.0e+00	o	
Cobs_03837	542	-0.2	1.45e-02	-	-	-	-	Cflo_N_g3303t1	1.0e-17	o	
Cobs_03869	83	0.3	9.26e-03	FBgn0028499	CG7985	2.0e-91	h	Cflo_N_g212t1	0.0e+00	o	
Cobs_03905	2602	-0.3	4.31e-02	FBgn0264695	Mhc	0.0e+00	o	Cflo_N_g12890t3	0.0e+00	o	
Cobs_03910	11	-0.6	3.08e-04	FBgn0024920	Ts	9.4e-01	h	Cflo_N_g12892t1	6.0e-31	o	
Cobs_03913	489	-0.2	2.93e-02	FBgn0039626	Slu7	0.0e+00	o	Cflo_N_g15017t2	0.0e+00	o	
Cobs_03929	50	0.4	1.21e-02	FBgn0038165	Task6	2.0e-23	h	Cflo_N_g9212t1	0.0e+00	o	

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015		
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h
Cobs_03948	5002	-0.1	7.58e-03	FBgn0263120	Acsl	0.0e+00	o	Cflo_N_g2404t1	0.0e+00	o
Cobs_04026	4192	0.2	2.16e-03	FBgn0260439	Pp2A-29B	4.0e-05	h	Cflo_N_g3075t1	0.0e+00	o
Cobs_04045	362	0.2	4.01e-02	FBgn0038692	CG3773	2.0e-45	o	Cflo_N_g6385t1	3.0e-108	o
Cobs_04068	188	-0.9	2.96e-27	FBgn0031307	MFS3	2.0e-180	o	Cflo_N_g180t1	0.0e+00	o
Cobs_04077	2005	0.6	5.35e-15	FBgn0031313	CG5080	5.0e-40	o	Cflo_N_g209t1	0.0e+00	o
Cobs_04126	1052	0.2	2.93e-02	FBgn0021872	Xbp1	2.0e-24	o	Cflo_N_g9239t1	0.0e+00	o
Cobs_04236	33	-1.2	2.19e-17	FBgn0250839	CG2016	3.0e-79	o	Cflo_N_g13479t1	1.0e-159	o
Cobs_04237	917	0.3	6.62e-03	FBgn0260964	Vmat	1.0e-08	h	Cflo_N_g12063t1	0.0e+00	o
Cobs_04252	1048	-0.4	2.35e-06	FBgn0030706	CG8909	1.0e-31	h	Cflo_N_g12062t1	0.0e+00	o
Cobs_04253	47	-0.4	9.76e-03	FBgn0261930	vnd	7.0e-43	o	Cflo_N_g7335t1	0.0e+00	h
Cobs_04257	4451	-0.3	2.12e-03	FBgn0000042	Act5C	0.0e+00	o	Cflo_N_g12390t1	0.0e+00	o
Cobs_04270	1429	-0.2	4.70e-02	FBgn0087008	e(y)3	5.0e-62	o	Cflo_N_g14440t2	0.0e+00	o
Cobs_04277	876	0.2	6.47e-03	FBgn0039508	CG3368	2.0e-25	o	Cflo_N_g14436t1	0.0e+00	o
Cobs_04281	497	0.2	1.81e-04	FBgn0038755	Hs6st	1.0e-173	o	Cflo_N_g14980t1	0.0e+00	o
Cobs_04285	455	0.2	7.16e-04	FBgn0033887	St4	1.0e-97	o	Cflo_N_g157t1	2.0e-175	o
Cobs_04290	967	0.2	1.63e-03	FBgn0036999	isoQC	1.0e-111	o	Cflo_N_g11674t2	0.0e+00	o
Cobs_04293	37	-0.4	2.12e-03	-	-	-	-	Cflo_N_g12535t1	0.0e+00	o
Cobs_04325	209	0.8	2.98e-10	FBgn0031110	Obp19b	3.2e-01	h	Cflo_N_g11628t1	8.0e-03	o
Cobs_04329	1038	0.1	2.11e-02	FBgn0031213	galactin	2.0e-19	h	Cflo_N_g11664t1	0.0e+00	o
Cobs_04357	71	-0.2	4.69e-02	FBgn0039776	PH4aeFB	0.0e+00	o	Cflo_N_g14062t1	0.0e+00	o
Cobs_04389	2184	-0.2	2.76e-02	FBgn0028685	Rpt4	0.0e+00	o	Cflo_N_g14178t1	0.0e+00	o
Cobs_04390	1963	-0.1	3.74e-02	FBgn0010348	Arf79F	1.0e-129	o	Cflo_N_g11218t1	2.0e-131	o
Cobs_04401	254	0.5	3.28e-07	FBgn0265512	mlt	3.0e-127	o	Cflo_N_g12845t1	0.0e+00	h
Cobs_04406	75	0.4	1.62e-04	FBgn0011676	Nos	4.2e+00	h	Cflo_N_g12459t1	5.9e+00	h
Cobs_04423	12892	-0.2	2.07e-02	FBgn0013733	shot	0.0e+00	o	Cflo_N_g13212t1	0.0e+00	o
Cobs_04429	72	0.4	4.11e-03	FBgn0259982	I(2)35Cc	5.0e-21	o	Cflo_N_g10088t1	1.0e-84	o
Cobs_04431	27	0.3	3.08e-02	-	-	-	-	-	-	-
Cobs_04454	27	-0.4	2.90e-02	-	-	-	-	Cflo_N_g11669t1	0.0e+00	o
Cobs_04614	181	-0.3	8.51e-03	FBgn0265042	Irk1	0.0e+00	o	Cflo_N_g4523t1	0.0e+00	o
Cobs_04617	1768	0.2	2.36e-03	FBgn0033224	Nop17l	6.0e-121	o	Cflo_N_g1965t2	0.0e+00	h
Cobs_04628	128	0.2	3.47e-02	FBgn0040344	CG3711	7.0e-21	h	Cflo_N_g1956t1	0.0e+00	o
Cobs_04632	650	-0.2	2.96e-02	FBgn0036662	CG9706	0.0e+00	o	Cflo_N_g1953t1	0.0e+00	o
Cobs_04663	3954	0.8	1.16e-11	FBgn0053160	CG33160	2.4e-01	h	Cflo_N_g14777t1	4.0e-18	o
Cobs_04724	1918	0.2	4.25e-02	FBgn0032192	CG5731	0.0e+00	o	Cflo_N_g15021t1	0.0e+00	o
Cobs_04733	277	0.2	1.21e-02	FBgn0086674	Tango13	0.0e+00	o	Cflo_N_g11492t1	0.0e+00	o
Cobs_04756	897	0.2	1.27e-02	FBgn0004406	tam	0.0e+00	o	Cflo_N_g11518t1	0.0e+00	o
Cobs_04773	1169	0.2	4.33e-02	FBgn0019890	Smg5	9.0e-35	o	Cflo_N_g14653t1	0.0e+00	o
Cobs_04783	352	-0.2	1.62e-02	FBgn0035529	CG1319	5.0e-51	o	Cflo_N_g14656t1	7.0e-63	h
Cobs_04790	124	0.2	4.47e-02	FBgn0052296	Mrtf	1.5e+00	h	Cflo_N_g11735t1	2.3e-02	h
Cobs_04792	1137	-0.3	8.91e-07	FBgn0027572	CG5009	0.0e+00	o	Cflo_N_g6147t1	0.0e+00	o
Cobs_04816	1021	0.3	2.07e-02	FBgn0036316	CG10960	9.0e-143	o	Cflo_N_g1714t3	0.0e+00	o
Cobs_04820	133	-0.5	5.37e-09	FBgn0003390	shf	4.0e-73	o	Cflo_N_g9739t1	7.0e-150	o
Cobs_04828	725	0.2	3.57e-02	FBgn0036824	CG3902	0.0e+00	o	Cflo_N_g7023t1	0.0e+00	o
Cobs_04922	1379	0.2	2.93e-03	FBgn0017418	ari-1	0.0e+00	o	Cflo_N_g7677t1	0.0e+00	o
Cobs_04923	332	-0.4	8.48e-03	FBgn0029896	CG3168	0.0e+00	o	Cflo_N_g719t2	0.0e+00	o
Cobs_04931	231	0.2	1.70e-02	FBgn0037679	CG8866	4.0e-154	o	Cflo_N_g7674t1	0.0e+00	o
Cobs_04989	459	-0.2	3.93e-02	FBgn0001218	Hsc70-3	0.0e+00	o	Cflo_N_g13858t1	0.0e+00	o
Cobs_04998	136	-0.3	1.06e-02	FBgn0085470	ImgB	6.0e-11	o	Cflo_N_g7992t1	0.0e+00	o
Cobs_05011	22	-0.4	7.17e-03	FBgn0033052	SCAP	3.8e-01	h	Cflo_N_g4281t1	3.0e-01	h
Cobs_05022	133	0.3	1.67e-02	FBgn0086673	CG13272	4.0e-10	o	Cflo_N_g9019t1	0.0e+00	o
Cobs_05027	6464	-0.2	1.79e-03	FBgn0027835	Dp1	0.0e+00	o	Cflo_N_g2447t1	9.0e-68	o
Cobs_05111	813	-0.2	2.41e-03	FBgn0010300	brat	0.0e+00	o	Cflo_N_g7605t1	0.0e+00	o
Cobs_05159	397	0.2	2.14e-02	FBgn0042083	CG3267	0.0e+00	o	Cflo_N_g13361t1	0.0e+00	o
Cobs_05170	15	-0.8	1.69e-07	FBgn0022355	Tsfl	1.7e+00	h	Cflo_N_g4811t2	1.0e+00	h
Cobs_05172	38	-0.9	2.59e-13	FBgn0031449	CG31689	3.0e-173	o	Cflo_N_g3711t3	0.0e+00	h
Cobs_05201	135	-0.3	7.01e-03	FBgn0032287	CG6415	3.0e-124	o	Cflo_N_g716t1	0.0e+00	o
Cobs_05204	95	-0.4	8.48e-03	FBgn0262636	Lin29	2.0e-73	o	Cflo_N_g7680t1	0.0e+00	o
Cobs_05210	220	0.2	1.51e-02	FBgn0034817	Art7	3.0e-21	h	Cflo_N_g13445t1	0.0e+00	o
Cobs_05211	481	0.2	1.88e-02	FBgn0035945	CG5026	2.0e-167	o	Cflo_N_g12285t1	0.0e+00	o
Cobs_05219	262	-0.5	9.27e-04	FBgn0034911	CG5549	1.0e-149	o	Cflo_N_g2632t1	2.0e-173	o
Cobs_05237	729	0.2	2.46e-03	FBgn0263594	lost	6.0e-88	o	Cflo_N_g8284t1	0.0e+00	o



	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_05244	39	0.5	9.13e-04	FBgn0034072	Dg	4.5e+00	h	Cflo_N_g1061t1	2.1e+00	h	
Cobs_05253	468	-0.2	1.55e-02	FBgn0028554	x16	1.0e-35	o	Cflo_N_g899t1	0.0e+00	o	
Cobs_05257	169	0.2	1.44e-02	FBgn0032117	FucTB	7.0e-89	o	Cflo_N_g11715t1	0.0e+00	o	
Cobs_05259	16	-0.4	3.73e-02	FBgn0000384	cta	1.6e-01	h	Cflo_N_g13880t1	2.0e-01	h	
Cobs_05260	630	-0.2	9.44e-03	FBgn0263077	CG43340	7.0e-10	o	Cflo_N_g11704t2	0.0e+00	o	
Cobs_05272	39	-0.4	2.11e-02	FBgn0035019	Ir60e	3.8e+00	h	Cflo_N_g5488t1	6.2e-01	h	
Cobs_05281	90	0.5	6.93e-08	FBgn0053993	CG33993	6.0e-63	o	Cflo_N_g3125t1	0.0e+00	o	
Cobs_05296	25	-0.5	1.53e-03	FBgn0030884	CG6847	7.7e-01	h	Cflo_N_g8918t1	5.9e+00	h	
Cobs_05303	241	0.4	3.51e-02	FBgn0036742	CG7497	1.0e-64	o	Cflo_N_g496t2	4.0e-161	o	
Cobs_05331	25944	0.1	9.03e-03	FBgn0039114	Lsd-1	2.0e-98	o	Cflo_N_g4068t1	0.0e+00	o	
Cobs_05352	2051	-0.2	7.44e-03	FBgn0036663	CG9674	0.0e+00	o	Cflo_N_g6265t1	0.0e+00	o	
Cobs_05358	152	-0.2	4.59e-02	FBgn0033710	CG17739	0.0e+00	o	Cflo_N_g6092t1	0.0e+00	o	
Cobs_05393	169	0.5	6.80e-06	FBgn0029092	ced-6	2.0e-05	h	Cflo_N_g6916t1	9.0e-168	o	
Cobs_05399	2596	-0.2	3.58e-02	FBgn0004654	Pgd	0.0e+00	o	Cflo_N_g2667t1	0.0e+00	o	
Cobs_05404	96	-0.6	3.91e-07	FBgn0003068	per	2.0e-110	o	Cflo_N_g2669t2	0.0e+00	o	
Cobs_05410	1115	0.2	4.33e-02	FBgn0034611	MFS16	7.0e-133	o	Cflo_N_g2661t1	3.0e-95	o	
Cobs_05419	1233	-0.1	1.72e-02	FBgn0037504	CG1142	7.0e-32	o	Cflo_N_g9200t1	2.0e-113	o	
Cobs_05429	16837	-0.2	1.46e-02	FBgn0023213	eIF4G	6.0e-102	o	Cflo_N_g12681t2	0.0e+00	o	
Cobs_05437	637	0.2	1.31e-02	-	-	-	-	Cflo_N_g9297t1	0.0e+00	o	
Cobs_05493	8	0.4	3.22e-02	FBgn0263598	Vha68-2	8.0e+00	h	Cflo_N_g10488t1	8.0e-20	h	
Cobs_05553	4249	0.5	6.07e-04	FBgn0041181	Tep3	0.0e+00	o	Cflo_N_g9745t2	0.0e+00	o	
Cobs_05556	17	-0.4	7.58e-03	FBgn0039749	CG11498	2.0e-74	o	Cflo_N_g5920t1	0.0e+00	o	
Cobs_05571	304	-0.2	4.36e-04	FBgn0029693	CG6379	0.0e+00	o	Cflo_N_g12546t1	0.0e+00	o	
Cobs_05573	433	0.3	2.81e-05	FBgn0036897	CG8786	6.0e-52	o	Cflo_N_g12553t2	0.0e+00	o	
Cobs_05579	904	-0.3	1.13e-05	FBgn0262870	axo	0.0e+00	o	Cflo_N_g12555t3	0.0e+00	o	
Cobs_05614	3826	-0.2	1.37e-04	FBgn0052479	CG32479	2.0e-134	o	Cflo_N_g5333t1	0.0e+00	o	
Cobs_05662	10	-0.4	3.47e-02	FBgn0036900	CG8765	1.0e-04	h	Cflo_N_g3819t1	4.0e-106	o	
Cobs_05675	1126	0.3	2.63e-03	FBgn0027611	LM408	0.0e+00	o	Cflo_N_g8960t2	0.0e+00	o	
Cobs_05712	1356	0.1	2.99e-02	FBgn0051265	CG31265	6.0e-49	o	Cflo_N_g9718t2	0.0e+00	o	
Cobs_05750	2239	-0.3	5.08e-04	FBgn0250788	beta-Spec	0.0e+00	o	Cflo_N_g14369t1	4.0e-12	h	
Cobs_05777	637	-0.2	1.76e-03	FBgn0038111	CG12360	4.0e-77	o	Cflo_N_g13423t1	1.0e-179	o	
Cobs_05797	5905	0.2	3.23e-04	FBgn0039120	Nup98-96	0.0e+00	o	Cflo_N_g11590t1	3.0e-06	h	
Cobs_05818	36	0.7	3.89e-06	FBgn0041180	Tep4	4.4e-02	h	Cflo_N_g7788t1	1.0e-44	h	
Cobs_05826	1694	0.7	1.04e-09	-	-	-	-	Cflo_N_g7789t1	2.0e-96	o	
Cobs_05834	815	-0.3	7.57e-03	FBgn0034718	wdp	7.0e-16	o	Cflo_N_g8587t1	5.0e-50	o	
Cobs_05849	18380	-0.3	5.17e-04	FBgn0001233	Hsp83	0.0e+00	o	Cflo_N_g7863t1	0.0e+00	o	
Cobs_05869	28	-0.4	4.03e-02	FBgn0264432	CG43850	7.5e+00	o	Cflo_N_g3317t1	2.0e-79	o	
Cobs_05943	283	0.4	1.31e-09	FBgn0031359	CG18317	3.0e-114	o	Cflo_N_g12900t1	7.0e-100	o	
Cobs_05947	352	-0.2	4.45e-02	FBgn0259734	CG42388	3.0e-111	o	Cflo_N_g3442t1	0.0e+00	o	
Cobs_05985	693	0.2	4.94e-03	FBgn0034897	Sesn	7.0e-173	o	Cflo_N_g43t1	0.0e+00	o	
Cobs_05988	23	-0.6	2.19e-05	FBgn0031876	Atac1	6.1e+00	h	Cflo_N_g6065t5	3.8e+00	h	
Cobs_05993	48	0.6	4.06e-05	FBgn0051344	CG31344	3.0e-18	h	Cflo_N_g6748t1	5.0e-110	o	
Cobs_05995	86	0.2	4.15e-02	FBgn0016919	nompB	8.0e-109	o	Cflo_N_g41t1	0.0e+00	o	
Cobs_06002	480	0.3	3.54e-02	FBgn0031251	CG4213	4.0e-68	o	Cflo_N_g15620t1	0.0e+00	o	
Cobs_06024	74	-0.3	4.36e-02	FBgn0053978	CG33978	1.0e-67	o	Cflo_N_g798t1	2.0e-117	o	
Cobs_06038	5402	-0.1	4.41e-02	FBgn0250789	$\alpha$ -Spec	0.0e+00	o	Cflo_N_g10544t1	0.0e+00	o	
Cobs_06066	252	0.2	4.19e-02	FBgn0033373	CG8080	4.0e-82	o	Cflo_N_g10428t1	0.0e+00	o	
Cobs_06086	141	0.3	5.74e-03	FBgn0011674	insc	3.0e-15	o	Cflo_N_g862t1	0.0e+00	o	
Cobs_06094	169	0.2	4.29e-02	FBgn0039751	CG1983	1.0e-81	o	Cflo_N_g1416t1	2.0e-143	o	
Cobs_06102	18	0.4	4.65e-03	FBgn0030664	CG8119	7.3e-01	h	Cflo_N_g11808t1	4.0e-15	h	
Cobs_06126	30	-0.9	4.94e-13	FBgn0050418	nord	7.0e-12	o	Cflo_N_g1648t1	1.2e+00	h	
Cobs_06135	771	0.2	1.72e-02	FBgn0037647	RagA-B	2.0e-162	o	Cflo_N_g13282t1	0.0e+00	o	
Cobs_06146	11	-0.4	4.39e-02	FBgn0265991	Zasp52	5.0e-14	h	Cflo_N_g8933t4	4.0e-20	h	
Cobs_06148	997	0.3	1.48e-02	FBgn0000121	Arr2	0.0e+00	o	Cflo_N_g8938t1	0.0e+00	o	
Cobs_06158	331	0.2	2.82e-02	FBgn0011676	Nos	0.0e+00	o	Cflo_N_g5430t1	0.0e+00	o	
Cobs_06197	40	-0.3	3.54e-02	FBgn0002466	sti	3.4e+00	h	Cflo_N_g8182t1	4.0e-11	h	
Cobs_06210	29	1.1	2.90e-18	FBgn0030590	CG9518	0.0e+00	o	Cflo_N_g3669t1	0.0e+00	o	
Cobs_06213	103	0.3	3.17e-02	FBgn0266435	CG45065	0.0e+00	o	Cflo_N_g3668t1	0.0e+00	o	
Cobs_06217	678	0.2	1.46e-02	FBgn0038890	CG7956	0.0e+00	o	Cflo_N_g5964t2	0.0e+00	o	
Cobs_06251	2475	-0.2	9.13e-04	FBgn0031990	CG8552	0.0e+00	o	Cflo_N_g14277t2	0.0e+00	o	
Cobs_06314	469	0.3	4.22e-02	FBgn0034487	efhc1.2	1.3e+00	h	Cflo_N_g273t1	1.0e-105	h	

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_06357	2796	0.4	1.79e-03	FBgn0011289	TfIIA-L	8.2e+00	h	Cflo_N_g12045t1	0.0e+00	o	
Cobs_06372	296	0.3	9.60e-04	FBgn0266268	FeCH	0.0e+00	o	Cflo_N_g2445t1	0.0e+00	h	
Cobs_06476	1893	0.2	5.17e-04	FBgn0032006	Pvr	0.0e+00	o	Cflo_N_g13896t2	0.0e+00	o	
Cobs_06477	130	-0.3	4.00e-02	FBgn0032076	CG9510	3.0e-171	o	Cflo_N_g6399t2	1.0e-87	h	
Cobs_06481	1693	-0.2	4.83e-02	-	-	-	-	Cflo_N_g6423t1	0.0e+00	o	
Cobs_06490	649	-0.2	1.43e-02	FBgn0265182	CG44247	3.0e-54	o	Cflo_N_g14233t1	1.0e-17	h	
Cobs_06498	3304	-0.5	2.03e-08	FBgn0002526	LanA	0.0e+00	o	Cflo_N_g1199t1	0.0e+00	o	
Cobs_06514	917	0.4	3.90e-08	FBgn0028984	Spn88ea	1.0e-62	o	Cflo_N_g10213t1	0.0e+00	h	
Cobs_06515	135	-0.4	3.85e-06	FBgn0037846	CG6574	2.0e-115	o	Cflo_N_g10213t1	0.0e+00	o	
Cobs_06516	3920	0.2	4.30e-02	FBgn0037874	Tctp	2.0e-99	o	Cflo_N_g8017t1	2.0e-114	o	
Cobs_06559	100	0.4	4.37e-02	FBgn0025595	AkhR	2.0e-105	o	Cflo_N_g9393t2	0.0e+00	o	
Cobs_06560	96	0.3	3.58e-03	-	-	-	-	Cflo_N_g9397t1	6.0e-100	o	
Cobs_06569	2258	0.1	7.39e-03	FBgn0051999	CG31999	7.0e-127	o	Cflo_N_g5898t1	0.0e+00	o	
Cobs_06570	1329	0.1	4.68e-02	FBgn0039731	Sas-6	1.0e-13	o	Cflo_N_g5895t1	0.0e+00	o	
Cobs_06576	174	0.3	6.72e-03	FBgn0050295	lpk1	3.0e-15	o	Cflo_N_g5893t1	0.0e+00	o	
Cobs_06578	92	0.3	2.28e-02	FBgn0243514	eater	4.0e-105	o	Cflo_N_g9696t1	0.0e+00	o	
Cobs_06587	1101	0.2	1.03e-02	FBgn0027505	Rab3-GAP	4.0e-165	o	Cflo_N_g12937t1	0.0e+00	o	
Cobs_06605	33	-0.4	2.59e-03	FBgn0039475	CG6277	1.0e-46	h	Cflo_N_g10098t1	3.0e-60	h	
Cobs_06627	1142	0.2	4.52e-02	FBgn0036876	CG9451	2.0e-56	h	Cflo_N_g8091t1	8.0e-156	o	
Cobs_06641	21	0.4	2.99e-02	FBgn0020309	crol	3.0e-45	h	Cflo_N_g2695t1	6.0e-121	o	
Cobs_06655	62	-0.3	1.70e-02	-	-	-	-	Cflo_N_g4430t1	0.0e+00	o	
Cobs_06675	498	0.2	2.20e-02	FBgn0052702	Cubn	0.0e+00	o	Cflo_N_g7592t1	0.0e+00	o	
Cobs_06690	510	-0.4	1.28e-03	FBgn0034394	CG15096	9.0e-122	o	Cflo_N_g845t1	0.0e+00	o	
Cobs_06729	133	-0.2	4.46e-02	FBgn0031879	uif	4.0e-42	h	Cflo_N_g881t1	0.0e+00	o	
Cobs_06738	1123	0.2	2.34e-02	FBgn0038816	Lrrk	0.0e+00	o	Cflo_N_g4611t1	0.0e+00	o	
Cobs_06845	1061	-0.2	1.06e-02	FBgn0032643	CG6453	2.0e-130	o	Cflo_N_g4767t1	0.0e+00	o	
Cobs_06846	24	1.0	5.10e-13	FBgn0034145	CG5065	2.0e-114	h	Cflo_N_g11540t1	4.0e-134	h	
Cobs_06864	136	0.3	7.03e-03	FBgn0004462	Pk17e	6.0e-70	o	Cflo_N_g7888t1	2.0e-169	o	
Cobs_06870	84	-0.4	3.98e-02	FBgn0031737	obst-e	1.0e-74	o	Cflo_N_g3525t2	0.0e+00	o	
Cobs_06894	438	0.2	3.52e-02	FBgn0032601	yellow-b	1.0e-40	h	Cflo_N_g11018t1	9.0e-135	o	
Cobs_06897	1806	0.2	1.29e-02	FBgn0039209	CG13624	1.0e-120	o	Cflo_N_g3366t1	0.0e+00	o	
Cobs_06946	2132	-0.2	4.41e-02	FBgn0011206	bol	2.0e-45	o	Cflo_N_g11916t1	0.0e+00	o	
Cobs_06953	747	0.2	6.62e-03	FBgn0031478	CG8814	2.0e-41	o	Cflo_N_g11920t1	4.0e-133	o	
Cobs_06959	86	-0.5	1.33e-03	FBgn0086129	sname	4.1e-01	h	Cflo_N_g11927t1	3.0e-141	o	
Cobs_06967	62	0.7	2.82e-05	FBgn0051344	CG1344	7.0e-40	h	Cflo_N_g5981t1	6.0e-90	o	
Cobs_06980	1222	0.2	6.84e-04	FBgn0036621	roq	1.0e-128	o	Cflo_N_g9026t1	0.0e+00	o	
Cobs_07040	1101	0.2	9.91e-03	FBgn0262738	norpA	0.0e+00	h	Cflo_N_g8784t1	0.0e+00	o	
Cobs_07046	103	0.2	2.79e-02	FBgn0036510	CG7427	4.0e-123	o	Cflo_N_g13235t1	0.0e+00	o	
Cobs_07047	212	0.3	1.82e-02	FBgn0037723	SpdS	6.0e-122	o	Cflo_N_g8777t1	7.0e-162	o	
Cobs_07052	168	0.5	2.82e-05	FBgn0053998	CG33998	2.1e-01	h	Cflo_N_g5161t1	3.0e-88	o	
Cobs_07056	5637	0.3	7.49e-06	FBgn0053852	His1	4.0e-32	o	Cflo_N_g5158t1	2.0e-56	o	
Cobs_07085	1008	-0.2	2.22e-02	FBgn0033661	CG13185	0.0e+00	o	Cflo_N_g8124t1	0.0e+00	o	
Cobs_07093	2926	-0.3	7.03e-03	FBgn0033661	CG13185	1.0e-163	h	Cflo_N_g8121t1	0.0e+00	o	
Cobs_07103	93	0.3	6.05e-03	FBgn0004509	Fur1	2.0e-03	h	Cflo_N_g13147t1	1.0e-29	o	
Cobs_07104	407	0.3	3.27e-03	FBgn0000038	nAChRβ1	0.0e+00	o	Cflo_N_g13144t1	0.0e+00	o	
Cobs_07105	291	0.2	2.50e-02	FBgn0004509	Fur1	0.0e+00	o	Cflo_N_g9068t1	0.0e+00	o	
Cobs_07180	16	-0.5	6.55e-03	FBgn0263750	CG43675	1.8e+00	h	Cflo_N_g4919t1	3.0e-23	h	
Cobs_07192	630	0.8	4.17e-07	FBgn0036299	Tsf2	1.0e-03	h	Cflo_N_g7714t1	3.0e-50	h	
Cobs_07199	51	0.3	2.37e-02	FBgn0027571	CG3523	2.0e-16	h	Cflo_N_g7690t1	9.0e-24	h	
Cobs_07207	403	0.4	1.82e-04	FBgn0040001	CG17374	1.0e-80	h	Cflo_N_g264t3	8.0e-121	h	
Cobs_07211	111	0.3	1.71e-03	FBgn0040001	CG17374	2.0e-12	h	Cflo_N_g1283t1	4.0e-38	o	
Cobs_07231	258	0.3	1.36e-02	FBgn0040001	CG17374	4.0e-55	h	Cflo_N_g497t1	6.0e-91	h	
Cobs_07244	537	0.3	1.48e-03	FBgn0040001	CG17374	2.0e-109	h	Cflo_N_g9156t1	3.0e-140	h	
Cobs_07245	368	0.3	1.23e-04	FBgn0042627	v(2)k05816	8.0e-83	h	Cflo_N_g497t1	0.0e+00	h	
Cobs_07246	268	0.3	1.63e-02	FBgn0040001	CG17374	3.0e-18	h	Cflo_N_g1907t3	2.0e-55	h	
Cobs_07286	1363	-0.2	6.77e-03	FBgn0026259	eIF5B	0.0e+00	o	Cflo_N_g12042t2	0.0e+00	o	
Cobs_07337	1482	0.1	3.58e-02	FBgn0261823	Asx	1.0e-56	o	Cflo_N_g5627t1	0.0e+00	o	
Cobs_07340	6424	0.2	8.27e-03	FBgn0035978	UGP	0.0e+00	o	Cflo_N_g4534t2	0.0e+00	o	
Cobs_07345	231	0.2	2.34e-02	FBgn0038725	CG6184	2.0e-115	o	Cflo_N_g3476t1	4.0e-106	o	
Cobs_07351	495	0.2	8.51e-03	FBgn0032078	C1GalTA	2.0e-147	o	Cflo_N_g2296t1	4.0e-178	o	
Cobs_07353	475	-0.2	1.11e-02	FBgn0264005	Hmx	2.0e-40	h	Cflo_N_g185t2	0.0e+00	o	

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015		
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h
Cobs_07375	155	0.3	1.20e-02	FBgn0001168	h	9.0e-57	o	Cflo_N_g4662t1	0.0e+00	o
Cobs_07380	1251	0.6	5.12e-08	-	-	-	-	Cflo_N_g11776t1	0.0e+00	o
Cobs_07381	993	0.2	5.60e-03	FBgn0037391	CG2017	0.0e+00	o	Cflo_N_g14411t1	0.0e+00	o
Cobs_07433	55	-0.4	8.37e-03	FBgn0000492	Dr	7.0e-46	o	Cflo_N_g1285t1	9.0e-36	h
Cobs_07447	333	-0.3	4.98e-03	FBgn0004397	Vinc	0.0e+00	o	Cflo_N_g14615t3	0.0e+00	o
Cobs_07462	2936	0.3	5.10e-06	FBgn0260635	th	4.0e-53	o	Cflo_N_g2127t1	2.0e-154	o
Cobs_07509	423	0.2	2.70e-02	FBgn0038769	CG10889	3.0e-37	h	Cflo_N_g1242t1	0.0e+00	o
Cobs_07517	920	0.2	1.36e-02	FBgn0022787	Hel89B	2.0e-86	h	Cflo_N_g14508t1	0.0e+00	o
Cobs_07528	1832	0.2	1.69e-03	FBgn0025836	RhoGAP1A	0.0e+00	o	Cflo_N_g4320t1	0.0e+00	o
Cobs_07532	1335	-0.6	5.54e-14	FBgn0010246	Myo61F	0.0e+00	o	Cflo_N_g10337t2	0.0e+00	o
Cobs_07560	901	0.8	2.95e-13	FBgn0040323	GNBP1	4.0e-57	o	Cflo_N_g5742t1	0.0e+00	o
Cobs_07561	111	0.3	3.85e-03	FBgn0036485	FucTA	2.0e-162	o	Cflo_N_g5745t1	0.0e+00	o
Cobs_07583	708	0.2	3.93e-02	FBgn0052533	CG32533	0.0e+00	o	Cflo_N_g15586t1	0.0e+00	o
Cobs_07603	77	0.7	1.79e-05	FBgn0039151	CG13607	1.0e-120	o	Cflo_N_g12023t1	0.0e+00	o
Cobs_07607	39	-0.5	1.63e-03	FBgn0038740	CG4562	0.0e+00	h	Cflo_N_g14515t1	0.0e+00	o
Cobs_07608	335	0.4	1.13e-02	FBgn0019940	Rh6	4.0e-145	o	Cflo_N_g11672t1	0.0e+00	o
Cobs_07609	3582	0.2	1.79e-05	FBgn0043362	bchs	0.0e+00	o	Cflo_N_g12022t1	0.0e+00	o
Cobs_07617	774	0.2	2.69e-04	FBgn0035877	CG7083	0.0e+00	o	Cflo_N_g7499t1	2.0e-156	o
Cobs_07649	351	-0.3	1.32e-03	FBgn0011571	caz	7.0e-41	o	Cflo_N_g7479t1	0.0e+00	o
Cobs_07687	511	0.6	3.54e-08	FBgn0028990	Spn27A	4.0e-66	o	Cflo_N_g4516t1	3.0e-14	o
Cobs_07689	400	0.2	8.72e-04	FBgn0038889	CG7922	2.0e-150	h	Cflo_N_g13648t1	0.0e+00	h
Cobs_07706	1791	0.2	2.28e-02	FBgn0039994	CG17082	2.0e-102	o	Cflo_N_g2512t1	0.0e+00	o
Cobs_07839	985	0.3	7.57e-03	FBgn0037146	CG7470	0.0e+00	o	Cflo_N_g7805t1	0.0e+00	o
Cobs_07841	204	-0.4	4.70e-04	FBgn0032618	CG31743	3.0e-80	o	Cflo_N_g197t1	0.0e+00	o
Cobs_07903	157	-0.3	1.62e-04	FBgn0263973	juv	8.0e-30	o	Cflo_N_g9151t1	0.0e+00	o
Cobs_07928	3341	0.2	7.93e-03	FBgn0052626	AMPdeam	0.0e+00	o	Cflo_N_g9919t1	0.0e+00	o
Cobs_07940	235	-0.5	5.13e-04	FBgn0015032	Cyp4c3	2.0e-88	h	Cflo_N_g10727t1	0.0e+00	o
Cobs_08041	108	0.4	1.37e-04	FBgn0023171	rnhl	8.0e-04	h	Cflo_N_g10661t1	3.0e-83	h
Cobs_08113	3106	0.3	7.58e-03	FBgn0001186	Hex-A	0.0e+00	o	Cflo_N_g6068t2	0.0e+00	o
Cobs_08116	1071	0.2	3.26e-03	FBgn0025681	CG3558	8.0e-08	h	Cflo_N_g5136t1	0.0e+00	o
Cobs_08128	1345	-0.2	4.60e-03	FBgn0262515	VhaAC45	1.0e-17	o	Cflo_N_g8461t1	2.0e-119	o
Cobs_08154	2421	0.2	9.61e-03	FBgn0086346	ALiX	0.0e+00	o	Cflo_N_g14195t1	0.0e+00	h
Cobs_08155	21	0.3	3.33e-02	FBgn0052447	CG32447	2.0e-93	o	Cflo_N_g384t1	0.0e+00	o
Cobs_08161	1987	0.1	3.77e-02	FBgn0039260	Smg6	1.0e-08	h	Cflo_N_g11790t1	0.0e+00	o
Cobs_08169	194	-0.3	2.87e-02	FBgn0053281	CG33281	9.0e-77	o	Cflo_N_g7985t1	0.0e+00	o
Cobs_08177	113	0.4	7.66e-05	FBgn0036485	FucTA	4.0e-39	h	Cflo_N_g375t1	0.0e+00	o
Cobs_08183	417	0.3	2.64e-04	FBgn0030099	CG12056	1.0e-48	o	Cflo_N_g374t1	1.0e-123	o
Cobs_08188	320	0.2	2.86e-02	FBgn0086768	Pemt	3.0e-62	o	Cflo_N_g10013t1	0.0e+00	o
Cobs_08210	1332	0.1	2.50e-02	FBgn0004574	Rop	0.0e+00	o	Cflo_N_g698t3	0.0e+00	o
Cobs_08211	499	-0.3	1.46e-02	FBgn0026565	CG1315	2.0e-173	o	Cflo_N_g697t1	0.0e+00	o
Cobs_08233	1008	-0.4	6.02e-12	FBgn0250732	gfzf	3.0e-91	o	Cflo_N_g6258t1	6.0e-138	o
Cobs_08284	169	0.3	1.62e-03	FBgn0031893	CG4495	0.0e+00	o	Cflo_N_g12917t3	0.0e+00	o
Cobs_08293	697	0.2	3.93e-02	FBgn0027291	l(1)G0156	0.0e+00	o	Cflo_N_g13089t2	3.0e-123	o
Cobs_08305	1015	0.2	1.06e-02	FBgn0265187	CG44252	0.0e+00	o	Cflo_N_g12659t1	0.0e+00	o
Cobs_08306	856	0.3	5.24e-07	FBgn0086779	step	0.0e+00	o	Cflo_N_g13578t1	0.0e+00	o
Cobs_08317	64	0.3	1.50e-02	FBgn0259749	myy	3.9e+00	h	Cflo_N_g4562t1	3.4e-01	h
Cobs_08321	46	-0.4	7.84e-03	FBgn0041713	yellow-c	3.0e-107	h	Cflo_N_g5479t1	0.0e+00	o
Cobs_08323	68	0.3	6.25e-03	FBgn0034479	CG8654	2.0e-103	h	Cflo_N_g5480t1	0.0e+00	o
Cobs_08335	148	-0.3	4.82e-02	FBgn0053725	CG33725	1.0e+00	h	Cflo_N_g12254t1	0.0e+00	o
Cobs_08377	661	-0.3	3.14e-10	FBgn0033296	Mal-A7	1.0e-29	h	Cflo_N_g7757t1	0.0e+00	o
Cobs_08379	668	-0.4	4.21e-02	FBgn0031418	CG3609	3.0e-14	h	Cflo_N_g6974t1	0.0e+00	o
Cobs_08394	11445	-0.5	1.67e-10	FBgn0005585	Crc	0.0e+00	o	Cflo_N_g6968t1	0.0e+00	o
Cobs_08397	100	-0.4	1.11e-04	FBgn0052669	CG32669	0.0e+00	o	Cflo_N_g11914t2	0.0e+00	o
Cobs_08449	573	-0.2	3.30e-02	FBgn0020257	ppa	0.0e+00	o	Cflo_N_g7509t1	0.0e+00	o
Cobs_08475	416	-0.6	4.75e-05	FBgn0036381	CG8745	0.0e+00	o	Cflo_N_g8263t2	0.0e+00	o
Cobs_08477	87	-0.3	2.19e-03	FBgn0034106	CG9068	4.0e-78	h	Cflo_N_g12350t1	1.0e-145	o
Cobs_08487	244	-0.3	4.02e-03	FBgn0022268	KdelR	2.0e-126	o	Cflo_N_g7342t1	1.0e-120	o
Cobs_08493	277	0.6	2.73e-08	FBgn0000567	eip74eF	2.0e-18	h	Cflo_N_g4603t1	0.0e+00	o
Cobs_08502	407	-0.3	1.78e-04	FBgn0000964	tj	5.0e-37	o	Cflo_N_g3591t1	0.0e+00	o
Cobs_08504	1224	0.1	2.16e-02	FBgn0025681	CG3558	2.0e-171	o	Cflo_N_g9674t2	0.0e+00	o
Cobs_08514	1819	0.2	9.76e-03	FBgn0015618	Cdk8	0.0e+00	o	Cflo_N_g9422t1	0.0e+00	o

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_08525	363	0.4	9.20e-07	FBgn0019972	Ice	9.0e-121	o	Cflo_N_g11720t1	6.0e-163	o	
Cobs_08528	2207	0.3	3.67e-06	FBgn0034985	CG3328	4.0e-173	o	Cflo_N_g2303t2	0.0e+00	o	
Cobs_08535	4715	0.1	2.90e-02	FBgn0051716	Cnot4	4.0e-140	o	Cflo_N_g7374t1	0.0e+00	o	
Cobs_08569	311	-0.2	4.33e-02	FBgn0031479	Prx6005	4.0e-87	o	Cflo_N_g360t1	4.0e-143	o	
Cobs_08579	2700	0.1	4.72e-02	FBgn0015795	Rab7	4.0e-122	o	Cflo_N_g6412t1	0.0e+00	o	
Cobs_08584	292	0.2	3.83e-02	FBgn0016930	Dyrk2	0.0e+00	o	Cflo_N_g7253t1	0.0e+00	o	
Cobs_08603	4287	-0.1	3.83e-02	FBgn0003520	stau	2.0e-83	o	Cflo_N_g7309t1	0.0e+00	o	
Cobs_08605	1786	-0.2	3.78e-03	FBgn0031589	CG3714	0.0e+00	o	Cflo_N_g12528t1	0.0e+00	o	
Cobs_08617	27	-0.3	4.42e-02	FBgn0035957	CG5144	1.5e+00	h	Cflo_N_g13487t1	1.3e-01	h	
Cobs_08645	930	-0.2	1.27e-02	FBgn0034215	Mtap	8.0e-93	o	Cflo_N_g10446t1	1.0e-80	o	
Cobs_08653	54	-0.4	6.28e-03	-	-	-	-	Cflo_N_g14331t1	6.0e-75	o	
Cobs_08664	27	-0.6	1.01e-04	FBgn0259878	Fs	4.0e-30	o	Cflo_N_g2279t1	0.0e+00	o	
Cobs_08674	108	-0.6	3.13e-11	FBgn0028573	prc	2.0e-45	o	Cflo_N_g2586t1	0.0e+00	o	
Cobs_08682	502	0.2	4.69e-04	FBgn0016131	Cdk4	3.0e-102	o	Cflo_N_g5533t1	0.0e+00	o	
Cobs_08688	38	-0.4	1.06e-02	FBgn0003174	pwn	4.0e-162	o	Cflo_N_g5530t1	0.0e+00	o	
Cobs_08700	43	-0.5	3.94e-04	FBgn0037530	CG2943	1.3e+00	h	Cflo_N_g10082t1	1.2e-01	o	
Cobs_08706	2386	-0.2	1.53e-03	FBgn0003062	Fib	1.0e-145	o	Cflo_N_g12344t1	1.0e-116	o	
Cobs_08709	189	-0.4	1.11e-05	FBgn0034797	nahoda	2.0e-173	o	Cflo_N_g8881t1	0.0e+00	o	
Cobs_08750	1162	0.4	9.20e-07	FBgn0010173	RpA-70	2.0e-170	o	Cflo_N_g13541t1	0.0e+00	o	
Cobs_08765	110	-0.5	1.23e-03	FBgn0015336	CG15865	7.0e-27	o	Cflo_N_g2587t1	0.0e+00	o	
Cobs_08776	749	-0.4	3.13e-11	FBgn0003189	r	0.0e+00	o	Cflo_N_g9712t2	0.0e+00	o	
Cobs_08807	84	0.3	7.25e-03	FBgn0030640	CG6294	9.0e-46	o	Cflo_N_g5538t1	0.0e+00	o	
Cobs_08832	30	0.3	4.14e-02	FBgn0032536	Ance-3	2.0e-33	h	Cflo_N_g9690t1	2.0e-54	o	
Cobs_08845	650	0.5	2.07e-04	FBgn0243514	eater	7.0e-55	h	Cflo_N_g9695t1	0.0e+00	o	
Cobs_08849	917	0.4	2.15e-04	FBgn0259111	Ndae1	0.0e+00	o	Cflo_N_g6364t1	0.0e+00	o	
Cobs_08884	1775	0.2	1.31e-02	FBgn0031682	CG5828	2.0e-107	o	Cflo_N_g3154t1	0.0e+00	o	
Cobs_08910	26	-0.4	9.38e-03	FBgn0029082	hbs	4.6e+00	h	Cflo_N_g15240t1	1.7e+00	h	
Cobs_08914	1589	-0.2	4.76e-02	FBgn0039149	CG18428	2.0e-19	o	Cflo_N_g2850t1	0.0e+00	o	
Cobs_08946	6690	0.2	3.93e-02	FBgn0265137	Spn42Da	7.0e-76	o	Cflo_N_g13004t1	0.0e+00	o	
Cobs_08951	337	-0.2	2.21e-02	FBgn0031256	CG4164	6.0e-178	o	Cflo_N_g8022t1	3.0e-02	h	
Cobs_08985	129	0.2	1.82e-02	FBgn0035092	Nplp1	2.0e-10	o	-	-	-	
Cobs_08996	12	-0.5	4.84e-03	FBgn0040507	ACXD	5.8e+00	h	Cflo_N_g12403t2	9.9e-01	h	
Cobs_09019	973	0.2	1.55e-04	FBgn0025885	Inos	0.0e+00	o	Cflo_N_g2299t2	0.0e+00	o	
Cobs_09026	5455	0.2	4.05e-03	FBgn0010352	Nc73eF	0.0e+00	o	Cflo_N_g8928t3	0.0e+00	o	
Cobs_09038	92	-0.3	4.00e-02	FBgn0011693	Pdh	6.0e-39	h	Cflo_N_g8702t1	0.0e+00	o	
Cobs_09040	1076	-0.2	1.82e-02	FBgn0028737	eflbeta	5.0e-75	o	Cflo_N_g11620t1	6.0e-137	o	
Cobs_09050	828	-0.5	2.52e-10	FBgn0011693	Pdh	4.0e-37	h	Cflo_N_g12523t1	3.0e-180	o	
Cobs_09081	501	0.2	2.10e-03	FBgn0038549	CG17802	3.0e-05	h	Cflo_N_g3855t1	0.0e+00	o	
Cobs_09117	105	-0.3	2.75e-03	FBgn0004797	mdy	2.0e-99	o	Cflo_N_g12724t1	2.0e-129	o	
Cobs_09119	88	-0.4	2.60e-05	FBgn0004797	mdy	3.0e-13	h	Cflo_N_g12724t1	3.0e-31	h	
Cobs_09152	1712	0.5	5.22e-18	FBgn0013984	InR	8.0e-63	h	Cflo_N_g2770t1	0.0e+00	o	
Cobs_09155	555	0.2	1.21e-02	FBgn0002901	mus304	3.0e-06	o	Cflo_N_g3798t1	0.0e+00	o	
Cobs_09158	253	0.5	3.48e-10	FBgn0000479	dnc	5.5e+00	h	Cflo_N_g10244t2	1.0e+00	h	
Cobs_09206	1006	0.2	4.31e-03	FBgn0037382	Hpr1	0.0e+00	o	Cflo_N_g8432t1	0.0e+00	o	
Cobs_09207	2187	0.2	3.42e-03	FBgn0029006	lack	0.0e+00	o	Cflo_N_g8431t1	0.0e+00	o	
Cobs_09261	13	-0.5	5.84e-03	FBgn0000299	Cg25C	7.0e-18	h	Cflo_N_g8680t2	0.0e+00	o	
Cobs_09271	1179	-0.5	2.53e-10	FBgn0051145	CG31145	5.0e-06	h	Cflo_N_g6817t1	5.0e-71	o	
Cobs_09276	461	-0.6	4.81e-10	FBgn0051145	CG31145	4.0e-140	o	Cflo_N_g10243t1	0.0e+00	o	
Cobs_09278	60	-0.5	6.86e-05	FBgn0051145	CG31145	5.0e-05	h	Cflo_N_g2136t1	6.0e-32	o	
Cobs_09297	247	-0.2	2.70e-02	FBgn0004237	Hrb87F	2.0e-93	h	Cflo_N_g4699t1	3.0e-153	o	
Cobs_09317	30	0.4	1.80e-02	FBgn0035060	eps-15	2.0e-03	h	Cflo_N_g12945t1	6.0e-57	o	
Cobs_09318	1057	0.3	2.35e-03	FBgn0003502	Btk29A	2.0e-106	o	Cflo_N_g8501t1	7.0e-167	o	
Cobs_09424	108	-0.3	1.94e-02	FBgn0039920	CG11360	1.0e-68	o	Cflo_N_g1606t1	2.0e-143	o	
Cobs_09473	411	-0.2	2.53e-03	FBgn0029167	Hml	0.0e+00	o	Cflo_N_g11181t1	0.0e+00	o	
Cobs_09475	1591	0.2	1.42e-05	FBgn0037897	CG5270	2.0e-124	o	Cflo_N_g8206t1	0.0e+00	o	
Cobs_09485	259	0.2	1.53e-02	FBgn0036819	Dysb	9.0e-30	o	Cflo_N_g3967t1	5.0e-102	o	
Cobs_09487	2043	0.1	4.63e-02	FBgn0038418	pad	1.2e-01	h	Cflo_N_g12473t1	4.0e-173	o	
Cobs_09492	1540	-0.1	1.62e-02	FBgn0004860	ph-d	6.0e-22	o	Cflo_N_g3966t1	0.0e+00	o	
Cobs_09508	557	-0.3	7.57e-03	FBgn0038098	CG7381	1.0e-10	h	Cflo_N_g14472t1	0.0e+00	o	
Cobs_09511	449	0.2	3.09e-02	FBgn0041627	Ku80	6.0e-16	o	Cflo_N_g4178t1	0.0e+00	o	
Cobs_09583	187	-0.6	1.66e-07	FBgn0034883	CG17664	2.0e-63	o	Cflo_N_g3250t1	1.0e-139	o	

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015		
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h
Cobs_09593	1484	0.2	1.32e-03	FBgn0033052	SCAP	0.0e+00	o	Cflo_N_g10634t1	1.0e-85	o
Cobs_09605	1029	-0.2	7.47e-03	FBgn0260742	CG12213	4.0e-07	o	Cflo_N_g7485t1	0.0e+00	o
Cobs_09606	185	-0.2	3.63e-02	FBgn0041630	Hexo1	0.0e+00	o	Cflo_N_g13990t1	0.0e+00	o
Cobs_09662	651	-0.2	8.87e-04	FBgn0039977	CG17454	3.0e-59	o	Cflo_N_g6655t1	3.0e-171	o
Cobs_09671	170	-0.6	7.30e-05	FBgn0052645	CG32645	6.0e-78	h	Cflo_N_g272t1	9.0e-110	h
Cobs_09724	263	0.2	4.71e-02	FBgn0034970	yki	2.0e-15	h	Cflo_N_g6908t3	1.0e-122	o
Cobs_09769	2159	-0.2	1.57e-02	FBgn0003887	$\beta$ Tub56D	0.0e+00	o	Cflo_N_g11520t1	0.0e+00	o
Cobs_09776	353	-0.2	1.75e-02	FBgn0027518	CG7609	3.4e+00	h	Cflo_N_g8075t1	1.0e+00	o
Cobs_09777	20	-0.4	2.18e-02	FBgn0000463	DI	3.5e-01	h	Cflo_N_g14268t1	7.0e-51	h
Cobs_09786	3500	0.5	2.79e-22	FBgn0041203	LIMK1	0.0e+00	o	Cflo_N_g13784t1	0.0e+00	o
Cobs_09804	52	0.3	4.77e-02	FBgn0033716	Den1	2.0e-43	o	Cflo_N_g1060t1	2.0e-93	o
Cobs_09813	734	0.2	4.25e-02	FBgn0029893	CG14442	3.0e-09	o	Cflo_N_g12102t1	0.0e+00	o
Cobs_09844	93	0.3	1.32e-02	FBgn0034184	CG9646	0.0e+00	o	Cflo_N_g8755t1	0.0e+00	o
Cobs_09857	334	-0.6	2.27e-04	FBgn0022359	Sodh-2	9.0e-156	o	Cflo_N_g12072t1	3.0e-169	o
Cobs_09863	99	0.2	1.58e-02	FBgn0036126	CG6272	1.0e-06	o	Cflo_N_g8097t1	9.0e-64	o
Cobs_09888	257	0.2	4.50e-02	FBgn0036927	CG7433	0.0e+00	o	Cflo_N_g814t1	0.0e+00	o
Cobs_09899	75	0.3	1.24e-02	FBgn0034032	CG8195	2.8e+00	h	Cflo_N_g13253t1	5.2e+00	h
Cobs_09902	253	0.3	4.49e-03	FBgn0259728	CG42382	5.0e-27	o	Cflo_N_g804t1	6.0e-125	o
Cobs_09904	1019	-0.2	2.64e-02	FBgn0263396	sqd	7.0e-95	o	Cflo_N_g805t1	1.0e-127	o
Cobs_09939	4274	0.2	8.89e-04	FBgn0250848	26-29-p	0.0e+00	o	Cflo_N_g851t1	0.0e+00	o
Cobs_09944	1753	0.3	1.21e-04	FBgn0033352	CG8232	0.0e+00	o	Cflo_N_g2527t2	0.0e+00	o
Cobs_09948	1132	0.2	1.33e-02	FBgn0000320	eya	2.0e-131	o	Cflo_N_g12689t1	6.0e-139	o
Cobs_09949	304	0.3	1.56e-03	FBgn0038042	Scgbeta	1.0e-43	o	Cflo_N_g8443t1	0.0e+00	o
Cobs_09954	872	-0.1	4.47e-02	FBgn0039773	CG2224	2.0e-119	o	Cflo_N_g10673t1	0.0e+00	o
Cobs_09972	156	0.2	4.20e-02	FBgn0058263	MFS17	0.0e+00	o	Cflo_N_g2036t1	0.0e+00	o
Cobs_10031	62	-0.3	1.23e-02	FBgn0263997	CG43740	9.7e-02	h	Cflo_N_g5602t1	2.0e-30	o
Cobs_10036	75	0.8	6.14e-09	FBgn0013811	Dhc62B	2.0e+00	h	Cflo_N_g1411t1	9.0e-01	h
Cobs_10049	5534	0.2	1.10e-02	FBgn0021818	cnk	4.0e-90	o	Cflo_N_g3783t1	0.0e+00	o
Cobs_10055	1306	0.2	4.15e-03	FBgn0030412	Tomosyn	0.0e+00	o	Cflo_N_g2721t1	0.0e+00	o
Cobs_10060	507	0.4	2.59e-13	FBgn0263776	CG43693	0.0e+00	o	Cflo_N_g729t1	0.0e+00	o
Cobs_10064	289	0.4	3.60e-09	FBgn0031773	Fbw5	2.8e-01	h	Cflo_N_g14403t1	3.0e-146	o
Cobs_10079	3106	-0.3	8.70e-03	FBgn0261836	Msp300	0.0e+00	o	Cflo_N_g98t1	8.0e-116	o
Cobs_10083	3999	-0.4	9.19e-04	FBgn0261836	Msp300	0.0e+00	o	Cflo_N_g14914t1	0.0e+00	o
Cobs_10110	3047	0.2	2.29e-06	FBgn0004395	unk	0.0e+00	o	Cflo_N_g497t1	1.0e-88	h
Cobs_10123	1576	-0.2	4.40e-02	-	-	-	-	Cflo_N_g1259t1	0.0e+00	o
Cobs_10152	1178	-0.2	4.56e-02	FBgn0040001	CG17374	0.0e+00	h	Cflo_N_g6717t1	0.0e+00	o
Cobs_10233	1393	-0.2	3.04e-02	FBgn0036801	MYPT-75D	1.0e-177	o	Cflo_N_g13753t1	0.0e+00	o
Cobs_10249	1659	0.2	2.63e-03	FBgn0010812	unc-45	0.0e+00	o	Cflo_N_g12270t1	0.0e+00	o
Cobs_10258	995	0.2	4.11e-02	FBgn0051064	CG31064	2.0e-11	h	Cflo_N_g3432t1	0.0e+00	o
Cobs_10259	1962	0.2	2.50e-02	FBgn0015279	Pi3K92e	0.0e+00	o	Cflo_N_g3416t1	0.0e+00	o
Cobs_10268	1195	0.1	2.37e-02	FBgn0037836	CG14692	6.0e-01	h	Cflo_N_g3415t1	0.0e+00	o
Cobs_10275	88	0.6	9.20e-08	FBgn0015546	spell	2.0e-33	h	Cflo_N_g3421t1	0.0e+00	o
Cobs_10288	120	0.5	5.46e-06	FBgn0028550	Atf3	2.0e-28	o	Cflo_N_g1860t2	0.0e+00	o
Cobs_10324	412	-0.2	1.06e-03	FBgn0039674	CG1907	2.0e-113	o	Cflo_N_g11458t1	2.0e-167	o
Cobs_10331	1303	0.4	5.10e-13	FBgn0032955	CG2201	1.0e-58	o	Cflo_N_g6595t1	0.0e+00	o
Cobs_10338	393	-0.2	2.34e-02	FBgn0051637	CG31637	2.0e-126	o	Cflo_N_g11411t1	0.0e+00	o
Cobs_10344	175	-0.2	1.17e-02	FBgn0031738	CG9171	7.0e-169	o	Cflo_N_g15178t1	0.0e+00	o
Cobs_10389	360	-0.2	1.01e-02	FBgn0034931	CG2812	3.0e-163	o	Cflo_N_g3063t1	0.0e+00	o
Cobs_10421	55	0.4	1.98e-04	FBgn0039420	CG6154	3.0e-165	o	Cflo_N_g2955t1	0.0e+00	o
Cobs_10427	1513	0.1	2.50e-02	FBgn0265140	Meltrin	0.0e+00	o	Cflo_N_g6182t1	0.0e+00	o
Cobs_10429	2855	-0.2	1.61e-03	FBgn0035370	CG1240	9.0e-40	o	Cflo_N_g6180t1	9.0e-143	o
Cobs_10459	169	0.2	4.23e-02	FBgn0259210	prom	9.0e-32	o	Cflo_N_g2443t1	0.0e+00	o
Cobs_10483	105	-0.3	2.02e-02	FBgn0011640	lark	2.8e-01	h	Cflo_N_g6086t1	1.0e-03	h
Cobs_10547	2929	-0.1	2.90e-02	FBgn0020622	Pi3K21B	9.0e-74	o	Cflo_N_g6277t1	0.0e+00	o
Cobs_10555	8	-0.4	3.31e-02	-	-	-	-	Cflo_N_g5609t1	5.2e-01	h
Cobs_10558	458	-0.5	3.07e-04	FBgn0031461	daw	7.0e-78	o	Cflo_N_g8385t1	0.0e+00	o
Cobs_10624	24324	-0.1	4.47e-02	FBgn0266599	Hsc70-4	0.0e+00	o	Cflo_N_g3612t1	0.0e+00	o
Cobs_10659	35	0.5	1.23e-04	FBgn0036101	NijA	3.0e-50	o	Cflo_N_g12733t1	5.0e-89	o
Cobs_10665	54	-0.3	9.77e-03	FBgn0263397	lh	2.0e-50	h	Cflo_N_g12731t1	0.0e+00	o
Cobs_10768	291	0.3	2.31e-02	FBgn0028400	Syt4	6.0e-157	o	Cflo_N_g12264t1	0.0e+00	o
Cobs_10774	428	0.2	3.77e-03	FBgn0034141	CG8311	8.0e-62	o	Cflo_N_g12265t1	5.0e-96	o

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_10851	213	-0.3	7.30e-05	FBgn0265988	mv	4.3e-01	h	Cflo_N_g13072t1	1.8e+00	h	
Cobs_10898	39	-0.5	5.86e-03	FBgn0263705	Myo10A	1.9e-01	h	Cflo_N_g785t2	0.0e+00	o	
Cobs_10899	13	-0.5	4.20e-03	FBgn0037288	CG14661	6.9e-01	h	Cflo_N_g11171t3	1.4e+00	h	
Cobs_10903	21	-0.4	2.73e-02	-	-	-	-	Cflo_N_g3560t3	0.0e+00	o	
Cobs_10905	634	0.1	3.54e-02	FBgn0043458	CG12084	8.0e-58	h	Cflo_N_g8919t1	0.0e+00	o	
Cobs_10906	19	-0.4	2.79e-02	FBgn0052457	CG32457	1.7e+00	h	Cflo_N_g7841t1	9.0e+00	h	
Cobs_10984	683	0.1	1.09e-02	FBgn0037561	CG9630	0.0e+00	o	Cflo_N_g12365t1	5.0e-172	o	
Cobs_10986	5784	-0.2	4.45e-03	FBgn0041188	Atx2	2.0e-57	o	Cflo_N_g12363t2	0.0e+00	o	
Cobs_10997	29	0.8	8.41e-09	FBgn0034136	DAT	3.0e-46	h	Cflo_N_g1106t1	5.0e-51	h	
Cobs_11011	494	-0.2	6.93e-04	FBgn0004003	wbl	8.0e-39	o	Cflo_N_g12352t1	9.0e-163	o	
Cobs_11047	1016	-0.3	1.63e-03	FBgn0259168	mnb	0.0e+00	o	Cflo_N_g7551t1	0.0e+00	o	
Cobs_11048	2649	0.2	1.19e-02	FBgn0016034	mael	6.0e-27	o	Cflo_N_g2858t1	0.0e+00	o	
Cobs_11065	52	-0.3	1.80e-02	FBgn0014135	bnl	2.0e-05	h	Cflo_N_g6538t1	2.0e-134	o	
Cobs_11114	90	0.2	4.62e-02	FBgn0261811	pico	1.0e-03	h	Cflo_N_g9943t2	3.0e-120	h	
Cobs_11121	1253	0.6	2.46e-04	FBgn0010435	emp	1.0e-88	h	Cflo_N_g9950t1	0.0e+00	o	
Cobs_11130	225	0.7	1.02e-14	FBgn0030357	Scfp	6.0e-19	o	Cflo_N_g6458t1	5.0e-63	o	
Cobs_11131	1935	-0.2	4.76e-02	FBgn0003165	pum	0.0e+00	o	Cflo_N_g1447t1	1.0e-41	o	
Cobs_11157	298	0.2	2.93e-02	FBgn0052699	CG32699	1.0e-106	o	Cflo_N_g3624t1	2.0e-130	h	
Cobs_11195	309	-0.6	1.14e-12	FBgn0053087	LRP1	4.0e-13	h	Cflo_N_g1333t2	0.0e+00	h	
Cobs_11200	4616	-0.3	2.00e-07	FBgn0261451	trol	0.0e+00	o	Cflo_N_g1333t4	0.0e+00	o	
Cobs_11205	354	0.4	2.74e-03	FBgn0035025	uri	9.3e-02	h	Cflo_N_g6593t1	8.0e-133	o	
Cobs_11213	44	0.4	6.55e-03	FBgn0085407	Pvf3	1.0e-13	h	Cflo_N_g11996t1	0.0e+00	o	
Cobs_11490	2380	-0.3	7.24e-03	FBgn0261934	dikar	1.0e-70	o	Cflo_N_g8873t1	0.0e+00	o	
Cobs_11492	557	-0.2	1.70e-02	FBgn0036191	Sug	3.0e-115	o	Cflo_N_g8874t1	0.0e+00	o	
Cobs_11502	529	0.2	4.76e-02	FBgn0015924	crq	7.0e-118	o	Cflo_N_g12197t1	0.0e+00	o	
Cobs_11504	950	-0.2	4.14e-02	FBgn0262717	Skeletor	0.0e+00	o	Cflo_N_g8867t1	0.0e+00	o	
Cobs_11651	2137	-0.2	1.70e-02	-	-	-	-	Cflo_N_g6873t1	5.0e-146	o	
Cobs_11661	203	0.4	4.45e-04	FBgn0033913	CG8468	2.0e-173	o	Cflo_N_g4422t3	0.0e+00	h	
Cobs_11700	50	0.5	1.21e-03	FBgn0001992	Cyp303a1	1.0e-94	h	Cflo_N_g4960t1	0.0e+00	o	
Cobs_11704	29	0.4	4.62e-03	-	-	-	-	Cflo_N_g5405t2	2.5e-01	h	
Cobs_11706	232	0.8	4.94e-13	FBgn0010909	msn	3.6e+00	h	Cflo_N_g9127t1	0.0e+00	o	
Cobs_11710	777	0.3	2.13e-06	FBgn0264695	Mhc	3.3e+00	h	Cflo_N_g4959t1	0.0e+00	o	
Cobs_11711	127	0.8	4.72e-16	FBgn0027654	jdp	3.0e-75	o	Cflo_N_g4958t1	5.0e-123	o	
Cobs_11725	177	0.2	2.73e-02	FBgn0263773	fok	2.0e+00	o	Cflo_N_g586t1	2.0e-53	o	
Cobs_11726	576	-0.2	4.00e-02	FBgn0002022	Catsup	2.0e-90	o	Cflo_N_g8041t1	3.0e-39	o	
Cobs_11734	1276	0.3	1.94e-02	FBgn0020764	Alas	0.0e+00	o	Cflo_N_g13654t1	0.0e+00	o	
Cobs_11738	495	-0.1	4.76e-02	FBgn0030007	CG2263	0.0e+00	o	Cflo_N_g13650t1	0.0e+00	o	
Cobs_11766	163	0.2	2.27e-02	FBgn0052112	CG32112	0.0e+00	o	Cflo_N_g1469t1	0.0e+00	o	
Cobs_11812	86	1.3	4.50e-35	FBgn0020909	Rtc1	1.2e+00	h	Cflo_N_g13226t3	1.6e+00	h	
Cobs_11824	631	-0.3	6.14e-04	FBgn0003257	r-1	2.0e-148	o	Cflo_N_g7753t1	0.0e+00	o	
Cobs_11836	2296	-0.2	4.23e-02	FBgn0033663	eRp60	0.0e+00	o	Cflo_N_g14619t1	0.0e+00	o	
Cobs_11839	23439	1.5	1.44e-33	FBgn0041182	Tep2	5.0e-114	h	Cflo_N_g7345t1	0.0e+00	o	
Cobs_11847	18	-0.4	1.48e-02	FBgn0037924	CG14712	3.5e+00	h	Cflo_N_g8789t1	0.0e+00	o	
Cobs_11849	61	0.4	3.76e-04	FBgn0085224	CG34195	4.0e-95	o	Cflo_N_g8791t2	1.0e-132	o	
Cobs_11853	29	-0.4	3.15e-02	-	-	-	-	Cflo_N_g8790t1	5.0e-07	h	
Cobs_11864	1910	0.3	1.21e-05	FBgn0020386	Pdk1	0.0e+00	o	Cflo_N_g8541t1	0.0e+00	o	
Cobs_11868	488	0.4	6.76e-04	-	-	-	-	Cflo_N_g8541t1	6.2e-02	h	
Cobs_11948	1415	-0.1	2.20e-02	FBgn0259214	PMCA	0.0e+00	o	Cflo_N_g14013t1	0.0e+00	o	
Cobs_11950	1838	-0.7	8.04e-21	FBgn0038349	CG6045	0.0e+00	o	Cflo_N_g14017t1	0.0e+00	o	
Cobs_11967	6508	-0.3	1.59e-03	FBgn0261260	mg1	0.0e+00	o	Cflo_N_g243t1	0.0e+00	o	
Cobs_11994	338	-0.2	3.83e-02	FBgn0032120	CG33298	0.0e+00	o	Cflo_N_g5113t1	0.0e+00	o	
Cobs_12013	160	-0.2	3.18e-02	FBgn0087007	bbg	9.0e-31	h	Cflo_N_g2766t1	1.0e-25	h	
Cobs_12014	1091	-0.2	3.58e-02	FBgn0050069	CG30069	0.0e+00	o	Cflo_N_g5127t1	0.0e+00	o	
Cobs_12024	925	0.6	1.19e-16	FBgn0031220	CG4822	2.0e-152	o	Cflo_N_g3099t1	0.0e+00	o	
Cobs_12025	1283	0.4	9.99e-06	FBgn0025683	CG3164	0.0e+00	o	Cflo_N_g3098t1	0.0e+00	o	
Cobs_12027	298	0.3	2.22e-02	FBgn0020762	Atet	0.0e+00	o	Cflo_N_g3096t1	0.0e+00	o	
Cobs_12047	1376	-0.2	4.40e-02	FBgn0004618	gl	3.0e-06	h	Cflo_N_g3080t1	0.0e+00	o	
Cobs_12060	769	0.6	5.10e-13	FBgn0025697	santa-maria	1.0e-105	o	Cflo_N_g10397t2	0.0e+00	o	
Cobs_12074	26	0.4	3.15e-02	-	-	-	-	Cflo_N_g7114t1	1.0e+00	h	
Cobs_12102	482	-0.2	6.86e-03	FBgn0036147	Plod	0.0e+00	o	Cflo_N_g14033t1	0.0e+00	o	
Cobs_12117	13	-0.4	3.47e-02	FBgn0036117	CG6321	2.9e+00	h	Cflo_N_g8960t2	1.6e-01	h	

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_12142	65	0.4	3.35e-03	FBgn0037363	Atg17	9.0e-21	h	Cflo_N_g4875t1	8.0e-52	h	
Cobs_12202	1884	0.6	3.60e-09	FBgn0032213	CG5390	5.0e-112	o	Cflo_N_g6513t1	1.1e+00	h	
Cobs_12204	372	0.3	3.04e-07	FBgn0029824	CG3726	7.0e-32	h	Cflo_N_g497t1	0.0e+00	h	
Cobs_12205	32	0.4	9.50e-03	FBgn0029824	CG3726	2.0e-23	h	Cflo_N_g7694t1	2.0e-30	h	
Cobs_12211	42	-0.3	2.78e-02	FBgn0039068	CG13827	2.0e-50	o	Cflo_N_g7706t1	2.0e-54	o	
Cobs_12253	2897	0.2	2.30e-03	FBgn0266019	rudhira	0.0e+00	o	Cflo_N_g14238t1	1.0e-32	h	
Cobs_12254	3421	0.4	3.21e-05	FBgn0032631	CG15140	2.0e-08	o	Cflo_N_g8350t1	4.0e-133	o	
Cobs_12257	146	0.3	2.16e-04	FBgn0031116	CG1695	7.5e-01	h	Cflo_N_g1956t1	5.9e+00	h	
Cobs_12258	978	0.2	1.53e-03	FBgn0031955	CG14535	2.7e+00	h	Cflo_N_g14426t1	1.1e+00	h	
Cobs_12269	186	-0.4	2.13e-06	FBgn0030662	CG9220	0.0e+00	o	Cflo_N_g5518t1	0.0e+00	o	
Cobs_12287	148	0.3	2.23e-02	FBgn0026255	clumsy	5.0e-04	h	Cflo_N_g12299t1	0.0e+00	o	
Cobs_12300	190	0.2	7.25e-03	FBgn0052121	CG32121	1.0e-42	o	Cflo_N_g13912t1	4.0e-125	o	
Cobs_12301	948	0.3	1.04e-04	FBgn0040066	wds	1.0e-19	h	Cflo_N_g13914t1	0.0e+00	o	
Cobs_12314	974	0.2	9.60e-04	FBgn0051357	CG31357	4.0e-158	o	Cflo_N_g1837t1	0.0e+00	o	
Cobs_12321	526	0.3	1.95e-04	FBgn0037960	mthl5	2.0e-115	o	Cflo_N_g7234t1	0.0e+00	o	
Cobs_12325	9851	-0.2	1.59e-02	FBgn0261710	nocte	5.0e-21	o	Cflo_N_g7240t5	0.0e+00	o	
Cobs_12330	1342	0.2	2.85e-02	FBgn0037443	Dmtn	6.0e-175	o	Cflo_N_g7237t2	0.0e+00	o	
Cobs_12336	38	0.4	1.14e-02	FBgn0034002	CG8079	6.7e-01	h	Cflo_N_g6005t3	7.6e-01	h	
Cobs_12338	192	-0.5	3.32e-05	FBgn0010638	Sec61beta	4.0e-37	o	Cflo_N_g12019t1	3.0e-61	o	
Cobs_12348	309	-0.5	4.30e-08	FBgn0034276	CG6385	0.0e+00	o	Cflo_N_g7242t1	0.0e+00	o	
Cobs_12353	3467	0.3	3.01e-08	FBgn0066101	LpR1	0.0e+00	o	Cflo_N_g9796t1	0.0e+00	o	
Cobs_12360	291	0.3	2.76e-04	FBgn0027581	CG6191	3.0e-131	o	Cflo_N_g9797t1	0.0e+00	o	
Cobs_12361	479	0.9	8.11e-11	FBgn0034136	DAT	0.0e+00	o	Cflo_N_g1106t1	0.0e+00	o	
Cobs_12428	436	0.2	2.59e-02	FBgn0051274	CG31274	1.0e-19	o	Cflo_N_g792t1	4.0e-125	o	
Cobs_12435	3526	-0.3	4.63e-02	FBgn0002926	ndl	8.0e-121	o	Cflo_N_g785t2	0.0e+00	h	
Cobs_12438	61	-0.4	5.86e-03	FBgn0031879	uif	0.0e+00	o	Cflo_N_g14366t1	9.1e-01	h	
Cobs_12445	149	0.3	1.35e-02	FBgn0005614	trpl	0.0e+00	o	Cflo_N_g777t1	0.0e+00	o	
Cobs_12457	549	0.4	4.81e-10	FBgn0032882	Ns4	2.0e-159	o	Cflo_N_g6816t1	0.0e+00	o	
Cobs_12482	202	-0.5	1.71e-05	FBgn0043792	CG30427	0.0e+00	o	Cflo_N_g10245t1	0.0e+00	o	
Cobs_12491	19	-0.4	2.50e-02	-	-	-	-	Cflo_N_g1056t1	6.9e-01	h	
Cobs_12495	328	-0.2	3.38e-02	FBgn0016754	sba	3.0e-28	o	Cflo_N_g7752t1	5.0e-04	h	
Cobs_12528	996	-0.3	1.36e-04	FBgn0023441	fus	0.0e+00	o	Cflo_N_g622t1	7.0e-125	o	
Cobs_12529	228	-0.5	1.48e-10	FBgn0023441	fus	2.0e-05	h	Cflo_N_g7650t1	3.0e-53	h	
Cobs_12530	1834	0.2	6.37e-03	FBgn0036257	RhoGAP68F	2.0e-108	o	Cflo_N_g11998t2	0.0e+00	o	
Cobs_12551	1487	-0.2	3.62e-03	FBgn0003053	peb	4.0e-59	o	Cflo_N_g13232t1	0.0e+00	o	
Cobs_12590	16	0.5	3.03e-03	-	-	-	-	Cflo_N_g254t1	1.4e+00	h	
Cobs_12718	2320	0.2	4.77e-02	FBgn0262579	ect4	0.0e+00	o	Cflo_N_g3635t1	0.0e+00	o	
Cobs_12815	9	-0.4	1.56e-02	-	-	-	-	Cflo_N_g12997t1	2.0e-37	h	
Cobs_12879	4596	0.2	4.64e-02	FBgn0032456	MRP	0.0e+00	o	Cflo_N_g10628t5	0.0e+00	o	
Cobs_12915	628	-0.4	1.09e-05	FBgn0266084	Fhos	0.0e+00	o	Cflo_N_g10704t1	0.0e+00	o	
Cobs_12925	19	-0.5	3.40e-04	FBgn0010470	Fkbp14	4.0e-84	o	Cflo_N_g10706t1	7.0e-110	o	
Cobs_12929	14	-0.4	1.93e-02	FBgn0045495	Gr28b	8.1e-01	h	Cflo_N_g4611t1	4.3e+00	h	
Cobs_12936	800	0.3	3.38e-03	FBgn0051974	CG31974	2.0e-50	o	Cflo_N_g1631t1	0.0e+00	o	
Cobs_12963	218	-0.5	1.37e-04	FBgn0030013	GIIIspla2	2.0e-37	o	Cflo_N_g13334t1	7.0e-157	o	
Cobs_12971	558	0.2	4.14e-03	FBgn0030183	CG15309	2.0e-47	o	Cflo_N_g12988t1	0.0e+00	o	
Cobs_12974	19	-0.5	7.31e-03	FBgn0040208	Kat60	7.1e+00	h	Cflo_N_g2081t1	1.5e-01	h	
Cobs_12977	8	-0.4	2.41e-02	FBgn0023508	Ocrl	2.6e+00	h	Cflo_N_g9179t1	3.5e+00	h	
Cobs_12980	13	-0.4	2.25e-02	FBgn0035942	CG5660	5.4e+00	h	Cflo_N_g4197t1	4.2e+00	h	
Cobs_13000	225	-0.3	6.79e-03	FBgn0265487	mbl	9.0e-37	h	Cflo_N_g13888t1	3.0e-43	o	
Cobs_13006	17	-0.4	2.58e-02	-	-	-	-	-	-	-	
Cobs_13018	1591	0.2	3.09e-02	FBgn0016126	CaMKI	0.0e+00	o	Cflo_N_g5919t1	0.0e+00	o	
Cobs_13021	1298	0.1	7.58e-03	FBgn0040475	SH3PX1	5.0e-154	o	Cflo_N_g6800t1	0.0e+00	o	
Cobs_13033	2568	0.1	4.57e-02	FBgn0028371	jbug	0.0e+00	o	Cflo_N_g7854t1	0.0e+00	o	
Cobs_13039	1469	0.2	3.98e-04	FBgn0037442	CG10277	8.0e-80	o	Cflo_N_g2700t1	7.0e-130	o	
Cobs_13044	1329	0.3	4.81e-04	FBgn0004606	zfh1	5.0e-49	o	Cflo_N_g8967t1	0.0e+00	o	
Cobs_13094	181	0.8	2.17e-08	FBgn0265001	ppk18	1.9e+00	h	Cflo_N_g5816t1	3.0e-29	o	
Cobs_13105	109	-1.4	1.53e-60	FBgn0053196	dp	1.0e-26	h	Cflo_N_g5826t2	0.0e+00	o	
Cobs_13108	10	-0.6	7.98e-05	FBgn0260642	Antp	6.2e+00	h	Cflo_N_g5826t1	3.0e-36	h	
Cobs_13111	11	-0.5	1.34e-03	FBgn0037051	CG10565	4.3e-01	h	Cflo_N_g10407t1	0.0e+00	o	
Cobs_13119	3237	0.2	4.70e-03	FBgn0022768	Pp2C1	3.0e-142	o	Cflo_N_g4196t1	0.0e+00	o	
Cobs_13169	1093	0.2	2.99e-03	FBgn0050104	NT5e-2	2.0e-162	o	Cflo_N_g3583t1	0.0e+00	o	

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015		
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h
Cobs_13195	91	0.3	2.73e-02	-	-	-	-	Cflo_N_g10322t1	2.6e+00	h
Cobs_13203	436	0.2	4.36e-02	FBgn0053156	CG33156	0.0e+00	o	Cflo_N_g5594t1	0.0e+00	o
Cobs_13242	73	-0.4	7.85e-03	FBgn0086917	spok	5.0e-116	o	Cflo_N_g2995t1	0.0e+00	o
Cobs_13269	615	-0.3	4.58e-05	FBgn0016977	spen	2.3e+00	h	Cflo_N_g14121t1	1.0e-135	o
Cobs_13295	56	-0.3	6.55e-03	FBgn0030040	CG15347	2.5e-01	o	Cflo_N_g10041t1	0.0e+00	o
Cobs_13313	9	-0.4	3.13e-02	FBgn0051133	Slimp	3.0e+00	h	Cflo_N_g12322t1	1.0e-27	h
Cobs_13320	129	0.3	9.38e-03	FBgn0040001	CG17374	2.0e-79	h	Cflo_N_g264t2	2.0e-130	h
Cobs_13322	37	-0.5	1.06e-03	-	-	-	-	Cflo_N_g10235t1	1.0e-97	h
Cobs_13325	23	-0.3	4.41e-02	-	-	-	-	Cflo_N_g15226t1	9.0e-31	h
Cobs_13490	2193	-0.2	9.64e-03	FBgn0028327	l(1)G0320	2.0e-83	o	Cflo_N_g3788t1	3.0e-153	o
Cobs_13499	33	-0.4	2.85e-03	FBgn0265726	NnaD	0.0e+00	o	Cflo_N_g12033t1	0.0e+00	o
Cobs_13538	185	0.6	5.23e-08	FBgn0023540	CG3630	2.0e-58	o	Cflo_N_g1798t1	2.0e-95	o
Cobs_13541	201	0.2	4.47e-02	FBgn0034566	CG9313	5.0e-13	h	Cflo_N_g9250t1	6.0e-107	o
Cobs_13547	1654	0.2	1.32e-03	FBgn0033266	Socs44A	4.0e-20	o	Cflo_N_g10988t1	0.0e+00	o
Cobs_13550	1002	-0.2	2.63e-03	FBgn0261872	scaf6	1.0e-81	o	Cflo_N_g1794t1	0.0e+00	o
Cobs_13553	803	0.3	1.50e-03	FBgn0032167	CG5853	3.0e-137	h	Cflo_N_g5454t1	0.0e+00	o
Cobs_13613	523	0.7	2.12e-06	FBgn0034647	pirk	1.0e-08	o	Cflo_N_g7396t1	5.0e-89	o
Cobs_13626	2138	0.2	1.58e-02	FBgn0267002	unc-104	0.0e+00	o	Cflo_N_g1096t2	0.0e+00	o
Cobs_13643	2517	0.3	1.98e-04	FBgn0013984	InR	0.0e+00	h	Cflo_N_g30t1	0.0e+00	o
Cobs_13644	72	0.3	2.06e-02	-	-	-	-	Cflo_N_g31t1	3.0e-67	o
Cobs_13682	1167	0.2	2.93e-03	FBgn0029891	Pink1	8.0e-126	o	Cflo_N_g10799t1	0.0e+00	o
Cobs_13685	11	-0.4	4.81e-02	-	-	-	-	Cflo_N_g14044t1	1.7e-01	h
Cobs_13692	31	0.4	9.97e-03	FBgn0034568	CG3216	0.0e+00	o	Cflo_N_g3375t2	0.0e+00	o
Cobs_13694	1008	0.2	1.94e-02	FBgn0027498	CG2061	5.0e-110	o	Cflo_N_g3213t1	0.0e+00	o
Cobs_13700	701	0.5	4.19e-08	FBgn0036053	iPLA2-VIA	4.0e-30	h	Cflo_N_g3376t1	0.0e+00	o
Cobs_13708	270	0.2	1.51e-02	FBgn0020503	CLIP-190	7.0e-06	h	Cflo_N_g3471t1	0.0e+00	o
Cobs_13721	4506	-0.5	5.13e-04	FBgn0036995	CG5195	2.0e-23	h	Cflo_N_g2894t1	7.0e-73	o
Cobs_13723	1031	0.2	1.11e-04	FBgn0020647	KrT95D	2.0e-178	o	Cflo_N_g9436t1	0.0e+00	h
Cobs_13731	23	-0.7	7.48e-06	FBgn0266557	kis	1.6e+00	h	Cflo_N_g3486t1	5.0e-28	o
Cobs_13830	8	-0.6	4.93e-04	FBgn0033447	dila	5.7e-02	h	Cflo_N_g12890t3	2.8e-01	h
Cobs_13879	538	0.2	4.69e-04	FBgn0261788	Ank2	1.0e-27	h	Cflo_N_g6254t1	2.0e-28	h
Cobs_13888	696	-0.3	2.62e-05	FBgn0051268	CG31268	9.3e-01	h	Cflo_N_g3882t1	2.0e-133	o
Cobs_13902	1946	0.3	7.52e-06	FBgn0052451	SPoCk	0.0e+00	o	Cflo_N_g15212t1	0.0e+00	o
Cobs_13903	1640	0.2	7.10e-04	FBgn0033814	CG4670	6.0e-99	o	Cflo_N_g5418t1	0.0e+00	o
Cobs_13915	167	0.2	7.57e-03	FBgn0259676	DNApol- $\alpha$ 60	3.0e-80	h	Cflo_N_g5153t1	0.0e+00	o
Cobs_13919	45	0.4	1.20e-02	FBgn0266801	CG45263	7.5e-01	h	Cflo_N_g6706t4	1.1e+00	h
Cobs_13933	1338	0.1	4.73e-02	FBgn0034948	Gadd34	8.0e-19	o	Cflo_N_g12185t1	4.0e-23	o
Cobs_13941	11	-0.4	3.08e-02	-	-	-	-	Cflo_N_g12153t1	7.0e-40	o
Cobs_13944	39	-0.5	1.09e-04	FBgn0015905	ast	7.8e+00	h	Cflo_N_g9861t3	1.3e+00	h
Cobs_13954	607	0.2	9.77e-03	FBgn0262871	lute	2.0e-12	h	Cflo_N_g8975t1	0.0e+00	o
Cobs_14009	3065	0.2	8.97e-04	FBgn0033375	CG8078	4.0e-03	h	Cflo_N_g5077t1	2.4e-01	h
Cobs_14010	103	0.2	3.08e-02	-	-	-	-	Cflo_N_g10661t1	5.0e-28	h
Cobs_14018	2439	-0.3	3.23e-02	FBgn0037913	fabp	8.0e-37	o	Cflo_N_g12156t1	7.0e-67	o
Cobs_14037	121	-0.7	2.17e-08	FBgn0003965	v	1.0e-165	o	Cflo_N_g12143t1	1.0e-180	o
Cobs_14078	451	-0.8	4.45e-20	FBgn0031307	MFS3	2.0e-97	h	Cflo_N_g556t1	0.0e+00	o
Cobs_14088	1029	1.0	7.28e-16	FBgn0000808	gd	8.0e-50	o	Cflo_N_g931t1	1.0e-154	h
Cobs_14094	213	0.3	1.27e-02	FBgn0033302	Cyp6a14	1.0e-131	h	Cflo_N_g11774t2	0.0e+00	h
Cobs_14096	22	-0.5	7.95e-04	FBgn0031573	CG3407	8.2e-01	h	Cflo_N_g7260t1	1.0e-87	o
Cobs_14122	1112	0.1	3.74e-02	FBgn0028467	CG11070	0.0e+00	o	Cflo_N_g4585t1	0.0e+00	o
Cobs_14158	137	-0.2	2.53e-02	FBgn0013799	Deaf1	1.0e-75	o	Cflo_N_g10934t1	0.0e+00	o
Cobs_14189	5636	-0.3	1.89e-04	FBgn0014002	Pdi	0.0e+00	o	Cflo_N_g2034t1	2.0e-154	o
Cobs_14208	454	0.2	3.79e-02	FBgn0001297	kay	7.0e-25	o	Cflo_N_g4719t2	0.0e+00	o
Cobs_14214	406	0.2	9.25e-04	FBgn0025830	IntS8	1.4e+00	h	Cflo_N_g13971t1	0.0e+00	o
Cobs_14221	3353	0.3	6.22e-04	FBgn0003231	ref(2)P	1.0e-09	o	Cflo_N_g12868t1	6.0e-176	o
Cobs_14228	3048	0.4	2.23e-09	FBgn0036449	bmm	1.0e-159	o	Cflo_N_g11733t1	4.0e-173	o
Cobs_14237	373	-0.3	2.07e-02	FBgn0053181	CG33181	1.0e-171	o	Cflo_N_g14917t1	0.0e+00	h
Cobs_14247	12	-0.6	5.08e-04	FBgn0034276	CG6385	4.4e+00	h	Cflo_N_g10504t1	3.0e-127	h
Cobs_14307	928	0.1	1.30e-02	FBgn0028509	CenG1A	9.0e-103	o	Cflo_N_g2053t1	0.0e+00	o
Cobs_14423	10	-0.4	4.42e-02	FBgn0263391	hts	2.2e-01	h	Cflo_N_g11653t1	3.0e-31	h
Cobs_14426	13	-0.5	6.85e-03	FBgn0034429	CG18607	1.3e-02	h	Cflo_N_g1826t1	5.0e-26	h
Cobs_14436	147	-0.2	4.77e-02	FBgn0260005	wtrw	7.0e-77	h	Cflo_N_g218t1	3.0e-64	o



	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_14445	598	0.2	6.55e-03	FBgn0085409	CG34380	2.0e-74	h	Cflo_N_g8022t1	4.0e-38	h	
Cobs_14468	75	-0.4	1.62e-03	FBgn0052666	Drak	7.0e-80	o	Cflo_N_g9986t1	0.0e+00	o	
Cobs_14490	516	-0.3	4.59e-03	FBgn0033358	CG8216	6.0e-17	o	Cflo_N_g4560t1	2.0e-104	o	
Cobs_14497	4019	0.1	2.20e-02	FBgn0004401	Pep	4.0e-50	o	Cflo_N_g13640t1	0.0e+00	o	
Cobs_14516	710	0.1	1.21e-02	FBgn0086909	CG31751	2.0e-87	o	Cflo_N_g9112t1	0.0e+00	o	
Cobs_14546	15	-0.5	9.20e-04	FBgn0053481	dpr7	2.0e-80	o	Cflo_N_g2802t2	2.0e-142	o	
Cobs_14548	757	-0.3	4.74e-02	FBgn0039311	CG10513	1.0e-57	o	Cflo_N_g2808t1	6.0e-132	o	
Cobs_14569	267	0.8	3.54e-08	FBgn0063923	Kaz1-ORFB	7.0e-08	o	Cflo_N_g13647t1	0.0e+00	o	
Cobs_14589	168	0.3	6.07e-03	FBgn0030257	CG11160	8.0e-72	h	Cflo_N_g12801t1	0.0e+00	o	
Cobs_14590	65	-0.4	5.03e-04	FBgn0038244	CG7987	6.2e+00	h	Cflo_N_g4500t1	0.0e+00	o	
Cobs_14629	245	-0.3	2.41e-03	FBgn0261380	mRpL37	1.0e-76	o	Cflo_N_g760t1	3.0e-180	o	
Cobs_14633	136	-0.4	1.02e-02	FBgn0029896	CG3168	1.0e-89	h	Cflo_N_g769t1	0.0e+00	o	
Cobs_14710	578	1.0	1.25e-11	FBgn0005626	ple	0.0e+00	o	Cflo_N_g5222t2	0.0e+00	o	
Cobs_14837	458	0.3	7.50e-03	FBgn0036980	RhoBTB	0.0e+00	o	Cflo_N_g4853t1	5.0e-140	o	
Cobs_14890	786	0.2	4.16e-02	FBgn0030892	CG7206	1.0e-11	o	Cflo_N_g8038t1	0.0e+00	o	
Cobs_15045	229	0.6	6.56e-11	FBgn0052372	lfl	2.0e-92	o	Cflo_N_g7352t1	0.0e+00	o	
Cobs_15052	1378	-0.3	3.10e-09	FBgn0033159	Dscam1	0.0e+00	o	Cflo_N_g4811t6	0.0e+00	o	
Cobs_15053	52	-0.4	7.70e-03	FBgn0033159	Dscam1	1.0e-56	h	Cflo_N_g4816t1	6.0e-156	o	
Cobs_15103	44	-0.5	5.48e-05	FBgn0013811	Dhc62B	0.0e+00	h	Cflo_N_g1069t1	0.0e+00	o	
Cobs_15115	173	0.5	6.18e-06	FBgn0030189	CG2909	6.0e-102	o	Cflo_N_g1062t2	0.0e+00	o	
Cobs_15135	502	0.2	1.08e-02	FBgn0030749	AnxB11	2.0e-126	o	Cflo_N_g4003t1	0.0e+00	o	
Cobs_15143	700	-0.3	7.68e-08	FBgn0260388	CG42514	1.0e-168	o	Cflo_N_g6983t1	0.0e+00	o	
Cobs_15154	384	-0.2	1.23e-02	FBgn0033348	Spt	6.0e-16	o	Cflo_N_g8895t1	0.0e+00	h	
Cobs_15159	389	-0.3	3.30e-03	FBgn0000409	Cyt-c-p	7.0e-55	o	Cflo_N_g12294t1	4.0e-73	o	
Cobs_15168	1036	0.1	3.81e-02	FBgn0067783	att-ORFA	6.0e-102	o	Cflo_N_g8882t1	0.0e+00	o	
Cobs_15174	28	-0.4	2.86e-02	FBgn0265767	zyd	1.0e-106	h	Cflo_N_g13823t1	0.0e+00	o	
Cobs_15186	1012	0.2	4.72e-02	FBgn0038418	pad	8.0e-31	o	Cflo_N_g12191t1	1.0e-153	o	
Cobs_15197	1690	0.2	4.22e-06	FBgn0027280	l(1)G0193	1.0e-173	o	Cflo_N_g4327t1	0.0e+00	o	
Cobs_15208	113	0.2	1.86e-02	FBgn0261976	Psf2	3.0e-60	o	Cflo_N_g3023t1	5.0e-108	o	
Cobs_15216	54	-0.3	3.86e-02	FBgn0051324	CG31324	5.0e-08	o	Cflo_N_g9999t1	5.0e-09	o	
Cobs_15242	2041	-0.2	3.47e-02	FBgn0011726	tsr	3.0e-93	o	Cflo_N_g10477t1	8.0e-35	o	
Cobs_15254	267	0.2	1.34e-03	FBgn0029713	CG11436	5.0e-25	o	Cflo_N_g5808t1	3.2e+00	h	
Cobs_15262	101	0.3	2.29e-03	FBgn0038804	CG10877	1.0e-168	o	Cflo_N_g11562t1	0.0e+00	o	
Cobs_15272	10	-0.6	4.25e-04	FBgn0086916	snky	4.7e+00	h	Cflo_N_g8529t1	1.3e+00	h	
Cobs_15341	501	0.2	4.79e-03	FBgn0038453	CG10326	4.0e-12	o	Cflo_N_g5850t1	4.0e-97	o	
Cobs_15382	70	-0.4	7.58e-04	FBgn0031821	KFase	3.0e-27	o	Cflo_N_g109t1	6.0e-159	o	
Cobs_15388	50	-0.3	1.05e-02	FBgn0263846	CG43707	7.1e+00	h	Cflo_N_g14608t2	2.0e-01	h	
Cobs_15391	126	-0.3	2.63e-02	FBgn0250871	pot	0.0e+00	o	Cflo_N_g117t1	0.0e+00	o	
Cobs_15401	452	-0.4	1.07e-08	FBgn0004646	ogre	4.0e-180	o	Cflo_N_g7714t1	0.0e+00	o	
Cobs_15424	713	-0.2	1.33e-02	FBgn0032015	Osty	9.0e-153	o	Cflo_N_g852t2	0.0e+00	o	
Cobs_15427	365	0.2	1.07e-03	FBgn0265464	Traf6	2.0e-68	o	Cflo_N_g13894t1	2.0e-167	o	
Cobs_15428	737	-0.2	9.51e-04	FBgn0262139	trh	2.6e+00	h	Cflo_N_g4238t1	8.0e-72	o	
Cobs_15497	2927	0.8	1.07e-08	FBgn0022355	Tsf1	1.0e-112	o	Cflo_N_g114t1	0.0e+00	o	
Cobs_15498	237	-0.2	5.60e-03	FBgn0031619	CG3355	1.0e-45	h	Cflo_N_g1261t1	0.0e+00	o	
Cobs_15511	6913	0.6	6.86e-05	-	-	-	-	Cflo_N_g6817t1	5.0e-20	h	
Cobs_15528	343	-0.2	6.45e-03	FBgn0265178	CG44243	8.0e-129	o	Cflo_N_g11959t1	0.0e+00	o	
Cobs_15530	805	-0.3	1.90e-05	FBgn0020238	14-3-3ε	2.0e-163	o	Cflo_N_g5870t1	8.0e-178	o	
Cobs_15538	123	-0.3	4.79e-03	FBgn0037330	mRpL44	1.9e+00	h	Cflo_N_g3424t1	9.7e-01	h	
Cobs_15561	2264	-0.2	2.98e-02	FBgn0001248	ldh	0.0e+00	o	Cflo_N_g11170t1	0.0e+00	o	
Cobs_15591	18	-0.5	4.98e-03	FBgn0034494	CG10444	7.0e-149	h	Cflo_N_g3709t1	0.0e+00	o	
Cobs_15595	12	-0.7	3.58e-06	FBgn0000287	salr	4.6e-01	h	Cflo_N_g5290t1	7.5e+00	h	
Cobs_15599	207	-0.6	2.59e-13	FBgn0026379	Pten	4.0e-17	h	Cflo_N_g3662t1	0.0e+00	o	
Cobs_15602	561	-0.4	6.65e-11	FBgn0000244	by	8.0e-89	o	Cflo_N_g3711t3	0.0e+00	o	
Cobs_15609	15	-0.4	9.42e-03	FBgn0036623	CG4729	1.4e+00	h	Cflo_N_g10680t1	3.9e+00	h	
Cobs_15636	536	0.2	1.99e-02	FBgn0035436	CG12016	1.0e-19	o	Cflo_N_g12995t1	1.0e-103	o	
Cobs_15639	147	0.7	1.19e-11	FBgn0030051	spirit	1.0e-55	o	Cflo_N_g8989t1	7.0e-162	h	
Cobs_15710	4691	0.1	1.72e-02	-	-	-	-	Cflo_N_g5961t1	0.0e+00	o	
Cobs_15768	2064	0.2	2.23e-02	FBgn0013984	InR	0.0e+00	o	Cflo_N_g8682t2	0.0e+00	o	
Cobs_15773	11	0.4	4.36e-02	FBgn0037797	CG12420	9.0e-07	o	Cflo_N_g11014t1	2.0e-84	o	
Cobs_15776	21	0.4	2.20e-02	FBgn0030715	Or13a	5.0e-13	h	Cflo_N_g8690t1	3.0e-156	o	
Cobs_15795	800	-0.3	5.86e-04	FBgn0031681	pgant5	0.0e+00	o	Cflo_N_g125t1	0.0e+00	o	

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_15805	1247	0.2	2.92e-02	FBgn0030087	CG7766	0.0e+00	o	Cflo_N_g5181t1	0.0e+00	o	
Cobs_15816	353	-0.5	8.13e-07	FBgn0264087	Slob	4.0e-56	o	Cflo_N_g11793t1	7.0e-132	o	
Cobs_15823	6593	0.2	5.74e-03	FBgn0032940	Mio	1.0e-62	o	Cflo_N_g10017t1	0.0e+00	o	
Cobs_15891	199	-0.4	1.36e-03	FBgn0020294	ko	4.0e-106	o	Cflo_N_g752t1	0.0e+00	o	
Cobs_15893	68	-0.3	1.37e-02	FBgn0051224	CG31224	2.0e-06	h	Cflo_N_g14428t1	3.0e-14	h	
Cobs_15914	516	0.2	2.14e-02	FBgn0003209	raw	2.0e-44	o	Cflo_N_g225t2	0.0e+00	o	
Cobs_15946	1245	0.9	4.23e-11	-	-	-	-	Cflo_N_g6700t1	3.0e-39	o	
Cobs_15971	99	-0.5	6.18e-06	FBgn0036576	CG5151	3.6e-02	h	Cflo_N_g12615t1	5.0e-43	o	
Cobs_16005	54	-0.3	4.41e-02	FBgn0000560	eg	3.0e-56	h	Cflo_N_g12589t1	4.0e-76	o	
Cobs_16081	1226	0.2	1.22e-02	FBgn0011296	l(2)efl	3.0e-53	h	Cflo_N_g14106t1	5.0e-106	o	
Cobs_16120	757	-0.2	2.70e-02	FBgn0020255	Ran	9.0e-149	o	Cflo_N_g9185t1	1.0e-127	h	
Cobs_16135	675	0.3	9.79e-04	FBgn0033302	Cyp6a14	1.0e-129	o	Cflo_N_g11706t1	0.0e+00	o	
Cobs_16180	611	0.4	5.23e-10	FBgn0086901	cv-c	0.0e+00	o	Cflo_N_g490t2	0.0e+00	o	
Cobs_16244	792	0.2	2.07e-02	FBgn0033484	CG2269	9.0e-21	o	Cflo_N_g9925t1	0.0e+00	o	
Cobs_16278	932	-0.4	2.00e-07	FBgn0000454	Dip-B	2.0e-158	o	Cflo_N_g8061t1	0.0e+00	o	
Cobs_16284	116	-0.6	5.37e-13	FBgn0011828	Pxn	0.0e+00	o	Cflo_N_g3502t1	0.0e+00	o	
Cobs_16313	194	-0.3	1.29e-03	FBgn0261436	DhpD	3.0e-139	o	Cflo_N_g11634t1	0.0e+00	o	
Cobs_16363	110	0.2	2.37e-02	FBgn0039915	CG1732	0.0e+00	h	Cflo_N_g1596t1	0.0e+00	o	
Cobs_16370	6608	-0.3	1.13e-02	FBgn0034976	CG4049	3.0e-122	o	Cflo_N_g1811t2	0.0e+00	o	
Cobs_16375	90	-0.8	9.09e-08	FBgn0039156	CG6178	3.0e-66	h	Cflo_N_g9349t1	5.0e-149	h	
Cobs_16379	108	0.4	5.69e-03	FBgn0013983	imd	2.0e-17	o	Cflo_N_g7081t1	1.0e-150	o	
Cobs_16398	19	-0.7	2.75e-06	FBgn0014343	mirr	2.0e-16	h	Cflo_N_g3325t1	1.0e-160	o	
Cobs_16404	293	0.2	8.70e-03	FBgn0033236	CG14764	2.0e-67	o	Cflo_N_g14913t1	0.0e+00	o	
Cobs_16405	200	0.4	1.09e-03	FBgn0036169	Fuca	5.0e-172	o	Cflo_N_g9344t1	0.0e+00	o	
Cobs_16410	34	-0.4	4.91e-03	FBgn0038630	CG14305	6.0e-103	h	Cflo_N_g14516t1	0.0e+00	o	
Cobs_16423	1341	-0.2	9.03e-03	FBgn0259979	CG17337	0.0e+00	o	Cflo_N_g9343t1	0.0e+00	o	
Cobs_16426	2710	0.3	1.63e-03	FBgn0261016	clos	3.0e-04	o	Cflo_N_g4849t1	0.0e+00	o	
Cobs_16428	3314	0.4	4.93e-03	FBgn0035976	PGRP-LC	2.0e-51	h	Cflo_N_g103t1	6.0e-103	o	
Cobs_16441	246	0.2	4.03e-02	FBgn0015000	betaggt-I	3.0e+00	h	Cflo_N_g2715t1	5.3e-01	h	
Cobs_16477	25	0.4	3.18e-02	FBgn0040001	CG17374	9.0e-17	h	Cflo_N_g2944t1	2.0e-58	h	
Cobs_16478	295	0.4	5.04e-07	FBgn0040001	CG17374	9.0e-105	h	Cflo_N_g264t3	4.0e-161	o	
Cobs_16482	10	0.4	2.21e-02	FBgn0051388	CG31388	5.5e+00	h	Cflo_N_g1076t1	0.0e+00	o	
Cobs_16484	11	0.4	2.65e-02	FBgn0031900	CG13786	6.2e+00	h	Cflo_N_g2837t1	3.2e+00	h	
Cobs_16486	593	0.3	8.21e-04	FBgn0265002	CG44153	1.0e-107	o	Cflo_N_g3993t1	0.0e+00	o	
Cobs_16487	2795	0.2	2.02e-02	FBgn0086768	Pcmt	1.0e-11	h	Cflo_N_g3996t1	0.0e+00	o	
Cobs_16494	51	0.3	2.31e-02	FBgn0263132	Cht6	1.1e+00	h	Cflo_N_g259t1	0.0e+00	o	
Cobs_16501	101	0.2	1.57e-02	FBgn0030506	Lig4	3.8e-01	h	Cflo_N_g14598t1	4.7e+00	h	
Cobs_16504	19	0.4	4.22e-02	FBgn0040001	CG17374	7.0e-91	h	Cflo_N_g2467t1	6.0e-74	o	
Cobs_16505	233	0.4	3.04e-07	FBgn0042627	v(2)k05816	8.0e-80	h	Cflo_N_g7694t1	0.0e+00	o	
Cobs_16506	108	0.4	2.29e-06	FBgn0040001	CG17374	1.0e-49	h	Cflo_N_g2078t3	0.0e+00	o	
Cobs_16507	42	0.5	7.30e-05	FBgn0040001	CG17374	2.0e-41	h	Cflo_N_g1135t1	0.0e+00	h	
Cobs_16508	130	0.6	6.68e-10	FBgn0027571	CG3523	5.0e-86	h	Cflo_N_g1907t2	7.0e-112	h	
Cobs_16511	13	0.4	4.77e-02	FBgn0039927	CG11155	1.7e+00	h	Cflo_N_g8226t1	0.0e+00	o	
Cobs_16516	37	0.6	5.97e-05	FBgn0035543	CG15020	5.1e+00	h	Cflo_N_g4919t1	9.0e-36	h	
Cobs_16517	78	0.7	2.51e-07	FBgn0038918	CG6690	4.5e+00	h	Cflo_N_g14055t1	1.0e-34	h	
Cobs_16521	1025	0.2	2.92e-02	FBgn0033188	Drat	4.0e-143	o	Cflo_N_g689t4	0.0e+00	o	
Cobs_16530	124	-0.6	1.38e-10	FBgn0036715	Cad74A	0.0e+00	o	Cflo_N_g2691t1	0.0e+00	o	
Cobs_16577	119	-0.2	2.52e-02	FBgn0037912	sea	2.0e-166	o	Cflo_N_g11837t1	0.0e+00	o	
Cobs_16603	1215	-0.5	2.94e-06	FBgn0031913	CG5958	1.0e-98	o	Cflo_N_g7429t1	3.0e-153	o	
Cobs_16610	1110	0.1	3.09e-02	FBgn0037716	CG8273	2.0e-111	o	Cflo_N_g10524t1	0.0e+00	h	
Cobs_16660	43	-0.8	2.75e-09	FBgn0038610	CG7675	1.0e-49	h	Cflo_N_g7215t1	3.0e-89	o	
Cobs_16692	481	-0.2	2.90e-02	FBgn0250823	gish	0.0e+00	o	Cflo_N_g14010t2	0.0e+00	o	
Cobs_16708	252	-0.2	4.16e-02	FBgn0015558	tty	3.0e-149	o	Cflo_N_g14243t2	0.0e+00	o	
Cobs_16711	693	-0.3	6.55e-03	FBgn0000052	ade2	0.0e+00	o	Cflo_N_g9080t2	0.0e+00	o	
Cobs_16725	602	0.1	4.24e-02	FBgn0032210	CYLD	0.0e+00	o	Cflo_N_g692t2	0.0e+00	o	
Cobs_16731	1224	0.6	1.89e-04	FBgn0003961	Uro	4.0e-102	o	Cflo_N_g10606t1	0.0e+00	o	
Cobs_16732	62	0.3	4.20e-02	FBgn0027865	Tsp96F	3.0e-09	h	Cflo_N_g7588t1	0.0e+00	o	
Cobs_16739	390	0.5	2.01e-07	FBgn0031265	CG2794	1.0e-98	o	Cflo_N_g2035t1	0.0e+00	o	
Cobs_16743	27	0.8	8.23e-10	FBgn0050099	CG30099	1.7e+00	h	Cflo_N_g4375t1	1.4e-01	h	
Cobs_16766	455	0.3	3.26e-03	FBgn0261549	rdgA	0.0e+00	o	Cflo_N_g2403t1	0.0e+00	o	
Cobs_16769	453	-0.5	1.22e-04	FBgn0010452	trn	4.0e-21	h	Cflo_N_g3766t1	3.0e-179	o	

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_16813	6760	0.2	1.70e-02	FBgn0032026	CG7627	0.0e+00	h	Cflo_N_g14817t1	0.0e+00	o	
Cobs_16815	1553	-0.2	5.55e-03	-	-	-	-	Cflo_N_g14811t1	3.0e-64	o	
Cobs_16819	2428	-0.2	5.08e-04	FBgn0029709	CHOp24	7.0e-80	o	Cflo_N_g8698t2	0.0e+00	o	
Cobs_16827	3600	-0.2	6.07e-03	FBgn0053196	dp	0.0e+00	o	Cflo_N_g6310t2	0.0e+00	o	
Cobs_16847	20	-0.4	4.12e-02	FBgn0001139	gro	5.0e-15	h	Cflo_N_g1604t2	3.0e-22	h	
Cobs_16874	40	-0.4	2.27e-03	FBgn0032298	CG6724	1.7e+00	h	Cflo_N_g10755t1	3.0e-03	h	
Cobs_16955	145	-0.3	4.02e-03	FBgn0026533	Dek	3.0e-02	h	Cflo_N_g12507t1	4.5e-02	h	
Cobs_16978	1537	0.2	5.84e-03	FBgn0031752	CG9044	2.0e-116	o	Cflo_N_g2612t2	5.0e-170	o	
Cobs_16983	204	-0.4	6.23e-07	FBgn0011824	CG4038	2.0e-43	o	Cflo_N_g9743t1	0.0e+00	o	
Cobs_16984	960	0.4	6.93e-08	FBgn0014018	Rel	6.0e-91	o	Cflo_N_g6082t1	0.0e+00	o	
Cobs_16998	4081	-0.2	2.12e-03	FBgn0023529	CG2918	0.0e+00	o	Cflo_N_g14522t1	3.4e+00	h	
Cobs_17032	227	-0.3	4.45e-03	FBgn0250876	Sema-5c	0.0e+00	o	Cflo_N_g7720t1	0.0e+00	o	
Cobs_17033	17	-0.4	2.12e-02	-	-	-	-	Cflo_N_g1177t1	2.7e-02	h	
Cobs_17034	12	-0.4	1.35e-02	FBgn0250876	Sema-5c	4.0e-03	h	Cflo_N_g7720t1	3.0e-12	h	
Cobs_17036	196	-1.2	1.49e-17	FBgn0036975	CG5618	1.0e-148	o	Cflo_N_g6700t1	6.0e-08	h	
Cobs_17043	2793	-0.2	1.33e-02	FBgn0034277	OstDelta	6.0e-149	o	Cflo_N_g6773t1	0.0e+00	o	
Cobs_17055	66	-0.7	5.49e-11	FBgn0000449	dib	9.0e-154	o	Cflo_N_g4358t1	0.0e+00	o	
Cobs_17058	102	0.3	1.84e-02	FBgn0051522	CG31522	1.0e-39	h	Cflo_N_g6730t1	9.0e-42	h	
Cobs_17061	132	-0.4	3.17e-03	FBgn0033644	Tret1-2	5.0e-61	h	Cflo_N_g14351t1	0.0e+00	o	
Cobs_17084	3747	0.2	1.14e-04	FBgn0037944	CG6923	1.0e-27	o	Cflo_N_g11587t1	0.0e+00	o	
Cobs_17086	478	0.9	1.08e-20	FBgn0035501	CG1299	3.0e-79	o	Cflo_N_g11587t1	1.0e-169	h	
Cobs_17099	9	-0.6	3.40e-04	FBgn0052296	Mrtf	3.9e-01	h	Cflo_N_g9998t1	7.0e-11	h	
Cobs_17118	82	-0.3	3.54e-02	FBgn0011281	Obp83a	2.0e-11	h	Cflo_N_g13617t1	3.0e-65	o	
Cobs_17126	4743	-0.4	6.02e-03	FBgn0032414	CG17211	1.3e-01	h	Cflo_N_g14857t1	1.0e-93	o	
Cobs_17138	7151	0.2	4.70e-04	FBgn0015623	Cpr	0.0e+00	o	Cflo_N_g4977t1	0.0e+00	o	
Cobs_17142	3328	0.3	1.03e-02	-	-	-	-	Cflo_N_g5701t1	0.0e+00	o	
Cobs_17144	742	0.3	3.30e-07	FBgn0015522	olf186-M	6.0e-21	o	Cflo_N_g5697t1	0.0e+00	o	
Cobs_17182	1187	0.3	8.13e-05	FBgn0025574	Pli	0.0e+00	o	Cflo_N_g7000t2	0.0e+00	o	
Cobs_17186	850	-0.3	1.56e-03	FBgn0039756	CG9743	1.0e-142	o	Cflo_N_g10065t1	0.0e+00	o	
Cobs_17196	75	-0.4	2.00e-03	FBgn0033628	CG13203	1.0e-11	o	Cflo_N_g15052t1	0.0e+00	o	
Cobs_17204	3071	-0.2	1.17e-03	FBgn0002528	LanB2	0.0e+00	o	Cflo_N_g9869t1	0.0e+00	o	
Cobs_17221	2482	0.1	2.19e-02	FBgn0037541	CG2747	0.0e+00	o	Cflo_N_g11414t2	0.0e+00	o	
Cobs_17224	769	-0.3	1.37e-02	FBgn0000308	chic	8.0e-54	o	Cflo_N_g10302t1	1.0e-16	o	
Cobs_17235	498	0.3	4.51e-05	FBgn0010350	CdsA	0.0e+00	o	Cflo_N_g10124t1	0.0e+00	o	
Cobs_17248	217	0.3	9.05e-04	FBgn0053892	His2B	1.0e-82	o	Cflo_N_g6334t1	5.0e-85	o	
Cobs_17251	2215	0.3	5.36e-04	FBgn0036824	CG3902	2.0e-178	h	Cflo_N_g6340t1	0.0e+00	h	
Cobs_17252	553	0.2	2.13e-02	FBgn0034674	CG9304	0.0e+00	o	Cflo_N_g6342t1	0.0e+00	o	
Cobs_17280	119	0.3	2.76e-02	FBgn0038889	CG7922	4.0e-07	h	Cflo_N_g13648t1	1.0e-64	h	
Cobs_17285	1436	-0.4	2.95e-04	FBgn0025700	CG5885	6.0e-91	o	Cflo_N_g6348t1	3.0e-78	o	
Cobs_17305	212	0.3	1.37e-03	FBgn0085412	CG34383	1.9e+00	h	Cflo_N_g2414t1	1.0e-55	o	
Cobs_17313	41	-0.4	1.80e-02	FBgn0001341	l(1)1Bi	8.7e-01	h	Cflo_N_g1500t1	0.0e+00	o	
Cobs_17348	50	-0.3	4.19e-02	FBgn0261836	Msp300	1.2e+00	h	Cflo_N_g11653t1	2.0e-79	h	
Cobs_17380	131	1.1	1.49e-17	FBgn0034200	CG11395	1.0e-06	h	Cflo_N_g7719t1	0.0e+00	o	
Cobs_17437	428	-0.5	2.45e-07	FBgn0011016	SsRbeta	1.0e-70	o	Cflo_N_g10531t1	1.0e-114	o	
Cobs_17438	829	0.2	2.65e-02	FBgn0051301	CG31301	1.0e-38	o	Cflo_N_g10535t2	9.0e-130	o	
Cobs_17465	198	-0.4	1.67e-06	FBgn0000139	ash2	1.1e+00	h	-	-	-	
Cobs_17466	4063	-0.2	7.19e-04	FBgn0000667	Actn	0.0e+00	o	Cflo_N_g8159t2	0.0e+00	o	
Cobs_17490	11435	-0.3	4.57e-03	FBgn0023518	trr	0.0e+00	o	Cflo_N_g9253t1	0.0e+00	o	
Cobs_17500	242	-0.2	4.36e-02	FBgn0030341	p24-1	6.0e-89	o	Cflo_N_g8687t1	1.0e-18	h	
Cobs_17504	296	-0.4	5.74e-06	FBgn0022288	l(2)09851	3.0e-176	o	Cflo_N_g12540t1	0.0e+00	o	
Cobs_17528	1732	0.1	3.67e-03	FBgn0024251	bbx	1.0e-43	o	Cflo_N_g9495t1	0.0e+00	o	
Cobs_17552	1125	0.2	1.71e-02	FBgn0034491	Hsl	0.0e+00	o	Cflo_N_g9488t1	0.0e+00	h	
Cobs_17564	5788	0.9	5.43e-23	FBgn0013770	Cp1	2.0e-164	o	Cflo_N_g1655t1	0.0e+00	o	
Cobs_17572	997	-0.4	6.17e-08	FBgn0000721	for	0.0e+00	o	Cflo_N_g9781t1	0.0e+00	o	
Cobs_17574	1937	0.2	5.55e-03	FBgn0024248	chico	3.0e-70	o	Cflo_N_g3508t1	0.0e+00	o	
Cobs_17596	936	0.2	3.29e-02	FBgn0052484	Sk2	4.0e-163	o	Cflo_N_g3534t1	0.0e+00	o	
Cobs_17600	2410	0.1	5.38e-03	FBgn0028475	Hrd3	0.0e+00	o	Cflo_N_g3537t2	0.0e+00	o	
Cobs_17616	286	0.2	2.53e-02	FBgn0032231	CG5056	2.0e-13	o	Cflo_N_g10156t1	4.0e-141	o	
Cobs_17617	160	-0.3	1.27e-02	FBgn0250820	meigo	4.0e-111	o	Cflo_N_g6417t1	5.0e-135	o	
Cobs_17620	6039	-0.2	3.98e-02	FBgn0000212	brm	0.0e+00	o	Cflo_N_g9088t1	1.0e-151	o	
Cobs_17632	139	0.2	1.21e-02	FBgn0260756	CG42554	4.0e-03	o	Cflo_N_g8104t1	0.0e+00	o	

	Injury versus Control			<i>Drosophila melanogaster</i> r5.56				<i>Camponotus floridanus</i> 2015			
	baseMean	logFC	padj	ID	Name	blastp	o/h	ID	blastp	o/h	
Cobs_17646	31	0.5	1.47e-03	-	-	-	-	Cflo_N_g9207t1	6.2e-01	h	
Cobs_17648	1396	0.7	2.66e-06	FBgn0003598	Su(var)3-7	7.2e+00	h	Cflo_N_g15153t1	2.6e+00	h	
Cobs_17658	964	0.3	5.94e-05	FBgn0030966	CG7280	0.0e+00	o	Cflo_N_g4888t1	0.0e+00	h	
Cobs_17668	321	0.6	5.06e-09	FBgn0029831	CG5966	4.0e-131	o	Cflo_N_g3196t1	0.0e+00	o	
Cobs_17720	25	-0.4	2.92e-02	FBgn0003867	tsl	4.0e-113	o	Cflo_N_g4469t1	0.0e+00	o	
Cobs_17737	61	-0.3	4.46e-02	FBgn0037057	CG10512	1.0e-120	o	Cflo_N_g3894t1	0.0e+00	o	
Cobs_17742	62	0.4	6.04e-04	FBgn0002937	ninaB	0.0e+00	o	Cflo_N_g13106t1	0.0e+00	o	
Cobs_17746	1152	0.2	1.14e-03	FBgn0052672	Atg8a	3.0e-76	o	Cflo_N_g3889t1	2.0e-82	o	
Cobs_17756	56	-0.3	2.18e-02	-	-	-	-	Cflo_N_g8528t1	1.1e-01	h	
Cobs_17758	17	-0.4	3.00e-02	FBgn0262534	CG43088	2.7e-01	h	Cflo_N_g14764t1	1.0e-11	h	
Cobs_17779	21	-0.4	1.44e-02	FBgn0031401	papi	1.0e-07	h	Cflo_N_g3226t1	5.0e-28	h	
Cobs_17807	8556	-0.3	3.03e-02	FBgn0029899	CG14438	3.0e-28	o	Cflo_N_g5914t1	0.0e+00	o	
Cobs_17812	1252	-0.5	4.73e-03	FBgn0039420	CG6154	2.0e-133	h	Cflo_N_g4435t1	0.0e+00	o	
Cobs_17829	614	-0.3	7.47e-03	FBgn0001098	Gdh	0.0e+00	o	Cflo_N_g10931t1	0.0e+00	o	
Cobs_17854	261	-0.9	4.93e-11	FBgn0040255	Ugt86De	2.0e-68	h	Cflo_N_g4454t3	0.0e+00	o	
Cobs_17944	138	0.4	2.88e-04	FBgn0052263	CG32263	2.0e-37	o	Cflo_N_g3192t1	9.0e-109	h	
Cobs_17958	559	-0.2	4.67e-03	FBgn0032167	CG5853	0.0e+00	o	Cflo_N_g7480t1	0.0e+00	o	
Cobs_17963	1048	-0.2	3.80e-02	FBgn0032167	CG5853	2.0e-149	h	Cflo_N_g9603t1	0.0e+00	o	
Cobs_17972	610	0.8	5.40e-14	FBgn0051217	modSP	2.0e-29	h	Cflo_N_g7438t1	2.0e-134	o	
Cobs_17985	3306	0.3	4.83e-06	FBgn0260462	CG12163	3.0e-135	o	Cflo_N_g5304t1	0.0e+00	o	
Cobs_18050	841	-0.2	4.89e-03	FBgn0000083	AnxB9	0.0e+00	o	Cflo_N_g247t1	4.0e-87	o	
Cobs_18054	389	-0.3	1.08e-04	FBgn0032703	CG10343	1.0e-62	o	Cflo_N_g4726t1	6.0e-126	o	
Cobs_18069	26	-0.4	2.95e-02	FBgn0032050	CG13096	2.8e-01	h	Cflo_N_g3701t1	1.7e+00	o	
Cobs_18077	1774	-0.2	1.11e-04	FBgn0010825	Gug	1.0e-71	o	Cflo_N_g10860t1	0.0e+00	o	
Cobs_18094	10	-0.4	1.80e-02	-	-	-	-	Cflo_N_g5267t1	2.0e-16	o	
Cobs_18106	3305	0.2	1.07e-02	FBgn0261862	whd	0.0e+00	o	Cflo_N_g5229t1	0.0e+00	o	
Cobs_18111	594	0.2	3.43e-02	FBgn0025615	Torsin	3.0e-08	h	Cflo_N_g5943t1	0.0e+00	o	
Cobs_18126	8464	-0.2	4.75e-03	FBgn0026427	Su(var)2-HP2	2.9e+00	h	Cflo_N_g8906t3	0.0e+00	o	
Cobs_18148	48	0.5	1.62e-03	FBgn0038839	CG10830	5.0e-109	o	Cflo_N_g7311t1	2.0e-174	o	
Cobs_18160	57	0.7	7.48e-06	FBgn0265102	Oseg1	9.2e+00	h	Cflo_N_g15337t1	1.4e+00	h	
Cobs_18172	729	0.2	3.83e-02	FBgn0030114	CG17754	0.0e+00	o	Cflo_N_g12526t1	0.0e+00	o	
Cobs_18173	297	0.3	4.65e-03	FBgn0262516	Trpml	0.0e+00	o	Cflo_N_g7310t1	0.0e+00	o	
Cobs_18187	51	-0.3	8.37e-03	FBgn0053177	CG33177	3.0e-13	h	Cflo_N_g14205t1	0.0e+00	o	
Cobs_18195	554	-0.2	1.78e-03	FBgn0026869	Thd1	5.0e-71	o	Cflo_N_g14539t1	0.0e+00	o	
Cobs_18203	1201	0.3	8.83e-07	FBgn0039714	Zip99C	8.0e-64	o	Cflo_N_g11137t1	0.0e+00	o	
Cobs_18227	3236	0.2	6.23e-07	FBgn0262473	Tl	0.0e+00	o	Cflo_N_g12885t1	9.0e-46	o	
Cobs_18228	278	0.4	6.04e-03	FBgn0041181	Tep3	8.0e-42	h	Cflo_N_g9745t2	4.0e-86	h	
Cobs_18277	1363	0.1	4.53e-02	FBgn0038651	CG14299	0.0e+00	o	Cflo_N_g9179t1	0.0e+00	o	
Cobs_18298	80	0.4	2.30e-03	FBgn0034856	yellow-d2	1.0e-16	h	Cflo_N_g8861t2	0.0e+00	o	

## 7.2.4 Lists of significant GO terms of the category biological process

Functional enrichments in genes differently expressed due to injury. Count, number of genes in list annotated for the corresponding term; Pop Hits, total number of annotated genes; FE, fold enrichment.

Genes upregulated in injured queens compared with control.

Term	Count	%	P value	Pop Hits	FE
GO:0001558~regulation of cell growth	7	2.2	5.6e-04	22	6.3
GO:0002376~immune system process	12	3.8	9.9e-03	99	2.4
GO:0002682~regulation of immune system process	8	2.5	4.9e-03	43	3.7
GO:0006027~glycosaminoglycan catabolic process	3	1.0	2.3e-02	5	11.9
GO:0006066~alcohol metabolic process	13	4.1	4.9e-03	103	2.5
GO:0006464~protein modification process	31	9.9	4.5e-02	438	1.4
GO:0006468~protein amino acid phosphorylation	16	5.1	1.7e-02	165	1.9
GO:0006508~proteolysis	27	8.6	2.5e-03	292	1.8
GO:0006950~response to stress	28	8.9	1.6e-04	258	2.2
GO:0006952~defense response	14	4.5	1.2e-05	64	4.3
GO:0006955~immune response	12	3.8	3.6e-04	66	3.6
GO:0007165~signal transduction	35	11.1	9.1e-04	393	1.8
GO:0007166~cell surface receptor linked signal transduction	26	8.3	2.4e-02	332	1.6
GO:0007169~transmembrane receptor protein tyrosine kinase signaling pathway	9	2.9	2.8e-02	73	2.4
GO:0007242~intracellular signaling cascade	18	5.7	2.6e-02	205	1.7
GO:0007431~salivary gland development	11	3.5	8.6e-03	84	2.6
GO:0007435~salivary gland morphogenesis	11	3.5	2.0e-03	69	3.2
GO:0007568~aging	9	2.9	1.6e-02	66	2.7
GO:0007602~phototransduction	6	1.9	1.3e-02	29	4.1
GO:0008063~Toll signaling pathway	5	1.6	1.3e-02	19	5.2
GO:0008219~cell death	11	3.5	3.0e-02	102	2.1
GO:0008286~insulin receptor signaling pathway	4	1.3	1.1e-02	10	7.9
GO:0008340~determination of adult life span	9	2.9	1.6e-02	66	2.7
GO:0008361~regulation of cell size	10	3.2	1.1e-03	53	3.7
GO:0009056~catabolic process	25	8.0	3.1e-02	323	1.5
GO:0009057~macromolecule catabolic process	17	5.4	3.2e-02	194	1.7
GO:0009314~response to radiation	10	3.2	6.3e-03	68	2.9
GO:0009416~response to light stimulus	8	2.5	2.2e-02	57	2.8
GO:0009581~detection of external stimulus	8	2.5	4.3e-03	42	3.8
GO:0009582~detection of abiotic stimulus	7	2.2	1.4e-02	40	3.5
GO:0009583~detection of light stimulus	7	2.2	7.1e-03	35	4.0
GO:0009605~response to external stimulus	15	4.8	1.6e-03	115	2.6
GO:0009607~response to biotic stimulus	14	4.5	8.1e-06	62	4.5
GO:0009617~response to bacterium	9	2.9	2.5e-05	26	6.9
GO:0010259~multicellular organismal aging	9	2.9	1.6e-02	66	2.7
GO:0012501~programmed cell death	11	3.5	2.7e-02	100	2.2
GO:0016049~cell growth	7	2.2	1.1e-02	38	3.7
GO:0016063~rhodopsin biosynthetic process	3	1.0	2.3e-02	5	11.9
GO:0016265~death	11	3.5	3.2e-02	103	2.1
GO:0022612~gland morphogenesis	11	3.5	2.0e-03	69	3.2
GO:0030307~positive regulation of cell growth	6	1.9	2.8e-04	13	9.2
GO:0031347~regulation of defense response	6	1.9	2.0e-03	19	6.3
GO:0032535~regulation of cellular component size	11	3.5	2.8e-03	72	3.0
GO:0032868~response to insulin stimulus	4	1.3	1.1e-02	10	7.9
GO:0032869~cellular response to insulin stimulus	4	1.3	1.1e-02	10	7.9
GO:0035070~salivary gland histolysis	6	1.9	4.8e-02	40	3.0
GO:0035071~salivary gland cell autophagic cell death	6	1.9	4.8e-02	40	3.0
GO:0035272~exocrine system development	11	3.5	8.6e-03	84	2.6
GO:0040007~growth	10	3.2	1.8e-02	80	2.5
GO:0040008~regulation of growth	11	3.5	4.6e-03	77	2.8
GO:0040014~regulation of multicellular organism growth	5	1.6	1.1e-02	18	5.5
GO:0040018~positive regulation of multicellular organism growth	4	1.3	8.3e-03	9	8.8
GO:0042592~homeostatic process	13	4.1	7.8e-03	109	2.4
GO:0042594~response to starvation	3	1.0	4.4e-02	7	8.5
GO:0042742~defense response to bacterium	7	2.2	3.2e-04	20	7.0
GO:0043434~response to peptide hormone stimulus	4	1.3	1.1e-02	10	7.9
GO:0043687~post-translational protein modification	26	8.3	4.5e-02	352	1.5
GO:0045087~innate immune response	6	1.9	1.2e-02	28	4.3
GO:0045088~regulation of innate immune response	5	1.6	3.7e-04	8	12.4
GO:0045793~positive regulation of cell size	7	2.2	5.0e-05	15	9.3
GO:0045927~positive regulation of growth	8	2.5	8.9e-05	23	6.9
GO:0046154~rhodopsin metabolic process	3	1.0	2.3e-02	5	11.9
GO:0046620~regulation of organ growth	5	1.6	2.6e-02	23	4.3
GO:0046622~positive regulation of organ growth	3	1.0	2.3e-02	5	11.9
GO:0048102~autophagic cell death	6	1.9	4.8e-02	40	3.0

Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0048732~gland development	12	3.8	1.1e-02	100	2.4
GO:0050776~regulation of immune response	6	1.9	5.9e-03	24	5.0
GO:0050789~regulation of biological process	91	29.0	1.7e-02	1495	1.2
GO:0050829~defense response to Gram-negative bacterium	4	1.3	1.5e-02	11	7.2
GO:0050830~defense response to Gram-positive bacterium	4	1.3	2.2e-03	6	13.2
GO:0050896~response to stimulus	47	15.0	5.3e-04	572	1.6
GO:0051606~detection of stimulus	8	2.5	8.2e-03	47	3.4
GO:0051704~multi-organism process	16	5.1	2.3e-05	88	3.6
GO:0051707~response to other organism	13	4.1	1.4e-05	56	4.6
GO:0051716~cellular response to stimulus	17	5.4	2.8e-02	191	1.8
GO:0065007~biological regulation	99	31.5	1.7e-02	1651	1.2
GO:0065008~regulation of biological quality	32	10.2	1.6e-02	419	1.5
GO:0080134~regulation of response to stress	8	2.5	1.3e-02	51	3.1

Genes downregulated in injured queens compared with control.

Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0006082~organic acid metabolic process	23	9.4	2.3e-05	181	2.7
GO:0006457~protein folding	8	3.3	3.6e-02	68	2.5
GO:0006519~cellular amino acid and derivative metabolic process	17	7.0	9.5e-04	145	2.5
GO:0006520~cellular amino acid metabolic process	16	6.6	2.7e-04	117	2.9
GO:0006525~arginine metabolic process	3	1.2	1.2e-02	4	16.1
GO:0006613~cotranslational protein targeting to membrane	4	1.6	2.9e-02	15	5.7
GO:0006753~nucleoside phosphate metabolic process	11	4.5	2.3e-02	105	2.2
GO:0007016~cytoskeletal anchoring at plasma membrane	4	1.6	2.4e-02	14	6.1
GO:0007026~negative regulation of microtubule depolymerization	3	1.2	3.8e-02	7	9.2
GO:0007155~cell adhesion	14	5.7	2.3e-03	115	2.6
GO:0007275~multicellular organismal development	64	26.2	3.8e-02	1115	1.2
GO:0007281~germ cell development	12	4.9	3.2e-02	127	2.0
GO:0007308~oocyte construction	8	3.3	3.6e-02	68	2.5
GO:0007409~axonogenesis	13	5.3	3.7e-02	146	1.9
GO:0007411~axon guidance	10	4.1	4.5e-02	102	2.1
GO:0007417~central nervous system development	13	5.3	1.5e-02	129	2.2
GO:0007420~brain development	11	4.5	7.9e-04	66	3.6
GO:0007472~wing disc morphogenesis	13	5.3	4.2e-02	149	1.9
GO:0007475~apposition of dorsal and ventral imaginal disc-derived wing surfaces	5	2.0	4.1e-03	15	7.2
GO:0007476~imaginal disc-derived wing morphogenesis	13	5.3	3.8e-02	147	1.9
GO:0007610~behavior	16	6.6	4.3e-02	200	1.7
GO:0007611~learning or memory	7	2.9	2.7e-02	50	3.0
GO:0007616~long-term memory	4	1.6	2.9e-02	15	5.7
GO:0008037~cell recognition	6	2.5	2.4e-02	36	3.6
GO:0008038~neuron recognition	6	2.5	2.1e-02	35	3.7
GO:0008587~imaginal disc-derived wing margin morphogenesis	5	2.0	4.3e-02	29	3.7
GO:0008643~carbohydrate transport	3	1.2	5.0e-02	8	8.1
GO:0008652~cellular amino acid biosynthetic process	5	2.0	3.0e-02	26	4.1
GO:0009063~cellular amino acid catabolic process	6	2.5	7.1e-03	27	4.8
GO:0009064~glutamine family amino acid metabolic process	7	2.9	2.1e-04	20	7.5
GO:0009117~nucleotide metabolic process	11	4.5	2.3e-02	105	2.2
GO:0009165~nucleotide biosynthetic process	9	3.7	3.5e-02	82	2.4
GO:0009308~amine metabolic process	22	9.0	1.3e-04	188	2.5
GO:0009310~amine catabolic process	6	2.5	1.1e-02	30	4.3
GO:0009605~response to external stimulus	11	4.5	3.9e-02	115	2.1
GO:0009798~axis specification	11	4.5	4.5e-02	118	2.0
GO:0009887~organ morphogenesis	29	11.9	1.9e-02	406	1.5
GO:0009994~oocyte differentiation	9	3.7	2.7e-02	78	2.5
GO:0016054~organic acid catabolic process	7	2.9	4.8e-03	35	4.3
GO:0016198~axon choice point recognition	4	1.6	2.0e-02	13	6.6
GO:0016199~axon midline choice point recognition	4	1.6	1.6e-02	12	7.2
GO:0019752~carboxylic acid metabolic process	23	9.4	2.3e-05	181	2.7
GO:0022610~biological adhesion	14	5.7	2.3e-03	115	2.6
GO:0031111~negative regulation of microtubule polymerization or depolymerization	3	1.2	3.8e-02	7	9.2
GO:0031114~regulation of microtubule depolymerization	3	1.2	5.0e-02	8	8.1
GO:0032501~multicellular organismal process	76	31.1	3.3e-02	1352	1.2
GO:0032507~maintenance of protein location in cell	8	3.3	1.3e-04	26	6.6
GO:0032787~monocarboxylic acid metabolic process	7	2.9	4.0e-02	55	2.7
GO:0034404~nucleobase, nucleoside and nucleotide biosynthetic process	9	3.7	4.7e-02	87	2.2
GO:0034654~nucleobase, nucleoside, nucleotide and nucleic acid biosynthetic process	9	3.7	4.7e-02	87	2.2
GO:0035220~wing disc development	16	6.6	2.8e-02	189	1.8
GO:0042180~cellular ketone metabolic process	25	10.2	7.7e-06	195	2.8
GO:0043436~oxoacid metabolic process	23	9.4	2.3e-05	181	2.7
GO:0044106~cellular amine metabolic process	16	6.6	1.2e-03	134	2.6
GO:0044271~nitrogen compound biosynthetic process	13	5.3	4.4e-02	150	1.9

Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0045185~maintenance of protein location	9	3.7	3.4e-05	29	6.7
GO:0045727~positive regulation of translation	3	1.2	5.0e-02	8	8.1
GO:0046395~carboxylic acid catabolic process	7	2.9	4.8e-03	35	4.3
GO:0048513~organ development	41	16.8	4.5e-02	667	1.3
GO:0048599~oocyte development	8	3.3	4.2e-02	70	2.5
GO:0048731~system development	52	21.3	1.5e-02	829	1.3
GO:0048856~anatomical structure development	56	23.0	4.0e-02	957	1.3
GO:0051235~maintenance of location	9	3.7	1.2e-04	34	5.7
GO:0051258~protein polymerization	4	1.6	9.2e-03	10	8.6
GO:0051651~maintenance of location in cell	8	3.3	1.3e-04	26	6.6
GO:0055086~nucleobase, nucleoside and nucleotide metabolic process	13	5.3	8.4e-03	119	2.3
GO:0055114~oxidation reduction	23	9.4	2.4e-02	306	1.6
GO:0065008~regulation of biological quality	30	12.3	1.7e-02	419	1.5

## 7.3 Appendix for Chapter 4

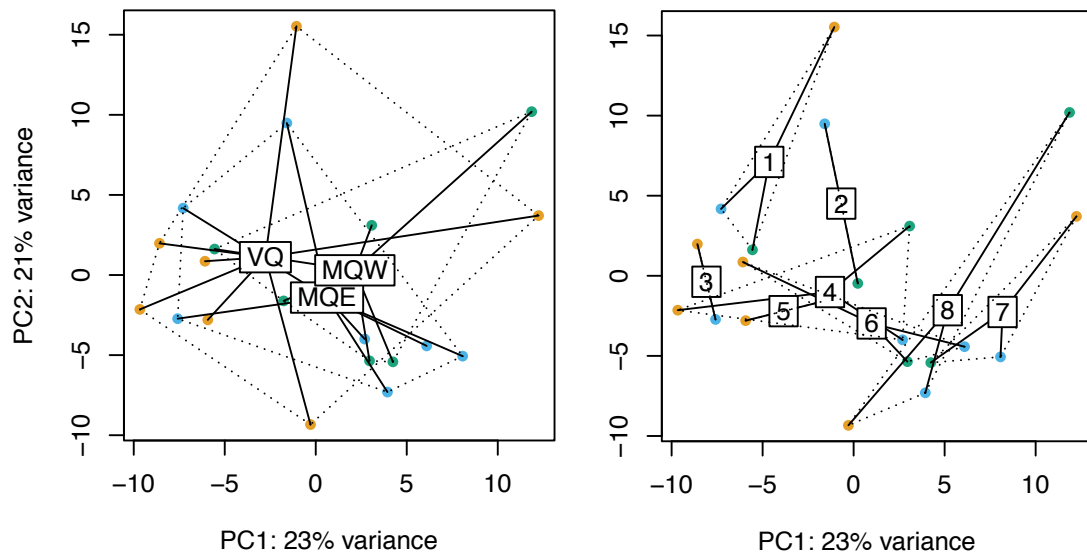
### 7.3.1 Mapping statistics of reads

Sample-wise numbers of raw and mapped reads are shown.

Sample	Treatment	Raw reads	Mapped reads	Mapped (%)
qe_5	MQE	36546316	11733378	32
qe_6	MQE	57677818	16318460	28
qe_9	MQE	36866997	11589379	31
qe_10	MQE	54635027	19088587	35
qe_13	MQE	57615421	23442385	41
qe_18	MQE	40524500	16896124	42
qe_22	MQE	37136707	8526999	23
qw_3	MQW	35986383	16204019	45
qw_5	MQW	46167534	17645426	38
qw_14	MQW	49572777	11286707	23
qw_22	MQW	46242996	15592877	34
qw_29	MQW	46247689	15697349	34
qw_34	MQW	41525145	16728049	40
qw_42	MQW	36646025	10669756	29
qv_3	VQ	33590577	11781621	35
qv_5	VQ	28852212	7751838	27
qv_6	VQ	54443498	15950242	29
qv_12	VQ	46352444	11312710	24
qv_17	VQ	53694150	11300920	21
qv_22	VQ	39107557	13746827	35
qv_23	VQ	40569444	14487919	36
	Mean	43809582	14178646	33



## 7.3.2 Principal component plots of all samples



The graphs were generated with normalized and transformed (VST) counts and illustrate the assignment of samples according to treatment (left) and lane (right).

## 7.3.3 List of DEGs

List of DEGs with corresponding homologs in *Drosophila melanogaster*. Mean, mean of counts per gene across replicates; logFC, log2-transformed fold change; *P*-adj, BH corrected *P* value; o, ortholog; h, homolog.

	Mean	VQ vs. MQe		VQ vs. MQW		MQW vs. MQe		<i>Drosophila melanogaster</i>	
		logFC	<i>P</i> -adj	logFC	<i>P</i> -adj	logFC	<i>P</i> -adj	ID	o/h
Cobs_00039	2442	-0.23	4.16e-02					FBgn0260749	o
Cobs_00066	1839	-0.33	6.96e-03					FBgn0032363	o
Cobs_00112	3898	-0.41	8.06e-03	-0.40	4.43e-02			FBgn0016977	o
Cobs_00115	1396	0.24	1.34e-02					FBgn0035134	h
Cobs_00193	11525	-0.45	5.11e-04	-0.45	6.31e-04			FBgn0004167	o
Cobs_00199	248	0.28	4.06e-02					FBgn0043012	o
Cobs_00214	880	-0.37	2.17e-02					FBgn0051151	h
Cobs_00223	412	0.22	4.64e-02					FBgn0039859	o
Cobs_00449	1945	0.37	2.63e-02					FBgn0261599	o
Cobs_00566						0.41	3.05e-02	FBgn0030090	o
Cobs_00634	814	-0.29	1.32e-02					FBgn0086679	o
Cobs_00659				-0.42	2.45e-02			FBgn0039640	h
Cobs_00699	297	0.27	3.35e-02					FBgn0029914	o
Cobs_00708	157	0.38	2.10e-02					FBgn0020309	h
Cobs_00777	10703	-0.24	4.35e-02					FBgn0029176	o
Cobs_00870	641	-0.31	3.55e-02					FBgn0250819	o
Cobs_00872	930	0.27	1.91e-02					FBgn0032509	o
Cobs_00880	769	-0.30	1.77e-02					FBgn0030613	o
Cobs_00888	973	0.38	7.64e-03						
Cobs_00931	202	-0.32	3.66e-02					FBgn0052333	o
Cobs_00984	2009	-0.23	4.79e-02					FBgn0005674	o
Cobs_01008	6069	-0.28	4.75e-02					FBgn0020309	h
Cobs_01011	1583	-0.32	2.67e-02					FBgn0265523	o
Cobs_01070	118	0.46	8.20e-03					FBgn0041712	h
Cobs_01100	239	0.32	8.35e-03					FBgn0035640	o
Cobs_01129	3299	-0.28	4.59e-02					FBgn0034854	o
Cobs_01176	3356	-0.29	1.43e-03					FBgn0019968	o
Cobs_01230	257	0.28	4.10e-02					FBgn0033669	o
Cobs_01234	1137	-0.33	1.32e-02					FBgn0031294	o
Cobs_01255	243	0.35	1.18e-02					FBgn0030081	h
Cobs_01259	791	0.22	3.65e-02					FBgn0003118	h
Cobs_01356	484	-0.32	3.39e-02					FBgn0033062	h
Cobs_01406	7784	-0.40	5.98e-03					FBgn0265434	o
Cobs_01662	350	0.31	2.69e-02					FBgn0020306	h
Cobs_01668	768	-0.21	4.16e-02					FBgn0035763	o
Cobs_01676	288	-0.36	3.09e-02					FBgn0011764	h
Cobs_01678	3982	-0.23	4.14e-02					FBgn0031374	o
Cobs_01767	685	0.25	6.79e-03					FBgn0029976	h
Cobs_01769	2004	-0.27	1.01e-02					FBgn0004611	o
Cobs_01780	125	0.35	2.65e-02					FBgn0030766	h
Cobs_02027	601	0.25	2.14e-02					FBgn0037566	o
Cobs_02125	578	-0.29	7.64e-03					FBgn0003076	o
Cobs_02126	885	0.31	6.12e-03					FBgn0260858	o
Cobs_02128	3680	-0.27	2.92e-02					FBgn0024277	o
Cobs_02140	196	-0.37	3.94e-03					FBgn0026059	o
Cobs_02142	666	-0.57	1.32e-06					FBgn0026059	o
Cobs_02158	155	0.37	2.08e-02					FBgn0044020	o
Cobs_02169	958	0.27	1.89e-02					FBgn0034372	o
Cobs_02243	2845	-0.31	2.35e-02					FBgn0005386	o
Cobs_02404	2055	0.25	2.54e-02					FBgn0024807	o
Cobs_02542	795	0.23	3.39e-02					FBgn0033810	o
Cobs_02550	724	0.32	6.96e-03					FBgn0025741	h
Cobs_02646	512	-0.29	1.55e-02					FBgn0266098	o
Cobs_02739	1894	-0.24	4.06e-02					FBgn0266696	o
Cobs_02815	6134	-0.24	4.57e-02					FBgn0262582	o
Cobs_02962	982	-0.22	4.03e-02					FBgn0034733	o
Cobs_02998	1957	-0.35	3.76e-02					FBgn0036340	h
Cobs_03026	2524	-0.28	8.06e-03					FBgn0264693	o
Cobs_03119	1329	-0.27	3.74e-02					FBgn0085447	o
Cobs_03166	168	0.27	4.79e-02					FBgn0032215	h
Cobs_03240	518	-0.27	4.87e-02					FBgn0027594	o
Cobs_03282	279	-0.29	4.16e-02					FBgn0024234	o
Cobs_03346	1881	-0.34	8.93e-03					FBgn0034742	o
Cobs_03493	421	0.35	2.65e-03					FBgn0033429	o
Cobs_03506	4162	-0.31	2.26e-02					FBgn0015589	o

	Mean	VQ vs. MQe		VQ vs. MQW		MQW vs. MQe		<i>Drosophila melanogaster</i>	
		logFC	P-adj	logFC	P-adj	logFC	P-adj	ID	o/h
Cobs_03515	154	0.29	4.64e-02					FBgn0035532	o
Cobs_03549				-0.31	8.38e-04			FBgn0035173	o
Cobs_03704	504	-0.30	3.35e-02					FBgn0036862	o
Cobs_03739	358	0.33	2.50e-02					FBgn0038983	o
Cobs_03748	887	0.29	1.06e-02					FBgn0031639	o
Cobs_03774	1494	-0.37	5.05e-03					FBgn0037363	o
Cobs_03799	411	0.26	9.77e-03					FBgn0020766	o
Cobs_03824	532	0.26	3.68e-02					FBgn0030239	h
Cobs_03827	269	0.35	2.42e-02					FBgn0030239	o
Cobs_03850	466	0.22	4.57e-02					FBgn0035591	o
Cobs_03876	5325	-0.33	4.64e-02					FBgn0013756	o
Cobs_03907	472	0.24	2.67e-02					FBgn0038301	h
Cobs_03909	492	0.26	4.79e-02					FBgn0032600	o
Cobs_03925	187	0.33	3.07e-02					FBgn0051363	o
Cobs_04029	1059	-0.29	1.34e-02					FBgn0260003	o
Cobs_04032	1563	0.27	4.18e-03					FBgn0035499	o
Cobs_04042	176	-0.29	4.07e-02					FBgn0260003	h
Cobs_04051	3094	-0.28	1.73e-02					FBgn0260003	h
Cobs_04118	118	0.42	9.09e-03					FBgn0039876	o
Cobs_04144	457	0.26	3.23e-02					FBgn0037384	o
Cobs_04252	1086	-0.33	2.17e-02					FBgn0030706	h
Cobs_04278	2366	-0.30	8.06e-03					FBgn0002121	o
Cobs_04345	777	-0.25	4.44e-02					FBgn0002887	o
Cobs_04388	549	0.25	4.53e-02					FBgn0033309	o
Cobs_04423	12558	-0.38	1.10e-03	-0.33	2.42e-02			FBgn0013733	o
Cobs_04588	301	0.28	3.04e-02					FBgn0005660	o
Cobs_04685	1708	-0.21	3.79e-02					FBgn0010380	o
Cobs_04714	416	-0.31	3.39e-02					FBgn0053196	h
Cobs_04773	1039	-0.23	4.75e-02					FBgn0019890	o
Cobs_04800	831	-0.21	2.97e-02					FBgn0001316	o
Cobs_04808	185	-0.28	4.62e-02					FBgn0052099	o
Cobs_04812	1371	0.22	4.53e-02					FBgn0261385	h
Cobs_04836	6875	-0.42	7.87e-03					FBgn0026577	o
Cobs_04845	1318	-0.29	4.53e-02					FBgn0031170	o
Cobs_04887	209	-0.26	4.58e-02					FBgn0085384	o
Cobs_04940	1207	0.24	4.94e-02					FBgn0029839	o
Cobs_04983	786	-0.33	1.22e-02					FBgn0010246	h
Cobs_04991	1117	0.23	4.82e-02					FBgn0000259	o
Cobs_05005	803	0.21	1.99e-02					FBgn0036005	o
Cobs_05213	373	-0.27	4.52e-02					FBgn0261015	o
Cobs_05219				-0.51	1.05e-04	0.46	1.31e-02	FBgn0034911	o
Cobs_05260	606	-0.48	1.61e-06	-0.49	9.74e-07			FBgn0263077	o
Cobs_05338	421	-0.36	4.69e-03					FBgn0036975	h
Cobs_05342	187	0.28	4.06e-02					FBgn0037580	h
Cobs_05351	4038	-0.23	4.79e-02					FBgn0015905	o
Cobs_05352	2049	-0.27	1.24e-02					FBgn0036663	o
Cobs_05416	551	0.25	3.09e-02					FBgn0039690	o
Cobs_05440	313	0.36	8.93e-03						
Cobs_05499	552	0.30	3.71e-02					FBgn0033060	o
Cobs_05579	930	-0.39	2.65e-03					FBgn0262870	o
Cobs_05724	1860	-0.22	4.92e-02					FBgn0020443	o
Cobs_05750	2223	-0.38	2.76e-05	-0.35	1.90e-04			FBgn0250788	o
Cobs_05834	826	-0.27	3.76e-02					FBgn0034718	o
Cobs_05849	18939	-0.27	2.65e-02					FBgn0001233	o
Cobs_05861	632	0.27	2.71e-02					FBgn0003978	o
Cobs_06038	5315	-0.22	2.26e-02					FBgn0250789	o
Cobs_06051	4273	-0.39	1.47e-02					FBgn0033636	o
Cobs_06078	806	0.27	2.42e-02					FBgn0087005	o
Cobs_06160	537	-0.23	4.94e-02					FBgn0015828	o
Cobs_06163	119	-0.30	4.13e-02					FBgn0263258	o
Cobs_06229	117	0.37	1.44e-02					FBgn0020304	h
Cobs_06352	439	0.23	4.94e-02						
Cobs_06507	2932	-0.29	1.56e-02					FBgn0260442	o
Cobs_06531	811	0.34	5.11e-04					FBgn0034345	o
Cobs_06565	808	-0.32	2.17e-02					FBgn0000455	o
Cobs_06607	1303	0.23	3.35e-02					FBgn0031037	o
Cobs_06684	1409	0.23	3.65e-02					FBgn0030693	o
Cobs_06732	273	0.38	2.65e-03					FBgn0024330	o
Cobs_06740	381	0.31	1.15e-02					FBgn0027095	o
Cobs_06751	120	0.34	3.70e-02					FBgn0003277	o
Cobs_06852	853	-0.39	1.32e-02					FBgn0261617	h
Cobs_06859	307	0.36	1.17e-02					FBgn0035853	o
Cobs_06895	1418	-0.30	3.09e-02					FBgn0030974	h
Cobs_06917	443	-0.28	3.74e-02					FBgn0031869	o
Cobs_06955	591	0.24	3.35e-02					FBgn0000032	o

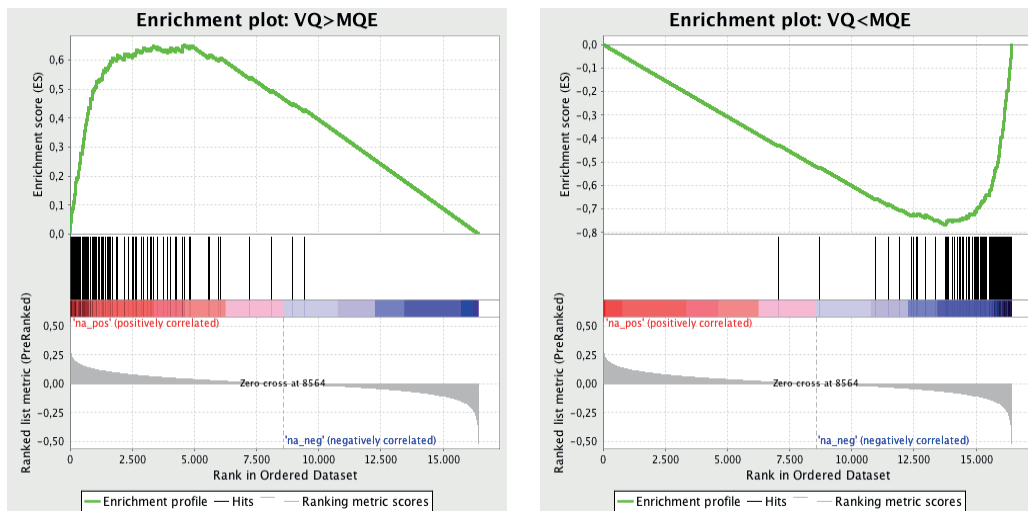
	Mean	VQ vs. MQe		VQ vs. MQW		MQW vs. MQe		<i>Drosophila melanogaster</i>	
		logFC	P-adj	logFC	P-adj	logFC	P-adj	ID	o/h
Cobs_07056	5082	0.28	4.16e-02					FBgn0053852	o
Cobs_07283	8552	0.41	5.29e-03					FBgn0053892	h
Cobs_07318	107	0.31	3.50e-02					FBgn0260779	h
Cobs_07441	452	0.28	4.44e-02					FBgn0035475	o
Cobs_07451	793	0.34	2.50e-02					FBgn0037715	o
Cobs_07473	13273	-0.32	3.48e-03					FBgn0261797	o
Cobs_07504	1501	-0.23	4.06e-02					FBgn0027094	o
Cobs_07625	2658	-0.32	4.18e-03					FBgn0011661	o
Cobs_07673	2378	-0.20	4.79e-02					FBgn0046704	o
Cobs_07701	679	0.33	3.50e-03					FBgn0000546	h
Cobs_07725	1776	-0.31	1.99e-02					FBgn0265045	o
Cobs_07875	225	-0.28	4.66e-02					FBgn0021768	o
Cobs_07894	531	0.22	4.86e-02					FBgn0040305	o
Cobs_07937	611	-0.20	4.37e-02					FBgn0037329	o
Cobs_08101	403	-0.25	4.79e-02					FBgn0010434	h
Cobs_08102	4298	-0.24	7.87e-03					FBgn0010434	o
Cobs_08154	2126	-0.23	3.66e-02					FBgn0086346	o
Cobs_08157	301	0.24	4.06e-02					FBgn0259794	h
Cobs_08165	1833	-0.20	4.39e-02					FBgn0038826	h
Cobs_08196	3640	-0.35	2.69e-02					FBgn0053519	o
Cobs_08279	962	-0.32	6.98e-03					FBgn0263873	o
Cobs_08283	712	-0.26	3.55e-02					FBgn0263873	h
Cobs_08296	372	-0.23	3.79e-02					FBgn0030468	o
Cobs_08354	561	0.28	2.81e-02					FBgn0034654	o
Cobs_08365	406	0.37	8.97e-03					FBgn0031822	o
Cobs_08445	330	0.27	1.78e-02					FBgn0029175	o
Cobs_08524	1382	-0.30	2.71e-02					FBgn0263968	o
Cobs_08568	730	-0.23	4.74e-02					FBgn0032398	o
Cobs_08575	5389	-0.38	2.76e-02					FBgn0266557	o
Cobs_08593	234	0.31	1.35e-02					FBgn0034314	h
Cobs_08675	132	0.43	3.62e-03					FBgn0035144	o
Cobs_08680	628	0.32	4.18e-03					FBgn0047135	o
Cobs_08752	589	0.30	2.65e-03					FBgn0259704	o
Cobs_08777	193	-0.36	8.06e-03					FBgn0003189	h
Cobs_08806	305	-0.28	2.71e-02					FBgn0036133	o
Cobs_08908	242	0.25	4.20e-02					FBgn0051516	h
Cobs_09064	127	0.46	3.48e-03					FBgn0033808	o
Cobs_09067	247	0.32	3.70e-02					FBgn0032236	o
Cobs_09104	3023	-0.20	4.06e-02					FBgn0000163	o
Cobs_09303	241	0.26	3.04e-02					FBgn0027903	h
Cobs_09406	3119	-0.27	2.69e-02					FBgn0040299	h
Cobs_09518	1053	0.24	4.75e-02					FBgn0037756	o
Cobs_09585	2840	-0.19	3.73e-02					FBgn0025724	o
Cobs_09621	116	0.31	4.78e-02					FBgn0069913	o
Cobs_09680	328	-0.46	3.71e-04					FBgn0263772	o
Cobs_09699	2350	-0.24	2.35e-02					FBgn0023388	o
Cobs_09954	936	0.22	3.35e-02					FBgn0039773	o
Cobs_10056	6490	-0.26	2.65e-02					FBgn0000319	o
Cobs_10079	3181	-0.26	4.29e-02					FBgn0261836	o
Cobs_10083	4218	-0.40	4.18e-03					FBgn0261836	o
Cobs_10112	1395	0.34	7.05e-03					FBgn0014857	h
Cobs_10116				-0.43	4.82e-02			FBgn0028886	h
Cobs_10134	356	-0.26	4.58e-02					FBgn0264272	o
Cobs_10185	687	0.30	1.55e-02					FBgn0032050	o
Cobs_10221	871	-0.24	4.58e-02					FBgn0250906	o
Cobs_10249	1445	-0.28	9.45e-03					FBgn0010812	o
Cobs_10271	3033	-0.30	6.79e-03					FBgn0031118	o
Cobs_10500	149	-0.49	2.65e-03						
Cobs_10546	226	0.29	3.09e-02					FBgn0036911	o
Cobs_10547	2915	-0.24	3.08e-02					FBgn0020622	o
Cobs_10626	2124	-0.36	3.24e-02					FBgn0034240	o
Cobs_10647	368	0.31	2.10e-02					FBgn0044823	o
Cobs_10671	1870	-0.21	3.39e-02					FBgn0028671	o
Cobs_10941	794	-0.46	6.79e-03	-0.47	2.28e-02			FBgn0265045	h
Cobs_10951	258	-0.26	4.99e-02						
Cobs_11062	1061	-0.22	2.35e-02					FBgn0000057	h
Cobs_11095	522	-0.27	2.63e-02					FBgn0036702	o
Cobs_11688	1198	-0.22	3.09e-02					FBgn0004449	o
Cobs_11736	18938	-0.26	4.52e-02					FBgn0030674	o
Cobs_11921	321	0.41	3.99e-03					FBgn0053199	o
Cobs_12090	1328	0.29	3.48e-03						
Cobs_12253	2492	-0.27	3.39e-02					FBgn0266019	o
Cobs_12322	373	0.34	3.50e-03					FBgn0051249	o
Cobs_12335	286	0.26	3.09e-02					FBgn0030590	h
Cobs_12343	3742	-0.29	2.65e-02					FBgn0031107	o

	Mean	VQ vs. MQe		VQ vs. MQW		MQW vs. MQe		<i>Drosophila melanogaster</i>	
		logFC	P-adj	logFC	P-adj	logFC	P-adj	ID	o/h
Cobs_12362	645	0.22	4.70e-02					FBgn0014029	o
Cobs_12470	1115	0.31	3.13e-02					FBgn0032010	o
Cobs_12471	672	0.35	1.15e-02					FBgn0039303	o
Cobs_12475	1073	0.23	4.87e-02					FBgn0031145	o
Cobs_12550	559	0.30	9.77e-03					FBgn0035558	o
Cobs_12564	6560	-0.32	2.03e-02	-0.45	5.67e-04			FBgn0032381	h
Cobs_12720	2115	-0.29	3.35e-02					FBgn0020503	o
Cobs_12915	661	-0.28	3.09e-02					FBgn0266084	o
Cobs_12970	1373	0.21	2.71e-02					FBgn0001222	o
Cobs_13051	479	0.27	3.09e-02					FBgn0037340	o
Cobs_13104	135	-0.45	6.12e-03					FBgn0014417	o
Cobs_13169	950	-0.27	2.91e-02					FBgn0050104	o
Cobs_13309	122	0.36	4.66e-02					FBgn0042627	h
Cobs_13716	702	0.30	3.16e-02					FBgn0031401	o
Cobs_13769	1639	-0.30	2.62e-03					FBgn0034792	o
Cobs_13950	147	0.34	2.65e-02					FBgn0040238	h
Cobs_14213	861	0.25	4.25e-02					FBgn0002924	o
Cobs_14340	655	0.27	2.91e-02					FBgn0029503	o
Cobs_14397	468	-0.22	4.06e-02					FBgn0038313	o
Cobs_14437	206	0.36	3.13e-02					FBgn0260005	h
Cobs_14463	5593	-0.24	4.16e-02					FBgn0001308	o
Cobs_14478	652	-0.22	2.42e-02					FBgn0039240	h
Cobs_14540	548	0.32	8.20e-03					FBgn0040285	o
Cobs_14647	1134	0.24	2.92e-02					FBgn0035542	o
Cobs_14649	782	0.40	5.11e-04					FBgn0037262	o
Cobs_14672	5637	-0.25	1.66e-02					FBgn0263352	o
Cobs_14706	1165	-0.31	2.92e-02					FBgn0036821	o
Cobs_14853	1135	-0.36	1.31e-02					FBgn0052529	h
Cobs_15054	193	-0.31	3.31e-02					FBgn0028970	o
Cobs_15056	329	-0.44	3.48e-03					FBgn0260487	o
Cobs_15294	936	0.24	1.10e-02					FBgn0032536	h
Cobs_15341	483	0.23	3.79e-02					FBgn0038453	o
Cobs_15450	930	-0.29	2.57e-02					FBgn0085370	o
Cobs_15514	193	-0.32	1.56e-02					FBgn0265082	h
Cobs_15589	2002	-0.23	2.71e-02					FBgn0264953	o
Cobs_15622	5901	-0.29	4.11e-02					FBgn0031052	o
Cobs_15627	2095	0.21	2.77e-02					FBgn0050349	o
Cobs_15721	334	-0.32	3.66e-02					FBgn0036480	h
Cobs_15738	422	0.29	8.20e-03					FBgn0037569	o
Cobs_15809	247	-0.46	3.63e-03					FBgn0000414	o
Cobs_15835	518	0.35	3.48e-03					FBgn0032189	o
Cobs_15838	7224	-0.27	4.09e-02					FBgn0035001	o
Cobs_15886	163	0.40	6.79e-03					FBgn0029718	o
Cobs_15897	2749	-0.19	2.71e-02					FBgn0032242	o
Cobs_15953	106	0.45	2.65e-03					FBgn0029943	o
Cobs_16041	504	-0.27	4.90e-02					FBgn0260799	o
Cobs_16087	210	-0.31	3.39e-02					FBgn0032252	o
Cobs_16136	289	0.30	3.35e-02					FBgn0031282	o
Cobs_16187	172	0.31	3.07e-02					FBgn0036356	o
Cobs_16491	1370	0.29	7.64e-03					FBgn0034791	o
Cobs_16702	617	0.27	1.24e-02					FBgn0040336	o
Cobs_16746	700	0.24	3.24e-02					FBgn0010894	o
Cobs_16795	969	0.33	1.21e-02					FBgn0036928	o
Cobs_16923	146	0.32	3.91e-02					FBgn0000330	o
Cobs_16924	632	-0.23	4.53e-02					FBgn0031883	h
Cobs_16982	1558	-0.21	4.06e-02					FBgn0027836	o
Cobs_16990	950	0.27	1.07e-02					FBgn0005655	o
Cobs_17094	248	0.33	1.10e-02					FBgn0039617	h
Cobs_17154	3715	0.18	4.06e-02					FBgn0005630	o
Cobs_17202	4900	-0.26	6.62e-03					FBgn0015245	o
Cobs_17223	2269	-0.21	2.35e-02					FBgn0036762	o
Cobs_17232	626	0.36	1.07e-02					FBgn0053815	o
Cobs_17272	983	-0.39	2.65e-03					FBgn0004839	o
Cobs_17278	371	0.28	2.26e-02					FBgn0040005	o
Cobs_17491	462	0.25	4.35e-02					FBgn0243516	o
Cobs_17500	269	0.27	4.44e-02					FBgn0030341	o
Cobs_17522	678	-0.22	3.87e-02					FBgn0032907	o
Cobs_17527	291	0.36	1.15e-02					FBgn0026749	o
Cobs_17533	10517	-0.24	4.87e-02					FBgn0262519	o
Cobs_17537	178	-0.30	3.79e-02					FBgn0033504	o
Cobs_17572	1068	-0.25	2.35e-02					FBgn0000721	o
Cobs_17583	675	0.23	4.73e-02					FBgn0263832	o
Cobs_17639	266	0.33	9.77e-03						
Cobs_17739	321	-0.28	7.64e-03					FBgn0051116	o
Cobs_17828	189	0.33	3.74e-02					FBgn0035534	o

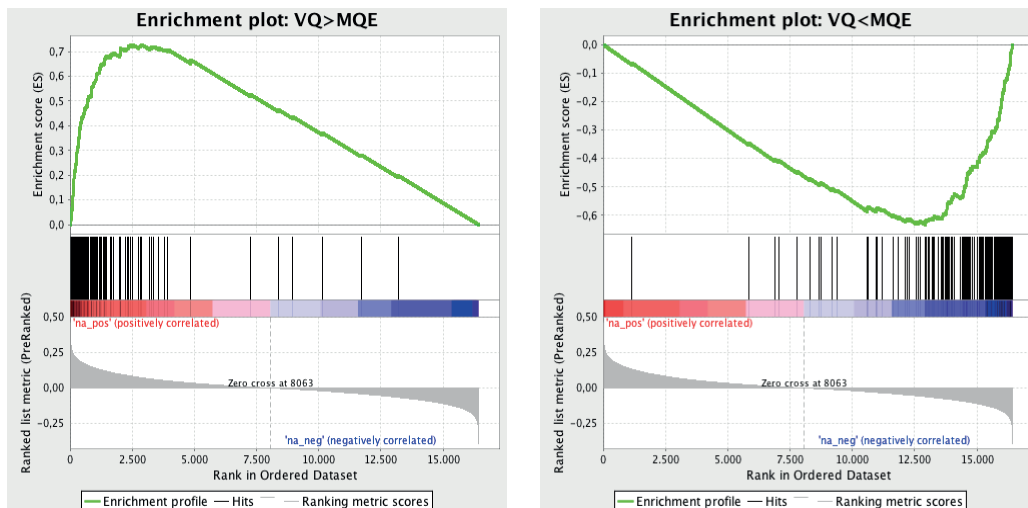
	Mean	VQ vs. MQe		VQ vs. MQW		MQW vs. MQe		<i>Drosophila melanogaster</i>	
		logFC	P-adj	logFC	P-adj	logFC	P-adj	ID	o/h
Cobs_17966	1389	0.26	2.65e-03					FBgn0038722	o
Cobs_18019	3206	-0.24	4.25e-02					FBgn0250786	o
Cobs_18040	1200	0.21	3.39e-02					FBgn0024841	o
Cobs_18089	673	-0.34	2.65e-03					FBgn0035802	o
Cobs_18173	274	0.28	3.67e-02					FBgn0262516	o
Cobs_18232	463	0.32	1.72e-02					FBgn0038829	o
Cobs_18234	283	0.32	3.55e-02					FBgn0264695	h
Cobs_18262	909	-0.38	7.64e-03					FBgn0259246	o

### 7.3.4 Gene Set Enrichment Analysis (GSEA)

#### Pre-ranked list: VQ vs. MQW



#### Pre-ranked list: MQW vs. MQE



Gene set enrichment plots illustrating the overrepresentation of gene sets in the corresponding pre-ranked list. Genes upregulated (VQ > MQE) and downregulated (VQ < MQE) in VQ compared to MQE were tested for an enrichment.

### 7.3.5 Lists of significant GO terms of the category biological process

Functional enrichments in genes differently expressed due to mating with one of both male types (MQE and MQW versus VQ) or due to mating with only the ergatoid type (MQE versus VQ). Count, number of genes in list annotated for the corresponding term; Pop Hits, total number of annotated genes; FE, fold enrichment.

Genes upregulated in MQE and MQW compared to VQ.

Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0000902~cell morphogenesis	3	75.0	1.58e-02	315	10.1
GO:0000904~cell morphogenesis involved in differentiation	3	75.0	7.32e-03	213	14.9
GO:0006928~cell motion	3	75.0	6.40e-03	199	15.9
GO:0007009~plasma membrane organization	2	50.0	4.96e-03	7	302.2
GO:0007026~negative regulation of microtubule depolymerization	2	50.0	4.96e-03	7	302.2
GO:0007399~nervous system development	3	75.0	3.26e-02	458	6.9
GO:0007409~axonogenesis	3	75.0	3.47e-03	146	21.7
GO:0007411~axon guidance	3	75.0	1.70e-03	102	31.1
GO:0008037~cell recognition	2	50.0	2.53e-02	36	58.8
GO:0008038~neuron recognition	2	50.0	2.46e-02	35	60.4
GO:0010639~negative regulation of organelle organization	2	50.0	1.55e-02	22	96.2
GO:0016043~cellular component organization	4	100.0	2.31e-02	1205	3.5
GO:0016198~axon choice point recognition	2	50.0	9.19e-03	13	162.7
GO:0016199~axon midline choice point recognition	2	50.0	8.49e-03	12	176.3
GO:0022008~neurogenesis	3	75.0	1.90e-02	347	9.1
GO:0030030~cell projection organization	3	75.0	1.09e-02	261	12.2
GO:0030154~cell differentiation	4	100.0	3.38e-03	636	6.7
GO:0030182~neuron differentiation	3	75.0	1.31e-02	287	11.1
GO:0031110~regulation of microtubule polymerization or depolymerization	2	50.0	6.37e-03	9	235.1
GO:0031111~negative regulation of microtubule polymerization or depolymerization	2	50.0	4.96e-03	7	302.2
GO:0031114~regulation of microtubule depolymerization	2	50.0	5.66e-03	8	264.4
GO:0031175~neuron projection development	3	75.0	6.79e-03	205	15.5
GO:0032501~multicellular organismal process	4	100.0	3.26e-02	1352	3.1
GO:0032502~developmental process	4	100.0	2.38e-02	1218	3.5
GO:0032886~regulation of microtubule-based process	2	50.0	7.78e-03	11	192.3
GO:0032989~cellular component morphogenesis	3	75.0	2.00e-02	356	8.9
GO:0032990~cell part morphogenesis	3	75.0	8.73e-03	233	13.6
GO:0043242~negative regulation of protein complex disassembly	2	50.0	7.08e-03	10	211.5
GO:0043244~regulation of protein complex disassembly	2	50.0	9.19e-03	13	162.7
GO:0048468~cell development	4	100.0	1.41e-03	475	8.9
GO:0048638~regulation of developmental growth	2	50.0	2.11e-02	30	70.5
GO:0048666~neuron development	3	75.0	9.71e-03	246	12.9
GO:0048667~cell morphogenesis involved in neuron differentiation	3	75.0	6.72e-03	204	15.6
GO:0048699~generation of neurons	3	75.0	1.75e-02	332	9.6
GO:0048812~neuron projection morphogenesis	3	75.0	6.72e-03	204	15.6
GO:0048858~cell projection morphogenesis	3	75.0	8.37e-03	228	13.9
GO:0048869~cellular developmental process	4	100.0	4.30e-03	689	6.1
GO:0051129~negative regulation of cellular component organization	2	50.0	2.46e-02	35	60.4
GO:0051493~regulation of cytoskeleton organization	2	50.0	2.39e-02	34	62.2
GO:0051494~negative regulation of cytoskeleton organization	2	50.0	7.78e-03	11	192.3
GO:0051960~regulation of nervous system development	2	50.0	4.88e-02	70	30.2
GO:0070507~regulation of microtubule cytoskeleton organization	2	50.0	7.78e-03	11	192.3

Genes exclusively downregulated in MQE compared to VQ.

Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0006412~translation	8	8.2	3.23e-02	237	2.6
GO:0009059~macromolecule biosynthetic process	17	17.3	1.46e-02	705	1.8
GO:0009987~cellular process	48	49.0	3.33e-02	3139	1.2
GO:0015031~protein transport	8	8.2	1.55e-02	204	3.0
GO:0034645~cellular macromolecule biosynthetic process	17	17.3	1.37e-02	700	1.8
GO:0043277~apoptotic cell clearance	2	2.0	3.85e-02	3	50.4
GO:0043652~engulfment of apoptotic cell	2	2.0	2.58e-02	2	75.6
GO:0045184~establishment of protein localization	8	8.2	1.75e-02	209	2.9

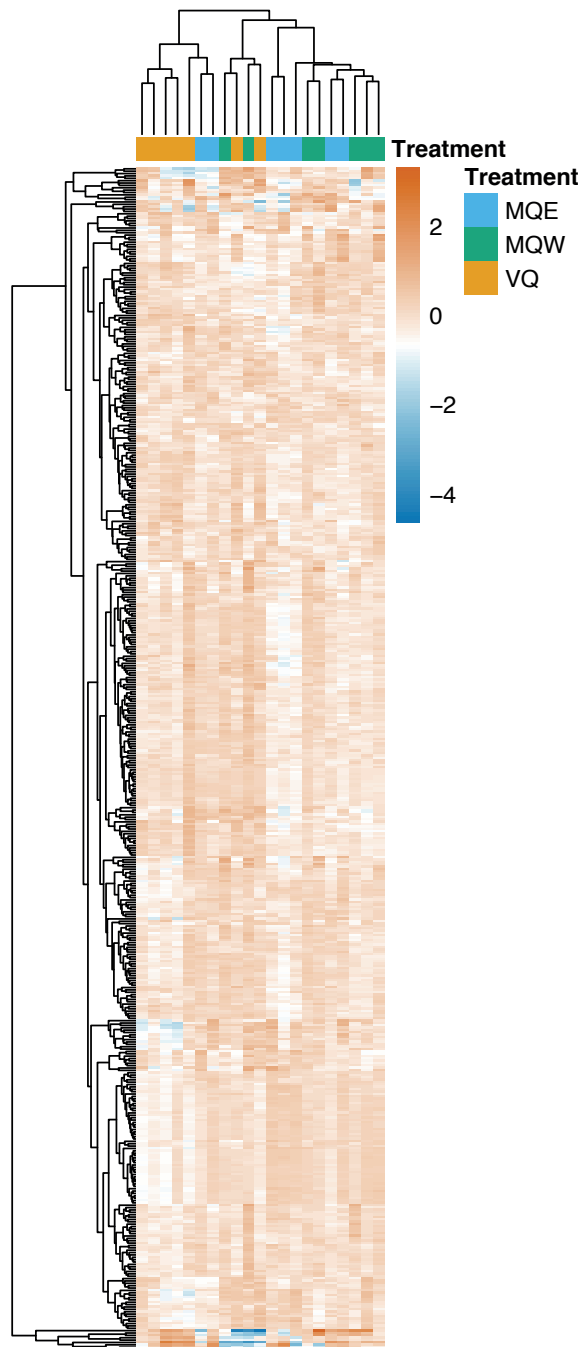
## Genes exclusively upregulated in MQE compared to VQ.

Term	Count	%	P value	Pop Hits	FE
GO:0001505~regulation of neurotransmitter levels	8	8.0	4.61e-04	82	5.6
GO:0001751~compound eye photoreceptor cell differentiation	6	6.0	1.36e-02	83	4.1
GO:0001754~eye photoreceptor cell differentiation	7	7.0	3.20e-03	85	4.7
GO:0002164~larval development	5	5.0	1.26e-02	53	5.4
GO:0002168~instar larval development	4	4.0	2.70e-02	38	6.0
GO:0003001~generation of a signal involved in cell-cell signaling	8	8.0	2.88e-04	76	6.0
GO:0003002~regionalization	10	10.0	3.40e-02	261	2.2
GO:0003008~system process	13	13.0	2.51e-03	278	2.7
GO:0006338~chromatin remodeling	4	4.0	2.34e-02	36	6.4
GO:0006836~neurotransmitter transport	8	8.0	4.96e-04	83	5.5
GO:0006886~intracellular protein transport	7	7.0	2.22e-02	128	3.1
GO:0006900~membrane budding	3	3.0	2.67e-02	15	11.4
GO:0006996~organelle organization	21	21.0	5.64e-03	651	1.8
GO:0007010~cytoskeleton organization	13	13.0	1.07e-02	333	2.2
GO:0007154~cell communication	12	12.0	2.27e-04	181	3.8
GO:0007155~cell adhesion	6	6.0	4.72e-02	115	3.0
GO:0007167~enzyme linked receptor protein signaling pathway	6	6.0	2.93e-02	101	3.4
GO:0007267~cell-cell signaling	11	11.0	1.89e-04	148	4.2
GO:0007268~synaptic transmission	11	11.0	7.72e-05	133	4.7
GO:0007269~neurotransmitter secretion	8	8.0	2.65e-04	75	6.1
GO:0007276~gamete generation	12	12.0	3.85e-02	354	1.9
GO:0007281~germ cell development	7	7.0	2.14e-02	127	3.2
GO:0007292~female gamete generation	11	11.0	2.37e-02	286	2.2
GO:0007308~oocyte construction	6	6.0	5.97e-03	68	5.0
GO:0007309~oocyte axis specification	5	5.0	2.50e-02	65	4.4
GO:0007314~oocyte anterior/posterior axis specification	4	4.0	3.29e-02	41	5.6
GO:0007389~pattern specification process	11	11.0	2.12e-02	281	2.2
GO:0007399~nervous system development	17	17.0	4.14e-03	458	2.1
GO:0007416~synaptogenesis	3	3.0	2.67e-02	15	11.4
GO:0008104~protein localization	12	12.0	1.01e-02	290	2.4
GO:0008358~maternal determination of anterior/posterior axis. embryo	4	4.0	3.50e-02	42	5.4
GO:0009653~anatomical structure morphogenesis	21	21.0	2.07e-02	734	1.6
GO:0009887~organ morphogenesis	13	13.0	4.32e-02	406	1.8
GO:0009987~cellular process	63	63.0	1.97e-02	3139	1.1
GO:0009994~oocyte differentiation	6	6.0	1.06e-02	78	4.4
GO:0010646~regulation of cell communication	11	11.0	4.36e-03	222	2.8
GO:0016043~cellular component organization	29	29.0	4.23e-02	1205	1.4
GO:0019226~transmission of nerve impulse	11	11.0	1.06e-04	138	4.6
GO:0019953~sexual reproduction	12	12.0	4.27e-02	360	1.9
GO:0021700~developmental maturation	6	6.0	1.11e-02	79	4.3
GO:0022008~neurogenesis	16	16.0	6.69e-04	347	2.6
GO:0022603~regulation of anatomical structure morphogenesis	7	7.0	1.79e-02	122	3.3
GO:0022604~regulation of cell morphogenesis	6	6.0	2.23e-02	94	3.6
GO:0022607~cellular component assembly	12	12.0	4.27e-02	360	1.9
GO:0022610~biological adhesion	6	6.0	4.72e-02	115	3.0
GO:0030010~establishment of cell polarity	3	3.0	2.34e-02	14	12.3
GO:0030029~actin filament-based process	6	6.0	3.51e-02	106	3.2
GO:0030036~actin cytoskeleton organization	6	6.0	3.39e-02	105	3.3
GO:0030154~cell differentiation	18	18.0	3.94e-02	636	1.6
GO:0030182~neuron differentiation	11	11.0	2.42e-02	287	2.2
GO:0031644~regulation of neurological system process	3	3.0	1.73e-02	12	14.3
GO:0032501~multicellular organismal process	34	34.0	1.15e-02	1352	1.4
GO:0032940~secretion by cell	8	8.0	7.59e-04	89	5.1
GO:0033036~macromolecule localization	16	16.0	1.01e-03	361	2.5
GO:0034613~cellular protein localization	7	7.0	2.45e-02	131	3.1
GO:0035023~regulation of Rho protein signal transduction	4	4.0	3.34e-03	18	12.7
GO:0035088~establishment or maintenance of apical/basal cell polarity	3	3.0	4.57e-02	20	8.6
GO:0042051~compound eye photoreceptor development	4	4.0	3.29e-02	41	5.6
GO:0042462~eye photoreceptor cell development	4	4.0	3.29e-02	41	5.6
GO:0044057~regulation of system process	4	4.0	7.69e-03	24	9.5
GO:0045197~establishment or maintenance of epithelial cell apical/basal polarity	3	3.0	2.34e-02	14	12.3
GO:0045595~regulation of cell differentiation	7	7.0	3.07e-02	138	2.9
GO:0046530~photoreceptor cell differentiation	7	7.0	5.00e-03	93	4.3
GO:0046903~secretion	8	8.0	1.05e-03	94	4.9
GO:0046907~intracellular transport	9	9.0	4.02e-02	227	2.3
GO:0048468~cell development	16	16.0	1.39e-02	475	1.9
GO:0048469~cell maturation	6	6.0	8.52e-03	74	4.6
GO:0048477~oogenesis	11	11.0	2.22e-02	283	2.2
GO:0048489~synaptic vesicle transport	5	5.0	2.63e-02	66	4.3
GO:0048592~eye morphogenesis	7	7.0	4.35e-02	150	2.7
GO:0048599~oocyte development	6	6.0	6.75e-03	70	4.9
GO:0048699~generation of neurons	15	15.0	1.33e-03	332	2.6
GO:0048731~system development	22	22.0	3.70e-02	829	1.5



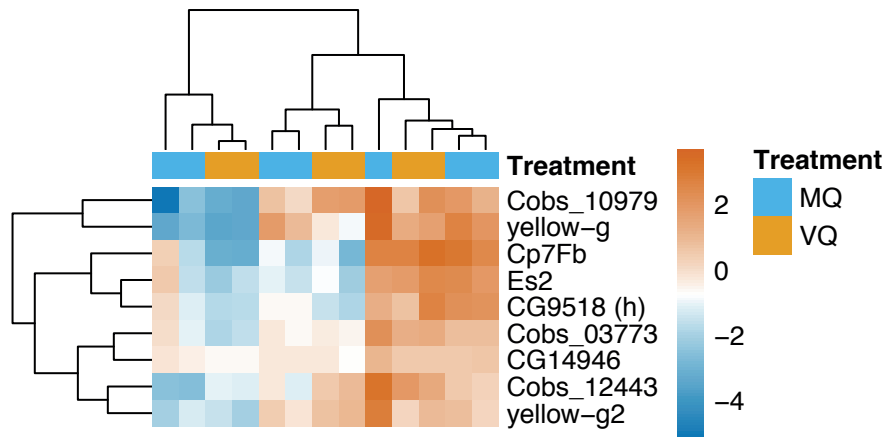
Term	Count	%	<i>P</i> value	Pop Hits	FE
GO:0048856~anatomical structure development	25	25.0	2.78e-02	957	1.5
GO:0048869~cellular developmental process	19	19.0	4.15e-02	689	1.6
GO:0050793~regulation of developmental process	9	9.0	4.68e-02	234	2.2
GO:0050804~regulation of synaptic transmission	3	3.0	1.73e-02	12	14.3
GO:0050877~neurological system process	13	13.0	1.84e-03	268	2.8
GO:0051128~regulation of cellular component organization	9	9.0	2.50e-02	207	2.5
GO:0051179~localization	26	26.0	3.17e-02	1020	1.5
GO:0051239~regulation of multicellular organismal process	11	11.0	2.44e-03	205	3.1
GO:0051640~organelle localization	5	5.0	9.62e-03	49	5.8
GO:0051641~cellular localization	19	19.0	3.36e-05	368	3.0
GO:0051649~establishment of localization in cell	17	17.0	6.44e-05	315	3.1
GO:0051656~establishment of organelle localization	5	5.0	7.12e-03	45	6.4
GO:0051960~regulation of nervous system development	5	5.0	3.18e-02	70	4.1
GO:0051969~regulation of transmission of nerve impulse	3	3.0	1.73e-02	12	14.3
GO:0060284~regulation of cell development	7	7.0	5.84e-03	96	4.2
GO:0065008~regulation of biological quality	17	17.0	1.66e-03	419	2.3
GO:0070727~cellular macromolecule localization	9	9.0	6.75e-03	164	3.1

## 7.3.6 Hierarchical clustering of 500 most variable genes



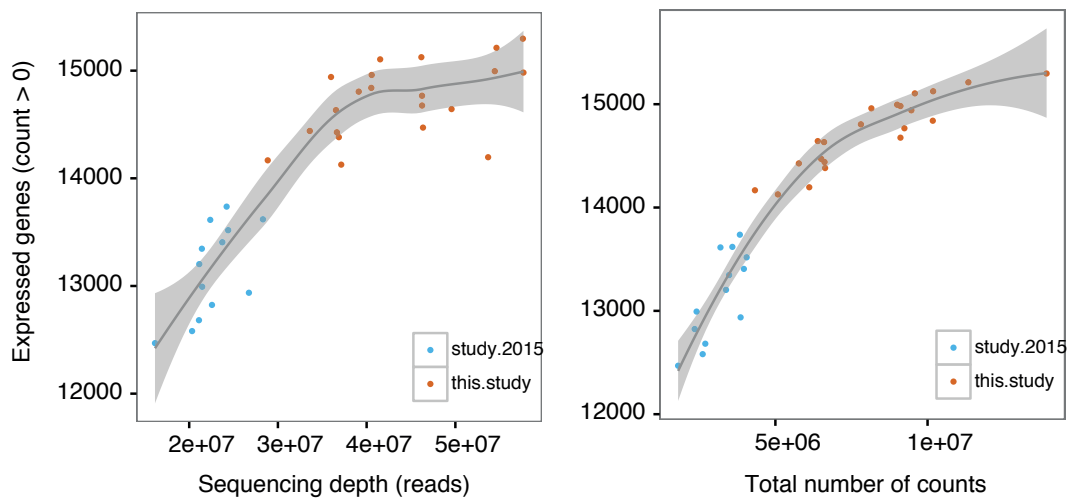
Hierarchical clustering of the top 500 genes with the highest variance across all samples. Nine genes including the most variable gene *Cobs\_10979* formed one separated clade (bottom). Normalized counts were transformed (VST), corrected for the batch effect and subtracted by the gene's mean expression prior to clustering.

### 7.3.7 Hierarchical clustering of coexpressed candidates



Hierarchical clustering of coexpressed candidate genes (Table 4.4) on the basis of gene counts obtained in the framework of a previous RNA-Seq study. Normalized counts from von Wychetzki *et al.* (2015) were transformed (VST) and subtracted by the gene's mean expression value prior to clustering. MQ, queen mated with ergatoid male; VQ, virgin queen.

### 7.3.8 Comparison of library sizes



The number of detectable genes increases with the number of sequenced reads (left) and gene counts (right). Samples from this and a former RNA-Seq study (Wychetzki *et al.* 2015) were plotted.

### 7.3.9 Comparison with previous studies

Overlap of this and previous comparisons of mated and virgin queens on the level of orthologous genes. +, upregulated in virgin; -, downregulated in virgin; V/VQ, virgin; M, mated; MQE, mated with ergatoid male.

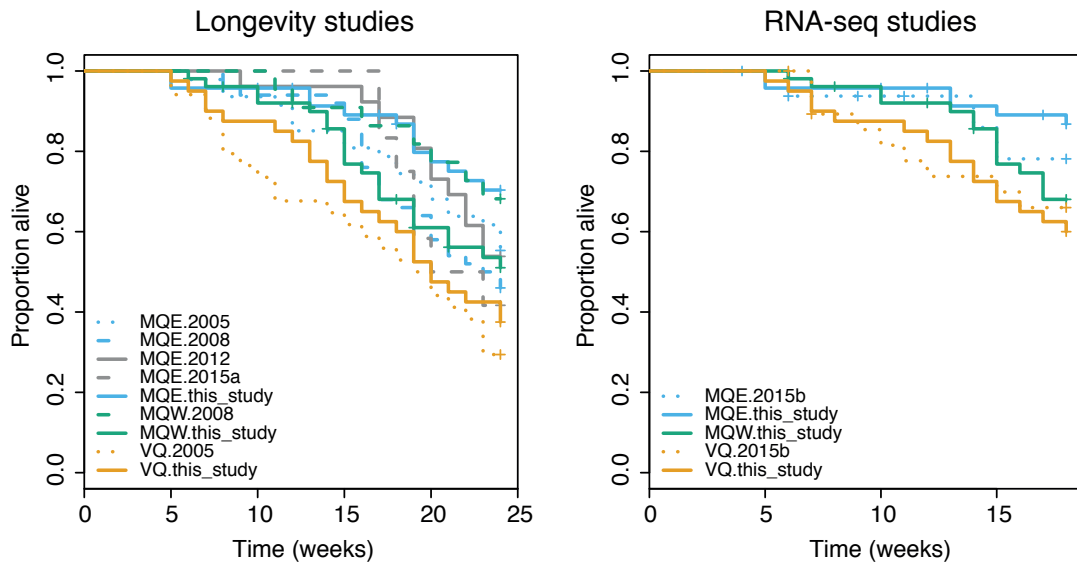
			DEGs	VQ vs. MQE	
				+	-
<i>Cardiocondyla obscurior</i>					
Queen whole body	VQ vs. MQE	+	33	0	1
(von Wyszczetki <i>et al.</i> 2015)		-	4	0	0
<i>Apis mellifera</i>					
Queen brain	V vs. M	+	42	0	0
(Kocher <i>et al.</i> 2008)		-	30	0	0
Queen ovary	V vs. M	+	74	1	1
(Kocher <i>et al.</i> 2008)		-	11	1	0
Queen brain	V vs. M	+	84	0	2
(Kocher <i>et al.</i> 2010)		-	45	0	1
Queen brain	V vs. M	+	95	1	1
(Manfredini <i>et al.</i> 2015)		-	386	2	6

### 7.3.10 Overview of all longevity studies performed in *C. obscurior* queens

MQE, queen mated with ergatoid male; MQW, queen mated with winged male; VQ, virgin queen. Both colony collection sites Ilheus and Una are located in Bahia, Brazil.

Queen mating type	Median lifespan	N	Author of study	Year	Collection site	Collection year
MQE	25	47	Schrempf <i>et al.</i>	2005	Ilheus	2004
VQ	20	34				
MQE	24	50	Schrempf & Heinze	2008	Ilheus	2004
MQW	33	22				
MQE	25	25	Heinze & Schrempf	2012	Una	2004
MQE	22	12	Schrempf <i>et al.</i>	2015	Ilheus	2004
MQE	-	17	von Wyszczetki <i>et al.</i>	2015b	Una	2009
VQ	-	29				
MQE	-	47	this study		Una	2009
MQW	24	52				
VQ	20	40				

## 7.3.11 Cross-study comparison of queen survival



Comparison of queen survival in this study with survival until 24 weeks in previous longevity studies (left) and survival until 18 weeks in the former RNA-Seq study (right). Labels refer to the year of publication as indicated in the table above.

## Chapter 8

### Summary

Why organisms age and why some species do so at a faster rate than others are fundamental questions in biology. The queens of perennial eusocial insects (ants, honeybees and termites) are extraordinarily long-lived compared with females of solitary insects. Similar to the reproductive females of eusocial mammals, they do not exhibit signs of functional senescence and terminate reproduction only shortly before they die. In contradiction to the widespread fecundity/longevity trade-off, lifespan and reproductive success seem to be positively associated in eusocial animals. Evolutionary theories explain the long lifespan of queens from their low extrinsic mortality. They live in sheltered, often subterranean nests, and are cared for by the workers. Furthermore, the queens of eusocial insects use the sperm of only one or a few males to fertilize all their eggs. The lifelong pair bond between males and females predicts that both partners benefit from an increased lifespan of the queen.

If and how the reproductive females of eusocial insects avoid the costs of reproduction are open questions. In this study, the myrmicine ant *Cardiocondyla obscurior* served as a model to investigate the regulation of queen longevity on the proximate level. Due to their relative short life expectancy, the survival and lifetime reproductive success of *C. obscurior* queens could be monitored in the laboratory.

This study is the first to report age-related changes in the transcriptome of mature social insect queens and shows that these changes are exactly opposite to what has previously been reported to aging females of fruit flies, *Drosophila melanogaster*. The results match the opposing reproductive and mortality patterns observed in social and solitary species and provide a first mechanistic explanation for the simultaneous increase of fecundity and longevity in ant queens (chapter 2).

The compensation of putative reproductive costs has not been thoroughly investigated in social insect queens. To test the prediction that reproduction competes for energy and nutrients with other processes, ant queens were forced to increase their investment into somatic repair. This experiment provides clear evidence on the phenotypic and transcriptome level that queens reallocate resources between the reproductive and immune systems (chapter 3).

The positive effect of mating on queen longevity was addressed in three analyses (chapter 2, 4 and 5). Physiological changes could be identified which are attributable to mating independent of reproduction (chapter 2) and the male type with whom the queen had mated (chapter 4 and 5).

In conclusion, this study suggests an alternative regulation of the conserved pathways that mediate the interplay among reproduction, metabolism and longevity. Queens might not avoid the costs of reproduction, but the costs of self-maintenance which are possibly borne by the workers.

## Chapter 9

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## Chapter 10

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