ABSTRACT

EDWARDS, ALEXIS CHRISTINE. The Genomic Architecture of Aggressive Behavior in *Drosophila melanogaster*. (Under the direction of Trudy F.C. Mackay).

Aggressive behavior is observed throughout the animal kingdom, and can be used to obtain or defend mates, food, or territory, or to establish and maintain social structure. However, aggression can be selectively disadvantageous, in that it can result in bodily harm, can detract from time spent mating or foraging, and is metabolically expensive. Furthermore, aggression can reach pathological levels in humans, and is often a component of psychiatric disorders such as schizophrenia, conduct disorder, and Alzheimer's Disease. Aggressive behavior is a complex, quantitative trait, influenced by multiple interacting genes whose effects vary in magnitude and direction, and are sensitive to environmental perturbations. An understanding of the genetics underlying aggression is important from an evolutionary perspective and in the context of human health. We used a suite of approaches in *Drosophila melanogaster* to identify genes affecting aggressive behavior: artificial selection to derive lines of flies divergent in their aggression levels, followed by whole genome expression analysis; a screen of *P*-element insertional mutants and characterization of a subset of candidate genes; quantitative trait locus (QTL) mapping to identify segregating variation affecting aggression; and behavioral and transcriptional analysis of a panel of wild-derived inbred lines to assess natural genetic variation and correlated modules of genes.

Artificial selection for 25 generations followed by expression profiling revealed that a substantial portion of the genome – more than 1,500 genes – exhibited differential expression that was correlated with the selection response. These candidate genes have been implicated in a wide variety of biological processes and molecular functions, including metabolism, learning and memory, and nervous system development. Many are computationally

predicted genes with no previous annotation. Functional tests of 19 *P*-element mutants confirmed 15 of these candidates, none of which have been previously implicated in aggressive behavior.

A screen of 170 *P*-element insertional mutants in a co-isogenic background identified 59 lines with aberrant aggressive behavior relative to the control. These genes fell into diverse gene ontology categories, including metabolism and localization. qPCR of nine candidate genes revealed disruption of gene expression throughout development. Morphometric analysis of the alpha and beta lobes of the mushroom bodies showed subtle yet significant differences in length and/or width relative to controls. *P*-element remobilization rescued the mutant behavioral phenotype in nearly every case, confirming these nine candidate genes as affecting aggression.

QTL mapping of introgression lines uncovered five regions contributing to variation in aggression levels between the two parental stocks. Deficiency complementation mapping was used to refine a region spanning two of these QTL, which fractionated into seven smaller regions. Mutant complementation tests to 58 positional candidate genes resulted in the identification of four genes affecting variation in aggressive behavior.

Finally, behavioral assessment of a reference panel of 40 wild-derived inbred lines revealed abundant natural variation in aggression. Transcriptome profiling was used to identify quantitative trait transcripts and single feature polymorphisms. Five hundred sixty unique genes met significance criteria in at least one of these categories, and these candidate genes have roles in biological processes such as olfaction, metabolism, and stress response. Tests of mutants in candidate genes suggest that this is a highly effective manner in which to identify genes influencing aggressive behavior. Genes implicated in affecting aggression

through transcript abundance fell into nine correlated modules: genes in each module are potentially co-regulated, and may be functionally related.

The Genomic Architecture of Aggressive Behavior in *Drosophila melanogaster*

by
Alexis Christine Edwards

A dissertation submitted to the Graduate Faculty of
North Carolina State University
In partial fulfillment of the
Requirements for the degree of
Doctor of Philosophy

Genetics

Raleigh, North Carolina

2008

APPROVED BY:

Trudy F.C. Mackay Chair of Advisory Committee Genetics	Robert R. Anholt Committee Member Zoology
Patricia Estes Committee Member Genetics	Robert M. Grossfeld Committee Member Zoology

Jane Lubischer
Committee Member
Zoology

DEDICATION

When I was young, I admired clever people. Now that I am old, I admire kind people.

- Abraham Joshua Heschel

BIOGRAPHY

Alexis Edwards was born on September 12, 1977 and was raised in Lake Wylie, South Carolina. After graduating from Clover High School in 1995, she matriculated to the South Carolina Honors College of the University of South Carolina. During her undergraduate studies, she developed an abiding interest in the biological and social sciences, particularly behavior. She earned a Bachelor of Science degree in Biology with a minor in Anthropology, and her Honors thesis was entitled "Anthropomorphism in Primatology", and focused on the behavioral parallels between humans and non-human primates.

After graduating in 1999, Alexis relocated to Raleigh, North Carolina, working for Paradigm Genetics in Research Triangle Park and volunteering at the Duke University Primate Center (now the Duke Lemur Center). In 2002, she began graduate school in the Genetics Department at North Carolina State University. During her graduate career under the mentorship of Dr. Trudy F.C. Mackay, she participated in conferences and symposia locally, nationally, and internationally; served in the Genetics Graduate Student Association; and published her work in scientific journals. After completing her doctorate, she will hold a post-doctoral position in Richmond, Virginia, at the Virginia Institute for Psychiatric and Behavioral Genetics, focusing on the genetics underlying alcoholism and depression.

ACKNOWLEDGMENTS

I would like to thank Dr. Trudy F.C. Mackay for her guidance and support during my graduate career. As a member of her lab, I have had opportunities and resources available to me that most young scientists can only dream of. I would also like to thank my committee members, Drs. Robert Anholt, Pat Estes, Robert Grossfeld, and Jane Lubischer. The knowledge I have gained from you (and my fear of your disappointment!) has improved my scientific abilities, and hopefully made this dissertation more interesting. To the members of the W.M. Keck Center for Behavioral Biology, I am grateful for the lively discussions I have witnessed and taken part in at evening meetings, for feedback on my research, and for funding and professional development opportunities I would not have otherwise had. I have also been lucky enough to receive funding from the NCSU Genetics Department, the US Department of Education, and the National Institute of Mental Health.

The members of the Mackay and Anholt laboratories have been invaluable to me over the years. In a large lab environment, it is easy to get lost (being in the maze that is Gardner Hall doesn't help), but I was able to trudge through my working days and nights with help from colleagues. In particular, I am indebted to Stephanie Rollmann and Ted Morgan for their technical expertise and help, but perhaps more importantly, for setting steadfast examples of scientific integrity. Harland Patch is responsible for broadening my perspective on innumerable topics, helping me think about my work contextually, and for keeping my spirits up when I was cursing the day I lay eyes on a fly. Sadly, he is also responsible for countless bad jokes.

There have been many times when I set out to disprove the notion that "there are no stupid questions", and I am very grateful to Mary Anna Carbone, Aki Yamamoto, Charlene Couch, and Christina Grozinger for answering those questions patiently, without condescension or sarcasm. Thanks also to Richard Lyman, who answered such queries with both sarcasm *and* condescension, thereby thickening my skin a bit. Richard's near-comic level of disappointment in me is likely due in no small part to my refusal to wear close-toed shoes in the lab.

Dr. Katie Jordan has been by my side in the Mackay lab since the beginning. She has been a friend, sounding board, co-conspirator, fly room entertainer, and then some. She made me feel a part of the lab, and has been a true friend through thick and thin. I will honestly miss her greeting of "Good Morning Sunshine!" even though I claim otherwise. My friend and fellow student Julien Ayroles is also responsible for making work enjoyable. I've benefited immensely from his presence in the lab, not just because he is funny and friendly, but also because he contributed a great deal to one of my projects. By the time he graduates, Julien will have completed enough work for several dissertations.

I have been immensely fortunate to have so many wonderful friends who have supported me here in Raleigh and elsewhere. Erica Marsh Hunkin started out as a classmate and turned out to be one of the most inspiring people I've ever met. I admire her especially for her strength, kindness, and integrity at all times, in all situations, toward everyone in her life. Vincent Simone has quietly become an integral part of my life, and I look forward to our journey to Virginia together. The friendships of Jessica

Gaus Barb, Katherine Graham, Jeni Reininga, Madalyn Sierra, Christina Strom, Noa Davidai, Katie Stokes, and Sarah Ayroles have made it possible for me to maintain my sanity and my social life all these years, and I will continue depending on them in the years to come. My parents and sister have been supportive of my scientific curiosity throughout this endeavor, and I owe huge thanks to them…even though they ignored my request that they never utter the sentence "When are you finishing?" Finally, I must mention my kitty Oblio. When human interaction has proved just a bit too demanding, she has never failed to warm my heart.

I am profoundly grateful to you all.

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CHAPTER ONE

Introduction to Aggressive Behavior

INTRODUCTION

Aggression is an important component of nearly every animal's behavioral repertoire. It can be employed in a variety of situations. Animals might display aggression in order to defend themselves against predators or conspecifics; food – prey or otherwise – might be secured or defended through aggressive means; access to mates might depend on the outcome of an aggressive encounter; and territory, mates, or progeny might require defense or protection. For some species, social structures or dominance hierarchies are determined in part through aggressive interactions.

Counterbalancing the potential for obtaining a selective advantage through expression of aggression is the fact that it can be energetically expensive. Metabolic costs arise from the physical exertion of aggressive displays. There is a risk associated with participation in aggressive encounters, especially in organisms with natural weapons such as claws or teeth; this is true even for the "winner" of such interactions. Besides the potential risk of injury, there is the possibility of losing one's territory, food, or mate. Finally, the time and energy invested in aggression detract from time spent locating resources such as food or mates.

Among humans, aggression often manifests as violent behavior, and can be an indicator of an underlying pathology. It is frequently a component of a number of psychiatric disorders, including schizophrenia, conduct disorder, alcoholism, bipolar disorder, and Alzheimer's Disease. It exacts a heavy toll on the human population: in the United States, an individual has an 80% chance of being the victim of a violent crime. The American Institute on Domestic Violence estimates that the annual cost of domestic

violence is $5.8 billion; work productivity is also severely affected. Aggressive behavior can also be self-directed, as with suicide. Suicide rates vary dramatically worldwide, and by gender. The World Health Organization estimates that nearly one million people commit suicide every year, and suicide attempts are approximately 20 times more common.

Because of the potentially dramatic and negative cost of aggressive behavior, both economically and to human health, it is critical that we identify its underlying causes to improve prediction, prevention, and treatment. As with other behaviors, the aggressive phenotype observed is the output of a complicated biological network of genetic pathways, neural systems, and environmental influences. Vast amounts of research support the notion that there is a substantial genetic component to behavioral disorders, but that they do not segregate as Mendelian traits: we should not expect to find "the aggression gene". Rather, susceptibility at the genetic level to pathological behaviors is conferred by alleles of many genes, each of which varies in the magnitude and direction of its effect; furthermore, these genes are subject to modulation by the proximate and developmental environment, and to epistatic interactions. The neural systems within which they exert their effects are also relevant to behavioral output. The same is true of non-pathological aggression, both in humans and other organisms. Aggressive behavior can best be understood as a complex, quantitative trait.

Although technological advances are being made daily that improve the ability to address behavioral traits in humans, model organisms continue to provide a valuable resource for studying the genetics of complex traits such as aggression.

Furthermore, assessing traits of interest in a variety of organisms can be quite informative as to their evolution, mechanisms, and variation. To this end, neurobiological and genetic experiments have been conducted in several organisms particularly well-suited to research on aggression: lobsters or crayfish, mice, and the fruit fly *Drosophila melanogaster*. Each of these animals can be considered a model system for different reasons. Lobsters and crayfish have a behavioral ecology that involves well-characterized patterns of aggression. Their neurons are large and easily accessible, making them ideal for studying the neural pathways and neural chemistry relevant to this behavior. Mice also exhibit aggressive behavior in their natural interactions, and have long been considered a good model for human health issues. Finally, Drosophila is an ideal model system for many practical reasons: its generation time is short; flies can be maintained inexpensively in a controlled environment; their physiological, behavioral, and developmental traits are often easy to observe and quantify; there is a high level of genetic homology between flies and humans; and there are ecologically diverse species of Drosophila, making them ideal for studies of speciation as well. Recent technological advances have made Drosophila even more attractive as a model organism: the *D. melanogaster* genome was sequenced in 2000 (ADAMS *et al.* 2000), and other species of flies have been sequenced more recently (CLARK *et al.* 2007). Currently, there are multiple efforts to create mutants for every gene in the Drosophila genome (BELLEN *et al.* 2004; THIBAULT *et al.* 2004). Tools at the disposal of Drosophila geneticists include recombinant inbred lines, co-isogenic lines, deficiency stocks, and various kinds of single-mutant stocks. Forward and reverse genetic screens are easily undertaken in

Drosophila, and researchers now routinely employ methods such as quantitative trait locus (QTL) mapping, *P*-element screening, whole genome expression profiling, and high density genotyping. Importantly, aggression is a component of the natural behavioral repertoire of Drosophila, and is easily characterized in a laboratory setting. Here, I review our current understanding of the neurobiology and genetics of aggressive behavior in these model organisms and in humans.

Neurobiology of Aggression

Rodent Models

Rodents are an excellent model system in which to address aggression. The ability to conduct genetic manipulations allows the assessment of gene function within the neural pathways implicated in aggression. Much is known about wild-type aggression in rodents, and behavioral assays have been developed that yield unambiguous results. The organization of the rodent central nervous system (CNS) is comparable to that of humans. As such, a vast literature on the neurobiology of aggression in mice is available. A substantial portion of the literature discusses the effect of hormones and pheromones on murine aggression, but those topics will not be addressed here in the interest of space.

Neurobiological aggression research focuses on three primary neurotransmitters (NT's): serotonin (5-hydroxytryptamine, or 5-HT), dopamine (DA), and norepinephrine (NE). These are involved in the modulation of aggressive behavior but specific roles and interactions are still being dissected. 5-HT generally has an inhibitory effect on

aggression (HOLMES *et al.* 2002), while DA and NE have more permissive or facilitative roles (MICZEK *et al.* 2002). Unfortunately, the contribution of each of these NT's is not consistent.

Regions under investigation for their role in aggression include the amygdala, prefrontal cortex (PFC), nucleus accumbens, ventral striatum, anterior cingulate cortex, and various regions of the hypothalamus (NELSON and CHIAVEGATTO 2001). Activity in these regions is likely interrelated; disturbance in one portion of a neural network could have far-reaching consequences. NELSON and CHIAVEGATTO propose that 5-HT is central to the expression of aggressive behavior but is elaborately modulated by other NT systems.

5-HT neurotransmission has been implicated in the normal expression of aggression via the stimulation and appropriate response of 5-HT receptors. The 5-HT_{1A} and 5-HT_{1B} receptors have been of particular interest in mice. Null mutants have been created to elucidate these genes' roles in aggression. Males lacking 5-HT_{1B} exhibit higher levels of aggression than controls (SAUDOU *et al.* 1994). Conversely, males lacking 5-HT_{1A} are less reactive and aggressive (WELLER *et al.* 2003). Both receptors have postsynaptic sites and contribute to serotonergic tone, but are differentially expressed throughout the brain and contribute differently to inhibition (NELSON and CHIAVEGATTO 2001). Pharmacological treatments directed toward specific receptor subtypes suggest that 5-HT levels increase briefly during aggressive bouts in rats, but that in the case of pathological aggression, this increase is preceded by a general state of inhibited serotonergic tone (DE BOER and KOOLHAAS 2005). Additional information

could be garnered by tissue-specific knock-out of receptor subtypes to ascertain whether the effects of receptor activation differ by location (NELSON and TRAINOR 2007).

The 5-HT transporter (5-HTT) has been associated with regulation of serotonergic activity and its involvement in aggression. HOLMES *et al.* (2002) generated 5-HTT$^{+/-}$ and 5-HTT$^{-/-}$ mice to assess the role of the transporter in aggression and observed that the 5-HTT$^{-/-}$ mice exhibited decreased aggression relative to controls and heterozygotes. Lack of a functioning 5-HT transporter increases extracellular levels of 5-HT due to its normal role in reuptake of 5-HT from the synapse. Reduced 5-HT$_{1A}$ mRNA in the dorsal raphe (where 5-HT is synthesized, PURVES *et al*. 2001) and results obtained in locomotor assays indicate that the homozygous mutants had reduced functioning of their 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors. While the increased extracellular 5-HT would logically result in increased inhibition of aggression, the cause of the aberrant function of the 5-HT receptors and the resulting phenotype is unclear.

VAN ERP and MICZEK (2000) examined the transmission of 5-HT, DA, and NE in the nucleus accumbens and medial PFC of rats during aggressive encounters. There were no concurrent changes in these NT systems in the nucleus accumbens, yet DA increased in the hour following the aggressive encounter. Medial PFC 5-HT levels decreased during the encounter and DA increased immediately after the encounter. Rats habituated to experiencing an aggressive encounter at a specific time each day exhibited the same NT alterations even in the absence of a fight at the anticipated time (FERRARI *et al.* 2003; VAN ERP and MICZEK 2000). This suggests that DA might be involved in the preparation for anticipated aggressive interactions or in activation of the necessary neural pathways.

Taken together, these results underscore the temporal and spatial dissociation between the dopaminergic and serotonergic systems. However, this does not mean that their effects are independent of one another.

Adding another layer of complexity to the function of the serotonergic system is monoamine oxidase A (MAOA), which inactivates monoamines such as 5-HT, DA, and NE. Mice lacking a functional MAOA gene have greatly increased levels of all these NT's in the brain (CASES *et al.* 1995), with 5-HT showing the most dramatic increase (nine-fold at some stages of development). Such an increase in 5-HT would presumably decrease aggressive behavior, but mutants showed higher levels of offensive aggression. The cortical structure of mutants was altered, which could be a confounding factor in the expression of behaviors involving the cortex. Elevated levels of permissive monoamines could potentially outweigh the inhibitory effects of 5-HT on aggression in this study, resulting in a counterintuitive behavioral phenotype.

Another component of serotonergic neurotransmission that has been investigated is α-calcium-calmodulin kinase II (α-CaMKII), a component of the signal transduction pathway. α-CaMKII is found both pre- and post-synaptically, and influences NT release. It also activates tryptophan hydroxylase (TPH), the rate-limiting enzyme in 5-HT synthesis (NELSON and CHIAVEGATTO 2001). CHEN *et al*. (1994) generated a knockout mutation of α-CaMKII and found that the mutants were deficient in their fear response and both offensive and defensive aggression. Mutants had reduced 5-HT release in the dorsal raphe, which would be expected to cause increased aggression. The investigators could not resolve this apparent contradiction.

As stated previously, DA and NE have permissive roles in aggressive behavior, in contrast to 5-HT (MICZEK and FISH 2006). Levels of 5-HT in the PFC decrease after initiation of an aggressive encounter, while DA levels there and in the nucleus accumbens increase in this time concurrently (MICZEK *et al.* 2002). A functional dopaminergic system in the mesocorticolimbal areas is necessary for the initiation and perpetuation of aggression. Evidence suggests that the D_2 receptor has an integral role in the permissive activity of DA; dopaminergic circuits in which this receptor is expressed show activation in response to aggression. Evidence also suggests that aberrant aggression results from disruption of the D_1 receptors (DE ALMEIDA *et al.* 2005) or the dopamine transporter (DAT) (RODRIGUIZ *et al.* 2004). The contribution to aggression of the DA- and NE-synthesizing enzyme tyrosine hydroxylase (TH) has been investigated as well. Inhibition of TH results in a decrease in aggression, which is thought to be primarily due to reduced levels of NE rather than DA (MICZEK *et al.* 2002). Furthermore, reduced aggression is observed in mice that cannot produce NE (MARINO *et al.* 2005).

Perturbations of the γ-aminobutyric acid (GABA) system reveal that this NT inhibits aggression at low or high doses, but intermediate levels enhance aggressive behavior (MICZEK *et al.* 2003; MICZEK *et al.* 2002). However, some evidence suggests that this mediation of agonistic behavior depends on the compound stimulating particular receptors: stimulation of $GABA_A$ by allopregnanolone increases aggression (MICZEK *et al.* 2003). The causal mechanism for this effect has not yet been identified. An alternative hypothesis is that the role of GABA is context-dependent: offensive

aggression appears to be more sensitive to stimulation by GABA, whereas GABA inhibits defensive aggression (MICZEK *et al.* 2002).

Mice have been used to investigate the role of nitric oxide (NO) in aggression. NO amplifies presynaptic NT release via retrograde diffusion (KANO *et al.* 1998). Stimulation of post-synaptic receptors, such as those involved in glutamatergic or GABAergic neurotransmission, triggers activation of NO synthase (NOS) to synthesize NO for use in intensification of presynaptic glutamate or GABA release. KANO *et al.* examined the effects of knockouts of neuronal NOS (nNOS) and endothelial NOS (eNOS) on glutamate and GABA release in the cortex, striatum, and hippocampus of mice. Mice lacking functional nNOS showed significant decreases in cortical and striatal glutamatergic release, while $eNOS^{-/-}$ mice showed significant decreases in GABA release in the cortex and striatum.

Additional work has been performed to further characterize the role of NO in aggression. DEMAS *et al*. (1999) assayed $eNOS^{-/-}$ male mice and found nearly complete abolishment of aggressive behaviors. This is counterintuitive considering the implications of the results from work done by KANO *et al*., whereby eNOS was demonstrated to amplify inhibitory neurotransmission through its synthesis of NO. Given those results, the expectation would be that abolishment of eNOS would result in increased aggression.

Further confounding the role of NO in aggression are results from experiments with nNOS. Male $nNOS^{-/-}$ mice exhibit drastically increased levels of aggressive behavior (CHIAVEGATTO *et al.* 2001). While glutamate's effect on aggression has not

been directly investigated, it is generally excitatory rather than inhibitory (PURVES *et al.* 2001). As such, one would expect that nNOS knockouts, which were shown to have decreased glutamate release in the cortex and striatum, would be less aggressive than controls. However, this contradiction might be resolved by examining the indirect effects of the absence of functional nNOS on the serotonergic system: these mutants showed reduced turnover of 5-HT in the cortex, hypothalamus, and midbrain. This deficit could be rescued by treatment with the 5-HT precursor, and the rebound of the serotonergic system coincides with a reversal of the aggressive phenotype. Serotonergic transmission in $nNOS^{-/-}$ mice was apparently compromised by alteration of $5\text{-}HT_{1A}$ and $5\text{-}HT_{1B}$ receptors. Agonists of both receptors were shown to reduce aggression, but $nNOS^{-/-}$ mice required significantly higher concentrations of the agonist to achieve this reduction. The combination of reduced function in 5-HT receptors and reduced turnover of 5-HT is likely the proximal cause of the increased aggression. How the lack of functional nNOS causes these deficits is unknown.

The impact of histamine on aggression has also been investigated. Histaminergic projections are involved in attention and arousal, and so are similar to those of NE (PURVES *et al.* 2001). Aggressive behavior is significantly decreased in mice lacking histamine H_1 receptors (YANAI *et al.* 1998), but not to the extent observed in $eNOS^{-/-}$ mice. In contrast to $nNOS^{-/-}$ mice, $H_1^{-/-}$ mice had significantly increased 5-HT turnover in the cortex and hippocampus. While a mechanism for the aberrant 5-HT turnover due to lack of the H_1 receptor has not been proposed, the behavioral results are in agreement with what would be expected in mice with augmented 5-HT transmission.

Extensive interconnections exist among different brain regions involved in aggression. Research on golden hamsters has revealed that the anterior hypothalamus and regions of the limbic system are activated at the onset of aggression (DELVILLE *et al.* 2000). Also implicated are regions of the amygdala, which is involved in fear (DAVIS and SHI 2000); the bed nucleus of the stria terminalis; and the central gray region. All of these areas have been hypothesized to be involved in some component of emotionality. In hamsters exhibiting differential levels of aggression, DAVID *et al.* (2004) observed higher levels of neural activity in the anterior hypothalamus of high-aggression animals. Reciprocal projections exist between the anterior hypothalamus and the amygdala, among other candidate regions for involvement in regulation of aggressive behavior. The NT(s) utilized by these neurons was not determined. Further elucidation of these networks is warranted.

Crustacean Models

A substantial amount of research on aggressive behavior has been performed in lobsters and crayfish. These animals are excellent for direct experimentation due to their modular, well-mapped neural systems; defined behavioral neural circuits; easily manipulated and monitored neuronal amine levels; and quantifiable aggressive behaviors (KRAVITZ 2000; KRAVITZ and HUBER 2003). However, as little research has been devoted to the genetics underlying these neural systems, that topic will not be addressed here.

Lobsters and crayfish exhibit stereotyped patterns of agonistic interactions in the establishment and maintenance of dominance hierarchies. Size discrimination is one

factor in determination of dominance, but animals that are relatively close in size must employ other measures. The intensity of aggression increases over the duration of the encounter, beginning with an approach with claws extended and escalating to lashing of antennae, ejection of urine, tussling, and offensive tail flips. A decision of dominance can be reached at any point during the fight, resulting in the retreat of the subordinate animal (EDWARDS *et al.* 2003).

The general trends observed in the neurobiology of crustacean aggression contrast with those observed in vertebrate systems. As in vertebrates and other systems, the biogenic amine systems have been repeatedly implicated as having a crucial role in aggression. In particular, 5-HT and octopamine (OA, the invertebrate analogue of NE) have been extensively investigated. The main disparity between most crustaceans and vertebrate systems is the inverse relationship between these amines and aggression: 5-HT injections into the relevant crustacean neural circuits confers the postures and behaviors typified by dominant individuals, i.e. increased aggression; OA injections trigger behaviors usually observed in subordinate animals (HUBER *et al.* 1997).

Evidence indicates that once an individual's position in the dominance hierarchy has been established through agonistic behaviors, the modulation of serotonergic neurons is altered. In dominants, 5-HT will enhance communication between sensory input and the lateral giant neurons. The opposite trend is observed in subordinates (HUBER *et al.* 1997; KRAVITZ 2000). The lateral giant neuron is integral in tail-flip-mediated retreats and possibly in offensive tail flips during an attack (KRAVITZ 2000). Experimental manipulations of 5-HT levels can reverse behaviors that have been previously established

by the hierarchy. Established subordinates can be injected with 5-HT and paired with a larger animal and will engage in aggressive interactions rather than retreating (HUBER *et al.* 1997). Such changes are ephemeral but significant.

The behavioral effects of 5-HT injection can be reversed by acute treatment with fluoxetine, a 5-HT reuptake inhibitor (KRAVITZ 2000); however, prolonged treatment yields the same results as the initial infusions, i.e., an unwillingness of the subordinate animal to retreat. Pharmacological depletion of 5-HT yields behavioral phenotypes that are not significantly different than controls, suggesting a possible compensatory mechanism that could be acting at any level of neural control – synthesis, metabolism, or release (PANKSEPP *et al.* 2003).

Lobsters have 120 serotonergic neurons, located primarily among the ganglia of the nerve cord (KRAVITZ 2000). The A1-5HT cells are of particular interest: they receive inhibitory input from GABAergic neurons and excitatory input from the lateral and medial giant neurons. It is hypothesized that 5-HT signal transduction between the lateral giant and A1-5HT is facilitated in dominants due to the involvement of the lateral giant in aggressive behavior (EDWARDS and KRAVITZ 1997).

Crustacean NT systems occasionally exhibit unpredictable neural and behavioral plasticity. One crayfish species exhibits postures not exemplified by dominants or subordinates during agonistic encounters when injected with 5-HT, and these injections actually decreased aggression levels during such encounters. Likewise, some experimental observations indicate that 5-HT depletion can increase aggression levels. The NT release/exposure patterns and endocrinological context is also important in

determining behavioral response. In crayfish, the rate of 5-HT application impacts which receptors are activated, thereby influencing signal transduction. Extremely high concentrations of 5-HT can actually inhibit lateral giant response, rather than enhance communication between the lateral giant and A1-5HT neuron (EDWARDS *et al.* 2003). Such results suggest that a narrow window of serotonergic function, or a fine balance between opposing neural inputs, is necessary to maintain normal behavioral patterns (KRAVITZ and HUBER 2003).

Drosophila Models

Recently, an effort has been made to establish Drosophila as a model system for the study of aggression. Although widely heralded as an excellent system for genetic research, the utility of the fruit fly for dissecting neurobiological processes has been largely unnoticed. The movement to exploit genetic manipulations as part of the elucidation of such processes has helped move Drosophila aggression research closer to the forefront of the field.

Research conducted by HOFFMANN (1987, 1988) and HOFFMANN and CACOYIANNI (1989) focused on the role of aggression in establishing and defending territory, and consequently, access to mates and food. Like crustaceans, fruit flies exhibit characteristic aggressive behaviors: charging, tussling, chasing, kicking, and a wing threat have been described (CHEN *et al.* 2002; DOW and VON SCHILCHER 1975; JACOBS 1960). As is true in many other species, females exhibit low levels of aggression relative to males (NILSEN *et al.* 2004). Patterns of aggressive interactions become detectable in

lengthy assays (CHEN *et al.* 2002), suggesting a complex underlying neurobiology of the behavior that unfolds as encounters proceed.

The biogenic amine systems in fruit flies have been well-characterized (MONASTIRIOTI 1999), facilitating research in this realm. The amine NT's 5-HT, DA, and OA have been the primary foci in aggression studies. The genetic tools at the disposal of Drosophila researchers make selective manipulation of gene expression in these NT pathways practical. Such an approach might help define the window within which "normal" behaviors are observed, and reveal how subtle perturbations of neural systems affect aggression.

Although research on the roles of different NT's in Drosophila aggression is still in relative infancy, preliminary results indicate that OA and DA affect aggression levels. Flies that do not produce OA exhibit virtually no aggression relative to controls (BAIER *et al.* 2002), which contrasts with the effects of OA observed in crustaceans. HOYER *et al*. (2007) specifically found that the absence of OA nearly abolished the lunging behavior that is frequently observed in males. Relative to flies with low levels of DA, those with high levels are less likely to be involved in an encounter, but more likely to respond aggressively should they be approached. Both NT's are utilized in the mushroom bodies of the brain, as evidenced by high expression of OA and DA receptors there.

Flies in which neuronal output from the mushroom bodies has been blocked exhibit behavioral phenotypes nearly identical to those of OA-deficient flies. Aberrant mushroom body morphology has also been observed in hyper-aggressive Drosophila with mutations in the *neuralized* gene (ROLLMANN *et al.* 2008). The mushroom bodies are

known to be involved in learning and memory in the fly, and are considered the site of sensory integration (WADDELL and QUINN 2001). There were no significant changes in aggression in flies with altered levels of 5-HT (BAIER *et al.* 2002). However, those results contrast with a later experiment demonstrating that pharmacologically or genetically increasing 5-HT levels corresponded to increased aggression levels (DIERICK and GREENSPAN 2007). Conversely, silencing the neuropeptide F neural circuitry increased aggression, indicating an opposing role of the invertebrate homolog to neuropeptide Y (DIERICK and GREENSPAN 2007). The implications of these results need to be clarified by more extensive testing and additional experimental approaches.

The *fruitless* gene has been associated with aggression levels as well. This gene has many alternative transcripts, some of which are sex-specific; these transcripts are denoted fru^{M} (male) and fru^{F} (female) and their protein products are responsible for downstream cascades specifying male or female development. Using transgenic lines to specify which sex-specific gene product was expressed, CHAN and KRAVITZ (2007) were able to implicate four subsets of neurons as being especially relevant to the effect of FRU on gender-specific aggression: *fru*-aSP2, located in the anterior superior protocerebrum; *fru*-mAL, over the medial antennal lobe; *fru*-mcAL, anterior to the antennal lobe; and *fru*-PrMs, located between the pro- and mesothoracic ganglia. A complete picture of how these neurons affect aggression has yet to be determined.

Human Neurobiology

Human aggressive behavior is generally studied only at pathological levels. Some individuals express high levels of aggression as part of a behavioral disorder, such as

borderline personality disorder (GOODMAN and NEW 2000), conduct disorder (FRICK and DICKENS 2006), or intermittent explosive disorder (OLVERA 2002). However, inappropriate aggression is often independent of a psychiatric diagnosis, and might be triggered by environmental or social conditions such as alcohol consumption or inordinate levels of stress. Information on the neurobiological causes of aggression gleaned from experimental animals such as mice can be used to identify similar neural states in human case studies. Association studies, which attempt to correlate behavioral phenotypes with genotypes or endophenotypes, are also employed in an effort to explain the neural mechanisms of aggression. Finally, the effects of pharmacological treatments can be used to ascertain the function of many gene products or neural pathways.

A number of brain regions have been implicated in modulating aggressive behavior in humans. A general theme is that hyperactivation of the limbic system, which regulates emotion, is often observed in highly impulsively aggressive and violent individuals; in particular, the amygdala exhibits increased activation in response to negative stimuli (HERPERTZ *et al.* 2001). In contrast, some research suggests that individuals who exhibit instrumental, or goal-directed, aggressive behavior, actually have reduced amygdala responsiveness (BLAIR 2004; KIEHL *et al.* 2001). This lack of affect is suggestive of a different type of psychopathology. Furthermore, the inhibitory function of cortical output is often reduced in aggressive individuals. As classically exemplified by the Phineas Gage case, damage to the frontal cortex can result in irritability, aggression, and poor social judgment (DAMASIO *et al.* 1994). The combination of limbic hyperactivity and reduced cortical inhibition seems to create a potentially dangerous

situation in which stimuli are reacted to in an inappropriate manner; namely, aggressive or violent responses that would normally be suppressed by cortical control. More specific dissections of how "normal" neural activation can be perturbed are discussed below.

Monoamine neurotransmitters are thought to be involved in both other-directed and self-directed forms of aggression and impulsivity (PLACIDI *et al.* 2001). In general, 5-HT activity in the CNS is negatively correlated with aggression and impulsive behavior (MANUCK *et al.* 2000). Abnormalities in the 5-HT system have been implicated in both self- and other-directed aggression (HEN 1996). These abnormalities can take a variety of forms and be present in various brain regions. The ventromedial PFC appears to be the most affected brain region. Damage to this region results in deficits in decision-making skills, cognitive inhibitions, and behavioral deficits (BECHARA 2004; NEW *et al.* 2004; SHALLICE and BURGESS 1996). Neurons in the PFC activated by anger send inhibitory projections to the amygdala, resulting in inhibition of impulsive responses that arise there (DAVIDSON *et al.* 2000). This is logical given the hypothesized, and to some extent empirically verified, role of the PFC in behavioral control and comprehension of consequences. Also of interest are the dorsal and medial raphe nuclei, which innervate the PFC via serotonergic neurons (KAMALI *et al.* 2001); and the temporal lobe (ITO *et al.* 2007; TONKONOGY and GELLER 1992).

The serotonergic activity of a healthy ventromedial PFC inhibits aggressive and impulsive behavior. Low levels of 5-hydroxyindoleacetic acid (5-HIAA, a metabolite of 5-HT) and a blunted response to the fenfluramine challenge are indicative of dysfunction

of the serotonergic system. Low cerebrospinal fluid levels of 5-HIAA have been correlated with violent and impulsive suicide attempts and impulsive aggression (PLACIDI *et al.* 2001; SPREUX-VAROQUAUX *et al.* 2001).

Perturbation of MAOA activity has also been implicated in aggressive behavior due to the impact this enzyme has on the 5-HT, DA, and NE systems. Some male members of a Dutch family lack functional MAOA and exhibit borderline mental retardation and extreme levels of aggression (BRUNNER *et al.* 1993). This enzyme will be discussed in further detail below, but its effect on such a range of integral neurotransmitters has far-reaching consequences.

The NE system is hypothesized to have a facilitative effect on impulsive behavior in the PFC such that it is antagonistic to 5-HT. This effect appears to be dose-dependent; extremely high or low levels of NE inhibit aggression (HALLER *et al.* 1998). MANN (2003) notes that studies have shown increased NE levels and lower binding of α-adrenergic receptors in the PFC in suicidal patients. These results are indicative of NE hyperactivity in this region of the brain, which logically correlates with disinhibition of aggressive and impulsive behaviors. α_1-adrenoceptors and β-adrenoceptors are post-synaptic; α_2-adrenoceptors are both pre- and post-synaptic; all appear to modulate aggression, which attests to the complexity of the feedback systems in place for NE (HALLER *et al.* 1998). Hyperactivity of the dopaminergic system is also observed in aggressive/impulsive patients (RUJESCU *et al.* 2003). In summary, a decrease in serotonergic transmission or an increase in the activity of DA or NE can all adversely affect aggression, suicidal behavior, and suicide lethality (OQUENDO and MANN 2000).

Genetics of Aggression

Rodent Models

Aggression was an early topic of study in mouse behavioral genetics (SCOTT 1942). Initial studies suggested that major effect loci existed, but did not rule out the possibility of aggression having a more complex genetic basis (KESSLER *et al.* 1977). Again, genes involved in biogenic amine systems are often the topic of study; since this system has been extensively addressed above, it will not be directly reiterated here. The ability to conduct genetic experiments in mice facilitates unbiased approaches, enabling researchers to pursue avenues besides those that are already well established.

YOUNG *et al*. (2002) identified a mutation in a gene they named *fierce*, which encodes a nuclear receptor, and which results in extremely high levels of aggression in mice. The mutation also results in stunted growth and aberrant brain development. Specifically, the olfactory bulb and cerebrum were disproportionately smaller in mutants, even controlling for the reduction in overall body size. Little is known about how or why this mutation influences aggression; however, it is clear that the effects are dependent on the genetic background, which supports the assertion that epistatic interactions influence behavioral phenotypes.

Mice lacking the neural cell adhesion molecule also exhibit increased aggression (STORK *et al.* 1997). Knock-outs ($NCAM^{-/-}$) attacked sooner and far more frequently in the resident-intruder aggression assay; heterozygotes showed intermediate phenotypes. These mice also had increased levels of corticosterone, which is often considered an indicator of stress response. The authors suggest that mice with NCAM mutations might

be more sensitive to social stress: such a gene-environment interaction (GEI) would not be unusual for a behavioral trait. The developmental role of NCAM likely also contributes to the mutant phenotypes.

The cytokine interleukin-6, which has been demonstrated to impact brain function and development, the hypothalamic-pituitary-adrenal axis, and some behaviors (BESEDOVSKY and DEL REY 1996; LYSON and MCCANN 1991; ROTHWELL *et al.* 1996), has also been implicated in affecting aggressive behavior. Mutants with decreased expression of the IL-6 gene were more aggressive than controls, while mice over-expressing IL-6 demonstrated a tendency toward affiliative behaviors (ALLEVA *et al.* 1998). Interestingly, dopamine turnover seems to be increased in the hypothalamus of mice lacking IL-6. Here too, the authors note that the genetic background of the mice was relevant to the behavioral phenotype.

Cathepsin E is another immune system related gene in which mutations result in aberrant aggression. In this case, the change in behavior was dependent on rearing environment: socially housed mice behaved normally, while individually housed mice were more aggressive than controls in a territorial challenge assay (SHIGEMATSU *et al.* 2008). The spatial and temporal expression patterns of *CatE* suggest that it functions in pathophysiological processes in the CNS, although this has not been specifically addressed. Results from SHIGEMATSU and colleagues found that substance P signaling is enhanced in the amygdala and hypothalamus of $CatE^{-/-}$ mice, suggesting that the increased aggression in mutants is due in part to increased stress sensitivity.

A similar study by IBI *et al*. (2008) also investigated the effect of solitary rearing on aggressive behavior, and found that isolated mice were more aggressive than group housed mice. They found that the genes *Nurr1* and *Npas4* were expressed at significantly reduced levels in the dentate gyrus of the hippocampus in socially isolated mice. *Nurr1* is required for survival and differentiation of dopaminergic precursor neurons (SAUCEDO-CARDENAS *et al.* 1998), and *Npas4* regulates patterning and function of the limbic system (MOSER *et al.* 2004). The behavioral abnormalities in these mutants could be partially to completely rescued with Fluoxetine, a selective-serotonin reuptake inhibitor, unsurprisingly suggesting that the 5-HT system influences the aggressive phenotype.

Drosophila Models

Although there has long been evidence of aggressive behavior in Drosophila, it has only been since the late 1990's that the fly was embraced as a model for studying the genetics underlying this trait. As discussed previously, the biogenic amine system is relatively conserved between flies and mammals (MONASTIRIOTI 1999), and has been of interest to Drosophila researchers. However, the experimental opportunities in Drosophila genetics are wide-ranging, and researchers should not limit themselves to the dissection of *a priori* candidate genes.

The first publication addressing a genetic factor contributing to aggressive behavior in flies examined the effects of β-alanine on the trait. JACOBS (1978) observed *black* mutants, which have decreased β-alanine synthesis, and found that their levels of aggression were reduced relative to wild-type flies; in contrast, *ebony* mutants were more

aggressive than wild-type flies, and have increased levels of β-alanine. BAIER *et al*. (2002) were able to replicate these results.

The *fruitless* gene is responsible for many components of the sex-determination hierarchy in Drosophila, and has long been of interest in the study of courtship or mating behavior. VILLELLA *et al*. (1997) observed unusually high frequency head-to-head encounters when investigating the courtship behavior of *fruitless* mutant males. LEE and HALL (2000) followed up on and confirmed this observation years later, and further noted that the behavior differed among distinct mutant lines, with some lines appearing to be more sensitive to social factors. These mutations have been demonstrated to manifest in the abdominal muscles (ANAND *et al.* 2001; ITO *et al.* 1996; OHSHIMA *et al.* 1997; RYNER *et al.* 1996; TAYLOR and KNITTEL 1995) and result in aberrant (reduced) levels of serotonin in the abdominal ganglion (LEE and HALL 2001). More recently, elegant transgenic approaches have been utilized to demonstrate that by directing expression of the male- or female-specific transcript of *fruitless*, flies exhibit aggressive behavior typical of the sex specified by the transcript (CHAN and KRAVITZ 2007; VRONTOU *et al.* 2006). In combination with the neural structures relevant to *fruitless* expression and aggression (CHAN and KRAVITZ 2007), this avenue of research is quite intriguing. However, it is important to remember that *fruitless* is a sex-determination pathway gene, and its effect on aggressive behavior might be indirect; that is, it might only influence aggression insomuch as aggression is a sexually dimorphic trait. Furthermore, it does not

have any apparent human homologues, and so might not be especially informative of human aggressive behavior.

Two research groups have utilized whole genome expression profiling on lines of flies artificially selected for divergent levels of aggression. One (EDWARDS *et al.* 2006) is part of the current research project and will be discussed in Chapter 2. The other (DIERICK and GREENSPAN 2006) found that approximately 80 genes were differentially expressed between unselected lines and those selected for increased aggression, specifically, for rapid escalation during an aggressive encounter. Genes involved in calcium signaling, muscle contraction, cuticle formation and metabolism were frequently expressed at higher levels in the aggressive lines. Furthermore, subsequent analysis confirmed that flies carrying a mutation in a cytochrome P450 gene, *Cyp6a20*, exhibited increased aggression. This class of genes is involved in electron carrier activity, iron ion binding, and monooxygenase activity (WILSON *et al.* 2008). That many genes were found to be differentially regulated, and that those genes fell into a broad range of ontological processes, supports the notion that the genetics underlying aggressive behavior is quite complex: the phenotype is influenced by many genes, which are likely to be pleiotropic and interact with one another.

Human Genetics

Experimental approaches in human genetics are obviously limited relative to model systems. In light of this fact, much of what is known about genes affecting aggressive behavior in humans has been determined by candidate gene approaches, which focus heavily on the aforementioned biogenic amine systems. Recent work has also

made an effort to incorporate the considerable impact of interactions between one's genetics and environment on behavior.

The somewhat serendipitous discovery of a Dutch family in which affected males exhibited aggressive or violent behavior (BRUNNER *et al.* 1993) proved very informative for research on the genetics of aggression. Further assessment of these males revealed a genetic mutation that ultimately mapped to the aforementioned MAOA gene, which is located on the X chromosome in humans, and which resulted in perturbed monoamine metabolism. These men effectively lacked the gene product altogether, since their only copy was non-functional. This is a dramatic example of how perturbation of the monoamine system can affect aggression, and we must consider the probability that abnormal development played a role in the phenotypes of these men (HEN 1996).

Subsequent work has taken into consideration common genetic variation in MAOA, which might be more relevant to our understanding of its effect in the general population than the mutation. A variable number tandem repeat polymorphism in the promoter region of the gene appears to confer high or low levels of gene expression, and some evidence exists suggesting that the "high" activity allele has a protective effect against violent behavior. CASPI *et al*. (2002) identified a gene-by-environment interaction between childhood maltreatment and adult antisocial behavior; here, the effect of the risk-conferring "low" activity allele was not detectable in the absence of childhood abuse. Caveats to these results are that they might not be applicable to all populations, or might only be predictive in the absence of other environmental stressors (WIDOM and BRZUSTOWICZ 2006); furthermore, the effects of genotype and environment are not

necessarily dependent on one another (REIF *et al.* 2007). It is also worth noting that, as is often the case, results of such association studies are difficult to replicate or are even contradictory. For example, a study investigating potential associations between this polymorphism and aggression using a smaller sample size, and without considering environmental factors, found that men with the "low" activity allele were less inclined toward impulsivity and aggression (MANUCK *et al.* 2000).

Another polymorphism that appears to modulate aggressive behavior is in the gene encoding catechol-O-methyltransferase (COMT), which inactivates catecholamines such as dopamine and noradrenaline. A functional single nucleotide polymorphism (SNP) in this gene changes a valine to a methionine, and is associated with a three- to four-fold difference in activity of the enzyme. RUJESCU *et al.* (2003) found that the allele conferring low activity was over-represented in subjects attempting violent forms of suicide; in addition, results of self-report questionnaires indicate that subjects homozygous for that allele were more likely to direct their anger outwardly than were individuals homozygous for the high activity allele.

Another polymorphism that has been investigated for its association with aggression, and that is relevant to the dopaminergic system, is the dopamine receptor DRD4. FRESAN *et al*. (2007) found that, among schizophrenic patients, aggressive individuals carried a long allele of DRD4 significantly more frequently than did non-aggressive individuals. Evidence of GEI also exists for DRD4: NOBILE *et al*. (2007) found that adolescents of low socioeconomic status who carried the long allele were more aggressive than other children. Polymorphisms in another dopamine receptor, DRD2,

and the dopamine transporter (DAT1) have also been associated with pathological aggression by adolescents (CHEN *et al.* 2005). As noted above, it is believed that increased dopaminergic signaling has a permissive effect on aggression, but the results of association studies are not always consistent, and must be considered in the context of different populations and different environments.

The gene encoding the serotonin transporter (SERT) is of interest for many behavioral traits, aggression among them. The polymorphism receiving the most attention is in the promoter, and confers relatively low (the short allele) or high (the long allele) transcript abundance. Although not all results are consistent among studies, there does appear to be evidence that the short allele can be permissive toward aggressive behavior. Males carrying the short allele have been identified as more likely to behave aggressively and/or have conduct disorder (CADORET *et al.* 2003); HABERSTICK *et al*. (2006) and SAKAI *et al*. (2006) obtained similar results. However, SAKAI *et al*. (2007) were unable to replicate these findings in another sample. Polymorphisms in serotonin receptors have also been implicated in aggressive behavior. Multiple SNPs in the 5-HT_{2A} receptor have been associated with impulsive behavior and/or aggression (BRUCE *et al.* 2005; GIEGLING *et al.* 2006; NOMURA and NOMURA 2006), as has a SNP in the 5-HT_{1B} promoter (ARANGO *et al.* 2003; ZOUK *et al.* 2007).

As in mice, the neurotransmitter nitric oxide appears to regulate aggression in humans. Polymorphisms in two versions of NOS have been associated with increased aggression (RUJESCU *et al.* 2008). Genetic variation in tryptophan hydroxylase (MANUCK

et al. 1999) and genes involved in noradrenergic signaling, such as the $ADRA_{2A}$ receptor (COMINGS *et al.* 2000), have also been implicated in aggressive behavior.

While the work cited here is not exhaustive, it does provide an overview of the primary targets for those investigating the genetics of aggression in humans. It also underscores the fundamental value of model systems. It is easy to experience a sense of dissatisfaction with association studies in humans since they are frequently not replicated in other populations, and are fraught with caveats. However, there are some promising advancements in the approaches taken in these studies: for example, environmental factors are more now commonly included in statistical models, and multiple loci are genotyped to investigate potential epistatic interactions. In addition, the field is generally hopeful about the potential of genome-wide association studies (THE WELLCOME TRUST CASE CONTROL CONSORTIUM 2007), although initial results are perhaps less fruitful than was expected.

Despite the limitations of research on the genetics of human aggression, there is a general agreement between principles established in model systems and results of human studies. The biogenic amine systems are consistently implicated in the modulation of aggressive behavior, with additional modifiers affecting the phenotype as well. Results from model systems, including those presented here, indicate that genes outside the biogenic amine system are quite important, and these results should increasingly be incorporated in the design of human studies. Model systems continue to provide vast resources and experimental opportunities that might never be available in human research, and should therefore be exploited to their fullest potential.

Relevance of the Research Project

As discussed previously, aggressive behavior is observed throughout the animal kingdom, and has the potential to be selectively advantageous despite its energetic costs and the inherent risk of injury. However, aggression exacts a dramatic toll in human societies, both economically and in terms of mental and physical health. The phenotype is ultimately determined by genetics, neurobiology, and environmental factors, and is therefore quite complex. This research project addresses the genetic architecture of aggressive behavior, using *Drosophila melanogaster* as a model.

The present work takes advantage of a variety of the resources available in Drosophila research. One well-established method for identifying genes contributing to variation in complex traits is quantitative trait locus (QTL) mapping. We used introgression lines constructed from parental lines whose levels of aggression differed significantly, and for which we had determined what regions of the genome derived from each parent. These introgression lines were behaviorally assessed to identify broad QTL. By implementing deficiency complementation mapping (PASYUKOVA *et al.* 2000), we dramatically reduced the region of interest. We then conducted quantitative complementation tests using mutants in positional candidate genes (DE LUCA *et al.* 2003; FANARA *et al.* 2002; HARBISON *et al.* 2004; MOEHRING and MACKAY 2004). If segregating variation exists in those genes between the mapping populations, we can detect it through a statistical test revealing failure to complement.

Another approach to identifying genes affecting aggression is to screen a panel of mutants. We capitalized on the genomic resources available in Drosophila, behaviorally

assessing a number of *P*-element mutants in a co-isogenic background (HARBISON and SEHGAL 2008; SAMBANDAN *et al.* 2006). After identifying mutants with extreme levels of aggression, we examined a subset in closer detail to better understand how they affect aggressive behavior in Drosophila. This was accomplished through precise gene expression measurements, examination of brain morphology, and analysis of gene ontologies.

Finally, we combined a classical approach with a more technologically recent advance to identify candidate genes. By applying artificial selection to a genetically heterogeneous base population, we produced lines of flies that are divergent in their levels of aggressive behavior. After 25 generations, segregating alleles will have had sufficient time to respond to the selective pressure, and spontaneous mutations affecting aggression can also contribute to the selection response (ANHOLT and MACKAY 2004). We then subjected these lines to whole genome transcriptional expression profiling. Genes whose expression levels correlate with response to selection are potentially causally affecting aggressive behavior, or are co-regulated by such genes. Gene ontology analysis provides unbiased information about the biological processes and functions relevant to aggressive behavior. We also assessed the efficacy of transcript profiling by confirming candidate genes by testing mutants in these genes.

A fundamental advantage to the broad, genomic approaches available in *Drosophila melanogaster* is that our experimental designs are essentially unbiased. This allows us to identify genes, pathways, and processes that have previously not been examined for their potential effects on manifestation of or variation in aggressive

behavior, rather than relying on *a priori* candidate genes and assumptions. Furthermore, by employing a variety of approaches, we are able to address the effects of mutations, segregating genetic variation, and variation in gene expression on aggression. The implementation of several experimental designs in this research project enables us to accomplish these goals and further our understanding of the genomic architecture of aggressive behavior and apply our findings to other systems.

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CHAPTER TWO

Quantitative Genomics of Aggressive Behavior in *Drosophila melanogaster*

Quantitative Genomics of Aggressive Behavior in *Drosophila melanogaster*

Alexis C. Edwards, Stephanie M. Rollmann, Theodore J. Morgan, and Trudy F.C. Mackay

Department of Genetics and W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina, 27695

Reference:
Edwards AC, Rollmann SM, Morgan TJ, Mackay TF. Quantitative genomics of aggressive behavior in *Drosophila melanogaster*. PLoS Genet. 2006 Sep 15;2(9):e154. Epub 2006 Aug 2.

OPEN ACCESS Freely available online PLoS GENETICS

Quantitative Genomics of Aggressive Behavior in *Drosophila melanogaster*

Alexis C. Edwards, Stephanie M. Rollmann, Theodore J. Morgan, Trudy F. C. Mackay*

Department of Genetics and W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina, United States of America

Aggressive behavior is important for animal survival and reproduction, and excessive aggression is an enormous social and economic burden for human society. Although the role of biogenic amines in modulating aggressive behavior is well characterized, other genetic mechanisms affecting this complex behavior remain elusive. Here, we developed an assay to rapidly quantify aggressive behavior in *Drosophila melanogaster,* and generated replicate selection lines with divergent levels of aggression. The realized heritability of aggressive behavior was approximately 0.10, and the phenotypic response to selection specifically affected aggression. We used whole-genome expression analysis to identify 1,539 probe sets with different expression levels between the selection lines when pooled across replicates, at a false discovery rate of 0.001. We quantified the aggressive behavior of 19 mutations in candidate genes that were generated in a common co-isogenic background, and identified 15 novel genes affecting aggressive behavior. Expression profiling of genetically divergent lines is an effective strategy for identifying genes affecting complex traits.

Citation: Edwards AC, Rollmann SM, Morgan TJ, Mackay TFC (2006) Quantitative genomics of aggressive behavior in *Drosophila melanogaster*. PLoS Genet 2(9): e154. DOI: 10.1371/journal.pgen.0020154

Introduction

Animal aggression is a near-universal survival trait. Aggressive behavior is important for acquisition and defense of food, mates, and progeny; predator avoidance and defense; and, in some animals, the establishment and maintenance of stable social hierarchies. Understanding the genetic, neurobiological, and environmental bases of aggressive behavior is of great importance to human health and society, as this could lead to more effective treatments for the increased levels of aggression observed among patients suffering from many behavioral disorders. Aggressive behavior is a complex, quantitative trait, with population variation attributable to multiple interacting loci with individually small effects, whose expression is contingent on the social and physical environment.

Other than the well-characterized role of biogenic amines in modulating aggressive behavior, little is known of the genetic architecture of this complex trait. In vertebrates, low levels of serotonin (5-hydroxytryptophan [5-HT]) and its metabolites (5-hydroxyindoleacetic acid [5-HIAA]) are associated with increased levels of aggression and impulsivity [1]. Male mice in which the 5-HT_{1B} receptor gene has been ablated are more aggressive than wild type [2]; and mice with a non-functioning 5-HT_{1A} receptor gene are more anxious and less reactive than wild type [3]. These effects are mimicked by pharmacological treatments: increasing levels of 5-HT using 5-HT precursors, 5-HT reuptake inhibitors, and 5-HT_{1A} and 5-HT_{1B} receptor agonists decrease aggressive behavior in rodents [4–8]. Polymorphisms in tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis, affect aggressive disposition in humans [9,10]. 5-HT also mediates aggressive behavior in lobsters and crayfish, but the effects of the serotonergic system in invertebrates are opposite to vertebrates. Crustaceans injected with 5-HT exhibit increased aggression and are less likely to retreat from an aggressive encounter, whereas high levels of octopamine, the invertebrate counterpart of noradrenaline, are associated with subordinate status [11–14].

Monoamine oxidase A (MAOA) oxidizes and degrades 5-HT and dopamine. Inhibition of MAOA activity in mice leads to decreased aggression [15], consistent with increased levels of 5-HT. An allele of MAOA that leads to reduced enzyme activity has been associated with an increase in violent behavior in humans, but only if the individual was abused as a child [16]. In contrast, MAOA deficiency in humans [17] and mice [18] leads to increased aggression, despite the resulting increase in 5-HT levels. Other neurotransmitters and neuromodulators associated with aggressive behavior include nitric oxide (NO) [19,20]; dopamine [21], γ-aminobutyric acid [22], and androgens and estrogens [1].

The role of bioamines is an important focus for studies on aggression, but our current knowledge represents only "the tip of the iceberg" of the complex genetic architecture that subserves aggressive behavior. Hints of this underlying complexity come from studies showing that mice with null mutations in the neural cell adhesion molecule [23], interleukin 6 [24], and the *Nr2e1* nuclear receptor gene [25] are all more aggressive than wild-type litter mates. Further understanding of the genetic basis of aggressive behavior will be greatly facilitated by studies in a genetic model system, such as *Drosophila melanogaster*. The *Drosophila* biogenic amines have been well characterized [26], and homologous mecha-

Editor: Wayne N. Frankel, The Jackson Laboratory, United States of America

Received June 9, 2006; **Accepted** August 1, 2006; **Published** September 15, 2006

A previous version of this article appeared as an Early Online Release on August 1, 2006 (DOI: 10.1371/journal.pgen.0020154.eor).

DOI: 10.1371/journal.pgen.0020154

Abbreviations: ANOVA, analysis of variance; C, control; GO, gene ontology; H, high aggression; L, low aggression; MAOA, monoamine oxidase A; SE, standard error; 5-HT, 5-hydroxytryptophan

* To whom correspondence should be addressed. E-mail: trudy_mackay@ncsu.edu

Synopsis

Aggressive behavior is a complex trait affected by numerous interacting genes whose expression depends on the environment. Aggression can be selectively advantageous in the pursuit of mates, territory, or food; however, excessive aggression may be deleterious. Pathological levels of aggression in humans create an enormous burden to society. Although dysfunction of the biogenic amine systems is often associated with alterations in aggressive behavior, this represents only the "tip of the iceberg" of the complex genetic architecture of aggressive behavior. The fruit fly *Drosophila melanogaster* is an excellent model genetic system for exploring the genetic basis of aggressive behavior. The authors have developed a rapid assay to quantify *Drosophila* aggression, and have used it to select genetically divergent replicate lines for increased and decreased behavior from a genetically heterogeneous base population. They used whole-genome expression profiling to identify variation in gene expression among these lines, and identified 1,539 transcripts that differed between the selection lines, illustrating the complex genomic basis of aggressive behavior. The authors evaluated aggressive behavior of flies with mutations in 19 genes that were implicated by the analysis of differential transcript abundance, and identified 15 novel candidate genes affecting this complex trait, eight of which have human orthologs.

nisms may operate in flies and vertebrates, including humans. *Drosophila* males exhibit aggressive behaviors in defense of territory and females [27–31]. Female territorial aggressive behavior has also been quantified [32]. There is substantial naturally occurring genetic variation for levels of aggression, as demonstrated by divergence in behavior among geographical populations [33] and rapid response to artificial selection for increased aggression [34–36]. However, surprisingly little is known about the genes regulating aggressive behavior in *Drosophila*, and none of the loci contributing to naturally occurring variation in aggression have been mapped. However, a handful of genes have mutational effects on aggressive behavior. Mutations in *fruitless* and *dissatisfaction*, two genes involved in the sex determination hierarchy, are associated with increased levels of inter-male aggression [30]. In addition, β-alanine, which can be conjugated to bioamine neurotransmitters, has also been implicated in *Drosophila* aggression. *ebony* mutants have elevated levels of β-alanine and are more aggressive than wild type, whereas *black* mutants have reduced levels of β-alanine and are less aggressive than wild type [28,37]. The neurotransmitters octopamine and dopamine also modulate aggressive behavior in *Drosophila* [37], although the role of serotonin has not been documented.

A major impediment for using *Drosophila* to study the genetic networks underpinning aggressive behavior, and variation in aggressive behavior, has been the lack of a high-throughput assay to quantify this behavior. Most previous studies confounded territorial behavior with mating behavior, and relied on analysis of long-term video recordings to quantify aggressive encounters [31,32]. We developed a rapid and highly reproducible assay to quantify aggressive behavior, and used it to generate replicate lines selected for divergent levels of aggression. We used whole-genome expression analysis to identify candidate genes with different expression levels among the selection lines, an approach that has been fruitful in identifying candidate genes for other behavioral traits [38–40]. Subsequent functional tests of aggressive behavior in lines containing mutations in these candidate genes revealed several novel genes affecting aggressive behavior in *Drosophila*.

Results

Direct Phenotypic Response to Selection for Aggressive Behavior

We developed an assay to rapidly measure aggressive behavior of individual *Drosophila*. Briefly, we deprived animals of food for a short period, and then allowed them to compete for and defend a limited food resource. We quantified the aggressive behavior of the focal individual as the total number of aggressive encounters [31] in a 2-min period. We derived a heterogeneous base population from isofemale lines derived from a single natural population, and used artificial selection to create genetically divergent replicate lines with high (H) and low (L) levels of male aggression (Figure 1A). From generation 25–28, the H and L replicate lines diverged by 4.1 aggressive encounters in a 2-min interval, or 3.3 phenotypic standard deviation units.

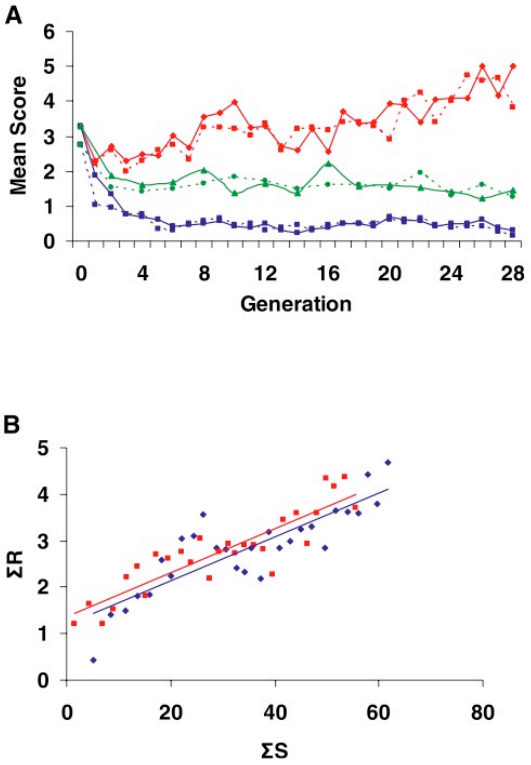

Figure 1. Phenotypic Response to Selection for Aggressive Behavior

(A) Mean aggression score of selection lines. Squares (■) indicate L lines; triangles (▲) indicate C lines; diamonds (♦) indicate H lines; solid lines indicate Replicate 1; and dashed lines indicate Replicate 2.

(B) Regressions of cumulative response on cumulative selection differential for divergence between high and low selection lines. Diamonds (♦) and blue line indicate Replicate 1; squares (■) and red line indicate Replicate 2.

DOI: 10.1371/journal.pgen.0020154.g001

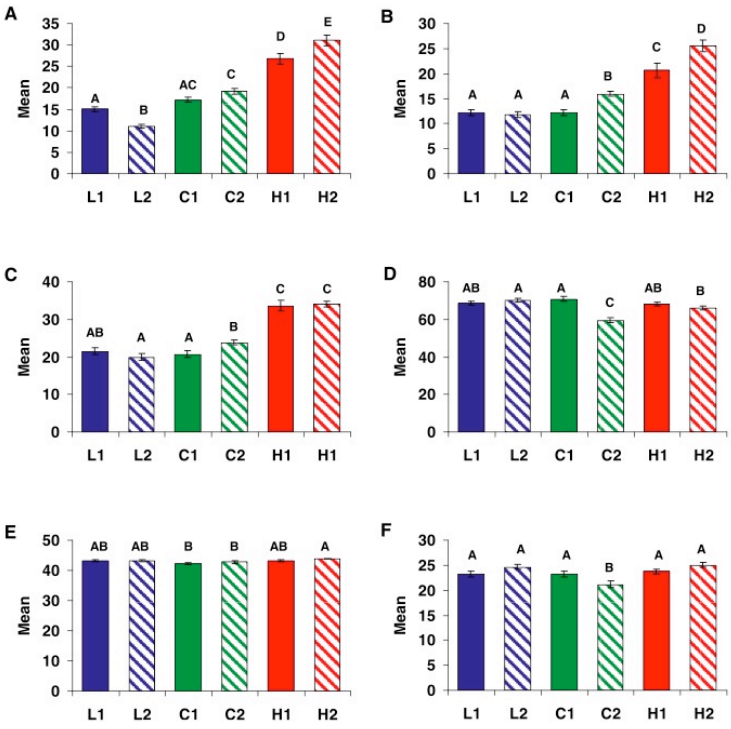

Figure 2. Correlated Phenotypic Responses to Selection

All scores are pooled across three successive generations. Lines with the same letter are not significantly different from one another at $p < 0.05$.
(A) Male aggression in eight-fly assay.
(B) Female aggression in eight-fly assay, after a 90-min food deprivation period.
(C) Female aggression in eight-fly assay, after a 120-min food deprivation period.
(D) Starvation stress resistance.
(E) Locomotor reactivity.
(F) Climbing behavior.
DOI: 10.1371/journal.pgen.0020154.g002

We estimated realized heritability ($h^2 \pm$ standard error [SE] of the regression coefficient) of aggressive behavior from the regressions of cumulated response on cumulated selection differential [41]. Heritability estimates from the divergence between H and L lines over 28 generations were $h^2 = 0.094 \pm 0.0057$ ($p < 0.0001$) for Replicate 1 and $h^2 = 0.095 \pm 0.0048$ ($p < 0.0001$) for Replicate 2 (Figure 1B). The selection response was symmetrical. Estimates of realized heritability were $h^2 = 0.098 \pm 0.0070$ ($p < 0.0001$) and $h^2 = 0.107 \pm 0.0061$ ($p < 0.0001$) for H Replicates 1 and 2, respectively; and $h^2 = 0.092 \pm 0.0458$ ($p = 0.0018$) and $h^2 = 0.058 \pm 0.0074$ ($p = 0.0006$) for L Replicates 1 and 2, respectively. There was little inbreeding depression for aggressive behavior: the regression of aggressive behavior in the control (C) lines over 28 generations was $b = -0.017 \pm 0.0097$ ($p = 0.10$) and $b = -0.033 \pm 0.0125$ ($p = 0.02$) for C1 and C2, respectively.

Correlated Phenotypic Response to Selection for Aggressive Behavior

Drosophila females are typically less aggressive than males [32]. We assessed whether female aggressive behavior changed as a correlated response to selection for divergence in male aggression. Here we used a multiple-fly assay for aggression for three consecutive generations. As expected, there was a significant difference in male aggressive behavior between the selection lines when assessed using this assay ($F_{2,3} = 19.16$, $p = 0.02$, Figure 2A). There was a marginally significant correlated response in female aggressive behavior when females were deprived of food for 90 min ($F_{2,3} = 11.21$, $p = 0.0405$, Figure 2B). However, the correlated response in female aggression was more pronounced after a 2-h deprivation period ($F_{2,3} = 52.81$, $p = 0.0046$, Figure 2C), although the selection group by time interaction term was not significant ($F_{2,3} = 1.01$, $p = 0.46$).

Since we assessed aggressive behavior after a period of starvation, it was important to determine whether the differences in aggressive behavior between the selection lines were not a reflection of underlying differences in sensitivity to starvation stress. There were no significant differences in starvation resistance among the selection lines ($F_{2,3} = 0.41$, $p = 0.70$, Figure 2D). In addition, it was possible that the differences in aggressive behavior were attributable to differences in general locomotion. There were no differences in locomotor behavior among the selection lines using an

assay for locomotor reactivity ($F_{2,3} = 5.47$, $p = 0.10$, Figure 2E) and a climbing assay ($F_{2,3} = 1.97$, $p = 0.28$, Figure 2F). Neither were there significant differences among the lines selected for divergent aggressive behavior for mating behavior ($F_{2,3} = 1.56$, $p = 0.34$), cold tolerance ($F_{2,3} = 0.30$, $p = 0.76$), ethanol tolerance ($F_{2,3} = 0.67$, $p = 0.58$), or longevity ($F_{2,3} = 0.29$, $p = 0.7662$) (Figure S1). Thus, the response to selection seems specific for aggressive behavior.

Transcriptional Response to Selection for Aggressive Behavior

We assessed transcript abundance in the H, L, and C selection lines using Affymetrix high-density oligonucleotide whole-genome microarrays, for flies of the same age and physiological state as selected individuals. We performed these analyses using whole bodies, rather than heads alone, as we wanted to include categories of genes potentially affecting aggressive behavior with more general expression (e.g., genes affecting metabolism and muscle function). Raw expression data are given in Table S1. We used factorial analysis of variance (ANOVA) to quantify statistically significant differences in transcript level for each probe set on the array. Using a false discovery rate [42] of $Q < 0.001$, we found 9,485 probe sets were significant for the main effect of sex, 1,593 were significant for the main effect of line, and 69 were significant for the line × sex interaction (Table S2). All 69 probe sets that were significant for the interaction term were also significant for the main effect of line.

We used ANOVA contrast statements on the 1,593 probe sets with differences in transcript abundance between selection lines to detect probe sets that were consistently up- or down-regulated in replicate lines [40]. We found 1,539 probe sets (8.2%) that differed between the selection lines when pooled across replicates (Table S3). Most (1,480) of these probe sets were significant in both sexes, consistent with divergence in aggression levels in both sexes. We found 1,116 probe sets that were divergent between H and C, 1,062 probe sets that were divergent between L and C, and 1,083 probe sets that were divergent between H and L. Although there was a widespread transcriptional response to selection for aggressive behavior, the magnitude of the changes of transcript abundance was not great, with the vast majority much less than 2-fold (Figure 3).

We identified 12 transcripts that exhibited sexually antagonistic expression (Table S4). Of these, four differed between the H and L selection lines (*sarcoplasmic calcium-binding protein* was up-regulated in H females and down-regulated in H males; *Transferrin 1*, *CG8093*, and *CG3239* were up-regulated in H males and down-regulated in H females). *Esterase-10* and *CG4199* had sexually antagonistic effects between the H and C groups (both up-regulated in H females and down-regulated in H males). Finally, six transcripts demonstrated antagonism between the C and L groups (*CG11523* was up-regulated in L males and down-regulated in L females; *twin of eyeless*, *CG15825*, *CG8093*, *CG7598*, and *CG4199* were up-regulated in L females but down-regulated in L males). These probe sets do not share obvious molecular functions or biological processes.

Since we selected for divergence in male aggressive behavior, we tested whether there was a disproportionate contribution of X-linked genes. We assessed whether the 1,539 differentially regulated probe sets were randomly distributed across the five major chromosome arms using a χ^2 test [40]. Indeed, the distribution of probe sets was not random ($\chi_4^2 = 19.66$, $p = 0.0006$). However, the deviation from random expectation was attributable to fewer, not more, probe sets than expected on the X chromosome ($\chi_1^2 = 13.31$, $p = 0.0003$), but not the other chromosome arms (Figure S2). The paucity of probe sets on the X chromosome was due to probe sets that were differentially expressed between males in the contrasts between H and L ($\chi_1^2 = 16.52$, $p = 0.00005$), H and C ($\chi_1^2 = 14.53$, $p = 0.00014$), and L and C ($\chi_1^2 = 13.49$, $p = 0.00024$).

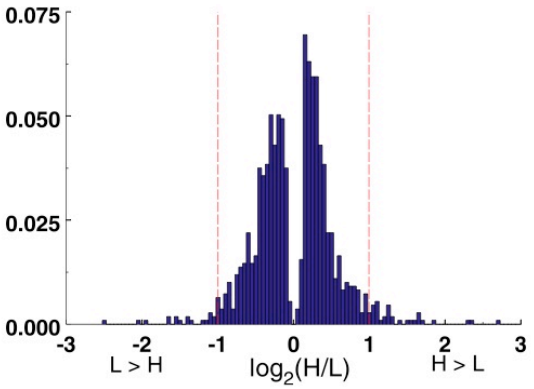

Figure 3. Histogram Showing Frequency of Relative Fold-Change in Probe Sets with Significant Differences in Transcript Abundance between H and L Selection Lines, Pooled over Sexes

The vertical red lines demarcate 2-fold changes in transcript abundance.

DOI: 10.1371/journal.pgen.0020154.g003

The probe sets with altered transcript abundance between the selection lines fell into all major biological process and molecular function gene ontology (GO) categories (Tables S5 and S6). We used χ^2 tests to determine which categories were represented more or less frequently than expected by chance, based on representation on the microarray. One interpretation of these analyses is that over-represented GO categories contain probe sets for which transcript abundance has responded to artificial selection, whereas under-represented GO categories contain probe sets for which transcript abundance is under stabilizing natural selection [40]. Highlights of the transcriptional response to artificial selection for aggressive behavior are given in Table 1. For example, the H lines are enriched for up-regulated genes affecting metabolism, response to biotic stimulus, and stress response; whereas the L lines are enriched for up-regulated genes affecting learning and memory and defense response. Probe sets in the biological process categories of morphogenesis and system development are consistently under-represented among up-regulated transcripts in the H and C lines.

Table 2 gives a sample of candidate genes affecting aggressive behavior that are up-regulated in the high or low selection lines, and which have proven functions in other processes. All are novel candidate genes for aggressive behavior. Conspicuously missing from this list are genes that have been previously implicated in *Drosophila* aggressive behavior *(fruitless, dissatisfaction, ebony,* and *black)* as well as

Table 1. Differentially Represented Biological Process GO Categories

H ≠ L		H ≠ C		C ≠ L	
H > L	**L > H**	**H > C**	**C > H**	**C > L**	**L > C**
Response to chemical stimulus 8.30×10^{-7}	**Oenocyte differentiation 9.99×10^{-5}**	**Response to chemical stimulus 5.92×10^{-4}**	**Nitrogen compound metabolism 3.03×10^{-10}**	**Nitrogen compound metabolism 3.36×10^{-10}**	**Response to oxidative stress 9.13×10^{-3}**
Response to biotic stimulus 4.86×10^{-3}	**Neuron differentiation 2.89×10^{-3}**	**Response to biotic stimulus 1.09×10^{-5}**	**Response to oxidative stress 3.04×10^{-3}**	**Catabolism 3.40×10^{-6}**	**Response to hormone stimulus 2.44×10^{-2}**
Secondary metabolism 2.59×10^{-6}	**Ectoderm development 8.42×10^{-5}**	**Response to stress 4.92×10^{-2}**	**Muscle contraction 3.82×10^{-3}**	**Feeding behavior 3.14×10^{-3}**	**Defense response 1.93×10^{-5}**
Regulation of metabolism 2.74×10^{-7}	**Response to oxidative stress 3.45×10^{-2}**	*Morphogenesis 6.73×10^{-4}*	**Feeding behavior 4.01×10^{-3}**	*Cell organization and biogenesis 3.58×10^{-5}*	**Response to external biotic stimulus 2.12×10^{-3}**
Neurophysiological process 3.16×10^{-4}	**Learning and/or memory 1.17×10^{-3}**	*Segmentation 9.76×10^{-9}*	**Embryonic development 1.81×10^{-3}**	*Regulation of physiological process 2.10×10^{-7}*	**Response to chemical stimulus 1.05×10^{-2}**
Cell communication 1.51×10^{-5}	*Locomotion 5.06×10^{-3}*	**Secondary metabolism 3.46×10^{-2}**	**Exocrine system development 5.78×10^{-3}**	*Cel lcommunication 1.24×10^{-4}*	**Autophagy 1.97×10^{-2}**

Bold and italic fonts indicate over- and under-represented categories, respectively, in contrasts (sexes pooled) of H, L, and C lines. *p*-Values are not corrected for multiple tests.
DOI: 10.1371/journal.pgen.0020154.t001

Table 2. Pleiotropic Candidate Genes Affecting Aggressive Behavior

Biological Process	Gene	Contrast	Fold-Change
Circadian Rhythm	*minibrain*[a]	L > H ♂	1.31
	PAR-domain protein1[a]	L > H	1.89
Learning and/or Memory	*Adh transcription factor 1*	H > L	1.78
	downstream of receptor kinase[a]	L > H	1.18
	derailed	L > H	1.27
	no extended memory[a]	L > H	1.36
	pastrel[a]	L > H	1.48
	schnurri[a]	L > H	2.18
Courtship Behavior	*doublesex*[a]	L > H	1.33
	Esterase-6	H > L	1.15
	yellow	L > H	1.78
	Btk family kinase at 29A	L > H ♂	1.57
Neurotransmitter Secretion/Transport	*CG31106*[a]	L > H	1.21
	Syntaxin 5	L > H	1.10
	Calcineurin B	L > H	1.30
Response to Stress	*p38b*	H > L	1.06
	CG7182	H > L	1.21
	methuselah-like 4	L > H ♀	1.26
	Lethal(2) tumorous imaginal discs[a]	H > L	1.22
Nervous System Development	*trachealess*	L > H	1.68
	POU domain protein 2	L > H	1.71
	barren	H > L	1.22
	couch potato[a]	L > H	1.27
	twin of eyeless	L > H	1.24
	CG31352	L > H	1.34
	neuralized	L > H ♂	1.71
	heartless	L > H	1.62
	LIM-kinase 1	H > L	1.31

Contrasts are for H, L and C lines; pooled over sexes unless otherwise specified. The ♀ symbol indicates female, and ♂ indicates male.
[a]Transcript abundance also altered in lines selected for fast and slow copulation latency [40].
DOI: 10.1371/journal.pgen.0020154.t002

Table 3. Functional Tests of Candidate Genes

Line	Gene	Mean Aggression Score (SE)	$F_{1,58}$	*p*-Value	Human Ortholog
Canton S B	Control	25.2 (0.37)	N/A	N/A	N/A
BG01014	*CG12292*	14.9 (0.94)	121.25	<0.0001	*non-imprinted in Prader-Willi/Angelman syndrome 1*
BG01127	*muscleblind*	32.8 (1.06)	62.65	<0.0001	*muscleblind-like 1*, isoform b
BG01245	*CG17154*	46.8 (1.69)	374.84	<0.0001	N/A
BG01491	*tramtrack*	20.1 (1.39)	24.23	<0.0001	*kelch-like 12 variant*
BG01510	*CG1623*	9.6 (2.02)	165.57	<0.0001	N/A
BG01720	*frizzled*	23.8 (0.65)	2.48	0.12	*frizzled 7*
BG01736	*CG5966*	33.4 (1.36)	63.67	<0.0001	*Pancreatic triacylglycerol lipase precursor*
BG02098	*CG30015*	37.3 (1.21)	148.63	<0.0001	N/A
BG02104	*CG13512*	10.2 (1.90)	162.41	<0.0001	N/A
BG02117	*SP71*	19.4 (0.69)	42.08	<0.0001	N/A
BG02248	*Btk family kinase at 29A*	27.2 (1.08)	4.30	0.04	*Tec protein tyrosine kinase*
BG02389	*couch potato*	26.0 (0.92)	0.74	0.39	*RNA-binding protein with multiple splicing 2*
BG02498	*Darkener of apricot*	34.4 (1.51)	74.33	<0.0001	*CDC-like kinase 2*, isoform 1
BG02501	*longitudinals lacking*	3.8 (0.49)	605.44	<0.0001	KLHL3 protein
BG02690	*CG14478*	45.5 (1.20)	419.76	<0.0001	N/A
BG02753	*scribbler*	14.7 (0.73)	135.90	<0.0001	KIAA1281 protein
BG00524	*Male-specific RNA 87F*	13.0 (0.58)	192.35	<0.0001	N/A
BG00668	*arginase*	25.2 (0.61)	0.00	1.00	*nonhepatic arginase*
BG02867	*kismet*	16.9 (1.22)	69.53	<0.0001	KIAA1416 protein

Bonferroni significance threshold = 0.0026. Human orthologs have BLAST scores of $E<10^{-10}$.
N/A, not applicable.
DOI: 10.1371/journal.pgen.0020154.t003

genes affecting bioamines (e.g., genes encoding tyrosine and tyramine hydroxylases; *Dopa decarboxylase;* and dopamine, serotonin, and octopamine; and associated transporters and receptors). Indeed, the only gene affecting bioamine levels that is differentially expressed between the selection lines is *Catecholamines up* (Table S3).

Functional Tests of Candidate Genes

To assess the extent to which transcript profiling of divergent selection lines accurately predicts genes that directly affect the selected trait, we evaluated the aggressive behavior of lines containing *P*-element insertional mutations in 19 candidate genes that were implicated by the analysis of differential transcript abundance. All of the *P*-element insertions were derived in a common isogenic background, and are viable and fertile as homozygotes [43,44]. The *P*-elements are inserted in, or immediately adjacent to, each candidate gene. The candidate genes are involved in diverse biological processes, including electron transport *(Male-specific RNA 87F)*, catabolism *(arginase)*, nervous system development *(longitudinals lacking, tramtrack,* and *muscleblind)*, and G-protein coupled receptor signaling *(frizzled)*. Seven of the mutations are in computationally predicted genes *(CG1623, CG5966, CG12292, CG13512, CG14478, CG17154,* and *CG30015)*. Remarkably, 15 of the mutations exhibited significant differences in aggressive behavior from the co-isogenic control line, after Bonferroni correction for multiple tests (Table 3, Figure 4). Mutations in *muscleblind, CG17154, CG5966, CG30015, Darkener of apricot,* and *CG14478* had higher levels of aggression than the control, and mutations in *CG12292, tramtrack, CG1623, CG13512, SP71, longitudinals lacking, scribbler, Male-specific RNA 87F,* and *kismet* were less aggressive than the control. None of these genes have been previously implicated to affect aggressive behavior.

Discussion

Phenotypic Response to Selection for Aggressive Behavior

Drosophila melanogaster exhibits a robust response to artificial selection for high and low levels of aggressive behavior. The heritability of aggressive behavior is relatively low (~0.1). However, if we express the genetic and environmental variances of aggressive behavior as genetic and environmental coefficients of variation (CV_G and CV_E, respectively [45]), we find that $CV_G = 23.2$ and $CV_E = 71.9$. Thus, the low heritability is not due to a lack of segregating genetic variation, which is abundant, but to a high level of environmental variance, as is typical for behavioral traits [45]. Although the phenotypic response to artificial selection appears to be greater in the direction of increased levels of aggression, the genetic response to selection as inferred from

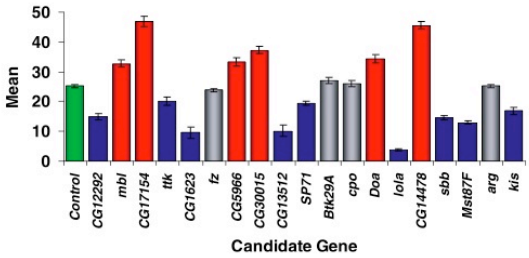

Figure 4. Mean Aggression Scores (±SE) of Lines Containing *P*-Element Insertional Mutations in Candidate Genes

The green bar denotes the Canton S B co-isogenic control line; grey bars indicate lines with scores not significantly different from the control; red bars indicate lines with significantly higher levels of aggression than the control; and blue bars indicate lines with significantly lower levels of aggression than the control.
DOI: 10.1371/journal.pgen.0020154.g004

realized heritability is symmetrical. The apparent discrepancy is attributable to the low phenotypic variance, and hence selection differential, in the L lines. The symmetrical selection response is consistent with natural selection for an intermediate optimum level of aggression, since fitness traits under directional natural selection typically exhibit asymmetrical responses to artificial selection, in the direction of reduced fitness [46]. This is consistent with the intuitive notion that both highly aggressive and very passive individuals would be at a selective disadvantage. Time spent in aggressive interactions cannot be at the expense of locating food and mates; further, aggressive behavior is energetically expensive, and must be limited accordingly.

The phenotypic response to selection appears to be specific for aggressive behavior. In particular, the differences in aggressive behavior among the selection lines are not due to general differences in activity or differential sensitivity to starvation stress. The lack of correlation with cold tolerance and longevity also indicates that the selection response is not directly related to differences in general stress response or physiological robustness. Neither do increased or decreased levels of aggression in the context of a limited food resource affect mating behavior, as measured by copulation latency. The latter observation is not necessarily at variance with previous reports that males with increased levels of territorial aggression appear to have a mating advantage [27,36]. The correlated response of copulation latency to selection for aggression is expected to be $i\, h_{CL}\, h_{AG}\, r_A\, \sigma_{PCL}$, where i is the selection intensity; h_{CL} and h_{AG} are the square roots of the heritabilities of copulation latency and aggression, respectively; r_A is the genetic correlation between the two traits; and σ_{PCL} is the phenotypic standard deviation of copulation latency [41]. From a previous study of response to selection for copulation latency from the same base population, we have estimates of $h^2_{CL} = 0.067$ and $\sigma_{PCL} = 18.7$, whereas this study gives estimates of $h^2_{AG} = 0.0945$ and $i = 0.951$. Thus, after 28 generations of selection for divergent aggressive behavior, we would expect a correlated response in copulation latency of $39.62 r_A$ min. We would only have the power to detect this correlated response if r_A was very high, but not if $r_A < 0.3$.

Transcriptional Response to Selection for Aggressive Behavior

We observe a profound transcriptional response to selection for aggressive behavior, with changes in expression of over 1,500 probe sets (~10% of the genome) between the selection lines, using a stringent false discovery rate of 0.001. Similarly, transcript abundance of over 3,700 probe sets evolved as a correlated response to artificial selection from the same base population for increased and decreased copulation latency [40]. This is in contrast to an analysis of transcriptional response to selection for geotaxis behavior [38] in which 5% of the genes assessed exhibited 2-fold or greater differences in expression between the selection lines. The discrepancy is likely attributable to the different methods for identifying differentially regulated transcripts. We find that transcripts exhibiting much less than 2-fold differences are often highly statistically significant.

We selected for divergence in male aggressive behavior, and therefore expected an increased selection response from X-linked genes affecting variation in male aggression. In contrast, at the level of transcript abundance, there were fewer than expected male-specific differences in expression between the selection lines for X-linked genes. Underrepresentation of genes that are up-regulated in males on the *Drosophila* X chromosome is apparently a general phenomenon [40,47,48]. X chromosome demasculinization is perhaps attributable to selection against genes that are advantageous in males but deleterious to females [47].

The large number of genes exhibiting parallel changes in transcript abundance among replicate selection lines implies that genes affecting complex behaviors are highly pleiotropic: if 10% of the genome affects any one trait, the same genes must affect multiple traits. Thus, genes affecting behavior are also likely to be involved in neurogenesis, metabolism, development, and general cellular processes, and many of the same genes may affect multiple behaviors. In a previous study, we observed a total of 3,727 probe sets that were differentially expressed between lines selected for increased and decreased copulation latency, from the same base population. A total of 878 probe sets with different expression levels between selection lines were common between lines selected for divergent mating behavior and aggression, which is significantly more overlap than expected by chance ($\chi^2 = 1{,}072.108$, $p < 0.0001$). For example, *Pigment dispersing factor* and *cryptochrome* were initially defined based on their involvement in circadian rhythm. Expression levels of these genes were up-regulated in lines selected for positive geotaxis, and confirmed to affect geotaxis behavior in functional tests [38]. *Pigment dispersing factor* and *cryptochrome* were also differentially expressed between the lines selected for increased and decreased mating speed, and here between lines selected for different levels of aggressive behavior (Table S3).

The dual observations of specific responses to artificial selection at the level of trait phenotype and large scale pleiotropy at the level of transcript abundance are not incongruent. Correlated responses to selection can only occur if the genetic correlation between the selected and the correlated trait is non-zero. Significant genetic correlations result from linkage and from net directional pleiotropic effects of genes affecting both traits [41]. We speculate therefore that pleiotropic genes affecting multiple complex traits may not be directional. Further, genetic correlations arise from the segregation of polymorphic alleles affecting both traits. The transcriptional response to selection is attributable to genes that have causally responded to selection, and to genes that are co-regulated by these genes. Since the transcriptional response to single mutations with subtle phenotypic effects can involve over 100 co-regulated genes [36], the number of selected loci causing the changes in transcript abundance between the selection lines could well be rather modest. It will be necessary to map the quantitative trait loci (QTLs) causing divergence between the selection lines in order to disentangle causal versus consequential transcriptional responses and correlated responses to selection.

Candidate Genes for Aggressive Behavior

Regardless of whether or not the observed changes in genes expression are causally associated with genetic divergence in aggression between the selection lines, the genes exhibiting altered expression levels are candidate genes affecting aggressive behavior. We tested for aggression levels of 19 mutations in candidate genes that were generated in a

common co-isogenic background, and identified 15 novel genes affecting aggressive behavior. Aggressive behavior is the first annotated biological function for seven of these mutations, which were in computationally predicted genes. *Male-specific RNA 87F* is involved in electron transporter activity and iron ion binding. The energetic costs of aggression are presumably high, and the mutant tested exhibited low aggression, suggesting that disruption of normal energetic processes adversely affects the ability to engage in costly behaviors. *muscleblind* encodes a protein with a zinc-finger domain involved in muscle development. Proper muscle development could directly affect the frequency and/or intensity of aggressive interactions, which can involve tussling or other elaborate postures. The remaining mutations with effects on aggressive behavior *(tramtrack, Darkener of apricot, longitudinals lacking,* and *scribbler)* were in genes affecting nervous system development. The expression of aggression requires integration of environmental and internal signals for effective behavioral output. Both afferent and efferent signaling can be perturbed by changes in neural activity or functioning; although the effects of these mutations on the neural circuitry involved in aggression remains to be elucidated. Further analyses are required to formally prove the involvement of these genes in aggression, including but not limited to generating multiple mutant alleles, assessment of temporal and spatial patterns of gene expression, transgene rescue, and evaluation of aggressive behavior in animals in which the genes are over-expressed or reduced.

The high success rate of these functional tests validates using expression profiling on genetically divergent lines in directed mutagenesis screens to identify genes affecting complex traits. This strategy is complementary to traditional strategies and cannot supplant them, since many key genes will not be detected as differentially expressed. Specifically, we will not detect genes that are differentially expressed at a different developmental period or if the magnitude of the difference in transcript abundance is too small to be detected; genes that affect protein abundance or activity; or genes affecting the trait that are not regulated at the level of transcription. Notably, we did not detect differences in transcript abundance between the selection lines for genes for which mutations are known to affect aggressive behavior. This observation highlights the difference between the complementary approaches of forward genetic screens and assessing natural variants for inferring the genetic architecture of complex behaviors. The former approach is invaluable for determining the full spectrum of genes affecting the manifestation of behavior, whereas the latter focuses on the subset of genes in which variants have survived the sieve of natural selection. Thus, mutations in genes that were previously determined to affect aggressive behavior may be too deleterious to remain segregating in nature.

Many of the genes with mutational effects on aggressive behavior are evolutionarily conserved and have human orthologs (Table 3). For example, *CG12292* is orthologous to *nonimprinted gene in prader-willi syndrome/angelman syndrome chromosome region 1*. Prader-Willi/Angelman syndrome is a developmental disorder in which many affected individuals exhibit dramatic behavioral phenotypes. It is thus possible that the genes and pathways affecting aggression in *Drosophila* will elucidate corresponding mechanisms in other organisms, including humans.

Materials and Methods

Drosophila stocks. Flies were reared on cornmeal/molasses/agar medium under standard culture conditions (25 °C, 12:12 h light/dark cycle). CO_2 was used as an anesthetic. Behavioral assays were conducted in a behavioral chamber (25 °C, 70% humidity) between 8 A.M. and 11 A.M.

Quantitative assay for individual aggressive behavior. Behavioral assays were performed on socially experienced, 3–7-d-old males. Flies were not exposed to anesthesia for at least 24 h prior to the assay. Aggression of single individuals was quantified by placing one experimental male, with wild-type eye color, with three reference white-eyed isogenic w^{1118} Canton S males. The flies were placed in a vial without food for 90 min, after which they were transferred (without anesthesia) to a test arena containing a droplet of food and allowed to acclimate for 2 min. After the acclimation period, the flies were observed for 2 min. The following behaviors were scored as aggressive encounters: kick—leg extension from one fly to another resulting in physical contact; chase; charge—rapid approach leading to head-to-head orientation; wing-raise—extension of wings in response to proximity/approach of another fly; and box—high impact interaction involving front legs of both flies [31]. The score of the experimental fly was the number of encounters in which it exhibited an aggressive behavior, including interactions initiated by the experimental fly and those in which he responded aggressively to a reference fly.

Artificial selection for aggressive behavior. The base population was generated from 60 isofemale lines established from flies collected in Raleigh, North Carolina, in 1999. The isofemale lines were crossed in a round robin design (Line 1 female × Line 2 male, Line 2 female × Line 3 male,...Line 60 female × Line 1 male). Single fertilized females from each cross were placed in each of two culture bottles. In the following generation (G0), the aggressive behavior of 50 virgin males of each replicate was scored using the single-fly assay. The 20 most aggressive males from each replicate were placed with 20 unselected virgin females in bottles to initiate the two H lines (H1 and H2); and the 20 least aggressive males from each replicate were placed with 20 unselected virgin females to initiate the two L lines (L1 and L2). The two C lines were initiated with 20 random, unselected males mated with 20 virgin females. In the following (G1) and all subsequent generations, the same procedure was repeated: 50 males from each line (H, L, and C) were scored, and the 20 highest-scoring males from the H lines and the 20 lowest-scoring males from the L lines were selected as parents for the next generation. The first 20 C line males scored were used as parents. C lines were scored every other generation.

Estimates of realized heritability (h^2) were calculated by regression of the cumulative selection response (ΣR) on the cumulative selection differential (ΣS) [41]. The coefficients of genetic (CV_G) and environmental (CV_E) variation were calculated as $CV_G = 100(\sqrt{V_G})/\bar{x}$ and $CV_E = 100(\sqrt{V_E})/\bar{x}$. V_G was estimated as h^2V_P, where V_P was the average phenotypic variance of the control lines in generations 1–10. V_E was estimated as $V_P - V_G$. The mean ($\bar{x}$) was estimated as the mean aggression score of the control lines from generations 1–10.

Correlated responses to selection. To assess the generality of the selection response, we also assessed male aggression levels in groups of eight 3–7-d-old flies of the same genotype. The aggression score for each replicate was the total number of aggressive interactions observed among all eight flies in the 2-min observation period. We also examined correlated responses in female aggression using the eight-fly assay.

We assessed female aggression after 90-min and 120-min starvation periods. These assays were performed on ten replicates per line per sex for each of three generations; males were assessed at generations 19–21; females were assessed at generations 20–22 (90-min food deprivation) and generations 23–35 (120-min food deprivation).

Starvation resistance was assessed as previously described [49]. Single-sex groups of ten 2-d-old flies were placed in vials containing non-nutritive media (1.5% agar and 5-ml water). Survival was scored every 8 h. This assay was conducted for generations 24–26, with five replicate measurements per line per sex per generation.

Locomotor behavior was assessed using two different assays. Locomotor reactivity was assessed as described previously [50]. A single 3–7-d-old fly was placed in a vial with approximately 3-ml standard medium, and subjected to gentle mechanical disturbance by tapping on the bench top. The vial was placed horizontally, and the total amount of time (in seconds) the fly remained mobile for the 45-s period immediately following the disturbance was the locomotor reactivity score of the individual. This assay was performed at generations 23–25, with 20 replicate measurements per line per sex

per generation. In the second assay, individual flies were transferred without anesthesia into an empty glass vial, with the height of the vial demarcated in 5-mm intervals from 0 to 27. The fly was tapped to the bottom of the vial, which was then placed vertically. The climbing score was the maximum height reached within the 10-s observation period. Twenty replicates per line per sex were tested at generations 24–46.

Chill-coma recovery was quantified as previously described [51]. Twenty-five 3–7-d-old flies were transferred without anesthesia into an empty vial and placed on ice for 3 h. The flies were then transferred to room temperature, and the recovery time was recorded as the length of time necessary for an individual to right itself and stand on its legs. The assay was performed at generations 26–28.

Ethanol sensitivity was measured using an inebriometer [52]. Briefly, approximately50–60 same-sex flies were aspirated into a glass column with mesh partitions, which was filled with saturated ethanol vapors. The flies lose postural control due to ethanol exposure and fall down the partitions to the bottom of the column where they were collected at 1-min intervals. The elution time was recorded as the measure of ethanol sensitivity. This assay was conducted for generations 24–26.

Longevity of mated males and females was quantified as previously described [53]. Three male and three female 2-d-old flies were placed in a vial containing approximately 3-ml standard culture medium, and scored for survival every other day until all were dead. Animals were transferred to fresh vials every 2 d. This assay was performed at generations 21–23, with ten replicate vials per line per generation.

Copulation latency was scored as previously described [40]. For each selection line, 20 pairs of 3–7-d-old virgin flies were aspirated into vials containing approximately 3-ml standard culture medium. The score recorded for a pair was the number of minutes from introduction to the vial until initiation of copulation. Reciprocal matings (females from the low selection group mated to males from the high selection group, and vice versa) were also performed. Assays were performed at generations 24–26.

Statistical analysis of correlated responses. Differences between the selection lines for the correlated traits were assessed using a nested mixed model ANOVA:

$$\begin{aligned} Y = \mu &+ Group + Line(Group) + Sex + Gen + Group \times Sex \\ &+ Group \times Gen + Line(Group) \times Sex + Line(Group) \times Gen \\ &+ Sex \times Gen + Group \times Sex \times Gen + Line(Group) \times Sex \times Gen + \varepsilon, \end{aligned} \quad (1)$$

where Y is the phenotypic score, μ is the overall mean, *Group* is the fixed effect of the selection treatment (H, C, or L), *Line*(*Group*) is the random effect of the replicate within each selection group, *Sex* is the fixed effect of sex, *Gen* is the fixed effect of generation, and ε is the error variance. The terms of most interest in the model are *Group*, *Line*(*Group*), *Group*×*Sex*, and *Line*(*Group*)×*Sex*. A significant *Group* term is indicative of a correlated response in the trait being tested to selection for aggressive behavior. The *Line*(*Group*) term reveals whether replicate lines responded similarly or divergently, giving an idea of the effects of random genetic drift within a replicate line. Interaction terms including the main effect of *Sex* are of interest in part because only males were directly subjected to selection.

Whole-genome expression profiling. At generation 25, two replicates of 12 3–7-d-old virgin males and females were collected from each selection line, and deprived of food for 90 min (i.e., the same age and physiological state as the flies prior to selection). Total RNA was extracted from the 24 samples (six lines × two sexes × two replicates) using the Trizol reagent (Gibco BRL, San Diego, California, United States). Biotinylated cRNA probes were hybridized to high-density oligonucleotide microarrays (Drosophila GeneChip 2.0; Affymetrix, Santa Clara, California, United States) and visualized with a streptavidin–phycoerythrin conjugate, as described in the Affymetrix GeneChip Expression Analysis Technical Manual (2000), using internal references for quantification. The quantitative estimate of expression of each probe set is the *Signal (Sig)* metric, as described in the Affymetrix Microarray Suite, version 5.0.

Microarray data analysis. The 18,800 probe sets on the Affymetrix Drosophila GeneChip 2.0 are represented by 14 perfect-match (PM) and 14 mismatch (MM) pairs. The *Sig* metric is computed using the weighted log(PM–MM) intensity for each probe set, and was scaled to a median intensity of 500. A detection call of Present, Absent, or Marginal is also reported for each probe set. We excluded probe sets with more than half of the samples called "Absent" from the analysis, leaving 11,666 probe sets. This filter retained sex-specific transcripts, but eliminated probe sets with very low and/or variable expression levels [40]. On the remaining probe sets, we conducted two-way fixed effect ANOVAs of the Signal metric, using the following model:

$$Y = \mu + Line + Sex + Line \times Sex + \varepsilon, \quad (2)$$

where *Sex* and *Line* are the fixed effects of sex and selection line, and ε is the variance between replicate arrays. We corrected the p-values computed in these ANOVAs for multiple tests using a stringent false discovery rate criterion [42] of $Q < 0.001$. We used contrast statements [40] to assess whether expression levels of probe sets with *Line* and/or *Line*×*Sex* terms at or below the $Q = 0.001$ threshold were significantly different between selection groups (H, C, and L) at the $p < 0.05$ level, both within each sex and pooled across sexes. GO categories were annotated using Affymetrix (http://www.affymetrix.com) and FlyBase (http://flybase.bio.indiana.edu) compilations.

Functional tests of mutations in candidate genes. We tested whether mutations in 19 of the candidate genes with altered transcript abundance between the selection lines affected aggressive behavior. The mutations were homozygous $P\{GT1\}$ elements inserted within the candidate genes, and all were generated in a common co-isogenic background (Canton S, B background [44]). Male aggressive behavior was assessed for all mutant lines using the eight-fly assay, with ten replicates per line, and for 50 replicates of the co-isogenic control line (Canton S, B background). We used t-tests to determine whether the aggressive behavior of the mutant lines differed significantly from the control.

Supporting Information

Figure S1. Correlated Phenotypic Responses to Selection

Found at DOI: 10.1371/journal.pgen.0020154.sg001 (32 KB DOC).

Figure S2. Genomic Distribution of Probe Sets

Found at DOI: 10.1371/journal.pgen.0020154.sg002 (40 KB DOC).

Table S1. Raw Microarray Data

Found at DOI: 10.1371/journal.pgen.0020154.st001 (4.8 MB ZIP).

Table S2. Probe Sets Differing Significantly by Selection Line Term

Found at DOI: 10.1371/journal.pgen.0020154.st002 (847 KB XLS).

Table S3. Probe Sets with Significant Differences in Contrast Statements

Found at DOI: 10.1371/journal.pgen.0020154.st003 (414 KB XLS).

Table S4. Sexually Antagonistic Probe Sets

Found at DOI: 10.1371/journal.pgen.0020154.st004 (19 KB XLS).

Table S5. Biological Process GO Categories

Found at DOI: 10.1371/journal.pgen.0020154.st005 (521 KB XLS).

Table S6. Molecular Function GO Categories

Found at DOI: 10.1371/journal.pgen.0020154.st006 (290 KB XLS).

Accession Numbers

Raw microarray data have been deposited in Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo) under accession number GSE5405.

Acknowledgments

We thank S. Heinsohn for help with the mating assays, R. Lyman for statistical advice, and M. J. Zanis for his Perl programming skills. This is a publication of the W. M. Keck Center for Behavioral Biology.

Author contributions. ACE and TFCM conceived and designed the experiments. ACE and SMR performed the experiments. ACE and TJM analyzed the data. ACE and TFCM wrote the paper.

Funding. This work was funded by grants from the National Institutes of Health (GM54146, GM58260, and MH074161), and from the University of North Carolina.

Competing interests. The authors have declared that no competing interests exist.

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CHAPTER THREE

Mutations in Many Genes Subtly Affect Aggressive Behavior in *Drosophila melanogaster*

Mutations in Many Genes Subtly Affect Aggressive Behavior in *Drosophila melanogaster*

Alexis C. Edwards[*,‡], Liesbeth Zwarts[§], Akihiko Yamamoto[†,‡], Patrick Callaerts[§], Trudy F.C. Mackay[*,‡]

[*]Department of Genetics, Campus Box 7614, North Carolina State University, Raleigh, NC, 27695-7614, United States of America. [†]Department of Zoology, Campus Box 7617, North Carolina State University, Raleigh, NC, 27695-7617, United States of America. [‡]W. M. Keck Center for Behavioral Biology, Campus Box 7617, North Carolina State University, Raleigh, NC 27695-7617, United States of America. [§]Laboratory of Developmental Genetics, VIB-PRJ8 & Katholieke Universiteit Leuven, Center for Human Genetics, Onderwijs & Navorsing, Herestraat 49, bus 602, B-3000 Leuven, Belgium.

This chapter consists of a manuscript that will be submitted for publication. I conducted behavioral assays on all lines. I verified sequences of revertant lines. I extracted RNA and conducted qRT-PCR. I conducted all data analyses. I wrote the manuscript. Liesbeth Zwarts conducted immunohistochemistry, in situ hybridizations, and brain morphometry. Akihiko Yamamoto maintained the *P*-element library and constructed

revertant lines. Trudy Mackay supervised the research project and provided extensive comments on the organization and content of the manuscript. All co-authors have provided critical review of the manuscript.

Running Head: Drosophila Aggression Mutants

Key Words: *P*-element mutations; mushroom bodies; aggressive behavior; qPCR

Corresponding Author:

Trudy F. C. Mackay
Campus Box 7614
North Carolina State University
Raleigh, NC 27695-7614
USA
Phone: 919-515-5810
Fax: 919-515-3355
E-mail: trudy_mackay@ncsu.edu

ABSTRACT

Drosophila melanogaster has recently been established as an excellent model organism for studying the genetic basis of aggressive behavior. Here, we present the results of a screen of 170 *P*-element insertional mutants for quantitative differences in aggressive behavior from their co-isogenic control line. Thirty-two of these mutants exhibited increased aggression, while 27 lines were less aggressive than the control. Gene ontology analysis reveals that these genes represent a range of processes, but are particularly enriched for development and metabolism. We characterized nine of these mutations in greater detail by assessing transcript levels throughout development, morphological changes in the mushroom bodies, and restoration of control levels of aggression in revertant alleles. This study reveals that many pleiotropic genes affect aggressive behavior, and substantiates the utility of *Drosophila melanogaster* in the investigation of analogous behaviors in humans.

INTRODUCTION

Aggressive behavior is important for survival and reproduction, and is near universal among animals. Aggression is used for self-defense against con-specifics and predators, in acquisition of territory, food and mates, and in defense of progeny. However, aggressive behaviors are energetically expensive, and there is likely an intermediate optimum level of aggression in natural populations from a balance between the energy and risk associated with territory defense and the need to find food and mates. In social organisms such as humans or other primates, an extremely high level of aggression can be disadvantageous or even pathological.

Aggressive behaviors are quantitative traits, with continuous variation in natural populations due to segregating alleles at multiple interacting loci, with effects that are sensitive to developmental and environmental conditions. Identifying the underlying genes and environmental contexts that affect aggressive behavior is necessary if we are to understand the evolutionary forces acting to maintain variation for aggressive behavior in natural populations, and to develop therapeutic interventions to modulate extreme levels of aggressive behavior in humans. Much of the work on the neurobiology and genetics of aggressive behavior to date has used the candidate gene approach to establish the role of neurotransmitters in mediating and modulating levels of aggression. In particular, biogenic amines and genes affecting their biosynthesis and metabolism have been associated with aggressive behavior in mammals (BRUNNER *et al.* 1993; HALLER *et al.* 1998; MANUCK *et al.* 1999; NELSON and CHIAVEGATTO 2001; HAN *et al.* 2004; DE

ALMEIDA *et al.* 2005; ALIA-KLEIN *et al.* 2008) and invertebrates (JACOBS 1978; EDWARDS and KRAVITZ 1997; HUBER *et al.* 1997; BAIER *et al.* 2002; CERTEL *et al.* 2007; DIERICK and GREENSPAN 2007; HOYER *et al.* 2008). The neurotransmitters nitric oxide and γ-aminobutyric acid also modulate aggressive behavior in mammals (MICZEK *et al.* 2003; NELSON *et al*. 1995; RUJESCU *et al.* 2008). Neuropeptide Y affects aggression in mammals (GAMMIE *et al.* 2007; KARL and HERZOG 2007; KARL *et al.* 2004) and its invertebrate homolog, neuropeptide F, affects aggression in Drosophila (DIERICK and GREENSPAN 2007). In Drosophila, correct expression of the male-specific transcript of *fruitless*, a gene in the sex-determination pathway, is required for executing male aggressive behaviors (CERTEL *et al.* 2007; CHAN and KRAVITZ 2007; LEE and HALL 2000; VRONTOU *et al.* 2006).

Drosophila exhibit territorial behavior in wild populations (HOFFMANN 1988; 1989), and therefore represents an excellent model system for investigating the genetic basis of aggressive behavior. Recent studies have revealed a much more complex genetic architecture of Drosophila aggression than suggested by targeted evaluation of candidate genes in biologically plausible pathways. Many novel loci affecting aggressive behavior have been implicated from widespread correlated responses in gene expression to selection for divergent levels of aggressive behavior (DIERICK and GREENSPAN 2006; EDWARDS *et al.* 2006). Subsequent evaluation of aggressive behavior of mutations in a sample of these candidate genes revealed that a large number indeed affected aggressive behavior, including mutations in a member of the cytochrome P450 gene family (DIERICK

and GREENSPAN 2006); and genes involved in electron transport, catabolism, nervous system development, G-protein coupled receptor signaling, as well as computationally predicted genes (EDWARDS *et al.* 2006; ROLLMANN *et al.* 2008). Analysis of quantitative trait loci (QTLs) affecting variation in aggression between two wild type strains also identified a complex genetic basis for natural variation in aggressive behavior, characterized by extensive epistasis among QTLs (EDWARDS and MACKAY 2008). Complementation tests to mutations at positional candidate genes in the QTL regions also revealed four additional novel loci affecting aggressive behavior (EDWARDS and MACKAY 2008). These results motivate a broader screen for mutations affecting Drosophila aggression.

Previously, we developed a highly reproducible and rapid assay to quantify levels of aggression in *D. melanogaster* males (EDWARDS *et al*. 2006). Here, we employed a modified version of this assay to screen 170 *P{GT1}* transposable element (*P*-element) mutant lines that were generated in the same co-isogenic background. All of these lines are viable and fertile as homozygote; therefore, the mutations are unlikely to be genetic null alleles. This is obviously an essential criterion for evaluating effects of mutations in essential genes on behavioral traits expressed in adults, and the quantitative assay enables detection of mutations with subtle as well as large effects. Further, the exact insertion site of the transposon, and thus the identity of the candidate gene(s) it disrupts, can be readily determined. The same panel of lines has been screened for mutations affecting numbers of sensory bristles (NORGA *et al.* 2003), resistance to starvation stress (HARBISON *et al.* 2004), sleep (HARBISON and SEGAL 2008) and olfactory (SAMBANDAN

et al. 2006) and locomotor (YAMAMOTO *et al.* 2008) behavior, enabling us to assess pleiotropic mutational effects. We identified 59 mutations in 57 genes that affect aggressive behavior, none of which had been previously implicated to affect aggression. While many of the genes affect the development and function of the nervous system, and are thus plausibly relevant to the execution of complex behaviors, others affect basic cellular and metabolic processes, or computationally predicted genes for which aggressive behavior is the first biological annotation. Most of the mutations had pleiotropic effects on other complex traits. More detailed characterization of nine of the mutations indicated that the *P*-element insertions affected the tagged genes, and that the mutations had pleiotropic effects on brain morphology.

MATERIALS AND METHODS

Drosophila stocks: Flies were reared on cornmeal/molasses/agar medium under standard culture conditions (25°C, 12:12 hour light/dark cycle). CO_2 was used as an anesthetic. All mutant lines are homozygous and contain single *P{GT1}* transposable element inserts in the w^{1118} *Canton-S B* co-isogenic background, and were constructed as part of the Berkeley Drosophila Gene Disruption Project (BELLEN *et al*. 2004). Male w^{1118} *Canton-S B* flies were used as the control line.

Quantitative assay for aggressive behavior: Assays were performed on socially experienced, 3-7 day-old male flies. Groups of eight males from the same mutant line were anesthetized 24 hours prior to the assay and placed in vials with food. On the day of the assay, the males were transferred without anesthesia to an empty vial and were

deprived of food for 90 minutes, after which they were exposed to a food droplet and given one minute to acclimate to this disturbance. The flies were then observed for an additional one minute, and the total number of aggressive encounters scored as described previously (EDWARDS *et al*. 2006). Behavioral assays were conducted in a behavioral chamber (25°C, 70% humidity) between 8 a.m. and 11 a.m.

The screen was conducted in 34 blocks of 1-7 mutant lines and the contemporaneous control, with 20 replicate vials for each mutant line and the control line per block. *P*-element insert lines with significantly different levels of aggression than the control were identified using a one-way fixed effect ANOVA model. Post-hoc Tukey HSD tests were used to determine whether aggression levels of mutant lines in a block differed significantly from that of the control after correcting for multiple tests. In addition, a one-way random effects ANOVA was performed on the entire data set, expressing the aggressive behavior of the mutant lines as deviations from their contemporaneous control. The among line (σ_L^2) and within line (σ_E^2) variance components were computed, and the broad sense mutational heritability estimated as $H_M^2 = \sigma_L^2/(\sigma_L^2 + \sigma_E^2)$.

Bioinformatics: Gene ontology categories among *P*-element insert lines associated increased or decreased levels of aggression were assessed using DAVID (DENNIS *et al*. 2003) and Babelomics (AL-SHAHROUR *et al*. 2006). Only gene ontology categories that applied to greater than five percent of the genes queried were considered

in these analyses. Human orthologues of the genes tagged by the *P*-elements were assessed using FlyAtlas (CHINTAPALLI *et al*. 2007).

Generation and verification of revertant lines: Genetic revertants were generated using standard crossing schemes, while preserving the co-isogenic background of the parental and revertant strains (HARBISON and SEHGAL, 2008). PCR products were sequenced to ascertain whether revertants were genetically precise. Primers were chosen to span either the 5' or 3' site of the original insertion. PCR products were run on 2% agarose gels and compared to a DNA ladder to determine whether they were of the appropriate length. Sequencing reactions were run on the PCR products, and the sequence of each *P*[-] line was compared to that of the control w^{1118} *Canton-S B* to determine whether the excision of the *P*-element was precise.

RNA extraction and cDNA generation: Samples were collected in triplicate from each of the following developmental stages: embryos aged 12-14 hours after egg laying (AEL); third instar larvae; pupae aged 8-9 days AEL; and male adults aged 3-5 days post-eclosion, with heads and bodies separated. Whole RNA was extracted using Trizol (GIBCO-BRL, Gaithersburg, MD) and purified using standard procedures. cDNA was generated using 500ng of whole RNA with reagents from Applied Biosystems (Foster City, CA).

Quantitative RT-PCR: Primers for qPCR were obtained from Sigma (St. Louis, MO) and targeted gene regions common to all transcripts. cDNA was diluted 1:6 for a concentration of 41.7 ng/μl, and 2μl cDNA were used for each 10μl qPCR reaction.

Each biological replicate was assessed in three technical replicates. An ABI-7900 sequence detector and protocols from Applied Biosystems were used to perform the quantitative RT-PCR with the SYBR Green detection method. Relative mRNA quantities were standardized using the housekeeping gene *Glyceraldehyde-3-phosphate dehydrogenase 1* (*Gapdh1*). Standardization was conducted on Ct values reported by the ABI-7900 software. Since Ct values are relative exponential measures, standardized values were converted to linearized values as described in LIVAK and SCHMITTGEN (2001) for statistical tests. Differences in gene expression level between the *P*-element insert line and the control line were tested for statistical significance using two-tailed Student's *t*-tests.

Whole-mount *in situ* hybridization: cDNA clones for *ed* (LD11008), *sgl* (SD09476), *emc* (LD10532), *pbx* (RE16319), *Syx4* (RE02884), *CG13377* (RE15974), *CG32572* (AT02481) and *CG3638* (LD20542) were ordered from the Berkeley Drosophila Genome Project. *Act5c* cDNA was obtained using the High Fidelity PCR System (Roche Applied Sciences) on *Canton-S* genomic DNA using the following primers: 5' ATGTGTGACGAAGAAGTTGCTG, 5' CACGTGGCGTTCACGAAGATT. The 1131bp fragment was cloned into the *pCR®II-TOPO* vector (Invitrogen). Digoxigenin-labeled sense and antisense RNA probes for were synthesized by *in vitro* transcription using the DIG RNA labeling kit (Roche Applied Science). The probes were hydrolyzed for X min at 60°C in buffer containing 200mM Na_2CO_3 and 200 mM $NaHCO_3$ ($X = (Lo - Ld)/(0.11 \times Lo \times Ld)$ with Lo = original length of the transcript in kb; Ld = desired 0.2 kb length), precipitated in ethanol and resuspended in RNase-free

water. Whole-mount *in situ* hybridization was performed using a variation of the protocol described by TAUTZ and PFEIFLE (1989). Signal detection was carried out using anti-digoxigenin-AP Fab fragments (Roche Applied Science). Color development was performed in the dark using 0.5 mg/ml NBT (Roche Applied Science) and 0.25 mg/ml BCIP (Roche Applied Science). Embryos were 0-17 h old. Images were obtained using a light microscope (model BX61; Olympus) and Cell^D 2.6 imaging software.

Immunohistochemistry and morphometric analysis: Immunohistochemical labeling of adult Drosophila brains with anti-fasciclin II MAb 1D4 (Developmental Studies Hybridoma Bank; under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA 52242) and morphometric analyses of mushroom body lobes and ellipsoid body were done as previously described (ROLLMAN *et al*. 2008). Measurements were taken for each hemisphere of 10 brains per line. Statistical significance was determined using *t*-tests for differences between mutant lines and the *Canton-S B* control line.

RESULTS AND DISCUSSION

Mutations affecting aggressive behavior: We quantified aggressive behavior of *P*-element insertional mutations that had been generated in a common isogenic background (*Canton S B*, BELLEN *et al*. 2004), as well as the control line (Supplementary Table 1). The 170 *P*-element lines represented insertions in 148 genes. Approximately one-half of these lines were chosen because they represent mutations in genes that exhibited changes in transcript abundance as a correlated response to artificial selection

for aggressive behavior (EDWARDS *et al*. 2006). Others were included if they had previously been shown to have a mutant phenotype for a different behavior, or if the *P*-element tagged a computationally predicted gene.

The broad sense mutational heritability (H_M^2) for aggressive behavior was rather high: $H_M^2 = 0.432$. The high mutational heritability could be due to a few mutants of large effect, or many mutants with smaller effects. Analysis of the effects of individual mutations revealed that the latter was the case. A total of 59 (~35%) of the *P*-element insert lines exhibited levels of aggression that differed significantly from the control; 27 lines were less aggressive, and 32 lines were more aggressive than the control line (Table 1, Fig.1). The absolute values of the standardized mutational effects (a/σ, where a is one-half of the difference in the mean aggression score of the *P*-element insert line and the control line, and σ is the phenotypic variance of the control line) of the 59 lines with significantly increased or decreased levels of aggression ranged from 0.28 – 2.27, with a mean of 0.77 (Table 1).

The high proportion of mutations associated with alterations in aggressive behavior is likely in part because the screen was enriched for mutations in candidate genes previously implicated to affect aggression (EDWARDS *et al*., 2006) and with mutations affecting other quantitative traits (NORGA *et al.* 2003; HARBISON *et al.* 2004; SAMBANDAN *et al.* 2006; HARBISON and SEHGAL, 2008, YAMAMOTO *et al.* 2008). However, the large mutational target size for aggressive behavior is consistent with a growing body of evidence that large numbers of loci can affect most quantitative traits

(ANHOLT *et al.* 1996; LYMAN *et al.* 1996; NORGA *et al.* 2003; HARBISON *et al.* 2004; MACKAY *et al*. 2005; EDWARDS *et al*. 2006; MOROZOVA *et al.* 2006; 2007; JORDAN *et al*. 2007; SAMBANDAN *et al.* 2006; HARBISON and SEHGAL, 2008, YAMAMOTO *et al.* 2008).

Gene ontology analysis: The genes tagged by the *P*-element inserts associated with increased or decreased levels of aggression spanned a variety of gene ontology categories (DENNIS *et al*. 2003; AL-SHAHROUR *et al*. 2006) (Fig. 2). Many of these genes affect early development, including the development of the nervous system, and are involved in transcriptional regulation, signal transduction, and ATP binding. There is a trend towards differential representation of some gene ontology categories between the lines associated with increased versus decreased levels of aggression (Fig. 2), although the differences are not significant due to the small numbers of mutations. For example, ~42% of the mutations with low levels of aggression are in genes affecting metabolism, but only ~26% of mutations with high levels of aggression fall into this category. A plausible interpretation is that dysfunction of metabolic processes can lead to a lower propensity to expend energy on demanding behaviors. Over 24% of mutations with low levels of aggression affect 'localization'; no mutations with high levels of aggression affect localization. The connection between this biological process and aggressive behavior is not intuitively obvious. Nearly all 59 genes tagged by *P*-elements that were associated with increased or decreased levels of aggression have orthologues that been implicated in human diseases or disorders (Supplementary Table 2), including susceptibility to schizophrenia, diabetes, deafness, and mental retardation (CHINTAPALLI *et al*. 2007).

Pleiotropic effects on other behaviors: Many of the *P*-element lines included in this screen have previously been examined for mutational effects on numbers of sensory bristles (NORGA *et al.* 2003), resistance to starvation stress (HARBISON *et al*. 2004), olfactory behavior (SAMBANDAN *et al*. 2006), 24-hour sleep (HARBISON and SEHGAL 2008) and locomotor reactivity (a startle response, YAMAMOTO *et al*. 2008). Mutational correlations (r_M) between aggressive behavior and male abdominal bristle number (n = 160, r_M = 0.09, p = 0.293), male sternopleural bristle number (n = 160, r_M = 0.11, p = 0.183), olfactory avoidance behavior (n = 158, r_M = 0.01, p = 0.94), starvation resistance (n = 88, r_M = 0.09, p = 0.40), and 24-hour sleep (n = 28, r_M = 0.20, p = 0.30) were not significantly different from zero. Mutational correlations could be non-significant if mutations specifically affect aggressive behavior, or if there are pleiotropic effects of mutations affecting aggression on other traits, but the effects are not in the same direction. It is the second explanation which is true – the mutations affecting aggressive behavior are highly pleiotropic, but mutations associated with increases (decreases) in aggressive behavior are not consistently associated with increases (decreases) of resistance to bristle number, starvation stress, olfactory behavior or sleep (Table 1).

The mutational correlation between aggressive behavior and locomotor reactivity, although weak, was significantly different from zero and positive (n = 157, r_M = 0.29, p = 0.0002; Fig. 2). The mutational correlation for the subset of 58 lines with significantly increased and decreased levels of aggression and for which locomotor reactivity data was available was not significantly different from that estimated from all 157 lines (r_M = 0.40,

$p = 0.0019$). Overall, variation in locomotor reactivity only explains 8% of the variation in aggressive behavior. This is largely attributable to a few insert lines with decreased levels of both aggression and locomotor reactivity. However, many lines with increased levels of aggression had reduced locomotor reactivity.

Analysis of *P*-element excision alleles: Nine lines were chosen for more detailed analysis that had large effects on aggressive behavior, and in which the *P*-element insertion site was located within the gene or in the presumed 5' regulatory region (Fig. 4). The *P*-element insertions in *Actin 5C* (*Act5C*), *extra macrochaetae* (*emc*), *CG32572*, and *Syntaxin 4* (*Syx4*) were associated with decreased levels of aggression; while *P*-element insertions in *pxb*, *echinoid* (*ed*), *sugarless* (*sgl*), *CG3638*, and *CG13377* were associated with increased levels of aggression. We attempted to generate precise revertant alleles of each of these *P*-element tagged genes, in order to map the mutant phenotype to the *P*-element insertion. The revertant alleles were sequenced, and at least one precise revertant was identified for each line except *CG3638*. The effects of imprecise excision alleles were also evaluated for the lines in which no or only a single precise revertant allele was generated.

The aggressive behavior of the revertant alleles was quantified, and for seven of the nine lines the behavior of the precise excision alleles also reverted to control levels, thus mapping the mutant phenotype to the *P*-element insertion in the tagged gene (Fig. 5). The exceptions were *emc* and *CG3638*. The *emc* precise revertant allele only showed partial phenotypic reversion. The behavior of one of the *CG3638* imprecise revertants

differed only marginally from that of the control ($p = 0.04$). The failure of the behavior of precise excision alleles to revert to the level of the control could indicate that the insertions of the *P*-elements in these loci do not cause the mutant phenotype. However, the observation of partial phenotypic reversion in conjunction with reduced levels of expression of *CG3638* and *emc* in the respective mutant alleles is consistent with a complex mutation in the precise excision alleles; for example, local hopping of the *P*-elements elsewhere in *emc*.

Analysis of gene expression: Quantitative reverse transcription PCR (qPCR) analyses were used to assess the effect of the *P*-element insertion on transcript levels of the tagged genes in the nine lines selected for further characterization. Since many of these genes have roles in development, expression was evaluated in embryos, larvae, pupae, and adults. The adult tissues were separated into heads and headless bodies (with the exception of the *sgl* mutant line, for which insufficient tissue was obtained to conduct qPCR on pupae or embryos, due to poor viability). All mutant lines were associated with alterations in transcript abundance in one or more developmental stages, confirming that the *P*-element insertions affect the expression of all tagged genes (Fig. 6).

There was no consistent pattern of gene expression changes in mutations associated with decreased levels of aggression. *Act5C* and *CG32572* mutants were associated with increased transcript levels – in embryos, pupae, and adult bodies for *Act5C*, and in embryos and adult heads for *CG32572*. Transcript levels in *emc* mutants were decreased throughout development, but increased in adult bodies. *Syx4* mutants had

reduced levels of gene expression in embryos and adult bodies, but increased expression in pupae. Mutations associated with increased aggression tended to have decreased levels of transcript abundance at one or more developmental stages. Gene expression was reduced in embryos and adult bodies of *CG3638* mutants; in larvae, pupae, and adult heads of *ed* mutants; in pupae and adult bodies of *pxb* mutants; and in adult heads of *sgl* mutants. In contrast, there was an increase of transcript abundance in adult heads of *CG13377* mutants.

This analysis shows that none of the mutations affecting aggressive behavior are transcriptional null alleles. The effects of all of the mutations on gene expression varied across development, and between adult heads and bodies. Depending on the developmental time point and/or adult tissue assessed, *CG3638*, *ed*, *pxb* and *sgl* are hypomorphic mutations; *Act5C* and *CG13377* are hypermorphic mutations; and *CG32572*, *emc* and *Syx4* are both hypomorphs and hypermorphs. All of the mutations showed significant differences in gene expression from the control in adults, but these differences were apparent in heads of only four of the mutations (*CG13377*, *CG32572*, *ed* and *sgl*). Further, many of the alterations in gene expression between mutant and control lines were of the order of two fold or less. These results indicate that even subtle mutational effects on transcription can be associated with large changes in behavior. Because the effects of the mutations on gene expression in pre-adult stages are often much larger than observed for adults, we cannot rule out the possibility that changes in gene expression during development affect adult behavior (SAMBANDAN *et al.* 2006).

The lack of a common pattern of gene expression differences among the mutations affecting increased and decreased levels of aggression suggests that there are multiple mechanisms by which this complex behavior can be altered. Finally, the observation that only four of the nine mutations show changes in gene expression in heads of adult flies indicates that only assessing changes in transcript abundance in heads of lines that are genetically divergent for behavioral traits will underestimate the number of transcripts associated with differences in the trait phenotype (TOMA *et al.* 2002; CIRELLI *et al*. 2005; DIERICK and GREENSPAN, 2006).

We further characterized the patterns of expression of the nine *P*-element tagged genes affecting aggressive behavior in wild type embryos. Consistent with previous results (BURN *et al.* 1989; TOBIN *et al.* 1990; CUBAS *et al.* 1994; BINARI *et al.* 1997; HAERRY *et al.* 1997), *Act5C*, *emc* and *sgl* were expressed in multiple tissues, including the ventral nerve cord for *Act5C* and *emc*. *CG32572*, *CG13377*, *ed* and *Syx4* were expressed in the central nervous system (Fig. 7). Expression outside the nervous system was also observed for most of the genes (Supplementary Fig. 1).

Morphometric analysis of central brain neuropils: Mushroom bodies and the ellipsoid body are central brain neuroplils that have been previously implicated in Drosophila aggressive behavior. Disruption of mushroom body output results in near abolishment of aggression (BAIER *et al.* 2002), and aberrant morphology of the mushroom bodies and ellipsoid body have been observed in hyper-aggressive mutants (ROLLMANN *et al*. 2008). Therefore, we measured the length and width of the alpha and

beta lobes of the mushroom bodies, and the surface area of the ellipsoid body, standardizing the values to overall brain size as a function of distance between peduncles (Table 2). There were significant quantitative changes in the length or width of one or both lobes of the mushroom bodies in all mutants except *emc*, further linking mushroom bodies and aggressive behavior. No significant differences in ellipsoid body area were observed for any of the mutations. The most frequently detected difference in mutants relative to control was an increase in the width of the alpha lobe. Only two of the mutations were associated with decreases in size: *Syx4* mutants had shorter beta lobes than controls, and *sgl* mutants had shorter alpha lobes. Increases in beta lobe measurements were only observed for mutations associated with increased levels of aggression. However, there was no overall correlation between any of the quantitative measurements of brain morphology and aggressive behavior, consistent with previous studies (ROLLMANN *et al.* 2008; YAMAMOTO *et al.* 2008) showing that there is no simple relationship between aggressive behavior and brain structure.

In addition to quantitative alterations in brain morphology, we also observed qualitative morphological defects in both alpha and beta lobes for five of the mutant lines (Fig. 8). One of ten brains examined for mutations of *CG32572*, *emc* and *CG13377* had, respectively, a missing beta lobe, shorter alpha lobe and an enlarged alpha lobe tip. Two of the ten *ed* mutant brains had qualitatively thicker beta lobes and thinner alpha lobes than the control, and three of the ten *sgl* brains had fused beta lobes. Although not completely penetrant, these defects were never observed in the control line.

Candidate genes affecting aggressive behavior: None of the candidate genes identified in this screen have been previously implicated to affect aggressive behavior. Three of the nine candidate genes characterized in greater detail are computationally predicted genes. *CG13377* is predicted to function in binding and metabolism (WILSON *et al*. 2008). RNAi-knockdown mutations of *CG3638* display reduced phagocytic immune response to *Candida albicans* cells (STROSCHEIN-STEVENSON *et al*. 2006). All that is known about *CG32572* is that it is expressed in the testis (CHINTAPALLI *et al*. 2007). *Act5C* is involved in ATP and protein binding (WILSON *et al*. 2008), and also has roles in cytokinesis (ECHARD *et al*. 2004, EGGERT *et al*. 2004) and spermatogenesis (NOGUCHI and MILLER 2003). *ed* has many developmental functions, including the negative regulation of neurogenesis (AHMED *et al*. 2003), appendage formation (LAPLANTE and NILSON 2006), and negative regulation of epidermal growth factor signaling (BAI *et al*. 2001). In adults, *ed* is expressed in ovaries, crop and male accessory glands (CHINTAPALLI *et al*. 2007). *emc* is also highly pleiotropic, and functions in peripheral nervous system (PROKOPENKO *et al*. 2000), midgut (SAITO *et al*. 2002), and spermatid development (CASTRILLON *et al*. 1993). *pxb* mutants have been implicated in olfactory learning and memory (DUBNAU *et al*. 2003) and in the *smoothened* signaling pathway (INAKI *et al*. 2002). *sgl*, like *pxb*, appears to be involved in *smoothened* signaling (NYBAKKEN and PERRIMON 2002), metabolism (WILSON *et al*. 2008), and biosynthesis (PERRIMON and BERNFIELD 2000, GHABRIAL *et al*. 2003). *Syx4* has been implicated in synaptic functions (LITTLETON 2000).

Given that we were able to map the behavioral mutant phenotype to the *P*-element insertion for seven of the nine mutations characterized in greater detail, and that all of the mutations affected gene expression of the tagged gene, we can predict that the majority of the remaining 48 *P*-element mutations associated with increased or decreased levels of aggression will also affect the genes into or near which they have inserted. Many of these genes are plausible candidates as they affect the development or the functioning of the nervous system (*Guanine nucleotide exchange factor GEF64C*, *NMDA receptor 1*, *schizo*, *tramtrack*, *Laminin A*, *longitudinals lacking*), and the effects of mutations in *neuralized* on aggressive behavior have been independently confirmed (ROLLMANN *et al.* 2008). Many other genes affect other aspects of development, metabolism or basic cellular processes, or are computationally predicted – these loci would not have been detected had we only concentrated on examining aggressive behavior for mutations in 'plausible' candidate genes.

The general picture emerging from the analysis of quantitative effects of *de novo* mutations that have been induced in a defined isogenic background is that a large fraction of the genome can potentially affect most quantitative traits, including complex behaviors (this study; NORGA *et al.* 2003; HARBISON *et al.* 2004; SAMBANDAN *et al.* 2006; HARBISON and SEGAL 2008; YAMAMOTO *et al.* 2008). Consequently, we expect that most genes have pleiotropic effects on multiple traits, and indeed, 55 of the 59 mutations associated with a significant difference in aggressive behavior from the control line had pleiotropic effects on one (19 lines), two (22 lines), three (11 lines) or four (three lines)

additional quantitative traits (Table 1). Further, different mutations in the same gene can have a different spectrum of pleiotropic effects (ROLLMANN *et al.* 2006; 2008), and the mutational effects on any one trait can be contingent on genetic background and the environment (FEDOROWICZ *et al.* 1998; SAMBANDAN *et al.* 2006; 2008; ROLLMANN *et al.* 2006). Given these complexities, an exhaustive mutational dissection of any complex behavior (or indeed, any quantitative trait) is not feasible. However, the collection of over 70 mutations affecting aggressive behavior that have been generated in the same isogenic background (this study, EDWARDS *et al.* 2006) are valuable molecular probes that can be used to gain insight into the key pathways and mechanisms affecting this trait using systems biology approaches (ANHOLT *et al.* 2003).

Acknowledgements: This work was supported by grants National Institutes of Health grants F31 MH-074161 to A. C. E. and R01 GM-076083 to T. F. C. M. This is a publication of the W. M. Keck Center for Behavioral Biology.

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Table 1 *P{GT1}* lines with aberrant aggressive behavior. MAS = Mean Aggression Score. Significant MAS deviations from the control line after correcting for multiple tests are indicated by asterisks. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

† Indicates lines characterized in greater detail. Pleiotropic effects of the mutations are given for numbers of sensory bristles (B, NORGA *et al.* 2003), locomotor reactivity (L, YAMAMOTO *et al.* 2008); olfactory avoidance behavior (O, SAMBANDAN *et al.* 2006); male 24-hour sleep (HARBISON and SEGAL 2008) and male starvation stress resistance (HARBISON *et al.* 2004). The arrows indicate significant positive (↑) and negative (↓) deviations from the control.

***P*-Element Line**	**Disrupted Locus**	**Cytogenetic Location**	**Mutational Effects**		**Pleiotropic Effects**					**Gene Ontology**
			MAS	a/σ_P	**B**	**L**	**O**	**Sl**	**St**	
BG00151	*CG9894*	23A3-23A3	16.35**	0.96						unknown
BG00336	*Guanine nucleotide exchange factor GEF64C*	64B13-64B17	6.1***	-1.45					↓	axon guidance; regulation of Rho protein signal transduction
BG00372	*CG1678*	20A1-20A1	17.2***	0.94				↑		unknown
BG00375	*Odorant-binding protein 99d*	99B8-99B8	14.8**	0.53	↑				↓	odorant binding; autophagic cell death; transport
BG00376†	*CG3638*	1E4-1E4	18.45***	1.16	↑			↑	↓	unknown

Table 1 (continued)

***P*-element Line**	**Disrupted Locus**	**Cytogenetic Location**	**Mutational Effects**		**Pleiotropic Effects**					**Gene Ontology**
			MAS	a/σ_P	**B**	**L**	**O**	**Sl**	**St**	
BG00386	*NMDA receptor 1*	83A6-83A7	20.65***	1.53					↓	long-term memory; olfactory learning; calcium-mediated signaling; nerve-nerve synaptic transmission; nervous system development
BG00670	*CG32541*	17F3-18A2	13.35**	0.28	↑					unknown
BG00735	*schizo*	78A5-78B1	16.25***	0.78					↑	central nervous system development; guanyl-nucleotide exchange factor activity
BG00986†	*extra macrochaetae*	61C9-61C9	7.05***	-0.90	↑	↓		↑	↓	nervous system development
BG01011	*Spinophilin*	62E4-62E5	17.2***	0.69		↓		↑	↑	phosphopantetheine binding
BG01043	*Gp150*	58D3-58D3	18.0*	0.91						ATP binding; transmembrane receptor protein tyrosine phosphatase signaling pathway
BG01046	*CG3587*	2B16-2B16	7.9***	-0.60	↑	↓			↓	unknown
BG01130	*alan shepard*	64C8-64C11	16.55***	0.86				↑	↓	mRNA processing; gravitaxis
BG01214†	*sugarless*	65D4-65D5	17.1***	0.93				↑	↓	cell communication; signal transduction; transmembrane receptor protein tyrosine kinase signaling pathway

Table 1 (continued)

***P*-element Line**	**Disrupted Locus**	**Cytogenetic Location**	**Mutational Effects**		**Pleiotropic Effects**					**Gene Ontology**
			MAS	a/σ_P	**B**	**L**	**O**	**Sl**	**St**	
BG01215	*CG11299*	59F6-59F7	8.7**	-0.48	↑	↓			↓	regulation of progression through cell cycle; cell cycle arrest
BG01299†	*Actin 5C*	5C7-5C7	3.6***	-1.75	↓	↓				structural constituent of cytoskeleton; ATP binding; protein binding
BG01354	*CG30492*	43E5-43E7	18.55**	1.01	↑					zinc ion binding
BG01402	*CG32345*	61C7-61C7	15.55***	0.68		↓			↓	unknown
BG01433	*CG13791*	28B1-28B2	16.75**	0.60		↓			↓	unknown
BG01469†	*Syntaxin 4*	3B4-3B4	7.9***	-0.65		↓			↓	t-SNARE activity; neurotransmitter secretion; vesicle-mediated transport; synaptic vesicle docking during exocytosis
BG01491	*tramtrack*	100D1-100D1	14.45*	0.48	↑				↓	zinc ion binding; peripheral nervous system development; transmission of nerve impulse
BG01498	*Casein kinase Iα*	11B7-11B7	8.85*	-0.43	↑				↓	receptor signaling protein serine/threonine kinase activity; ATP binding
BG01536	*Beadex*	17C3-17C4	7.25***	-0.60	↑	↓			↓	zinc ion binding; locomotory behavior; response to cocaine; regulation of metabolism
BG01566	*arrest*	33D3-33D5	6.75***	-0.75						negative regulation of oskar mRNA translation

Table 1 (continued)

***P*-element Line**	**Disrupted Locus**	**Cytogenetic Location**	**Mutational Effects**		**Pleiotropic Effects**					**Gene Ontology**
			MAS	a/σ_P	**B**	**L**	**O**	**Sl**	**St**	
BG01596†	*CG13377*	1A1-1A1	19.55***	1.74		↓	↑	↑	↓	metabolism
BG01654	*pickpocket 23*	16B4-16B4	9.55**	-0.57		↓			↑	sodium channel activity
BG01662	*Laminin A*	65A8-65A9	15.55**	0.68				↑	↓	receptor binding; locomotion; central nervous system development
BG01683†	*CG32572*	15A3-15A3	7.65***	-0.66		↓			↓	unknown
BG01693	*CG10777*	7C3-7C4	4.5***	-1.11		↓	↑		↓	RNA helicase activity; nucleic acid binding; ATP binding; ATP-dependent helicase activity
BG01713	*4EHP*	95E1-95E1	8.9**	-0.50		↓				translation initiation
BG01733	*CG6175*	68C1-68C2	14.55*	0.51	↑	↓			↓	unknown
BG01757	*CG17323*	37B1-37B1	12.75*	0.50		↓				defense response; polysaccharide metabolism; response to toxin; steroid metabolism
BG01765	*Tehao*	34C1-34C1	8.35**	-0.70	↓					transmembrane receptor activity; signal transduction; Toll signaling pathway
BG01893	*Splicing factor 1*	90B4-90B4	7.2*	-0.51	↑	↓			↓	transcription cofactor activity; nucleic acid binding; zinc ion binding
BG01900	*mir-317*	85F10-85F10	6.6**	-0.62	↑	↓			↓	microRNA
BG01909	*CG14035*	25C6-25C6	21.45***	2.27	↓	↓	↑		↓	unknown

Table 1 (continued)

***P*-element Line**	**Disrupted Locus**	**Cytogenetic Locus**	**Mutational Effects**		**Pleiotropic Effects**					**Gene Ontology**
			MAS	a/σ_P	**B**	**L**	**O**	**Sl**	**St**	
BG01912†	*pxb*	89A1-89A2	17.3***	0.85	↑	↓				learning and/or memory; olfactory learning
BG01916	*no ocelli*	35B2-35B2	13.65**	0.36				↑	↓	zinc ion binding
BG01949	*ade5*	11B16-11B16	16.2*	0.57					↓	purine base metabolism; 'de novo' IMP biosynthesis
BG02019	*CG9171*	25F4-25F5	13.3**	0.60						transferase activity
BG02022	*CG34460*	2R	7.15***	-0.91		↓	↑			unknown
BG02077	*Rtnl1*	25B9-25C1	9.0*	-0.65	↑			↓	↓	receptor signaling protein activity; calcium ion binding
BG02081	*Rtnl1*	25B9-25C1	16.6***	1.19		↓	↑			receptor signaling protein activity; calcium ion binding
BG02095†	*echinoid*	24D4-24D6	14.8**	0.68					↑	epidermal growth factor receptor signaling pathway; negative regulation of neurogenesis; sensory organ development
BG02128	*lethal (1) G0007*	12E3-12E5	5.55***	-0.86		↓			↓	ATP-dependent RNA helicase activity; ATP-dependent helicase activity; ATP binding
BG02188	*eclair*	85E4-85E4	15.8***	0.56	↑					intracellular protein transport
BG02217	*plexus*	58E4-58E8	4.6***	-0.99	↓	↓				wing vein morphogenesis
BG02276	*lethal (3) L1231*	88C10-88C10	10.95*	0.44	↑					unknown

Table 1 (continued)

***P*-element Line**	**Disrupted Locus**	**Cytogenetic Locus**	**Mutational Effects**		**Pleiotropic Effects**					**Gene Ontology**
			MAS	a/σ_P	**B**	**L**	**O**	**Sl**	**St**	
BG02377	*CG14478*	54B16-54B16	4.2**	-0.56	↓/↑					unknown
BG02420	*CG5946*	68E1-68E1	3.85**	-0.61	↑					cholesterol metabolism; electron transport; fatty acid desaturation
BG02470	*CG8963*	53E4-53E4	7.6*	-0.52	↓	↓	↑			unknown
BG02495	*ade5*	11B16-11B16	13.35*	0.33				↑	↓	purine base metabolism; 'de novo' IMP biosynthesis
BG02501	*longitudinals lacking*	47A11-47A13	7.9*	-0.61	↑	↓				axon guidance; axonogenesis; transmission of nerve impulse
BG02522	*CG42270*	16C1-16C8	6.1***	-0.80		↓	↑			Ras GTPase activator activity; receptor binding; G-protein coupled receptor protein signaling pathway;
BG02523	*lamina ancestor*	64C12-64C13	7.95***	-0.59	↑					unknown
BG02539	*Basigin*	28E3-28E5	15.5**	-0.58				↑	↓	spermatid development
BG02542	*neuralized*	85C2-85C3	11.35*	-0.60	↑					ubiquitin-protein ligase activity; protein binding; zinc ion binding; nervous system development; sensory organ development; regulation of Notch signaling pathway
BG02644	*Fkbp13*	57E6-57E6	12.0**	0.59	↑					calcium ion binding; protein folding
BG02731	*longitudinals lacking*	47A11-47A13	13.6**	0.63	↓					axon guidance; axonogenesis; transmission of nerve impulse

Table 2 Mushroom body measurements. Measurements of the length and width of alpha and beta lobes (±SE) for *Canton S B* (the control line) and mutant lines associated with increased or decreased levels of aggression. Measurements are standardized to overall brain size. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Mutant	Alpha Lobes		Beta Lobes	
	Length (SE)	**Width (SE)**	**Length (SE)**	**Width (SE)**
Canton S B	6.03 (0.09)	0.6925 (0.0170)	4.28 (0.03)	0.7901 (0.0236)
Act5C	6.35 (0.13)*	0.6648 (0.0185)	4.18 (0.04)	0.7746 (0.0282)
CG3638	6.07 (0.13)	0.8372 (0.0323)***	4.26 (0.06)	0.8866 (0.0303)*
CG13377	5.99 (0.12)***	0.8036 (0.0222)	4.22 (0.04)	0.8273 (0.0342)
CG32572	6.56 (0.09)***	0.8218 (0.0169)***	4.23 (0.04)	0.8085 (0.0345)
ed	5.99 (0.16)	0.8330 (0.0299)***	4.09 (0.08)*	0.8683 (0.0389)
emc	6.23 (0.17)	0.7174 (0.0216)	4.29 (0.05)	0.8622 (0.0300)
pxb	6.12 (0.08)	0.8070 (0.0230)***	4.15 (0.05)*	0.8729 (0.0281)*
sgl	5.60 (0.13)**	0.7631 (0.0214)*	4.25 (0.07)	0.8263 (0.0312)
Syx4	5.94 (0.14)	0.7044 (0.0170)	4.13 (0.05)*	0.8504 (0.0194)

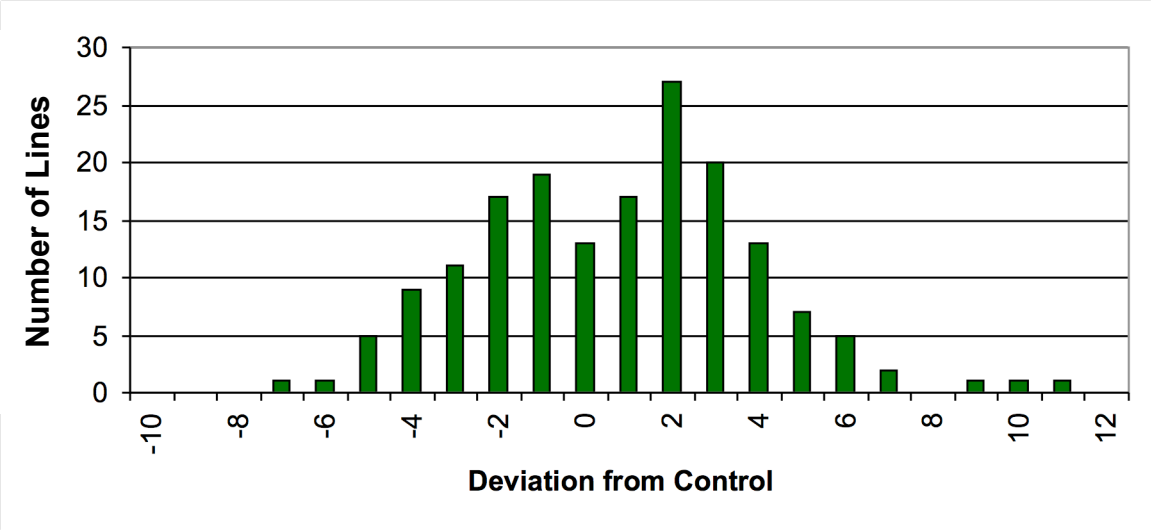


Figure 1. Distribution of mean aggression scores among 170 *P{GT1}*-element insertion lines, expressed as deviations from the co-isogenic *Canton-S B* control line.

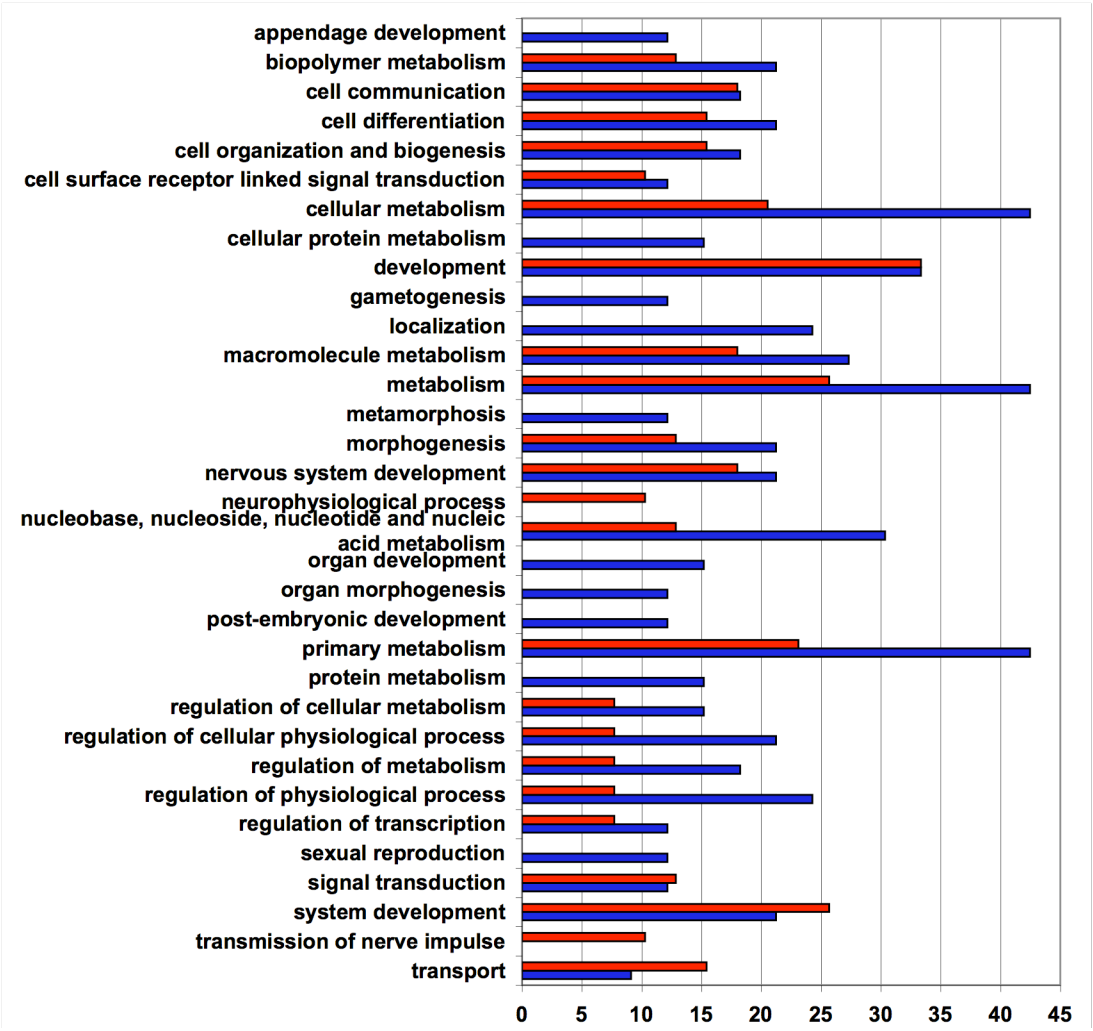


Fig. 2A

Figure 2. Gene ontologies of candidate genes with mutations associated with aggressive behavior. (A) Biological Process gene ontology categories. (B) Molecular Function gene ontology categories. The percentage of genes in each category is shown for mutations increasing (red bars) and decreasing (blue bars) aggressive behavior.

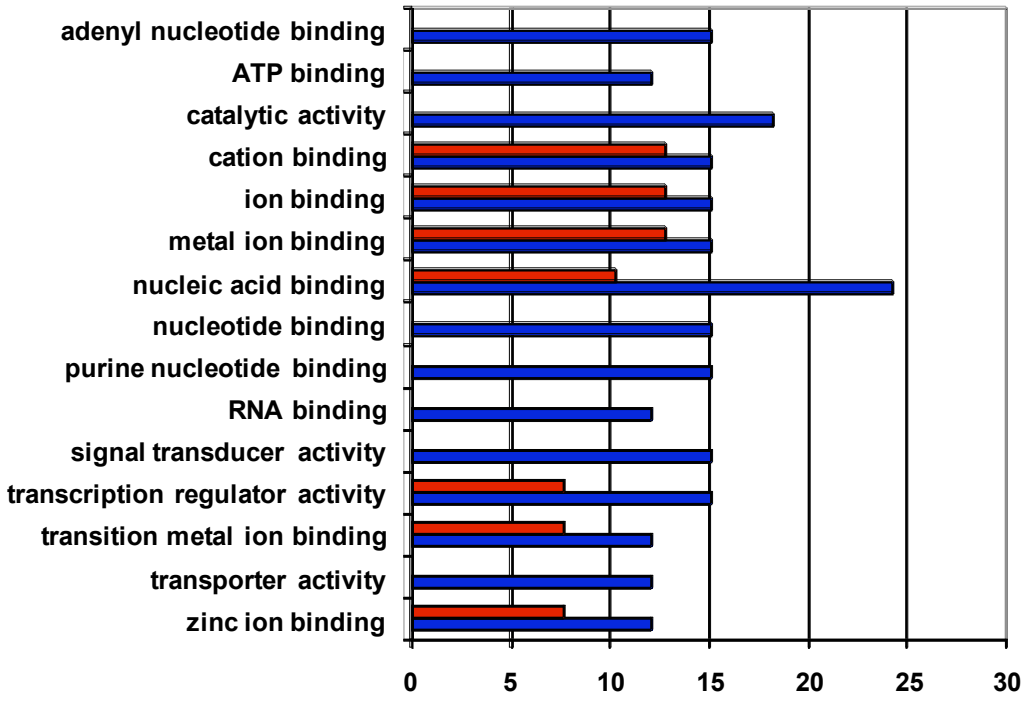

adenyl nucleotide binding
ATP binding
catalytic activity
cation binding
ion binding
metal ion binding
nucleic acid binding
nucleotide binding
purine nucleotide binding
RNA binding
signal transducer activity
transcription regulator activity
transition metal ion binding
transporter activity
zinc ion binding
0
5
10
15
20
25
30

Fig. 2B

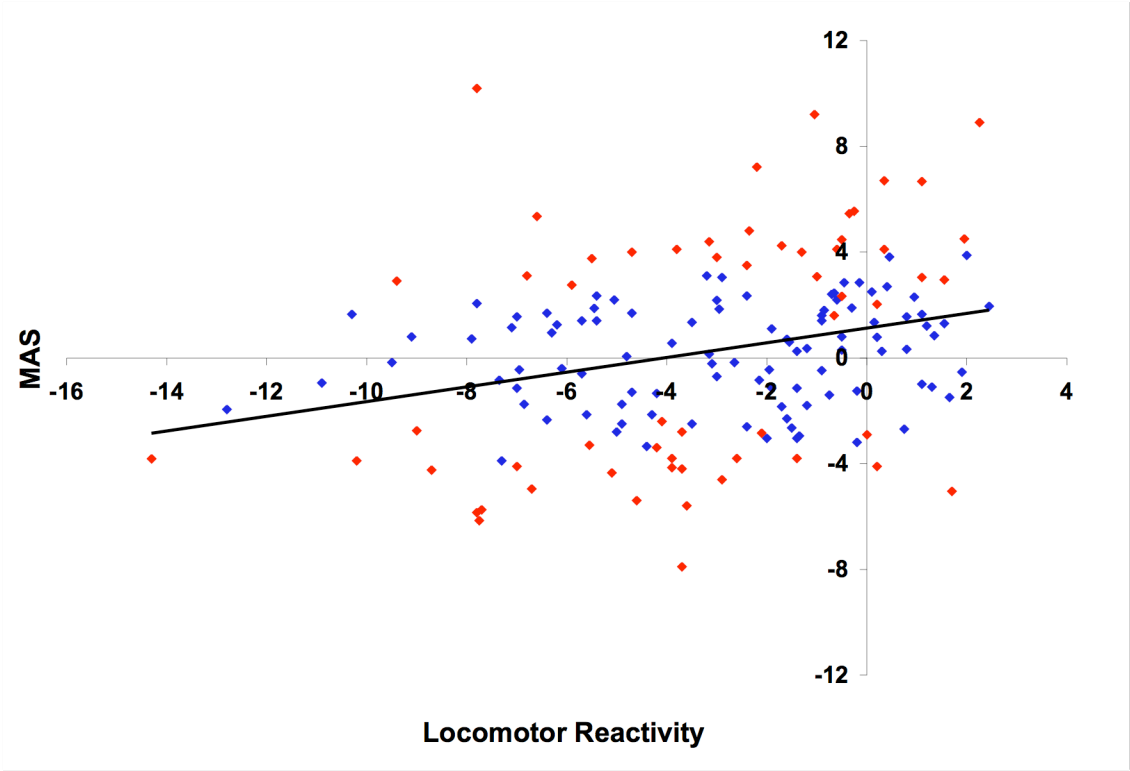
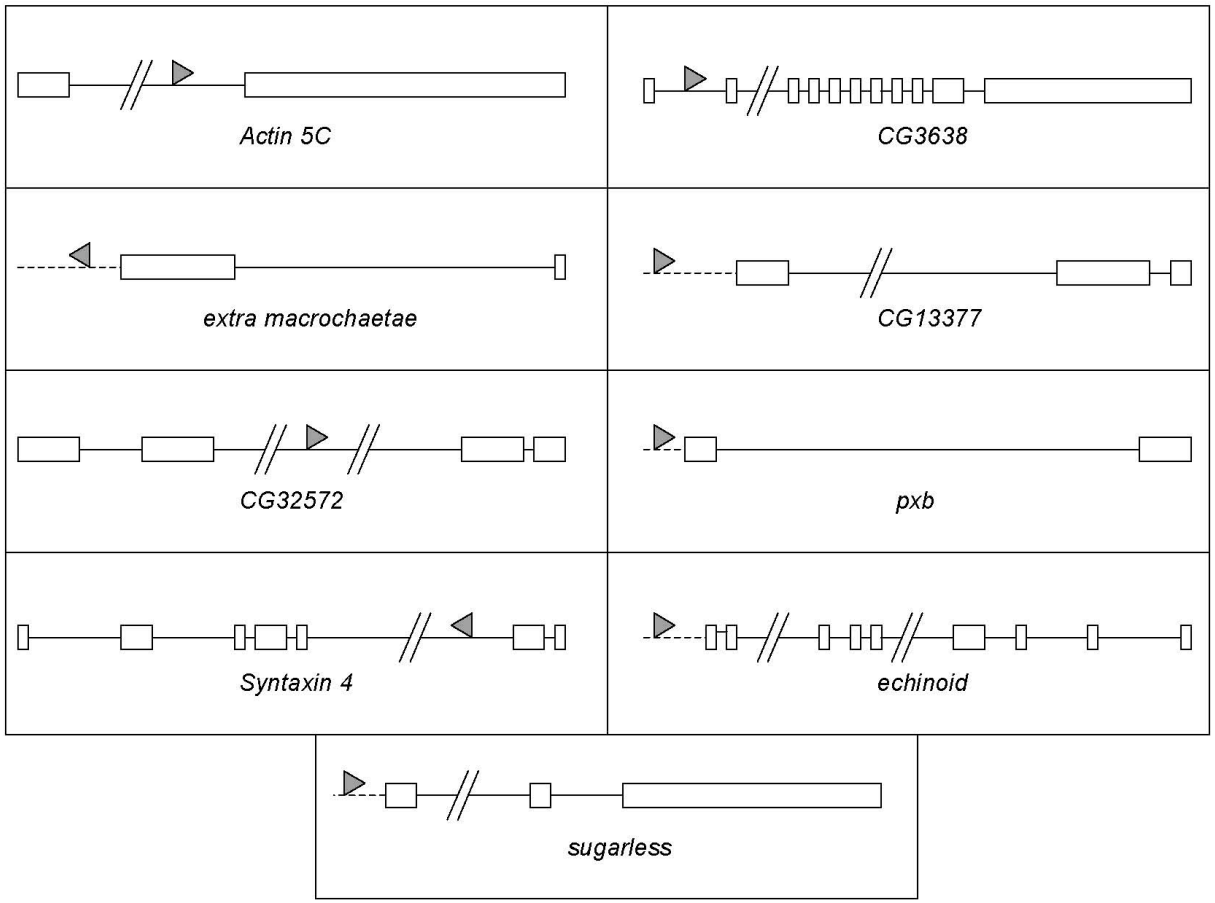


Figure 3. Correlation between mean aggression score (MAS) and locomotor reactivity in *P*-element insertion lines. Scores are given as a deviation from the control line. Data points in red represent lines with levels of aggressive behavior that are significantly different from the control.

Figure 4. Structure of nine genes in which mutations affect male aggressive behavior. All genes are oriented 5' to 3', with boxes indicating exons and solid lines indicating introns. Dashed lines represent 5' putative promoter regions. Solid triangles indicate the location of the *P*-element insertion, with the direction of the triangle indicating the orientation of the insertion.


Actin 5C
CG3638
extra macrochaetae
CG13377
CG32572
pxb
Syntaxin 4
echinoid
sugarless

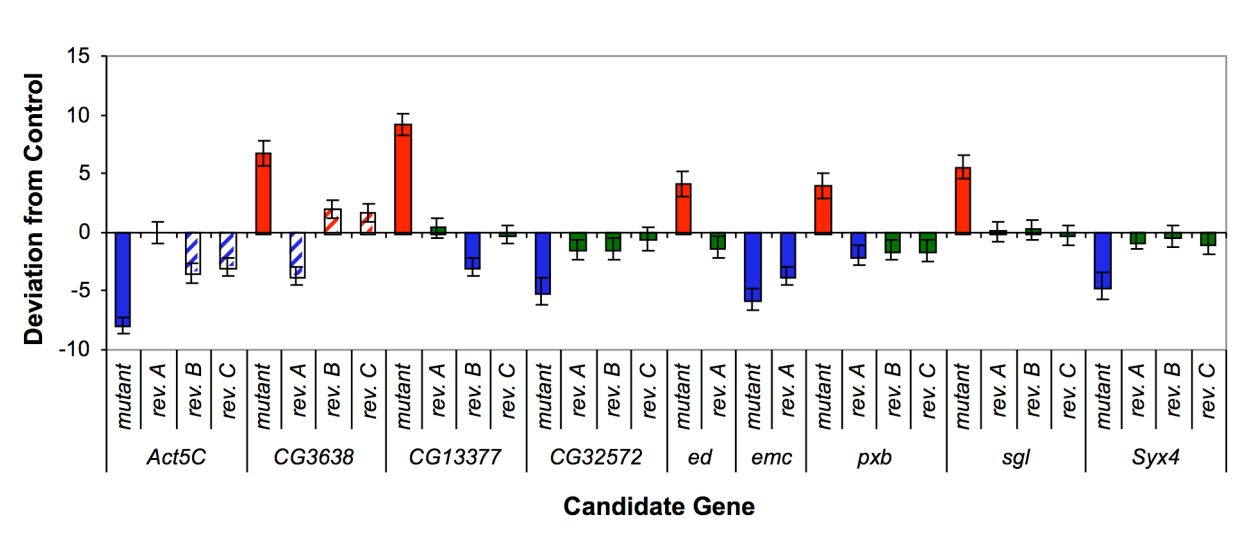
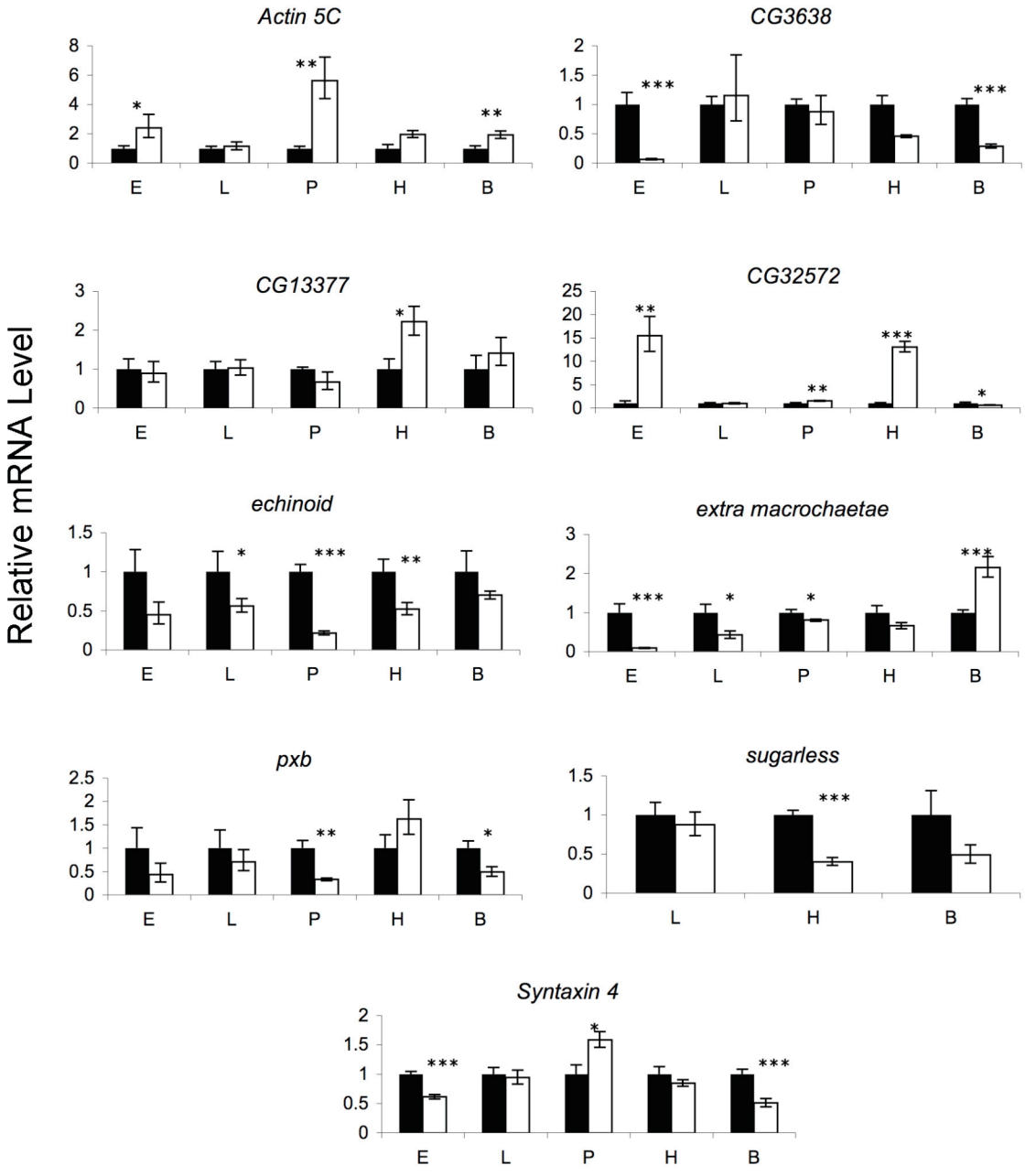


Figure 5. Mean aggression scores of *P*-element mutations affecting aggressive behavior and revertant alleles. The mean aggression score is given as the deviation from the contemporaneously tested control line for the mutant lines and up to three revertant alleles. Blue bars indicate significantly ($p < 0.05$) lower levels of aggression than the control; red bars indicate higher levels if aggression than the control; and green bars indicate no significant difference in mean aggression score from the control. Hatched bars indicate imprecise revertant alleles.

Figure 6. Quantitative RT-PCR analysis of candidate genes affecting aggressive behavior. Levels of mRNA for each gene (white bars) are depicted relative to the level in the co-isogenic control (black bars). mRNA levels were assessed at four developmental time periods: embryos aged 10-12 hours AEL (E), third instar larvae (L), pupae (P), and adults (heads [H] and headless bodies [B]). Only larvae and adults could be obtained for *sgl* mutants. Standard errors were obtained using Ct values normalized to an internal control (*Gapdh1*). The significance of two-tailed Student's *t*-tests conducted on linearized Ct values are depicted by asterisks (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).


Relative mRNA Level
Actin 5C
CG3638
CG13377
CG32572
echinoid
extra macrochaetae
pxb
sugarless
Syntaxin 4
E
L
P
H
B

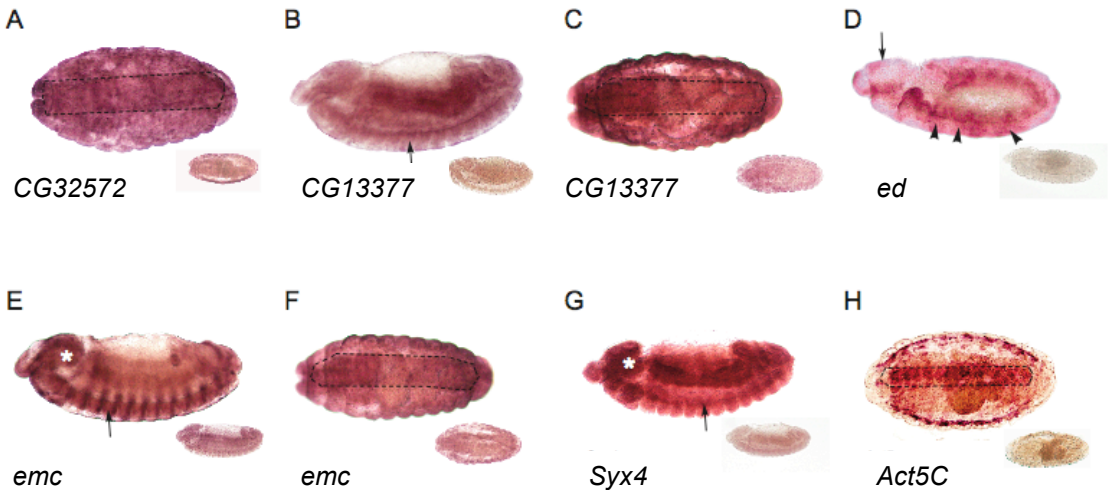


Figure 7. Expression of candidate genes affecting aggressive behavior in the embryonic nervous system. The insets show the sense control probes. (A) *CG32572*, stage 17, ventral view: expression in the ventral cord (highlighted with dotted line). (B) *CG13377*, stage 14, lateral view: expression in the ventral nerve cord (arrow) (C) *CG13377*, stage 17, ventral view: expression in the ventral nerve cord (highlighted with dotted line). (D) *ed*, stage 11, lateral view: expression in the procephalic neuroblasts (arrow) and the neuroblasts forming the ventral nerve cord (arrowheads). (E) *emc*, stage 14, lateral view: expression in the ventral nerve cord (arrow) and the brain (asterisk). (F) *emc*, stage 17, ventral view: expression in the ventral nerve cord (highlighted with dotted line). (G) *Syx4*, stage 13, lateral view: expression in the ventral nerve cord (arrow) and the brain (asterisk). (H) *Act5C*, stage 16, ventral view: expression in the ventral nerve cord (highlighted with dotted line).

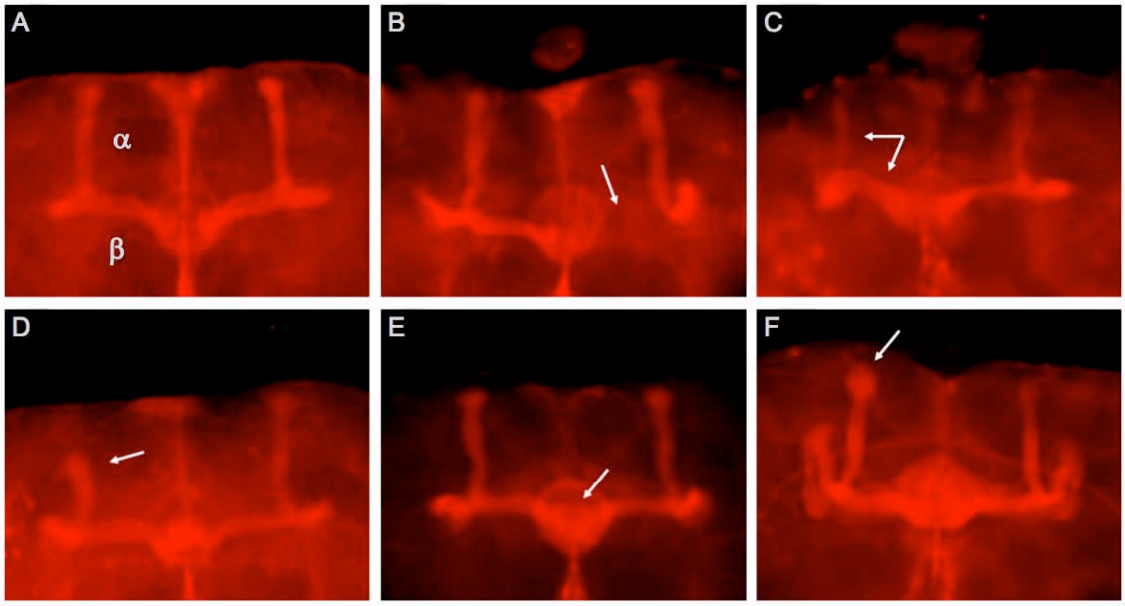


Figure 8. Gross morphological defects in the mushroom bodies in mutations of candidate genes affecting aggressive behavior. A-H, Anti-fasciclin 2 staining of adult brains using the 1D4 monoclonal antibody. Defects are indicated by the white arrows. (A) *Canton S B* control line. α, alpha lobes of mushroom bodies; β, beta lobes of mushroom bodies. (B) Missing beta lobe in *CG32572* mutation. (C) Misrouting of some of the alpha lobe axons leads to thicker beta lobes and thinner alpha lobes in *ed* mutation. (D) Shorter alpha lobe in *emc* mutation. (E) Overextension resulting in fusion of the beta lobes in *sgl* mutation. (F) Alpha lobe tip defect resulting in enlargement of the tip in *CG13377* mutation.

CHAPTER FOUR

Quantitative trait loci for aggressive behavior in *Drosophila melanogaster*

Quantitative Trait Loci for Aggressive Behavior in *Drosophila melanogaster*

Alexis C. Edwards and Trudy F.C. Mackay

Department of Genetics, Box 7614, and W. M. Keck Center for Behavioral Biology, Box 7617, North Carolina State University, Raleigh, North Carolina 27695, USA.

This chapter consists of a manuscript that has been prepared for submission for publication. I conducted behavioral assays on all lines. I conducted all data analyses. I wrote the manuscript. Trudy Mackay supervised the research project and provided extensive comments on the organization and content of the manuscript.

Running Head: Aggression in *Drosophila melanogaster*

Key Words: QTL mapping, deficiency mapping, mutant complementation test, quantitative trait genes

Corresponding Author:
Trudy F. C. Mackay
Department of Genetics
Campus Box 7614
North Carolina State University
Raleigh, NC 27695-7614 USA
Phone: 919-515-5810
Fax: 919-515-3355
E-mail: trudy_mackay@ncsu.edu

ABSTRACT

Aggressive behavior is observed across animal taxa and is likely to be evolutionarily conserved. Although potentially advantageous, aggression can have social and health consequences in humans, and is a component of a number of psychiatric disorders. As a complex genetic trait, it is modulated by numerous quantitative trait loci (QTLs) with allelic effects that can vary in direction and magnitude, and that are sensitive to environmental perturbations. Assays to quantify aggressive behavior in *Drosophila melanogaster* have been developed, making this an ideal model system in which to dissect the genomic architecture underlying manifestation of and variation in aggressive behavior. Here, we map QTLs affecting variation in aggression between two wild type Drosophila strains. We identified a minimum of five QTLs in a genome-wide scan: two on chromosome *2* and three on chromosome *3*. At least three and possibly all five of these QTLs interact epistatically. We used deficiency complementation mapping to subdivide two linked, epistatically interacting QTLs of large effect on chromosome *3* into at least six QTLs. Complementation tests to mutations identified four candidate quantitative trait genes (*CG11006*, *CG10754*, *mutagen-sensitive 312*, *Ral guanine nucleotide exchange factor 2*), none of which have been previously implicated to affect aggressive behavior.

INTRODUCTION

Aggressive behavior is observed throughout the animal kingdom and can be essential for survival. Aggression is used to gain access to territory, food, or mates; in defense against predators; to protect offspring; and to gain social status in a dominance hierarchy. On the other hand, aggression can be costly, since violent interactions with others can cause injury, and aggressive displays are energetically expensive and interfere with other essential behaviors, such as feeding and mating. The persistence of high levels of genetic variation for aggression within populations (DOW and VON SCHILCHER 1975; HOFFMANN 1988; HOFFMANN 1989; HOFFMANN and CACOYIANNI 1989) suggests that a balance of evolutionary forces act to maintain the variation. Theoretical modeling has suggested that frequency dependent selection could be generating genetic variation in aggression (MAYNARD SMITH and HARPER 1988). The observation that many behavioral phenotypes vary widely in a population is often attributed to life history trade-offs (CABRAL *et al.* 2008; MANEY 2008); recent work also suggests that animal "personalities" within a population might be adaptive (WOLF *et al.* 2007). Another potential explanation is that the pleiotropic nature of many genes affecting aggressive behavior subjects them to opposing or multi-directional selective pressures, maintaining variation.

In humans, aggression is often associated with impulsive behavior, and can reach pathological levels. It is a component of a number of behavioral or psychiatric disorders, such as alcoholism, borderline personality disorder, conduct disorder, and Alzheimer's disease. It can also constitute a behavioral pathology in and of itself (e.g., intermittent explosive disorder). Therefore, an understanding of the genetic basis of variation in

aggressive behavior is important from the dual perspectives of evolutionary genetics and human health.

Considerable work on the neurobiology and genetics of aggressive behavior in mammals and invertebrates has revealed key molecules required to mediate and modulate aggression, in particular biogenic amines. Normal levels of serotonin (5-hydroxytryptophan, 5-HT) inhibit aggressive behavior in vertebrates, and decreased levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, are associated with increased levels of impulsivity and aggression (MANUCK *et al.* 1999; NELSON and CHIAVEGATTO 2001). In invertebrates, increased 5-HT results in increased aggressive behavior (BAIER *et al.* 2002; EDWARDS and KRAVITZ 1997; HUBER *et al.* 1997; DIERICK and GREENSPAN 2007). Monoamine oxidase A (MAOA), which catabolizes 5-HT as well as dopamine and norepinephrine, has also been associated with aggressive behavior in humans; males lacking a functional copy of MAOA (which is *X*-linked) are highly aggressive (BRUNNER *et al.* 1993), and expression levels of brain MAOA are inversely correlated with aggression (ALIA-KLEIN *et al.* 2008). The noradrenergic (DE ALMEIDA *et al.* 2005) and dopaminergic (HALLER *et al.* 1998) systems also modulate aggressive behavior. The Drosophila homolog of noradrenaline, octopamine, also appears to modulate aggression, since males lacking octopamine display hardly any aggressive behaviors (BAIER *et al.* 2002; HOYER *et al.* 2008). Polymorphisms in catecholamine-O-methyltransferase, which degrades dopamine and norepinephrine, are also associated variation in aggression in schizophrenic men (HAN *et al.* 2004).

The neurotransmitters nitric oxide (NO) and γ-aminobutyric acid (GABA, MICZEK *et al.* 2003) also modulate aggressive behavior. In humans, haplotypes in the gene encoding nitric oxide synthase (NOS) are associated with increased aggression and/or suicidal behavior (RUJESCU *et al.* 2008). In mice, knocking out expression of neuronal NOS is associated with increased aggression (NELSON *et al*., 1995), although this may be attributable to the effect of the knockout on 5-HT (CHIAVEGATTO *et al.* 2001). Neuropeptide Y and its invertebrate homolog, Neuropeptide F, affect aggression in mammals (GAMMIE *et al.* 2007; KARL and HERZOG 2007; KARL *et al.* 2004) and Drosophila (DIERICK and GREENSPAN 2007). In Drosophila, correct expression of the male-specific transcript of *fruitless*, a gene in the sex-determination pathway, is required for executing male aggressive behaviors (CERTEL *et al.* 2007; CHAN and KRAVITZ 2007; LEE and HALL 2000; VRONTOU *et al.* 2006).

As a quantitative trait, we expect natural variation in levels of aggression to be influenced by segregating alleles at multiple interacting loci with varying effects that depend on the social and physical environment. Recent studies documenting correlated responses of the transcriptome to artificial selection for divergent levels of aggressive behavior from wild-derived (EDWARDS *et al.* 2006) and laboratory (DIERICK and GREENSPAN 2006) strains of Drosophila hint that the genetic basis of natural variation in this behavior may be very complex. However, these studies are unable to discriminate which of the correlated transcriptional responses are caused by polymorphic alleles at loci directly affecting aggressive behavior, and which are trans-regulated by the causal

polymorphisms, or hitch-hike along with the selected causal polymorphisms. Therefore, we need to use QTL mapping to identify causal loci affecting variation in aggressive behavior between wild type lines.

Here, we map QTLs affecting the difference in aggressive behavior between two laboratory stocks: *Oregon-R* (*Ore*), a standard laboratory wild type strain (LINDSLEY and ZIMM 1992), and *2b*, a Russian strain selected for low male mating ability (KAIDANOV 1990). Recombinant inbred lines (RILs) derived from these strains have been used previously to map QTLs affecting a wide variety of quantitative traits: life span (NUZHDIN *et al*. 1997; LEIPS and MACKAY, 2000; PASYUKOVA *et al*. 2000; VIEIRA *et al*. 2000; LEIPS and MACKAY, 2002; REIWITCH and NUZHDIN, 2002; LAI *et al*. 2007); starvation (VIEIRA *et al*. 2000; HARBISON *et al*. 2004), heat and cold stress resistance (MORGAN and MACKAY 2006); reproductive success (FRY *et al*. 1998; WAYNE *et al*. 2001); numbers of sensory bristles (GURGANUS *et al*. 1998), sex comb teeth (NUZHDIN and REIWITCH, 2000) and ovarioles (WAYNE *et al*. 2001); flight velocity and metabolic traits (MONTOOTH *et al*. 2003); courtship song (GLEASON *et al*. 2003); and olfactory (FANARA *et al.* 2002), male mating (MOEHRING and MACKAY, 2004) and locomotor (JORDAN *et al.* 2006) behavior. We used a population of introgression lines derived from the recombinant inbred lines to infer a minimum of five QTLs affecting aggressive behavior in these strains, at least three of which interact epistatically. We used complementation tests to deficiencies to fractionate two linked epistatic QTLs on the left arm of the third chromosome into six QTLs; and complementation tests to mutations to implicate four novel candidate genes affecting variation in aggression between *Ore* and

2b. The expression and function of these genes informs our understanding of the genetic influences on aggression and lays the foundation for future research.

MATERIALS AND METHODS

Drosophila stocks: We derived 20 introgression lines from the population of 98 *Ore* × *2b* RILs (NUZHDIN et al., 1997). We screened the RIL genotypes, one chromosome at a time, for partially overlapping segments of the *2b* genome in an otherwise *Ore* background, and chose 20 segmental introgressions that together constitute the entire *2b* genome. We then substituted each of the chromosomes containing a *2b* introgressed fragment into the *Ore* background, by standard crosses using balancer chromosomes that had themselves previously been substituted into *Ore*. The cytological limits of the introgressed portion of the *2b* genome, as assessed by insertion sites of *roo* transposable element markers, are given in Supplementary Table 1.

We obtained DrosDel (RYDER *et al*. 2007) and Exelixis (PARKS *et al.* 2004) deficiency (*Df*) stocks with defined molecular breakpoints that had been generated in a w^{1118} isogenic background and stocks with mutations in positional candidate genes from the Bloomington Drosophila Stock Center, Bloomington, Indiana.

Assay to quantify aggressive behavior: Eight male flies of the same genotype, aged 3-7 days, were transferred without anesthesia to an empty vial and deprived of food for ninety minutes. They were then exposed to a droplet of standard food and allowed to acclimate for two minutes, after which they were observed for two minutes. The number

of aggressive encounters observed during this period was recorded as the aggression score for the replicate. The average of these scores is the Mean Aggression Score (MAS). All assays were performed between 8-11 a.m., at 25°C and 75% humidity.

QTL mapping using introgression lines: We scored 20 replicate samples of each introgression line for aggressive behavior. We partitioned variance in aggressive behavior between (*L*) and within (*E*) introgression lines using the one-way random effects ANOVA model $Y = \mu + L + E$. We estimated the broad sense heritability (H^2) of aggressive behavior as $H^2 = \sigma_L^2/(\sigma_L^2 + \sigma_E^2)$, where σ_L^2 and σ_E^2 are the among- and within-line variance components, respectively. We then used *t*-tests to assess whether the mean level of aggression in each introgression line was significantly different from *Ore* and/or *2b*. Introgression lines with significantly different MAS scores from *Ore* contain a *2b* QTL allele affecting aggressive behavior.

Deficiency complementation mapping: The DrosDel and Exelixis deficiency stocks have isogenic genomic background control lines (w^{1118}). We crossed *Ore* and *3-88Я* to each deficiency stock and to the control, and assessed aggressive behavior for 10 replicates of each of four genotypes: *Df*/*Ore*, *Df*/*3-88Я*, w^{1118}/*Ore*, and w^{1118}/*3-88Я*. The statistical model used to analyze the behavioral data is a two-way ANOVA: $Y = \mu + L + G + L \times G + E$, where μ is the overall mean, *L* is the main effect of line (*Ore* or *3-88Я*), *G* is the main effect of genotype (*Df* or w^{1118}), and *E* is the environmental variance. A significant $L \times G$ term indicates quantitative failure to complement. We excluded lines that met this criterion but for which the difference in means between the w^{1118}/*Ore* and

w^{1118}/*3-88Я* genotypes was not significantly different from that between the *Df*/*Ore* and *Df*/*3-88Я* genotypes, since this result is difficult to interpret as an allelic interaction. As for all complementation tests, failure to complement could arise from different effects of *Ore* and *2b* alleles at one locus in the region uncovered by the deficiency, or non-allelic interactions. Note that failure to complement due to epistasis is minimized in these tests, because the *Df* strains have no additional mutations and a co-isogenic control strain rather than a balancer chromosome is used as the wild type allele. Also, the introgression design limits epistasis to other QTLs in the introgressed region.

Mutant complementation tests: The test for quantitative failure to complement using mutants is similar to that for deficiencies. However, since no genomic control line is available, we instead assessed aggressive behavior for 10 replicates of each of the following genotypes: *mutant*/*Ore*, *mutant*/*3-88Я*, *Bal*/*Ore*, and *Bal*/*3-88Я*, where *Bal* is the balancer chromosome over which the mutation is maintained. The same ANOVA model was used, and the same criteria were applied to assess quantitative complementation. Again, we excluded cases of failure to complement where the difference between *Bal*/*Ore* and *Bal*/*3-88Я* means was not significantly different than that between *mutant*/*Ore* and *mutant*/*3-88Я* means. Mutants meeting these criteria were retested using 20 replicates per genotype. Results from both blocks of testing were pooled and tested together using the model

$$y = \mu + L + G + B + L \times G + L \times B + G \times B + L \times G \times B + E,$$

where L is the line term, G is the genotype term and B is the block term.

All statistical analyses were conducted using Statistical Analysis Software Version 8.2 or JMP Version 7.0 (SAS; Cary, NC).

RESULTS AND DISCUSSION

QTL mapping using introgression lines: A mapping population of RILs derived from *Ore* and *2b* has been used to map QTLs affecting a large number of complex traits. We assessed whether these strains also differed in aggressive behavior, and found that the MAS of *Ore* males (20.82 ± 2.25) was greater than twice that of *2b* males (8.55 ± 1.17) ($t = 2.09$, $p < 0.0001$). We hypothesized that we could improve the power to map QTLs while reducing the number of lines to be scored if we constructed a population of segmental introgression lines, each of which contains a fragment of the *2b* genome in an otherwise *Ore* background, but which together comprise the entire 2b genome (Figure 1, Supplementary Table 1).

There was significant variation in mean aggression scores among the 20 introgression lines ($F_{19, 359} = 10.72$, $p < 0.0001$; Figure 1). The estimate of broad sense heritability of aggression levels in the introgression lines is $H^2 = 0.33$. All but two lines (*2-16* and *3-34Я*) exhibited significantly ($p < 0.05$) higher levels of aggression than *2b*. Five lines differed from *Ore*: *2-16*, *3-17*, *3-31Я*, and *3-34Я* were less aggressive, and line *3-88Я* was more aggressive.

We used these data to infer the locations of QTLs affecting the difference in aggressive behavior between *Ore* and *2b*. None of the *X* chromosome introgressions were significantly different from *Ore*, and all were different from *2b*. The most parsimonious

inference is that there are no *X*-linked QTLs affecting the difference in aggressive behavior between these strains (Figure 1).

The MAS of chromosome *2* introgression line *2-16* is significantly different from *Ore*, but not *2b*, and therefore contains at least one QTL affecting aggressive behavior. However, the introgressions in lines *2-18* and *2-81Я* together include the entire *2b* fragment in *2-16*, which is not consistent with a single QTL model. There are three possible two-QTL models that explain this observation (Figure 1). (1) There is a QTL with a negative effect on aggressive behavior between 29F;30D and another with an equal positive effect in the region of 27B. Line *2-16* contains only the negative effect QTL, while lines *2-78Я* and *2-18* contain both QTLs. (2) There is a QTL with a negative effect on aggressive behavior between 34EF;38E and another with an equal positive effect between 39A;50B. Line *2-16* contains only the negative effect QTL, while line *2-81Я* contains both QTLs. (3) Line *2-16* contains two QTLs between 29F;30D and 34EF;38E that interact epistatically to reduce the MAS; neither QTL on its own has a detectable effect on aggressive behavior. These models are indistinguishable based on the available data.

There are at least three QTLs on chromosome *3*. Lines *3-34Я*, *3-31Я*, *3-17* and *3-27* do not have statistically different MAS from each other (data not shown); *3-34Я*, *3-31Я*, and *3-17* have significantly lower MAS than *Ore*, and *3-31Я*, *3-17* and *3-27* have significant higher MAS than *2b*. Taken together, these results are consistent with a QTL affecting reduced aggressive behavior in the *2b* region common to all these introgressions, from 97D;100F. Surprisingly, the MAS of line *3-88Я*, which includes

only the 65D;69D region of the *2b* genome, is significantly different from both *Ore* and *2b*, and is nearly twice as aggressive as *Ore*. Therefore, the effect of one or more QTLs in this interval must be suppressed by another QTL elsewhere on the *2b* chromosome *3*. Further, this introgression is completely overlapped by the introgressions in *3-15* and *3-5*, indicating that there must be a least two epistatic QTLs in the 65D;69D region, one between 65D;67D and another in the region of 69D (the region of non-overlap of *3-15* and *3-5*). We must further postulate that neither epistatic QTL has an effect on its own, but that the effect of both are suppressed by the QTL at 97D;100F, since line *3-34Я* contains all three QTLs, but is not significantly different from lines *3-17* and *3-27*, which only contain the 97D;100F QTL (Figure 1).

Therefore, of the five QTLs affecting aggressive behavior, at least three and possibly all five interact epistatically. We previously inferred that epistatic interactions were prevalent among loci affecting natural variation in aggressive behavior, since the estimate of additive genetic variance from response to artificial selection (EDWARDS *et al.* 2006) was much less than the estimate of the total genetic variance among inbred lines derived from the same base population as the selection lines (EDWARDS *et al.* 2008). In the light of extensive epistasis shaping the genetic architecture of aggressive behavior, we cannot be certain that there are no QTLs for aggressive behavior on the *X* chromosome, only that we cannot detect them with the available genotypes. Furthermore, this experimental design enables us to detect QTLs affecting aggressive behavior and infer the presence of interactions among them, but we do not have enough information to estimate all additive and epistatic effects.

Deficiency complementation mapping: There are at least two epistatically interacting QTLs with a large effect on aggressive behavior in the 65D;69D region. Therefore, we used complementation to deficiencies (PASYUKOVA *et al.* 2000) to map these QTLs with higher resolution. Deficiency mapping has been used to successfully fine-map QTL affecting variation between *Ore* and *2b* for many quantitative traits (DE LUCA *et al.* 2003; PASYUKOVA *et al.* 2000; FANARA *et al.* 2002; HARBISON *et al.* 2004; JORDAN *et al.* 2006; MOEHRING and MACKAY 2004). These analyses typically reveal that single QTLs fractionate into multiple linked QTLs.

The precise breakpoints of this QTL are somewhere between 65A;65D and 69D;70C; therefore we used deficiencies spanning the 65A;70C cytological interval. We introduced two improvements over previous deficiency complementation tests. First, introgression line *3-88Я* contains this region of the *2b* genome in an otherwise *Ore* genetic background. Therefore we used *3-88Я* and *Ore* as the parent lines for the complementation tests. This limits the potential for non-complementation due to epistatic interactions to interactions between QTLs in this region only. Second, we used deficiency stocks from the DrosDel and Exelixis (PARKS *et al*. 2004, RYDER *et al*. 2007) collections. These deficiencies have been constructed in isogenic backgrounds and do not contain any additional mutations, which reduces the likelihood of identifying a region that fails to complement due to epistatic interactions. Furthermore, the breakpoints of the deficiencies are molecularly defined, enabling more precise mapping.

We crossed *Ore* and *3-88Я* to 27 deficiency stocks (*Df*/*Bal*, where *Bal* is a balancer chromosome) with overlapping breakpoints spanning the QTL region (Figure 2);

and to a w^{1118} control line, which had the appropriate isogenic background in which the deficiency stocks were constructed. We scored the aggressive behavior of F_1 males of four genotypes: *Df*/*Ore*, *Df*/*3-88Я*, w^{1118}/*Ore* and w^{1118}/*3-88Я-3*. We analyzed the data for each deficiency by factorial ANOVA (Table 1), which partitions variation among the genotypes into the cross-classified main effects of Line (*Ore*, *3-88Я*), Genotype (*Df* , w^{1118}) and the Line × Genotype interaction. We inferred complementation if the Line × Genotype interaction term was not significant, as this indicates that the difference in phenotype between *Ore* and *3-88Я* was the same in the *Df* and w^{1118} chromosome backgrounds. We inferred failure to complement if the Line × Genotype interaction was significant, which indicates the difference in aggressive behavior between *Ore* and *3-88Я* varied between the *Df* and w^{1118} chromosome backgrounds. We then delineated the QTL locations by the region of non-overlap of deficiencies complementing the trait phenotype with those that fail to complement the trait phenotype.

Six of the deficiency stocks exhibited significant ($p < 0.05$) quantitative failure to complement (22% of those tested). Thus, the QTL region was fractionated into multiple smaller QTL. Four of the QTL were in the first of the epistatic QTL inferred from the analyses of the introgression lines (64E5;64F5, 65E8;65F4, 67B10;67C5 and 67D1;67D2), while the other two QTL were in the region of the second epistatic QTL (69B5;69C4, and 69C4;69F6). The effects of all six of these QTL were in the same direction, with the *Df/3-88Я* genotype, which elucidates the effect of the *2b* allele in that region, exhibiting higher aggression than the *Df/Ore* genotype. An additional QTL in the region of the second epistatic QTL, 70A3;70C10, approached formal significance (p =

0.0554). We included candidate genes from this region in complementation tests to mutants, although the direction of effect of this QTL was opposite to the others, with the *Df/Ore* allele exhibiting higher levels of aggression.

The 65A;70C cytological interval encompasses nearly 1000 genes mapped to the sequence. Deficiency complementation mapping narrowed the QTL intervals to approximately 300 genes: 43 in 64E5;F5, 17 in 65E7;F4, 49 in 67B10;C5; 14 in 67D1;D2, 22 in 69B5;C4, 86 in 69C4;F6 and 70 in 70A3;70C10. Many of the genes in these regions affect metabolism and development.

Mutant complementation tests: We used quantitative complementation tests to mutations in positional candidate genes in QTL intervals defined by deficiency complementation tests to identify candidate genes corresponding to QTLs affecting the difference in aggressive behavior between *Ore* and *2b*. The logic of quantitative complementation tests to mutations is the same as that for deficiency complementation tests. However, the mutations are maintained over balancer chromosomes with visible markers, and they are not all available in a common isogenic background. Therefore, we crossed each mutant stock to both *3-88Я* and *Ore*, and measured the aggressive behavior of F_1 males of the four genotypes: *mutant/3-88Я*, *mutant/Ore*, *Bal/3-88Я* and *Bal/Ore*, where *Bal* is the balancer chromosome.

We initially screened 58 mutants in positional candidate genes from all seven QTLs defined by the deficiency complementation tests (Supplementary Table 2). Where possible, we used *P*-element insertional mutations that were generated in the same background. We also chose stocks with few additional mutations, and included

mutations in computationally predicted genes. Of the 58 mutants tested, 12 (~21%) exhibited quantitative failure to complement, inferred by a significant ($p < 0.05$) Line × Genotype interaction term. We excluded five of these mutations from further analyses since the pattern of failure to complement did not meet our strict criterion for allelic non complementation. We retested the remaining seven mutants, using twice the number of replicates as in the initial screen, and confirmed four mutations that exhibited quantitative failure to complement the QTL alleles affecting the difference in aggressive behavior between *Ore* and *3-88Я*: *CG11006*, *CG10754*, *mutagen-sensitive 312* and *Ral guanine nucleotide exchange factor 2* (Figure 3). None of these loci have been previously associated with aggressive behavior. The *3-88Я/mutant* genotype exhibited higher levels of aggression than the *Ore/mutant* genotype for *CG10754*, *CG11006*, and *Rgl*; this observation was reversed for the *mus312* mutant. That flies hemizygous for the *88Я-3* allele at all loci are not the more aggressive underscores the complex nature of QTL affecting aggression. The cumulative effects and potential epistatic interactions at these and additional, unidentified, loci must be teased apart in great detail, as they are neither necessarily predictable nor intuitive.

We did not identify candidate genes in the 64E5;64F5, 67B10;67C5, and 67D1;67D2 QTLs, and it is possible there are additional candidate genes in the QTLs in which we did identify a candidate gene. Ideally, all positional candidate genes should be screened, but there were many of the 300 genes for which no mutation was available, and we did not test mutations in candidate genes when the stock contained multiple additional mutations.

Candidate quantitative trait genes for aggressive behavior: *CG11006* has four predicted transcripts and is annotated as being involved in 'cellular component organization and biogenesis' (LYNE *et al*. 2007). *CG11006* is highly expressed in the brain, ovary, and male accessory glands (CHINTAPALLI *et al*. 2007). The CG11006 protein interacts with ten other gene products, many of which are also computationally predicted (GIOT *et al.* 2003). However, those that have been described function in central nervous system development, bristle morphogenesis, and calcium ion binding. It is orthologous to the human DMD gene, which is implicated in both Becker and Duchenne muscular dystrophy (BOYCE *et al*. 1991, WOOD *et al.* 1987).

CG10754 is adjacent to and partially overlapping with *CG11006*. *CG10754* has one predicted transcript, is annotated to function in RNA splicing, metabolism, and zinc binding (LYNE *et al*. 2007), and is expressed in the ovary and male accessory glands (CHINTAPALLI *et al*. 2007). No gene ontology information is available for the two predicted gene products with which *CG10754* interacts (GIOT *et al.* 2003). Variation in *CG10754* transcript abundance is associated with chill coma recovery time (AYROLES *et al.* 2008) and sleep behavior (HARBISON *et al.* 2008) in a reference panel of inbred lines derived from a natural population.

mutagen-sensitive 312 (*mus312*) has three transcripts, and functions in DNA damage recognition and repair, protein binding, and metabolism (LYNE *et al*. 2007). *mus312* is expressed in the ovary and testes, as well as other tissues (CHINTAPALLI *et al*. 2007). The MUS312 protein interacts with *CG5410* and *CG8942* gene products, which

are involved in mitochondrial transport and Wnt receptor signaling, respectively (GIOT *et al.* 2003).

Ral guanine nucleotide exchange factor 2 (*Rgl*) encodes four transcripts and is involved in G-protein coupled receptor and tyrosine kinase signaling (LYNE *et al*. 2007). It is highly expressed in the brain, crop, ovary, and male accessory glands (CHINTAPALLI *et al*. 2007). RGL interacts with CG2091, encoded by a computationally predicted gene with no known function (GIOT *et al.* 2003). *Rgl* is orthologous to the human gene SOS1, which is implicated in the dysmorphic Noonan Syndrome (ROBERTS *et al.* 2007, TARTAGLIA *et al.* 2007).

The four candidate quantitative trait genes affecting the difference in aggressive behavior between *Ore* and *3-88Я* have little in common other than that they are all expressed in reproductive tissues. *CG10754* and *Rgl* are potentially involved in neurotransmission through their roles in zinc binding and as a G-protein coupled receptor, respectively.

Complex genetic architecture of aggressive behavior: We have identified four novel candidate genes affecting natural variation in aggressive behavior in a small genomic region, in two laboratory strains not selected for aggressive behavior. The QTL regions interact epistatically: it remains to be seen how the candidate genes interact, and what are the gene(s) in the *2b* 97D;100F QTL region that suppress the effect of the *2b* 65D;69D QTLs on aggressive behavior. These loci were not implicated in previous studies that identified large numbers of potential candidate genes affecting aggressive behavior from whole genome expression profiling of genetically divergent strains

recently derived from nature (EDWARDS *et al*. 2006; EDWARDS *et al*. 2008). This is not surprising, since different loci can affect variation in complex traits in different strains. Further, the four loci described in this report may not affect aggression through differences in gene expression; they may differ in expression but these differences were too small to detect in the previous studies; or differences in gene expression at a different developmental stage than young adults (when the transcriptional profiles were obtained) are important. Nevertheless, these results highlight the utility of unbiased genome scans for naturally segregating QTLs for identifying novel genes and genetic networks affecting complex behaviors.

Acknowledgements: This work has been supported by NIH grants GM R01 GM076083 to T. F. C. M. and F31 MH 74161 to A. C. E. This is a publication of the W. M. Keck Center for Behavioral Biology.

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Table 1. Deficiency complementation tests. Deficiency stocks used in quantitative complementation tests. ED denotes DrosDel deficiencies and Exel indicates Exelixis deficincies. The isogenic background stocks for both sets of deficiencies are listed as controls. Bold font indicates deficiencies that failed to complement the QTLs for aggressive behavior (*p*- values of the L×G term from ANOVA < 0.05). *Df(3L)ED4502* was marginally significant and was therefore included in the QTL mapping analysis.

Deficiency Genotype	**Deficiency Breakpoints**	**Mean Aggression Score (SE)**		**ANOVA *p*-values**		
		***Df/88* MAS**	***Df/Ore* MAS**	**Line (*L*)**	**Genotype (*G*)**	***L* × *G***
w^{1118}; Df(3L)ED4341/TM6C, Sb^1	63F6;64B11	12.1 (1.12)	12.0 (1.21)	0.9820	0.0082	0.9224
w^{1118}; Df(3L)ED4342/TM6C, Sb^1	64B1;64B13	15.6 (1.54)	16.1 (1.89)	0.8175	0.8947	0.9054
w^{1118}; Df(3L)ED210/TM6C, Sb^1	64B11;64D1	22.0 (1.75)	18.3 (1.86)	0.2237	0.0026	0.1850
w^{1118}; Df(3L)Exel6105/TM6B, Tb^1	64D1;64D6	16.9 (0.98)	19.9 (0.66)	0.0499	0.0103	0.4879
w^{1118}; Df(3L)Exel6106/TM6B, Tb^1	64D6;64E2	22.8 (1.04)	22.5 (1.83)	0.6360	<.0001	0.4709
w^{1118}; Df(3L)Exel6107/TM6B, Tb^1	**64E5;64F5**	**30.3 (2.30)**	**21.8 (1.87)**	**0.0089**	**<.0001**	**0.0003**

Table 1 (continued)

Deficiency Genotype	Deficiency Breakpoints	Mean Aggression Score (SE)		ANOVA *p*-values		
		Df/88 MAS	*Df/Ore* MAS	Line (*L*)	Genotype (*G*)	$L \times G$
w^{1118}; Df(3L)Exel7210/TM6B, Tb^1	65A1;65A5	17.5 (0.98)	20.2 (2.23)	0.0997	0.0080	0.6151
w^{1118}; Df(3L)Exel8101/TM, Tb^1	65A3;65A9	21.1 (1.84)	18.5 (1.46)	0.6404	0.0007	0.1048
w^{1118}; Df(3L)ED211/TM6C, Sb^1	65A9;65B4	11.9 (1.57)	11.3 (0.94)	0.8709	0.0036	0.7789
w^{1118}; Df(3L)ED212/TM6C, Sb^1	65A9;65D5	21.4 (2.77)	18.8 (1.05)	0.4203	0.0043	0.3622
w^{1118}; Df(3L)Exel6110/TM6B, Tb^1	65E4;65E8	16.5 (1.15)	17.0 (1.11)	0.4007	0.2697	0.6828
w^{1118}; Df(3L)Exel6111/TM6B, Tb^1	**65E7;65F4**	**24.3 (1.24)**	**16.9 (1.02)**	**0.0114**	**<.0001**	**0.0002**
w^{1118}; Df(3L)Exel8104/TM6B, Tb^1	65F7;66A4	19.3 (1.63)	15.7 (1.16)	0.4032	0.1167	0.0519
w^{1118}; Df(3L)ED4408/TM6C, Sb^1	66A22;66C5	12.8 (1.32)	10.2 (1.44)	0.3737	0.0032	0.3146
w^{1118}; Df(3R)Exel7317/TM6B, Tb^1	66C5;66D3	17.8 (0.88)	16.2 (1.12)	0.9453	0.1820	0.1838
w^{1118}; Df(3L)ED4421/TM6C, Sb^1	66D14;67B1	11.9 (1.89)	8.8 (1.89)	0.3173	0.0005	0.2679

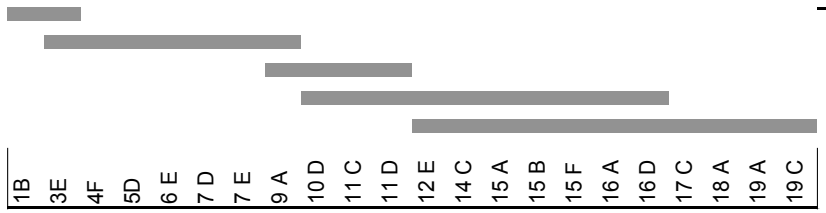
Table 1 (continued)

Deficiency Genotype	Deficiency Breakpoints	Mean Aggression Score (SE)		ANOVA *p*-values		
		Df/88 MAS	*Df/Ore* MAS	Line (*L*)	Genotype (*G*)	$L \times G$
w^{1118}; Df(3L)Exel6114/TM6B, Tb^{1}	**67B10;67C5**	**18.5 (1.21)**	**15.3 (0.87)**	**0.4438**	**0.2143**	**0.0450**
w^{1118}; Df(3L)Exel9048/TM6B, Tb^{1}	**67D1;67D2**	**32.2 (1.33)**	**18.1 (1.27)**	**<.0001**	**<.0001**	**<.0001**
w^{1118}; Df(3L)ED4470/TM6C, Sb^{1}	68A6;68E1	14.0 (1.86)	11.6 (2.20)	0.4551	0.0590	0.3935
w^{1118}; Df(3L)ED4483/TM6C, Sb^{1}	69A4;69D3	17.7 (1.17)	17.0 (2.03)	0.8489	0.2353	0.7615
w^{1118}; Df(3L)ED215/TM6C, Sb^{1}	**69B5;69C4**	**16.9 (1.93)**	**10.9 (0.72)**	**0.0372**	**0.2946**	**0.0282**
w^{1118}; Df(3L)ED4486/TM6C, Sb^{1}	**69C4;69F6**	**23.9 (2.16)**	**14.3 (1.59)**	**0.0018**	**0.0212**	**0.0013**
w^{1118}; Df(3L)Exel6261/TM6B, Tb^{1}	69F6;70A3	18.5 (1.44)	15.8 (1.09)	0.5931	0.1556	0.0805
w^{1118}; Df(3L)ED4502/TM6C, Sb^{1}	**70A3;70C10**	**14.9 (1.08)**	**20.3 (1.45)**	**0.0424**	**0.1539**	**0.0554**
w^{1118}; Df(3L)ED4543/TM6C, Sb^{1}	70C6;70F4	14.6 (2.34)	11.9 (1.18)	0.3929	0.1078	0.3366
w^{1118}; Df(3L)ED217/TM6C, Sb^{1}	70F4;71E1	20.0 (1.75)	17.8 (1.43)	0.4725	0.0267	0.4068

Deficiency Genotype	Deficiency Breakpoints	Mean Aggression Score (SE)		ANOVA *p*-values		
		***Df/88* MAS**	***Df/Ore* MAS**	**Line (*L*)**	**Genotype (*G*)**	***L* × *G***
w^{1118}; Df(3L)ED218/TM6C, Sb^{1}	71B1;71E1	17.6 (0.90)	15.6 (0.91)	0.4800	0.4708	0.4072
w^{1118} (Exel Control)	n/a	14.8 (0.67)	16.2 (0.96)	n/a	n/a	n/a
w^{1118} (ED Control)	n/a	15.6 (0.80)	15.7 (1.16)	n/a	n/a	n/a

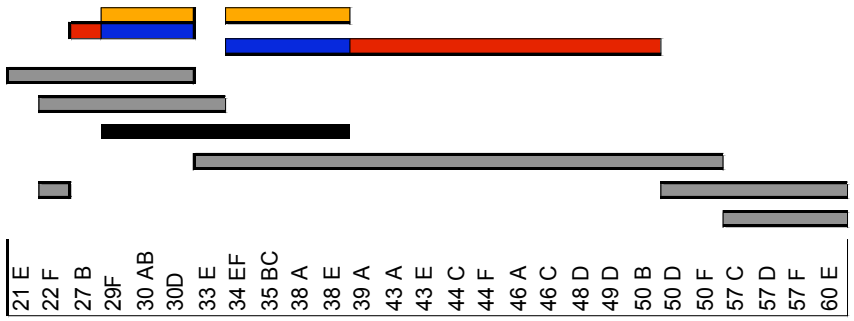
Figure 1. QTL mapping using introgression lines. Grey bars represent the region of the genome derived from the *2b* parental line for each introgression line on (A) the *X* chromosome, (B) chromosome *2*, and (C) chromosome *3*. The cytological locations of *roo* transposable element markers are depicted below each set of lines. Grey bars denote introgressions with a mean aggression score (MAS) that are not significantly different from *Ore* but are different from *2b*; black bars denote introgressions with MAS that are significantly different from *Ore* but not significantly different from *2b*, and grey/black bars denote introgressions with MAS that are intermediate between *Ore* and *2b*, and significantly different from both parental lines. The green bar denotes an introgression with a MAS that is significantly greater than either parent line. The QTL locations are inferred using this physical map in conjunction with the MAS of each introgression line and statistical comparisons of the MAS between the introgression line and the parental lines. Orange bars represent QTLs with epistatic effects; red bars indicate QTLs with positive additive effects; and blue bars represent QTL with negative additive effects. Three possible models explain the QTL on chromosome *2*, shown here in different rows (see text for explanation). Significance levels were determined using Student's *t*-tests. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

A



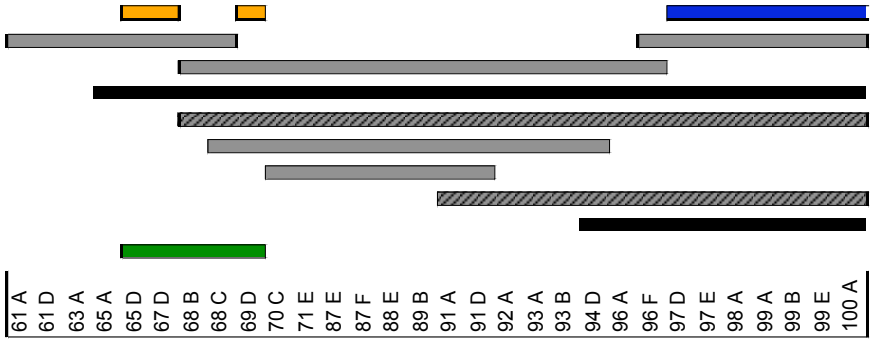
Line	MAS ± SE	*p*-value *Ore*	*p*-value *2b*
X-20	24.15 ± 1.69	ns	****
X-27	17.12 ± 1.64	ns	****
X-21	21.75 ± 1.45	ns	****
*X-43_*	21.85 ± 1.47	ns	****
X-76	23.7 ± 1.68	ns	****

B



Line	MAS ± SE	*p*-value *Ore*	*p*-value *2b*
*2-78_*	19 ± 2.34	ns	***
2-18	26.3 ± 1.67	ns	***
2-16	9.08 ± 1.82	***	ns
*2-81_*	23.4 ± 2.22	ns	***
*2-6_*	17.3 ± 2.04	ns	**
2-22	22.6 ± 1.57	ns	***

C



Line	MAS ± SE	*p*-value *Ore*	*p*-value *2b*
3-15	18.6 ± 1.50	ns	***
3-5	18.4 ± 1.65	ns	***
*3-34_*	11.55 ± 1.43	**	ns
*3-31_*	13.65 ± 1.72	*	*
3-22	19.2 ± 16.9	ns	***
3-16	18.95 ± 1.27	ns	***
3-17	13.67 ± 1.71	*	*
3-27	14.95 ± 1.95	ns	*
*3-88_*	34.5 ± 1.89	***	***

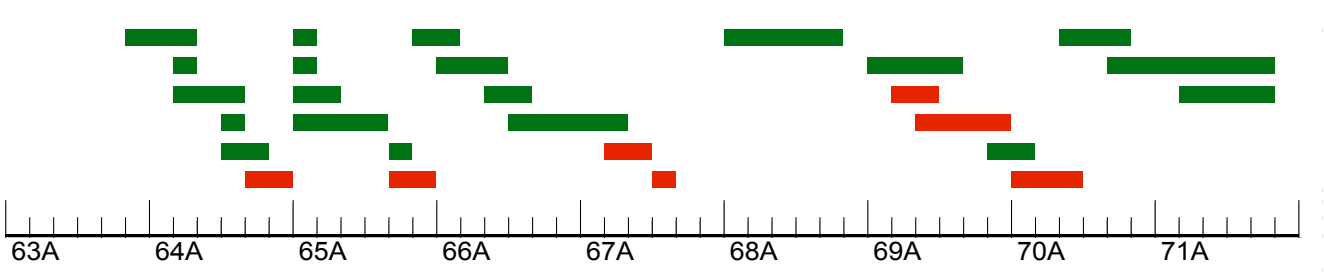
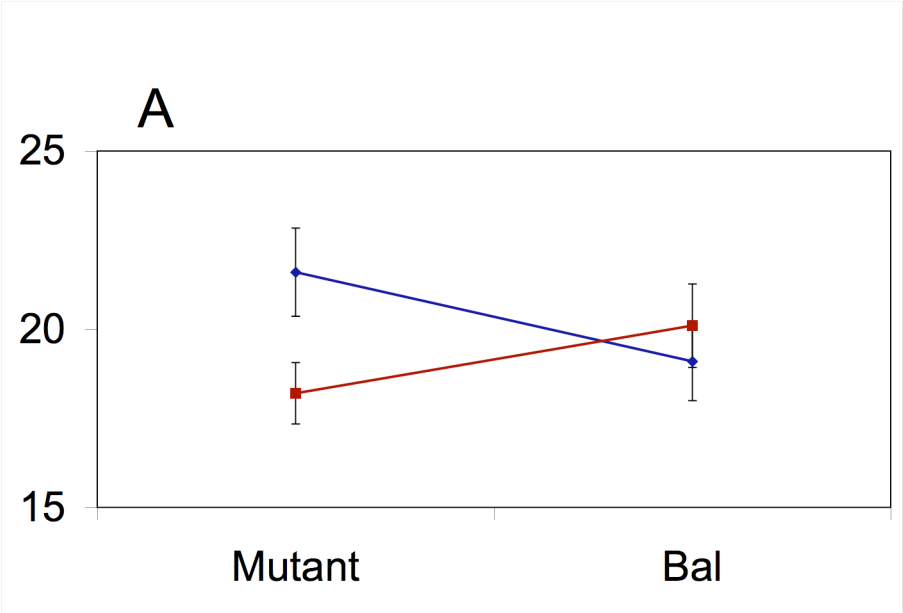


Figure 2. Deficiency complementation mapping. The bars depict the approximate cytological locations of deficiencies used for complementation mapping. Red bars indicate deficiencies that fail to complement QTLs affecting aggressive behavior; green bars represent deficiencies that complement QTLs affecting aggression.

Figure 3. Mutant complementation tests. Means and standard errors of aggression scores (*y*-axis) are depicted for the four genes what exhibit quantitative failure to complement *Ore* and *3-88Я* alleles. The blue lines represent the *3-88Я* line, and the red lines represent the *Ore* line. Genotype is depicted on the x-axis, such that four genotypes are shown: *Ore/Bal, 3-88Я/Bal, Ore/mutant,* and *3-88Я/mutant*. (A) $CG10754^{f03161}$. (B) $CG11006^{EY12079}$. (C) $mus312^{D1}$. (D) $Rgl^{GT-000359}$.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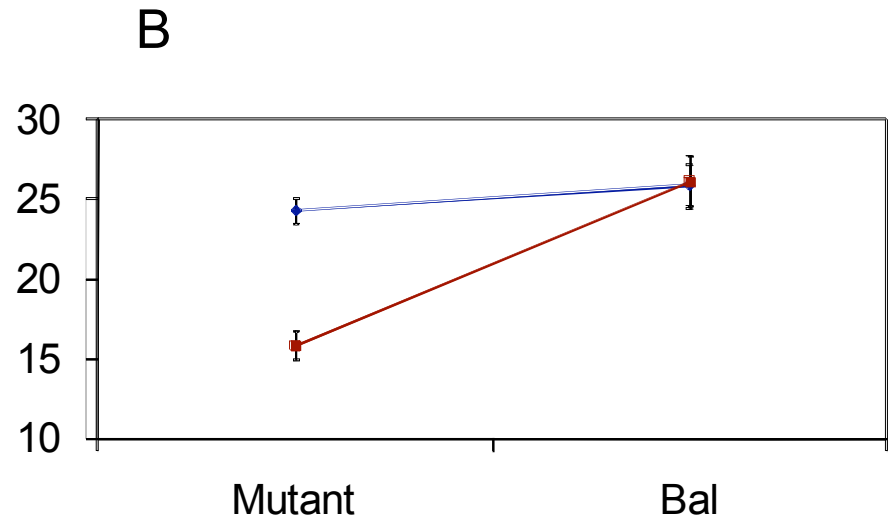
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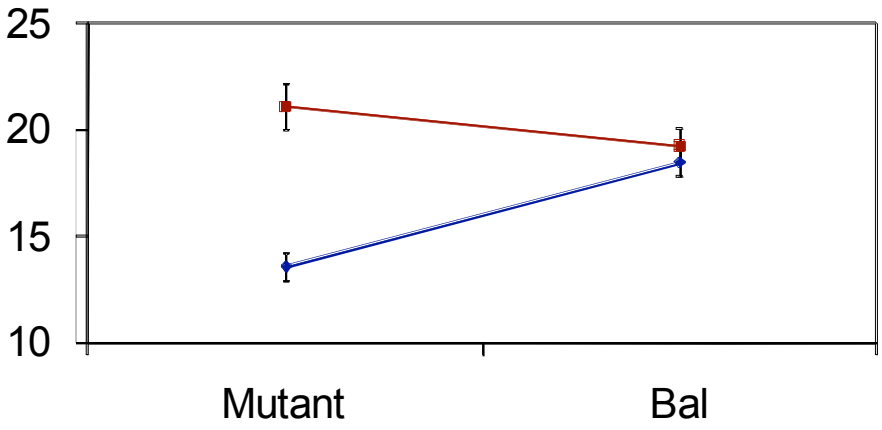

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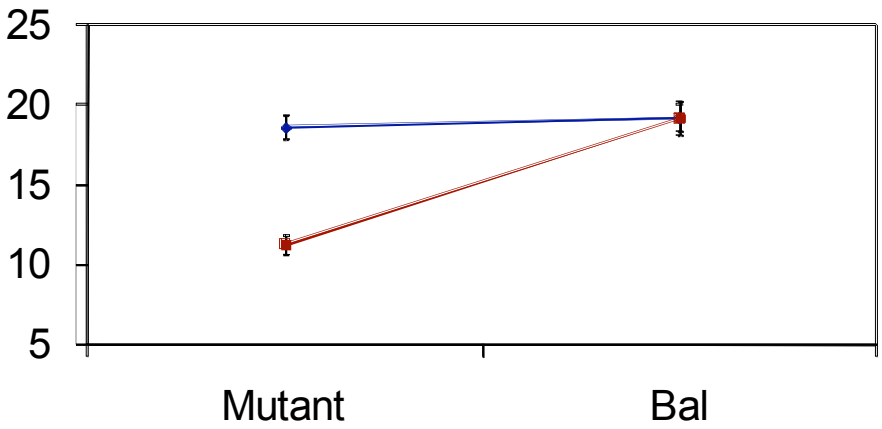

CHAPTER FIVE

A Transcriptional Network Associated With Natural Variation in *Drosophila* Aggressive Behavior

BIOLOGICAL SCIENCES: Genetics

A Transcriptional Network Associated With Natural Variation in *Drosophila* Aggressive Behavior

Alexis C. Edwards[*,‡], Julien F. Ayroles[*,‡], Eric A. Stone[†,‡] and Trudy F.C. Mackay[*,‡,§]

[*] Department of Genetics, Box 7614, North Carolina State University, Raleigh, North Carolina 27695, USA. [†] Department of Statistics, Box 7566, North Carolina State University, Raleigh, North Carolina 27695, USA. [‡] W. M. Keck Center for Behavioral Biology, Box 7617, North Carolina State University, Raleigh, North Carolina 27695, USA.

[§] To whom correspondence should be addressed: Dr. Trudy F. C. Mackay, Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614, USA. Tel. 919-515-5810; Fax.919-515-3355; E-mail: trudy_mackay@ncsu.edu

25 pages, 7 figures, 2 supplementary figures, 0 tables, 5 supplementary tables

This chapter consists of a manuscript that has been prepared for submission for publication. I conducted behavioral assays on all lines. I conducted all bioinformatics. I wrote the manuscript. Trudy Mackay supervised the research project and provided extensive comments on the organization and content of the manuscript.

ABSTRACT

Aggressive behavior is an important component of fitness in most animals, and pathological levels of aggression pose serious problems in human society. Aggressive behavior is genetically complex, with natural variation attributable to multiple segregating loci with allelic effects that are sensitive to the physical and social environment. However, we know little about the genes and genetic networks affecting population variation in aggressive behavior. Populations of *Drosophila melanogaster* harbor substantial quantitative genetic variation in aggressive behavior, providing an excellent model system for dissecting the genetic basis of naturally occurring variation in aggression. Correlating variation in transcript abundance with variation in complex trait phenotypes is a rapid method for identifying candidate genes affecting quantitative traits. Here, we quantified aggressive behavior in 40 wild-derived inbred lines of *D. melanogaster* and performed a genome wide association screen for quantitative trait transcripts (QTTs) and single feature polymorphisms (SFPs) affecting aggression. We identified 266 novel candidate genes, many of which have pleiotropic effects on metabolism, development, and/or other behavioral traits. We performed behavioral tests of mutations in 12 of these candidate genes, and showed that nine indeed affected aggressive behavior. We used the genetic correlations among the QTTs to derive a transcriptional genetic network associated with natural variation in aggressive behavior. The network consists of nine modules of correlated transcripts that are enriched for genes affecting common functions, expressed in the same tissues, and/or sequence motifs. This

approach establishes a foundation for understanding natural variation for complex behaviors in terms of networks of interacting genes.

INTRODUCTION

Animals display aggressive behaviors in defense of territory, to secure and defend food and mates, and to establish dominance hierarchies. These behaviors are, however, energetically costly and individually risky, suggesting that excessive aggression may be deleterious. In humans, aggression often manifests as violent behavior with attendant costs to society, and is frequently a component of psychiatric disorders, including schizophrenia, conduct disorder, alcoholism, bipolar disorder, and Alzheimer's Disease (1-4). Analysis of mutations and pharmacological treatments have established that aggressive behavior is evolutionarily conserved and is modulated by the neurotransmitters serotonin, dopamine, norepinephrine, γ-aminobutyric acid, histamine and nitric oxide; their receptors, transporters and key enzymes in their biosynthetic pathways in mammals (5) and invertebrates (6). However, these molecules are not the only players. In mice, mutations in *fierce*, a nuclear receptor (7), *neural cell adhesion molecule* (8), *interleukin-6* (9) and *Cathepsin E* (10) affect aggressive behavior. In *Drosophila*, aggressive behavior is correlated with levels of β-alanine (11, 12), correct expression of sex-specific transcripts of *fruitless* (13, 14), biogenic amines (11, 15), and expression of neuropeptide F (15).

Levels of aggression vary continuously in natural populations, due to the segregation of alleles at multiple loci with effects that depend on the social and physical environment: aggressive behavior is thus a typical quantitative trait (16). In contrast to our understanding of the neurobiological and genetic mechanisms responsible for the manifestation of aggressive behavior, we know very little of the genes and genetic networks affecting natural variation in aggression. Hints that the genetic architecture of aggressive behavior may be complex come from studies examining correlated responses of the *Drosophila* transcriptome to artificial selection for aggressive behavior in a laboratory stock (17) and a population recently derived from nature (18). These studies showed that the expression of 80 (17) – 1,539 transcripts (18) involved in a wide variety of biological processes and molecular functions varied between the selected and control lines. Subsequent analysis of the effects of mutations in genes encoding some of these transcripts showed that *Cyp6a20* (17) and 15 other novel genes (18) (*muscleblind*, *CG17154*, *CG5966*, *CG30015*, *Darkener of apricot*, *CG14478*, *CG12292*, *tramtrack*, *CG1623*, *CG13512*, *SP71*, *longitudinals lacking*, *scribbler*, *Male-specific RNA 87F*, *kismet*) affect aggressive behavior. However, the genotypes created by artificial selection are different from any naturally segregating genotype, and it is possible that novel combinations of alleles perturb the transcriptome beyond the range of variation that would be found in a population of wild type alleles. In addition, selection induces linkage disequilibrium between selected and linked loci, raising the possibility that some correlated transcriptional responses to selection are due to linkage drag.

Here, we quantified male aggressive behavior for 40 inbred lines derived from the same population, and performed a genome wide association scan for QTTs (19) and SFPs (20) associated with aggressive behavior in wild type genotypes. This unbiased genomic approach reveals natural genetic variation that is correlated with aggression at the level of allelic differences and networks of genetically correlated transcripts.

RESULTS AND DISCUSSION

Natural variation in aggressive behavior. We quantified aggressive behavior of 40 wild-derived inbred lines, using a rapid and high-throughput behavioral assay (18). Variation in aggressive behavior was continuously distributed among these lines, as expected for a quantitative trait. There was significant genetic variation in aggression among lines ($F_{40, 779} = 73.0168$, $p < 0.0001$, Fig. 1). Estimates of among line (σ_L^2) and within line (σ_E^2) variance components were $\sigma_L^2 = 0.783$ and $\sigma_E^2 = 0.217$, for a broad-sense heritability (H^2) of aggressive behavior of $H^2 = 0.78$. Surprisingly, there was a 25-fold range of aggressive behavior in these lines: from an average of 3.3 to 76.9 aggressive encounters for eight flies in a two minute observation period.

The variation among the inbred lines far exceeds that of lines selected for 21 generations for increased and decreased aggressive behavior, which only differ less than three-fold (with a mean of 14.2 and 34.2 encounters in the high and low selection lines using the same assay) (18). Under a strictly additive model, we expect variation among fully inbred lines to be twice the additive genetic variation in the base population from which they were derived (16). Thus, under strict additivity, the estimate of the narrow

sense heritability (h^2) in this population would be $h^2 = 0.64$. This is much greater than the estimate of realized h^2 from response to selection ($h^2 \approx 0.09$) (18), indicating that alleles affecting natural variation are recessive and/or interact epistatically.

Candidate genes for aggressive behavior. Previously, we quantified variation in gene expression among these wild-derived inbred lines (21). A total of 7,508 transcripts were significantly variable among lines in males at an FDR < 0.01 and 3,316 probes contained SFPs. We identified 133 QTTs ($p < 0.01$) associated with variation in aggressive behavior (Supplementary Table 1). In addition, 167 SFPs ($p < 0.05$) with a minor allele frequency of at least 10% were associated with variation in aggression; these represent 137 independent genes (Supplementary Table 2). Four of the QTTs were also implicated as candidates from the SFP analysis (*CG1146*, *CG2556*, *CG31038* and *methuselah-like 8*). No gene ontology information is available for three of these genes (*CG1146*, *CG2556*, and *CG31038*). *methuselah-like 8* is a predicted G protein coupled receptor that may affect the determination of life span (22).

In total, these analyses implicate 266 unique candidate genes affecting natural variation in aggressive behavior. These candidate genes are involved in a broad spectrum of biological processes, including vision, olfaction, learning and memory, and the development and function of the nervous system (Supplementary Tables 1 and 2, Supplementary Figs. 1 and 2). However, the candidate genes are also involved in transcription, protein modification, mitosis and other basic cellular processes (Supplementary Tables 1 and 2, Supplementary Figs. 1 and 2). More than half of the

genes with annotations are involved in metabolism, nearly 60% have protein binding functions, and approximately 25% are implicated in development (Fig. 2) (23).

Two categories of candidate genes are worthy of mention. We found a member of the Cytochrome P450 gene family associated with aggressive behavior, *Cyp4p2*. Members of this gene family have also been associated with aggressive behavior in previous studies (17, 18). Cytochrome P450s are generally involved in oxidation, metabolism, protection from xenobiotics, and possibly pheromone recognition (24). The repeated implication of this class of genes suggests that some or all of these functions mediate aggressive behavior, although it remains unclear precisely how. We also found three genes that have been previously implicated in learning and/or memory to be associated with natural variation in aggression in this screen: *nord*, *visgun*, and *klingon* (25), consistent with a previous report that Drosophila aggressive behavior is associated with learning and memory (26). Perhaps variation in these genes affects the fly's learning ability, which could subsequently influence the behavioral response to aggressive encounters. Assessment of these wild-derived lines in a learning and memory assay could inform our understanding of the relevance and variation of social memory in wild Drosophila.

A total of 26 of the 266 candidate genes identified in this study overlapped with the candidate genes implicated from the correlated response of the transcriptome to selection for divergent level of aggressive behavior (18), from a different sample of the same base population as the one from which the inbred lines were derived (Supplementary Table 3). This is no more overlap than expected by chance ($\chi_1^2 = 0.36$, p

> 0.05). There are several possible – and not mutually exclusive – reasons why the degree of overlap between the two experiments is not more extensive. First, the control line was the most extreme for many of the transcripts that were divergent among the selection lines; this type of transcript-phenotype association will not be detected in a linear regression. Second, selection causes linkage disequilibrium between the selected locus and linked unselected loci; changes in transcript abundance among these linked loci between the selection lines are false positive associations. In contrast, the rapid decay of linkage disequilibrium in regions of normal recombination in unselected Drosophila (27, 28) minimizes false positive associations of transcript abundance of linked loci in the unselected inbred lines. Third, a greater fraction of the genetic variation among the inbred lines than the selection lines is due to dominance and epistasis. The transcriptional signature of a homozygous recessive allele in the inbred lines is likely to be different from the same allele as a heterozygote in the selection lines. Thus, the overlap of genes between the two studies may be enriched for loci with additive effects that causally affect natural variation in aggressive behavior.

Functional tests. To evaluate whether the candidate genes suggested from these analyses potentially affect aggressive behavior, we assessed aggression levels of *P*-element insertional mutations in 12 of the candidate genes, and their co-isogenic control lines. Nine of the mutant alleles had significantly different aggression levels from the control (Fig. 3). This high "success" rate shows that expression profiling of wild-derived

genetically divergent lines is an efficient method for identifying candidate genes affecting complex traits, as has been observed previously (17, 18, 29-31).

Mutations in *CG11448*, *CG13760*, *CG2556*, *CG31038*, *CG32425*, *late bloomer* and *skuld* are all more aggressive than their controls, while mutations in *GTPase-activating protein 1* (*Gap1*) and *schizo* are less aggressive than the control strain. No gene ontology information is available for the predicted genes tested; however, *CG11448* is homologous to the amyloid beta A4 precursor protein, which is implicated in Alzheimer's Disease. *late bloomer* has a role in nervous system development and synapse biogenesis. It is homologous to TSPAN7, a tetraspanin protein implicated in mental retardation (32). *skuld* is involved in numerous transcription-related processes, and also has roles in metabolism and development. *Gap1* has roles in the cell cycle, and is also involved in signal transduction and numerous developmental processes, such as axis specification and sensory organ development. Finally, *schizo* is involved in several signal transduction pathways, the development of the central nervous system, and muscle development. It is homologous to the human protein ADP-ribosylation factor guanine nucleotide exchange factor 2, in which dysfunctions are associated with microcephaly (33).

Transcriptional network associated with aggression. The transcriptome is highly genetically inter-correlated (21). This correlation structure can be used to infer modules of genetically correlated transcripts associated with aggressive behavior, after removing the correlations among the transcripts attributable to their association with aggression

itself. The number and contents of modules are determined such that the average correlation of probe sets within a module is maximized, while the average correlation among probe sets in different modules is minimized. The 133 QTTs grouped into nine modules, ranging in size from two to 54 probe sets (Fig. 4*A*, Supplementary Table 4). The correlated transcript modules associated with aggressive behavior can also be represented as an interaction network, with edges between transcripts in the network determined by genetic correlations in transcript abundance exceeding a threshold value (Fig. 4*B* represents $|r| \geq 0.7$). Note that these are at present undirected networks. We do not know which transcripts are causally associated with variation in aggression, due to functional polymorphisms in *cis*-regulatory regions; and which transcripts are *trans*-regulated and change expression as a consequence of *cis*-regulatory variation at another locus (34).

We evaluated the biological plausibility of the modules by querying whether genes in the modules are enriched for shared gene ontology categories, tissue-specific expression patterns, or DNA sequence motifs (Multiple EM for Motif Elicitation, or MEMES). Approximately one third of the transcripts in Module 6 affect ion binding, relative to ~2% of the probe sets in the genomic background; this is a significant enrichment ($p = 0.0087$). Nearly 50% of the annotated genes in Module 6 are involved in establishment of localization, compared to ~13% of the background; 25-30% of the genes in Modules 6 and 7 are involved in cell communication, whereas only 13% of the background falls into that category (Fig. 5). Module 7 is enriched for several categories related to development (Fig. 5). Transcripts in Modules 6 and 7 are enriched in the brain,

head, and thoracicoabdominal ganglion (Fig. 6*A*), indicating that these genes function primarily in central nervous system functions. However, the fact that they fall into distinct modules suggests that their specific functions differ, or that they are differentially regulated in a temporally or spatially specific manner.

Additional support for the hypothesis that genes in a module are co-regulated is generated by shared MEMEs among members of a module (35) (Fig. 7*A-B*). The *p* value for each gene containing the consensus sequence represents the probability of a random sequence having the same match score or higher. Twenty-nine of 35 genes in Module 6 share a motif with a 20 base pair consensus sequence, and the significance values for genes containing this motif range from $p = 2.68 \times 10^{-4}$ to $p = 1.82 \times 10^{-10}$ (Fig. 7*A*). Eighteen of 54 genes in Module 7 share a 14 base pair motif, with *p* values ranging from $p = 9.32 \times 10^{-6}$ to $p = 4.73 \times 10^{-9}$ (Fig. 7*B*). These conserved motifs are potentially transcription factor binding sites.

Although many of the QTTs lack annotation, we can infer potential functions based on the characterized genes that fall into the same correlated module. Three of the four QTTs in Module 1 belong to a large transcriptional module enriched for male biased transcripts (21), and these genes are highly expressed in the testis (36); perhaps this module is related specifically to male reproductive functions. Of the five QTTs in Module 4, three are involved in visual perception. Their correlated expression implies that the others, *CG13928* and *CG6403*, might share a similar function. The fact that all of these transcripts are highly expressed in the head supports this possibility (Fig. 6*A*).

Three of the four annotated genes in Module 8 are involved in metabolic functions, suggesting a similar role for the uncharacterized genes in that module. Additional tests can help us tease apart the relationships among genes within a module. For example, manipulation of a single gene and assessment of the effects on other genes within the same module can elucidate causality and direction of effects.

Pleiotropy. The wild-derived inbred lines have been assessed for variation in other complex traits: longevity, starvation stress resistance, chill coma recovery time, locomotor reactivity (a startle response), copulation latency, competitive fitness, sleep traits, and alcohol sensitivity and tolerance (21, 37, 38). At the level of organismal phenotype, only locomotor reactivity was significantly genetically correlated with aggressive behavior ($r_G = 0.49$, $p < 0.001$). However, organismal genetic correlations can only be significant if alleles affect both traits in the same direction (16). There can be substantial pleiotropy in the absence of genetic correlation if alleles at many loci affect both traits, but the sign of the effects is not correlated. This motivated us to ask whether particular modules of transcripts associated with aggressive behavior were associated with modules of transcripts associated with the other traits (Supplementary Table 5). Many of the probe sets implicated in multiple traits correspond to predicted genes about which little is known. However, transcript abundance of *synaptogyrin*, which in involved in synaptic vesicle exocytosis (39), is associated with variation in starvation resistance and fitness (21). *Rab9* is associated with chill coma recovery (21) and sleep (37). *GRHRII*, which is a predicted G-protein coupled receptor (40) and gonadotropin-

releasing hormone receptor (41), is associated with starvation resistance (21) and sleep (37). *Cad96Ca*, a kinase involved in calcium-dependent cell adhesion (39) is associated with the response to ethanol exposure and development of tolerance, as are *Tyrosine kinase-related protein* and *activin-*β (38).

In addition to examining genetic correlations between QTTs affecting aggressive behavior, we can ask which of the genes affecting aggressive behavior are most highly correlated ($r \geq 0.70$) to the transcriptome (Fig. 4*B*). Three QTTs stand out as being highly connected. *miple* transcript abundance is highly correlated with 22 other transcripts. It is highly pleiotropic, and is thought to affect locomotor behavior, muscle development, ATP binding, synapse biogenesis, and response to stimulus (22). *VAChT* expression is correlated with 21 other transcripts. It is described as an acetylcholine transporter, and is also involved in the response to a chemical stimulus (22). Another "hub" gene is *unc-104*, which falls into many of the gene ontology categories described for *miple*; it is also involved in nucleotide binding. Mutations in human homologues have been implicated in spastic paraplegia and Charcot-Marie-Tooth disease (22). Additional highly connected genes are the computationally predicted genes *CG2790*, *CG13928*, *CG14853*, and *CG6156*, about which little annotation information is available, although *CG2790* and *CG13928* are reportedly involved in zinc ion and protein binding.

Expression of all of these integral genes is highly enriched in the brain, head, and thoracicoabdominal ganglion. Furthermore, the male accessory glands exhibit enrichment of *unc-104*; and *CG6156* is up-regulated in the crop, tubule, larval tubule, and

larval fat body (26). Their high degree of connectivity implies that these genes might be central to networks involved in aggressive behavior. The range of biological processes and molecular functions in which they are involved makes it difficult to isolate which are relevant to aggression, but their high expression levels in the head and nervous system unsurprisingly implicate those tissues in the modulation of aggression. We can also use this data to develop hypotheses about the highly connected yet uncharacterized genes, *CG2790*, *CG13928*, *CG14853*, and *CG6156*.

Insights about the genetic architecture of aggressive behavior. Aggression is clearly a highly complex trait – we have identified 266 candidate genes associated with natural variation in aggressive behavior, none of which have been previously implicated to affect aggression. It is likely that many of these candidate genes are false positives; however, 26 of these genes were previously correlated with response to selection for aggression from a different sample from the same base population, and 75% of *P*-element insertional mutations tested in these candidate genes indeed affect aggression. The candidate genes embrace a wide range of biological functions with plausible connections to aggressive behavior (sensory perception and chemosensation, function and development of the nervous system), as well as other general functions with less obvious relationships to aggression *per se* (metabolism, protein modification, mitosis). Analysis of natural variants affecting complex traits that have survived the sieve of natural selection thus gives insights about the genetic basis of complex behaviors that is not possible from analysis of mutations of large effect. That none of the genes previously implicated to

affect aggression was detected in this screen is somewhat surprising. There are several possible explanations. The known candidate genes may not be genetically variable at the level of transcription; we could not detect genetically variable transcripts at these loci because they are expressed at low levels or at a different developmental stage; our SFP map only detects a small fraction of polymorphic variants; and the candidate genes may not tolerate functional variation due to strong purifying selection. For example, variation in *fruitless* was not associated with variation in aggressive behavior in this study or previous studies (17, 18). Only one of the seven probe sets on the array that target *fruitless* was genetically variable, and variation in *fruitless* expression for this probe set was not associated with variation in aggressive behavior.

The QTTs associated with natural variation in aggressive behavior groups into genetically correlated modules with shared functional annotations, sequence motifs, and tissue-specific expression. These modules are into turn correlated with other traits, providing insight about the molecular basis of pleiotropy between aggression and other behavioral and fitness-related traits. These results provide the foundation for a systems genetics analysis of natural variation in aggressive behavior. The future availability of whole genome DNA sequence variation for these lines will enable us to discriminate *cis*-from *trans*-acting polymorphisms, and infer the direction of the flow of information through the network. The entire suite of 266 candidate genes provides a focal point for linkage analysis of segregating populations derived from the inbred lines. Further, the inbred lines can be characterized for other quantitative traits, including components of metabolism, which will enable us to interpret the balance of selective forces maintaining

variation for aggressive behavior in natural populations on a genome wide scale. Finally, it is not inconceivable that our understanding the genetic underpinnings of variation in aggressive behavior in Drosophila could be used to develop novel pharmacological therapies for treatment of pathological aggression in humans and domestic animals.

MATERIALS AND METHODS

Drosophila strains. The 40 inbred lines were derived by 20 generations of full-sib mating from isofemale lines that were collected from the Raleigh, NC farmer's market in 2003 (21). Flies were reared under standard culture conditions on cornmeal-molasses-agar medium at 25^{o}C, 60-75% relative humidity, on a 12h light-dark cycle. *P*-element insertional mutations and their co-isogenic control lines were obtained from Bloomington Drosophila Stock Center, Bloomington, Indiana.

Behavioral assay. Behavioral assays were performed as described previously (18) on socially experienced, 3-7 day old males. Flies were not exposed to anesthesia for at least 24 hours prior to the assay. A total of 20 replicate assays were performed for each line, with one replicate per line per day for a total of 20 days. Each replicate consisted of a group of eight 3-7 day old flies of the same genotype. The flies were placed in a vial without food for 90 minutes, after which they were transferred (without anesthesia) to a test arena containing a droplet of food and allowed to acclimate for two minutes. After the acclimation period, the flies were observed for two minutes; the aggression score of each replicate was the total number of aggressive interactions observed among all eight

flies in the two minute observation period. Behavioral assays were conducted in a behavioral chamber (25°C, 70% humidity) between 8 a.m. and 11 a.m.

Whole genome expression analysis. The gene expression analysis has been described previously (21). Briefly, RNA was extracted from two independent pools of 25 3-5 day old flies/sex/line that were frozen at the same time of day; labeled; and hybridized to Affymetrix Drosophila 2.0 arrays, using a strictly randomized experimental design. The raw array data was normalized using a median standardization. The measure of expression was the median log2 signal intensity of the probes in the perfect match probe sets, after removing probes containing SFPs between the wild-derived lines and the reference strain sequence used to design the array. Negative control probes were used to estimate the level of background intensity; probe sets with expression levels below this threshold were considered to be not expressed.

Quantitative genetic analyses. The ANOVA model $Y = \mu + L + \varepsilon$ was used to partition variation in male aggressive behavior and transcript abundance between lines (L, random) and the variation within lines (ε). A false discovery rate (FDR) < 0.01 (42) was used to assess significance of the L term in the analyses of natural variation in gene expression, to account for multiple testing. Broad sense heritabilities (H^2) were estimated as $H^2 = \sigma_L^2/(\sigma_L^2 + \sigma_E^2)$, where σ_L^2 and σ_E^2 are the among line and within line variance components, respectively. Estimate of cross-trait genetic correlations were $r_G = cov_{ij}/\sigma_i\sigma_j$, where cov_{ij} is the covariance of line means between trait i and trait j, and σ_i and σ_j are the

square roots of the among line variance components for the two traits. Differences in aggressive behavior between *P*-element insert lines and their co-isogenic controls were assessed by *t*-tests, with significance levels based on Bonferroni-corrected *p*-values. Simple linear regressions were used to identify QTTs significantly associated ($p < 0.01$) with variation in aggressive behavior across the 40 lines. Similarly, ANOVA models ($Y = \mu + M + \varepsilon$, where M denotes SFP presence or absence) were used to identify SFPs significantly associated ($p < 0.05$) with variation in aggressive behavior.

Transcriptional networks. The genetic correlations between all transcripts significantly associated with aggressive behavior were computed after removing the correlation between these transcripts and the phenotype. This was achieved by fitting the model $Y = \mu + E + \varepsilon$ (Y is the phenotype and E is the covariate median log2 expression level) and extracting the residuals to compute the genetic correlations for module construction (21). Modules of transcripts associated with aggressive behavior with coordinated patterns of expression across the forty lines were then quantified as described previously (21) by transforming the pairwise genetic correlations among transcripts into Euclidean-like distances, which were used to construct an affinity matrix. The transcripts were partitioned into modules using a graph-theoretical approach that envisions the transcripts as nodes in an undirected graph whose edges are weighted by the entries of the affinity matrix. Transcriptional modules common to aggressive behavior and other phenotypes measured on the 40 wild-derived inbred lines (21, 37, 38) were identified by comparing the transcripts in each aggression module to the transcripts in each module from the other

phenotypes, and determining whether the overlap between the modules exceed what is expected by chance using a Fisher's Exact Test (21).

Bioinformatics. Statistical analyses were performed using JMP 7.0 software. Functional annotations of genes are based on FlyBase (flybase.bio.indiana.edu) (39) annotations; additional information was obtained using FlyMine v12.0 (www.flymine.org) (22) and Babelomics v2 and v3 (babelomics.bioinfo.cipf.es) (23). Categories that were represented by fewer than 5% of the gene list queried were excluded. Statistically significant over- or under-representation was determined by the online software used when available; otherwise, a chi-square test was performed, using the appropriate genomic background to determine the expected values.

ACKNOWLEDGEMENTS

This work was funded by NIH grants F31 MH-074161 to A. C. E. and R01 GM-076083 to T. F. C. M. This is a publication of the W. M. Keck Center for Behavioral Biology.

Author contributions: T. F. C. M. and A.C. E designed research; A. C. E performed research; E. A. S. contributed analytic tools; A. C. E. and J. F. A. analyzed data; T. F. C. M. and A. C. E. wrote the paper.

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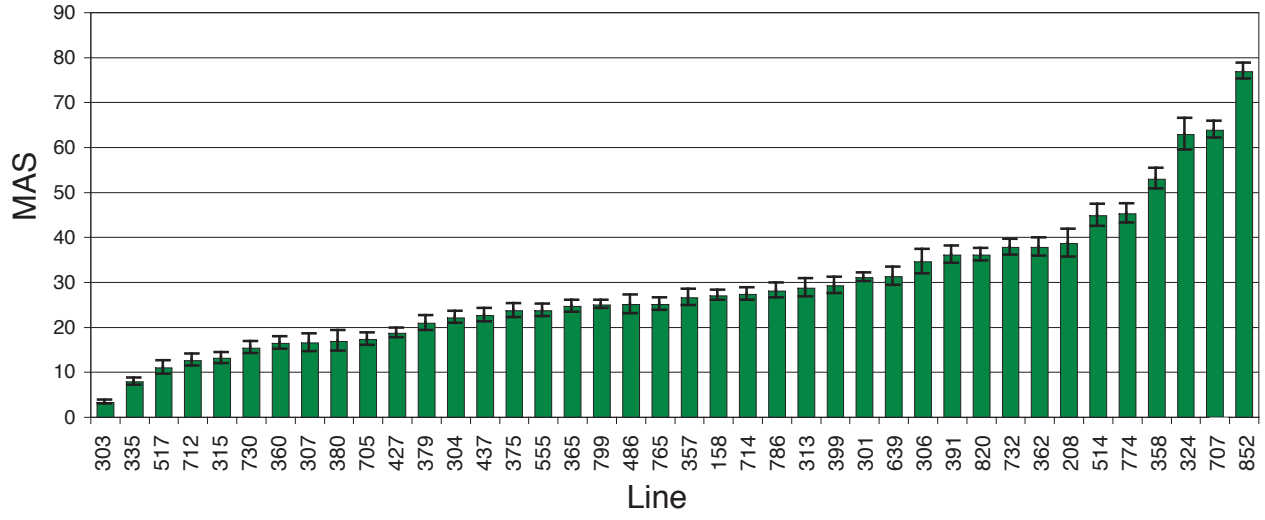

Figure 1. Variation in aggressive behavior among 40 wild-derived inbred lines. The line number is indicated on the x-axis, and the mean aggression score (MAS) on the y-axis. Error bars are SE.

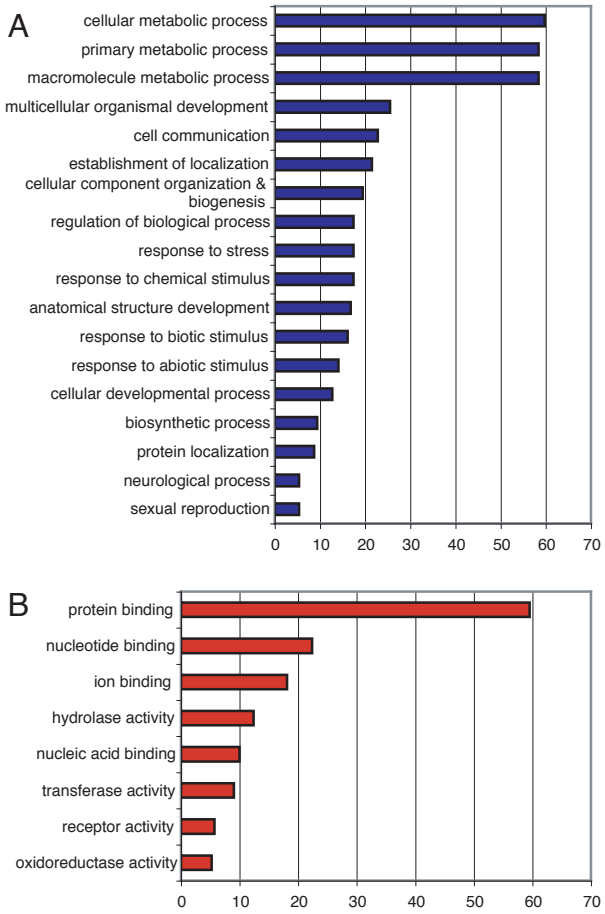

Figure 2. Gene ontologies categories represented by genes associated with male aggressive behavior through either the identification of SFPs or transcript abundance. Categories in (A) are Level 3 biological processes; those in (B) are Level 3 molecular functions. The percent of genes falling into a given category is depicted on the y-axis.

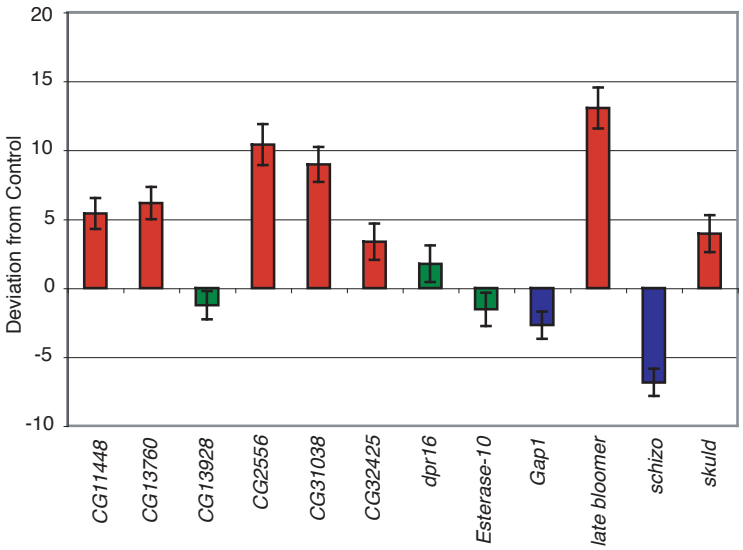

Fig. 3 – Aggression levels in *P*-element mutants. Mean deviation from control levels of aggression is depicted, ±SE. Red bars indicate significantly higher aggression ($p < 0.05$); blue bars indicate significantly lower aggression; and green bars indicate lines that did not differ significantly from control.

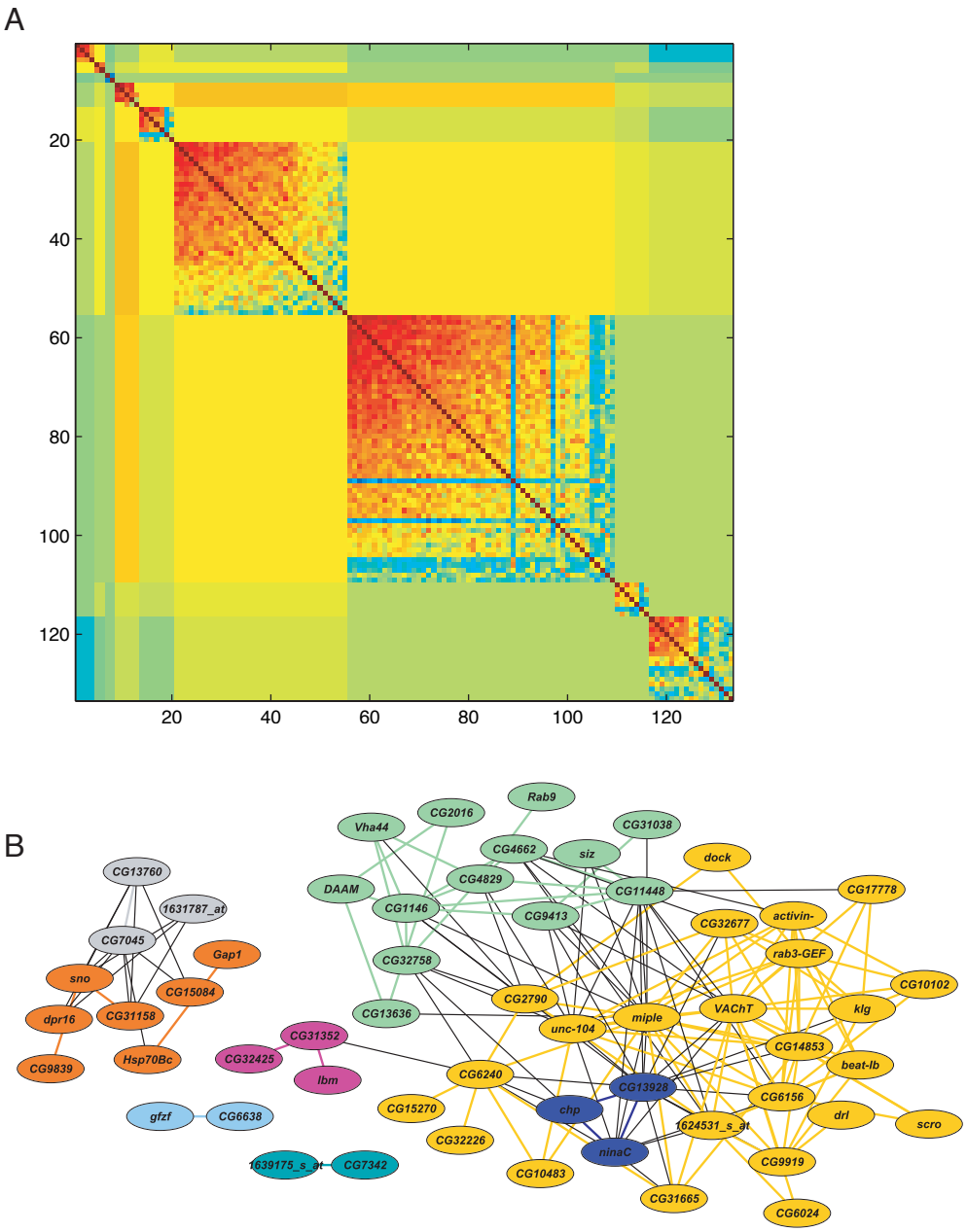

Figure 4. Modules of correlated transcripts associated with variation in aggressive behavior. (A) Heat map of correlated probe sets after module formation. The strength of the module decreases down the diagonal. (B) Network view of the most highly correlated ($r \geq 0.7$) probe sets where the edges represent correlated transcripts and the color-coding of nodes represents the different modules depicted in (A).

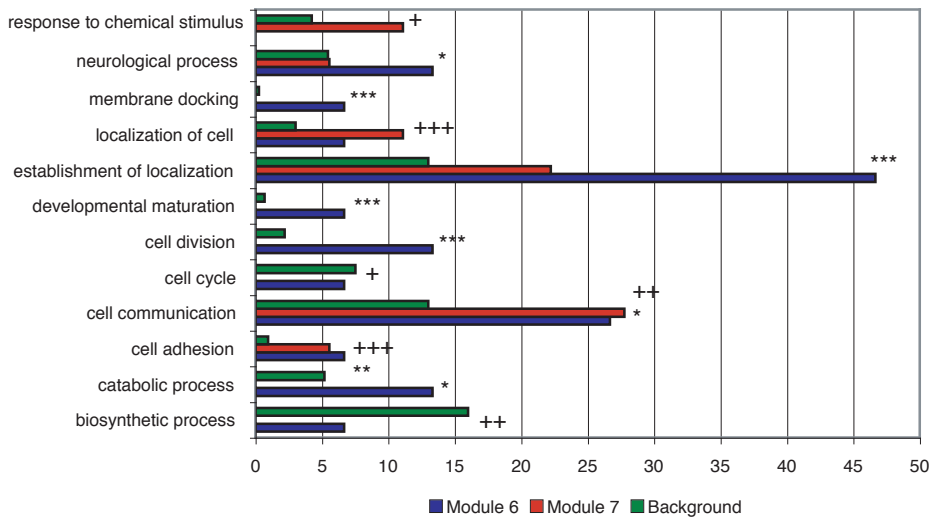

Figure 5. Differences in gene ontology representation between modules. All categories depicted are statistically over- or under-represented in Module 6 and/or 7 relative to the appropriate genomic background. Asterisks (*) indicate significance levels in Module 6, while plus-symbols (+) indicate significance in Module 7. For example, genes involved in the cell cycle are significantly ($p < 0.05$) under-represented in Module 7. */+: $p < 0.05$; **/++: $p < 0.01$; ***/+++: $p < 0.001$.

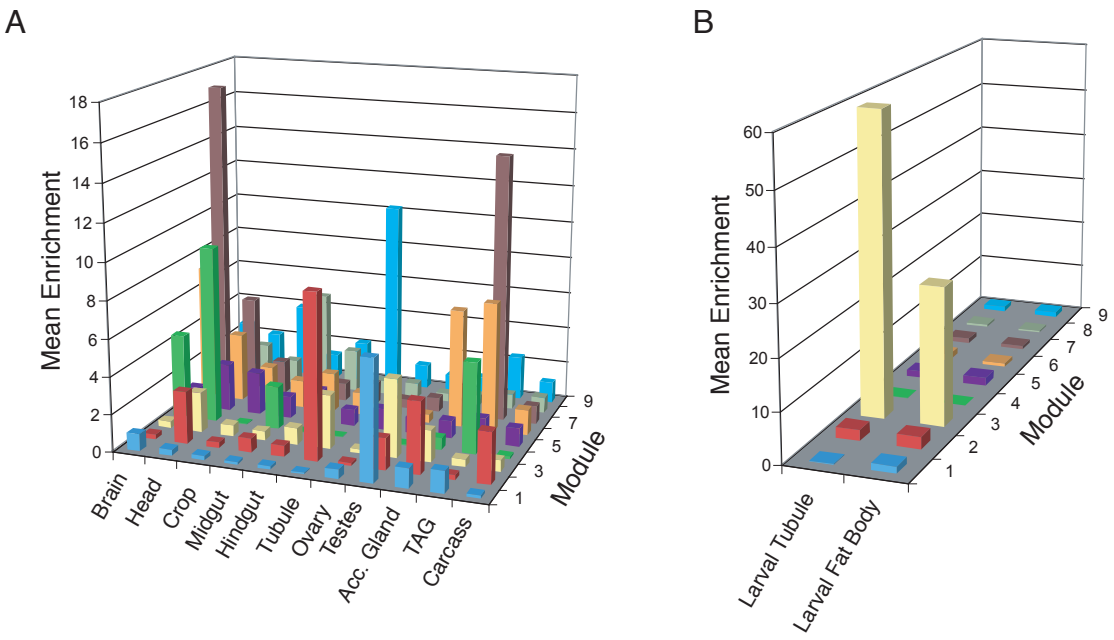

Figure 6. Module-specific tissue enrichment scores from FlyAtlas. (A) Enrichment in adult tissues and (B) larval-specific tissues.

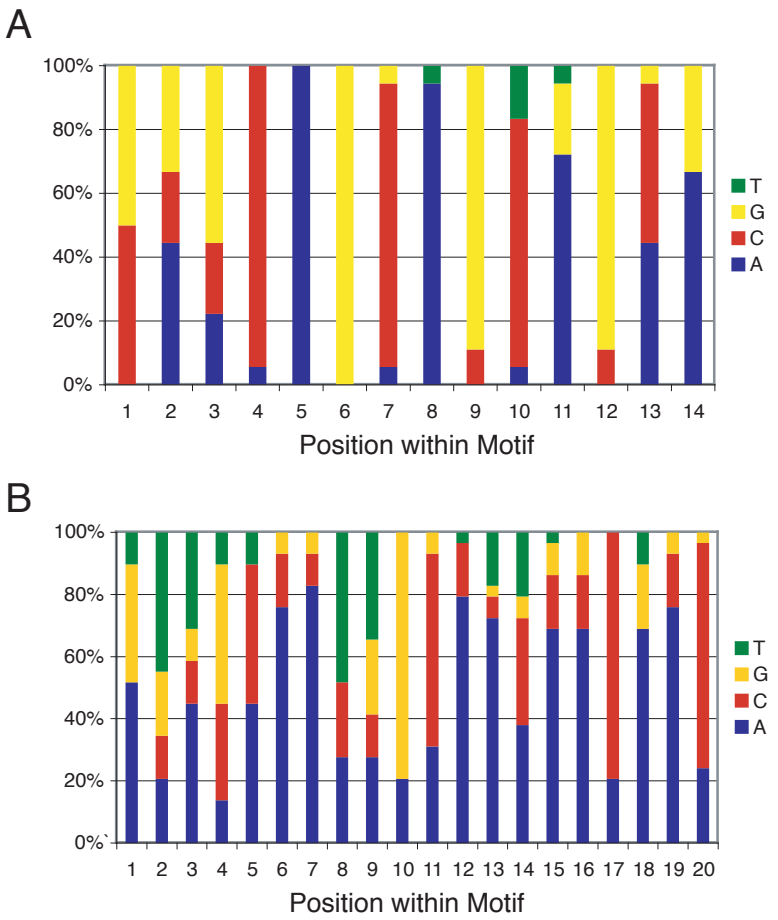

Figure 7. Conserved Motifs in Modules 6 and 7. The motifs most frequently found among genes in modules 6 (A) and 7 (B) are shown. The frequency of each nucleotide at each position is depicted on the y-axis, with the nucleotide position within the consensus sequence depicted on the x-axis. The motif in (A) was contained in 29 of 35 genes in Module 6; the motif in (B) was contained within 18 of 54 genes in Module 7. Significant level of adherence to the consensus sequence was at least $p = 2.68 \times 10\text{-}4$ for (A) and $p = 9.32 \times 10\text{-}6$ for (B).

CHAPTER SIX

Conclusions

Aggressive behavior is nearly ubiquitous throughout the animal kingdom and is often essential for survival. It may be employed to defend mates, progeny, or oneself; to obtain access to food or territory; or to establish or maintain a social hierarchy. The potential selective advantage conferred by aggression is counterbalanced by potential costs: violent encounters can result in injury; time spent engaged in aggressive interactions cannot be used to mate or find food; and aggression is often energetically expensive. Furthermore, aggression can have adverse consequences in human society, as excessive aggression can exact a steep toll on physical and mental health. Indeed, it is often symptomatic of psychiatric disorders such as schizophrenia and conduct disorder.

The majority of research addressing aggressive behavior has focused on the role of biogenic amines. Genes affecting the synthesis and function of serotonin, dopamine, and noradrenaline have been implicated in the modulation of human aggression (NELSON and CHIAVEGATTO, 2001; HALLER *et al*. 1998; DE ALMEIDA *et al*. 2005). The evolutionary conservation of these genes and pathways allows us to address many questions using model organisms. Furthermore, in this post-genomic age, we are in a position to widen the scope of our research, employing unbiased approaches to questions regarding the genetics of aggression.

In order to address the complex genetics underlying a quantitative trait, a variety of experimental approaches are necessary. We capitalized on the genomic resources available in *Drosophila melanogaster* to dissect the genetic architecture of aggressive behavior. We conducted an artificial selection experiment to derive lines of flies with

divergent aggression levels, then performed whole genome expression analysis to identify genes whose transcript abundance was correlated with the selection response. We carried out a behavioral screen of mutants in a co-isogenic background to identify genes in which mutations cause aberrant aggressive behavior. Using quantitative trait locus mapping, we were able to identify genes in which alleles segregating between two populations differentially affect aggression. In addition, we used a reference panel of wild-derived inbred lines to assess natural variation in aggression, then used microarrays to determine which genes' transcript levels correlated with aggression levels and which genes harbor single feature polymorphisms. The results of these experiments are summarized below.

Artificial Selection for Aggressive Behavior

We observed a substantial divergence in aggression between lines selected for high or low aggression levels, with "High" and "Low" aggression lines differing by 3.3 phenotypic standard deviation units after 25-28 generations of selection. Heritability estimates were approximately h^2=0.1. We assessed the selection lines in a variety of other fitness-related traits and found no evidence of directional phenotypic pleiotropy. However, this is not to say that genes involved in aggressive behavior do not also influence other traits; indeed, many pleiotropic candidate genes were identified.

We collected whole flies at generation 25 and extracted RNA for whole genome expression profiling. We found 1,539 transcripts whose expression level was correlated with the selection response, taking into consideration probe sets that differed between the

High and Low lines, the High and Control lines, and the Low and Control lines. By using this approach, we were able to see that some transcripts respond to only one direction of selection. Of the 1,539 differentially regulated transcripts, 416 were correlated only with a response to selection for high aggression, and 362 only with selection for low aggression. Thus, different genes might regulate aggression through different methods; it is also possible that different alleles of the same gene impact aggression differently.

These genes fall into a wide variety of gene ontology categories, with processes related to metabolism, development, stress response, and behavior statistically over-represented. This finding underscores the fact that there is far more to the genetics of aggressive behavior than allelic variation in biogenic amine-related genes. We selected 19 candidate genes to assess using behavioral tests of mutants in a co-isogenic background, and found that 15 of these exhibited aberrant aggressive behavior. Indeed, the functions of these were also varied, including electron transport and nervous system development.

In summary, we found that there is abundant segregating genetic variation contributing to phenotypic variation in aggression. As with other quantitative traits, artificial selection can be imposed to maximize divergence between phenotypes, and we can use an unbiased genomic approach such as expression profiling to identify genes whose expression is correlated with aggression levels. Follow-up through behavioral tests of mutations in candidate genes confirms that this is an effective and informative experimental approach.

P-Element Mutant Screen

Although in the past decade Drosophila has been established as a model organism for the study of aggressive behavior, a forward genetic screen for mutants affecting aggression had not been carried out. We took advantage of a library of *P*-element mutations available in a co-isogenic background, a gift from Hugo Bellen (BELLEN *et al*. 2004), to conduct such a screen. *P*-element mutants are ideal in that they often have subtle effects that seldom result in lethality. We chose 170 lines based on the results of the previously described artificial selection experiment, as well as results of screens on other fitness traits; additionally, a subset of randomly selected lines were included in the screen.

We found that 59 of the lines screened exhibited aberrant aggression levels relative to the control. Thirty-two of these had increased aggression, and 27 were less aggressive. Again, the previously described functions of these genes were quite diverse, with metabolic and developmental functions frequently represented. We chose nine of these candidates for further characterization, including several computationally predicted genes about which little to nothing is known. Quantitative PCR revealed that expression of these genes was disrupted at various developmental stages, and to varying degrees. The effects of the P-element insertion were not always consistent throughout development, nor were there correlations between expression level and adult aggression level; i.e., expression was not necessarily increased in hyper-aggressive mutants or vice versa.

In addition, we performed a morphometric analysis of the alpha and beta lobes of the mushroom bodies, which have been previously implicated in aggressive behavior (ROLLMANN *et al*., 2008; BAIER *et al*. 2002). We observed aberrant mushroom body morphology in all but one mutant, *extra macrochaetae*; there was no correlation between mushroom body phenotype and aggressive phenotype. Gross morphological defects were also observed in many mutants, but never in the control line. We did not see significant changes in ellipsoid body measurements. These results suggest that the relationship between brain morphology and aggressive behavior is quite complex, and behavioral output cannot be predicted based on neuropil size.

The results of the *P*-element screen imply that quite subtle expression changes in many different kinds of genes can have dramatic effects on aggression. The effects of these mutations on expression might be most pronounced during early development but still impact adult behavior. Furthermore, while many of the candidate genes we identified are pleiotropic, we did not observe directional pleiotropy; i.e., most hyper-aggressive mutants had been identified as hypoactive in a locomotor reactivity assay. Overall, we found that there are many different ways in which aggressive behavior can be disrupted, and these behavioral changes are often accompanied by morphological aberrations in the mushroom bodies.

Quantitative Trait Locus Mapping

QTL mapping has proven an effective means for determining segregating allelic differences that contribute to phenotypic differences. We applied this approach to a

series of introgression lines constructed from parental lines, *Oregon-R* and *2b*, with divergent aggression levels. We conservatively identified five QTL, two on the second chromosome and three on the third. Epistatic interactions are pervasive among these lines for this trait, and we cannot rule out the possibility that more QTL exist.

We elected to conduct high-resolution mapping in the region of the *2b* genome represented in line *3-88Я*, 65D;69D and the flanking regions, as this line exhibited the highest aggression level. This rather large portion of the genome fractionated into seven smaller regions when we employed quantitative complementation testing to 27 deficiency stocks. We then performed complementation tests to mutations in 58 positional candidate genes, of which seven met our criteria for non-complementation. Retesting using twice as many replicates confirmed four of these mutants as exhibiting failure to complement: these mutations were in *CG11006*, *CG10754*, *mus312*, and *Ral guanine nucleotide exchange factor 2* (*Rgl*). The *3-88Я/mutant* genotype exhibited higher levels of aggression than the *Ore/mutant* genotype for *CG10754*, *CG11006*, and *Rgl*; this observation was reversed for the *mus312* mutant. Expression of all of these genes is enriched in reproductive tissues; *CG11006* and *Rgl* are also enriched in the brain. Their functions vary; *Rgl* is involved in G-protein coupled receptor signaling, *mus312* and *CG10754* have metabolic functions, and *CG11006* is involved in biogenesis.

Again, we find a complicated story in the dissection of the genomic architecture of aggressive behavior. We observe evidence of abundant epistasis, making it difficult to accurately predict how a particular allele will affect aggression. Even in our small sample of four replicated loci, not all alleles affected aggression in the expected direction.

We also see that genes previously implicated in developmental and metabolic functions impact adult behavior. In summary, this unbiased approach to candidate gene identification yields results that, while not inconsistent with what is already known about genes affecting aggressive behavior, add a layer of complexity to its underlying causes.

Transcriptional Networks and Natural Variation

We used a reference panel of 40 wild-derived, inbred lines of Drosophila to address natural variation in aggressive behavior. We found significant ($p<0.0001$) variation in aggression levels among the lines. We conducted whole genome expression profiling and employed two approaches to candidate gene identification. First, we identified probe sets whose transcript abundance was correlated with aggression level across lines; 133 probe sets met our significance criteria and were termed quantitative trait transcripts (QTT). Second, we identified probes with differential binding among lines, a characteristic indicative of a single feature polymorphism (SFP); we found 513 such probes that met our criteria, representing 435 independent genes. Eight genes overlapped between our approaches. Thus, we identified 560 unique candidate genes affecting aggressive behavior.

These genes have been implicated in olfaction, learning and memory, nervous system development, metabolism, regulation of transcription, and cell cycle regulation, among many other processes. Among genes with SFPs, several metabolic processes were statistically over-represented, as was protein localization. Furthermore, we find overlap between our QTT candidate genes with those for other traits identified using the same

technique. These findings substantiate the assertion that genes affecting aggression are often pleiotropic, although not necessarily in a directional manner. Many of our candidates are computationally predicted and lack annotation. Some of these were included in functional tests we conducted on mutants in 12 candidate genes. We found nine mutants with aberrant aggression, seven of which showed increased aggression levels relative to controls, and two of which were hypo-aggressive.

Next, we asked whether the QTTs were inter-correlated. Using the method described in AYROLES *et al*. (2008), we found nine modules, ranging in size from two to 54 probe sets. Probe sets within each module are statistically correlated and potentially co-regulated. Furthermore, genes in different modules are often enriched in different tissues, and are differentially implicated in biological processes and metabolic functions.

This study provides further evidence that a substantial portion of the genome can be involved in the modulation of a quantitative behavioral trait. We can use whole genome expression profiling to identify candidate genes in an unbiased manner. Additionally, we can identify modules of transcripts whose expression is correlated and might be co-regulated and/or share functional roles. Results of functional tests suggest that this is an effective way to identify candidate genes. Our findings are particularly interesting given that we used wild-derived lines of flies and captured natural genetic variation, which will likely be quite informative for the identification of relevant allelic variation affecting aggression.

Future Directions

This body of work provides a strong foundation for our understanding of the genomic architecture of aggressive behavior. However, there is always more work to be done. Many candidate genes were identified through transcriptome analysis of artificially selected lines, and our functional tests of *P*-element mutants in 19 of these genes validates the approach. A question unanswered by the current data is whether gene expression level and aggression level are always correlated in the same direction: that is, if increased expression of a particular gene is observed in high aggression lines, does increased expression always confer higher aggression, or is this pattern particular to genomic background? To address this question, quantitative PCR could be performed on candidate genes in a variety of backgrounds.

Another question of interest is whether *cis* or *trans* variation is the source of variation in transcript abundance. The answer is likely both, and identification of the relevant sequence variation could be quite informative. Are there transcription factor binding sequences that responded to selection? Are there coding regions within transcription factors themselves that responded to selection, which might in turn affect the expression of numerous target genes? To what degree is allelic variation in coding regions of other genes responsible for the phenotypic response to selection? Have allelic differences in some genes become fixed between the high and low lines? Unfortunately, the behavioral phenotypes of our selection lines reverted toward control levels in the absence of selection pressure and we did not maintain the lines, making it impossible to address these questions in this particular case. However, replicating the selection

experiment would enable these questions to be addressed while also providing insight as to which genes are most integral to the selection response. We would not expect an exact replication of results: replication is dependent on the presence of the same variation that was latent in the base population of the initial experiment, as well as identical implementation of selection pressures and environmental controls. In addition to these complications, we could not distinguish between expression that was causal to the selection response versus merely correlated. With these caveats in mind, we could be particularly confident that genes replicated between two experiments are quite robust candidates.

An expansion of the *P*-element mutant screen is quite feasible. Nearly all of the mutant lines screened were included due to their implication in another relevant trait, or as candidate genes from the artificial selection experiment. As such, we likely enriched our screen for lines likely to exhibit aberrant aggression. An unbiased approach would improve our understanding of how much of the genome influences aggressive behavior. More than 30% of the lines we screened were aggression mutants. While large proportions of the genome have been shown to affect behavioral traits (MACKAY *et al*. 2005; JORDAN *et al*. 2007, MOROZOVA *et al*. 2006; MOROZOVA *et al*. 2007; SAMBANDAN *et al*. 2006), this result might be biased due to our selection criteria. A limitation to this type of screen is that it identifies the effects of mutations on behavior rather than that of natural variation, although this does not negate the utility of mutant screens.

An additional avenue of future research would be to further characterize the effects of mutations in candidate genes on brain morphology. We did not see a linear

correlation between mushroom body size and aggression level, but mushroom bodies have previously been associated with aggression (ROLLMANN *et al*. 2008; BAIER *et al*. 2002) and this correlation could be quite informative. Which candidate genes are expressed specifically in the mushroom bodies? Is their effect on aggression due to acute expression or is it relevant only at certain developmental stages? The availability of GAL4 lines allows us to temporally and spatially control gene expression. This resource could be exploited to determine when and where candidate gene expression is relevant to aggressive behavior.

The QTL mapping approach could be expanded upon in different ways. We did not test every positional candidate gene in our QTL, and this task would be time-consuming but possible. However, a more interesting approach may be to map loci segregating in different populations. The parental strains used in our experiment are laboratory lines, and do not necessarily reflect natural variation. By conducting QTL mapping on lines derived from natural populations, we could obtain information not only about what genes and types of genes affect aggression, but also what alleles are segregating in the natural population. These alleles are potentially of more interest because they have been subjected to natural selection, and might be informative in our understanding of the evolution of aggressive behavior in a natural environment.

The transcriptome analysis of the reference panel of 40 wild-derived inbred lines has the pronounced strength of being an unbiased genome-wide approach addressing natural variation. Future work could take advantage of the vast amount of data generated by a project of this ilk. Many of the questions left unanswered at this point can be

addressed with genome sequence. With such information, it should be possible to overlay a biological directionality onto the current statistical relationships. A different approach is to further characterize a subset of candidate genes. We used functional tests of candidate gene mutants to validate our findings, but this evidence could be bolstered by quantitative PCR. Furthermore, RNA interference could be employed to determine if perturbation of a "hub" gene impacts the genes with which it shares a statistical relationship. Investigating these relationships in a different population of flies could also validate results.

Finally, in order to understand behavioral output, it is necessary to combine the genetic networks affecting aggression with neural networks. This is a sizeable task, but the use of genome-wide approaches and other technological advances brings it within our grasp. Ultimately, we hope that the dissection of aggressive behavior using model organisms such as *Drosophila melanogaster* will lead to effective treatments and prevention of pathological human aggression.

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APPENDICES

Appendix 2: Supplementary Tables and Figures for Chapter 2

Appendix 2

The publication, supplementary figures, and tables from Chapter Two can be downloaded online using the addresses below:

Publication:
http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.0020154

Supplementary Figure 1:
http://www.plosgenetics.org/article/fetchFirstRepresentation.action?uri=info:doi/10.1371/journal.pgen.0020154.sg001

Supplementary Figure 2:
http://www.plosgenetics.org/article/fetchFirstRepresentation.action?uri=info:doi/10.1371/journal.pgen.0020154.sg002

Table S1 (only available online):
http://www.plosgenetics.org/article/fetchFirstRepresentation.action?uri=info:doi/10.1371/journal.pgen.0020154.st001

Table S2:
http://www.plosgenetics.org/article/fetchFirstRepresentation.action?uri=info:doi/10.1371/journal.pgen.0020154.st002

Table S3:
http://www.plosgenetics.org/article/fetchFirstRepresentation.action?uri=info:doi/10.1371/journal.pgen.0020154.st003

Table S4:
http://www.plosgenetics.org/article/fetchFirstRepresentation.action?uri=info:doi/10.1371/journal.pgen.0020154.st004

Table S5 (only available online):
http://www.plosgenetics.org/article/fetchFirstRepresentation.action?uri=info:doi/10.1371/journal.pgen.0020154.st005

Table S6 (only available online):
http://www.plosgenetics.org/article/fetchFirstRepresentation.action?uri=info:doi/10.1371/journal.pgen.0020154.st006

Raw microarray data are available through Gene Expression Omnibus under accession number GSE5405 (http://www.ncbi.nlm.nih.gov/geo).

Appendix 3: Supplementary Tables and Figures for Chapter 3

Appendix 3.1

Supplementary Table 1. Complete Results of Behavioral Screen.

† Indicates mutant line included in additional analyses.

Significant deviations from control line are denoted as follows: *, p<0.05; **, p<0.01; ***, p<0.001.

MAS=Mean Aggression Score

P-Element Line	Disrupted Locus	FlyBase #	MAS	SE	Deviation from Control	a/σ_p	Relevant Gene Ontology
BG00016	*Lipid storage droplet-2*	FBgn0030608	14.5	1.12507	0.7	0.163375377	
BG00111	*Guanine nucleotide exchange factor GEF64C*	FBgn0035574	15.15	1.1222	3.825	0.400657629	
BG00151	*CG9894*	FBgn0031453	16.35	0.6336	4.1**	0.964969523	unknown
BG00228	*CG33692*	FBgn0064121	11	1.31589	-1.25	-0.29419803	
BG00291	*retinal degeneration B*	FBgn0003218	15.1	0.62786	2.85	0.670771498	
BG00336	*Guanine nucleotide exchange factor GEF64C*	FBgn0035574	6.1	0.92024	-6.15***	-1.44745428	axon guidance; regulation of Rho protein signal transduction
BG00369	*CG13334*	FBgn0033856	10.9	0.80426	-0.85	-0.14657007	
BG00372	*CG1678*	FBgn0031176	17.2	0.98569	5.45***	0.939772806	unknown
BG00373	*CG11226*	FBgn0037195	13.2	0.8448	-0.6	-0.14003604	
BG00375	*Odorant-binding protein 99d*	FBgn0039684	14.8	0.55536	3.05**	0.5259279	odorant binding; autophagic cell death; salivary gland cell autophagic cell death; transport
BG00376†	*CG3638*	FBgn0026875	18.45	0.90459	6.7***	1.155317027	unknown
BG00386	*NMDA receptor 1*	FBgn0010399	20.65	1.01379	8.9***	1.534674857	long-term memory; olfactory learning; calcium-mediated signaling; nerve-nerve synaptic transmission; nervous system development
BG00489	*Osiris 9*	FBgn0037416	13.55	0.79629	1.3	0.305965946	
BG00524	*pncr001:3R*		10.5	0.76606	-1.4	-0.3150456	
BG00528	*Osiris 9*	FBgn0037416	14.95	0.86291	2.7	0.635467735	
BG00670	*CG32541*	FBgn0052541	13.35	0.74436	1.6**	0.275896604	unknown
BG00675	*cropped*	FBgn0001994	11.65	1.01896	-2.15	-0.5017958	
BG00735	*schizo*	FBgn0026179	16.25	0.61932	4.5***	0.775959197	central nervous system development; guanyl-nucleotide exchange factor activity
BG00816	*pumilio*	FBgn0003165	14.4	0.61302	1.6	0.249589827	
BG00986†	*extra macrochaetae*	FBgn0000575	7.05	0.54519	-5.75***	-0.89696344	nervous system development
BG00987	*smell impaired 35A*	FBgn0016930	15.65	0.89817	2.85	0.44458188	
BG00990	*wing blister*	FBgn0004002	13.75	0.59327	2.3	0.378981201	
BG01008	*CG11133*	FBgn0037205	13.4	1.00105	0.6	0.093596185	
BG01011	*Spinophilin*	FBgn0010905	17.2	0.69812	4.4***	0.686372025	phosphopantetheine binding
BG01014	*CG12292*	FBgn0032451	11.45	0.87201	-0.45	-0.10126466	
BG01018	*forkhead box, sub-group O*	FBgn0038197	14.6	0.8124	1.8	0.280788556	
BG01024	*CG9650*	FBgn0029939	14.75	0.90866	1.95	0.304187602	
BG01027	*like-AP180*	FBgn0026210	16.1579	1.02673	1.40789	0.240842968	
BG01028	*Trithorax-like*	FBgn0013263	10.25	0.75	-1.75	-0.25598137	
BG01037	*βυ integrin*	FBgn0010395	10.5	0.72001	0.15	0.028395414	
BG01043	*Gp150*	FBgn0013272	18	2.08503	6.675*	0.908157292	ATP binding; transmembrane receptor protein tyrosine phosphatase signaling pathway
BG01044	*putative noncoding RNA 001:3R*		9.7	0.8243	-2.2	-0.49507166	
BG01046	*CG3587*	FBgn0023521	7.9	0.55678	-4.1***	-0.59972779	unknown
BG01047	*frizzled*	FBgn0001085	12.75	0.63194	0.85	0.191277687	
BG01068	*Guanine nucleotide exchange factor GEF64C*	FBgn0035574	11.7	0.67317	0.375	-0.21368407	
BG01092	*roundabout*	FBgn0005631	13.9	1.04856	-0.85	-0.13448531	
BG01097	*CG12078*	FBgn0035426	13.3	0.7817	0.975	0.071228023	
BG01127	*muscleblind*	FBgn0053197	13.15	0.97137	2.45	0.409291864	
BG01130	*alan shepard*	FBgn0052423	16.55	0.91616	4.8***	0.86064549	mRNA processing; gravitaxis
BG01131	*tramtrack*	FBgn0003870	13.75	0.78094	2.425	0.151359549	
BG01173	*Odorant receptor 83b*	FBgn0037324	11.55	0.7521	-0.45	-0.06582378	
BG01196	*CG41475*		13.85	0.66203	1.85	0.270608881	
BG01214†	*sugarless*	FBgn0010851	17.1	0.73592	5.55***	0.925135264	cell communication; signal transduction; transmembrane receptor protein tyrosine kinase signaling pathway
BG01215	*CG11299*	FBgn0034897	8.7	0.5717	-3.3**	-0.48270773	regulation of progression through cell cycle; cell cycle arrest
BG01223	*Glutamine synthetase 2*	FBgn0001145	13.55	0.66283	2.05	0.453643611	
BG01226	*CG32130*	FBgn0052130	9.65	0.85924	-1.85	-0.4093857	
BG01228	*CG33681*	FBgn0053681	12.1	0.88228	1.4	0.233881065	

BG01232	*CG11940*	FBgn0031079	12.75	0.66442	1.25	0.276611958	
BG01245	*Sema-5c*	FBgn0028679	14.25	1.20498	1.55	0.25026742	
BG01259	*ken and barbie*	FBgn0011236	12.1	0.89413	1.4	0.233881065	
BG01280	*CG5468*	FBgn0039434	8.9	0.70673	-2.6	-0.57535287	
BG01290	*Btk family kinase at 29A*	FBgn0003502	15.2	1.10882	3.875	0.409561131	
BG01294	*GDP dissociation inhibitor*	FBgn0004868	13.6	0.66253	-1.15	-0.18195071	
BG01299†	*Actin 5C*	FBgn0000042	3.6	0.50471	-7.9***	-1.74818757	structural constituent of cytoskeleton; ATP binding; protein binding
BG01314	*CG6540*	FBgn0030943	9.7	1.00289	-1	-0.1670579	
BG01336	*diminutive*	FBgn0000472	14	1.01307	2.5	0.553223916	
BG01354	*CG30492*	FBgn0050492	18.55	1.34844	7.225**	1.006095823	zinc ion binding
BG01380	*Oseg4*	FBgn0035264	11.55	0.67852	-0.95	-0.13506303	
BG01389	*Laminin A*	FBgn0002526	11.4	0.86268	-2.8	-0.51855804	
BG01402	*CG32345*	FBgn0052345	15.55	0.86595	4.1***	0.675575184	unknown
BG01411	*Glutathione S transferase S1*	FBgn0010226	11.65	0.94109	1.65	0.301777306	
BG01424	*frizzled*	FBgn0001085	11.45	0.71258	0.5	0.111832832	
BG01433	*CG13791*	FBgn0031923	16.75	0.82677	4.25**	0.604229332	unknown
BG01438	*CG31531*	FBgn0051531	12.8	0.73842	0.3	0.042651482	
BG01469†	*Syntaxin 4*	FBgn0024980	7.9	0.86115	-4.6***	-0.65398939	t-SNARE activity; neurotransmitter secretion; vesicle-mediated transport; synaptic vesicle docking during exocytosis
BG01472	*CG9028*	FBgn0036389	14.9	0.81402	2.4	0.341211858	
BG01486	*Myosin 31DF*	FBgn0011673	12.15	0.73368	-1.15	-0.24321054	
BG01491	*tramtrack*	FBgn0003870	14.45	0.72357	2.9*	0.483404012	zinc ion binding; peripheral nervous system development; transmission of nerve impulse
BG01498	*Casein kinase Iα*	FBgn0015024	8.85	0.7549	-2.85*	-0.43047834	receptor signaling protein serine/threonine kinase activity; ATP binding
BG01510	*CG1623*	FBgn0033448	7.35	0.65404	-3.35	-0.55964398	
BG01536	*Beadex*	FBgn0000242	7.25	0.88815	-3.9***	-0.60020565	zinc ion binding; locomotory behavior; response to cocaine; regulation of metabolism
BG01546	*CG14035*	FBgn0031685	11.65	0.92131	0.325	0.175026317	
BG01548	*alpha-Esterase-10*	FBgn0015569	11.25	0.54229	-2.5	-0.33938096	
BG01564	*CG14430*	FBgn0029927	11.3	1.02623	-0.4	-0.06041801	
BG01566	*arrest*	FBgn0000114	6.75	0.69915	-4.95***	-0.7476729	negative regulation of oskar mRNA translation;
BG01586	*CG40470*		14	1.04881	0.25	0.033938096	
BG01587	*CG13917*	FBgn0035237	9.65	0.79232	-3.05	-0.41459459	unknown
BG01596†	*CG13377*	FBgn0040369	19.55	0.73797	9.2***	1.741585363	metabolism
BG01609	*CG30152*		13.2105	1.04176	-0.5395	-0.14204255	
BG01613	*canoe*	FBgn0000340	11.4	0.77595	-2.35	-0.3190181	
BG01645	*faint sausage*	FBgn0000633	10.85	0.61248	-0.475	0.101331026	
BG01646	*Basigin*	FBgn0011219	11.6	0.76914	-1.1	-0.17760914	
BG01654	*pickpocket 23*	FBgn0030844	9.55	0.67072	-4.2**	-0.57016002	sodium channel activity;
BG01655	*CG32038*	FBgn0052038	10.8	0.97495	-2.95	-0.40046954	
BG01661	*raspberry*	FBgn0003204	14.05	0.69386	1.35	0.177683397	oogenesis, purine base metabolism
BG01662	*Laminin A*	FBgn0002526	15.55	0.70515	3.8**	0.681344346	receptor binding; locomotion; central nervous system development
BG01672	*CG14591*	FBgn0033054	10.9	0.72873	0.55	0.104116516	
BG01683†	*CG32572*	FBgn0052572	7.65	0.79232	-5.05***	-0.66466752	unknown
BG01693	*CG10777*	FBgn0029979	4.5	0.58264	-5.85***	-1.10742113	RNA helicase activity; nucleic acid binding; ATP binding; ATP-dependent helicase activity
BG01697	*CG33523*	FBgn0053523	14.4	0.82844	1.7	0.223749463	structural molecule activity
BG01709	*kermit*	FBgn0010504	14.25	0.73225	1.55	0.204006863	GPCR receptor protein signaling pathway
BG01713	*4EHP*	FBgn0053100	8.9	0.69168	-3.8**	-0.50014586	translation initiation
BG01720	*frizzled*	FBgn0001085	11.2	0.83855	-0.7	-0.1575228	
BG01724	*Calreticulin*	FBgn0005585	13.9	1.12834	2.17778	0.280460794	
BG01733	*CG6175*	FBgn0036152	14.55	0.67072	3.1*	0.510800749	unknown
BG01735	*big brain*	FBgn0000180	12.45	0.87502	0.72778	0.066424925	
BG01736	*CG5966*	FBgn0029830	9.4	0.80263	-1.3	-0.21717527	
BG01757	*CG17323*	FBgn0032713	12.75	0.65242	2.75*	0.502962176	defense response; polysaccharide metabolism; response to toxin; steroid metabolism
BG01765	*Tehao*	FBgn0026760	8.35	0.54423	-4.35**	-0.7023634	transmembrane receptor activity; signal transduction; Toll signaling pathway
BG01893	*Splicing factor 1 OR l(3)07882*	FBgn0025571	7.2	0.70562	-2.8*	-0.51210694	transcription cofactor activity; nucleic acid binding; zinc ion binding
BG01900	*mir-317*	FBgn0067687	6.6	0.56847	-3.4**	-0.62184415	microRNA
BG01909	*CG14035*	FBgn0031685	21.45	0.91039	10.2***	2.271839181	unknown
BG01912†	*pxb*	FBgn0053207	17.3	0.93499	4***	0.845949694	learning and/or memory; olfactory learning
BG01914	*sugarless*	FBgn0010851	15.8	0.96136	3.1	0.50053484	
BG01916	*no ocelli*	FBgn0005771	13.65	0.90692	2.325**	0.359264546	zinc ion binding
BG01949	*ade5*	FBgn0020513	16.2	0.91938	3.5*	0.565119981	purine base metabolism; 'de novo' IMP biosynthesis

BG01960	*buttonless*	FBgn0014949	8.85	0.94109	-1.25	-0.17236957	
BG01990	*CG30492*	FBgn0050492	13	0.83666	1.2	0.214113297	zinc ion binding
BG02019	*CG9171*	FBgn0031738	13.3	0.89766	3.3**	0.603554611	transferase activity
BG02022	*CG6301*	FBgn0034161	7.15	0.46609	-4.1***	-0.91319026	unknown
BG02029	*E(spl) region transcript m7*	FBgn0002633	11.5	0.89295	-0.2222	-0.07380547	
BG02053	*Sema-5c*	FBgn0028679	12.5	1.17092	0.77778	0.073805472	
BG02077	*Rtnl1*	FBgn0053113	9	0.69585	-2.9*	-0.65259446	receptor signaling protein activity; calcium ion binding
BG02081	*Rtnl1*	FBgn0053113	16.6	0.93302	5.35***	1.191601923	receptor signaling protein activity; calcium ion binding
BG02095[†]	*echinoid*	FBgn0000547	14.8	0.92224	4.1**	0.684937404	epidermal growth factor receptor signaling pathway; negative regulation of neurogenesis; sensory organ development
BG02104	*CG13511*	FBgn0034759	11.05	0.99333	0.35	0.058470266	
BG02113	*Laminin A*	FBgn0002526	13.25	0.91155	1.5	0.268951716	
BG02128	*lethal (1) G0007*	FBgn0026713	5.55	0.43815	-5.6***	-0.86183375	ATP-dependent RNA helicase activity; RNA splicing factor activity, transesterification mechanism; ATP-dependent helicase activity; ATP binding
BG02165	*CG32700*	FBgn0052700	10.35	0.73368	-0.175	-0.03603297	
BG02169	*High mobility group protein D*	FBgn0004362	11.95	0.65484	0.8	0.123119108	
BG02188	*eclair*	FBgn0069242	15.8	0.72038	4.475***	0.557320642	intracellular protein transport
BG02197	*Basigin*	FBgn0011219	11.25	0.8009	-0.5	-0.08965057	
BG02207	*lilliputian*	FBgn0041111	13.45	0.98535	2.2	0.490004529	
BG02210	*lamina ancestor*	FBgn0016031	14.05	0.93041	1.35	0.21797485	
BG02217	*plexus*	FBgn0003175	4.6	0.48341	-5.4***	-0.98763482	wing vein morphogenesis
BG02236	*Checkpoint suppressor homologue*	FBgn0029504	14.85	0.54423	3.05	0.54420463	transcription factor
BG02237	*putative noncoding RNA 001:3R*	FBgn0053327	10.85	0.77553	-1.05	-0.2362842	
BG02246	*CG32137*	FBgn0052137	10.3	0.76468	-1.5	-0.26764162	protein targeting, intracellular protein transport
BG02276	*CG7832*	FBgn0026578	10.95	1.43724	2.95*	0.435452285	unknown
BG02281	*failed axon connections*	FBgn0014163	10.35	0.76184	-0.175	-0.03603297	
BG02320	*Toll*	FBgn0003717	8.1	0.77085	-2.7	-0.56311779	
BG02345	*Guanine nucleotide exchange factor GEF64C*	FBgn0035574	13.45	1.28242	1.65	0.294405784	axon guidance; regulation of Rho protein signal transduction
BG02377	*CG14478*	FBgn0028953	4.2	0.5831	-3.8**	-0.56092159	unknown
BG02380	*Laminin A*	FBgn0002526	10.4	0.72693	-1.35	-0.24205654	
BG02386	*Sema-5c*	FBgn0028679	14.1	0.61516	2.35	0.36421382	
BG02420	*CG5946*	FBgn0036211	3.85	0.46609	-4.15**	-0.61258542	cholesterol metabolism; electron transport; fatty acid desaturation
BG02435	*Tollo*	FBgn0029114	11.5	0.71267	0.8	0.133646323	
BG02439	*CG32556*	FBgn0052556	8.6	0.70487	-3.2	-0.57096879	unknown
BG02464	*lamina ancestor*	FBgn0016031	13.95	0.69386	2.2	0.340966129	
BG02470	*CG8963*	FBgn0034181	7.6	0.80916	-2.75*	-0.52058258	unknown
BG02491	*Ras-related protein*	FBgn0015286	11.3	0.50315	0.05	0.289548131	
BG02495	*ade5*	FBgn0020513	13.35	0.64185	2.025*	0.331628811	purine base metabolism; 'de novo' IMP biosynthesis
BG02497	*mushroom-body expressed*	FBgn0014362	7.45	0.59593	-2.65	-0.36542349	
BG02498	*Darkener of apricot*	FBgn0053553	10.1	0.81402	-1.8	-0.40505863	
BG02501	*longitudinals lacking*	FBgn0005630	7.9	1.17854	-3.8222*	-0.60520487	axon guidance; axonogenesis; transmission of nerve impulse
BG02510	*chameau*	FBgn0028387	12.25	0.68777	1.1	0.169288773	
BG02518	*CG8920*	FBgn0027529	10.2	0.74551	2.2	0.324744077	
BG02522	*CG32560*	FBgn0052560	6.1	0.37627	-4.25***	-0.80453672	Ras GTPase activator activity; receptor binding; G-protein coupled receptor protein signaling pathway; MAPKKK cascade; negative regulation of Ras protein signal transduction
BG02523	*lamina ancestor*	FBgn0016031	7.95	0.69006	-3.8***	-0.5889415	unknown
BG02536	*abrupt*	FBgn0000011	12.45	1.00387	2.35	0.324054794	
BG02539	*Basigin*	FBgn0011219	15.5	0.7729	3.75**	0.581192266	spermatid development.
BG02542	*neuralized*	FBgn0002932	11.35	0.85924	-2.4*	-0.59838821	ubiquitin-protein ligase activity; protein binding; zinc ion binding; nervous system development; sensory organ development; tissue development; regulation of Notch signaling pathway
BG02560	*CG9674*	FBgn0036663	12.3	0.54338	1.15	0.176983717	
BG02563	*capricious*	FBgn0023095	13.8	0.66728	2.35	0.387219922	

BG02573	*CG31163*	FBgn0051163	6.9	0.55678	-3.9	-0.81339237	
BG02588	*tropomodulin*	FBgn0082582	7.6	0.57765	-2.5	-0.34473914	
BG02624	*CG9650*	FBgn0029939	10.3	0.60306	-1.15	-0.1894906	
BG02644	*Fkbp13*	FBgn0010470	12	0.63246	4**	0.590443777	calcium ion binding; protein folding
BG02646	*Calreticulin*	FBgn0005585	14.7	0.61601	0.95	0.104980387	
BG02684	no longer in database		13.6	0.66253	1.87778	0.236177511	
BG02715	*nebbish*	FBgn0004374	10.55	0.70515	-1.35	-0.30379397	
BG02724	*High mobility group protein D*	FBgn0004362	8.4	0.8059	-3.05	-0.50256203	
BG02731	*longitudinals lacking*	FBgn0005630	13.6	0.85962	3.075**	0.633150733	axon guidance; axonogenesis; transmission of nerve impulse
BG02735	*polychaetoid*	FBgn0003177	8.25	0.54229	0.25	0.036902736	
BG02749	*CG11033*	FBgn0037659	8.75	0.98375	-1.95	-0.32576291	
BG02777	*innexin 7*	FBgn0027106	12.85	0.52952	1.7	0.261628103	
BG02785	*dacapo*	FBgn0010316	9.3	0.8587	-2.15	-0.35426504	
BG02812	*Lipid storage droplet-2*	FBgn0030608	12.05	0.92188	-1.75	-0.40843844	
BG02818	*pipsqueak*	FBgn0004399	11.5	0.98275	-2.3	-0.53680481	
BG02844	*CG11226*	FBgn0037195	15.6923	1.40231	1.89231	0.52124525	
Canton S-B (control)			11.754	0.12994	n/a		

Appendix 3.2

Supplementary Table 2 Human Orthologues of *P[GT1]* lines with aberrant aggressive behavior.

† Indicates mutant line included in further analyses.

MAS=Aggression Score

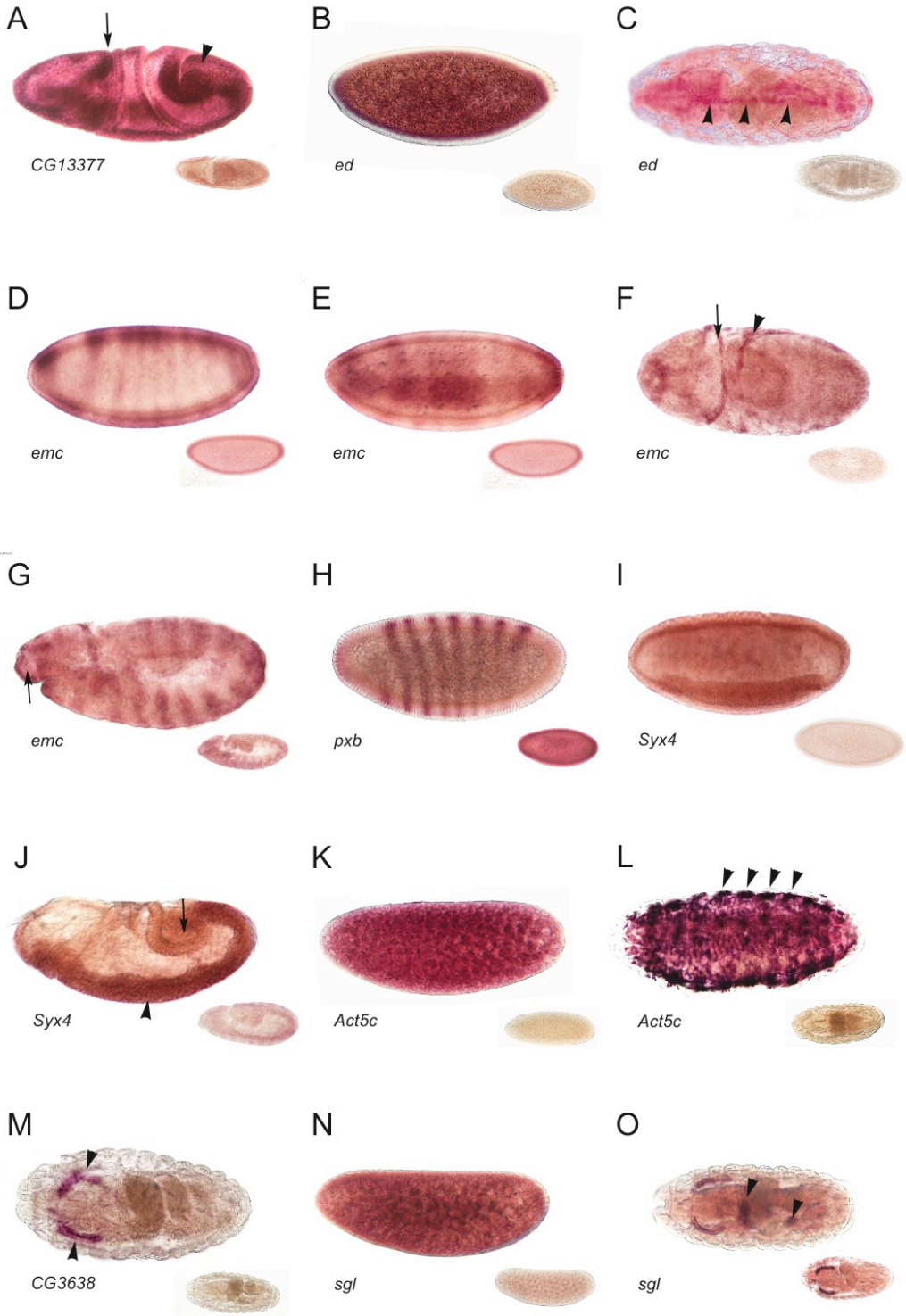
Significant MAS deviations from control line are denoted as follows: *, p<0.05; **, p<0.01; ***, p<0.001.

***P*-Element Line**	**Disrupted Locus**	**Mutational Effects**		**Human Orthologue**
		MAS	**a/σ_p**	
BG00151	*CG9894*	16.35**	0.96	n/a
BG00336	*Guanine nucleotide exchange factor GEF64C*	6.1***	-1.45	Mental retardation; Spinocerebellar ataxia
BG00372	*CG1678*	17.2***	0.94	n/a
BG00375	*Odorant-binding protein 99d*	14.8**	0.53	n/a
BG00376†	*CG3638*	18.45***	1.16	Speech-language disorder
BG00386	*NMDA receptor 1*	20.65***	1.53	Deafness; Hyperparathyroidism
BG00670	*CG32541*	13.35**	0.28	n/a
BG00735	*schizo*	16.25***	0.78	Susceptibility to multiple sclerosis; Hypercholesterolemia
BG00986†	*extra macrochaetae*	7.05***	-0.90	n/a
BG01011	*Spinophilin*	17.2***	0.69	Diabetes mellitus, noninsulin-dependent; Charcot-Marie-Tooth disease; Mental retardation; Spinocerebellar ataxia; Deafness; Autism
BG01043	*Gp150*	18*	0.91	Obesity, hyperphagia, and developmental delay; Epilepsy, partial, with auditory features; Susceptibility to schizophrenia; Tourette syndrome
BG01046	*CG3587*	7.9***	-0.60	n/a
BG01130	*alan shepard*	16.55***	0.86	Diabetes mellitus, noninsulin-dependent; Leukoencephalopathy
BG01214†	*sugarless*	17.1***	0.93	n/a
BG01215	*CG11299*	8.7**	-0.48	Lymphedema and ptosis
BG01299†	*Actin 5C*	3.6***	-1.75	n/a
BG01354	*CG30492*	18.55**	1.01	n/a
BG01402	*CG32345*	15.55***	0.68	n/a
BG01433	*CG13791*	16.75**	0.60	n/a
BG01469†	*Syntaxin 4*	7.9***	-0.65	Spastic paraplegia
BG01491	*tramtrack*	14.45*	0.48	Advanced sleep phase syndrome; Insulin resistance, severe, digenic; Giant axonal neuropathy; Cerebral cavernous malformations
BG01498	*Casein kinase Iα*	8.85*	-0.43	n/a
BG01536	*Beadex*	7.25***	-0.60	Pituitary hormone deficiency
BG01566	*arrest*	6.75***	-0.75	Obesity, variation in
BG01596†	*CG13377*	19.55***	1.74	Cortisone reductase deficiency
BG01654	*pickpocket 23*	9.55**	-0.57	Pseudohypoaldosteronism
BG01662	*Laminin A*	15.55**	0.68	Epidermolysis bullosa; Muscular dystrophy; Forebrain defects; Charcot-Marie-Tooth disease; Cerebellar hypoplasia; Susceptibility to bipolar disorder
BG01683†	*CG32572*	7.65***	-0.66	Thyroid hormone metabolism, abnormal
BG01693	*CG10777*	4.5***	-1.11	Susceptibility to insulin-dependent Diabetes mellitus
BG01713	*4EHP*	8.9**	-0.50	n/a
BG01733	*CG6175*	14.55*	0.51	n/a
BG01757	*CG17323*	12.75*	0.50	n/a
BG01765	*Tehao*	8.35**	-0.70	Mental retardation; Obesity, hyperphagia, and developmental delay; Endotoxin hyporesponsiveness; Susceptibility to schizophrenia; Tourette syndrome; Periventricular heterotopia with microcephaly
BG01893	*Splicing factor 1*	7.2*	-0.51	Deafness; Diabetes mellitus, noninsulin-dependent; Spinal muscular atrophy
BG01900	*mir-317*	6.6**	-0.62	n/a

BG01909	*CG14035*	21.45***	2.27	Spinocerebellar ataxia
BG01912†	*pxb*	17.3***	0.85	Mitochondrial complex I deficiency
BG01916	*no ocelli*	13.65**	0.36	Tourette syndrome
BG01949	*ade5*	16.2*	0.57	n/a
BG02019	*CG9171*	13.3**	0.60	Muscular dystrophy
BG02022	*CG34460*	7.15***	-0.91	n/a
BG02077	*Rtnl1*	9*	-0.65	Microphthalmia; Susceptibility to dyslexia
BG02081	*Rtnl1*	16.6***	1.19	Microphthalmia; Susceptibility to dyslexia
BG02095†	*echinoid*	14.8**	0.68	Jackson-Weiss syndrome; Mental retardation; Partial agenesis of corpus callosum; Obesity, hyperphagia, and developmental delay; Gaze palsy
BG02128	*lethal (1) G0007*	5.55***	-0.86	Charcot-Marie-Tooth disease; Sensory ataxia neuropathy
BG02188	*eclair*	15.8***	0.56	n/a
BG02217	*plexus*	4.6***	-0.99	Alzheimer disease; Deafness; Lissencephaly; Microcephaly
BG02276	*lethal (3) L1231*	10.95*	0.44	Mental retardation; Angelman syndrome; Susceptibility to autism
BG02377	*CG14478*	4.2**	-0.56	Diabetes mellitus, noninsulin-dependent; Parkinson disease; Spinocerebellar ataxia
BG02420	*CG5946*	3.85**	-0.61	n/a
BG02470	*CG8963*	7.6*	-0.52	Diabetes mellitus, noninsulin-dependent
BG02495	*ade5*	13.35*	0.33	Congenital disorder of glycosylation
BG02501	*longitudinals lacking*	7.9*	-0.61	n/a
BG02522	*CG42270*	6.1***	-0.80	Diabetes mellitus, noninsulin-dependent
BG02523	*lamina ancestor*	7.95***	-0.59	n/a
BG02539	*Basigin*	15.5**	0.58	Jackson-Weiss syndrome; Obesity, hyperphagia, and developmental delay; Gaze palsy
BG02542	*neuralized*	11.35*	-0.60	n/a
BG02644	*Fkbp13*	12**	0.59	Major depressive disorder and accelerated response to antidepressant drug treatment
BG02731	*longitudinals lacking*	13.6**	0.63	Deafness; Increased responsiveness to growth hormone; Giant axonal neuropathy; Carpenter syndrome

Appendix 3.3

Supplementary Figure 1. (A-I) Expression throughout embryonic development of the studied genes. The insets show the sense control probes. (A) CG13377: stage 8, lateral view: expression in the cephalic furrow (arrow), the hindgut and the posterior midgut rudiment (arrowhead). (B) ed, stage 1-4, lateral view: maternal contribution. (C) ed, stage 17, dorsal view: expression in the cardioblasts forming the dorsal vessel (arrowheads). (D) emc, stage 4-5, lateral view (E) emc stage 5, ventral view (F) emc stage 7, dorsal view: expression in the procephalic furrow (arrow) and the anterior tranversal furrow (arrowhead) (G) emc stage 10, lateral view: expression in the clypeolabrum (arrow), and in a segmental pattern (H) pxb: stage 5, lateral view: pair-rule expression pattern (I) syx4, stage 6, lateral view (J) syx4, stage 9, lateral view: expression in the posterior midgut primordium (arrow) and the mesoderm (arrowhead) (K) act5c, stage 1-4, lateral view: maternal contribution (L) act5c, stage 14, dorsal view: broad expression in many tissues including muscles (arrowheads) (M) CG3638, stage 16, dorsal view: expression in the salivary glands (arrowheads) (N) sgl, stage 1-4, lateral view: maternal contribution (O) sgl, stage 16, dorsal view: expression in the mid- and hindgut (arrowheads). Salivary glands are stained with the anti-sense as well as sense probes.


A
CG13377
B
ed
C
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D
emc
E
emc
F
emc
G
emc
H
pxb
I
Syx4
J
Syx4
K
Act5c
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Act5c
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CG3638
N
sgl
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Appendix 4: Supplementary Tables and Figures for Chapter 4

Appendix 4.1

Supplementary Table 1. Cytogenetic breakpoints of introgression lines. The portion of the genome deriving from each of the parental lines, *2b* (B) and *Oregon-R* (O), is described for each of the 20 introgression lines, along with the Mean Aggression Score (MAS) of each line.

Chromosome	Genotype	Line	MAS (SE)
X	1A-3E (B); 4F-20F (O)	*20*	24.15 (1.69)
	1B (O); 3E-9A (B); 10D-20F (O)	*27*	17.12 (1.64)
	1B-7E (O); 9A-11D (B), 12E-20F (O)	*21*	21.75 (1.45)
	1B-9A (O); 10D-16D (B); 17C-20F (O)	*43Я*	21.85 (1.47)
	1B-11D (O); 12E-20F (B)	*76Я*	23.7 (1.68)
2	21A-30D (B); 33E-60F (O)	*78Я*	19.0 (2.34)
	21A-21E (O); 22F-33E (B); 34EF-60F (O)	*18*	26.3 (1.67)
	21A-27B (O); 29F-38E (B); 39A-60F (O)	*16*	9.08 (1.82)
	21A-30D (O); 33E-50F (B); 57C-60F (O)	*81Я*	23.4 (2.22)
	21E (O); 22F (B); 27B-50B (O); 50D-60F (B)	*6Я*	17.3 (2.04)
	21A-50F (O); 57C-60F (B)	*22*	22.6 (1.57)
3	61A-63A (O); 65A-100F (B)	*34Я*	11.55 (1.43)
	61A-67D (O); 68B-100F (B)	*31Я*	13.65 (1.72)
	61A-68C (B); 69D-91D (O); 92A-96A (B); 96F-100A (O)	*15*	18.6 (1.50)
	61A-65A (O); 65D-69D (B); 70C-100A (O)	*88Я*	34.05 (1.89)
	61A-67D (O); 68B-96F (B); 97D-100A (O)	*5*	18.4 (1.65)
	61A-68B (O); 68C-94D (B); 96A-100F (O)	*22*	19.2 (1.69)
	61A-69D (O); 70C-91D (B); 92A-100F (O)	*16*	18.95 (1.24)
	61A-89B (O); 91A-100F (B)	*17*	13.67 (1.71)
	61A-93B (O); 94D-100A (B)	*27*	14.95 (1.95)

Appendix 4.2

Supplementary Table 2. Mutant Complementation Tests.
Mean Aggression Scores and ANOVA *p*-values are given for each gene. Genes that exhibited quantitative failure to complement (inferred from a significant line*genotype term) are in boldface. Results are from the initial screen of mutants.

			Mean Aggression Scores (SE)				ANOVA Output				
Stock #	**Gene**	**Cytological Position**	***mutant/88Я-3***	***Bal/88Я-3***	***mutant/Ore***	***Bal/Ore***	**Line *p*-value**	**Genotype *p*-value**	**L↔G *p*-value**	**L↔G F value**	**Genotype**
2460	*vn*	64E12-64F2	13.9 (1.36)	25.2 (1.53)	11.8 (1.65)	19.2 (2.39)	0.0288	<0.0001	0.28	1.2032	$vn^{C221}/TM3, Sb^{1}$
14976	*sif*	64E1-64E5	17.5 (1.09)	29.2 (0.96)	15.7 (1.87)	25.5 (1.82)	0.0738	<0.0001	0.5287	0.4048	$y^{1}\ w^{67c23};\ P\{y^{+mDint2}\ w^{BR.E.BR}=SUPor\text{-}P\}sif^{KG09067}\ ry^{506}$
16851	***CG10576***	**64E6-64E6**	**28.8 (2.15)**	**19.0 (1.29)**	**17.2 (1.93)**	**29.6 (1.91)**	**0.7885**	**0.4867**	**<0.0001**	**36.0146**	$y^{1}\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}CG10576^{EY07625}$
18136	*CG32418*	64E7-64E7	12.1 (1.13)	14.8 (1.25)	15.3 (1.30)	15.4 (1.05)	0.1174	0.2449	0.2797	1.2048	$w^{1118};\ PBac\{w^{+mC}=RB\}CG32418^{e03237}/TM6B, Tb^{1}$
11713	*S6k*	64E8-64E11	16.9 (1.85)	26.2 (2.77)	14.5 (1.49)	18.4 (0.83)	0.0098	0.0012	0.1572	2.0872	$P\{ry^{+t7.2}=PZ\}S6k^{07084}\ ry^{506}/TM3, ry^{RK}\ Sb^{1}\ Ser^{1}$
11318	*CG32409*	64F4-64F4	14.0 (1.77)	11.7 (1.56)	22.7 (1.78)	16.0 (1.24)	0.0003	0.0079	0.1778	1.8894	$w^{1118};\ PBac\{w^{+mC}=PB\}CG32409^{e03942}/TM6B, Tb^{1}$
19366	*CG10483*	64F5-64F5	18.3 (1.57)	20.2 (1.19)	24.6 (2.69)	28.1 (2.77)	0.0023	0.2212	0.7144	0.1361	$w^{*};\ PBac\{GAL4D,EYFP\}CG10483^{PL00048}\ P\{FRT(whs)\}2A\ P\{neoFRT\}82B$
18128	***CG33523***	**64F5-65A1**	**22.9 (2.17)**	**14.1 (1.23)**	**16.2 (0.87)**	**14.6 (0.83)**	**0.0316**	**0.0006**	**0.0135**	**6.7441**	$w^{1118};\ PBac\{w^{+mC}=RB\}CG33523^{e03176}$
16706	*Bj1*	65A1-65A1	18.5 (0.58)	26.8 (0.89)	17.3 (1.03)	26.2 (1.70)	0.431	<0.0001	0.7922	0.0705	$y^{1}\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}Bj1^{EY06262}$
4547	*melt*	65E4-65E5	14.5 (1.33)	25.0 (1.52)	16.6 (0.86)	26.3 (2.17)	0.2795	<0.0001	0.7976	0.0667	$y^{1}\ w^{67c23};\ P\{w^{+mC}=lacW\}melt^{S144114}/TM3, Sb^{1}\ Ser^{1}$
17844	***CG14830***	**65E9-65E10**	**16.7 (1.54)**	**24.0 (1.32)**	**20.2 (2.10)**	**19.8 (1.46)**	**0.8313**	**0.0414**	**0.0238**	**5.5741**	$w^{1118};\ PBac\{w^{+mC}=RB\}CG14830^{e00332}/TM6B, Tb^{1}$
21134	*RhoGEF4*	65F4-65F4	20.6 (1.87)	20.8 (2.63)	21.0 (0.82)	26.6 (1.75)	0.1079	0.1317	0.1596	2.0626	$y^{1}\ w^{67c23};\ P\{EPgy2\}RhoGEF4^{EY15427}$
17262	*CG8602*	65F4-65F4	18.6 (1.07)	17.3 (0.82)	20.5 (0.97)	20.4 (1.93)	0.063	0.6005	0.6547	0.2035	$w^{1118};\ P\{w^{+mC}=EP\}CG8602^{EP3102}$
329	***mus312***	**65F4-65F5**	**11.9 (1.09)**	**17.7 (1.17)**	**15.6 (1.09)**	**16.5 (0.89)**	**0.2434**	**0.003**	**0.0259**	**5.4036**	$mus312^{D1}$
17834	*CG32036*	67B10-67B10	24.1 (1.80)	27.6 (1.44)	29.6 (2.36)	27.2 (1.76)	0.1812	0.7704	0.1235	2.4878	$w^{1118};\ PBac\{w^{+mC}=RB\}CG32036^{e00275}$
14607	*path*	67B10-67B10	23.6 (2.18)	27.3 (1.44)	22.4 (1.33)	25.5 (2.11)	0.4078	0.066	0.8679	0.0281	$y^{1};\ P\{y^{+mDint2}\ w^{BR.E.BR}=SUPor\text{-}P\}path^{KG06640}\ ry^{506}/TM3, Sb^{1}\ Ser^{1}$
20067	*CG3654*	67B9-67B10	17.6 (1.09)	23.7 (2.01)	14.2 (0.77)	19.6 (1.01)	0.0068	<0.0001	0.7903	0.0718	$y^{1}\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}CG3654^{EY02717}$
20919	*CG8108*	67C11-67D1	19.2 (1.67)	31.5 (1.99)	23.5 (1.22)	32.7 (1.80)	0.1132	<0.0001	0.3662	0.8375	$y^{1}\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}CG8108E^{Y14316}/TM3, Sb^{1}\ Ser^{1}$
17737	*CG3335*	67C2-67C2	17.6 (2.05)	12.7 (1.41)	19.4 (1.58)	22.0 (2.56)	0.0073	0.5593	0.0626	3.6928	$w^{1118};\ PBac\{w^{+mC}=PB\}CG3335^{e05958}/TM6B, Tb^{1}$
11558	*fry*	67C3-67C4	15.8 (1.07)	22.9 (1.29)	16.5 (1.42)	17.8 (2.23)	0.1452	0.0084	0.0582	3.8425	$P\{ry^{+t7.2}=PZ\}fry^{02240}\ ry^{506}/TM3, ry^{RK}\ Sb^{1}\ Ser^{1}$
19575	*CG33696*	67C3-67C4	24.9 (1.12)	26.7 (1.96)	19.3 (1.31)	24.1 (1.73)	0.0128	0.042	0.3443	0.9184	$w^{*};\ PBac\{GAL4D,EYFP\}fry^{PL00631}\ CG33696^{PL00631}\ P\{FRT(whs)\}2A\ P\{neoFRT\}82B$
1750	*alphaTub67C*	67C4-67C4	17.1 (1.35)	21.0 (1.12)	12.3 (0.96)	17.2 (1.67)	0.0021	0.0017	0.7024	0.1484	$alphaTub67C^{1}\ kni^{ri\text{-}1}\ e^{1}/TM3, Sb^{1}$
15447	***UbcD4***	**67C5-67C5**	**17.4 (1.48)**	**29.2 (2.84)**	**21.2 (0.98)**	**20.3 (1.02)**	**0.1543**	**0.0037**	**0.0009**	**13.1284**	$y^{1}\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}UbcD4^{EY05497}$
17746	*defl*	67C5-67C5	23.3 (2.03)	18.7 (1.72)	25.3 (1.48)	15.3 (1.07)	0.6665	<0.0001	0.1024	2.8086	$w^{1118};\ PBac\{w^{+mC}=PB\}defl^{c06100}/TM6B, Tb^{1}$
14574	*vsg*	67C5-67C5	26.8 (1.61)	28.4 (2.26)	24.6 (2.43)	26.4 (1.66)	0.3163	0.4052	0.9527	0.0036	$w^{1118};\ P\{w^{+mGT}=GT1\}vsg^{BG00708}$
17422	***CalpB***	**67D1-67D1**	**15.1 (0.55)**	**21.4 (1.49)**	**8.1 (0.59)**	**27.5 (1.67)**	**0.7072**	**<0.0001**	**<0.0001**	**30.3615**	$y^{1}\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}CalpB^{EY08042}$
15027	***CG10616***	**69C2-69C2**	**21.5 (1.19)**	**27.7 (1.54)**	**14.5 (0.62)**	**29.7 (2.11)**	**0.0966**	**<0.0001**	**0.0041**	**202.5**	$y^{1}\ w^{67c23};\ P\{EPgy2\}CG10616^{EY00474}/TM3, Sb^{1}\ Ser^{1}$
503	*eyg*	69C2-69C2	20.6 (1.20)	34.6 (1.18)	16.7 (1.39)	30.0 (1.71)	0.0041	<0.0001	0.802	0.0638	$noc^{Sco}/CyO;\ eyg^{1}$
17916	*CG10638*	69C3-69C4	28.3 (0.94)	20.5 (1.63)	17.1 (1.23)	14.5 (1.86)	<0.0001	0.0011	0.0839	3.1605	$w^{1118};\ PBac\{w^{+mC}=RB\}CG10638^{e01020}$
18590	***Pcaf***	**69C4-69C4**	**23.9 (1.46)**	**11.8 (1.04)**	**22.9 (1.57)**	**17.7 (2.12)**	**0.1336**	**<0.0001**	**0.0374**	**4.6702**	$w^{1118};\ PBac\{w^{+mC}=WH\}Pcaf^{f02830}/TM6B, Tb^{1}$
17683	*CG10627*	69C4-69C4	18.5 (1.01)	17.1 (1.73)	23.1 (0.85)	17.4 (1.26)	0.0595	0.0078	0.0963	2.9159	$w^{1118};\ PBac\{w^{+mC}=PB\}CG10627^{e04986}/TM6B, Tb^{1}$
14933	*tral*	69C4-69C4	27.5 (1.25)	32.9 (3.19)	26.1 (1.52)	27.0 (1.84)	0.0797	0.1284	0.2739	1.2357	$y^{1};\ P\{y^{+mDint2}\ w^{BR.E.BR}=SUPor\text{-}P\}tral^{KG08052}\ ry^{506}/TM3, Sb^{1}\ Ser^{1}$
4871	*sti*	69C4-69C4	20.2 (1.11)	23.0 (1.32)	17.8 (1.17)	22.4 (2.03)	0.3093	0.0154	0.54	0.3829	$sti^{3}\ rn^{roe\text{-}1}\ p^{p}/TM3, Sb^{1}\ Ser^{1}$

17425	*eIF-2beta*	69C4-69C4	16.9 (0.96)	33.6 (1.16)	17.8 (1.24)	34.9 (1.50)	0.3767	<0.0001	0.8716	0.0265	$y^1\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}eIF\text{-}2beta^{EY08063}/TM3, Sb^1\ Ser^1$
13452	*Tsf2*	69C4-69C5	19.1 (1.97)	21.4 (1.79)	11.6 (0.58)	19.9 (1.77)	0.0088	0.0024	0.0731	3.4084	$y^1;\ P\{SUPor\text{-}P\}Tsf2^{KG01571}\ ry^{506}/TM3,\ Sb^1\ Ser^1$
18688	*CG32111*	69D1-69D1	18.1 (1.41)	17.8 (1.31)	23.8 (1.49)	22.8 (1.85)	0.0012	0.6729	0.82	0.0525	$w^{1118};\ PBac\{w^{+mC}=WH\}CG32111^{f03764}/TM6B,\ Tb^1$
12788	*caup*	69D1-69D1	16.7 (1.00)	26.7 (1.56)	14.6 (0.79)	24.2 (1.00)	0.0484	<0.0001	0.86	0.0316	$w^{1118};\ P\{GT1\}caup^{BG01626}$
2394	*mirr*	69D3-69D4	15.2 (1.16)	7.1 (0.78)	11.9 (1.04)	7.6 (1.07)	0.1791	<0.0001	0.0711	3.4582	$In(3L)D,\ D^1/Gl^1$
8486	*Ptp69D*	69D6-69E1	17.5 (1.80)	15.4 (1.53)	17.0 (1.15)	19.6 (1.57)	0.2346	0.8712	0.1334	2.3575	$w^{1118};\ Ptp69D^{10}/TM6B,\ Tb^1$
10883	***Klc***	**69E1-69E1**	**19.1 (2.51)**	**20.5 (1.57)**	**27.0 (0.91)**	**19.2 (1.52)**	**0.0637**	**0.0718**	**0.0114**	**7.1126**	$w^{1118};\ PBac\{w^{+mC}=PB\}Klc^{c02312}/TM6B,\ Tb^1$
11494	*Atg1*	69E2-69E4	18.7 (1.47)	29.3 (1.41)	16.4 (1.15)	26.0 (2.52)	0.1122	<0.0001	0.7729	2.5	$P\{ry^{+t7.2}=PZ\}Atg1^{00305}\ ry^{506}/TM3,\ ry^{RK}\ Sb^1\ Ser^1$
18626	***CG10754***	**69E4-69E4**	**27.5 (0.64)**	**23.5 (1.75)**	**16.8 (1.96)**	**26.6 (1.75)**	**0.0238**	**0.08**	**0.0001**	**18.3704**	$w^{1118};\ PBac\{w^{+mC}=WH\}CG10754^{f03161}/TM6B,\ Tb^1$
20342	***CG11006***	**69E4-69E4**	**25.8 (0.95)**	**31.7 (1.58)**	**20.6 (1.46)**	**34.7 (2.80)**	**0.5516**	**<0.0001**	**0.0314**	**5.0171**	$y^1\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}CG11006^{EY12079}/TM3,\ Sb^1\ Ser^1$
15721	*CG11008*	69E4-69E5	21.7 (2.01)	30.2 (1.97)	23.5 (0.91)	34.4 (2.51)	0.1288	<0.0001	0.5456	0.3724	$y^1\ w^{67c23};\ P\{EPgy2\}CG11008^{EY04154}/TM3,\ Sb^1\ Ser^1$
16855	*CG11255*	69F5-69F5	22.2 (1.85)	27.2 (3.12)	19.8 (1.19)	21.3 (1.45)	0.0498	0.1207	0.3977	0.7326	$y^1\ w^{67c23};\ P\{EPgy2\}CG11255^{EY07694}$
16978	*CG11267*	69F5-69F5	25.9 (1.42)	28.8 (1.31)	25.0 (1.82)	26.8 (1.55)	0.3517	0.1349	0.7225	0.1281	$y^1\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}CG11267^{EY10233}/TM3,\ Sb^1\ Ser^1$
12085	*RpS12*	69F5-69F6	16.0 (1.94)	19.3 (1.79)	15.9 (1.06)	23.7 (3.20)	0.3218	0.0137	0.3001	1.1051	$w^{1118};\ P\{w^{+mC}=lacW\}RpS12^{s2783}/TM3,\ Sb^1\ Ser^1$
11536	*Syx13*	69F6-69F6	23.7 (0.82)	26.7 (0.98)	17.4 (2.41)	23.2 (1.57)	0.0036	0.0082	0.3792	0.7926	$P\{ry^{+t7.2}=PZ\}Syx13^{01470}\ ry^{506}/TM3,\ ry^{RK}\ Sb^1\ Ser^1$
11579	*caps*	70A3-70A4	17.5 (1.21)	37.6 (1.55)	9.4 (0.72)	36.7 (4.15)	0.0607	<0.0001	0.13	2.4007	$P\{ry^{+t7.2}=PZ\}caps^{02937}\ ry^{506}/TM3,\ ry^{RK}\ Sb^1\ Ser^1$
5311	*sens*	70A8-70A8	24.6 (2.15)	24.5 (2.12)	18.5 (0.69)	16.5 (1.38)	0.0002	0.5398	0.5789	0.3136	$sens^{E2}\ red^1\ e^1/TM6B,\ Tb^+$
15808	*CG10724*	70A8-70B1	17.3 (0.86)	26.9 (1.19)	17.3 (1.98)	23.4 (2.03)	0.2808	<0.0001	0.2808	1.199	$y^1\ w^{67c23};\ P\{w^{+mC}\ y^{+mDint2}=EPgy2\}CG10724^{EY05812}/TM3,\ Sb^1\ Ser^1$
10850	*CG10154*	70B1-70B1	20.4 (1.05)	14.3 (0.96)	18.5 (1.15)	16.9 (1.66)	0.7783	0.0035	0.0765	3.3263	$w^{1118};\ PBac\{w^{+mC}=PB\}CG10154^{c02170}/TM6B,\ Tb^1$
10178	*26-29-p*	70C10-70C10	21.7 (0.63)	18.1 (1.03)	24.7 (1.67)	17.8 (1.35)	0.2835	0.0001	0.1853	1.8258	$w^{1118};\ P\{w^{+mC}=lacW\}26\text{-}29\text{-}p^{s3635}/TM6C,\ Antp^{Hu}\ Sb^1\ Tb^1$
18646	*Hml*	70C4-70C4	18.1 (1.25)	22.1 (1.75)	20.9 (1.04)	23.4 (0.62)	0.105	0.0123	0.5468	0.3701	$w^{1118};\ PBac\{w^{+mC}=WH\}Hml^{f03374}$
17912	*CG8745*	70C4-70C5	12.7 (0.63)	16.5 (1.49)	17.6 (1.89)	15.5 (2.18)	0.2458	0.6102	0.0827	3.1855	$w^{1118};\ PBac\{w^{+mC}=RB\}CG8745^{e00991}/TM6B,\ Tb1$
20984	***Rgl***	**70C5-70C5**	**16.2 (0.89)**	**23.6 (1.71)**	**9.1 (0.84)**	**23.3 (1.55)**	**0.0075**	**<0.0001**	**0.0133**	**6.7889**	$w^{1118};\ P\{w^{+mGT}=GT1\}Rgl^{GT\text{-}000359}$
510	*Gl*	70C5-70C6	11.0 (1.74)	23.1 (2.52)	11.9 (2.06)	24.3 (1.73)	0.6097	<0.0001	0.9417	0.0054	$Gl^1\ Sb^1/LVM$
19563	*CG32137*	70C6-70C7	22.4 (1.11)	27.5 (2.09)	20.8 (0.92)	27.3 (1.49)	0.5444	0.0004	0.637	0.2266	$w^*;\ PBac\{GAL4D,EYFP\}CG32137^{PL00607}P\{w^{+mW.hs}=FRT(w^{hs})\}2A\ P\{ry^{+t7.2}=neoFRT$

Appendix 5: Supplementary Tables and Figures for Chapter 5

Appendix 5.1

Supplementary Table 1. Transcripts significantly associated with variation in aggressive behavior among 40 wild-derived inbred lines (regression p-value < 0.01). Mean expression level, among (σ_{L2}) and within (σ_{E2}) line variance components, broad sense heritabilities (H_2) and the false discovery rate (FDR) for the line term are for males only.

Probe Set ID	Gene Title	Flybase ID	Module	Regression p-value	Fold change	Male/Female	Male mean expression level	Male H^2	Cytogenetic Location	Biological Process
1622920_at	transient receptor potential	FBgn0003861	4	0.00040874	1.4154	1.0412	12.6614	0.5832	99C6-99C7	0006810 // transport /// 0006811 // ion transport /// 0006816 // calcium ion transport /// 0007601 // visual perception /// 0007602 // phototransduction
1623190_at	CG9168	FBgn0035216	6	0.00020173	2.0351	1.1109	10.2679	0.8262	62A1-62A1	0008152 // metabolic process
1623191_at	Rab9	FBgn0032782	6	0.00519077	1.3060	0.9671	11.3391	0.6194	37E1-37E1	0006886 // intracellular protein transport /// 0006898 // receptor-mediated endocytosis /// 0007165 // signal transduction /// 0007264 // small GTPase mediated signal transduction /// 0015031 // protein transport /// 0017157 // regulation of exocytosis
1623241_s_at	CG30104	FBgn0050104	2	0.00755304	1.4232	1.0446	11.9340	0.8072	54B17-54B17	0006139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolic process /// 0009166 // nucleotide catabolic process
1623327_at	---	---	2	0.0029926	1.0236	1.0026	12.7848	0.6368		---
1623418_at	Cad96Ca	FBgn0022800	6	0.00759318	1.0771	1.0106	10.2448	0.7525	96C4-96C5	0006468 // protein amino acid phosphorylation /// 0007156 // homophilic cell adhesion /// 0007169 // transmembrane receptor protein tyrosine kinase signaling pathway
1623430_at	Peptidylglycine-hydroxylating monooxygenase	FBgn0019948	6	0.00498755	1.0228	1.0032	10.3136	0.8151	60A16-60A16	0001519 // peptide amidation /// 0006464 // protein modification process /// 0006518 // peptide metabolic process /// 0006584 // catecholamine metabolic process
1623896_a_at	CG6156	FBgn0038328	7	0.003076	1.1070	0.9859	10.2670	0.4598	88F1-88F1	---
1624297_at	derailed	FBgn0015380	7	0.00177861	1.1000	0.9865	10.0173	0.4612	37C7-37C7	0006468 // protein amino acid phosphorylation /// 0007165 // signal transduction /// 0007169 // transmembrane receptor protein tyrosine kinase signaling pathway /// 0007267 // cell-cell signaling
1624531_s_at	---	---	7	0.00652524	1.1498	1.0197	10.4070	0.5623		---
1624537_s_at	CG9866	FBgn0031420	5	0.00582936	1.4322	0.9521	10.3030	0.4290	22E1-22E1	---
1624541_at	RhoGAP19D	FBgn0031118	6	0.0063376	1.7560	0.9275	10.3842	0.4736	19D1-19D2	0007165 // signal transduction /// 0007242 // intracellular signaling cascade
1624763_at	CG2556	FBgn0030396	6	0.00059018	1.7007	0.9351	11.0434	0.6936	11A12-11A12	---
1624864_s_at	dreadlocks	FBgn0010583	7	0.00165407	1.2408	0.9710	10.4403	0.4583	21E2-21E2	0007186 // G-protein coupled receptor protein signaling pathway /// 0007242 // intracellular signaling cascade /// 0007409 // axonogenesis /// 0007411 // axon guidance /// 0007411 // axon guidance
1624881_at	CG11448	FBgn0024985	6	0.0015366	1.0729	1.0093	11.0410	0.5102	2A1-2A1	---
1625178_at	CG31038	FBgn0051038	6	0.0012961	1.1686	1.0196	11.6884	0.6117	99C5-99C6	---
1625454_at	G5	FBgn0030011	7	0.00739925	1.1529	1.0204	10.2688	0.5706	7D16-7D16	0007186 // G-protein coupled receptor protein signaling pathway
1625819_at	strawberry notch	FBgn0005410	9	0.00013916	2.2761	0.8960	10.2238	0.7474	11E3-11E3	0007173 // epidermal growth factor receptor signaling pathway /// 0007219 // Notch signaling pathway /// 0007560 // imaginal disc morphogenesis /// 0008587 // imaginal disc-derived wing margin morphogenesis
1625951_at	CG17778	FBgn0023534	7	0.00054789	1.0328	1.0046	10.1090	0.7001	1B5-1B5	---
1625981_at	rab3-GEF	FBgn0030613	7	0.0080454	1.1165	1.0159	10.1610	0.4694		0000187 // activation of MAPK activity /// 0006915 // apoptosis /// 0007269 // neurotransmitter secretion /// 0016192 // vesicle-mediated transport /// 0042981 // regulation of apoptosis /// 0051726 // regulation of cell cycle
1626285_at	CG13806	FBgn0035325	9	0.00047281	1.1002	0.9862	9.8779	0.7820	62D4-62D4	0006030 // chitin metabolic process
1626350_at	Esterase-10	FBgn0015569	8	0.00789901	1.2883	1.0329	11.4602	0.6617	84D8-84D9	---
1626565_at	CG2790	FBgn0027599	7	0.00617963	1.2835	0.9682	10.9689	0.4813	60E8-60E8	0006457 // protein folding
1626591_at	schizo	FBgn0026179	6	0.00706527	1.1942	0.9796	12.3116	0.5391	78A5-78B1	0006886 // intracellular protein transport /// 0006887 // exocytosis /// 0007417 // central nervous system development /// 0007520 // myoblast fusion /// 0032012 // regulation of ARF protein signal transduction
1626605_at	CG14075	FBgn0036835	8	0.00470837	1.2040	1.0249	11.0038	0.6226	75F2-75F2	---
1626619_at	CG9919	FBgn0030742	7	0.00021275	1.1117	1.0152	10.1755	0.6420	14B13-14B14	---
1626641_s_at	globin 1	FBgn0027657	9	0.00605259	1.1069	0.9892	13.3839	0.7321	89A8-89A8	0006810 // transport /// 0015671 // oxygen transport
1626667_at	miple	FBgn0027111	7	0.01003572	1.3383	1.0391	11.1779	0.4556	61B3-61B3	---
1626739_s_at	Glutamate receptor IIC	FBgn0046113	6	0.00535335	1.0935	1.0128	10.1769	0.7472	21E2-21E2	0006810 // transport /// 0006811 // ion transport /// 0006812 // cation transport /// 0006813 // potassium ion transport /// 0006936 // muscle contraction /// 0007268 // synaptic transmission /// 0007270 // nerve-nerve synaptic transmission /// 000

1627096_s_at	Tyrosine kinase-related protein	FBgn0003715	7	0.00931305	1.1342	0.9810	9.3826	0.6951	60F3-60F5	0042332 // gravitaxis
1627297_at	CG15270	FBgn0028879	7	0.0098614	1.2503	1.0324	10.2640	0.3915	35C1-35C2	---
1627499_at	CG2016	FBgn0037289	6	0.00222006	1.1426	1.0181	10.8101	0.3935	82E4-82E4	---
1627529_at	CG32726	FBgn0052726	7	0.00500986	1.0740	0.9899	10.0921	0.8302	7B2-7B2	---
1627736_at	activin-beta	FBgn0024913	7	0.00853021	1.1053	1.0149	9.8627	0.4244	102D4-102D4	0007178 // transmembrane receptor protein serine/threonine kinase signaling pathway /// 0007267 // cell-cell signaling /// 0016049 // cell growth /// 0048813 // dendrite morphogenesis
1627870_at	CG31900	FBgn0051900	7	0.00269957	1.3717	0.9561	9.9424	0.5793	28E8-28E9	---
1628005_at	CG31666	FBgn0051666	7	0.00217274	1.1006	1.0142	9.8502	0.4769	22A5-22A8	---
1628159_a_at	CG32206	FBgn0052206	7	0.0022075	1.0627	1.0092	9.5807	0.4548	76A6-76B2	---
1628496_at	dpr16	FBgn0037295	9	0.00080555	1.1235	1.0183	9.3470	0.7747	82F1-82F3	---
1628647_at	antennal protein 5	FBgn0011294	7	0.00122308	1.2619	1.0325	10.6703	0.7108	22A1-22A1	0007165 // signal transduction
1628662_at	CG13531	FBgn0034786	5	0.00244994	1.3564	0.9611	10.8690	0.4463	59B2-59B3	---
1628759_a_at	CG14073	FBgn0036814	6	0.00997876	1.4626	0.9497	10.3643	0.5654	75E1-75E1	---
1628909_at	CG9686	FBgn0030158	6	0.00148937	1.4845	1.0505	11.8501	0.6201	9A3-9A3	---
1629015_a_at	Dishevelled Associated Activator of Morphogenesis	FBgn0025641	6	0.00410737	2.0053	0.9167	11.0488	0.4155	1F2-1F3	0007067 // mitosis /// 0007242 // intracellular signaling cascade /// 0007283 // spermatogenesis /// 0007424 // open tracheal system development /// 0016043 // cellular component organization and biogenesis /// 0030036 // actin cytoskeleton organizat
1629066_at	GRHRII	FBgn0036278	7	0.00753343	1.2460	1.0301	10.8714	0.7528	69B2-69B2	0007186 // G-protein coupled receptor protein signaling pathway /// 0007292 // female gamete generation /// 0019226
1629189_at	sec5	FBgn0031537	6	0.00445204	1.2382	0.9716	10.5357	0.4631	23F3-23F3	0000910 // cytokinesis /// 0006810 // transport /// 0006887 // exocytosis /// 0007269 // neurotransmitter secretion /// 0015031 // protein transport /// 0016080 // synaptic vesicle targeting /// 0016081 //
1629417_s_at	CG14853	FBgn0038246	7	0.00023844	1.1816	1.0244	10.1092	0.5291	88C9-88C9	---
1629428_at	CG7342	FBgn0038716	3	8.3757E-05	1.4544	1.0556	10.2538	0.7953	92A10-92A10	0006810 // transport /// 0006812 // cation transport /// 0006858 // extracellular transport
1629517_at	CG5118	FBgn0031317	7	0.00515401	1.5107	0.9433	9.9043	0.4388	21F2-21F2	---
1629678_a_at	APP-like protein interacting protein 1	FBgn0040281	7	0.00868675	1.3332	1.0411	10.5159	0.5217	61F3-61F3	0007186 // G-protein coupled receptor protein signaling pathway /// 0007275 // multicellular organismal development /// 0019896 // axon transport of mitochondrion
1629842_at	GTPase-activating protein 1	FBgn0004390	9	0.00484083	2.2887	0.9023	11.0378	0.7133	67C10-67C11	0000165 // MAPKKK cascade /// 0007062 // sister chromatid cohesion /// 0007067 // mitosis /// 0007242 // intracellular signaling cascade /// 0007265 // Ras protein signal transduction
1629844_s_at	retina aberrant in pattern	FBgn0003200	6	0.00230983	2.5876	0.8856	10.6165	0.4178	4C11-4C12	0001745 // compound eye morphogenesis /// 0006508 // proteolysis /// 0007455 // eye-antennal disc morphogenesis /// 0008054 // cyclin catabolic process /// 0008347 // glial cell migration /// 0030163 // protein catabolic process
1629996_at	CG11910	FBgn0039332	6	0.00434493	1.5094	1.0569	11.0254	0.6535	96D2-96D3	0006952 // defense response /// 0007155 // cell adhesion /// 0007166 // cell surface receptor linked signal transduction
1630004_at	beaten path Ib	FBgn0028645	7	0.00493794	1.0589	1.0084	9.9026	0.5556	35D6-35D7	0007155 // cell adhesion /// 0007415 // defasciculation of motor neuron axon /// 0016198 // axon choice point recognition
1630043_a_at	alpha-catenin-related	FBgn0029105	7	0.00166104	1.1948	1.0262	10.0714	0.4305	60A14-60A14	0006928 // cell motility /// 0007010 // cytoskeleton organization and biogenesis /// 0007155 // cell adhesion /// 0007186 // G-protein coupled receptor protein signaling pathway
1630106_at	CG10362	FBgn0030358	7	0.00844909	1.1041	1.0146	9.9494	0.4732	10F7-10F7	0007242 // intracellular signaling cascade
1630212_at	CG2065	FBgn0033204	7	0.00069247	1.2118	1.0257	11.0691	0.9462	43E8-43E9	0008152 // metabolic process
1630335_s_at	CG7084	FBgn0038938	7	0.00315402	1.1648	1.0180	12.4416	0.6460	94A4-94A4	0006812 // cation transport /// 0006858 // extracellular transport
1630603_at	CG31352	FBgn0051352	5	0.00549803	1.2991	1.0340	11.4708	0.6416	85E4-85E5	0006928 // cell motility /// 0007010 // cytoskeleton organization and biogenesis /// 0007275 // multicellular organismal development /// 0007398 // ectoderm development /// 0007399 // nervous system development
1630658_at	CG32226	FBgn0052226	7	0.00466767	1.6208	0.9398	10.8698	0.4557	77B1-77B2	---
1630734_at	CG6240	FBgn0038714	7	0.00928024	1.0037	1.0006	9.5980	0.4181	92A10-92A10	---
1630961_at	CG17193	FBgn0040571	6	0.00114115	1.0350	1.0053	9.5106	0.4041	92C2-92C4	---
1631165_at	CG4688	FBgn0033817	5	0.00709942	1.1772	0.9769	9.9426	0.7987	49F12-49F12	0006952 // defense response /// 0009636 // response to toxin
1631455_at	CG30493	FBgn0050493	6	0.00056766	1.0431	0.9944	10.8904	0.5093	43E11-43E11	0006744 // ubiquinone biosynthetic process

1631516_s_at	skuld	FBgn0003415	7	0.00855323	2.2235	0.8961	9.9391	0.5846	78A2-78A2	0006366 // transcription from RNA polymerase II promoter /// 0006367 // transcription initiation from RNA polymerase II promoter /// 0009299 // mRNA transcription /// 0009790 // embryonic development /// 0045165 // cell fate commitment
1631532_at	CG5282	FBgn0036986	7	0.00577752	1.1241	1.0166	10.3092	0.4396	77C2-77C2	0006508 // proteolysis
1631604_at	CG9511	FBgn0031810	6	0.00120856	1.0916	1.0096	13.3373	0.7569	26D1-26D1	---
1631649_at	CG8271	FBgn0033657	7	0.0070645	1.1715	1.0230	10.1553	0.4464	48C4-48C5	0006810 // transport /// 0006812 // cation transport
1631787_at	---	---	1	0.00872907	25.7053	1.4964	14.1209	0.8931		---
1631956_at	CG15145	FBgn0032649	7	0.00474064	1.2937	1.0398	9.7120	0.4869	36C11-36C11	---
1632304_at	CG8026	FBgn0033391	6	0.0051349	1.0759	0.9890	9.4796	0.6174	45B3-45B3	0006810 // transport /// 0006839 // mitochondrial transport /// 0015884 // folic acid transport
1632406_at	CG9117	FBgn0031766	9	0.00872161	1.4276	0.9498	9.7190	0.4395	26B3-26B3	---
1632424_at	CG13995	FBgn0031770	7	0.0068789	1.1462	1.0203	9.9107	0.6164	26B3-26B4	0007186 // G-protein coupled receptor protein signaling pathway
1632550_at	unc-5	FBgn0034013	7	0.00248371	1.0952	0.9867	9.7467	0.5581	51F9-51F11	0007165 // signal transduction /// 0007275 // multicellular organismal development /// 0007398 // ectoderm development /// 0007411 // axon guidance /// 0007411 // axon guidance /// 0007432 // salivary gland boundary specification
1632554_at	CG9839	FBgn0037633	9	0.00106513	2.6433	0.8761	9.9172	0.5791	85B1-85B2	---
1632748_at	CG15084	FBgn0034402	9	0.00399273	1.3963	0.9532	9.8018	0.5842	55F7-55F7	---
1632841_x_at	Heat-shock-protein 70Bc	FBgn0013279	9	0.00511602	1.0409	0.9940	9.5407	0.9882	87B14-87B15	0006457 // protein folding /// 0006461 // protein complex assembly /// 0006952 // defense response /// 0006986 // response to unfolded protein /// 0009408 // response to heat
1633026_a_at	CG31158	FBgn0051158	9	0.00042259	1.7249	0.9318	10.7505	0.7278	94B5-94B6	0006886 // intracellular protein transport /// 0006887 // exocytosis /// 0032012 // regulation of ARF protein signal transduction
1633386_s_at	methuselah-like 8	FBgn0052475	9	0.00543643	1.0036	0.9995	9.7843	0.8849	61A5-61A5	0006950 // response to stress /// 0006950 // response to stress /// 0007165 // signal transduction /// 0007186 // G-protein coupled receptor protein signaling pathway
1633704_at	CG7045	FBgn0038978	1	0.00647428	21.3977	1.4799	13.6290	0.8115	94B1-94B1	0006355 // regulation of transcription, DNA-dependent
1633775_at	CG31665	FBgn0051665	7	0.00881367	1.3662	1.0454	10.3584	0.4024	22B3-22B4	0007154 // cell communication /// 0007165 // signal transduction /// 0007166 // cell surface receptor linked signal transduction /// 0007267 // cell-cell signaling /// 0007275 // multicellular organismal development /// 0007398 // ectoderm development
1633865_at	CG16734	FBgn0037667	9	0.0018565	2.0052	0.9090	10.0222	0.5368	85D4-85D4	---
1634054_at	neither inactivation nor afterpotential C	FBgn0002938	4	0.00123232	1.3746	1.0408	11.7084	0.5992	27F3-27F3	0006468 // protein amino acid phosphorylation /// 0006886 // intracellular protein transport /// 0007010 // cytoskeleton organization and biogenesis /// 0007601 // visual perception
1634073_at	CG13466	FBgn0036456	7	0.00915503	1.0361	1.0053	9.6647	0.4926	71A2-71A2	0007017 // microtubule-based process /// 0007067 // mitosis /// 0007242 // intracellular signaling cascade
1634134_at	CG6114	FBgn0036544	7	0.00073016	1.0520	1.0077	9.5707	0.5956	72A4-72B1	0006468 // protein amino acid phosphorylation /// 0007242 // intracellular signaling cascade
1634142_at	CG6403	FBgn0039453	4	0.00883972	1.1793	0.9765	9.8836	0.8674	97D1-97D1	0006030 // chitin metabolic process
1634200_a_at	CG9413	FBgn0030574	6	0.00407372	1.0695	1.0088	11.1434	0.4469	12F1-12F1	0006520 // amino acid metabolic process /// 0006810 // transport /// 0006865 // amino acid transport
1634291_at	CG11814	FBgn0035296	7	0.00150404	1.1511	0.9811	10.5360	0.6561	62B11-62B11	0006605 // protein targeting /// 0006952 // defense response /// 0007041 // lysosomal transport
1634315_a_at	CG32982	FBgn0052982	7	0.00330861	1.1039	1.0141	10.2184	0.6225	30A2-30A2	---
1635144_at	klingon	FBgn0017590	7	0.00491287	1.1275	1.0179	9.8639	0.4881	94D3-94D4	0007156 // homophilic cell adhesion /// 0007165 // signal transduction /// 0007465 // R7 cell fate commitment /// 0007611 // learning and/or memory /// 0008355 // olfactory learning /// 0045466 // R7 cell differentiation
1635198_at	CG10483	FBgn0035649	7	0.00291323	1.2808	1.0358	10.3212	0.5457	64F5-64F5	0007165 // signal transduction /// 0007166 // cell surface receptor linked signal transduction /// 0007186 // G-protein coupled receptor protein signaling pathway
1635298_at	CG13458	FBgn0036479	9	0.00543648	1.4866	1.0568	10.6483	0.5774	71B5-71B5	---
1635307_at	CG4662	FBgn0038735	6	0.00476084	1.0413	1.0059	10.0424	0.4142	92B3-92B3	---

1635352_s_at	Vacuolar H+ ATPase 44kD C subunit	FBgn0020611	6	0.00874143	1.4508	0.9612	13.3130	0.3910	53B5-53C1	0006754 // ATP biosynthetic process /// 0006810 // transport /// 0006811 // ion transport /// 0007557 // regulation of juvenile hormone biosynthetic process /// 0015986 // ATP synthesis coupled proton transport /// 0015992 // proton transport
1635607_at	CG31742	FBgn0051742	1	0.00900594	1.9120	1.0998	10.3016	0.8191	36E3-36E3	0006508 // proteolysis /// 0006511 // ubiquitin-dependent protein catabolic process
1635675_at	scarecrow	FBgn0028993	7	0.00214658	1.0503	0.9930	9.9815	0.4859		0006355 // regulation of transcription, DNA-dependent /// 0045449 // regulation of transcription
1635818_at	CG4713	FBgn0032342	6	0.00087918	1.4705	0.9518	10.9942	0.4058	32D3-32D4	---
1635996_at	CG6638	FBgn0035911	8	0.00852987	1.8830	0.9210	10.6439	0.6647	66D7-66D7	0006118 // electron transport /// 0006552 // leucine catabolic process /// 0006637 // acyl-CoA metabolic process /// 0008152 // metabolic process
1636264_at	VAChT	FBgn0015323	7	0.00822891	1.3201	1.0390	10.6746	0.4525	91C1-91C5	0006810 // transport /// 0006836 // neurotransmitter transport /// 0015893 // drug transport /// 0019226 // transmission of nerve impulse
1636291_at	Rae1	FBgn0034646	9	0.0018001	2.4710	0.8931	10.9059	0.7579	57F6-57F6	0006139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolic process /// 0006403 // RNA localization /// 0007049 // cell cycle /// 0007059 // chromosome segregation /// 0007067 // mitosis
1636344_at	chaoptic	FBgn0000313	4	0.00556746	1.4557	1.0439	12.8741	0.5596	100B5-100B6	0006952 // defense response /// 0007155 // cell adhesion /// 0007156 // homophilic cell adhesion /// 0007601 // visual perception /// 0019221 // cytokine and chemokine mediated signaling pathway
1636488_at	CG4468	FBgn0038749	9	0.00232993	1.3192	1.0370	11.1964	0.5068	92B6-92B6	---
1636583_at	CG5932	FBgn0036996	6	0.00790375	1.1953	0.9814	13.6059	0.7100	77C4-77C4	0006629 // lipid metabolic process
1636742_at	nord	FBgn0050418	7	0.00549929	1.3231	0.9606	9.8453	0.6009	60C3-60C4	0007611 // learning and/or memory /// 0008355 // olfactory learning
1636848_at	CG6024	FBgn0036202	7	0.00766779	1.0806	1.0112	10.0863	0.5113	68D3-68D4	---
1636865_at	Os-C	FBgn0010401	6	0.00455566	1.3662	1.0389	12.0239	0.5309	84E6-84E6	---
1636923_a_at	CG11198	FBgn0033246	6	0.00280785	1.0480	1.0049	13.9817	0.5301	44A1-44A2	0006633 // fatty acid biosynthetic process /// 0008152 // metabolic process
1637150_at	CG13928	FBgn0035246	4	0.00359258	1.4579	1.0486	11.7435	0.5438	62A9-62A9	---
1637506_at	CG32758	FBgn0052758	6	0.0096258	1.3121	0.9646	10.6908	0.4451	5B2-5B3	0007154 // cell communication /// 0007165 // signal transduction /// 0007242 // intracellular signaling cascade
1637605_s_at	CG1146	FBgn0035346	6	0.00336988	1.5363	1.0521	12.4988	0.6082	62E3-62E4	---
1637684_at	unc-104	FBgn0034155	7	0.00612717	1.3478	1.0368	12.1196	0.4556	53D6-53D7	0006605 // protein targeting /// 0006886 // intracellular protein transport /// 0007018 // microtubule-based movement
1637820_at	CG32677	FBgn0052677	7	0.00461257	1.1637	1.0220	10.1554	0.4589	9E1-9E1	0006605 // protein targeting
1637851_at	CG7422	FBgn0035815	9	0.00745268	1.1639	1.0223	10.0591	0.8220	66A11-66A11	0007155 // cell adhesion
1637869_at	Pray For Elves	FBgn0032661	7	0.00937174	1.1117	1.0160	9.7077	0.5883	36E3-36E3	0006464 // protein modification process /// 0006468 // protein amino acid phosphorylation /// 0006952 // defense response /// 0007155 // cell adhesion /// 0007165 // signal transduction /// 0007166 // cell surface receptor linked signal transduction
1638523_at	CG32633	FBgn0052633	8	0.00596862	1.2660	0.9684	10.4356	0.5694	12A4-12A4	---
1638678_at	CG6522	FBgn0034223	5	5.6085E-05	2.9235	0.8644	9.8621	0.6497	54B16-54B16	0007010 // cytoskeleton organization and biogenesis
1638807_s_at	CG4829	FBgn0030796	6	0.00077891	1.2059	1.0266	10.4327	0.5281	15A10-15A11	0006464 // protein modification process /// 0006508 // proteolysis /// 0006520 // amino acid metabolic process
1639175_s_at	---	---	3	0.0044279	1.1284	1.0188	9.4682	0.6957		---
1639406_at	late bloomer	FBgn0016032	5	0.00075138	1.2334	1.0287	10.8443	0.7833	42E6-42E6	0007398 // ectoderm development /// 0007399 // nervous system development /// 0007416 // synaptogenesis /// 0007416 // synaptogenesis /// 0019226 // transmission of nerve impulse
1639431_at	synaptogyrin	FBgn0033876	7	0.00567923	1.4298	1.0461	11.7152	0.6776	50C6-50C6	0016079 // synaptic vesicle exocytosis /// 0017158 // regulation of calcium ion-dependent exocytosis
1639534_at	CG6792	FBgn0032401	9	0.00453081	1.3691	0.9552	9.6676	0.8118	33B11-33B11	0006139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolic process /// 0006357 // regulation of transcription from RNA polymerase II promoter /// 0006366 // transcription from RNA polymerase II promoter /// 0008283 // cell proliferation
1639694_s_at	CG10102	FBgn0033927	7	0.00657816	1.6599	1.0600	12.9262	0.5311		---
1639846_at	CG13760	FBgn0040375	1	0.00100173	1.5428	0.9416	10.0809	0.6797	3A6-3A6	---
1639909_at	CG7058	FBgn0030961	7	0.00116774	1.0347	1.0052	9.4741	0.5273	17E1-17E1	---
1639923_at	CG30502	FBgn0050502	8	0.00628192	1.4518	0.9521	10.7017	0.6243	43C1-43C1	0008152 // metabolic process
1639951_at	CG2611	FBgn0032871	7	0.00359535	1.2882	0.9663	10.4608	0.5766	38D2-38D2	---

1640227_at	CG8801	FBgn0028473	6	0.00307855	2.0317	0.9302	13.6236	0.6085	45D2-45D3	0008283 // cell proliferation /// 0042254 // ribosome biogenesis and assembly
1640566_at	Cyp4p2	FBgn0033395	8	0.00634698	1.0754	0.9903	10.7041	0.5994	45B7-45B7	0006118 // electron transport /// 0008202 // steroid metabolic process
1640633_s_at	CG32425	FBgn0052425	5	0.00803031	1.1428	0.9843	12.0915	0.5324	77C6-77C6	---
1640913_at	GST-containing FLYWCH zinc-finger protein	FBgn0053546	8	0.00811355	2.0068	0.9079	9.9087	0.3939	84C6-84C6	0006952 // defense response /// 0009636 // response to toxin
1640974_a_at	CG33143	FBgn0053143	7	0.00466106	1.1646	1.0218	10.3118	0.4489	58F2-58F2	---
1641120_at	CG13506	FBgn0034723	6	0.00369758	1.1803	1.0191	12.7715	0.6252	58D4-58D4	---
1641427_at	fear-of-intimacy	FBgn0024236	6	0.00925176	2.8781	0.8762	10.7979	0.4583	66D5-66D6	0006810 // transport /// 0006811 // ion transport /// 0006829 // zinc ion transport /// 0007275 // multicellular organismal development /// 0007280 // pole cell migration /// 0007399 // nervous system development /
1641738_a_at	CG13636	FBgn0039232	6	0.00080227	3.3912	0.8625	11.0505	0.5909	96A24-96A25	---

Appendix 5.2

Supplementary Table 2. Associations of SFPs with aggressive behavior. MAF = Minor Allele Frequency. MAS = Mean Aggression Score. The *P*-value is from the ANOVA of the difference in trait means between the two SFP classes. *a* is one half the difference in trait mean between the SFP alleles.

Supplementary Table 2. Independent probe set targets containing single feature polymorphisms at a nominal *p* value of <0.05. All targets have a minor allele frequency of at least 10%.

Probe Set ID	Probe Number	Gene Title	Flybase ID	Parametric *p*-value	Major Allele MAS	Minor Allele MAS	*a*	Minor allele frequency	Cytogenetic Location	SFP calling score	Biological Process	Molecular Function
1623102_at	4	GIP-like	FBgn0011770	0.0110916	27.4946	50.5333	-11.5194	0.075	9B7-9B7	0.922521	5975 // carbohydrate metabolism // 6281 // DNA repair //	3677 // DNA binding // 4519 // endonuclease activity // 16853 // isomerase activity // 5515 // protein binding
1623458_at	11	CG14899	FBgn0038438	0.0110415	27.1986	47.4375	-10.1194	0.1	89B18-89B18	0.926643	---	5515 // protein binding
1623666_at	8	CG10516	FBgn0036549	0.0288915	34.7711	24.2024	5.2843	0.475	72C1-72C1	0.953118	---	---
1623737_a_at	13	Rab-protein 14	FBgn0015791	0.0134233	41.0625	26.2625	7.4000	0.2	35A4-35A4	0.903646	6886 // intracellular protein transport // 6898 // receptor mediated endocytosis // 6909 // phagocytosis // 7041 // lysosomal transport	3924 // GTPase activity // 5525 // GTP binding // 166 // nucleotide binding // 5525 // GTP binding
1623788_at	9	CG7461	FBgn0034432	0.0302589	33.4979	22.8094	5.3443	0.4	56C9-56C10	0.945059	6118 // electron transport // 6637 // acyl-CoA metabolism //	17099 // very-long-chain-acyl-CoA dehydrogenase activity // 17099 // very-long-chain-acyl-CoA dehydrogenase activity
1623991_s_at	9	coro	FBgn0033109	0.002461	26.8528	50.5500	-11.8486	0.1	42D6-42D6	0.94237	7010 // cytoskeleton organization and biogenesis //	3779 // actin binding // 3824 // catalytic activity // 5200 // structural constituent of cytoskeleton // 3779 // actin bind
1624033_at	13	amyloid protein precursor-like	FBgn0000108	0.0361004	24.1475	34.2975	-5.0750	0.5	1B9-1B10	0.968418	6917 // induction of apoptosis // 7010 // cytoskeleton organization and biogenesis // 7166 // cell surface receptor linked signal transduction	4872 // receptor activity // 5515 // protein binding // 5488 // binding //
1624042_at	12	G protein-coupled receptor kinase 2	FBgn0004834	0.0376391	23.3647	33.5522	-5.0937	0.425	100C3-100C4	0.960482	6468 // protein amino acid phosphorylation /// 6468 // protein amino acid phosphorylation /// 7165 // signal transduction	4674 // protein serine/threonine kinase activity // 4702 // receptor signaling protein serine/threonine kinase activity // 4703 // G-protein coupled receptor kinase activity
1624044_at	9	mitochondrial ribosomal protein L52	FBgn0033208	0.0224507	23.9929	35.0026	-5.5049	0.475	43E9-43E9	0.903845	---	3735 // structural constituent of ribosome
1624080_s_at	3	Transportin	FBgn0024921	0.038185	25.3320	35.7067	-5.1873	0.375	65A8-65A8	0.964951	59 // protein import into nucleus, docking // 6606 // protein import into nucleus /// 6606 // protein import into nucleus	8320 // protein carrier activity // 5488 // binding // 8565 // protein transporter activity
1624131_at	11	---	---	0.0074831	56.9500	27.7632	14.5934	0.05		0.965545	---	---
1624290_at	11	CG4752	FBgn0034733	0.0081349	40.1350	25.5850	7.2750	0.25	58E3-58E3	0.93402	6952 // defense response // 9636 // response to toxin //	17168 // 5-oxoprolinase (ATP-hydrolyzing) activity // 3824 // catalytic activity // 16787 // hydrolase activity // 17168 //
1624423_at	5	CG5945	FBgn0032494	0.0430972	26.9621	39.8786	-6.4582	0.175	34A11-34B1	0.912981	---	5515 // protein binding
1624731_s_at	13	CG33205	FBgn0053205	0.0358097	26.6813	39.3875	-6.3531	0.2	67C9-67C10	0.946883	7498 // mesoderm development	5515 // protein binding
1624763_at	5	CG2556	FBgn0030396	0.0152012	37.6115	25.1833	6.2141	0.325	11A12-11A12	0.971941	---	---
1624890_s_at	3	CG12393	FBgn0031768	0.0321336	33.0250	22.1607	5.4321	0.35	26B3-26B3	0.96857	---	---
1624957_a_at	13	Tequila	FBgn0023479	0.0067704	24.5019	37.9893	-6.7437	0.35	66F4-66F5	0.97442	6030 // chitin metabolism // 6508 // proteolysis /// 15986 // ATP synthesis coupled proton transport // 6508 //	4252 // serine-type endopeptidase activity // 4263 // chymotrypsin activity
1624981_a_at	11	CG10576	FBgn0035630	0.0132399	32.6467	18.9500	6.8483	0.25	64E6-64E6	0.938293	6412 // protein biosynthesis // 6464 // protein modification // 6508 // proteolysis //	3676 // nucleic acid binding // 4239 // methionyl aminopeptidase activity // 30528 // transcription regulator activity
1625034_s_at	12	transforming acidic coiled-coil protein	FBgn0026620	0.0050108	23.5109	36.9500	-6.7196	0.425	82D2-82D2	0.968182	226 // microtubule cytoskeleton organization and biogenesis // inferred from mutant phenotype /// 7026 // negative regulation of microtubule depolymerization	5515 // protein binding // 8017 // microtubule binding
1625113_s_at	10	CG6509	FBgn0032363	0.0195297	32.2484	18.8000	6.7242	0.225	32F2-32F2	0.946854	7010 // cytoskeleton organization and biogenesis //	5198 // structural molecule activity // 5515 // protein binding
1625178_at	7	CG31038	FBgn0051038	0.0230709	31.7439	17.3357	7.2041	0.175	99C5-99C6	0.912158	---	---
1625430_at	1	CG14022	FBgn0031700	0.024746	38.6000	26.0967	6.2517	0.25	25D6-25D6	0.922045	---	3998 // acylphosphatase activity /
1625430_at	2	CG14022	FBgn0031700	0.024746	38.6000	26.0967	6.2517	0.25	25D6-25D6	0.939694	---	3998 // acylphosphatase activity
1625491_at	12	brain washing	FBgn0045064	0.0385217	19.1938	31.7297	-6.2680	0.2	38B2-38B3	0.986627	6672 // ceramide metabolism // 7420 // brain development /// 6629 // lipid metabolism //	17040 // ceramidase activity // 16787 // hydrolase activity // 16811 // hydrolase activity, acting on carbon-nitrogen (but not peptide) bonds, in linear amides
1625568_a_at	13	CG15111	FBgn0034419	0.004196	25.8406	42.7500	-8.4547	0.2	56B5-56B5	0.947103	---	---
1625640_a_at	7	Tetraspanin 97E	FBgn0039465	0.0453607	26.9848	39.7714	-6.3933	0.175	97D11-97D11	0.958548	---	4872 // receptor activity //
1625640_a_at	8	Tetraspanin 97E	FBgn0039465	0.0453607	26.9848	39.7714	-6.3933	0.175	97D11-97D11	0.94348	---	4872 // receptor activity //

1625680_a_at	10	CG5792	FBgn0032455	0.0378008	31.9339	19.8833	6.0253	0.225	33F3-33F4	0.947416	---	5515 // protein binding
1625856_at	1	diminutive	FBgn0000472	0.0253118	25.0440	36.1867	-5.5713	0.375	3D2-3D2	0.909297	74 // regulation of progression through cell cycle /// 6355 // regulation of transcription, DNA-dependent // 8283 // cell proliferation /// 8361 // regulation	3677 // DNA binding // 3700 // transcription factor activity // 5515 // protein binding
1626203_at	5	---	---	0.0187752	36.1406	24.6104	5.7651	0.4	31E1-31E1	0.962731	6397 // mRNA processing // 16071 // mRNA metabolism //	17070 // U6 snRNA binding
1626221_at	2	Glycogenin	FBgn0034603	0.0381645	25.5365	36.0679	-5.2657	0.35	57D1-57D1	0.946246	7498 // mesoderm development /// 16051 // carbohydrate biosynthesis //	8466 // glycogenin glucosyltransferase activity // 5515 // protein binding // 16758 // transferase activity, transferring hexosyl
1626682_s_at	14	CG9135	FBgn0031769	0.0127068	32.4355	18.1556	7.1400	0.225	26B3-26B3	0.954163	6508 // proteolysis //	5085 // guanyl-nucleotide exchange factor activity // 5515 // protein binding/// 5085 // guanyl-nucleotide exchange factor activity
1626821_s_at	1	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.971944	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	10	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.960125	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	11	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.976618	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	12	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.979259	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	13	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.986102	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	14	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.975714	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	2	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.970296	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	3	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.988302	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	4	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.981737	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	5	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.964571	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	6	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.974246	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	7	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.972475	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	8	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.964238	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1626821_s_at	9	Heat-shock-protein-70Bc	FBgn0013279	0.0074831	56.9500	27.7632	14.5934	0.05	87B14-87B15	0.96595	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding
1627868_at	14	Phosphoribosylamidotransferase	FBgn0004901	0.0291891	32.0613	19.4444	6.3084	0.225	84E5-84E5	0.931591	5996 // monosaccharide metabolism // 6144 // purine base metabolism // 6189 // 'de novo' IMP biosynthesis	4044 // amidophosphoribosyltransferase activity // 4044 // amidophosphoribosyltransferase activity // 287 // magnesium ion binding
1627962_at	7	CG31749	FBgn0051749	0.0090839	35.7605	23.3071	6.2267	0.475	36F3-36F4	0.950178	---	---

1628285_a_at	14	Regena	FBgn0017550	0.036023	19.8000	31.9581	-6.0790	0.225	83B5-83B6	0.955037	6139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolism // 6366 // transcription from RNA polymerase II promoter // 9299 // mRNA transcription	30528 // transcription regulator activity
1628432_at	14	CG5590	FBgn0039537	0.0240343	24.7813	35.8844	-5.5516	0.4	98B1-98B1	0.926145	8152 // metabolism //	5498 // sterol carrier activity // 16616 // oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor // 16491 // oxidoreductase activity
1628494_a_at	1	Picot	FBgn0024315	0.0176507	24.5688	36.2031	-5.8172	0.4	53C15-53D1	0.936175	6796 // phosphate metabolism // 6812 // cation transport // 6817 // phosphate transport /// 6858 // extracellular transport	5316 // high affinity inorganic phosphate:sodium symporter activity
1628613_a_at	3	CG30492	FBgn0050492	0.0111286	22.5167	34.7091	-6.0962	0.45	43E5-43E7	0.916482	---	8270 // zinc ion binding
1628678_at	3	CG13920	FBgn0025712	0.0494617	31.4212	18.8571	6.2820	0.175	62A9-62A9	0.957068	---	---
1628723_at	6	CG4038	FBgn0011824	0.0001693	22.9442	40.8821	-8.9690	0.35	57B20-57B20	0.943915	6364 // rRNA processing // 6365 // 35S primary transcript processing /// 7046 // ribosome biogenesis	19843 // rRNA binding // 30515 // snoRNA binding // 30559 // rRNA pseudouridylation guide activity
1628726_s_at	6	Ral guanine nucleotide exchange factor 2	FBgn0026376	0.0288408	25.5593	36.8308	-5.6358	0.325	70C5-70C5	0.940288	7169 // transmembrane receptor protein tyrosine kinase signaling pathway // 7186 // G-protein coupled receptor protein signaling pathway	8321 // Ral guanyl-nucleotide exchange factor activity // 8321 // Ral guanyl-nucleotide exchange factor activity // 16220 // RAL GDP-dissociation stimulator activity //
1628763_at	7	Ptpmeg	FBgn0035133	0.0380492	31.3338	17.2583	7.0377	0.15	61C1-61C1	0.916569	6470 // protein amino acid dephosphorylation /// 7010 // cytoskeleton organization and biogenesis // 7242 // intracellular signaling cascade	4725 // protein tyrosine phosphatase activity // 5200 // structural constituent of cytoskeleton
1628763_at	8	Ptpmeg	FBgn0035133	0.0380492	31.3338	17.2583	7.0377	0.15	61C1-61C1	0.941916	6470 // protein amino acid dephosphorylation /// 7010 // cytoskeleton organization and biogenesis // 7242 // intracellular signaling cascade // /	4725 // protein tyrosine phosphatase activity // 5200 // structural constituent of cytoskeleton
1628821_at	10	CG17027	FBgn0036553	0.0435562	25.8241	36.2808	-5.2283	0.325	72C1-72C1	0.957271	6644 // phospholipid metabolism // 7242 // intracellular signaling cascade // 16311 // dephosphorylation /// 5975 // carbohydrate metabolism	8934 // inositol-1(or 4)-monophosphatase activity // 8934 // inositol-1(or 4)-monophosphatase activity // 4437 // inositol or phosphatidylinositol phosphatase activity
1629083_at	5	CG16704	FBgn0031558	0.0236151	35.2528	24.2886	5.4821	0.45	24B1-24B1	0.918761	6810 // transport //	4867 // serine-type endopeptidase inhibitor activity // 5215 // transporter activity
1629362_at	13	methuselah-like 8	FBgn0052475	0.022099	33.9609	22.8118	5.5746	0.425	61A5-61A5	0.936885	6950 // response to stress /// 7186 // G-protein coupled receptor protein signaling pathway /// 8340 // determination of adult life span	4930 // G-protein coupled receptor activity // 4871 // signal transducer activity // 4872 // receptor activity
1629362_at	7	methuselah-like 8	FBgn0052475	0.022099	33.9609	22.8118	5.5746	0.425	61A5-61A5	0.93648	6950 // response to stress /// 7186 // G-protein coupled receptor protein signaling pathway/// 8340 // determination of adult life span	4930 // G-protein coupled receptor activity // 4871 // signal transducer activity // 4872 // receptor activity
1629372_a_at	12	CG7578	FBgn0028538	0.0196512	26.4172	40.4438	-7.0133	0.2	34D1-34D1	0.956704	6886 // intracellular protein transport // 6887 // exocytosis // 6888 // ER to Golgi vesicle-mediated transport /	5086 // ARF guanyl-nucleotide exchange factor activity // 5086 // ARF guanyl-nucleotide exchange factor activity // 5488 //
1629469_s_at	14	CG10960	FBgn0036316	0.0208671	26.8868	42.4583	-7.7858	0.15	69E5-69E6	0.936737	5975 // carbohydrate metabolism // 8643 // carbohydrate transport // 6810 // transport //	5351 // sugar porter activity // 5355 // glucose transporter activity // 5215 // transporter activity
1629510_at	3	CG4294	FBgn0034742	0.0259079	33.6104	22.6406	5.4849	0.4	58F1-58F1	0.916468	8152 // metabolism //	287 // magnesium ion binding // 16462 // pyrophosphatase activity
1629563_at	3	CG17059	FBgn0040754	0.0272394	18.5625	31.8875	-6.6625	0.2	49F10-49F10	0.946404	---	---
1629578_at	5	CG8309	FBgn0033902	0.0298323	33.5083	22.7938	5.3573	0.4	50E1-50E1	0.936663	---	5515 // protein binding // inferred from physical interaction /// 3824 // catalytic activity //
1629676_at	13	Tat-binding protein-1	FBgn0028684	0.0142114	37.2286	24.9115	6.1585	0.35	95B1-95B1	0.967798	6508 // proteolysis /// 50875 // cellular physiological process /// 30163 // protein catabolism	166 // nucleotide binding // 4175 // endopeptidase activity // inferred from direct assay /// 4175 // endopeptidase activity // 4175 // endopeptidase activity
1629779_at	11	Macroglobulin complement-related	FBgn0020240	0.0439159	34.3632	24.5714	4.8959	0.475	28E1-28E3	0.954199	6508 // proteolysis // 6952 // defense response // 7165 // signal transduction // 7267 // cell-cell signaling	4866 // endopeptidase inhibitor activity // 4867 // serine-type endopeptidase inhibitor activity // 5125 // cytokine activity

1629827_s_at	1	Heat-shock-protein-70Bb	FBgn0013278	0.0074831	56.9500	27.7632	14.5934	0.05	87B12-87B12	0.952488	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding //
1629827_s_at	2	Heat-shock-protein-70Bb	FBgn0013278	0.0074831	56.9500	27.7632	14.5934	0.05	87B12-87B12	0.94185	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding //
1629827_s_at	4	Heat-shock-protein-70Bb	FBgn0013278	0.0074831	56.9500	27.7632	14.5934	0.05	87B12-87B12	0.936646	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat //	5524 // ATP binding // 166 // nucleotide binding //
1629843_s_at	14	Ubc-E2H	FBgn0029996	0.0278564	32.2883	20.0250	6.1317	0.25	7D6-7D6	0.98634	6464 // protein modification // 6512 // ubiquitin cycle // 19538 // protein metabolism //	4840 // ubiquitin conjugating enzyme activity // 16874 // ligase activity // 5515 // protein binding
1630052_s_at	10	CG8446	FBgn0034089	0.0307797	24.5000	34.9944	-5.2472	0.45	52F7-52F8	0.958707	6464 // protein modification // 6732 // coenzyme metabolism //	16874 // ligase activity // 17118 // lipoyltransferase activity // 3824 // catalytic activity
1630216_at	12	drosha	FBgn0026722	0.0012583	39.4607	23.7096	7.8755	0.35	43F2-43F3	0.922002	6396 // RNA processing // 16072 // rRNA metabolism // 31053 // primary microRNA processing	3725 // double-stranded RNA binding // 4525 // ribonuclease III activity
1630421_at	5	CG33170	FBgn0053170	0.0296617	28.0171	52.1250	-12.0539	0.05	80B1-80B1	0.915033	---	---
1630506_at	13	CG3077	FBgn0031457	0.0281295	20.0400	32.2833	-6.1217	0.25	23A5-23A5	0.93907	7242 // intracellular signaling cascade //	5515 // protein binding // 35091 // phosphoinositide binding //
1630521_at	5	Resistance to Juvenile Hormone	FBgn0002723	0.0336524	33.6413	23.2441	5.1986	0.425	10D1-10D1	0.93519	6357 // regulation of transcription from RNA polymerase II promoter // 7165 // signal transduction // 45449 // regulation of transcription	3700 // transcription factor activity // 4871 // signal transducer activity // 5500 // juvenile hormone binding
1630842_s_at	10	CG32641	FBgn0052641	0.0263465	25.5037	36.9462	-5.7212	0.325	11E3-11E3	0.920581	6457 // protein folding // 9408 // response to heat / /// 9408 // response to heat	51082 // unfolded protein binding //
1630946_at	13	---	---	0.035489	30.9194	13.9500	8.4847	0.1		0.988758	---	---
1631368_s_at	7	CG8108	FBgn0027567	0.0064164	34.5063	21.2969	6.6047	0.4	67C11-67D1	0.94001	---	3676 // nucleic acid binding // 8270 // zinc ion binding //
1631425_at	11	CG9231	FBgn0036887	0.0314804	33.4688	22.8531	5.3078	0.4	76B8-76B8	0.986604	---	---
1631425_at	13	CG9231	FBgn0036887	0.0314804	33.4688	22.8531	5.3078	0.4	76B8-76B8	0.90959	---	---
1631531_at	13	CG5434	FBgn0038810	0.0308862	32.2350	20.1850	6.0250	0.25	92F2-92F2	0.944423	6445 // regulation of translation // 6614 // SRP-dependent cotranslational protein targeting to membrane	8135 // translation factor activity, nucleic acid binding // 8312 // 7S RNA binding // 5488 // binding
1631691_at	14	CG16772	FBgn0032835	0.0084932	23.5523	36.1528	-6.3003	0.45	38A8-38A8	0.944796	---	---
1632087_at	6	CG9928	FBgn0032472	0.0120262	39.6250	25.7550	6.9350	0.25	34A6-34A6	0.965203	---	5515 // protein binding // inferred from physical interaction
1632089_at	10	CG4045	FBgn0025629	0.0460803	26.2397	37.0864	-5.4234	0.275	2C10-2C10	0.935204	6400 // tRNA modification /// 8033 // tRNA processing	8176 // tRNA (guanine-N7-)-methyltransferase activity // 5515 // protein binding // inferred from physical interaction /// 8168 // methyltransferase activity
1632220_s_at	11	Ribosomal protein S26	FBgn0004413	0.0230325	33.9304	22.8529	5.5387	0.425	36F4-36F4	0.977946	6412 // protein biosynthesis	3676 // nucleic acid binding // 3735 // structural constituent of ribosome // 3735 // structural constituent of ribosome
1632361_at	14	real-time	FBgn0031814	0.031382	37.1708	25.8161	5.6774	0.3	26D4-26D5	0.943455	6886 // intracellular protein transport //	5386 // carrier activity // 5488 // binding // 8526 // phosphatidylinositol transporter
1632667_s_at	11	Glyceraldehyde 3 phosphate dehydrogenase 2	FBgn0001092	0.0046157	27.6855	58.4250	-15.3697	0.05	13F17-13F17	0.97949	6096 // glycolysis /// 6006 // glucose metabolism	4365 // glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) activity
1633034_s_at	6	Ferritin 1 heavy chain homologue	FBgn0015222	0.0249258	33.8717	22.9324	5.4697	0.425	99F2-99F2	0.923079	6826 // iron ion transport // 6879 // iron ion homeostasis	8198 // ferrous iron binding // 8199 // ferric iron binding
1633034_s_at	8	Ferritin 1 heavy chain homologue	FBgn0015222	0.0169506	24.5417	36.2438	-5.8510	0.4	99F2-99F2	0.985271	6826 // iron ion transport // 6879 // iron ion homeostasis	8198 // ferrous iron binding // 8199 // ferric iron binding
1633074_a_at	4	Moesin	FBgn0011661	0.0192687	24.3848	35.7676	-5.6914	0.425	8B4-8B6	0.949967	2009 // morphogenesis of an epithelium /// 7010 // cytoskeleton organization and biogenesis // traceable author statement /// 7315 // pole plasm assembly	3779 // actin binding // 5200 // structural constituent of cytoskeleton // 5515 // protein binding // inferred from physical interaction /// 8092 // cytoskeletal protein binding //
1633224_at	13	CG9631	FBgn0027563	0.0421506	35.2594	25.1979	5.0307	0.4	88A12-88B1	0.919039	6508 // proteolysis /// 6952 // defense response //	4252 // serine-type endopeptidase activity // 4263 // chymotrypsin activity // 4295 // trypsin activity //

1633269_at	14	CG9140	FBgn0031771	0.0010169	28.0000	76.9000	-24.4500	0.025	26B4-26B4	0.91802	6120 // mitochondrial electron transport, NADH to ubiquinone /// 6118 // electron transport	3954 // NADH dehydrogenase activity // 8137 // NADH dehydrogenase (ubiquinone) activity // 5506 // iron ion binding
1633333_a_at	5	CG7231	FBgn0031968	0.0350496	21.8692	32.7630	-5.4469	0.325	28D3-28D3	0.909226	---	---
1633386_s_at	10	methuselah-like 8	FBgn0052475	0.022099	33.9609	22.8118	5.5746	0.425	61A5-61A5	0.957451	6950 // response to stress /// 7186 // G-protein coupled receptor protein signaling pathway /// 8340 // determination of adult life span	4930 // G-protein coupled receptor activity // 4871 // signal transducer activity // 4872 // receptor activity
1633613_at	7	CG2765	FBgn0035087	0.0212485	28.3346	63.8500	-17.7577	0.025	60E10-60E11	0.949682	---	---
1633935_at	4	skiff	FBgn0050021	0.040304	21.1500	32.2845	-5.5672	0.275	47E1-47E1	0.916283	6605 // protein targeting // 7165 // signal transduction // 8105 // asymmetric protein localization //	4385 // guanylate kinase activity // 5515 // protein binding // 5515 // protein binding
1633997_s_at	7	CG5938	FBgn0046247	0.04516	27.8378	46.3000	-9.2311	0.075	97F1-97F1	0.92042	---	---
1634072_s_at	7	HMG Coenzyme A synthase	FBgn0010611	0.0177162	30.8446	9.2167	10.8140	0.075	53C1-53C1	0.916674	1700 // embryonic development (sensu Insecta) /// 8203 // cholesterol metabolism // 6084 // acetyl-CoA metabolism //	4421 // hydroxymethylglutaryl-CoA synthase activity // 16829 // lyase activity
1634103_at	1	CG15122	FBgn0034457	0.0341025	26.4597	38.7389	-6.1396	0.225	56D15-56D15	0.937698	---	---
1634121_at	14	Odorant-binding protein 57c	FBgn0034509	0.0365842	34.8194	24.6432	5.0881	0.45	57A4-57A4	0.986472	6810 // transport // 7606 // sensory perception of chemical stimulus // 7608 // sensory perception of smell	5549 // odorant binding
1634209_at	9	pimples	FBgn0003087	0.0395716	35.6633	25.3580	5.1527	0.375	31D10-31D10	0.943496	70 // mitotic sister chromatid segregation	5515 // protein binding
1634304_a_at	5	CG17233	FBgn0036958	0.0468959	21.3864	32.1948	-5.4042	0.275	77A3-77A3	0.909209	---	---
1634443_a_at	13	Rab-protein 1	FBgn0016700	0.0317436	35.9267	25.2000	5.3633	0.375	93D2-93D2	0.967924	160 // two-component signal transduction system (phosphorelay) // 6355 // regulation of transcription, DNA-dependent // 6886 // intracellular protein transport	3677 // DNA binding // 3924 // GTPase activity // 5525 // GTP binding // 166 // nucleotide binding
1634452_s_at	3	endophilin A	FBgn0038659	0.0144454	26.5227	41.9500	-7.7136	0.175	91D4-91D4	0.920429	7269 // neurotransmitter secretion 48488 // synaptic vesicle endocytosis	5515 // protein binding // 42171 // lysophosphatidic acid acyltransferase activity
1634687_at	10	CG15019	FBgn0035541	0.0424428	22.5143	32.8346	-5.1602	0.35	64B4-64B4	0.946754	---	---
1634728_at	2	CG17821	FBgn0034383	0.0242267	34.6550	23.7900	5.4325	0.5	55E11-55E11	0.926937	6629 // lipid metabolism // 6631 // fatty acid metabolism //	8415 // acyltransferase activity // 16740 // transferase activity //
1634820_at	5	Proteasome 29kD subunit	FBgn0003150	0.0414849	36.7750	25.9857	5.3946	0.3	57B5-57B5	0.949966	6510 // ATP-dependent proteolysis /// 6511 // ubiquitin-dependent protein catabolism	5515 // protein binding /// 4175 // endopeptidase activity
1635283_at	13	CG31839	FBgn0028543	0.0003242	38.8088	22.1370	8.3359	0.425	34E5-34E5	0.968355	7160 // cell-matrix adhesion // 7165 // signal transduction // 7498 // mesoderm development	5102 // receptor binding // 5198 // structural molecule activity //
1635284_a_at	8	HDAC4	FBgn0041210	0.0235176	25.2173	36.6607	-5.7217	0.35	11E8-11E9	0.932716	74 // regulation of progression through cell cycle // 6357 // regulation of transcription from RNA polymerase II promoter //	3682 // chromatin binding // 4407 // histone deacetylase activity
1635771_a_at	8	CG32306	FBgn0052306	0.0143185	27.5514	49.8333	-11.1410	0.075	62E1-62E2	0.90005	---	---
1635887_at	14	CG30094	FBgn0050094	0.0118286	32.9293	19.4500	6.7397	0.275	52E10-52E11	0.928035	---	8270 // zinc ion binding //
1635903_at	11	Protein kinase at 92B	FBgn0014006	0.0332276	32.1967	20.3000	5.9483	0.25	92B4-92B4	0.960462	165 // MAPKKK cascade // 6468 // protein amino acid phosphorylation /// 6468 // protein amino acid phosphorylation	4702 // receptor signaling protein serine/threonine kinase activity // 4710 // MAP/ERK kinase kinase activity // 5524 // ATP binding
1635960_at	13	supernumerary limbs	FBgn0023423	0.0453607	26.9848	39.7714	-6.3933	0.175	93B13-93C1	0.908255	6508 // proteolysis /// 6512 // ubiquitin cycle /// 7611 // learning and/or memory /// 7623 // circadian rhythm	4842 // ubiquitin-protein ligase activity // 5515 // protein binding
1635989_at	9	Olfactory-specific 9	FBgn0014000	0.0144072	26.0629	40.1056	-7.0213	0.225	38A8-38A8	0.969508	---	---
1636348_s_at	6	Actin 87E	FBgn0000046	0.0092541	32.8050	18.4750	7.1650	0.25	87E11-87E11	0.931945	7010 // cytoskeleton organization and biogenesis	3774 // motor activity // 5200 // structural constituent of cytoskeleton // 5515 // protein binding //
1636363_s_at	12	Mapmodulin	FBgn0034282	0.0422633	25.2000	35.2563	-5.0281	0.4	54F4-54F4	0.952532	7017 // microtubule-based process /// 6913 // nucleocytoplasmic transport /// 7242 // intracellular signaling cascade	8017 // microtubule binding // 19212 // phosphatase inhibitor activity
1636398_at	10	CG10086	FBgn0037517	0.0355721	26.4806	38.6667	-6.0930	0.225	84E1-84E1	0.913928	---	5515 // protein binding
1636446_at	7	COP9 complex homolog subunit 6	FBgn0028837	0.0206993	36.0406	24.6771	5.6818	0.4	94B5-94B5	0.926667	74 // regulation of progression through cell cycle // 6508 // proteolysis // 338 // protein deneddylation /// 7275 // development	19781 // NEDD8 activating enzyme activity /// 5515 // protein binding

1636514_at	7	TGF- activated kinase 1	FBgn0026323	0.0096713	32.0652	15.8214	8.1219	0.175	19D2-19D2	0.947751	165 // MAPKKK cascade // 6468 // protein amino acid phosphorylation /// 6915 // apoptosis /// 6915 // apoptosis	4672 // protein kinase activity // 4706 // JUN kinase kinase kinase activity // inferred from mutant phenotype /// 4709 // MAP kinase kinase kinase activity
1636514_at	8	TGF- activated kinase 1	FBgn0026323	0.010057	32.5323	17.8222	7.3550	0.225	19D2-19D2	0.98076	165 // MAPKKK cascade // 6468 // protein amino acid phosphorylation /// 6915 // apoptosis	4672 // protein kinase activity // 4706 // JUN kinase kinase kinase activity // inferred from mutant phenotype /// 4709 // MAP kinase kinase kinase activity
1636630_s_at	12	CG5091	FBgn0032234	0.0173605	35.2289	23.7881	5.7204	0.475	31D11-31D11	0.961681	6487 // protein amino acid N-linked glycosylation7155 // cell adhesion // 7275 // development // 19226 // transmission of nerve impulse	16757 // transferase activity, transferring glycosyl groups // 42281 // dolichyl pyrophosphate Man9GlcNAc2 alpha-1,3-glucosyltransferase activity
1636934_at	11	CG32307	FBgn0052307	0.0037839	42.8875	25.8063	8.5406	0.2	62C4-62D1	0.937532	---	---
1637024_at	2	VhaPPA1-1	FBgn0028662	0.046804	33.1646	23.3094	4.9276	0.4	88D6-88D6	0.986058	6812 // cation transport // 15986 // ATP synthesis coupled proton transport //	8553 // hydrogen-exporting ATPase activity, phosphorylative mechanism // 46933 // hydrogen-transporting ATP synthase activity, rotational mechanism
1637193_at	14	eIF3-S8	FBgn0034258	0.029964	41.7083	27.0191	7.3446	0.15	54D4-54D4	0.990746	6413 // translational initiation /// 6445 // regulation of translation	3743 // translation initiation factor activity
1637276_at	8	Pendulin	FBgn0011823	0.0170223	40.6813	26.3578	7.1617	0.2	31A1-31A2	0.932082	59 // protein import into nucleus, docking /// 6606 // protein import into nucleus /// 6607 // NLS-bearing substrate import into nucleus	8320 // protein carrier activity // 5515 // protein binding // inferred from physical interaction /// 5488 // binding // 8565 // protein transporter activity //
1637300_at	12	---	---	0.0003598	25.7824	48.7167	-11.4672	0.15	44F11-44F11	0.90169	---	---
1637461_at	10	CG9527	FBgn0031813	0.0239496	35.5529	24.5435	5.5047	0.425	26D1-26D1	0.950169	6118 // electron transport // 6635 // fatty acid beta-oxidation // 6631 // fatty acid metabolism //	16402 // pristanoyl-CoA oxidase activity // 3995 // acyl-CoA dehydrogenase activity // 3997 // acyl-CoA oxidase activity // 50660 // FAD bin
1637605_s_at	7	CG1146	FBgn0035346	0.0149162	38.1375	25.4018	6.3679	0.3	62E3-62E4	0.92974	7010 // cytoskeleton organization and biogenesis //	3779 // actin binding //
1637736_a_at	10	UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2	FBgn0030930	0.0042198	26.1091	43.9000	-8.8955	0.175	17B6-17B6	0.953481	5976 // polysaccharide metabolism // 6486 // protein amino acid glycosylation // 9312 // oligosaccharide biosynthesis	4653 // polypeptide N-acetylgalactosaminyltransferase activity
1638310_at	12	Helicase	FBgn0001565	0.0143146	37.6808	25.1500	6.2654	0.325	20A1-20A1	0.933423	6139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolism //	3676 // nucleic acid binding // 4004 // ATP-dependent RNA helicase activity // 5524 // ATP binding
1638701_at	9	CG3825	FBgn0034948	0.0359185	26.4855	38.6500	-6.0823	0.225	60A15-60A16	0.988344	---	---
1638726_at	8	lethal (3) 03670	FBgn0010808	0.0105099	38.0269	24.9833	6.5218	0.325	100B1-100B1	0.9076	---	5515 // protein binding
1638872_at	3	Heat shock protein 68	FBgn0001230	0.0074831	56.9500	27.7632	14.5934	0.05	95D11-95D11	0.965994	6457 // protein folding /// 6461 // protein complex assembly // 6952 // defense response	5524 // ATP binding // 51082 // unfolded protein binding // 166 // nucleotide binding
1638872_at	6	Heat shock protein 68	FBgn0001230	0.0074831	56.9500	27.7632	14.5934	0.05	95D11-95D11	0.947457	6457 // protein folding /// 6461 // protein complex assembly // 6952 // defense response	5524 // ATP binding // 51082 // unfolded protein binding // 166 // nucleotide binding
1638872_at	8	Heat shock protein 68	FBgn0001230	0.0074831	56.9500	27.7632	14.5934	0.05	95D11-95D11	0.963819	6457 // protein folding /// 6461 // protein complex assembly // 6952 // defense response	5524 // ATP binding // 51082 // unfolded protein binding // 166 // nucleotide binding
1638889_at	9	CG3918	FBgn0029873	0.0071647	48.4000	27.0917	10.6542	0.1	6B1-6B1	0.964108	---	5515 // protein binding/// 3676 // nucleic acid binding // 8270 // zinc ion binding //
1638942_at	14	CG30499	FBgn0050499	0.0177375	25.2704	37.4308	-6.0802	0.325	43D7-43D7	0.967249	5975 // carbohydrate metabolism // 6098 // pentose-phosphate shunt //	4750 // ribulose-phosphate 3-epimerase activity // 16853 // isomerase activity // 16854 // racemase and epimerase activity //
1638994_at	6	CG10627	FBgn0036298	0.0247308	24.5674	35.5206	-5.4766	0.425	69C4-69C4	0.92765	5996 // monosaccharide metabolism // 5975 // carbohydrate metabolism //	4610 // phosphoacetylglucosamine mutase activity // 5515 // protein binding /// 16868 // intramolecular transferase activity, phosphotransferases
1639269_a_at	12	Photoreceptor dehydrogenase	FBgn0011693	0.0418904	24.2875	34.1575	-4.9350	0.5	72E2-72E2	0.964735	6629 // lipid metabolism // 8152 // metabolism //	4022 // alcohol dehydrogenase activity // 16616 // oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor // 5515 // protein binding
1639522_at	6	CG31950	FBgn0051950	0.042781	33.6682	23.7889	4.9396	0.45	23C1-23C1	0.933305	16071 // mRNA metabolism //	---

1639544_at	8	CG8010	FBgn0031008	0.014531	24.1870	36.0353	-5.9242	0.425	18B7-18B7	0.943228	---	5515 // protein binding
1639571_s_at	9	Heat-shock-protein-70Aa	FBgn0013275	0.0074831	56.9500	27.7632	14.5934	0.05	87A3-87A3	0.98285	6457 // protein folding // 6461 // protein complex assembly // 6952 // defense response // 9408 // response to heat	5524 // ATP binding // 166 // nucleotide binding //
1639646_at	8	CG30373	FBgn0050373	0.0390122	24.9217	35.0412	-5.0597	0.425	44B7-44B7	0.923177	---	---
1639788_at	10	Vacuolar H<up>+</up> ATPase 14kD subunit	FBgn0010426	0.0385692	30.6500	11.6167	9.5167	0.075	52B5-52B5	0.912296	15986 // ATP synthesis coupled proton transport // 15992 // proton transport /// 6754 // ATP biosynthesis // 6810 // transport	8553 // hydrogen-exporting ATPase activity, phosphorylative mechanism
1639806_at	10	chromosome bows	FBgn0021760	0.037361	32.9231	22.3500	5.2865	0.35	78C1-78C2	0.958415	22 // mitotic spindle elongation /// 70 // mitotic sister chromatid segregation /// 7051 // spindle organization and biogenesis /// 7052 // mitotic spind	5525 // GTP binding // inferred from direct assay /// 8017 // microtubule binding // 5488 // binding //
1640043_s_at	9	---	---	0.0484129	24.4275	34.0175	-4.7950	0.5		0.940339	74 // regulation of progression through cell cycle // 7049 // cell cycle 7067 // mitosis	---
1640318_at	11	dj-1	FBgn0039802	0.0230494	21.7607	33.2404	-5.7398	0.35	100A5-100A5	0.954002	6732 // coenzyme metabolism // 6766 // vitamin metabolism // 9110 // vitamin biosynthesis // 51189 // prosthetic group metabolism	3676 // nucleic acid binding // 3729 // mRNA binding // 3824 // catalytic activity //
1640380_a_at	12	Mediator complex subunit 14	FBgn0035145	0.045534	25.4620	35.4900	-5.0140	0.375	61C6-61C6	0.942123	6366 // transcription from RNA polymerase II promoter /// 6367 // transcription initiation from RNA polymerase II promoter	16455 // RNA polymerase II transcription mediator activity // 16455 // RNA polymerase II transcription mediator activity // 4872 // receptor activity
1640472_at	9	---	---	0.0010169	28.0000	76.9000	-24.4500	0.025		0.929783	---	---
1640567_at	11	CG7337	FBgn0031374	0.0467316	27.8473	46.1833	-9.1680	0.075	22B4-22B6	0.940113	---	---
1640834_at	2	mitochondrial ribosomal protein L27	FBgn0053002	0.0031852	21.9211	35.8286	-6.9538	0.475	24F3-24F3	0.934636	6412 // protein biosynthesis //	3735 // structural constituent of ribosome
1640834_at	6	mitochondrial ribosomal protein L27	FBgn0053002	0.0050581	21.0938	34.6417	-6.7740	0.4	24F3-24F3	0.906756	6412 // protein biosynthesis //	3735 // structural constituent of ribosome
1640991_at	13	CG32091	FBgn0052091	0.0424441	23.1938	33.2417	-5.0240	0.4	68D1-68D1	0.919751	6629 // lipid metabolism // 6810 // transport /// 6820 // anion transport // 6869 // lipid transport	166 // nucleotide binding // 5215 // transporter activity // 5524 // ATP binding
1640991_at	9	CG32091	FBgn0052091	0.0329449	21.3417	32.6000	-5.6292	0.3	68D1-68D1	0.956086	6629 // lipid metabolism // 6810 // transport /// 6820 // anion transport // 6869 // lipid transport	166 // nucleotide binding // 5215 // transporter activity // 5524 // ATP binding
1641006_s_at	8	Phosphotidylinositol 3 kinase 68D	FBgn0015278	0.0471917	26.8125	38.8625	-6.0250	0.2	68D4-68D6	0.930208	6605 // protein targeting // 6886 // intracellular protein transport // 6897 // endocytosis // 6952 // defense response	5543 // phospholipid binding /// 16303 // phosphatidylinositol 3-kinase activity
1641108_at	5	CG17928	FBgn0032603	0.0224851	41.1571	26.6909	7.2331	0.175	36B1-36B1	0.904631	---	16491 // oxidoreductase activity //
1641281_at	8	CG12391	FBgn0033581	0.0111193	19.3727	32.9586	-6.7929	0.275	47D5-47D5	0.927221	---	3676 // nucleic acid binding // 8270 // zinc ion binding // 5515 // protein binding /// 46872 // metal ion binding //
1641281_at	9	CG12391	FBgn0033581	0.0222675	18.2125	31.9750	-6.8813	0.2	47D5-47D5	0.906404	---	3676 // nucleic acid binding // 8270 // zinc ion binding // 5515 // protein binding /// 46872 // metal ion binding //
1641347_s_at	14	visgun	FBgn0045823	0.0128875	26.9729	44.9700	-8.9986	0.125	67C5-67C5	0.956839	7611 // learning and/or memory /// 8355 // olfactory learning	---
1641347_s_at	3	visgun	FBgn0045823	0.0148694	26.3031	40.9000	-7.2984	0.2	67C5-67C5	0.906809	7611 // learning and/or memory /// 8355 // olfactory learning	---
1641623_at	10	CG15917	FBgn0034199	0.040846	25.7815	36.3692	-5.2939	0.325	54A1-54A1	0.912258	---	---
1641623_at	9	CG15917	FBgn0034199	0.040846	25.7815	36.3692	-5.2939	0.325	54A1-54A1	0.927187	---	---
1641692_at	8	CG5886	FBgn0039379	0.0021525	25.0700	41.6800	-8.3050	0.25	96F3-96F4	0.913632	---	---
1641745_a_at	4	CG5558	FBgn0038684	0.0461172	33.8286	24.1316	4.8485	0.475	91F4-91F4	0.915584	6139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolism // 16072 // rRNA metabolism //	3676 // nucleic acid binding // 5515 // protein binding
1641747_s_at	2	CG3136	FBgn0033010	0.0151997	25.8617	39.3050	-6.7217	0.25	41D4-41E1	0.904757	6355 // regulation of transcription, DNA-dependent //	3677 // DNA binding // 42803 // protein homodimerization activity // 5515 // protein binding /// 3700 // transcription factor

Appendix 5.3

Supplementary Table 3. Candidate genes previously associated with aggressive behavior (18). SFP = Single Feature Polymorphism . QTT = Quantitative Trait Transcript. Average $|r|$ is the mean absolute value of the correlation of the transcript to all other variable transcripts. H^2 = broad sense heritability.

Probe Set ID	SFP	QTT	Gene Name	Flybase ID	Mean expression accross sexes	Average |r|	Chromosomal Location	Cytogenetic Location	Female Mean	Male Mean	H2	Gene Ontology Biological Process	Gene Ontology Molecular Function
1623191_at	yes	yes	Rab9	FBgn0032782	11.53170486	0.4526	Chr2L	37E1-37E1	11.7243	11.3391	0.48451	6886 // intracellular protein transport /// 6898 // receptor mediated endocytosis /// 7165 // signal transduction /// 7264 // small GTPase	3924 // GTPase activity /// 5525 // GTP binding /// 166 // nucleotide binding /// 3924 // GTPase activity
1623241_s_at		yes	CG30104	FBgn0050104	11.67948128	0.3288	chr2R	54B17-54B17	11.4249	11.934	0.77704	6139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolism /// 9166 // nucleotide catabolism	16791 // phosphoric monoester hydrolase activity /// 19204 // nucleotide phosphatase activity /// 166 // nucleotide binding
1623418_at		yes	Cad96Ca	FBgn0022800	10.19123354	0.2387	chr3R	96C4-96C5	10.1377	10.2448	0.60474	6468 // protein amino acid phosphorylation /// 7156 // homophilic cell adhesion	
1623788_at	yes		CG7461	FBgn0034432	14.01423465	0.3878	chr2R	56C9-56C10	14.1686	13.8599	0.38162	6118 // electron transport /// 6637 // acyl-CoA metabolism	17099 // very-long-chain-acyl-CoA dehydrogenase activity /// 17099 // very-long-chain-acyl-CoA dehydrogenase activity
1624297_at		yes	derailed	FBgn0015380	10.08605519	0.286	Chr2L	37C7-37C7	10.1548	10.0173	0.42827		
1626350_at		yes	Esterase-10	FBgn0015569	11.27742841	0.2957	chr3R	84D8-84D9	11.0947	11.4602	0.78126	---	4091 // carboxylesterase activity /// 4104 // cholinesterase activity
1626641_s_at		yes	globin 1	FBgn0027657	13.45717479	0.1598	chr3R	89A8-89A8	13.5304	13.3839	0.70291	15671 // oxygen transport /// 6810 // transport	5344 // oxygen transporter activity /// 5386 // carrier activity /// 5515 // protein binding
1628909_at		yes	CG9686	FBgn0030158	11.56514003	0.3983	chrX	9A3-9A3	11.2801	11.8501	0.6457	---	---
1629083_at	yes		CG16704	FBgn0031558	12.57150182	0.1227	Chr2L	24B1-24B1	12.6393	12.5037	0.81671	6810 // transport	4867 // serine-type endopeptidase inhibitor activity /// 5215 // transporter activity
1629469_s_at	yes		CG10960	FBgn0036316	12.89574876	0.351	chr3L	69E5-69E6	13.1313	12.6602	0.69422	5975 // carbohydrate metabolism /// 8643 // carbohydrate transport /// 6810 // transport	5351 // sugar porter activity /// 5355 // glucose transporter activity /// 5215 // transporter activity
1629779_at	yes		Macroglobulin complement-related	FBgn0020240	11.85791205	0.6098	Chr2L	28E1-28E3	12.3254	11.3904	0.41421	6508 // proteolysis /// 6952 // defense response /// 7165 // signal transduction /// 7267 // cell-cell signaling	4866 // endopeptidase inhibitor activity /// 4867 // serine-type endopeptidase inhibitor activity /// 5125 // cytokine activity
1630335_s_at		yes	CG7084	FBgn0038938	12.33160213	0.2456	chr3R	94A4-94A4	12.2216	12.4416	0.61369	6812 // cation transport /// 6858 // extracellular transport	8513 // organic cation porter activity /// 15144 // carbohydrate transporter activity
1630842_s_at	yes		CG32641	FBgn0052641	12.64364569	0.2398	chrX		12.7853	12.502	0.77456	6457 // protein folding /// 9408 // response to heat	51082 // unfolded protein binding
1631787_at		yes	---	---	11.77885337	0.6112	chr2R		9.4369	14.1209	0.77807	---	---
1632087_at	yes		CG9928	FBgn0032472	13.82466881	0.1229	Chr2L	34A6-34A6	13.7656	13.8837	0.63664	---	5515 // protein binding
1632550_at		yes	unc-5	FBgn0034013	9.812257017	0.3035	chr2R	51F9-51F11	9.8778	9.7467	0.53679	7398 // ectoderm development /// 7411 // axon guidance /// 8347 // glial cell migration	5042 // netrin receptor activity /// 5043 // repulsive netrin receptor activity /// 4872 // receptor activity
1633613_at	yes		CG2765	FBgn0035087	14.22499769	0.3088	chr2R	60E10-60E11	14.0587	14.3913	0.59034	---	---
1633704_at		yes	CG7045	FBgn0038978	11.41933107	0.6258	chr3R	94B1-94B1	9.2096	13.629	0.69286	6355 // regulation of transcription, DNA-dependent	3677 // DNA binding
1634072_s_at	yes		HMG Coenzyme A synthase	FBgn0010611	12.75610489	0.4451	chr2R	53C1-53C1	12.9472	12.565	0.38185	1700 // embryonic development (sensu Insecta) /// 8203 // cholesterol metabolism /// 6084 // acetyl-CoA metabolism	4421 // hydroxymethylglutaryl-CoA synthase activity /// 16829 // lyase activity
1634142_at		yes	CG6403	FBgn0039453	10.00262123	0.2281	chr3R	97D1-97D1	10.1216	9.8836	0.84409	6030 // chitin metabolism	8061 // chitin binding
1635284_a_at	yes		HDAC4	FBgn0041210	9.644324072	0.1049	chrX		9.632	9.6567	0.60368	74 // regulation of progression through cell cycle /// 6357 // regulation of transcription from RNA polymerase II promoter	3682 // chromatin binding /// 4407 // histone deacetylase activity

1636583_at		yes	CG5932	FBgn0036996	13.73454106	0.2081	chr3L	77C4-77C4	13.8632	13.6059	0.77553	6629 // lipid metabolism /// 16042 // lipid catabolism	4806 // triacylglycerol lipase activity /// 16789 // carboxylic ester hydrolase activity
1636923_a_at		yes	CG11198	FBgn0033246	13.94782173	0.1495	chr2R	44A1-44A2	13.914	13.9817	0.36505	6633 // fatty acid biosynthesis /// 8152 // metabolism	3989 // acetyl-CoA carboxylase activity /// 4075 // biotin carboxylase activity /// 5524 // ATP binding
1637869_at		yes	Pray For Elves	FBgn0032661	9.631328475	0.3513	Chr2L	36E3-36E3	9.555	9.7077	0.50467	6464 // protein modification /// 6468 // protein amino acid phosphorylation /// 6952 // defense response /// 7155 // cell adhesion	4672 // protein kinase activity /// 4675 // transmembrane receptor protein serine/threonine kinase activity /// 4872 // receptor activity
1640043_s_at	yes		---	---	11.65254342	0.3566	Chr2L		11.8469	11.4582	0.67147	74 // regulation of progression through cell cycle /// 7049 // cell cycle /// 7067 // mitosis	---
1640566_at		yes	Cyp4p2	FBgn0033395	10.75655064	0.1966	chr2R	45B7-45B7	10.809	10.7041	0.60319	6118 // electron transport /// 8202 // steroid metabolism	4497 // monooxygenase activity /// 16491 // oxidoreductase activity /// 5515 // protein binding /// 5506 // iron ion binding

Appendix 5.4

Supplementary Table 4. Analysis of modules of correlated transcripts associated with aggressive behavior. Degree = the average correlation of a transcript with all other transcripts in its module. Average Degree = the average correlation of all transcripts in the module.

Probe Set ID	Gene Name	Gene Symbol	GO Biological Process	Module	Degree	Average Degre	Disease Associated with Human Orthologue
1633704_at	*CG7045*	*CG7045 /// DsimCG7045*	0006355 // regulation of transcription, DNA-dependent // inferred from electronic annotation	1	0.64991	0.594	Microphthalmia, abnormalities of the central nervous system, Mental retardation
1639846_at	*CG13760*	*CG13760*	---	1	0.63652	0.594	
1631787_at	---	---	---	1	0.61571	0.594	
1635607_at	*AE003657 - proteasome beta subunit*	*CG31742*	0006508 // proteolysis // inferred from electronic annotation /// 0006511 // ubiquitin-dependent protein catabolic process // inferred from electronic annotation	1	0.47386	0.594	
1623241_s_at	*CG30104*	*CG30104*	0006139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolic process // inferred from electronic annotation /// 0009166 // nucleotide catabolic process // inferred from electronic annotation	2	0.57237	0.57237	
1623327_at	---	---	---	2	0.57237	0.57237	
1629428_at	*CG7342*	*CG7342*	0006810 // transport // inferred from electronic annotation /// 0006812 // cation transport // inferred from electronic annotation /// 0006858 // extracellular transport // inferred from electronic annotation	3	0.55944	0.55944	Diabetes mellitus, Deficiency in glucose transport across BBB
1639175_s_at	---	---	---	3	0.55944	0.55944	
1637150_at	*CG13928*	*CG13928*	---	4	0.63352	0.53335	
1634054_at	*Droninac*	*ninaC*	0006468 // protein amino acid phosphorylation // non-traceable author statement /// 0006468 // protein amino acid phosphorylation // inferred from electronic annotation /// 0006886 // intracellular protein transport // inferred from mutant phenotype /// 0007010 // cytoskeleton organization and biogenesis // inferred from mutant phenotype /// 0007601 // visual perception // inferred from electronic annotation /// 0007603 // phototransduction, visible light // inferred from mutant phenotype /// 0007604 // phototransduction, UV // inferred from mutant phenotype /// 0008104 // protein localization // inferred from mutant phenotype /// 0008104 // protein localization // traceable author statement /// 0016059 // deactivation of rhodopsin mediated signaling // inferred from mutant phenotype /// 0016059 // deactivation of rhodopsin mediated signaling // traceable author statement /// 0016062 // adaptation of rhodopsin mediated signaling // inferred from mutant phenotype /// 0016062 // adaptation of rhodopsin mediated signaling // traceable author statement	4	0.62153	0.53335	Ccardiomyopathy, Deafness, Obesity, Spinocerebellar ataxia, Mental retardation, Parkinson disease
1622920_at	*Cosens-Manning mutant*	*trp*	0006810 // transport // inferred from electronic annotation /// 0006811 // ion transport // inferred from electronic annotation /// 0006816 // calcium ion transport // inferred from sequence or structural similarity /// 0006816 // calcium ion transport // inferred from electronic annotation /// 0006816 // calcium ion transport // inferred from mutant phenotype /// 0007601 // visual perception // inferred from electronic annotation /// 0007602 // phototransduction // non-traceable author statement /// 0007608 // sensory perception of smell // inferred from electronic annotation /// 0008104 // protein localization // inferred from mutant phenotype /// 0008355 // olfactory learning // inferred from mutant phenotype /// 0008377 // light-induced release of internally sequestered calcium ion // inferred from direct assay /// 0009416 // response to light stimulus // inferred from mutant phenotype /// 0009416 // response to light stimulus // traceable author statement /// 0019722 // calcium-mediated signaling // traceable author statement /// 0030845 // phospholipase C inhibition //	4	0.59893	0.53335	Microphthalmia, Infantile neuroaxonal dystrophy, Neurodegeneration, Partial agenesis of Corpus callosum, deafness, Glomerulosclerosis
1636344_at	*chaoptin*	*chp*	0006952 // defense response // inferred from electronic annotation /// 0007155 // cell adhesion // inferred from electronic annotation /// 0007156 // homophilic cell adhesion // inferred from direct assay /// 0007156 // homophilic cell adhesion // non-traceable author statement /// 0007601 // visual perception // inferred from electronic annotation /// 0019221 // cytokine and chemokine mediated signaling pathway // inferred from electronic annotation /// 0042051 // compound eye photoreceptor development // non-traceable author statement /// 0042052 // rhabdomere development // inferred from genetic interaction /// 0050896 // response to stimulus // inferred from electronic annotation	4	0.55796	0.53335	Obesity, hyperphagia, Hypogonadotropic hypogonadism, Pseudohermaphroditism, male, Endotoxin hyporesponsiveness, Epilepsy, Susceptibility to schizophrenia, Tourette syndrome, Endotoxin hyporesponsiveness, Susceptibility to Legionnaire Disease
1634142_at	*CG6403*	*CG6403*	0006030 // chitin metabolic process // inferred from electronic annotation	4	0.25479	0.53335	

1630603_at	*CG31352*	*CG31352*	0006928 // cell motility // inferred from electronic annotation /// 0007010 // cytoskeleton organization and biogenesis // inferred from electronic annotation /// 0007275 // multicellular organismal development // inferred from electronic annotation /// 0007398 // ectoderm development // inferred from electronic annotation /// 0007399 // nervous system development // inferred from electronic annotation	5	0.49931	0.36847	Pituitary hormone deficiency
1624537_s_at	*CG9866*	*CG9866*	---	5	0.46327	0.36847	Charcot-Marie-Tooth disease
1640633_s_at	*CG32425*	*CG32425*	---	5	0.44498	0.36847	
1639406_at	*Late Bloomer*	*lbm*	0007398 // ectoderm development // inferred from electronic annotation /// 0007399 // nervous system development // inferred from electronic annotation /// 0007416 // synaptogenesis // inferred from mutant phenotype /// 0007416 // synaptogenesis // non-traceable author statement /// 0019226 // transmission of nerve impulse // inferred from electronic annotation	5	0.40918	0.36847	Mental retardation
1628662_at	*CG13531*	*CG13531*	---	5	0.39271	0.36847	Spastic paraplegia, Hepatic lipase deficiency
1631165_at	*CG4688*	*CG4688*	0006952 // defense response // inferred from electronic annotation /// 0009636 // response to toxin // inferred from electronic annotation	5	0.20567	0.36847	Charcot-Marie-Tooth disease
1638678_at	*CG6522*	*CG6522*	0007010 // cytoskeleton organization and biogenesis // inferred from electronic annotation	5	0.16416	0.36847	Scapuloperoneal myopathy, Pituitary hormone deficiency
1638807_s_at	*CG4829*	*CG4829*	0006464 // protein modification process // inferred from electronic annotation /// 0006508 // proteolysis // inferred from electronic annotation /// 0006520 // amino acid metabolic process // inferred from electronic annotation	6	0.50586	0.35753	Immunodeficiency
1637605_s_at	*CG1146*	*CG1146*	---	6	0.50487	0.35753	Aromatase deficiency, Adrenal hyperplasia, Hypertension
1624881_at	*CG11448*	*CG11448*	---	6	0.50305	0.35753	Alzheimer disease, Pachyonychia congenita, Deafness, Neuropathy, Spinocerebellar ataxia, Microcephaly
1635352_s_at	*vacuolar ATPase C-subunit*	*Vha44*	0006754 // ATP biosynthetic process // inferred from electronic annotation /// 0006810 // transport // inferred from electronic annotation /// 0006811 // ion transport // inferred from electronic annotation /// 0007557 // regulation of juvenile hormone biosynthetic process // traceable author statement /// 0015986 // ATP synthesis coupled proton transport // inferred from electronic annotation /// 0015992 // proton transport // inferred from sequence or structural similarity /// 0015992 // proton transport // inferred from electronic annotation	6	0.48272	0.35753	
1637506_at	*CG32758*	*CG32758*	0007154 // cell communication // inferred from electronic annotation /// 0007165 // signal transduction // inferred from electronic annotation /// 0007242 // intracellular signaling cascade // inferred from electronic annotation	6	0.47177	0.35753	Autism, Susceptibility to Crohn disease, Deafness, Mental retardation
1629015_a_at	*Dishevelled Associated Activator of Morphogenesis*	*DAAM*	0007067 // mitosis // inferred from electronic annotation /// 0007242 // intracellular signaling cascade // inferred from electronic annotation /// 0007283 // spermatogenesis // inferred from electronic annotation /// 0007424 // open tracheal system development // inferred from mutant phenotype /// 0016043 // cellular component organization and biogenesis // inferred from electronic annotation /// 0030036 // actin cytoskeleton organization and biogenesis // non-traceable author statement /// 0030036 // actin cytoskeleton organization and biogenesis // inferred from electronic annotation /// 0035017 // cuticle pattern formation // non-traceable author statement /// 0035152 // regulation of tube architecture, open tracheal system // inferred from mutant phenotype /// 0045011 // actin cable formation // inferred from mutant phenotype	6	0.46397	0.35753	Deafness, Premature ovarian failure, Cherubism, Spinocerebellar Ataxia

1626591_at	*schizo*	*siz*	0006886 // intracellular protein transport // inferred from electronic annotation /// 0006887 // exocytosis // inferred from electronic annotation /// 0007417 // central nervous system development // inferred from mutant phenotype /// 0007520 // myoblast fusion // inferred from mutant phenotype /// 0007520 // myoblast fusion // non-traceable author statement /// 0032012 // regulation of ARF protein signal transduction // inferred from electronic annotation	6	0.46374	0.35753	Periventricular heteropia with microcephaly
1624541_at	*RhoGAP19D*	*RhoGAP19D*	0007165 // signal transduction // inferred from electronic annotation /// 0007242 // intracellular signaling cascade // inferred from electronic annotation	6	0.44478	0.35753	Encephalopathy, Parkinson disease, Deafness, X-linked mental retardation
1634200_a_at	*CG9413*	*CG9413*	0006520 // amino acid metabolic process // inferred from electronic annotation /// 0006810 // transport // inferred from electronic annotation /// 0006865 // amino acid transport // inferred from electronic annotation	6	0.4439	0.35753	Spinal muscular atrophy, Cystinuria
1625178_at	*CG31038*	*CG31038*	---	6	0.44349	0.35753	Fragile X syndrome
1635307_at	*CG4662*	*CG4662*	---	6	0.43861	0.35753	Hypothyroidism
1641738_a_at	*CG13636*	*CG13636*	---	6	0.43677	0.35753	
1623191_at	*Rab9*	*Rab9*	0006886 // intracellular protein transport // inferred from electronic annotation /// 0006898 // receptor-mediated endocytosis // inferred from electronic annotation /// 0007165 // signal transduction // inferred from electronic annotation /// 0007264 // small GTPase mediated signal transduction // inferred from electronic annotation /// 0015031 // protein transport // inferred from electronic annotation /// 0017157 // regulation of exocytosis // inferred from electronic annotation /// 0042147 // retrograde transport, endosome to Golgi // traceable author statement	6	0.42007	0.35753	Carpenter Syndrome, Costello Syndrome, Charcot-Marie-Tooth Disease, Griscelli Syndrome
1635818_at	*CG4713*	*CG4713*	---	6	0.41335	0.35753	Mental retardation syndrome, Lissencephaly, Combined oxidative phosphorylation deficiency
1640227_at	*CG8801*	*CG8801*	0008283 // cell proliferation // inferred from electronic annotation /// 0042254 // ribosome biogenesis and assembly // inferred from electronic annotation	6	0.40338	0.35753	Carpenter syndrome
1631455_at	*CG30493*	*CG30493*	0006744 // ubiquinone biosynthetic process // inferred from electronic annotation /// 0006744 // ubiquinone biosynthetic process // inferred from sequence or structural similarity	6	0.40284	0.35753	Acromegaly
1641120_at	*CG13506*	*CG13506*	---	6	0.39412	0.35753	Jackson-Weiss syndrome, Mental retardation, Corpus callosum, partial agenesis of, obesity, Muscular dystrophy, Macular degeneration
1623418_at	*Cad96Ca*	*Cad96Ca*	0006468 // protein amino acid phosphorylation // inferred from sequence or structural similarity /// 0006468 // protein amino acid phosphorylation // non-traceable author statement /// 0006468 // protein amino acid phosphorylation // inferred from electronic annotation /// 0007156 // homophilic cell adhesion // inferred from electronic annotation /// 0007169 // transmembrane receptor protein tyrosine kinase signaling pathway // inferred from electronic annotation /// 0007398 // ectoderm development // inferred from electronic annotation /// 0007399 // nervous system development // inferred from electronic annotation /// 0008283 // cell proliferation // inferred from electronic annotation /// 0016339 // calcium-dependent cell-cell adhesion // inferred from sequence or structural similarity	6	0.3862	0.35753	Dopamine receptor D2, reduced brain density of; Obesity, Hyperphagia, and Developmental delay, Achondroplasia, Noonan Syndrome, Multiple endocrine neoplasia, Insensitivity to pain, Kallmann Syndrome 2, Coffin-Lowry Syndrome, Crouzon Syndrome, Parkinsons Disease
1641427_at	*fear of intimacy*	*foi*	0006810 // transport // inferred from electronic annotation /// 0006811 // ion transport // inferred from electronic annotation /// 0006829 // zinc ion transport // inferred from mutant phenotype /// 0006829 // zinc ion transport // inferred from electronic annotation /// 0007275 // multicellular organismal development // inferred from electronic annotation /// 0007280 // pole cell migration // traceable author statement /// 0007399 // nervous system development // inferred from electronic annotation /// 0007417 // central nervous system development // inferred from mutant phenotype /// 0007424 // open tracheal system development // inferred from mutant phenotype /// 0007506 // gonadal mesoderm development // inferred from mutant phenotype /// 0008354 // germ cell migration // inferred from mutant phenotype /// 0008406 // gonad development // traceable author statement /// 0016477 // cell migration // inferred from mutant phenotype /// 0016477 // cell migration // traceable author statement /// 0030001 // metal ion transport // inferred from electronic annotation /// 0030154 // cell	6	0.38422	0.35753	Susceptibility to Panic disorder, Susceptibility to Schizophrenia

1629996_at	*CG11910*	*CG11910*	0006952 // defense response // inferred from electronic annotation /// 0007155 // cell adhesion // inferred from electronic annotation /// 0007166 // cell surface receptor linked signal transduction // inferred from electronic annotation	6	0.37006	0.35753	Susceptibility to schizophrenia, Hypogonadotropic hypogonadism, Parkinson disease, Tourette syndrome
1636923_a_at	*Acetyl CoA carboxylase*	*CG11198*	0006633 // fatty acid biosynthetic process // inferred from electronic annotation /// 0008152 // metabolic process // inferred from electronic annotation	6	0.36615	0.35753	Pyruvate carboxylase deficiency
1628759_a_at	*Nirvana*	*CG14073*	---	6	0.35435	0.35753	Mental retardation-hypotonic facies syndrome, Lissencephaly, Spinocerebellar ataxia, Glaucoma, Parkinson disease, Infantile neuroaxonal dystrophy, Autism, Deafness, Reduced brain density of dopamine receptor D2, Microphthalmia
1627499_at	*CG2016*	*CG2016*	---	6	0.34416	0.35753	
1629844_s_at	*fizzy-related*	*rap*	0001745 // compound eye morphogenesis // inferred from mutant phenotype /// 0006508 // proteolysis // inferred from electronic annotation /// 0007455 // eye-antennal disc morphogenesis // inferred from mutant phenotype /// 0008054 // cyclin catabolic process // inferred from direct assay /// 0008347 // glial cell migration // inferred from mutant phenotype /// 0030163 // protein catabolic process // inferred from mutant phenotype /// 0031536 // positive regulation of exit from mitosis // inferred from mutant phenotype	6	0.33769	0.35753	Lissencephaly, Chediak-Higashi syndrome, Joubert syndrome, Glaucoma, Susceptibility to Crohn disease
1629189_at	*sec5*	*sec5*	0000910 // cytokinesis // inferred from mutant phenotype /// 0006810 // transport // inferred from electronic annotation /// 0006887 // exocytosis // inferred from electronic annotation /// 0007269 // neurotransmitter secretion // non-traceable author statement /// 0015031 // protein transport // inferred from electronic annotation /// 0016080 // synaptic vesicle targeting // inferred from sequence or structural similarity /// 0016080 // synaptic vesicle targeting // non-traceable author statement /// 0016081 // synaptic vesicle docking during exocytosis // inferred from sequence or structural similarity /// 0016081 // synaptic vesicle docking during exocytosis // non-traceable author statement /// 0016192 // vesicle-mediated transport // inferred from sequence or structural similarity /// 0032456 // endocytic recycling // inferred from mutant phenotype /// 0045087 // innate immune response // inferred from direct assay /// 0048599 // oocyte development // inferred from mutant phenotype	6	0.32212	0.35753	Cardioencephalomyopathy, Combined oxidative phosphorylation deficiency 3
1628909_at	*CG9686*	*CG9686*	---	6	0.27027	0.35753	Alzheimer disease
1636865_at	*Os-C*	*Os-C*	---	6	0.26537	0.35753	
1624763_at	*CG2556*	*CG2556 /// DsimCG2556*	---	6	0.22585	0.35753	
1636583_at	*CG5932*	*CG5932*	0006629 // lipid metabolic process // inferred from electronic annotation	6	0.22026	0.35753	Wolfram Syndrome
1632304_at	*CG8026*	*CG8026*	0006810 // transport // inferred from electronic annotation /// 0006839 // mitochondrial transport // inferred from sequence or structural similarity /// 0006839 // mitochondrial transport // inferred from electronic annotation /// 0015884 // folic acid transport // inferred from sequence or structural similarity	6	0.21885	0.35753	Obesity, Epilepsy, Microcephaly
1626739_s_at	*glutamate receptor*	*GluRIIC*	0006810 // transport // inferred from electronic annotation /// 0006811 // ion transport // inferred from electronic annotation /// 0006812 // cation transport // inferred from electronic annotation /// 0006813 // potassium ion transport // inferred from electronic annotation /// 0006936 // muscle contraction // inferred from electronic annotation /// 0007268 // synaptic transmission // inferred from mutant phenotype /// 0007270 // nerve-nerve synaptic transmission // inferred from electronic annotation /// 0007274 // neuromuscular synaptic transmission // inferred from electronic annotation /// 0045184 // establishment of protein localization // inferred from mutant phenotype	6	0.20708	0.35753	Mental retardation
1631604_at	*CG9511*	*CG9511*	---	6	0.18505	0.35753	
1623190_at	*CG9168*	*CG9168*	0008152 // metabolic process // inferred from electronic annotation	6	0.18403	0.35753	Defective flycerol release during exercise

1623430_at	*Peptidyl gycine alpha hydroxylating monooxygenase*	*Phm*	0001519 // peptide amidation // non-traceable author statement /// 0006464 // protein modification process // inferred from electronic annotation /// 0006518 // peptide metabolic process // inferred from electronic annotation /// 0006584 // catecholamine metabolic process // inferred from electronic annotation	6	0.12107	0.35753	Dopamine beta-hydroxylase activity
1630961_at	*CG17193*	*CG17193*	---	6	0.11355	0.35753	
1636264_at	*VAChT*	*VAChT*	0006810 // transport // inferred from electronic annotation /// 0006836 // neurotransmitter transport // non-traceable author statement /// 0006836 // neurotransmitter transport // inferred from electronic annotation /// 0015893 // drug transport // inferred from electronic annotation /// 0019226 // transmission of nerve impulse // inferred from electronic annotation	7	0.53495	0.35646	Allan-Herndon-Dudley syndrome
1626667_at	*miple*	*miple*	---	7	0.51879	0.35646	Hypoparathyroidism-retardation-dysmorphism Syndrome, X-linked mental retardation
1635144_at	*klingon*	*klg*	0007156 // homophilic cell adhesion // inferred from direct assay /// 0007165 // signal transduction // inferred from electronic annotation /// 0007465 // R7 cell fate commitment // inferred from genetic interaction /// 0007611 // learning and/or memory // inferred from mutant phenotype /// 0008355 // olfactory learning // inferred from mutant phenotype /// 0045466 // R7 cell differentiation // inferred from mutant phenotype	7	0.50898	0.35646	Macular degeneration, Spinocerebellar ataxia, Gaze palsy, Hydrocephalus
1625981_at	*rab3-GEF*	*rab3-GEF*	0000187 // activation of MAPK activity // inferred from sequence or structural similarity /// 0006915 // apoptosis // inferred from electronic annotation /// 0007269 // neurotransmitter secretion // non-traceable author statement /// 0016192 // vesicle-mediated transport // non-traceable author statement /// 0042981 // regulation of apoptosis // inferred from sequence or structural similarity /// 0051726 // regulation of cell cycle // inferred from sequence or structural similarity	7	0.50592	0.35646	Charcot-Marie-Tooth disease, Amyotrophy, Spinocerebellar ataxia
1629417_s_at	*CG14853*	*CG14853*	---	7	0.50085	0.35646	Leukemia
1626619_at	*CG9919*	*CG9919*	---	7	0.48879	0.35646	
1623896_a_at	*CG18496*	*CG34404*	---	7	0.48668	0.35646	
1637684_at	*unc-104*	*unc-104*	0006605 // protein targeting // inferred from electronic annotation /// 0006886 // intracellular protein transport // inferred from electronic annotation /// 0007018 // microtubule-based movement // inferred from electronic annotation	7	0.48626	0.35646	Spastic paraplegia, Aarskog-Scott syndrome, Mental retardation, Charcot-Marie-Tooth disease
1637820_at	*X11Lbeta*	*X11Lbeta*	0006605 // protein targeting // inferred from electronic annotation	7	0.47919	0.35646	Diabetes mellitus, Dementia, Tauopathy, Mental retardation, Susceptibility to Crohn disease, Deafness
1624531_s_at	---	---	---	7	0.4727	0.35646	
1630004_at	*beaten path Ib*	*beat-Ib*	0007155 // cell adhesion // non-traceable author statement /// 0007415 // defasciculation of motor neuron axon // non-traceable author statement /// 0016198 // axon choice point recognition // non-traceable author statement	7	0.47166	0.35646	
1625951_at	*CG17778*	*CG17778*	---	7	0.47078	0.35646	
1635675_at	*scarecrow*	*scro*	0006355 // regulation of transcription, DNA-dependent // inferred from electronic annotation /// 0045449 // regulation of transcription // inferred from electronic annotation	7	0.45848	0.35646	
1627736_at	*dActivin*	*activin-beta*	0007178 // transmembrane receptor protein serine/threonine kinase signaling pathway // inferred from electronic annotation /// 0007267 // cell-cell signaling // inferred from electronic annotation /// 0016049 // cell growth // non-traceable author statement /// 0048813 // dendrite morphogenesis // non-traceable author statement	7	0.45604	0.35646	Muscle hypertrophy

1624864_s_at	*dreadlocks*	*dock*	0007186 // G-protein coupled receptor protein signaling pathway // inferred from electronic annotation /// 0007242 // intracellular signaling cascade // inferred from electronic annotation /// 0007409 // axonogenesis // traceable author statement /// 0007411 // axon guidance // inferred from genetic interaction /// 0007411 // axon guidance // inferred from mutant phenotype /// 0007411 // axon guidance // traceable author statement /// 0008286 // insulin receptor signaling pathway // inferred from physical interaction	7	0.44173	0.35646	Autism, Mental retardation
1636742_at	*nord*	*nord*	0007611 // learning and/or memory // inferred from mutant phenotype /// 0008355 // olfactory learning // inferred from mutant phenotype	7	0.43629	0.35646	
1626565_at	*CG2790*	*CG2790*	0006457 // protein folding // inferred from electronic annotation	7	0.42381	0.35646	Spastic ataxia
1636848_at	*CG6024*	*CG6024*	---	7	0.41873	0.35646	
1633775_at	*fibropellin*	*CG31665*	0007154 // cell communication // inferred from electronic annotation /// 0007165 // signal transduction // inferred from electronic annotation /// 0007166 // cell surface receptor linked signal transduction // inferred from electronic annotation /// 0007267 // cell-cell signaling // inferred from electronic annotation /// 0007275 // multicellular organismal development // inferred from electronic annotation /// 0007398 // ectoderm development // inferred from electronic annotation /// 0007399 // nervous system development // inferred from electronic annotation /// 0008283 // cell proliferation // inferred from electronic annotation /// 0016337 // cell-cell adhesion // inferred from electronic annotation /// 0019226 // transmission of nerve impulse // inferred from electronic annotation	7	0.41684	0.35646	Deafness, Lissencephaly syndrome, Forebrain defects, Macular degeneration, Cerebral arteriopathy, Muscular dystrophy
1632424_at	*CG13995*	*CG13995*	0007186 // G-protein coupled receptor protein signaling pathway // inferred from sequence or structural similarity /// 0007186 // G-protein coupled receptor protein signaling pathway // inferred from electronic annotation	7	0.40543	0.35646	Autonomic nervous system dysfunction, Novelty seeking personality, Attention deficit-hyperactivity disorder, Protection against Parkinson disease, Susceptibility to alcohol dependence, Anorexia nervosa, Obesity
1624297_at	*linotte*	*drl*	0006468 // protein amino acid phosphorylation // non-traceable author statement /// 0006468 // protein amino acid phosphorylation // inferred from electronic annotation /// 0007165 // signal transduction // inferred from mutant phenotype /// 0007169 // transmembrane receptor protein tyrosine kinase signaling pathway // inferred from electronic annotation /// 0007267 // cell-cell signaling // inferred from electronic annotation /// 0007275 // multicellular organismal development // inferred from electronic annotation /// 0007398 // ectoderm development // inferred from electronic annotation /// 0007411 // axon guidance // inferred from mutant phenotype /// 0007482 // haltere development // inferred from genetic interaction /// 0007611 // learning and/or memory // inferred from mutant phenotype /// 0007613 // memory // inferred from mutant phenotype /// 0008355 // olfactory learning // inferred from mutant phenotype /// 0008355 // olfactory learning // traceable author statement /// 0016055 // Wnt receptor signaling pathway // inferred from genetic interaction ///	7	0.40369	0.35646	Jackson-Weiss syndrome, Diabetes mellitus, Parkinson disease, Obesity, Colon cancer, Insensitivity to pain
1627297_at	*Axs-like*	*CG15270*	---	7	0.40286	0.35646	Alzheimer disease, Glaucoma, Neuropathy, Susceptibility to Amyotrophic Lateral Sclerosis, Gnathodiaphyseal dysplasia
1635198_at	*CG10483*	*CG10483*	0007165 // signal transduction // inferred from electronic annotation /// 0007166 // cell surface receptor linked signal transduction // inferred from electronic annotation /// 0007186 // G-protein coupled receptor protein signaling pathway // inferred from electronic annotation	7	0.39595	0.35646	Mental retardation
1632550_at	*unc-5-like*	*unc-5*	0007165 // signal transduction // inferred from electronic annotation /// 0007275 // multicellular organismal development // inferred from electronic annotation /// 0007398 // ectoderm development // inferred from electronic annotation /// 0007411 // axon guidance // inferred from genetic interaction /// 0007411 // axon guidance // inferred from mutant phenotype /// 0007432 // salivary gland boundary specification // inferred from mutant phenotype /// 0008045 // motor axon guidance // inferred from mutant phenotype /// 0008347 // glial cell migration // inferred from mutant phenotype	7	0.39198	0.35646	Jackson-Weiss syndrome, Mental retardation, Muscular dystrophy, Macular degeneration
1630043_a_at	---	*alpha-catenin-related*	0006928 // cell motility // inferred from electronic annotation /// 0007010 // cytoskeleton organization and biogenesis // inferred from electronic annotation /// 0007155 // cell adhesion // inferred from electronic annotation /// 0007186 // G-protein coupled receptor protein signaling pathway // inferred from electronic annotation	7	0.37906	0.35646	Bardet-Biedl syndrome

1630106_at	*CG10362*	*CG10362*	0007242 // intracellular signaling cascade // inferred from electronic annotation	7	0.37796	0.35646	Macular degeneration, Diabetes mellitus, Microcephaly, Parkinson disease
1631532_at	*CG5282*	*CG5282*	0006508 // proteolysis // inferred from electronic annotation	7	0.36664	0.35646	
1631649_at	*CG8271*	*CG8271*	0006810 // transport // inferred from electronic annotation /// 0006812 // cation transport // inferred from electronic annotation	7	0.35991	0.35646	Allan-Herdon-Dudley Syndrome; susceptibility to Crohn disease
1629678_a_at	*APP-like protein interacting protein 1*	*Aplip1*	0007186 // G-protein coupled receptor protein signaling pathway // inferred from electronic annotation /// 0007275 // multicellular organismal development // inferred from electronic annotation /// 0019896 // axon transport of mitochondrion // inferred from mutant phenotype /// 0046328 // regulation of JNK cascade // inferred from physical interaction /// 0046328 // regulation of JNK cascade // inferred from sequence or structural similarity /// 0046328 // regulation of JNK cascade // non-traceable author statement /// 0048490 // anterograde synaptic vesicle transport // inferred from mutant phenotype /// 0048491 // retrograde synaptic vesicle transport // inferred from mutant phenotype	7	0.35347	0.35646	Susceptibility to Schizophrenia, Thyroid hormonogenesis, Spinocerebellar ataxia, Hydrocephalus, Muscular dystrophy, Epilepsy
1637869_at	*Pray For Elves*	*PFE*	0006464 // protein modification process // inferred from electronic annotation /// 0006468 // protein amino acid phosphorylation // inferred from electronic annotation /// 0006952 // defense response // inferred from electronic annotation /// 0007155 // cell adhesion // inferred from electronic annotation /// 0007165 // signal transduction // inferred from electronic annotation /// 0007166 // cell surface receptor linked signal transduction // inferred from electronic annotation /// 0007178 // transmembrane receptor protein serine/threonine kinase signaling pathway // inferred from electronic annotation /// 0019226 // transmission of nerve impulse // inferred from electronic annotation /// 0019538 // protein metabolic process // inferred from electronic annotation	7	0.34869	0.35646	Susceptibility to schizophrenia, Tourette syndrome
1639909_at	*CG7058*	*CG7058*	---	7	0.34721	0.35646	
1640974_a_at	*CG33143*	*CG33143*	---	7	0.33757	0.35646	
1628005_at	*CG31666 /// chronologically inappropriate morphogenesis*	*CG31666 /// chinmo*	---	7	0.33386	0.35646	Hypercholanemia
1639694_s_at	*CG10102 /// CG12505*	*CG10102 /// CG12505*	---	7	0.3263	0.35646	
1639431_at	*synaptogyrin*	*synaptogyrin*	0016079 // synaptic vesicle exocytosis // inferred from sequence or structural similarity /// 0017158 // regulation of calcium ion-dependent exocytosis // inferred from sequence or structural similarity	7	0.32479	0.35646	
1634134_at	*SNF1-related*	*CG6114*	0006468 // protein amino acid phosphorylation // non-traceable author statement /// 0006468 // protein amino acid phosphorylation // inferred from electronic annotation /// 0007242 // intracellular signaling cascade // inferred from electronic annotation	7	0.31098	0.35646	Rett Syndrome, Reduced brain density of dopamine receptor D2, Pseudohypoalsosteronism, Jackson-Weiss syndrome, Diabetes mellitus, Susceptibility to Schizophrenia, Spinocerebellar ataxia, Lissencephaly, Mental retardation, Obesity, Hyperphagia
1634291_at	*CG11814*	*CG11814 /// DmirCG11814*	0006605 // protein targeting // inferred from electronic annotation /// 0006952 // defense response // inferred from electronic annotation /// 0007041 // lysosomal transport // inferred from sequence or structural similarity	7	0.30234	0.35646	Lissencephaly, Chediak-Higashi Syndrome
1630335_s_at	*CG7084*	*CG7084*	0006812 // cation transport // inferred from electronic annotation /// 0006858 // extracellular transport // inferred from electronic annotation	7	0.29684	0.35646	Susceptibility to Crohn disease, Carnitine deficiency, Susceptibility to rheumatoid arthritis
1627096_s_at	*BTB-protein-III*	*Tkr*	0042332 // gravitaxis // inferred from mutant phenotype	7	0.29634	0.35646	Giant axonal neuropathy
1629066_at	*Drosophila Corazonin Receptor*	*GRHRII*	0007186 // G-protein coupled receptor protein signaling pathway // inferred from sequence or structural similarity /// 0007186 // G-protein coupled receptor protein signaling pathway // non-traceable author statement /// 0007186 // G-protein coupled receptor protein signaling pathway // inferred from electronic annotation /// 0007292 // female gamete generation // inferred from electronic annotation /// 0019226 // transmission of nerve impulse // inferred from electronic annotation /// 0019722 // calcium-mediated signaling // inferred from electronic annotation	7	0.29134	0.35646	Asthma susceptibility, Response to morphine-6-glucuronie, Hypogonadotropic hypogonadism, Susceptibility to ADHD, Susceptibility to schizophrenia, Susceptibility to OCD, Susceptibility to MDD
1625454_at	*Gbeta5*	*Gbeta5*	0007186 // G-protein coupled receptor protein signaling pathway // non-traceable author statement	7	0.29014	0.35646	Lissencephaly, X-linked mental retardation
1630734_at	*CG6240*	*Cpr92A*	---	7	0.26671	0.35646	Microphthalmia
1628647_at	*antennal protein 5*	*a5*	0007165 // signal transduction // inferred from electronic annotation	7	0.25309	0.35646	
1630658_at	*CG32226*	*CG32226 /// DmirCG32226*	---	7	0.24144	0.35646	Muscular dystrophy

1631516_s_at	*blind spot*	*skd*	0006366 // transcription from RNA polymerase II promoter // non-traceable author statement /// 0006367 // transcription initiation from RNA polymerase II promoter // inferred from sequence or structural similarity /// 0009299 // mRNA transcription // inferred from electronic annotation /// 0009790 // embryonic development // inferred from mutant phenotype /// 0045165 // cell fate commitment // inferred from genetic interaction /// 0045498 // sex comb development // inferred from genetic interaction /// 0045498 // sex comb development // inferred from mutant phenotype	7	0.23582	0.35646	Microcephaly, Deafness
1627529_at	*CG32726*	*CG32726*	---	7	0.22536	0.35646	
1634073_at	*CG13466*	*CG13466*	0007017 // microtubule-based process // non-traceable author statement /// 0007067 // mitosis // inferred from electronic annotation /// 0007242 // intracellular signaling cascade // inferred from electronic annotation	7	0.21463	0.35646	Susceptibility to Crohn disease, Lissencephaly
1634315_a_at	*CG32982*	*CG32982*	---	7	0.20261	0.35646	
1628159_a_at	*CG32206*	*CG32206*	---	7	0.20024	0.35646	Cerebellar hypoplasia
1631956_at	*CG15145*	*CG15145*	---	7	0.19451	0.35646	
1629517_at	*CG5118*	*CG5118*	---	7	0.18319	0.35646	Infantile neuroaxonal dystrophy
1639951_at	*CG2611*	*CG2611*	---	7	0.18237	0.35646	
1627870_at	*CG31900*	*CG31900*	---	7	0.12788	0.35646	
1630212_at	*CG2065*	*CG2065*	0008152 // metabolic process // inferred from electronic annotation	7	0.099923	0.35646	Cortisone reductase deficiency, Pseudohermaphroditism, male; Leber congenital amaurosis
1639923_at	*CG30502*	*CG30502*	0008152 // metabolic process // inferred from electronic annotation	8	0.35685	0.28997	
1640566_at	*Cyp4p2*	*Cyp4p2*	0006118 // electron transport // inferred from electronic annotation /// 0008202 // steroid metabolic process // inferred from electronic annotation	8	0.32246	0.28997	Aromatase deficiency, Adrenal hyperplasia, Codeine sensitivity, Glaucoma
1626605_at	*CG14075*	*CG14075*	---	8	0.31761	0.28997	
1635996_at	*CG6638*	*CG6638*	0006118 // electron transport // inferred from electronic annotation /// 0006552 // leucine catabolic process // inferred from sequence or structural similarity /// 0006637 // acyl-CoA metabolic process // inferred from electronic annotation /// 0008152 // metabolic process // inferred from electronic annotation	8	0.31263	0.28997	Glutaric acidemia I, SCAD deficiency, 2-alpha-methylbutyryl-CoA dehydrogenase deficiency
1638523_at	*CG32633*	*CG32633*	---	8	0.27695	0.28997	
1640913_at	*GST-containing FLYWCH zinc-finger protein*	*gfzf*	0006952 // defense response // inferred from electronic annotation /// 0009636 // response to toxin // inferred from electronic annotation	8	0.26514	0.28997	
1626350_at	*fragment K*	*alpha-Est10*	---	8	0.17816	0.28997	Susceptibility to X-linked autism, X-linked mental retardation
1625819_at	*Strawberry Notch*	*sno*	0007173 // epidermal growth factor receptor signaling pathway // traceable author statement /// 0007219 // Notch signaling pathway // inferred from genetic interaction /// 0007219 // Notch signaling pathway // traceable author statement /// 0007560 // imaginal disc morphogenesis // non-traceable author statement /// 0008587 // imaginal disc-derived wing margin morphogenesis // traceable author statement /// 0009790 // embryonic development // non-traceable author statement /// 0009790 // embryonic development // traceable author statement /// 0030162 // regulation of proteolysis // traceable author statement /// 0042676 // compound eye cone cell fate commitment // traceable author statement /// 0045944 // positive regulation of transcription from RNA polymerase II promoter // inferred from mutant phenotype	9	0.37831	0.24942	Muscular dystrophy
1629842_at	*sextra*	*Gap1*	0000165 // MAPKKK cascade // inferred from electronic annotation /// 0007062 // sister chromatid cohesion // inferred from mutant phenotype /// 0007067 // mitosis // inferred from mutant phenotype /// 0007242 // intracellular signaling cascade // inferred from electronic annotation /// 0007265 // Ras protein signal transduction // inferred from mutant phenotype /// 0007265 // Ras protein signal transduction // non-traceable author statement /// 0007476 // imaginal disc-derived wing morphogenesis // inferred from mutant phenotype /// 0008293 // torso signaling pathway // non-traceable author statement /// 0008595 // determination of anterior/posterior axis, embryo // traceable author statement /// 0016321 // female meiosis chromosome segregation // inferred from mutant phenotype /// 0045678 // positive regulation of R7 differentiation // inferred from genetic interaction /// 0046580 // negative regulation of Ras protein signal transduction // non-traceable author statement /// 0051056 // regulation of small GTPase mediated signal	9	0.37085	0.24942	Basal cell carcinoma
1633026_a_at	*CG31158*	*CG31158*	0006886 // intracellular protein transport // inferred from electronic annotation /// 0006887 // exocytosis // inferred from electronic annotation /// 0032012 // regulation of ARF protein signal transduction // inferred from electronic annotation	9	0.36378	0.24942	Periventricular heteropia with microcephaly

1632748_at	*CG15084*	*CG15084*	---	9	0.32969	0.24942	
1632841_x_at	*heat shock 70*	*Hsp70Bc*	0006457 // protein folding // inferred from electronic annotation /// 0006461 // protein complex assembly // inferred from electronic annotation /// 0006952 // defense response // inferred from electronic annotation /// 0006986 // response to unfolded protein // non-traceable author statement /// 0009408 // response to heat // inferred from mutant phenotype /// 0009408 // response to heat // non-traceable author statement /// 0035080 // heat shock-mediated polytene chromosome puffing // inferred from mutant phenotype	9	0.32562	0.24942	Cockayne syndrome
1628496_at	*dpr16*	*dpr16*	---	9	0.32559	0.24942	Cleft lip/palate
1632554_at	*CG9839*	*CG9839*	---	9	0.30824	0.24942	
1636291_at	*Rae1*	*Rae1*	0006139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolic process // inferred from electronic annotation /// 0006403 // RNA localization // inferred from electronic annotation /// 0007049 // cell cycle // inferred from electronic annotation /// 0007059 // chromosome segregation // inferred from electronic annotation /// 0007067 // mitosis // inferred from electronic annotation	9	0.28313	0.24942	Lissencephaly
1626285_at	*CG13806*	*CG13806*	0006030 // chitin metabolic process // inferred from electronic annotation	9	0.23751	0.24942	
1639534_at	*CG6792*	*CG6792*	0006139 // nucleobase, nucleoside, nucleotide and nucleic acid metabolic process // inferred from electronic annotation /// 0006357 // regulation of transcription from RNA polymerase II promoter // inferred from electronic annotation /// 0006366 // transcription from RNA polymerase II promoter // inferred from electronic annotation /// 0008283 // cell proliferation // inferred from electronic annotation	9	0.23298	0.24942	Mental retardation, Charcot-Marie-Tooth disease, Holoprosencephaly
1633865_at	*CG16734*	*CG16734*	---	9	0.19264	0.24942	
1626641_s_at	*Hemoglobin*	*glob1*	0006810 // transport // inferred from electronic annotation /// 0015671 // oxygen transport // inferred from electronic annotation	9	0.17377	0.24942	
1637851_at	*CG7422*	*CG7422*	0007155 // cell adhesion // inferred from electronic annotation	9	0.1587	0.24942	Malaria (protection AND susceptibility), Platelet glycoprotein IV deficiency
1635298_at	*CG13458*	*CG13458*	---	9	0.14595	0.24942	
1636488_at	*CG4468*	*CG4468 /// DereCG4468 /// DlitCG4468 /// DwilCG4468*	---	9	0.14415	0.24942	
1632406_at	*CG9117*	*CG9117*	---	9	0.14376	0.24942	Ethylmalonic encephalopathy
1633386_s_at	*Mth-like 8*	*mthl8*	0006950 // response to stress // inferred from sequence or structural similarity /// 0006950 // response to stress // inferred from electronic annotation /// 0007165 // signal transduction // inferred from electronic annotation /// 0007186 // G-protein coupled receptor protein signaling pathway // inferred from sequence or structural similarity /// 0007186 // G-protein coupled receptor protein signaling pathway // inferred from electronic annotation /// 0008340 // determination of adult life span // inferred from sequence or structural similarity	9	0.12545	0.24942	

Appendix 5.5

Supplementary Table 5. Pleiotropic genes affecting aggression. Expression of these genes has been correlated with aggressive behavior and the other traits listed. (a) Ref. 21. (b) Ref. 38. (c) Ref. 37.

a

Starvation Resistance
GRHRII
CG9117
CG6403
CG1146
synaptogyrin

Fitness
CG30502
synaptogyrin
CG14075
CG2016
antennal protein 5
CG5282
neither inactivation nor afterpotential C
chaoptic
CG6024
Os-C

Life Span
Peptidylglycine-hydroxylating monooxygenase

Chill Coma Recovery
Rab9
GTPase-activating protein 1
CG30493
CG9839
CG11198
CG30502
1639175_s_at

Locomotor Reactivity
CG13806
CG31900
CG2065
CG8026
CG10102

Copulation Latency
CG31666
CG11814

b

Initial Ethanol Exposure
Cad96Ca
CG9919
Tyrosine kinase-related protein
CG15270
activin-
CG14853
CG11910
beaten path Ib
klingon
nord
CG13928
CG7422
CG10102

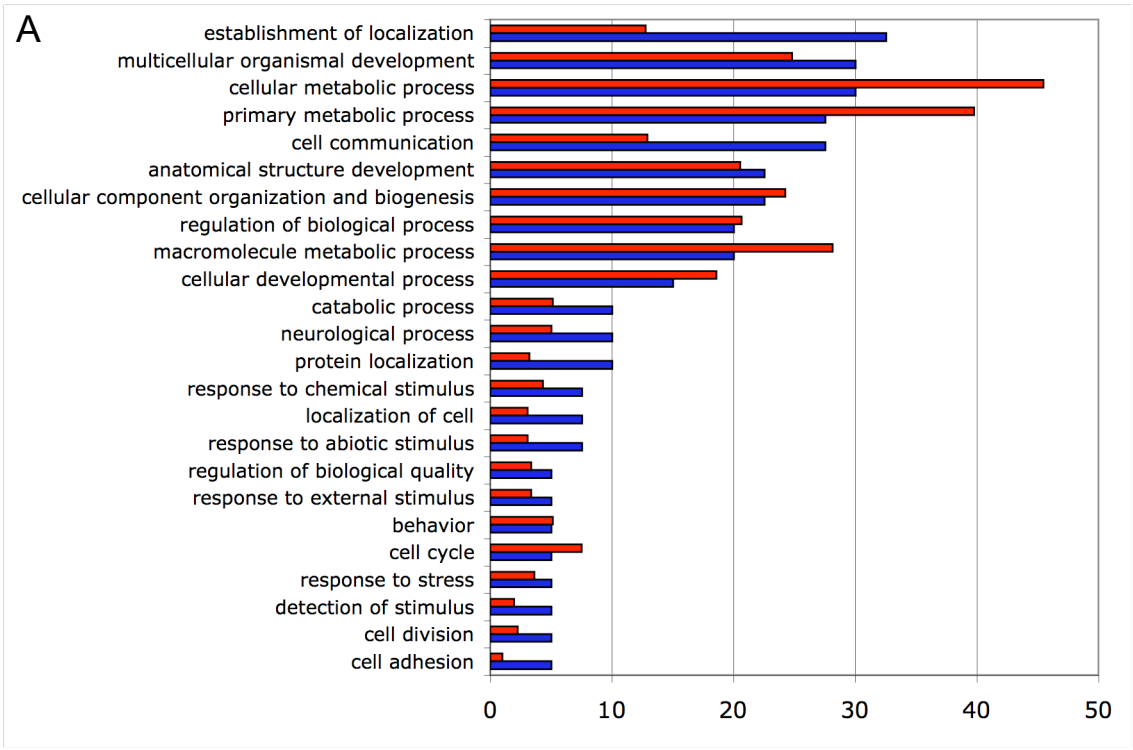
Ethanol Tolerance
1623327_at
1624531_s_at
Esterase-10
CG2065
unc-5
CG31158
CG4829
CG11198
Cad96Ca
Tyrosine kinase-related protein
activin-beta
CG11910
CG13928
CG7422

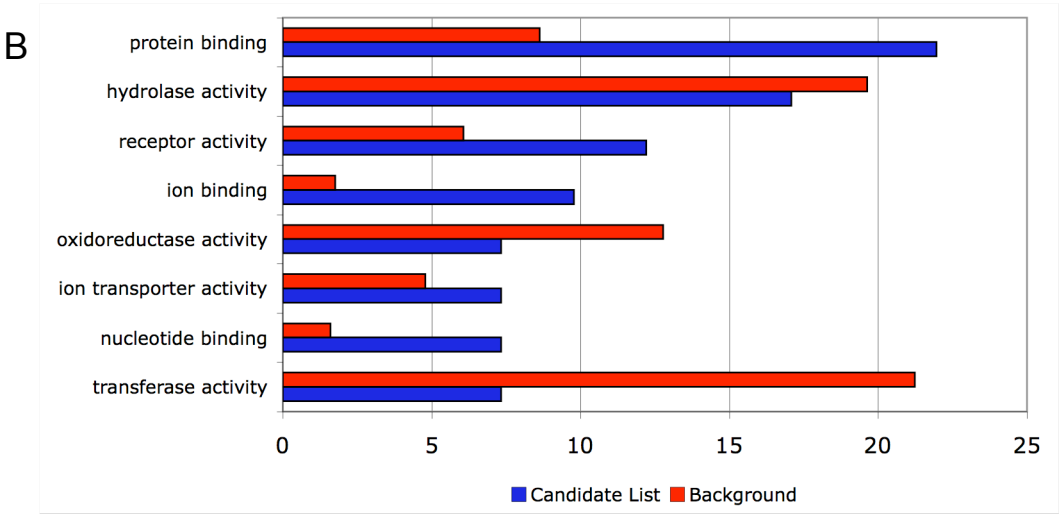
c

Sleep
Rab9
CG30104
CG2556
CG2790
CG13531
CG9686
GRHRII
CG9511
CG8026
CG9117
CG6403
CG4713
CG5932
CG1146
CG30502
CG32425
fear-of-intimacy

Appendix 5.6

Supplementary Figure 1. Gene ontologies represented by quantitative trait transcripts**.** Level 3 Biological Process (A) and Molecular Function (B) gene ontologies for genes whose transcript abundance is correlated with aggression level. The percentage of genes falling into a given category is depicted on the x-axis. The genomic background relevant to our data set includes 7510 probe sets that were differentially expressed in males at a FDR<0.01. No categories were significantly over-represented among Level 3 categories; however, the Level 4 "transport" biological process category was over-represented (adjusted p=0.0364, data not shown).

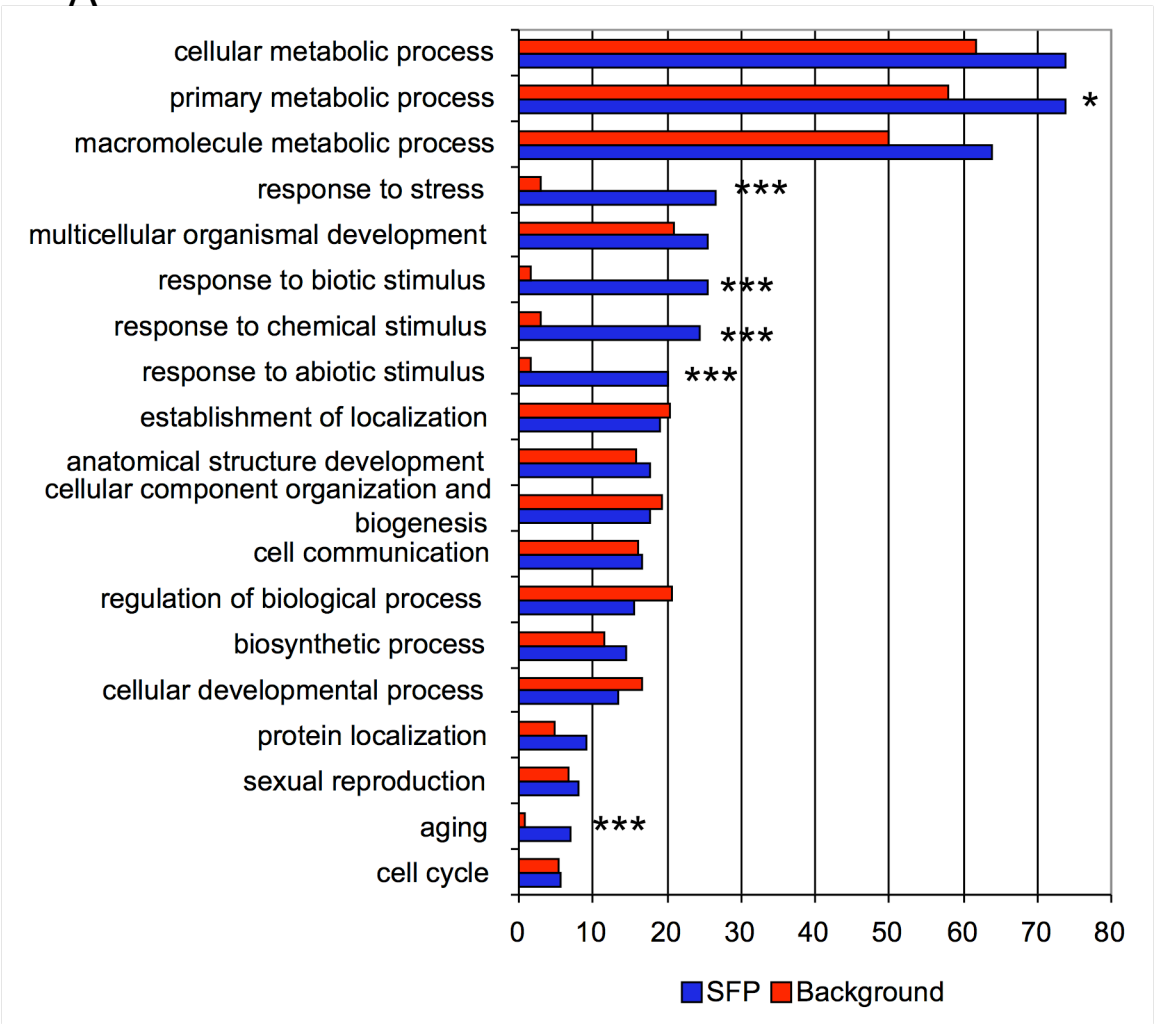

A
establishment of localization
multicellular organismal development
cellular metabolic process
primary metabolic process
cell communication
anatomical structure development
cellular component organization and biogenesis
regulation of biological process
macromolecule metabolic process
cellular developmental process
catabolic process
neurological process
protein localization
response to chemical stimulus
localization of cell
response to abiotic stimulus
regulation of biological quality
response to external stimulus
behavior
cell cycle
response to stress
detection of stimulus
cell division
cell adhesion
0
10
20
30
40
50


B
protein binding
hydrolase activity
receptor activity
ion binding
oxidoreductase activity
ion transporter activity
nucleotide binding
transferase activity
0
5
10
15
20
25
Candidate List
Background

Appendix 5.6

Supplementary Figure 2. Gene Ontologies represented by probe sets containing SFPs. (A) Level 3 Biological Process and (B) Level 4 Molecular Function categories. Only categories applying to at least 5% of a list are depicted. Categories significantly over-represented in the SFP list relative to the genomic background are denoted as follows: *=adjusted *p*-value<0.05; ** adjusted *p*-value<0.01; ***= adjusted *p*-value<0.001.

A


cellular metabolic process
primary metabolic process
macromolecule metabolic process
response to stress
multicellular organismal development
response to biotic stimulus
response to chemical stimulus
response to abiotic stimulus
establishment of localization
anatomical structure development
cellular component organization and biogenesis
cell communication
regulation of biological process
biosynthetic process
cellular developmental process
protein localization
sexual reproduction
aging
cell cycle
*
***
***
***
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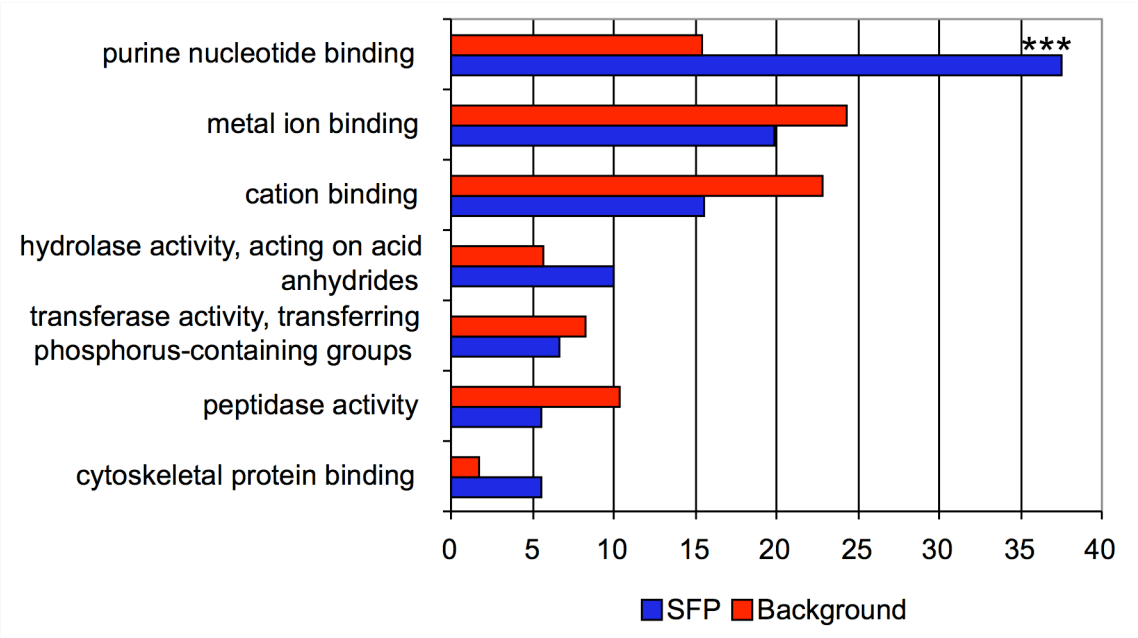
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Appendix 6: Pleiotropic effects of Drosophila *neuralized* on complex behaviors and brain structure

Pleiotropic effects of Drosophila *neuralized* on complex behaviors and brain structure

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DOI: 10.1534/genetics.108.088435

Pleiotropic Effects of Drosophila *neuralized* on Complex Behaviors and Brain Structure

Stephanie M. Rollmann,*,†,1,2 Liesbeth Zwarts,‡,1 Alexis C. Edwards,†,§ Akihiko Yamamoto,*,† Patrick Callaerts,‡ Koenraad Norga,‡, Trudy F. C. Mackay†,§ and Robert R. H. Anholt*,†,§,3**

**Department of Zoology, North Carolina State University, Raleigh, North Carolina 27695-7617, †W. M. Keck Center for Behavioral Biology, North Carolina State University, Raleigh, North Carolina 27695-7617, ‡Laboratory of Developmental Genetics, VIB-PRJ8 and Katholieke Universiteit Leuven, Center for Human Genetics, B-3000 Leuven, Belgium, §Department of Genetics, North Carolina State University, Raleigh, North Carolina 27695-7614 and **Children's Hospital, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

Manuscript received February 23, 2008
Accepted for publication April 24, 2008

ABSTRACT

Understanding how genotypic variation influences variation in brain structures and behavioral phenotypes represents a central challenge in behavioral genetics. In *Drosophila melanogaster*, the *neuralized* (*neur*) gene plays a key role in development of the nervous system. Different *P*-element insertional mutations of *neur* allow the development of viable and fertile adults with profoundly altered behavioral phenotypes that depend on the exact location of the inserted *P* element. The *neur* mutants exhibit reduced responsiveness to noxious olfactory and mechanosensory stimulation and increased aggression when limited food is presented after a period of food deprivation. These behavioral phenotypes are correlated with distinct structural changes in integrative centers in the brain, the mushroom bodies, and the ellipsoid body of the central complex. Transcriptional profiling of *neur* mutants revealed considerable overlap among ensembles of coregulated genes in the different mutants, but also distinct allele-specific differences. The diverse phenotypic effects arising from nearby *P*-element insertions in *neur* provide a new appreciation of the concept of allelic effects on phenotype, in which the wild type and null mutant are at the extreme ends of a continuum of pleiotropic allelic effects.

BEHAVIORS are complex traits. Their manifestation depends on interactions among multiple genes and their interplay with the environment. In contrast to other complex traits, behaviors are the quintessential expression of the nervous system, which mediate adaptive responses to changes in the environment. Previous studies have shown that the genetic architectures that shape behaviors are composed of modular ensembles of pleiotropic genes (Anholt *et al.* 2003; Anholt 2004; van Swinderen and Greenspan 2005). Furthermore, subtle disruptions of key genes within such ensembles have widespread effects on transcriptional regulation throughout the genome (Anholt *et al.* 2003) and can display a range of allelic effects that differentially affect different traits (Rollmann *et al.* 2006). For example, nearby *P*-element insertions in the *Tre1-Gr5a* region that interact epistatically with components of the insulin-signaling pathway differentially affect life span, resistance to heat stress and starvation, and preference for trehalose intake (Rollmann *et al.* 2006).

Understanding how genotypic variation results in variation in behavioral phenotypes requires corresponding insights into how variations in structure and function in the nervous system give rise to variation in these behaviors. To begin to understand pleiotropic effects of key genes in epistatic networks that orchestrate behaviors in the context of this "genes–brain–behavior" paradigm, we have studied *P*-element insertional mutants of *neuralized* (*neur*). The *Drosophila melanogaster neur* gene encodes a ubiquitin ligase, which processes the Notch ligand Delta and is involved in cell fate commitment during development of the nervous system (Dietrich and Campos-Ortega 1984; Yeh *et al.* 2000; Lai and Rubin 2001; Lai *et al.* 2001; Pavlopoulos *et al.* 2001; Timmusk *et al.* 2002). *P*-element insertions at *neur* can result in changes in the number of mechanosensory bristles (Norga *et al.* 2003) and reduced olfactory avoidance behavior (Sambandan *et al.* 2006). In addition, lines selected for increased and decreased aggression show altered transcriptional regulation of *neur* compared to unselected lines (Edwards *et al.* 2006).

[1]These authors contributed equally to this work.

[2]*Present address:* Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221.

[3]*Corresponding author:* W. M. Keck Center for Behavioral Biology, Campus Box 7617, North Carolina State University, Raleigh, NC 27695-7617. E-mail: anholt@ncsu.edu

We identified three co-isogenic *P*-element insertions in *neur* (Norga *et al.* 2003; Sambandan *et al.* 2006) and studied their effects on olfactory avoidance behavior, aggression, and locomotor reactivity in adult flies. We also performed morphometric neuroanatomical analyses to assess structural changes in integrative centers in the brain, the mushroom bodies, and the ellipsoid body of the central complex. Our observations demonstrate that mutations from nearby *P*-element insertions in a single gene can give rise to pleiotropic behavioral effects associated with neuroanatomical alterations.

MATERIALS AND METHODS

Drosophila stocks: The $neur^{BG02391}$, $neur^{BG02542}$, and $neur^{BG02587}$ *P*-element insertion lines each contain a single *p[GT1]* insertion (Lukacsovich *et al.* 2001; Bellen *et al.* 2004) in the *neur* (*CG11988*) gene region in the co-isogenic Canton-S (B) background. All flies were reared on an agar–yeast–molasses medium in vials at 25 and under a 12-hr light/dark cycle.

P-element excision lines: *P*-element excision lines were constructed in a controlled Canton-S (B) background by crossing *w;CS(B);*$neur^{BG02391}$ or *w;CS(B);*$neur^{BG02542}$ females to *w;Cy/Sp;SbD2-3/TM6,Tb* males. Male offspring of the genotype *w;Cy/CS(B);P/SbD2-3* were then crossed to *w;CS(B);H/TM3,Sb* females, and single male offspring, *w;CS(B);P/H,* were crossed to *w;CS(B);H/TM3,Sb* females. Progeny in which the *P* element has been excised, *w;CS(B);P/TM3,Sb,* were mated *inter se* to generate a homozygous *P*-element excision line.

Bristle numbers: Abdominal and sternopleural bristle numbers were scored for males and females. Abdominal bristle number is the number of microchaetae on the sixth sternite in females or the fifth sternite in males and sternopleural bristle number reflects the total number of macrochaetae and microchaetae on the right and left sternopleural plates. Four replicates of 10 flies per sex and line were counted. Abdominal and sternopleural bristle numbers were analyzed separately by two-way fixed effects ANOVA according to the model $Y = m + L + S + L \times S + E$, where L denotes line, S denotes sex, and E denotes environmental variation. Abdominal bristle number differed by line and sex as shown by a significant line-by-sex interaction term. Thus, abdominal bristle scores were subsequently analyzed separately for each sex according to the ANOVA model $Y = m + L + E$, where L denotes line and E denotes environmental variation. Post-hoc Tukey's tests were used to determine significant mean differences among the lines, where applicable.

Behavioral assays: Avoidance responses to benzaldehyde and locomotor reactivity assays were measured as described previously (Anholt *et al.* 1996; Jordan *et al.* 2006; Sambandan *et al.* 2006). Statistically significant differences from the Canton-S (B) control line were evaluated by two-way fixed effects ANOVA according to the model $Y = m + L + S + L \times S + E$, where L designates line, S designates sex, and E designates the environmental variation. A post-hoc Tukey's test was used to determine line differences in mean scores, where applicable. Male aggressive behavior was scored using the eight-fly assay described previously by measuring the number of aggressive encounters observed during a 2-min period in an arena with a droplet of food following a period of food deprivation (Edwards *et al.* 2006). Data were analyzed by a one-way fixed effects ANOVA according to the model $Y = m + L + E$, where L denotes line and E denotes environmental variation, with a subsequent post-hoc Tukey's test to determine significant mean differences among the lines. All *P*-element insertion lines and the Canton-S (B) control were tested contemporaneously for each behavior, and behavioral data were accumulated over multiple days or weeks to randomize environmental variation. Measurements for each behavior were always made during the same time of day to minimize experimental variation by avoiding differential sampling of circadian fluctuations.

Immunohistochemistry and morphometric analysis: Adult Drosophila brains were fixed in phosphate buffered saline (PBS)–37% formaldehyde for 15 min at room temperature, washed extensively with PBS, and blocked in PAXD (PBS containing 5% bovine serum albumin (Roche Biochemicals), 0.3% Triton X-100, 0.3% sodium deoxycholate for 10 min at room temperature. Incubation with a 100-fold dilution of antifasciclin II MAb 1D4 (Developmental Studies Hybridoma Bank; under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA) was done overnight at 4 . After washing with PAXD, brains were incubated with a 100-fold dilution of Cy™3-conjugated Affinipure goat anti-mouse IgG (H + L) (Jackson ImmunoResearch Laboratories, West Grove, PA) for 2 hr at room temperature, followed by washing with PAXD. Brain samples were mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and visualized under an Olympus BX61 epifluorescence microscope equipped with a DP70 digital camera controlled with analySIS Software. For ease of analysis, relevant dimensions (length and width of a- and b-lobes and diameters of ellipsoid body; see Figure 2g for color-coded schematic) were measured on screen and were subsequently converted to values (expressed as percentages) relative to the distance between the two mushroom body heels per brain. Statistical significance was determined using two-way ANOVA with post-hoc Tukey's tests. Images for Figure 2, a–d, were generated using a Leica TCS SE confocal laser microscope.

Transcriptional profiling: Flies were frozen on dry ice 5–7 days post eclosion and total RNA was isolated for two replicate groups of males and females for each line. First- and second-strand cDNA were synthesized followed by synthesis of biotinylated cRNA targets. These targets were hybridized to GeneChip Drosophila genome arrays (Affymetrix) and visualized with a streptavidin–phycoerythrin conjugate, as described in the Affymetrix GeneChip Expression Analysis Technical Manual (2000). An estimate of expression of each probe set is the signal metric, which is the weighted averaged signal from all probes within the probe set. Signal values were analyzed by two-way ANOVA according to the model $Y = m + L + S + L \times S + E$, where L denotes line, S denotes sex, and E denotes the environmental variation. Corrections for multiple testing were done using the false-discovery-rate q-statistic (Storey and Tibshirani 2003), with a false-discovery-rate threshold for significance set at $q < 0.05$.

Quantitative RT–PCR: RNA was isolated from three replicate groups of 25 animals each of control and *neuralized* mutant adults, as described above. cDNA was generated from 1 mg RNA of each sample using the Transcriptor First Strand cDNA synthesis kit (Roche Biochemicals). The qPCR Mastermix Plus for SYBR Green I (Eurogentec) was used in quantitative RT–PCR (qRT–PCR) reactions that were performed on an ABI7000 instrument. For each replicate group, four technical replicates were measured. Expression levels of transcripts from the various samples were normalized to actin5C expression. We used the following primers: Neur-AB F, 5′-GTCTCGAAGTTGTCGTCGTCGG, and Neur-AB R, 5′-AGCGATAGAGTTCTTCTTCG; Neur-CD F, 5′-GCTCACGGTGCACATAATATCG, and Neur-CD R, 5′-CAGCCACAACAACTAGGACACAC; actin5C F, AGTCCGGCCCCTCCATT, and actin5C R, CTGATCCTCTTGCCCAGACAA. Primers Neur-AB F and

Neur-AB R anneal to exon 2 (shared by transcripts A and B) and will allow quantification of the combined amounts of transcripts A and B, while primers Neur-CD F and Neur-CD R anneal to exon 1 (shared by transcripts C and D) and will allow quantification of the combined amounts of transcripts C and D. Further discrimination between transcripts A *vs.* B and C *vs.* D was technically not possible.

RESULTS

***P*-element insertions at the *neur* locus of *D.melanogaster*:** Drosophila *neur* is a neurogenic gene with a role in cell fate commitment. The Drosophila *neur* gene contains three exons, which generate four alternatively spliced transcripts: *neur*-RA, *neur*-RB, *neur*-RC, and *neur*-RD (NCBI accession no. AE014297). These transcripts differ in the first exon, but share their second and third exons with transcripts *neur*-RA and *neur*-RC differing by 3 bp in the lengths of their second intron and third exon compared to *neur*-RB and *neur*-RD, respectively (Figure 1a). The 3-bp difference is most likely due to alternative splice acceptor site usage. We identified three different *p[GT1]*-element insertions at the *neur* locus ($neur^{BG02391}$, $neur^{BG02542}$, and $neur^{BG02587}$; Bellen *et al.* 2004). The *p[GT1]* insertion sites are 70 bp upstream of exon 1 of transcripts *neur*-RA and *neur*-RB and in the first intron (5350 bp downstream of exon 1) of transcripts *neur*-RC and *neur*-RD. All insertions are in the same orientation (Figure 1a).

Previous studies indicated that *neur* mutations can affect numbers of sensory bristles, consistent with the role of *neur* in peripheral nervous system development (Norga *et al.* 2003). We observed a significant reduction of 1.84 sternopleural bristles in homozygous $neur^{BG02391}$ flies in both sexes, but the sternopleural bristle numbers of $neur^{BG02542}$ and $neur^{BG02587}$ were not significantly different from the control (Table 1). The line-by-sex interaction term was significant in the ANOVA of abdominal bristle number ($F_{4,390}$ ¼ 5.73, P ¼ 0.0002), indicating significant sex-specific effects of *neur* mutations on this trait. $neur^{BG02391}$ males and $neur^{BG02542}$ and $neur^{BG02587}$ females had reduced abdominal bristle numbers compared to the co-isogenic Canton-S (B) control (Table 1).

Pleiotropic behavioral effects of hypomorphic *neur* mutants: In a previous study we demonstrated that $neur^{BG02391}$ homozygotes display decreases in olfactory avoidance behavior in adult flies (Sambandan *et al.* 2006). We confirmed the aberrant olfactory avoidance behavior of $neur^{BG02391}$ mutant flies (Figure 1b). No significant differences in olfactory avoidance behavior from the control were observed for $neur^{BG02542}$ or $neur^{BG02587}$ (data not shown). To verify that the aberrant olfactory phenotype arose due to the insertion of the *p[GT1]* construct rather than from an independent mutation, we mobilized the *p[GT1]* transposon and showed that *P*-element excision restored normal olfactory avoidance behavior (Figure 1b). *P*-element excision also restored the wild-type sternopleural and abdominal bristle phenotype (Table 1).

Next, we asked whether reduced responsiveness to environmental cues was generalized or specific to particular sensory modalities by measuring a startle response (locomotor activity in response to a mechanical stimulus; Jordan *et al.* 2006) and male aggressive behavior (Edwards *et al.* 2006). We observed a significant reduction in locomotor reactivity for both sexes in $neur^{BG02542}$ ($F_{1,116}$ ¼ 33.45, $P < 0.0001$) and $neur^{BG02587}$ ($F_{1,116}$ ¼ 20.47, $P < 0.0001$) compared to the Canton-S (B) control (Figure 1c). However, $neur^{BG02391}$, which showed reduced olfactory avoidance responses to benzaldehyde, showed normal locomotor reactivity, which indicates that these behavioral phenotypes are dependent on the specific insertion site of the *P* element. To verify that altered locomotor reactivity was indeed attributable to the transposon insertions, we generated two *P*-element excision lines of $neur^{BG02542}$ ($neur^{BG02542Rev1}$ and $neur^{BG02542Rev2}$) and demonstrated restoration of wild-type locomotor reactivity ($F_{3,192}$ ¼ 14.28, $P < 0.0001$; Figure 1d).

Surprisingly, the average numbers of aggressive encounters by males competing for a limited food supply after a period of food deprivation were 1.8-fold greater in $neur^{BG02391}$ and $neur^{BG02587}$ than in the co-isogenic Canton-S (B) control ($F_{2,57}$ ¼ 20.60, $P < 0.0001$; Figure 1e). We did not observe a significant effect of $neur^{BG02542}$ on aggressive behavior. Again, *P*-element excision in a controlled genetic background restored the level of aggressive behavior of the $neur^{BG02391}$ mutant to wild-type levels (Figure 1e). The increased level of aggression in these flies demonstrates that their impaired locomotor reactivity is not due to physical limitations on mobility.

Effects of *P*-element insertions in *neur* on brain structure: Olfactory avoidance behavior, startle-induced locomotion, and aggression are behaviors that all involve input from different sensory modalities. This input is integrated and processed in higher brain structures and ultimately results in a motor response. We sought to determine whether the behavioral abnormalities that we observed in *neur* mutant flies could result from structural alterations in integrative centers in the brain, namely the mushroom bodies and the central complex. Sensorimotor coordination in Drosophila depends on the mushroom bodies and the central complex in the brain (Martin *et al.* 1998, 1999). The startle-induced locomotor response is distinct from spontaneous open-field locomotor activity, and not an *a priori* component of aggressive behavior. Thus, the neural circuits that mediate these different behaviors need not necessarily overlap.

In our analysis, we focused on the α- and β-lobes of the mushroom bodies and on the ellipsoid body of the central complex as they integrate information for the execution of complex behaviors. For example, the α-lobes of the mushroom bodies have been implicated in

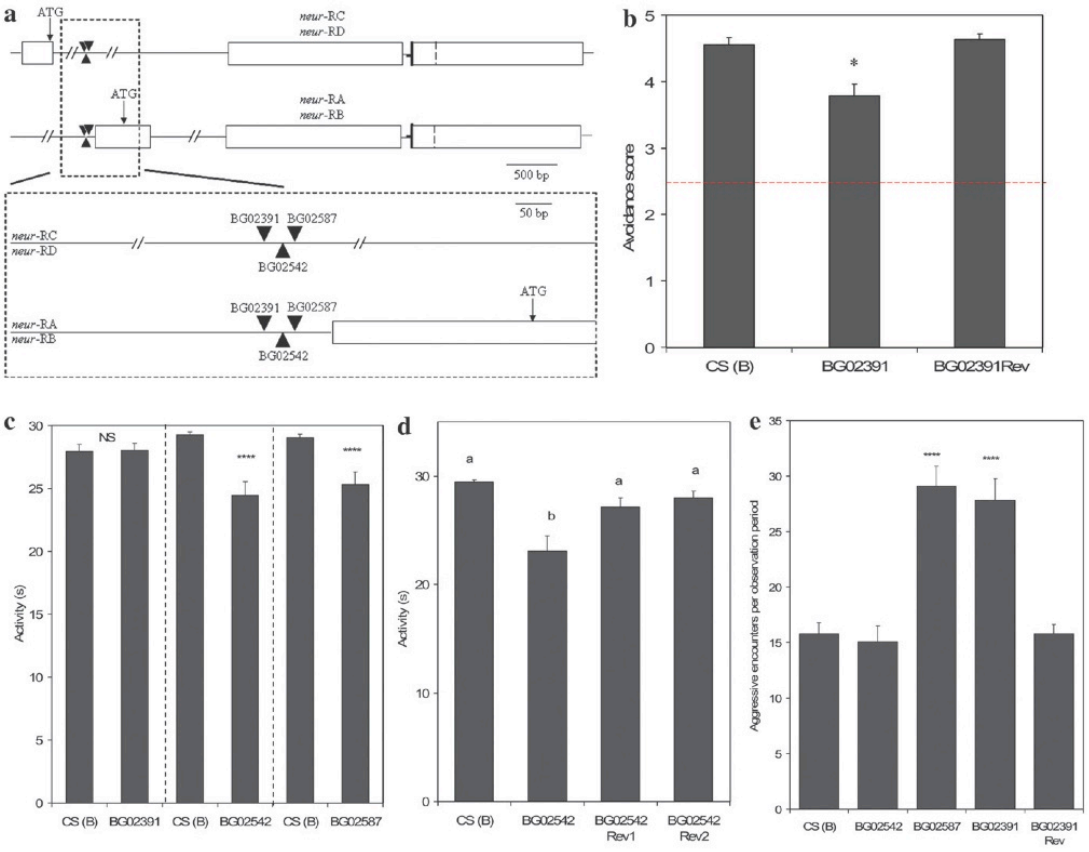

FIGURE 1.—Behavioral effects of transposon-mediated disruption of the *neur* gene. (a) *neur* gene structure and *P*-element insertion sites. The horizontal line represents genomic DNA with open boxes representing exons of *neur*. Alternative transcripts of *neur*-designated *neur*-RA, *neur*-RB, *neur*-RC, and *neur*-RD are shown with solid areas in intron 2 and exon 3 denoting small differences in the lengths of the transcripts. Three independent *p*[*GT1*] insertions in the first intron of *neur*-RC and *neur*-RD and upstream of the first exon of *neur*-RA and *neur*-RB were identified. *P*-element insertion sites are indicated by arrowheads. Translation initiation sites are denoted by ATG with an arrow. The end of the coding region is indicated by a vertical dashed line in exon 3. The inset shows a magnified view of the region containing the *P*-element insertion sites. Flanking sequences at the 3′-ends of the *P*-element insertions of BG02391 and BG02542 are CCAGTACTATCCGTTACTCTCCAGCTGAGCTGCGTCAGCGACGTCGCGC and CTCCAGCTGAGCTGCGTCAGCGACGTCGCGC, respectively. Flanking sequences at the 5′-ends of the *P*-element insertions of BG02542 and BG02587 are CAGTACTATCCGTTACT-CTCCAGCT and GCGCCAGTACTATCCGTTACTCTCCAGCTGAGCTG CGTCAGCGA, respectively. (b) Olfactory avoidance responses to benzaldehyde. The $neur^{BG02391}$ (BG02391) flies showed reduced behavioral avoidance responses to benzaldehyde. Excision of the *p*[*GT1*] construct (BG02391Rev) resulted in phenotypic reversion of olfactory avoidance behavior. The red dashed line denotes the expected avoidance score that corresponds to indifference to the presence of the odorant. Post-hoc Tukey's test was used to determine significant difference among line means; $*P$, 0.05. (c) Locomotor reactivity following mechanical stimulation. A significant reduction in locomotor reactivity was observed for $neur^{BG02542}$ (BG02542) and $neur^{BG02587}$ (BG02587). No significant difference was observed for $neur^{BG02391}$; $****P$, 0.0001. (d) Phenotypic reversion of locomotor reactivity after *P*-element excision. Locomotor reactivity was reduced in the *P*-element insertion line $neur^{BG02542}$ as compared to its Canton S (B) control. Precise excision of the *P* element in lines $neur^{BG02542Rev1}$ and $neur^{BG02542Rev2}$ restored locomotor reactivity to wild-type levels. Means designated by the same letter are not statistically significantly different from one another. (e) Aggressive behavior. A significant increase in aggressive encounters compared to the control was observed in $neur^{BG02391}$ and $neur^{BG02587}$. Excision of the *P* element (BG02391Rev) resulted in phenotypic reversion of aggressive encounters to wild-type Canton S (B) levels. $****P$, 0.0001 (post-hoc Tukey's test).

long-term memory formation, whereas short-term memory requires the gamma lobes (ZARS *et al.* 2000; PASCUAL and PREAT 2001; YU *et al.* 2006). α- and β-lobe outputs serve in olfactory memory retrieval, but not in its formation or storage (DUBNAU *et al.* 2001; MCGUIRE *et al.* 2001; SCHWAERZEL *et al.* 2002). The ellipsoid body is also implicated in long-term memory consolidation (WU *et al.* 2007). Here, we conducted morphological

TABLE 1

Effects of P-element insertions at the neur locus on average (± standard error) sternopleural and abdominal bristle numbers

Line	ST (♂ and ♀)	AB (♂)	AB (♀)
Canton S (B)	17.69 ± 0.22 (a, b)	17.8 ± 0.35 (a)	20.93 ± 0.32 (a)
$neur^{BG02391}$	15.85 ± 0.17 (c)	16.48 ± 0.31 (b)	20.13 ± 0.33 (a, b)
$neur^{BG02542}$	17.1 ± 0.18 (b)	17.38 ± 0.3 (a, b)	18.83 ± 0.37 (c)
$neur^{BG02587}$	18.3 ± 0.21 (a)	18.13 ± 0.31 (a)	19.43 ± 0.21 (b, c)
$neur^{BG02391Rev}$	17.33 ± 0.16 (b)	17.25 ± 0.23 (a, b)	19.93 ± 0.28 (a, b, c)

No difference between the sexes was observed for average sternopleural (ST) bristle number and therefore sternopleural bristle counts for the sexes were pooled. Significant differences were observed for the line-by-sex interaction for abdominal bristle (AB) number and therefore sexes were analyzed separately. Significant mean differences in bristle counts are designated by different lowercase letters (post-hoc Tukey's test).

and morphometric analyses of the ellipsoid body and the mushroom body a- and b-lobes of the adult brain following immunohistochemical labeling with the antifasciclin 2 antibody 1D4. This antibody strongly labels the a- and b-lobes of the mushroom bodies and, to a lesser extent, the gamma lobes (Figure 2a; Crittenden *et al.* 1998). We first determined whether any gross morphological alterations could be seen. Next we measured widths and lengths of the a- and b-lobes and diameters of the ellipsoid body. Mushroom bodies were scored individually (*i.e.*, per hemisphere) while ellipsoid body scores were scored per brain. A direct relationship between gross structural brain defects and behavior has been demonstrated previously (see, *e.g.*, Strauss 2002), but subtle alterations in brain neuroanatomy and behavioral changes have hardly been studied. The biological relevance of such subtle changes is illustrated by a recent study of the paper wasp, *Polistes instabilis*, where mushroom body volume was related to social aggression (Molina and O'Donnell 2007).

We observed gross morphological defects in some of the mushroom bodies of $neur^{BG02542}$ flies. These defects included shorter and thinner a-lobes (7/77; Figure 2b), missing a-lobes (2/77; Figure 2c), and aberrant projections of the a-lobe (1/77; Figure 2d). We did not observe such defects either in the co-isogenic controls (0/43) or in the $neur^{BG02391}$ (0/75) and $neur^{BG02587}$ (0/74) alleles. A *G*-test of independence (Sokal and Rohlf 1995) indicates that the frequency of aberrations is significantly different for the different genotypes ($G_3 = 26.04$, $P = 9.35 \times 10^{-6}$). In the central complex, the antifasciclin 2 antibody stains primarily the ellipsoid body, which is labeled in a characteristic pattern of two concentric rings (Figure 2e). We observed disorganized ellipsoid bodies (ranging from less distinct or poorly defined concentric rings to completely abnormal organization) or defects (ventral open) in 20 of 39 brains (Figure 2f) in the $neur^{BG02542}$ mutants. A comparable disorganization or poor structural definition but not the ventral open defect is occasionally observed in the wild-type control (4/22). We also did not see major disorganization in the $neur^{BG02391}$ (0/39) and $neur^{BG02587}$ (0/38) alleles. Again, a *G*-test reveals that the differences in frequency of ellipsoid body aberrations among these genotypes are significant ($G_3 = 56.60$, $P = 3.12 \times 10^{-12}$).

Whereas gross morphological defects were not always apparent, a detailed morphometric analysis revealed consistent subtle differences in neuroanatomical organization. We quantified widths and lengths of a- and b-lobes as well as surface areas of the ellipsoid bodies using the variables outlined in Figure 2g. To control for possible shrinkage effects due to fixation, all measurements were expressed as percentages relative to the distance between the two mushroom body heels (blue double arrow in Figure 2g). Behavioral alterations in the *neur* mutants reveal remarkable parallels with most neuroanatomical alterations. We found significant differences in a-lobe ($F_{6,327} = 2.991$; $P = 0.007$) and b-lobe widths ($F_{6,327} = 4.925$; $P < 0.001$). a- and b-lobe widths of $neur^{BG02391}$, which shows reduced olfactory responsiveness (Figure 1b) and hyperaggression (Figure 1e), were significantly different from the Canton-S (B) controls ($P < 0.05$; Figure 2i). Length measurements showed significant differences only for a-lobes ($F_{6,327} = 6.420$; $P < 0.001$). The a-lobe lengths of $neur^{BG02391}$ were significantly different from controls ($P < 0.05$) (Figure 2j). The analysis of ellipsoid body surfaces also revealed significant differences among *neur* alleles ($F_{6,165} = 2.153$; $P = 0.050$). The locomotor response-impaired $neur^{BG02542}$ mutant (Figure 1c) displayed a significantly reduced ellipsoid body surface relative to the Canton-S (B) control ($P < 0.05$). The other two *neur* alleles also showed a reduction in ellipsoid body surface, of which $neur^{BG02391}$ was also significantly different from Canton-S (B) (Figure 2k). We next asked whether the restoration of behavior to wild type seen for the $neur^{BG02391Rev}$, $neur^{BG02542Rev1}$, and $neur^{BG02542Rev2}$ alleles is accompanied by changes in morphology of mushroom bodies and ellipsoid bodies. None of the revertants displayed the gross morphological defects seen in the original alleles. Furthermore, we observed restoration of a- and b-lobe width as well as of ellipsoid body surface of $neur^{BG02391Rev}$

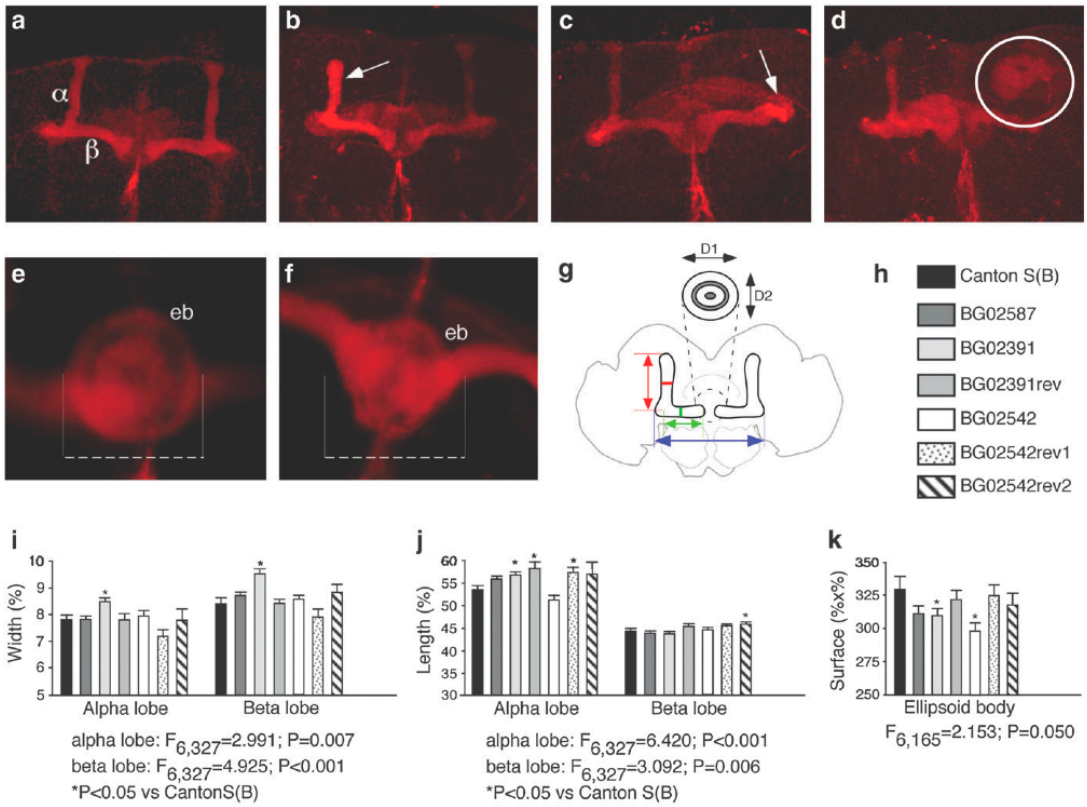

FIGURE 2.—Morphometric analysis of wild-type Canton S (B) controls and the *P*-element-tagged *neuralized* alleles BG02542, BG02391, and BG02587. (a–f) Antifasciclin 2 staining of adult brains using the 1D4 monoclonal antibody. (a) Canton S (B) control: α, α-lobes of mushroom bodies; β, β-lobes of mushroom bodies. (b–d) Mushroom body defects observed in $neur^{BG02542}$: short and thinner α-lobe (arrow in b); α-lobe missing (arrow in c); aberrant ball-shaped axonal projections instead of regular α-lobe (d). (e) Canton S (B) control: typical appearance of the ellipsoid body (eb) upon staining with antifasciclin 2 antibody reveals two concentric rings. (f) The ellipsoid body in $neur^{BG02542}$ often appears less organized and smaller. The dashed lines in e and f, which are at the same magnification, are of the same length, illustrating the fact that the ellipsoid body in $neur^{BG02542}$ is smaller. (g) Schematic of the measurements that were made for morphometry. α- and β-lobe diameters (red and green arrows, respectively) were measured and diameters D1 and D2 were determined for the ellipsoid body to calculate the ellipsoid body surface. The blue arrow indicates the distance between the α-lobe heels that was used to normalize the measurements for each brain. (h) Key for histograms in i–k. (i) Mushroom body α- and β-lobe widths in Canton S (B), $neur^{BG02542}$, $neur^{BG02391}$, $neur^{BG02587}$, $neur^{BG02542Rev1,2}$, and $neur^{BG02391Rev}$. (j) Mushroom body α- and β-lobe lengths in Canton S (B), $neur^{BG02542}$, $neur^{BG02391}$, $neur^{BG02587}$, $neur^{BG02542Rev1,2}$, and $neur^{BG02391Rev}$. (k) Ellipsoid body surfaces in Canton S (B), $neur^{BG02542}$, $neur^{BG02391}$, $neur^{BG02587}$, $neur^{BG02542Rev1,2}$, and $neur^{BG02391Rev}$. For the Canton S (B) controls, 43 hemispheres were scored for mushroom body defects and 22 brains for ellipsoid body defects. For the *neur* alleles, we examined 77 brain hemispheres for mushroom body defects and 39 brains for ellipsoid body defects for $neur^{BG02542}$, 75 brain hemispheres and 39 brains for $neur^{BG02391}$, and 74 brain hemispheres and 38 brains for $neur^{BG02587}$. For the $neur^{BG02542Rev1,2}$ and $neur^{BG02391Rev}$ revertant alleles, we analyzed 22, 23, and 21 brain hemispheres and 11, 12, and 11 brains, respectively. Lobe width and length were normalized to the distance between the α-lobe heels and are expressed as percentages. The ellipsoid body surface values shown in the *y*-axis in k were calculated by multiplying the radii (R1 = D1/2 and R2 = D2/2) expressed as percentages after normalization. The constant value Π of the formula surface = Π × R1 × R2 was omitted from the calculation.

to levels not different from Canton-S (B) controls (see Figure 2, i and k). Furthermore, we found that the ellipsoid body surface of both revertants for $neur^{BG02542}$ is restored to the size of the Canton-S (B) control (see Figure 2k). The one parameter that did not revert to wild-type values in the revertant lines was α-lobe length. We do not know the underlying reason for this observation, but our data indicate that the α-lobe phenotype seen in $neur^{BG02391}$ does not depend on the *P*-element insertion. It was previously shown that $neur^{BG02542}$ is associated with locomotor reactivity deficits and $neur^{BG02391}$ with aggressive behavior. The observation

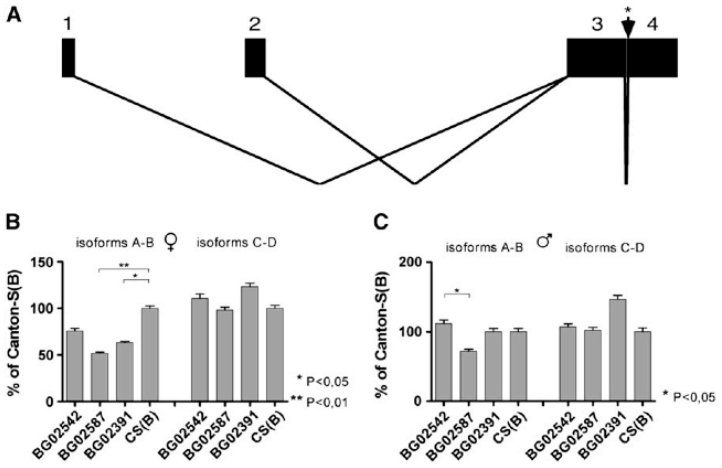

FIGURE 3.—qRT–PCR analysis of *neuralized* transcripts A-B and C-D in wild-type Canton S (B) controls and the *P*-element insertion alleles BG02542, BG02391 and BG02587. (A) Genomic structure of the *neuralized* gene. The *neuralized* gene consists of four exons (1–4). Alternative splice acceptor sites are located 3 bp apart at the 5′-end of exon 4 (arrow with asterisk; for the locations of the *P*-element insertion sites in the *neur* locus, see Figure 1a). (B) qRT–PCR analysis of A-B and C-D transcripts in RNA isolated from females of $neur^{BG02542}$, $neur^{BG02587}$, $neur^{BG02391}$, and Canton S (B). Transcript levels are expressed as percentages of the Canton S (B) control. The A-B transcript levels in $neur^{BG02587}$ and $neur^{BG02391}$ are significantly lower than in Canton S (B), whereas reduction in $neur^{BG02542}$ transcript levels does not reach statistical significance ($F_{3,8} = 11.399$; $P = 0.003$). The C-D transcripts do not differ. (C) qRT–PCR analysis of A-B and C-D transcripts in RNA isolated from males of $neur^{BG02542}$, $neur^{BG02587}$, $neur^{BG02391}$, and Canton S (B). Transcript levels are expressed as percentages of the Canton S (B) control. The A-B transcript levels in $neur^{BG02542}$ and $neur^{BG02587}$ differ significantly ($F_{3,8} = 4.570$; $P = 0.038$). The apparent reduction in the level of $neur^{BG02587}$ compared to $neur^{BG02391}$ and the Canton S (B) control is not statistically significant. For the C-D transcripts, no statistically significant differences were observed although the levels in $neur^{BG02391}$ appear higher. Statistical significance was determined with ANOVA and post-hoc analysis with Bonferroni correction.

that *P*-element excision results in reversion to wild-type levels of behavior and brain structure suggests that the alterations seen in the ellipsoid body and in ellipsoid body and mushroom body lobes, respectively, may be causally linked to the observed differences in behavior. This is consistent with the previously demonstrated roles of ellipsoid bodies and mushroom bodies in locomotor activity (MARTIN *et al.* 1998, 1999).

***P*-element insertions in *neuralized* affect ratios of alternative transcripts:** Small differences in the locations of the *P* elements might result in differences in expression levels or ratios of alternatively spliced transcripts which could impact different forms of adult behavior. To determine whether the different *P*-element insertions had differential effects on transcription, we performed qRT–PCR experiments, allowing us to discriminate transcripts A and B from transcripts C and D (Figure 3A). We found that there are indeed differential effects on these transcript pairs associated with the different *P*-element insertions (see Figure 3, B and C). This was most pronounced in females where transcripts A and B are significantly reduced in $neur^{BG02587}$ and $neur^{BG02391}$ when compared to the Canton-S (B) control. By contrast, no such differences were observed for the C and D transcripts in females. In males, a significant difference was seen between $neur^{BG02542}$ and $neur^{BG02587}$. The latter appears lower than $neur^{BG02391}$ and the Canton-S (B) control without, however, reaching statistical significance. The C and D transcripts again showed less variation although in $neur^{BG02391}$ an increase was seen that was, however, not statistically significant. These observations extend a previous study, in which we showed that *neur* transcript abundance is reduced in embryos and larvae of $neur^{BG02391}$ homozygotes and that this reduction resulted in decreased olfactory avoidance behavior in adult flies (SAMBANDAN *et al.* 2006).

Genes with altered transcriptional regulation in adult *neur* mutants: Previously, we showed that single *P*-element insertions cause genomewide alterations in expression of coregulated genes (ANHOLT *et al.* 2003). To determine to what extent the *p[GT1]*-element insertion alleles of *neur* alter the transcriptional context of *neur* expression, we examined genomewide transcriptional profiles in the Canton-S (B) control and $neur^{BG02391}$, $neur^{BG02542}$, and $neur^{BG02587}$ mutants using Affymetrix high-density oligonucleotide Drosophila GeneChips. Analysis of whole-genome transcriptional profiles resulted in 135 probe sets with altered expression levels at a false discovery rate of $q < 0.05$ (STOREY and TIBSHIRANI 2003; supplemental Table S1). We observed considerable overlap among the ensembles of genes with altered expression in the different mutants. Of the 135 probe sets, 85 (63%) were altered in two or more mutant lines compared to the co-isogenic Canton-S (B) control. However, 22 were altered only in $neur^{BG02391}$, 11 only in $neur^{BG02542}$, and 8 only in $neur^{BG02587}$, consistent with different behavioral, morphological, and neuroanatomical phenotypes observed for each mutant allele (supplemental Table S1). In addition, 9 lines showed a significant line-by-sex interaction (supplemental Tables S1 and S2). Precedence for such different phenotypic effects arising from nearby *P* elements, or even from *P* elements at the same insertion site but in different orientations, has been documented previously for *P*-element insertions in the

TABLE 2

Overrepresented gene ontology categories of coregulated transcripts in *neur* mutants

Biological process[a]	*P*	Genes
Carbohydrate metabolism (23)	2.80E−09	*Peptidoglycan-recognition protein-SC1a/b precursor, Amylase distal, Amylase proximal, Peptidoglycan-recognition protein-SD precursor, Lysozyme P, Lysozyme X, Lysozyme C, GIP-like, Lysozyme E, Peptidoglycan-recognition protein-S2 precursor, Isocitrate dehydrogenase, Serine pyruvate aminotransferase, Lyzozyme B,* 10 predicted transcripts
Hydrolase activity (43)[b]	1.40E−08	*Peptidoglycan-recognition protein-SC1a/b precursor, Amylase distal, Serine protease 6, Minichromosome maintenance 6, Serine protease 12, Amylase proximal, Lysozyme P, Peptidoglycan-recognition protein-SD precursor, GIP-like, Jonah 6Ci, Jonah 44E, Astray, Lysozyme C, Lysozyme X, Puromycin sensitive aminopeptidase, Jonah 74E, Lysozyme E, Tubulin at 67C, Jonah 65Ai, Peptidoglycan-recognition protein-SC2 precursor, Alkaline phosphatase 4, Lysozyme B,* 20 predicted transcripts
Antibacterial humoral response (8)	1.70E−07	*Cecropin A1, Attacin-D, Lysozyme P, Lysozyme B, Lysozyme C, Lysozyme X, Lysozyme E, Peptidoglycan-recognition protein-SD precursor*
Chymotrypsin activity (12)[b]	6.20E−07	*Serine protease 6, Jonah 66Ci, Jonah 65Ai, Jonah 44E, Serine protease 12, Jonah 74E,* 6 predicted transcripts
Immune response (10)	1.90E−06	*Peptidoglycan-recognition protein-SC1a/b precursor, Cecropin A1, Attacin-D Peptidoglycan-recognition protein-SC2 precursor, Lysozyme P, Lysozyme B, Peptidoglycan-recognition protein-SD precursor, Lysozyme C, Lysozyme X, Lysozyme E*

Analyses were performed with the DAVID program (Dennis *et al.* 2003) and the complete data output is presented in supplemental Table 3.

[a] The number of coregulated transcripts in each category is indicated in parentheses.

[b] The *jonah* genes indicated in this category are annotated as serine-type peptidases.

Tre1-Gr5a region that differentially affect starvation and heat stress resistance, gustatory behavior, and life span (Rollmann *et al.* 2006). In addition, 9 probe sets showed sex-specific differences in expression (supplemental Table S2). It is of interest that, among the genes with altered transcriptional regulation in one or both of our hyperaggressive *neur* mutants, 34 exhibited differential regulation in lines selected for increased or decreased aggression (Edwards *et al.* 2006; <15 genes would be expected by chance).

We assigned coregulated genes to the gene ontology categories of molecular function and biological process (Dennis *et al.* 2003). The five most significant biological process categories are highlighted in Table 2 and the complete data set is presented in supplemental Table S3. In contrast to transcripts expected to be associated with the function of *neur* in early development of the nervous system, such as *Notch* and *Delta*, transcripts with altered regulation in the *neur* mutant background in adult flies are predominantly associated with proteolysis. This is in line with the ubiquitin ligase function of the *neur* gene product and could reflect a role in the dynamics of synaptic organization, as implied previously for *Tequila*, which is transiently upregulated in the mushroom bodies during memory formation (Didelot *et al.* 2006) and shows altered regulation in the *neur* mutants.

DISCUSSION

We have shown that distinct *P*-element insertions at nearby locations in the *neur* gene give rise to profoundly different effects on adult behaviors and that aberrant startle-induced locomotor responses, olfactory responses, and aggression correlate with different structural alterations in integrative brain centers, the mushroom bodies, and the ellipsoid body of the central complex. Similar pleiotropic allelic effects with differential effects on life span, resistance to heat stress and starvation, and preference for trehalose intake were observed previously for *P*-element insertions in the *Tre1-Gr5a* region (Rollmann *et al.* 2006). In addition, different naturally occurring polymorphisms in *Catsup*, which encodes a negative regulator of tyrosine hydroxylase, are associated with phenotypic variation in sternopleural bristle number, environmental plasticity of abdominal bristle number, and starvation resistance (Carbone *et al.* 2006). The diverse phenotypic effects arising from nearby *P*-element insertions in *neur* contribute to an emerging new appreciation of the concept of allelic effects on phenotype, in which the wild type and null mutant are at the extreme ends of a continuum of pleiotropic allelic effects. Subtle alterations in transcript abundance for splice variants may contribute to these pleiotropic effects, which would be in line with the subtle regulatory variations that have been associated with phenotypic effects on human and rodent behaviors (*e.g.*, Krishnan *et al.* 2007; Jensen *et al.* 2008).

Previous studies have shown that the introduction of a single *P*-element in the genome gives rise to widespread altered transcriptional regulation and that about two-thirds of genes with altered transcriptional regulation in a *P*-element-disrupted background are candidate genes

affecting the trait (Anholt *et al.* 2003). Moreover, such altered transcriptional profiles can define a functional context for the disrupted gene (Rollmann *et al.* 2005). The results from our expression microarray analysis show that insertions of *P* elements in *neur* result in a genomewide cascade of transcripts with altered expression. Proteolytic and degradative enzymes feature prominently among coregulated genes. One protease-encoding transcript that features notably in these transcriptional profiles is *Tequila*, which has previously been implicated in synaptic plasticity in the mushroom bodies during memory formation (Didelot *et al.* 2006; supplemental Table S3). One could speculate that the different, yet overlapping patterns of transcriptional profiles with altered expression of proteolytic enzymes and peptidoglycan recognition precursor proteins (Table 2) may reflect alterations in neural connectivity, which could contribute to the different behavioral effects. In addition, changes in structure of the mushroom bodies and ellipsoid body could also arise from developmental effects of *neuralized* (Sambandan *et al.* 2006).

The behavioral phenotypes of *neur* mutant flies are reminiscent of those encountered in patients suffering from neuropsychiatric and neurodegenerative disorders, including reduced responsiveness to environmental stimuli and increased aggressive behavior. Neurodegenerative and neuropsychiatric disorders ranging from bipolar disorder, schizophrenia, and antisocial personality disorder to Alzheimer's and Parkinson's disease are often accompanied by behavioral alterations, such as indifference to stimuli, hypokinesia, hyperactivity, and aggression (Paveza *et al.* 1992; Aarsland *et al.* 1999; Moran 1999; Oquendo *et al.* 2000; Brieden *et al.* 2002; Haller and Kruk 2006). Apathy and aggressive behavior have a catastrophic impact on the social functioning of neuropsychiatric patients. In addition, these behaviors represent some of the most difficult to treat symptoms. Whereas it is likely that underlying genetic architectures that may predispose to such behavioral syndromes are heterogeneous and complex, we have demonstrated that reduced responsiveness to environmental stimuli together with increased aggressive behavior can arise from a single hypomorphic mutation at the *neur* locus in Drosophila and that these behavioral defects are associated with distinct subtle alterations in neuroanatomy.

We thank Richard Lyman for assistance in the analysis of bristle phenotypes, Stefanie Heinsohn and Stacy Williams for technical assistance, Ann Schellens for help with the qRT–PCR analyses, Mimi Deprez for help with preparing the figures and Zsuzsanna Callaerts-Vegh for help with the statistical analysis of the morphometric and qRT–PCR data. K.N. is a Senior Clinical Investigator of the Fonds voor Wetenschappelÿk Onderzoek-Flanders. L.Z. is supported by the Institute for the Promotion of Innovation through Science and Technology in Flanders (Instituut voor de Aanmoediging van Innovatie door Wetenschap en Technologie in Vlaandeven). This work was supported by grants from the National Institutes of Health (to R.R.H.A. and T.F.C.M.) and in part by a Belgian Federal Science Policy Return Grant (to K.N.), a Marie-Curie International Reintegration Grant (to K.N.), and Vlaams Instituut voor Biotechnologie. This is a publication of the W. M. Keck Center for Behavioral Biology at North Carolina State University.

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Communicating editor: L. Harshman