ABSTRACT

SHUFORD JR., DAVID TICE. THE GENETIC ANALYSIS OF NEGATIVE GEOTAXIS BEHAVIOR IN DROSOPHILA MELANOGASTER. (Direction provided by Dr. Trudy Mackay.)

Behaviors are complex traits, which exhibit continuous phenotypic variation in natural populations. The continuous variation is attributable to the segregation of multiple interacting loci with individually small effects on behavior, which are sensitive to the environment. In *Drosophila*, loci with small, environmentally sensitive effects on behavior can be identified by screening collections of *P*-element insertions that have been generated in a co-isogenic background. Here, we have used this approach to identify novel candidate genes affecting geotaxis. *Drosophila melanogaster* are negatively geotactic, i.e., flies move opposite the Earth's gravitational vector when disturbed. We developed a rapid assay to quantify this geotactic behavior. Individual flies are placed in a 15cm tube, and lightly tapped to the bottom. The vertical distance traveled in 10s is the measure of behavior. Using this assay, we quantified the behavior of 475 co-isogenic *P*-element insertion lines, generated in co-isogenic Canton-S backgrounds as part of the Berkeley Drosophila Gene Disruption Project. The most extreme scoring lines were also assayed for locomotor activity to control for pleiotropic effects associated with this quantitative trait. We found 24 lines with increased, and 15 lines with decreased geotaxis. Four lines had sex-specific effects on geotactic behavior. Seventeen of the mutations are in known genes, many of which affect neurogenesis (e.g. *Mushroom-body expressed* and *neuralized*). The remaining are insertions in predicted genes of unknown function. We tested a subset of lines in the classic geotaxis maze. Of the ten lines chosen to be tested, eight lines showed a significant difference from the parental line, and of these, six lines showed a phenotype

that corroborated our observations in the climbing assay. Thus, our approach identified new candidate genes that contribute to geotaxis in *Drosophila melanogaster*.

# THE GENETIC ANALYSIS OF NEGATIVE GEOTAXIS BEHAVIOR IN DROSOPHILA MELANOGASTER

By

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# BIOGRAPHY

I was born in Daytona Beach, Florida, on August 3, 1977. My brother was born on July 11, 1980, and we spent our formative years together getting into trouble and trying to establish who we would become. At Liberty Junior High School in Morganton, NC I began participating in extracurricular activities, competing in track & field, basketball, and most notably, Science Olympiad. Our team won the North Carolina competition and then traveled to Auburn, Alabama for the nationals. I enjoyed the competition, but looking back, I realize the camaraderie that comes with a team is what I really needed at that time. The need to work with others toward a common goal has a profound affect on my life and is how I solidify my friendships with others even as an adult. Since then I have gone to college, gotten myself into and out of trouble, and found the person with whom I want to spend the rest of my life. Many years have passed, and I am not exactly sure why, but science still has a stranglehold on my psyche and probably will for the foreseeable future.

**ACKNOWLEDGEMENTS**

I have heard that it takes a village to raise a child, but who knew that it would take a small army to get me to where I am today. Early in my life that army was led by my mother and father. They both deserve medals of valor for raising two boys. Recently, the manager of the 'David Shuford Project' has been my wife, Caroline. She deserves a lot of credit for where I am today and where I will be tomorrow.

I want to thank Dr. Mark Peifer, for taking a chance on a recent graduate in search of direction.

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Go Heels!

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# 1. INTRODUCTION

Gravity is the force of attraction that exists between any two masses. Because earth is an exceptionally large mass, all objects on earth are pulled towards the earth's center at the constant rate of 9.8m/s $^2$, known as the gravitational constant. As one travels away from earth, the gravitational constant lessens until it nears zero, indicated by the fact that astronauts experience weightlessness in space. Gravity also asserts a measurable influence on cellular events, physiological function, and even a *Drosophila melanogaster* behavior known as geotaxis, which is the fly's behavior to move opposite the gravitational vector. Geotaxis relates directly to how the fly senses and responds to its location in space. Understanding the genetic basis of behavioral response to normal gravity will yield insights as to the mechanisms underlying proprioception. In addition, it is important to understand the genetic architecture underlying mechanisms regulating responses to changes in the force of gravity in both humans and other organisms as prolonged space flight becomes a reality.

Because of gravity's effect on life forms, all life on earth has evolved and adapted to conditions of constant gravity, a fact made readily apparent when the gravitational field is altered like it is during space travel. During space travel, humans lose bone density as they no longer bear their skeletal weight (BIKLE *et al.* 2003), and experience muscular atrophy and cardiovascular degeneration (GRAEBE *et al.* 2004). Additionally, space travel causes body fluids to shift upward which the body senses as a "fluid-volume overload," and the body responds with a decrease in plasma volume and overall fluid deficits, a phenomenon known as the Henry-Gauer reflex (GAUER *et al.* 1970). So great is the impact on normal human function (GRAEBE *et al.*

2004) even the impact of gravity upon the pharmacokinetics and pharmacodynamics of medications taken in space must be taken into consideration.

Due to these severe effects on normal human function, investigating the role of gravity in normal molecular functions must be a priority as man looks to travel to space extensively. Given the wealth of genetic and genomic resources in model plants (*Arabidopsis*) and animals (*Drosophila*), a necessary first step is to investigate the genetic networks regulating responses to gravity in these organisms. Despite this need, little has been learned from space flight experiments aimed at studying the effects of microgravity on *Drosophila*. This is due to a multitude of factors, including the scarcity and expense of such experiments, small sample sizes, improper controls, and lack of proper statistical analysis (LE BOURG and MINOIS 1999).

The IML-2 experiment undertaken in July of 1994 is one of the few recent space experiments where solid information was gained. Flies spent 14.5 days in microgravity during space flight and exhibited increased longevity and locomotor activity (BENGURIA *et al.* 1996). Embryos laid in space were collected, and though development was slightly delayed in space, flies were able to mate and produce viable offspring upon returning to earth (MARCO *et al.* 1996). This was surprising because multiple cellular systems are affected by microgravity and it was hypothesized that space travel would have a noticeable effect on development (SPOONER *et al.* 1994).

The IML-2 experiment, while informative, points to some of the inherent deficiencies with experiments done in short-term space flight. First, the costs of the experiment were high, given what was learned. Second, the short duration may not allow for noticeable affects. Certain techniques to simulate microgravity, such as drop tubes, weightlessness towers, and

parabolic flights, do not allow for prolonged exposure to microgravity necessary for a genetic response.

To counter these deficiencies, hypergravity, a gravity force greater than normal 1xg usually applied by centrifuge exposure has been used to examine the effect of altered gravity on phenotypes such as aging. In fact, the motivation for much of this work was to test different theories of aging. Specifically, Pearl's rate of living theory (1928) proposes that energy expenditure and longevity are negatively correlated (PEARL 1928). Under this theory, therefore, hypergravity increases weight and microgravity decreases weight, and metabolic rate should change in the same manner. Therefore longevity should decrease in hypergravity and increase in microgravity (ECONOMOS *et al.* 1982). This theory is partly supported by studies of metabolic rate in rats under conditions of microgravity and hypergravity. In these studies, the change in metabolic rate was constant as the gravity load was altered from 0 to 2×g (PLAUT *et al.* 2003).

In other studies, exposure to hypergravity at a young age has been shown to increase longevity in *Drosophila* males (LE BOURG *et al.* 2000). After being exposed to hypergravity for the first two weeks of their life, *Drosophila* initially showed less spontaneous motor activity, less climbing behavior, and altered movement pattern in comparison to a 1×g control (LE BOURG and MINOIS 1999). This longitudinal study also showed that age-related changes in behavior were substantially slower in the aforementioned behaviors from exposure to 5×g hypergravity conditions. In addition, there were three experimental conditions (1×g, 3×g, and 5×g) that showed no linear relationship between longevity and gravity, thereby failing to support Pearl's theory. Le Bourg hypothesizes that the stress of hypergravity causes the fly to forego costly behaviors, something not taken into account in Pearl's rate of living theory.

Recently, O'Donnell and colleagues have examined the effects of mutations in genes regulating catecholamine biosynthesis in response to centrifuge induced hypergravity stress to measure the effect of catecholamine production on stress resistance (WANG 2003). *Catecholamines-up* (*Catsup*) is a negative regulator of the catecholamine biosynthesis pathway, and *Catsup* mutants have increased catecholamine pools. *Punch* (*Pu*), which encodes GTP-cyclohydrolase, is a positive regulator of catecholamine production, and *Pu* mutants have decreased levels of catecholamines. As catecholamine production is affected by stress, it was hypothesized that *Catsup* mutants would show increased resistance to hypergravity stress resistance, and that *Pu* mutants would be sensitive to hypergravity. This was indeed found when viability and mobility of *Catsup* and *Pu* mutants were assessed under a range (3×g, 15×g, and 30×g) of hypergravity conditions. The survival and mobility of *Catsup* mutants under hypergravity were increased relative to the control, while that of *Pu* mutants decreased relative to the control in a dose-dependent fashion. This was the first work to implicate a molecular pathway in the response to hypergravity stress.

With these experiments taken into consideration, another important issue is whether we can infer the effects of microgravity from the effects of hypergravity on organisms. Because these experiments applied only short-term exposure to hypergravity conditions, they reveal little about genetic effects due to prolonged microgravity. Though it is thought that lifetime exposure to hypergravity will decrease longevity and increase the aging process in both sexes (LE BOURG *et al.* 1993) this has not yet been demonstrated. This problem will be best addressed using a model organism with excellent genetic and genomic resources that has a clear behavioral response to gravity. Geotaxis behavior in *Drosophila melanogaster* is one such model system.

## 1.1 *Drosophila* geotaxis behavior

*Drosophila* provides an excellent model for studying the genetic mechanisms underlying a behavioral response (negative geotaxis) to the gravitational field that can be readily observed and easily quantified. This allows for experiments that can be done on earth which still effectively investigate the influence of gravity on behavior. Most important, there is extensive genetic homology between *Drosophila* and other organisms, including humans, such that the underlying mechanisms discovered in *Drosophila* are likely to be shared across diverse taxa. Second, the ability to control genetic background and environmental variation allows us to quantitatively assess the effects of subtle variation. A plethora of genetic tools, a sequenced genome, rapid generation time, easy propagation, and general amenability to genetic study also make *Drosophila* an ideal genetic model system.

There are two classical approaches to understand the genetic basis of behavior. The first examines the effects of naturally occurring variation (the 'Hirschian' approach), while the second studies the effects of induced mutations (the 'Benzerian' approach) (SOKOLOWSKI 2001; TOMA *et al.* 2002). While lively debate between proponents of these two approaches initially polarized the field of behavioral genetics (TOMA *et al.* 2002), we now recognize that the two approaches are complementary in that they address subtly different questions. Mutagenesis studies define the genes required to produce the behavior, while studies of naturally occurring variation determine the subset of these genes that are segregating in nature.

Hirsch and colleagues were the first to show that there is substantial naturally segregating variation for *Drosophila* geotaxis. Hirsch and Erlenmeyer-Kimling (HIRSCH and ERLENMEYER-KIMLING 1961) developed a novel assay for geotaxis: a 15-unit vertically placed maze that allowed quantification of positive and negative geotaxis responses for a population of flies.

Divergent selection for positive and negative geotaxis was applied to a base population derived by mixing the Formosa stock from Berkeley, California, with newly trapped Captown and Syosset stocks from Cold Spring Harbor, New York (HIRSCH and ERLENMEYER-KIMLING 1961). These lines eventually experienced 600+ generations of intermittent selection. By 1985, the lines had stabilized upon relaxation of selection pressure (RICKER and HIRSCH 1985). Subsequent genetic analyses of these lines revealed that geotaxis is a typical quantitative trait, affected by multiple quantitative trait loci (QTLs).

At Generation 52, low and high reverse selection lines were established from the high and low forward selection lines, respectively. After 56 generations of reverse selection, the geotaxis phenotypes were similar to those of the forward selection lines; i.e. the geotaxis of the low reverse line was similar to that of the high forward line, and the behavior of the high reverse line was similar to that of the low forward line (HOSTETTER and HIRSCH 1967). Complementation tests between lines of like phenotype indicated that the genotypes of the forward and reverse selection lines displaying similar geotaxis were not the same, and that different genes were responsible for geotaxis in each line.

Hirsch and Erlenmeyer-Kimling (1962) performed a chromosome substitution experiment to map the QTLs affecting response to selection to the three major chromosomes. Flies from the lines selected for positive geotaxis, negative geotaxis, and their unselected founding line were crossed and then backcrossed to a multiple balancer stock. All eight possible combinations of homozygous wild type and heterozygous balancer chromosomes in females from each of the crosses were assessed for geotaxis behavior, enabling the mapping of factors that affect variation in geotaxis to each chromosome. Each major chromosome had a marked effect on geotaxis, and this effect changed due to selection. The *X* and second chromosome

affected positive geotaxis, and chromosome *3* had a major effect on negative geotaxis. Little interaction (epistasis) was observed between chromosomes.

A more elaborate chromosome substitution experiment that enabled the estimation of dominance and male and female effects was conducted at Generation 133 of selection (HIRSCH and KSANDER 1969). This experiment utilized the line selected for extreme negative geotaxis behavior (H), the unselected control (W), and a multiple balancer stock. Again, the eight possible combinations of homozygous wild type and heterozygous balancer chromosomes were produced from the crosses to H and W. Then, crosses between these strains were conducted to produce all possible combination of homozygous and heterozygous genotypes for each chromosome – 27 female genotypes and 18 male genotypes. The behavior of each genotype was assessed, and a factorial analysis of variance was used to estimate the main effects of each chromosome and all possible interactions between chromosomes. Effects in both sexes were attributable to all three chromosomes, and were strictly additive with no significant dominance or epistasis. However, the magnitudes of effects of the different chromosomes were different in males and females: the effect of the *X* chromosome on negative geotaxis in males was twice as large as the *X* effect in females.

Ricker and Hirsch (RICKER and HIRSCH 1988) repeated the chromosome substitution design of Hirsch and Erlenmeyer-Kimling (1962) after approximately 600 generations of intermittent selection. In this assay, significant interactions were observed between chromosomes in addition to main effects of all three chromosomes for both the high and low selection lines.

The effect of the *Y* chromosome on male geotaxis behavior was examined by substituting *Y* chromosomes from the selected geotaxis lines into high- and low-selected lines, Canton-S and

Champaign wild-type backgrounds (STOLTENBERG and HIRSCH 1997). Intriguingly, the *Y* chromosome had a small effect on geotaxis in the genetic background of the selected lines, but not in wild type backgrounds, indicating the evolution of *Y*-autosome epistasis in the selection lines.

These studies established that *Drosophila* harbors considerable segregating genetic variation for geotaxis behavior, and that multiple, additive QTLs contribute to variation in behavior. However, the goal of exploiting natural variation to understand the genetic architecture of geotaxis can only be met if we know the individual genes affecting variation in the trait. QTLs can be mapped to broad genomic regions by linkage to molecular markers (FALCONER AND MACKAY 1996), and in *Drosophila*, quantitative complementation tests to deficiencies followed by complementation to positional candidate genes is an effective strategy for identifying candidate genes corresponding to the QTLs (MACKAY 2001). This approach has been used successfully to map QTLs for olfactory behavior (FANARA *et al.* 2002), courtship (GLEASON *et al.* 2002), flight (MONTOOTH *et al.* 2003) and mating behavior (MOEHRING *et al.* 2004; MOEHRING and MACKAY 2004), but has not yet been applied to the high and low geotaxis lines. An attempt to associate variation in geotaxis behavior with a change in frequency of allozyme markers between the selected lines implicated a QTL affecting geotaxis within one centimorgan of *Alcohol dehydrogenase* (*Adh*, 2-50.1) (STOLTENBERG and HIRSCH 1996). However, with only two selection lines, it is not possible to distinguish drift from selection as the cause of differences in gene frequency.

Toma *et al*. (2002) described a novel strategy for identifying candidate genes affecting variation in geotaxis. RNA was extracted from heads of the Hirsch high and low selected geotaxis lines, and hybridized to cDNA microarrays containing approximately one-third of the

predicted genes in the *Drosophila* genome. Expression differences were found for roughly 5% of these genes. Since these differences could be attributable to random genetic drift or to selection, six genes that displayed no expression differences, and four that showed differential gene expression, were chosen for further functional tests. Quantitative PCR confirmed the microarray expression results. Further, mutations at three of the four candidate genes exhibiting differential gene expression also directly affected geotaxis. These genes were *cryptochrome* (*cry*), *Pendulin* (*Pen*) and *Pigment-dispersing factor* (*Pdf*), none of which were previously implicated in geotaxis. *Pen* encodes a nuclear importin that is expressed in the central nervous system, and *cry* and *Pdf* affect circadian rhythm. Thus, genes affecting behavioral phenotypes have pleiotropic effects.

## 1.2 Mutagenesis and the genetic architecture of behavior

In contrast to studies of natural variation where identification of genes responsible for phenotypic variation is difficult, mutagenesis approaches have the advantage that gene identification is straightforward. The first *Drosophila* behavioral mutants were identified in a screen for "clock genes" in which the normal 24 hour circadian locomotion rhythm was altered (KONOPKA and BENZER 1971). This screen uncovered three alleles of the same gene, *period*, that changed the normal peaks of locomotor activity to 19hrs, 29hrs, and one allele that lacked any rhythm whatsoever. At this point seven *Drosophila* genes are known to contribute to the circadian clock: *period* (*per*), *timeless* (*tim*), *Clock* (*Clk*), *cycle* (*cyc*), *vrille* (*vri*), *double-time* (*dbt*), and *shaggy* (*sgg*) (STANEWSKY 2003). Mutagenesis has also been extremely successful in identifying genes affecting learning and memory (BOYNTON and TULLY 1992), sleep (HALL 2000) and olfactory behavior (ANHOLT *et al.* 1996).

Most early mutagenesis screens did not control for genetic background. This is especially true of screens for effects of *P*-element insertional mutations that are generated by crossing a line containing an engineered *P*-element with a line containing a transposase source (LUKACSOVICH *et al.* 2001). Since there is natural variation for most behaviors, only mutations with large, qualitative effects could be identified in such screens. Further, the behavioral effects of genes in which null mutations are homozygous lethal cannot be ascertained.

Mackay and colleagues (ANHOLT *et al.* 1996; LYMAN *et al.* 1996) proposed that combining *P*-element mutagenesis in an isogenic background with quantitative analysis of subtle effects of adult viable, hypomorphic mutations would be an efficient strategy for identifying novel candidate genes affecting complex traits, including complex behaviors. Anholt *et al*. (1996) first applied this strategy to identify genes affecting olfactory behavior. Approximately 400 co-isogenic *P*-element inserts in the Samarkand background were screened for olfactory avoidance behavior using a rapid and reproducible 'dip-stick' assay. Fourteen *smell-impaired* (*smi*) mutations with sex-specific effects on olfactory behavior were discovered (ANHOLT *et al.* 1996). Sex-specific effects of QTLs affecting naturally occurring variation in olfactory behavior were also observed in a survey of *X* and third chromosome substitution lines derived from a natural population (MACKAY *et al.* 1996), and for QTLs affecting variation in olfactory behavior between two wild type strains, Oregon and 2b (FANARA *et al.* 2002). Similar sex-specific effects have been found in quantitative analysis of bristle number (MACKAY and FRY 1996). Thus genotype by sex interaction is a possible mechanism for maintaining quantitative genetic variation if these sex-specific effects confer fitness advantages.

Because the *smi* mutations were co-isogenic, it was possible to perform a quantitative genetic analysis of epistasis between them (FEDOROWICZ *et al.* 1998). Twelve of the *smi*

mutations were crossed in all possible combinations, yielding 66 trans-heterozygote lines. These lines were assessed for olfactory avoidance behavior, and the data analyzed as a half-diallel cross, which partitioned variation among lines into that attributable to general combining ability (GCA) and specific combining ability (SCA). GCA is a measure of the effect of each mutation averaged over the background of all others. SCA measures epistasis – the degree to which the effect of the trans-heterozygote genotype deviates from that predicted from the average of the GCA of the two parent lines. Remarkably, eight of the *smi* lines could be ordered in an epistatic network (FEDOROWICZ *et al.* 1998).

Detailed genetic and molecular analysis of two of the *smi* mutations, *smi60E* (KULKARNI *et al.* 2002) and *smi97B* (GANGULY *et al.* 2003) revealed that these are mutations in *dsc1* and *scribble*, respectively. The *dsc1* gene encodes an ion channel of unknown function that is homologous to the *paralytic* (*para*) sodium channel that mediates neuronal excitability. *Scribble* is a pleiotropic gene required for establishing polarity in epithelial cells during embryonic development.

Taken together, these studies show that genes with pleiotropic effects affect behaviors, that alleles affecting behavior typically have different effects in males and females, and that genes affecting behavior interact in epistatic networks. Still, a major challenge is to discover all genes that contribute to complex behaviors and place them in genetic networks. Anholt *et al*. (2003) proposed that a systems biology approach (ANHOLT *et al.* 2003; IDEKER *et al.* 2001), in which whole-genome transcriptional profiling is applied to collections of mutations affecting the same behavior in a common isogenic background, would be an efficient strategy to accomplish this goal. Anholt *et al*. (2003) applied this strategy to five of the co-isogenic *smi* mutations that formed an epistatic network, and their control. A total of 530 genes were co-regulated in

response to one or more *smi* mutations. In addition, 63 co-regulated genes had sex-specific effects on transcription. Quantitative complementation tests of mutations in genes with altered transcript levels to *smi* mutations and their control strain showed epistatic interactions that paralleled *trans*-regulation at the transcription level, thereby identifying new candidate genes affecting olfactory behavior.

### 1. 3 A screen for mutations affecting *Drosophila* geotaxis

In this study, we use quantitative analysis of effects of single co-isogenic *P*-element insertions to identify new candidate genes affecting geotaxis. The goal of the Berkeley *Drosophila* Gene Disruption Project (BELLEN *et al.* 2004; SPRADLING *et al.* 1999) is to generate a library of single *P*-element insertion strains that will eventually cover all *Drosophila* open reading frames. Currently, 40% of the genome has been tagged in this manner (BELLEN *et al.* 2004). As part of this effort, the Bellen group constructed approximately 2000 *P{GT})* (LUKACSOVICH *et al.* 2001) BG insert lines in isogenic derivatives of the standard wild type strain, Canton–S (http://flypush.imgen.bcm.tmc.edu/pscreen/, Bellen *et al*. 2004).

The *P{GT1}* element is a dual-tag gene-trap vector, designed to recover only mutant lines that are inactivated by the insertion (LUKACSOVICH *et al.* 2001). The presence of two markers, *mini-w* and *Gal-4*, indicate integration of the insert downstream of the promoter of a gene, often in the gene itself. *mini-w* has a promoter but lacks a polyadenylation signal, and will only be expressed if the *P*-element inserts in a gene, enabling *mini-w* to be spliced to a downstream exon of the host gene and polyadenylated at the 3'end. The promoterless *Gal-4* reporter is expressed as a fusion mRNA only when integrated downstream of the promoter of the host gene. Tagged genes are readily identified using inverse PCR, and the system offers all the advantages of the

binary *UAS-Gal4* system (BRAND and PERRIMON 1993) for studying gene expression (ADAMS and SEKELSKY 2002; KENNERDELL and CARTHEW 1998). Previous screens for quantitative effects of the *P{GT1}* inserts in the BG lines uncovered novel loci affecting sensory bristle number (NORGA *et al.* 2003) and resistance to starvation stress (HARBISON *et al.* 2004).

We developed a rapid and highly reproducible climbing assay as a measure of geotaxis in 475 independent *P(GT1)* BG insertion lines generated in one of two isogenic derivatives of $w^{1118}$*;* Canton-S (B and F backgrounds) (BELLEN *et al.* 2004). Because behavioral traits are highly sensitive to the circadian clock, assays were restricted to the same four-hour period each day. Behavioral traits are also exquisitely sensitive to small environmental perturbations. Therefore, all measurements were conducted in an environmental chamber at a constant temperature and humidity. Measurements of the appropriate co-isogenic control lines (B or F) were taken at the same time as the insert lines to control for random day-to-day environmental influences on behavior. The 79 lines that exceeded the 99.9% confidence intervals were re-tested, giving 59 inserts in 54 candidate genes affecting performance in the rapid climbing assay. In contrast to olfactory behavior, these genes largely had the same effects in males and females. Performance in the rapid climbing assay has a strong locomotor component; therefore, we assessed locomotor behavior for the 59 insert lines that were deviant from the control in the rapid climbing assay. Overall, there was a moderate and significant positive correlation between performance in the climbing and locomotor reactivity assays, indicating that geotaxis as judged by the rapid climbing assay and locomotion behavior are partially influenced by the same genes. According to this analysis, 39 insert lines specifically affect upward climbing and cannot be explained by locomotor deficiencies, while 20 affect both traits to some degree.

We also tested a subset of lines in a classic geotaxis maze. The geotaxis maze also takes locomotor deficiency into account as the assay runs overnight, as opposed to a ten second period. Of the ten lines assayed in the maze, six showed a similar phenotype to the climbing assay. This is a remarkable outcome considering the differences between the two assays and the complexity of this behavior. The candidate genes affecting climbing and/or locomotor behavior fall into a large number of molecular function and biological process gene ontology categories.

# 2. MATERIALS AND METHODS

## 2.1 *Drosophila* stocks

475 independent homozygous viable *P{GT1}* (LUKACSOVICH *et al*. 2001) insert lines were generated in one of two isogenic derivatives of $w^{1118}$*;* Canton-S (Canton-S B and Canton-S F), as part of the Berkeley Drosophila Gene Disruption Project (NORGA *et al*. 2003; BELLEN *et al*. 2004; http://flypush.imgen.bcm.tmc.edu/pscreen/). Geotactic behavior was assessed for all 475 *P{GT1}* lines as homozygotes and compared to contemporaneous measurements of the appropriate co-isogenic control: $w^{1118}$; Canton-S B or F.

Stocks used for quantitative complementation testing, with either a hypomorphic mutation at the gene of interest or a deletion uncovering the candidate gene, were obtained from the Bloomington *Drosophila* Stock Center (Bloomington, IN).

## 2.2 Climbing assay: Initial screen

We used a rapid climbing assay to quantify a component of geotactic behavior of the *P*-element insert lines and their co-isogenic controls. Individual flies were placed in a 25 mm × 150 mm vertical tube (Fisherbrand borosilicate culture tubes with plain end) with 24 marked gradations spaced at 5 mm intervals. Each fly was tapped to the bottom of the tube, and after 10 seconds the vertical distance it traveled was scored from 1 to 24. A value of 1 is the lowest position in the tube, indicating positive geotaxis; and a value of 24 is the highest position, indicating negative geotaxis. The assays were performed between 1:00 p.m. and 5:00 p.m. under a red light, in a climate controlled room set at 24°C, ~40% humidity. The red light eliminates

any confounding phototactic component to the behavior. This assay allowed us to efficiently screen a large collection of *P*-element lines for geotaxis behavior.

*P*-element insert lines were assayed in blocks of 10-13 lines. The behavior of 10 males and 10 females from each line was measured on two different days, for a total sample size of 40 individuals per line. The behavior of 15 males and 15 females from the appropriate parental line was also assessed daily.

### 2.3 Statistical analysis: Initial screen

Analysis of variance (ANOVA) was used to determine the magnitude of mutational variance for rapid climbing behavior for the *P*{*GT1*} insertions. The behavior of each individual from the *P*-insert lines was expressed as the deviation from the mean of the contemporaneous control, for males and females separately. Two-way ANOVAs were run according to the mixed model $Y = \mu + S + L + S\times L + D(L) + S\times D(L) + Er$, where $\mu$ is the overall mean, *S* and *L* denote the cross-classified effects of Sex (fixed) and Line (random), *D* indicates replicate Days, and *Er* denotes variance in behavior between flies of the same sex and genotype, measured on the same day. Reduced models were also run for each sex. SAS GLM and VARCOMP procedures were used to compute the ANOVAs, F-ratio tests of significance and variance components (SAS Institute 1988).

Confidence limits were computed as $\pm z_\alpha \sigma/\sqrt{n}$, where $z_\alpha$ is the critical value of the normal distribution corresponding to the type I significance threshold, $\alpha$; $\sigma$ is the standard error derived from the total variance; and *n* is the number of individuals per line: $n = 40$ for the analysis pooled over sexes and $n = 20$ for the single sex analyses. Critical values of $z_\alpha$ are 1.96, 2.576, and 3.291 for the 95, 99, and 99.9% confidence limits, respectively. The total variance ($\sigma^2$) in climbing

behavior was estimated from the sum of the $L$, $S\times L$, $D(L)$, $S\times D(L)$ and $Er$ variance components from the ANOVAs of climbing behavior pooled across sexes, and from the sum of the $L$, $D(L)$, and $Er$ variance components from the ANOVAs for each sex.

### 2.4 Climbing assay: Re-test

We retested a total of 79 homozygous insert lines that exceeded the 99.9% confidence limits, for data pooled over sexes and for each sex separately. The re-test assays were the same as the original test, with two replicates of 10 flies for each sex per line. The climbing behavior of 15 flies per sex for the co-isogenic control strains was assessed daily.

### 2.5 Statistical analysis: Re-test

We used a three-way mixed model factorial ANOVA to assess the significance of the difference in behavior between each re-tested *P*-insert line and the control. The ANOVA model was $Y = \mu + S + L + T + S\times L + S\times T + L\times T + S\times L\times T + D(T) + S\times D(T) + L\times D(T) + S\times L\times D(T) + Er$, where $\mu$ is the overall mean, $S$ and $L$ are cross-classified effects of sex and line (insert line vs. control), $T$ is test, $D$ is replicate day and $Er$ denotes variance in behavior between flies of the same sex and genotype, measured on the same day. $D(T)$, $S\times D(T)$, $L\times D(T)$, and $S\times L\times D(T)$ are random effects, the rest are fixed. Reduced models were run for each sex. Insert lines with significant ($P < 0.05$) $L$ and/or $S\times L$ terms are strong candidates for mutations affecting geotaxis, as inferred from climbing behavior. The SAS GLM procedure was used to compute the ANOVAs and F-ratio tests of significance (SAS Institute 1988).

## 2.6 Locomotion assays

Each of the 59 *P*-element insert lines that remained significant after the re-test was assayed for locomotor behavior. The day before the locomotor assay, single flies were placed in a standard culture vial containing ~ 3 ml culture medium and stored in a climate controlled room with a constant temperature of 24°C and ~40% humidity. To quantify locomotor reactivity following a mechanical disturbance, each fly was gently tapped to the bottom of the vial, which was then placed horizontally for observation. The reactivity score was the total time in seconds that the fly was active in the 30 seconds immediately following the disturbance. *P*-element insert lines were assayed in blocks of 4-6 lines. The behavior of 10 males and 10 females from each line was measured on two different days, for a total sample size of 40 individuals per line. The behavior of 15 males and 15 females from the appropriate parental line was also assessed daily. All assays were performed in the morning, between 8:00 a.m. and 11:00 a.m.

## 2.7 Statistical analysis of locomotion assays

We used a three-way mixed model factorial ANOVA to assess the significance of the difference in behavior between each *P*-insert line and the control. The ANOVA model was $Y = \mu + S + L + D + S\times L + S\times D + L\times D + S\times L\times D + Er$, where $\mu$ is the overall mean, *S* (fixed), *L* (fixed) and *D* (random) are cross-classified effects of sex, line (insert line vs. control) and day, respectively. The SAS GLM procedure was used to compute the ANOVAs and F-ratio tests of significance (SAS Institute 1988). In addition, we used the SAS CORR procedure to quantify the association between performance in the locomotion and climbing assays, with data from both assays expressed as deviations from the contemporaneous control means, separately for males and females.

## 2.8 Geotactic maze assays

To further characterize geotactic behavior, we tested a subset of lines in a classic geotactic maze (HIRSCH and ERLENMEYER-KIMLING 1961; TOMA *et al.* 2002). We constructed eight-choice mazes from two pieces of plexiglass that are screwed together; one piece is one inch thick with the maze routed out and the other is flat. Groups of 80-100 virgin flies of the same sex and genotype were placed in the mazes in the late afternoon (~5:00 p.m.) and allowed to migrate through the maze overnight. A vertical halogen light was positioned in front of the maze to attract the flies to the collection tubes through their positive phototactic behavior. Flies were collected in 9 empty tubes, numbered 1 through 9, at the end of the maze. Flies scoring 1 have made 8 positively geotactic decisions, and flies with a score of 9 made 8 negatively geotactic choices. All assays were performed in a climate controlled room set at 24°C, ~40% humidity. The maze assays were conducted in same-sex groups of three *P*-element lines and the appropriate control line, with five replicate assays per line.

## 2.9 Statistical analysis of geotactic maze assays

ANOVA was used to assess the differences between each *P*-element line and the control in performance in the maze assays, using the raw scores (1-9) of each fly that migrated through the maze. The model was $Y = \mu + S + L + L\times S + R(L\times S) + Er$, where $\mu$ is the overall mean, $S$ and $L$ are cross-classified fixed effects of sex and line, $R$ is replicate (random), and $Er$ is the variance within replicates. Reduced models were also run for each sex separately. The SAS GLM procedure was used to compute the ANOVAs and F-ratio tests of significance (SAS Institute 1988).

## 2.10 Deficiency and mutant complementation tests

Quantitative complementation tests were performed to assess whether the genes tagged by the *P*-element insertions affected climbing behavior. We chose 10 candidate genes for complementation testing based on extreme scores in the climbing assay and the availability of mutants from the public stock center. Multiple mutant alleles were tested when available. Mutant stocks were obtained from the Bloomington Stock Center.

Mutant stocks (*m*) stocks (*m*/Balancer) were crossed to both the *P*-element stocks (*P*) and the parental line (Canton-S B and F, +), and the *m*/*P* and +/*P* offspring from each cross, respectively, were assayed for rapid climbing behavior. Assays were set up similarly to the re-test analysis, with two sets of tests, each with two replications.

The statistical analysis was the same as for the re-test analysis. We used a three-way mixed model factorial ANOVA to assess the significance of the difference in behavior between *m/P* offspring and *+/P* offspring. The ANOVA model was $Y = \mu + S + L + T + S\times L + S\times T + L\times T + S\times L\times T + D(T) + S\times D(T) + L\times D(T) + S\times L\times D(T) + Er$, where $\mu$ is the overall mean, *S* and *L* are cross-classified effects of sex and line (*m/P* offspring and *+/P* offspring), *T* is test, *D* is replicate day and *Er* denotes variance in behavior between flies of the same sex and genotype, measured on the same day. $D(T)$, $S\times D(T)$, $L\times D(T)$, and $S\times L\times D(T)$ are random effects, the rest are fixed. Reduced models were run for each sex. Complementation tests with significant ($P < 0.05$) *L* term shows failure to complement. The SAS GLM procedure was used to compute the ANOVAs and F-ratio tests of significance (SAS Institute 1988).

# 3. RESULTS

## 3.1 Climbing assay: Initial screen

We screened 475 co-isogenic single *P*{GT1} insert lines for performance in a rapid climbing assay to quantify geotactic behavior. ANOVA of geotaxis scores shows highly significant variation among *P*-element insert lines ($P < 0.0001$), pooled across sexes (Table 1), and for males and females separately (Table 2). There was strong sexual dimorphism for climbing behavior ($P < 0.0001$), with a mean difference from parental line for males of –0.56 and a female difference from parental line of 0.44 (male control line mean 11.44, female control line mean 10.03). The line × sex interaction term from the ANOVA pooled across sexes was not significant ($P = 0.5686$, Table 1); therefore, genetic mechanisms governing this behavior are largely similar in males and females.

The magnitude of mutational variance for climbing behavior induced by *P*-element insertions can be quantified by the mutational heritability, $h_M^2$, computed as $\sigma_L^2/\sigma_E^2$, where $\sigma_L^2$ is the among line variance component and $\sigma_E^2$ is the variance within lines. Estimates of $h_M^2$ were 0.071, 0.068, and 0.073, respectively, from the analyses pooled across sexes and for males and females separately. These estimates are large, along the same order of magnitude as heritabilities of behavioral traits in natural populations (ROFF & MOUSSEAU 1987), suggesting that some *P*-element insertions had large effects on climbing behavior. The distribution of mutational effects of the *P*-element insertions on climbing behavior, expressed as deviations from the contemporaneous control line means and pooled over sexes (Figure 1) confirms this.

We calculated the cross-sex genetic correlation, $r_{GS}$, as $\sigma^2_L/(\sigma^2_{LM} \times \sigma^2_{LF})^{1/2}$ (ROBERTSON 1959), where $\sigma^2_L$ is the variance among lines from the analysis pooled across sexes, and $\sigma^2_{LM}$

and $\sigma^2_{LF}$, are, respectively, the among-line variance components from the analyses of males and females separately (Tables 1 & 2). The estimate is $r_{GS} = 1.005$, indicating, as inferred above, that the mutations have largely the same effects on climbing behavior in both sexes.

We determined 95%, 99%, and 99.9% confidence interval thresholds for the deviation of *P*-element insert line means from the control, pooled across sexes, and separately for males and females (Figure 1). Remarkably, the scores of 206 *P*-element insertion lines (43.4%) exceeded the 95% confidence intervals. Such a large number of loci affecting climbing behavior must have other molecular and biological functions, pointing to substantial pleiotropy for this complex behavior. For the analysis pooled over sexes, 74 insertion lines had reduced and 75 insertion lines had increased geotaxis scores relative to the control. In the separate sex analyses, 28 insertion lines had male-specific effects on geotaxis (13 with reduced scores and 15 with increased scores) and 29 lines were female specific (14 with reduced scores and 15 with increased scores). Although *P*-element insertional effects on geotactic behavior were highly correlated in males and females for the whole data set, these data suggest that some inserts have differential effects between the sexes.

### 3.2 Climbing assay: Re-test

We performed a second phenotypic assessment on the 79 insertion lines with geotaxis scores that exceeded the 99.9% confidence limit in the initial test for males and females individually, and sexes pooled. Table 3 shows the mutational effects of the insertions that were significant after both assays, with the cytological location of the *P*-element, the putative candidate gene, any gene by line interaction, and the deviation from the parental mean for males, females, and sexes pooled. A total of 59 of the 79 insertion lines retested remained significant at

$P \leq 0.05$ following the second test. The mutational effects are expressed as the deviation of the insert line score from the control, averaged over both tests. Positive effects thus indicate negative geotaxis, whereby the insert line on average exceeds the control score. The line exhibiting the most extreme negative geotaxis (7.4, $P < 0.0001$) was *BG02785*, with an insert near *dacapo*. This insert also showed a significant line×sex interaction ($P = 0.0102$) with females being even more negatively geotactic (10, $P < 0.0001$). The line exhibiting the most extreme positive geotaxis (−6.2, $P < 0.0001$) was *BG00968*, with an insert near *mushroom-body expressed* (*mub*).

### 3.3 Locomotion assays

We assessed locomotor reactivity in the 59 lines that were significantly deviant from the control in the pooled analysis of both climbing assays. The results are given in Table 4. ANOVA of locomotion scores in the 59 lines that were significantly deviant from the control in the pooled analysis of both climbing assays shows highly significant variation among *P*-element insert lines ($P < 0.0001$), pooled across sexes (Table 5), and for males and females separately (Table 6). Surprisingly, there was no significant sexual dimorphism for this trait, with a non-significant effect of sex ($P = 0.310$). As with geotaxis behavior, the line by sex interaction term from the ANOVA pooled across sexes was not significant ($P = 0.0524$, Table 5), showing that genetic mechanisms governing locomotion are largely similar in males and females.

The magnitude of mutational variance for locomotion behavior induced by *P*-element insertions can be quantified by the mutational heritability, $h_M^2$, computed as $\sigma_L^2/\sigma_E^2$, where $\sigma_L^2$ is the among line variance component and $\sigma_E^2$ is the variance within lines. Estimates of $h_M^2$ were 0.255, 0.210, and 0.402, respectively, from the analyses pooled across sexes and for males and

females separately. These estimates are exceptionally large, especially considering that mutational variance estimates of bristle number are $10^{-3}$ times the environmental variance (NORGA *et al.* 2003). This is likely due to the sample of lines assayed for locomotion, which is biased by using insertion lines shown to be extremely mutant for climbing behavior. Pleiotropy is often seen with genes affecting quantitative traits due to the large number of loci involved (KEIGHTLEY *et al.* 1993), making it reasonable to imagine that these lines are biased towards large mutational variance.

We calculated the cross-sex genetic correlation, $r_{GS}$, as $\sigma^2_L/(\sigma^2_{LM} \times \sigma^2_{LF})^{1/2}$ (ROBERTSON 1959), where $\sigma^2_L$ is the variance among lines from the analysis pooled across sexes, and $\sigma^2_{LM}$ and $\sigma^2_{LF}$, are, respectively, the among-line variance components from the analyses of males and females separately (Tables 1 & 2). The estimate is $r_{GS} = 0.883$.

In the analysis pooled over sexes, line *BG01564*, with an insert near *CG14430*, showed the highest score for locomotion, remaining active on average 3.8 seconds longer than the control ($P = 0.0023$). This line also exhibited a significant ($P = 0.046$) line×sex interaction: female activity is on average 5.7 seconds greater than the control, but male activity is not significantly different from the control. The most non-reactive line was *BG02501*, with an insert near *longitudinals lacking* (*lola*), with a mean activity 6.1 seconds less than the control ($P < 0.0001$). This effect was strongly sex-specific (the line×sex interaction term was highly significant, $P < 0.0001$), and confined to males, which were less reactive than the control by 10.0 seconds.

Of the 59 lines assayed for both locomotor reactivity and rapid climbing behavior, 39 were statistically significant for climbing behavior only and 20 were significant for both rapid climbing and locomotor reactivity behavior (Table 7). Figure 2 is a scatter plot of the climbing and locomotion assay scores, all expressed as deviations from their control means. The two

assays are significantly positively correlated ($P = 0.0189$). The estimate of Pearson's product moment correlation ($r$) is $r = 0.37$. The locations of *P*-element inserts in each of the 39 lines with deviant climbing behavior, but not locomotor reactivity behavior, are given in Figure 3 I-XXX.

### 3.4 Geotactic maze assays

Most previous work on *Drosophila* geotaxis has used geotactic mazes to quantify behavior. In the geotaxis maze assays, many individuals are introduced to the maze and then make several up-or-down decisions as they migrate across the maze. The final geotaxis score is based upon the net number of 'up' decisions. The maze and climbing assays are different in two major aspects. First, the maze assay runs overnight, while the climbing assay lasts ten seconds per fly. Thus, the maze assay integrates behavioral response over a long period, where the climbing assay measures acute response. In this respect the geotaxis maze limits the effect of locomotor activity. The climbing assay is done in the afternoon and the geotaxis maze assay runs overnight which allows for different circadian times between the two assays. Further, it may be possible for animals to backtrack in our maze design. Lastly, 100 *Drosophila* are put in the maze together, allowing flies to interact, whereas the climbing assay does not allow for interaction between individuals.

To examine possible differences between genes affecting the two assays, we chose ten *P*-element insertion lines to assess in the geotaxis maze. Two insertion lines, *BG02542* and *BG00467,* assayed in the maze were not shown not to be significant for climbing behavior after retest, insertion line *BG00467* showing a highly significant positive geotaxis score in the geotaxis maze (Table 8). The remaining eight lines were significant in the climbing assay, and were chosen based upon multiple criteria: extreme positive and negative geotaxis scores,

significant line × sex interaction, multiple lines of a single gene showing significance, and/or interesting candidate genes. Eight of the ten lines tested in the geotactic maze had scores that were significantly different from the co-isogenic parent (Table 8). Figure 4 I-XX shows the distribution of performance in the geotactic maze for the insertion lines and their co-isogenic parental lines.

Six lines behaved similarly in both assays: *BG00372* (*CG1678*), *BG01564* (*CG14430*), *BG01799* (*CG14998*), *BG00320* (*CHES-1-like*), *BG02542* (*neuralized*, *neur*), and *BG02391* (*neur*). *BG00372* was strongly negatively geotactic in both sexes, *BG01799* exhibited female-specific negative geotaxis, *BG01564* was moderately negatively geotactic, *BG00320* was positively geotactic, and *BG02542* was not significantly different from the control. *BG02391* had a male-specific effect on negative geotaxis in the climbing assay, whereas in the maze assay the negative geotactic effect was not sex-specific (Table 6).

Given the aforementioned differences between the two assays, finding six lines out of ten that perform similarly in the climbing assay and the geotaxis maze is remarkable. The geotaxis maze separates the locomotor component from the assay and validates the climbing assay as efficient in identifying geotaxis mutants. This is important as *BG00372, BG01564*, and *BG00320* were found to be significant in the locomotor reactivity assay (Table 4).

The behavior of the remaining four lines was not consistent between the two assays. *BG02501* and *BG02571*, located 8.2kb and 10.6kb upstream of *lola*, were both strongly positively geotactic in the climbing assay, and not sex-specific. These lines were strongly negatively geotactic in the maze assay, and the effects were sex-specific, with the effect of *BG02501* greater in males, and the effect of *BG02572* greater in females. Interestingly, these lines also had sex-specific effects on locomotor reactivity, but in opposite directions. *BG02501*

males, but not females, were highly hypoactive, while *BG02572* flies were moderately hyperactive in females. One of the control lines, *BG00467* (*CG8620*) was strongly positively geotactic in the maze assay, and *BG00968* (*mub*), the line exhibiting the greatest positive geotaxis in the climbing assay, was not significantly different from the control in the maze.

Upon analyzing the geotaxis maze data we noted that the distribution of flies in the geotaxis mazes was bimodal, with more animals in the top and bottom tubes, and not the binomial distribution expected if the animals were behaving independently. To assay independence we looked at the distribution of the parental co-isogenic lines assayed during the insertion line maze assays. Also, to study whether flies act independently in the maze when gravity is not a factor, co-isogenic B-line flies were assayed in horizontal mazes. For the vertical maze, we computed the expected distributions assuming independence using the binomial expectation based on the mean number of up decisions made. We based the expected binomial distribution for the horizontal maze assays on the assumption that left and right decisions are equally made. We used $\chi^2$ goodness-of-fit tests to assess the significance of the departure of the observed distributions from the expected distributions. Figure 5 shows the results of five replications of this experiment. For the horizontal maze, $\chi^2_8 = 1103.3$, $P < 0.0001$, and for the vertical maze $\chi^2_7 = 48323.0$, $P < 0.0001$. Without question the flies are not acting independently within the mazes and tend to accumulate towards the extremes of the distribution.

### 3.5 Candidate gene complementation tests

Quantitative complementation tests were done to further establish candidate genes as the causative agent of aberrant behavior. Results of all complementation tests are given in Table 9. Six of the 20 known mutant alleles tested, representing 16 candidate genes, failed to complement

for sexes pooled: *dacapo, neuralized, CG1678, mub,* and *erectwing*. *Malic enzyme (Men)* exhibited sex-specific failure to complement. Figure 6 shows the results of the six genes that failed to complement. While failure to complement shows that the known mutant and *P*-element are the same locus, complementation between two hypomorphic mutations does not definitively show that the known mutant and *P*-element are not at the same loci. Interallelic complementation has been known to occur, where in the known mutant and *P*-element the gene's function is disrupted in a different fashion and together show a normal phenotype.

# 4. DISCUSSION

## 4.1 *P*-element screen

I have screened a collection of 475 homozygous *P*-element insertion lines in a rapid climbing assay, to identify mutants for geotaxis. Extreme lines were re-tested to eliminate false positives from the initial screen. The 59 lines that remained significantly different from the parental line upon re-test were then assayed for locomotor reactivity behavior, showing that 39 insertion lines had specific effects on climbing behavior. Therefore, these lines are precisely defined as defective in geotaxis, and the aberrant geotaxis phenotypes are not a side effect of a defect in locomotion. Quantitative complementation tests with deficiency stocks and mutant alleles further supported six candidate genes (*dacapo, neuralized, CG1678, mushroom-body expressed, Malic enzyme, erect wing*) that contribute to geotaxis. Lastly, geotaxis maze assays on a selection of insert lines showed similarity between the two assays which is surprising considering the differences between the two assays.

The 39 insertion lines with significant mutant effects for the rapid climbing component of geotaxis, but not locomotor reactivity, each have a single *P*-element inserted in or directly upstream of a candidate gene (Figure 3). Fourteen inserts are in the transcribed region of the candidate gene, twenty-one inserts are upstream of the candidate gene, and four insert locations were not identifiable by BLAST analysis of sequences downstream of the insertion.

The 20 lines significantly different from the parental line for both geotaxis and locomotion identify candidate genes with pleiotropic behavioral effects. We will not discuss these more general behavior candidate genes in detail, as we are interested in genes with specific effects on geotaxis.

Candidate genes affecting negative geotaxis serve a wide variety of roles in *Drosophila* development and normal adult function. A large number perform unknown molecular and biological functions; these data are the first to assign a mutant phenotype disruption of several genes of unknown function. The most prevalent molecular functions of candidate genes affecting negative geotaxis were transcription regulation, nucleic acid binding, and catalytic activity. The most common biological functions of the candidate genes were development and metabolism, followed by cell growth and/or maintenance, and response to external stimuli. Many of the candidate genes affecting geotaxis (as assessed by the climbing assay) are required for development of the sensory nervous system, suggesting pleiotropic effects of these loci on proprioception and response to gravity.

### 4.2 Candidate genes involved in development

Several insertions putatively affect genes involved in neurogenesis. Of these, a subset affects the development of mechanosensory bristles in the adult fly: *neuralized, HLHm7, SP71, tout-velu, capricious,* and *escargot*. *P*-element insertion line *BG02391* (*neur*), Figure 3 I, showed a male-specific increase in negative geotaxis (Table 3), and shows an increase in bristle number (NORGA *et al.* 2003). *neuralized* encodes a protein involved in mesoderm development that affects sensory organ precursor (SOP) cell formation (YEH *et al.* 2000), finding *neuralized* to be required for determining epidermal cell fates within the proneural cluster. *P*-element insertion line *BG02029* (*HLHm7*) showed significant decrease in negative geotaxis and decreased bristle number (NORGA *et al.* 2003). *E*(*spl*) *region transcript m7* (*HLHm7*), Figure 3 II, is a bristle suppressor (LIGOXYGAKIS *et al.* 1999) in addition to being required during early neurogenesis to give neuroectodermal cells access to the epidermal pathway of development (SCHRONS *et al.*

1992). *HLHm7* is involved in lateral inhibition, suppressing the neural fate pathway of ectodermal cells surrounding sensory organ precursor cells (GIAGTZOGLOU *et al.* 2003). Individual genes in the *E*(*spl*) cluster are functionally redundant, and mutations in individual genes in this cluster were not thought to have phenotypic effects (SCHRONS *et al.* 1992).

*BG01109,* Figure 3 III, contains an insertion near *tout-velu* (*ttv*), which means 'all hair', referring to its *Drosophila* segment polarity mutant phenotype. This line is associated with decreased geotaxis only in females, although the line by sex interaction term is not significant. In addition, *BG01109* shows a gain of bristles (NORGA *et al.* 2003). *tout-velu* is involved in heparan sulfate proteoglycan biosynthesis (TOYODA *et al.* 2000). It is required for Hedgehog diffusion, is a member of the EXT gene family, and likely affects bone morphogenesis (BELLAICHE *et al.* 1998).

Line *BG02415*, with a *P*-element insertion in *capricious* (*caps*) Figure 3 IV, shows a male-specific decrease in negative geotaxis, with a significant line by sex interaction term ($P = 0.0238$). Interestingly, *BG02415* has a gain of bristle number (NORGA *et al.* 2003). *capricious* acts as a recognition molecule in motor axon guidance in muscle development (TANIGUCHI *et al.* 2000) and is involved in correct patterning of motoneurons in the neuromuscular system of larvae (LANDGRAF *et al.* 1997; SCHMID *et al.* 1999). *Capricious* is a member of the cell-adhesion molecules (CAMs) family and interacts with *Kruppel* in correct axon pathfinding and muscle-specific synapsing of the SNb (one of five exit junctions from the segmental nerve root) derived RP5 motoneuron (ABRELL and JACKLE 2001).

Line *BG02297* contains an insertion in *escargot* (*esg*)(Figure 3 V) and shows a slight increase in negative geotaxis, and an extreme loss of bristles (NORGA *et al.* 2003). In earlier work *esg* was shown to affect the development of *Drosophila* sensory organs; lowered

expression results in loss of macrochaetae (ABDELILAH-SEYFRIED *et al.* 2000). *esg* plays an essential role in embryonic CNS development (ASHRAF *et al.* 1999) and is an important regulator of cell motility in tracheal morphogenesis (LLIMARGAS 2000).

It is no surprise that several candidate genes affecting geotaxis are required for development. What is rather unintuitive is that many insertions upstream of key development genes, where disruption likely results in improper development, actually results in flies that are more negatively geotaxic. For example, homozygous *P*-element insertion line *BG01596*, with an insertion near *erect wing* (*ewg*)(Figure 3 VI), shows increased negative geotaxis. *erect wing* encodes an RNA polymerase II transcription factor involved in muscle development (DE LA POMPA *et al.* 1989; DESIMONE *et al.* 1996). In addition, *erect wing* is necessary for neuronal development and is expressed throughout the developing nervous system (DESIMONE and WHITE 1993).

Line *BG02244*, with an insertion in *crooked legs* (*crol*)(Figure 3 VII), also shows an increase in negative geotaxis. *crooked legs* encodes an RNA polymerase II transcription factor that is ecdysone regulated and important in early development (D'AVINO and THUMMEL 1998). An insert in *1.28* (*BG02537*)(Figure 3 VIII) is associated with a slight increase in negative geotaxis. *1.28* encodes a protein product involved in specification of segmental identity and is active in the maxillary segment (PEDERSON *et al.* 2000). Although previous studies have not noted sex-specific expression, the effect of the *P*-element insertion on climbing behavior is much greater in the males.

The *P*-element insertion in line *BG00361* is upstream of *SP71* and is associated with decreased negative geotaxis. *SP71* encodes a protein with a hairpin loop containing domain of

hepatocyte growth factor (www.Flybase.com). This line was also associated with reduced numbers of bristles (NORGA *et al.* 2003).

A number of insertions that are associated with aberrant geotaxis (as inferred from the climbing assay) correspond to candidate genes with roles in the Ras pathway. Two lines, *BG02602* (*Rab27*)(Figure 3 IX) and *BG01367* (*Rab23*)(Figure 3 X), contain insertions upstream of genes in the Rab GTPase family, the largest family within the Ras superfamily, with a role in vesicle trafficking (STENMARK and OLKKONEN 2001). The effects of *P*-element insertions associated with *Rab27* and *Rab23* are different, with extreme increases and decreases in negative geotaxis scores, respectively. *Rab23* is orthologous to the mouse *open brain* (*opb*) gene, and plays an important role in the Hedgehog pathway of neural patterning (EGGENSCHWILER *et al.* 2001). Mutations in the human *Rab27A* gene cause Griscelli syndrome, a rare, autosomal recessive disorder (MENASCHE *et al.* 2000). Symptoms of Griscelli syndrome include dilution of pigmentation in the skin and hair, large clumps of pigment in hair shafts, variable immunodeficiency, and accumulation of melanosomes in melanocytes. *Rab27* regulates exocytosis of cell-specific store organelles (IZUMI *et al.* 2003). Line *BG02312* (Figure 3 XI) is associated with an increase in negative geotaxis, pooled over sexes. The *P*-element insertion in *BG02312* is upstream of *sprint* (*spri*). *sprint* is the *Drosophila* homologue of the human gene *RIN1*, a potential Ras effector protein *sprint* is expressed in a subset of differentiating neurons in the CNS (SZABO *et al.* 2001).

Developmental genes implicated in the rapid climbing component of geotaxis behavior likely exert the majority of their expression during development, but they are crucial in creating the organs, nervous system, and morphology necessary for geotaxis (SOKOLOWSKI 2001).

### 4.3 Candidate genes involved in metabolic function

Many candidate genes affecting climbing behavior are active in normal metabolic function. The insertion in line *BG01628* near *Malic enzyme* (*Men*)(Figure 3 XII), is associated with increased negative geotaxis. *Men* encodes a NADP-dependent protein product involved in the tricarboxylic acid cycle (VOELKER *et al.* 1981).

Line *BG0312* contains an insertion in *6-phosphofructo-2-kinase* (*Pfrx*)(Figure 3 XIII) that is associated with a decrease in negative geotaxis. *Pfrx* encodes a protein involved in fructose metabolism (LUKACSOVICH *et al.* 2001).

Two lines, *BG01327* and *BG01756*, contain *P*-element insertions upstream of *Protein kinase61C* (*Pk61C*) (Figure 3 XIV), both of which are associated with an increase in negative geotaxis. *BG01327* shows a gain of bristles while *BG01756* has a loss of bristles (NORGA *et al.* 2003). The insertion in *BG01756* is 500bp upstream of *Pk61C*; the insertion in *BG01327* is in the first intron of *Pk61C*.

### 4.4 Candidate genes involved in protein folding

A number of candidate genes identified in this screen encode chaperone proteins and genes involved in protein folding. What is more interesting is recent work associating defects in protein folding with neurogenic disease. Aggregation of misfolded proteins has been implicated in a number of neurogenerative diseases, such as Huntington's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis (ALS)(ROSS and POIRIER 2004). These protein aggregates are not thought to be causative, but the end result of the disease mechanism, which at some point likely

involves misfolding of proteins. Therefore, it is not hard to reconcile disruption of genes involved in protein folding affecting a complex behavior such as negative geotaxis.

Line *BG02646* is associated with an increase in negative geotaxis, has an insertion near *Calreticulin* (*Crc*), and has an extreme loss of bristle number (NORGA *et al.* 2003). *Calreticulin* encodes a calcium ion binding protein resident of the endoplasmic reticulum (SMITH 1992). As such, it plays a role as a molecular chaperone and is important for correct protein folding (DANILCZYK *et al.* 2000). In addition, it is also thought to be involved in peripheral nervous system development, as mutations affect the embryonic neuron and the embryonic peripheral nervous system.

Line *BG02644* contains a *P*-element insert near *Fkbp13* (Figure 3 XV)and is associated with increased negative geotaxis. *Fkbp13* is an FK506-binding protein that has been implicated in endoplasmic reticulum protein folding in a murine model (BUSH *et al.* 1994).

The insertion in *BG01741* is near *Protein disulfide isomerase* (*Pdi*) Figure 3 XVI, and is associated with an increase in negative geotaxis. *Pdi* encodes a protein product involved in protein folding and is found in the endoplasmic reticulum lumen (MANN *et al.* 1999; MCKAY *et al.* 1995). *Pdi* has a role in protein folding by assisting in the formation of disulphide bonds (CLISSOLD and BICKNELL 2003).

*P*-element insertions upstream or in multiple heat shock proteins were associated with increased negative geotaxis scores. Two independent insertions in lines *BG00737* and *BG01813* correspond to *Heat shock protein 27* (*Hsp27*), and the insertion in *BG02348* is near *Heat shock protein 23* (*Hsp23*) (Figure 3 XVII) results in an extreme increase in negative geotaxis.

### 4.5 Candidate genes involved in various other biological functions

Line *BG00489* contains an insertion in *Osiris9* Figure 3 XVIII and is associated with a slight decrease in negative geotaxis. *Osiris9* has a function that is insect or Arthropod specific and has endoplasmic reticulum signal peptides. The insertion in *BG00968* is in *mushroom-body expressed* (*mub*) (Figure 3 XIX) and is associated with an extreme decrease in negative geotaxis. *mub* encodes a product putatively involved in histone mRNA 3'-end processing (www.Flybase.com). It is expressed in the mushroom bodies and is thought to be important to learning and memory (GRAMS and KORGE 1998). Line *BG02084* has a *P*-element insertion in the first intron of *Vha16* (Figure 3 XX) and is associated with an extremely significant increase in negative geotaxis. *Vha16* is a major component of gap junctions and is highly conserved across taxa (FINBOW *et al.* 1994). Line *BG01625* is associated with a slight decrease in negative geotaxis, and contains an insert in the first intron of *jim* (Figure 3 XXI). *jim* is a zinc finger protein that is expressed during oogenesis in the follicular epithelium (DOERFLINGER *et al.* 1999)

Several other lines associated with significant effects on climbing behavior are first mutations in predicted genes. Lines *BG01006*, *BG01296*, and *BG02864* all contain inserts upstream of *CG4564* (Figure 3 XXII) and all are associated with sex-specific effects on climbing behavior. The insertions in *BG1006* and *BG01296* are in exactly the same place, 291bp upstream of *CG4564*, and both exhibit increased negative geotaxis in females. The insertion in *BG02864* is 5402bp upstream of *CG4564* and shows decreased negative geotaxis in males. These results are remarkably consistent and point to a sex-specific role in geotaxis for *CG4564*. Performing a BLAST analysis on the sequence data of *CG4564* sheds little light on the molecular properties or biological function of *CG4564*.

Two other lines, *BG02295* and *BG01799*, contain insertions upstream of previously undescribed candidate genes, *CG15321* and *CG14998,* respectively. Both lines are associated

with sex-specific effects on climbing behavior. Line *BG02295* shows an increase in negative geotaxis for males and has a significant line by sex interaction term. Line *BG01799* shows an increase in negative geotaxis for females and also has a significant line by sex interaction term.

Taken together, these results highlight the concept that subtle mutations in pleiotropic genes required for normal development can have profound effects on adult behavior (ANHOLT0. *et al.* 2003; SOKOLOWSKI 2001). We note that the microarray expression analysis of lines selected for increased and decreased geotactic behavior (TOMA *et al.* 2002) identified *cryptochrome*, *pigment-dispersing factor* and *Pendulin* as positively affecting geotaxis. We were unable to test these genes in our assays, since our *P*-element screen did not include insertions in these candidate genes.

### 4.6 Potential link between bristles, mechanosensation, and geotaxis behavior

In addition to the eyes and antennae, *Drosophila* senses its environment using mechanosensory bristles. Defects in mechanosensory behavior identify gene products that may be involved in mechanotransduction (KERNAN *et al.* 1994). Because geotaxis relates to how the fly responds to gravity, it is not surprising that many of the mutations in candidate genes implicated in climbing behavior are associated with loss or gain of sensory bristles (NORGA *et al.* 2003). Norga *et al.* (2003) assessed variation in abdominal and sternopleural bristle number among 1731 *P{GT1} P*-element insert lines. (The 475 lines of this study were a subset of these lines.) Insertions in *Vha16*, *capricious*, *tout-velu*, *Protein kinas 61C*, *neuralized*, and *HLHm7* were all associated with increases in bristle number; while insertions in *SP71*, *escargot* and *crooked legs* were associated with reductions in bristle number. The same lines were deviant in the climbing behavior assay in this screen.

*Escargot* also affects loss of macrochaetae in a gain-of-function screen (ABDELILAH-SEYFRIED *et al.* 2000). When *esg* expression was driven using a *sca-Gal4* driver line crossed to the *P*-element line, disruption was localized to the sensory organ precursor (SOP) cells during development.

Interestingly, *SP71* encodes a gene product that is very similar to *nompA*, which is a PNS-specific protein which is required for connection of mechanosensory dendrites to sensory bristles (CHUNG *et al.* 2001). The external bristle shaft and socket appear normal in *nompA* mutant bristles.

Finding a link between geotaxis and bristle number was a very positive result given the potential relationship between how *Drosophila* senses its environment and behaves in relation to its environment. Bristles have long been known to be important mechanosensory organs and finding a number of candidate genes in this screen that affect bristle number points to a mechanism for proprioception in particular.

### 4.7 Quantitative complementation tests

It is necessary to prove causation when identifying candidate genes in a *P*-element screen. There is potential for the *P*-element to move around the genome, with the potential to excise imprecisely and disrupt gene expression elsewhere in the genome. Demonstrating that *P*-element lines fail to complement mutations at candidate genes is a rapid method for identifying putative quantitative trait genes. However, because of the hypomorphic nature of most mutations, *P*-elements included, complementation does not eliminate a putative candidate gene from further consideration. We performed complementation tests to 20 mutations and deficiencies, representing 16 candidate genes. Candidate genes tested were based upon

availability of known mutants or deficiency stocks from the Bloomington Stock Center (Bloomington, IN). Of these, five mutations failed to complement the climbing behavior phenotype of the *P*-element insertional mutations in the analyses pooled across sexes: *dacapo*, *neuralized*, *CG1678*, *mub*, and *erect wing*. *Malic enzyme* (*Men*) exhibited female-specific failure to complement. This supports a causal relationship between the *P*-element insertions in these candidate genes and deviant climbing behavior, but direct proof that the *P*-elements cause the mutations will require correlation of precise *P*-element excisions with reversions of the behavioral phenotypes to wild type, and ultimately rescue of the mutant behavioral effects with a wild type transgene. Complementation does not rule out the other ten candidate genes, as intra-allelic complementation is not uncommon with hypomorphic mutations (e.g., Ganguly *et al*. 2003).

### 4.8 Maze assay

While our climbing assay is an efficient and precise assay for quantifying one component of geotaxis, we chose 10 insertion lines to assay in the classic geotaxis maze to look for similarities between the two assays (HIRSCH and ERLENMEYER-KIMLING 1961; TOMA *et al.* 2002). It was necessary to use the climbing assay in the mutant screen because the maze-assay is very time and labor intensive.

Comparison of both the climbing assay and the maze assay is given in Table 8. *BG02542 neur* was assayed in the maze as a control to make sure that lines that were not significant in the climbing assay were also not significant in the maze assay. It was very reassuring that this was the case. *BG02391 neur*, which showed a male specific and sexes pooled increase in the climbing assay also showed a significant increase in the maze assay. *BG01799*, candidate gene

*CG14998*, showed a female specific increase in geotaxis behavior in both assays. This result is remarkable considering the differences between the two assays and the subtlety of sex specific effects. *BG00372, CG1678,* and *BG01564, CG14430*, both show a significant increase in geotaxis for both assays and *BG00320, CHES-1-like,* shows a decrease in geotaxis in the climbing and maze assay.

The bimodal nature of the maze data began made us think that the flies were not acting independently in the maze. If they were behaving independently in the maze, we would expect a binomial distribution about the mean collection tube. We showed that the geotaxis behavior of individuals in both horizontal and vertically placed mazes was not independent. It should be noted that the climbing assay does not allow for interaction between individual flies. Thus, it is perhaps surprising that the congruence between the two assays was so strong.

# 5. CONCLUSION AND FUTURE DIRECTIONS

We have identified a number of candidate genes that affect climbing behavior, but not locomotor reactivity. The results of complementation tests to available mutations strongly supports six of these genes: *dacapo*, *neuralized*, *CG1678*, *mub*, *erect wing* and *Malic enzyme*. Many of the candidate genes we identified as influencing geotaxis are important for normal development, for neurogenesis, and normal bristle development. Perhaps most interesting is our identification of previously undescribed genes as candidate genes affecting geotaxis. These data add considerably to our understanding of the genetic architecture of this complex behavior, since previously only three genes have been implicated as affecting geotaxis (TOMA *et al.* 2002).

There are many routes of investigation to build upon what we have learned to better our understanding of the genetic architecture of geotaxis. This screen covered less than 5% of the total number of genes in the *Drosophila* genome. This only gives us a window to the complex nature of the genetic basis of geotactic behavior. Expanding the screen to include a larger proportion of the genome would give a more complete picture of the genetic networks affecting negative geotaxis. Once genes that influence geotaxis are identified, we can investigate what genes influence variation of geotaxis in natural populations. Studying the allelic effects of these genes will give us insight into how they interact, i.e. dominance and epistasis, and define the genetic architecture of geotaxis.

Another important question regarding geotaxis is how and why variation in natural populations is maintained, which is an important evolutionary question. Mapping QTLs affecting naturally occurring variation in geotactic behavior would define gene regions contributing to variation in nature. As these regions are sometimes quite large, deficiency

analysis of these regions would narrow the responsible area, and quantitative complementation assays with known mutants would show actual genes that maintain phenotypic variation in nature. Single nucleotide polymorphism (SNP) analysis could then correlate variation to nucleotide changes. This would go very far to increase our understanding of how genetic variation for a complex behavior is maintained in nature.

Another line of inquiry would be to investigate the physiological mechanism of geotaxis. The nature of the gene-trap system our *P*-element utilizes gives us the opportunity to study the expression patterns of the candidate genes we uncovered and to look at the effects of overexpression in some cell types. Also, imprecise excision of the *P*-element would allow us to create hypomorphic and null mutations to characterize. Because many genes have complex expression patterns with multiple transcripts (GOEKE *et al.* 2003), this gives us the opportunity to find in the adult what physiology is important for normal geotaxis.

Finding a model to study the effect of gravity on genetics on earth is imperative if we hope to make a meaningful contribution to the space program. This will provide us with a starting place for genetic study before we begin prolonged space flight, as opposed to the current approach of spending most of our resources on preliminary genetic studies while in space. With present opportunities to conduct research in space limited by time, financial considerations, and the decidedly sparse nature of spaceflight, study of geotaxis in *Drosophila* bypasses an imposing barrier to study genetics in relation to gravity. Furthermore, earthbound study allows us to make use of the *Drosophila* research community and the entire genetic tool chest available with the *Drosophila* model system. This earthbound approach to studying gravity's effect on genetic circuitry will prove productive as we begin to plot a course toward extended space travel.

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# TABLE 1

**Analysis of variance of initial climbing assay, sexes pooled.**

| Source | d. f. | MS | F | $\sigma^2$ | *P* |
|---|---|---|---|---|---|
| Line | 474 | 236.64 | 2.03 | 3.122 | <0.0001 |
| Sex | 1 | 4324.14 | 53.12 | - | <0.0001 |
| Line x Sex | 473 | 82.06 | 0.98 | 0 | 0.5686 |
| Day (Line) | 470 | 116.64 | 1.41 | 1.527 | 0.0001 |
| Sex × Day (Line) | 457 | 82.85 | 1.89 | 3.801 | <0.0001 |
| Error | 16946 | 43.89 | | 44.082 | |

**TABLE 2**

**Analysis of variance of initial climbing assay, sexes separately.**

| Source | d. f. | MS | F | $\sigma^2$ | *P* |
|---|---|---|---|---|---|
| **Males** | | | | | |
| Line | 474 | 168.18 | 3.70 | 3.126 | <0.0001 |
| Day (Line) | 467 | 108.49 | 2.39 | 5.851 | <0.0001 |
| Error | 8594 | 45.48 | | 45.704 | |
| **Females** | | | | | |
| Line | 473 | 151.96 | 3.60 | 3.087 | <0.0001 |
| Day (Line) | 460 | 91.37 | 2.16 | 4.782 | <0.0001 |
| Error | 8352 | 42.25 | | 42.424 | |

**TABLE 3**

***P*-element insert lines with significant effects on climbing behavior. Data are pooled from the initial test and the re-test.**

| Insert Line | Nearest Gene | Cyt. Location | *P*(*L*) | *P*(*L*×*S*) | Effect (Sexes Pooled) | Effect (Males) | Effect (Females) |
|---|---|---|---|---|---|---|---|
| *BG00968* | *mub* | 79A7-B1 | <0.0001 | 0.8686 | –6.200*** | –6.375*** | –6.025*** |
| *BG02501* | *lola* | 47A8-11 | <0.0001 | 0.0875 | –5.833*** | –7.725*** | –3.942* |
| *BG02572* | *lola* | 47A8-11 | <0.0001 | 0.8380 | –5.367*** | –5.575*** | –5.158*** |
| *BG02068* | *Unknown* | | <0.0001 | 0.0275 | –4.579*** | –7.092*** | –2.067 |
| *BG01101* | *CG32191* | 75B6 | <0.0001 | 0.0398 | –4.338*** | –6.575*** | –2.100 |
| *BG00320* | *CHES-1-like* | 7B6 | 0.0002 | 0.4157 | –4.158*** | –5.050** | –3.267* |
| *BG01367* | *Rab23* | 83B9 | 0.0038 | 0.8561 | –4.077*** | –4.921** | –3.328* |
| *BG01129* | *Adhr, Adh* | 35B3 | 0.0005 | 0.3711 | –3.877*** | –2.924 | –4.842** |
| *BG02356* | *Dally* | 66E1-3 | 0.0014 | 0.0006 | –3.833*** | –7.233*** | –0.157 |
| *BG00361* | *SP71* | 1A5-6 | 0.0009 | 0.7812 | –3.833*** | –4.150* | –3.517* |
| *BG02029* | *HLHm7* | 96F10 | 0.0003 | 0.9293 | –3.816*** | –3.960* | –3.732** |
| *BG02062* | *CG15312* | 9B1 | 0.0005 | 0.4778 | –3.717*** | –4.466** | –2.971 |
| *BG00312* | *Pfrx* | 18C8 | 0.0039 | 0.1442 | –3.289** | –1.624 | –4.953** |
| *BG02217* | *Px* | 58F2-59A1 | 0.0035 | 0.5369 | –3.204** | –2.533 | –3.875** |
| *BG00299* | *CG13697* | 75B6 | 0.0096 | 0.5027 | –3.135** | –3.921* | –2.349 |
| *BG00489* | *Osiris9* | 83E2 | 0.0289 | 0.8498 | –2.517* | –2.300 | –2.733 |
| *BG01636* | *CG12750* | 36E3 | 0.0288 | 0.04 | –2.461* | –4.777** | –0.150 |
| *BG01109* | *tout-velu* | 51B9-11 | 0.0963 | 0.0878 | –1.852 | –0.050 | –3.751* |
| *BG02415* | *capricious* | 70A4 | 0.1684 | 0.0238 | –1.633 | –4.325* | 1.058 |
| *BG02864* | *CG4564* | 6E2 | 0.1980 | 0.0321 | –1.330 | –3.555* | 0.887 |
| *BG01635* | *Willdieslowly, Ubx* | | 0.3589 | 0.258 | –0.913 | 1.542 | –3.367* |
| *BG02295* | *CG15321* | 8F9 | 0.5700 | 0.0029 | 0.487 | 3.699* | –2.650 |
| *BG01006* | *CG4564* | 6E2 | 0.6075 | 0.0025 | 0.558 | –2.775 | 3.892* |
| *BG01799* | *CG14998* | 64A7 | 0.0619 | 0.0071 | 2.058 | –0.925 | 5.042*** |
| *BG01813* | *Hsp27* | 67B1 | 0.0580 | 0.0085 | 2.092 | 5.040** | –0.842 |
| *BG01296* | *CG4564* | 6E2 | 0.0643 | 0.0043 | 2.290* | –0.970 | 5.392*** |
| *BG02291* | *CG14059* | 73E4 | 0.0352 | 0.2256 | 2.297* | 3.560* | 1.033 |
| *BG01756* | *Pk61C* | 61B1 | 0.0396 | 0.8041 | 2.433* | 2.142 | 2.725 |
| *BG02537* | *1.28* | 42B2 | 0.0301 | 0.1346 | 2.558* | 4.180* | 0.713 |
| *BG02297* | *escargot* | 35D2 | 0.0197 | 0.0780 | 2.637* | 4.603** | 0.672 |
| *BG00683* | *CG5127* | 96E2 | 0.0063 | 0.0565 | 3.033** | 0.933 | 5.167** |
| *BG00846* | *Invected* | 48A1 | 0.0031 | 0.5444 | 3.123** | 2.553 | 3.733* |
| *BG00737* | *Hsp27* | 67B1 | 0.0253 | 0.9658 | 3.148* | 2.617 | 3.692* |

| Insert Line | Nearest Gene | Cyt. Location | $P(L)$ | $P(L\times S)$ | Effect (Sexes Pooled) | Effect (Males) | Effect (Females) |
|---|---|---|---|---|---|---|---|
| *BG02391* | *neuralized* | 85C5 | 0.0021 | 0.0002 | 3.292** | 7.358*** | –0.775 |
| *BG01243* | *CG12537* | 88A4 | 0.0026 | 0.3711 | 3.325** | 4.300** | 2.35 |
| *BG02676* | *CG10990* | 12B6-8 | 0.0037 | 0.0804 | 3.384** | 1.367 | 5.433*** |
| *BG02312* | *Spri* | 9D3 | 0.0025 | 0.5081 | 3.502** | 2.701 | 4.322* |
| *BG01625* | *jim* | 80D1 | 0.0028 | 0.8625 | 3.509* | 3.700* | 3.296* |
| *BG02470* | *CG8963* | 53F11-12 | 0.0015 | 0.7095 | 3.533** | 3.125 | 3.942** |
| *BG01741* | *Pdi* | 71B5 | 0.0010 | 0.7831 | 3.731** | 4.053* | 3.428* |
| *BG01127* | *muscleblind* | 54B16 | 0.0015 | 0.0724 | 3.761** | 5.888*** | 1.506 |
| *BG02644* | *Fkbp13* | 57F4 | 0.0015 | 0.4087 | 3.835** | 2.858 | 4.846** |
| *BG01179* | *CG12489* | 59A1-3 | 0.0006 | 0.3754 | 3.840*** | 2.564 | 5.026** |
| *BG01564* | *CG14430* | 6E4 | 0.0008 | 0.7137 | 3.858*** | 3.400* | 4.310** |
| *BG00670* | *CG7378* | 18A1 | 0.0002 | 0.4441 | 3.929*** | 3.150* | 4.708** |
| *BG01628* | *Men* | 87C6-7 | 0.0004 | 0.2747 | 4.078*** | 5.324** | 2.866 |
| *BG02244* | *crooked legs* | 33A1-2 | 0.0003 | 0.9733 | 4.088*** | 4.125* | 4.050* |
| *BG01218* | *CG6767* | 67C4-5 | 0.0003 | 0.4765 | 4.092*** | 4.892** | 3.292* |
| *BG02646* | *Crc* | 85E1 | 0.0001 | 0.7439 | 4.308*** | 3.950* | 4.667** |
| *BG02602* | *Rab27* | 2B1 | 0.0001 | 0.1894 | 4.543*** | 5.986*** | 3.074 |
| *BG02348* | *Hsp23* | 67B1 | 0.0025 | 0.2916 | 4.702*** | 4.944** | 4.217* |
| *BG02084* | *Vha16* | 42C1 | <0.0001 | 0.8341 | 4.975*** | 4.717** | 5.238** |
| *BG01596* | *erect wing* | 1A1 | <0.0001 | 0.4280 | 5.168*** | 4.242* | 6.100*** |
| *BG01045* | *CG14150* | 67E7 | <0.0001 | 0.5402 | 5.246*** | 4.583** | 5.942*** |
| *BG01327* | *Pk61C* | 61B1 | <0.0001 | 0.6384 | 5.750*** | 5.292*** | 6.233*** |
| *BG02650* | *CG15711* | 53C5 | <0.0001 | 0.3134 | 5.817*** | 4.675** | 6.958*** |
| *BG00372* | *CG1678* | 20A1 | <0.0001 | 0.0831 | 6.243*** | 8.163*** | 4.350** |
| *BG00490* | *CG9894* | 23A3 | <0.0001 | 0.3309 | 6.603*** | 5.533*** | 7.630*** |
| *BG02785* | *dacapo* | 46A4 | <0.0001 | 0.0102 | 7.367*** | 4.733** | 10.000*** |

$P(L)$ and $P(L\times S)$ are from ANOVA pooled across the initial assay and the re-test assay (See Materials and Methods). Effects are expressed as deviations from the contemporaneous control line mean. *P*-values for estimated effects are from Dunnett's *t*-tests comparing the insert line to the control. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

## TABLE 4

**Locomotion assay results for *P*-element insert lines with significant effects on climbing behavior.**

| Insert Line | Nearest Gene | Cyt. Location | *P*(*L*) | *P*(*L*×*S*) | Effect (Sexes Pooled) | Effect (Males) | Effect (Females) |
|---|---|---|---|---|---|---|---|
| *BG01564* | *CG14430* | 6E4 | 0.0023 | 0.0460 | 3.783** | 1.233 | 5.733** |
| *BG00299* | *CG13697* | 75B6 | 0.0001 | 0.6426 | 3.675*** | 3.250* | 4.100** |
| *BG01636* | *CG12750* | 36E3 | 0.0036 | 0.6155 | 3.646** | 4.183* | 3.064 |
| *BG00372* | *CG1678* | 20A1 | 0.0072 | 0.0878 | 3.308** | 1.233 | 5.383** |
| *BG02062* | *CG15312* | 9B1 | 0.0029 | 0.3562 | 2.593** | 3.317* | 1.843 |
| *BG02785* | *dap* | 46A4 | 0.0005 | 0.6607 | 2.228** | 2.467** | 1.971* |
| *BG02646* | *Crc* | 85E1 | 0.0020 | 0.1326 | 2.059* | 3.033* | 1.0512 |
| *BG02572* | *lola* | 47A8-11 | 0.1202 | 0.2712 | 1.776 | 0.649 | 2.833* |
| *BG02644* | *Fkbp13* | 57F4 | 0.0635 | 0.2426 | 1.581 | 2.540 | 0.531 |
| *BG01101* | *CG32191* | 75B6 | 0.3739 | 0.7982 | 0.875 | 1.233 | 0.450 |
| *BG01367* | *Rab23* | 83B9 | 0.2116 | 0.2103 | 0.840 | 1.633 | 0.024 |
| *BG01109* | *tout-velu* | 51B9-11 | 0.5082 | 0.3549 | 0.750 | 1.800 | –0.300 |
| *BG00361* | *SP71* | 1A5-6 | 0.4421 | 0.0535 | 0.515 | 1.733 | –0.726 |
| *BG01006* | *CG4564* | 6E2 | 0.6225 | 0.5683 | 0.484 | 1.033 | –0.098 |
| *BG02602* | *Rab27* | 2B1 | 0.4289 | 0.9564 | 0.483 | 0.450 | 0.517 |
| *BG02244* | *crooked legs* | 33A1-2 | 0.4198 | 0.6162 | 0.483 | 0.783 | 0.183 |
| *BG02348* | *Hsp23* | 67B1 | 0.4700 | 0.6473 | 0.408 | 0.150 | 0.667 |
| *BG00670* | *CG7378* | 18A1 | 0.7073 | 0.3643 | 0.334 | –0.467 | 1.102 |
| *BG00489* | *Osiris9* | 83E2 | 0.6124 | 0.5810 | 0.301 | 0.633 | –0.031 |
| *BG01218* | *CG6767* | 67C4-5 | 0.7335 | 0.4371 | 0.233 | 0.767 | –0.300 |
| *BG00737* | *Hsp27* | 67B1 | 0.6611 | 0.3562 | 0.212 | –0.316 | 0.717 |
| *BG02029* | *HLHm7* | 96F10 | 0.7940 | 0.5604 | 0.084 | –0.089 | 0.233 |
| *BG01327* | *Pk61C* | 61B1 | 0.8961 | 0.4495 | 0.083 | 0.567 | –0.400 |
| *BG02864* | *CG4564* | 6E2 | 0.9893 | 0.4453 | 0.008 | 0.483 | –0.467 |
| *BG01741* | *Pdi* | 71B5 | 1.0000 | 0.4007 | 0.000 | –0.333 | 0.333 |
| *BG01179* | *CG12489* | 59A1-3 | 0.9841 | 0.2115 | –0.008 | 0.517 | –0.533 |
| *BG02391* | *neur* | 85C5 | 0.9497 | 0.0848 | –0.031 | –1.700 | 1.621 |
| *BG00490* | *sbb* | 55C4-6 | 0.6431 | 0.0954 | –0.127 | 0.350 | –0.617* |
| *BG02291* | *CG14059* | 73E4 | 0.7678 | 0.5131 | –0.192 | 0.233 | –0.617 |
| *BG00968* | *mub* | 79A7-B1 | 0.9167 | 0.4284 | –0.225 | 0.933 | –1.450 |
| *BG02537* | *1.28* | 42B2 | 0.7041 | 0.6351 | –0.267 | 0.067 | –0.600 |
| *BG01596* | *erect wing* | 1A1 | 0.6733 | 0.4226 | –0.286 | –0.844 | 0.233 |
| *BG02068* | *Unknown* | | 0.7089 | 0.2381 | –0.292 | 0.633 | –1.217 |
| *BG01296* | *CG4564* | 6E2 | 0.6275 | 0.7853 | –0.342 | –0.533 | –0.150 |
| *BG02084* | *Vha16* | 42C1 | 0.2551 | 0.0777 | –0.363 | –1.050 | 0.250 |
| *BG01756* | *Pk61C* | 61B1 | 0.1308 | 0.6125 | –0.475 | –0.633 | –0.317 |
| *BG01628* | *Men* | 87C6-7 | 0.1911 | 0.9651 | –0.500 | –0.483 | –0.517 |

| Insert Line | Nearest Gene | Cyt. Location | *P*(*L*) | *P*(*L*×*S*) | Effect (Sexes Pooled) | Effect (Males) | Effect (Females) |
|---|---|---|---|---|---|---|---|
| *BG02295* | *CG15321* | 8F9 | 0.1542 | 0.1428 | –0.575 | –1.100 | 0.038 |
| *BG01625* | *Jim* | 80D1 | 0.1650 | 0.1650 | –0.665 | –1.328 | 0.000 |
| *BG02415* | *capricious* | 70A4 | 0.2745 | 0.2986 | –0.684 | –1.322 | –0.033 |
| *BG00312* | *Pfrx* | 18C8 | 0.3163 | 0.0805 | –0.687 | 0.583 | –2.013 |
| *BG01799* | *CG14998* | 64A7 | 0.4383 | 0.3854 | –0.692 | –1.467 | 0.083 |
| *BG02297* | *escargot* | 35D2 | 0.1722 | 0.1096 | –0.813 | –1.728 | 0.158 |
| *BG02312* | *Spri* | 9D3 | 0.1080 | 0.3210 | –0.902 | –0.350 | –1.467 |
| *BG01243* | *CG12537* | 88A4 | 0.1758 | 0.8108 | –1.010 | –1.097 | –0.704 |
| *BG00846* | *invected* | 48A1 | 0.1893 | 0.2535 | –1.017 | –0.133 | –1.900* |
| *BG02650* | *CG15711* | 53C5 | 0.0599 | 0.0802 | –1.165 | –0.078 | –2.250 |
| *BG01813* | *Hsp27* | 67B1 | 0.3307 | 0.5001 | –1.300 | –0.400 | –2.200 |
| *BG02676* | *CG10990* | 12B6-8 | 0.0509 | 0.6289 | –1.553* | –1.225 | –1.883 |
| *BG00320* | *CHES-1-like* | 7B6 | 0.0059 | 0.0491 | –1.708** | –2.917* | –0.500 |
| *BG02356* | *Dally* | 66E1-3 | 0.0022 | 0.7153 | –1.783** | –1.572 | –1.983* |
| *BG01129* | *Adhr, Adh* | 35B3 | 0.0240 | 0.2892 | –1.867* | –2.733 | –1.000 |
| *BG01635* | *willdieslowly, Ubx* | | 0.0089 | 0.8704 | –2.042** | –2.167 | –1.917* |
| *BG00683* | *CG5127* | 96E2 | 0.0240 | 0.4492 | –2.049* | –2.717 | –1.381 |
| *BG01045* | *CG14150* | 67E7 | 0.0016 | 0.9723 | –2.095** | –2.143* | –2.050* |
| *BG02470* | *CG8963* | 53F11-12 | 0.0717 | 0.4820 | –2.485 | –5.690 | –1.067 |
| *BG01127* | *muscleblind* | 54B16 | 0.0002 | 0.3679 | –2.740*** | –3.378** | –2.100* |
| *BG02217* | *Px* | 58F2-59A1 | <0.0001 | 0.2211 | –4.950*** | –3.833* | –6.067*** |
| *BG02501* | *Lola* | 47A8-11 | <0.0001 | <0.0001 | –6.123*** | –10.022*** | –1.986 |

*P*(*L*) and *P*(*L*×*S*) are from ANOVA (See Materials and Methods). Effects are expressed as deviations from the contemporaneous control line mean. *P*-values for estimated effects are from Dunnett's *t*-tests comparing the insert line to the control. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

# TABLE 5

**Analysis of variance of locomotion assay, sexes pooled.**

| Source | d. f. | MS | F | $\sigma^2$ | *P* |
|---|---|---|---|---|---|
| Line | 58 | 247.26 | 4.86 | 4.95 | <0.0001 |
| Sex | 1 | 50.60 | 1.05 | - | 0.310 |
| Line x Sex | 58 | 48.29 | 1.54 | 0.76 | 0.0524 |
| Day (Line) | 58 | 33.72 | 1.08 | 0 | 0.3827 |
| Sex × Day (Line) | 58 | 31.17 | 1.63 | 1.06 | 0.0021 |
| Error | 2129 | 19.11 | | 19.41 | |

**TABLE 6**

**Analysis of variance of locomotion assay, sexes separately.**

| Source | d. f. | MS | F | $\sigma^2$ | *P* |
|---|---|---|---|---|---|
| **Males** | | | | | |
| Line | 58 | 127.20 | 6.07 | 4.486 | <0.0001 |
| Day (Line) | 58 | 34.34 | 1.64 | 1.034 | 0.0022 |
| Error | 1073 | 20.94 | | 21.343 | |
| **Females** | | | | | |
| Line | 58 | 167.08 | 9.69 | 6.999 | <0.0001 |
| Day (Line) | 58 | 30.44 | 1.76 | 1.015 | 0.0005 |
| Error | 1056 | 17.25 | | 17.403 | |

**TABLE 7**

***P*-element insert lines significant for the climbing assay but not the locomotion assay.**

| | | | Climbing Assay | | | | | Locomotion Assay | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Insert Line** | **Nearest Gene** | **Cyt. Location** | ***P*(*L*)** | ***P*(*L*×*S*)** | **Effect (Sexes Pooled)** | **Effect (Males)** | **Effect (Females)** | ***P*(*L*)** | ***P*(*L*×*S*)** | **Effect (Sexes Pooled)** | **Effect (Males)** | **Effect (Females)** |
| *BG02650* | *CG15711* | 53C5 | <0.0001 | 0.3134 | 5.817*** | 4.675** | 6.958*** | 0.0599 | 0.0802 | –1.165 | –0.0776 | –2.250 |
| *BG01327* | *Pk61C* | 61B1 | <0.0001 | 0.6384 | 5.750*** | 5.292*** | 6.233*** | 0.896 | 0.4495 | 0.083 | 0.567 | -0.400 |
| *BG01596* | *erect wing* | 1A1 | <0.0001 | 0.4280 | 5.168*** | 4.242* | 6.100*** | 0.6733 | 0.4226 | –0.286 | –0.844 | 0.233 |
| *BG02084* | *Vha16* | 42C1 | <0.0001 | 0.8341 | 4.975*** | 4.717** | 5.238** | 0.255 | 0.078 | –0.363 | –1.050 | 0.250 |
| *BG02348* | *Hsp23* | 67B1 | 0.0025 | 0.2916 | 4.702*** | 4.944** | 4.217* | 0.4700 | 0.6473 | 0.408 | 0.150 | 0.667 |
| *BG02602* | *Rab27* | 2B1 | 0.0001 | 0.1894 | 4.543*** | 5.986*** | 3.074 | 0.4289 | 0.9564 | 0.483 | 0.450 | 0.517 |
| *BG01218* | *CG6767* | 67C4-5 | 0.0003 | 0.4765 | 4.092*** | 4.892** | 3.292* | 0.7335 | 0.4371 | 0.233 | 0.767 | –0.300 |
| *BG02244* | *crooked legs* | 33A1-2 | 0.0003 | 0.9733 | 4.088*** | 4.125* | 4.050* | 0.4198 | 0.6162 | 0.483 | 0.783 | 0.183 |
| *BG01628* | *Men* | 87C6-7 | 0.0004 | 0.2747 | 4.078*** | 5.324** | 2.866 | 0.1911 | 0.9651 | –0.500 | –0.483 | –0.517 |
| *BG00670* | *CG7378* | 18A1 | 0.0002 | 0.4441 | 3.929*** | 3.150* | 4.708** | 0.7073 | 0.3643 | 0.334 | –0.467 | 1.102 |
| *BG01179* | *CG12489* | 59A1-3 | 0.0006 | 0.3754 | 3.840*** | 2.564 | 5.026** | 0.9841 | 0.2115 | –0.008 | 0.517 | –0.533 |
| *BG02644* | *Fkbp13* | 57F4 | 0.0015 | 0.4087 | 3.835** | 2.858 | 4.846** | | | | | |
| *BG01741* | *Pdi* | 71B5 | 0.0010 | 0.7831 | 3.731** | 4.053* | 3.428* | 1.0000 | 0.4007 | 0.000 | –0.333 | 0.333 |
| *BG02470* | *CG8963* | 53F11-12 | 0.0015 | 0.7095 | 3.533** | 3.125 | 3.942** | 0.0717 | 0.4820 | –2.485 | –5.690 | –1.067 |
| *BG01625* | *jim* | 80D1 | 0.0028 | 0.8625 | 3.509* | 3.700* | 3.296* | 0.1650 | 0.1650 | –0.665 | –1.328 | 0.000 |
| *BG02312* | *spri* | 9D3 | 0.0025 | 0.5081 | 3.502** | 2.701 | 4.322* | 0.1080 | 0.3210 | –0.902 | –0.350 | –1.467 |
| *BG01243* | *CG12537* | 88A4 | 0.0026 | 0.3711 | 3.325** | 4.300** | 2.350 | 0.1758 | 0.8108 | –1.010 | –1.097 | –0.704 |
| *BG02391* | *neuralized* | 85C5 | 0.0021 | 0.0002 | 3.292** | 7.358*** | –0.775 | 0.9497 | 0.0848 | –0.031 | –1.700 | 1.621 |
| *BG00737* | *Hsp27* | 67B1 | 0.0253 | 0.9658 | 3.148* | 2.617 | 3.692* | 0.6611 | 0.3562 | 0.212 | –0.316 | 0.717 |
| *BG02297* | *escargot* | 35D2 | 0.0197 | 0.0780 | 2.637* | 4.603** | 0.672 | 0.1722 | 0.1096 | –0.813 | –1.728 | 0.158 |
| *BG02537* | *1.28* | 42B2 | 0.0301 | 0.1346 | 2.558* | 4.180* | 0.713 | 0.7041 | 0.6351 | –0.267 | 0.067 | –0.600 |
| *BG01756* | *Pk61C* | 61B1 | 0.0396 | 0.8041 | 2.433* | 2.142 | 2.725 | 0.1308 | 0.6125 | –0.475 | –0.633 | –0.317 |

| | | | Climbing Assay | | | | | Locomotion Assay | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Insert Line** | **Nearest Gene** | **Cyt. Location** | ***P*(*L*)** | ***P*(*L*×*S*)** | **Effect (Sexes Pooled)** | **Effect (Males)** | **Effect (Females)** | ***P*(*L*)** | ***P*(*L*×*S*)** | **Effect (Sexes Pooled)** | **Effect (Males)** | **Effect (Females)** |
| *BG02291* | *CG14059* | 73E4 | 0.0352 | 0.2256 | 2.297* | 3.560* | 1.033 | 0.7678 | 0.5131 | –0.192 | 0.2333 | –0.617 |
| *BG01296* | *CG4564* | 6E2 | 0.0643 | 0.0043 | 2.290* | –0.970 | 5.392*** | 0.6275 | 0.7853 | –0.342 | –0.533 | –0.150 |
| *BG01813* | *Hsp27* | 67B1 | 0.0580 | 0.0085 | 2.092 | 5.040** | –0.842 | 0.3307 | 0.5001 | –1.300 | –0.400 | –2.200 |
| *BG02295* | *CG15321* | 8F9 | 0.5700 | 0.0029 | 0.487 | 3.699* | –2.650 | 0.1542 | 0.1428 | –0.575 | –1.100 | 0.038 |
| *BG01799* | *CG14998* | 64A7 | 0.0619 | 0.0071 | 2.058 | –0.925 | 5.042*** | 0.4383 | 0.3854 | –0.692 | –1.467 | 0.083 |
| *BG01006* | *CG4564* | 6E2 | 0.6075 | 0.0025 | 0.558 | –2.775 | 3.892* | 0.6225 | 0.5683 | 0.484 | 1.033 | –0.098 |
| *BG02415* | *capricious* | 70A4 | 0.1684 | 0.0238 | –1.633 | –4.325* | 1.058 | 0.2745 | 0.2986 | –0.684 | –1.322 | –0.033 |
| *BG02864* | *CG4564* | 6E2 | 0.1980 | 0.0321 | –1.330 | –3.555* | 0.887 | 0.9893 | 0.4453 | 0.008 | 0.483 | -0.467 |
| *BG01109* | *tout-velu* | 51B9-11 | 0.0963 | 0.0878 | –1.852 | –0.050 | –3.751* | 0.5082 | 0.3549 | 0.750 | 1.800 | –0.300 |
| *BG00489* | *Osiris9* | 83E2 | 0.0289 | 0.8498 | –2.517* | –2.300 | –2.733 | 0.6124 | 0.5810 | 0.301 | 0.633 | –0.031 |
| *BG00312* | *Pfrx* | 18C8 | 0.0039 | 0.1442 | –3.289** | –1.624 | –4.953** | 0.3163 | 0.0805 | –0.687 | 0.583 | –2.013 |
| *BG02029* | *HLHm7* | 96F10 | 0.0003 | 0.9293 | –3.816*** | –3.960* | –3.732** | 0.7940 | 0.5604 | 0.084 | –0.089 | 0.233 |
| *BG00361* | *SP71* | 1A5-6 | 0.0009 | 0.7812 | –3.833*** | –4.150* | –3.517* | 0.4421 | 0.0535 | 0.515 | 1.733 | –0.726 |
| *BG01367* | *Rab23* | 83B9 | 0.0038 | 0.8561 | –4.077*** | –4.921** | –3.328* | 0.2116 | 0.2103 | 0.840 | 1.633 | 0.024 |
| *BG01101* | *CG32191* | 75B6 | <0.0001 | 0.0398 | –4.338*** | –6.575*** | –2.100 | 0.3739 | 0.7982 | 0.875 | 1.233 | 0.450 |
| *BG02068* | *Unknown* | | <0.0001 | 0.0275 | –4.579*** | –7.092*** | –2.067 | 0.7089 | 0.2381 | –0.292 | 0.633 | –1.217 |
| *BG00968* | *Mub* | 79A7-B1 | <0.0001 | 0.8686 | –6.200*** | –6.375*** | –6.025*** | 0.9167 | 0.4284 | –0.225 | 0.933 | –1.450 |

*P*(*L*) and *P*(*L*×*S*) are from ANOVA (See Materials and Methods). Effects are expressed as deviations from the contemporaneous control line mean. *P*-values for estimated effects are from Dunnett's *t*-tests comparing the insert line to the control. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

**TABLE 8**

**Comparison between the performance of selected *P*-element insert lines in the classic geotactic maze and the climbing assay.**

| | | Climbing Assay | | | | Geotaxis Maze Assay | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Insert Line** | **Gene** | ***P*(*L*×*S*)** | **Effect (Sexes Pooled)** | **Effect (Males)** | **Effect (Females)** | ***P*(*L*×*S*)** | **Effect (Sexes Pooled)** | **Effect (Males)** | **Effect (Females)** |
| *BG02501* | *lola* | ns | –5.733*** | –7.725*** | –3.942* | <0.0001 | 1.751*** | 2.352*** | 0.921*** |
| *BG02572* | *lola* | ns | –5.367*** | –5.575*** | –5.158*** | 0.0064 | 1.184*** | 0.809*** | 1.564*** |
| *BG02542* | *neur* | ns | –0.449 | –1.499 | 0.567 | ns | –0.043 | –0.142 | –0.012 |
| *BG02391* | *neur* | 0.0002 | 3.292** | 7.358*** | –0.775 | ns | 1.459*** | 1.411*** | 1.613*** |
| *BG00372* | *CG1678* | ns | 6.243*** | 8.163*** | 4.350** | ns | 0.894*** | 0.893*** | 0.923*** |
| *BG00467* | *CG8620* | ns | 1.450 | 1.783 | –1.125 | ns | –1.249*** | –1.192*** | –1.347*** |
| *BG01799* | *CG14998* | 0.0071 | 2.058 | –0.925 | 5.042*** | <0.0001 | 0.951*** | 0.256 | 1.832*** |
| *BG01564* | *CG14430* | ns | 3.858*** | 3.400* | 4.310** | ns | 0.319* | 0.630** | 0.060 |
| *BG00320* | *CHES-1-like* | ns | –4.158*** | –5.050** | –3.267* | ns | –0.317* | –0.477* | –0.131 |
| *BG00968* | *mub* | ns | –6.200*** | –6.375*** | –6.025*** | ns | –0.022 | –0.013 | 0.031 |

*P*(*L*×*S*) is from ANOVA (See Materials and Methods). Effects are expressed as deviations from the contemporaneous control line mean. *P*-values for estimated effects are from Dunnett's *t*-tests comparing the insert line to the control. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

**TABLE 9**

**Results of quantitative complementation tests with deficiencies and known mutants**

| Line | Gene | Deficiency/Allele | Bloomington stock # | Sexes pooled Line | Line x Sex | Male Line | Female Line |
|---|---|---|---|---|---|---|---|
| *BG02062* | *CG15312* | *Df(1)C52*, $flw^{C52}$/*FM6* | 952 | | | | 0.5834 |
| *BG02646* | *Calreticulin* | *Df(3R)GB104*, $red^{1}$/*TM3*, $Sb^{1}$ $Ser^{1}$ | 1937 | 0.2295 | 0.1488 | 0.8396 | 0.1044 |
| *BG02646* | *Calreticulin* | $y^{1}$ $w^{67c23}$; *P*{$w^{+mC}$=$lac^{W}$}$Crc^{S114307}$/*TM3*, $Sb^{1}$ $Ser^{1}$ | 4545 | 0.737 | 0.3638 | 0.6369 | 0.4328 |
| *BG02646* | *Calreticulin* | $w^{1118}$; *P*{$w^{+mGT}$=*GT1*}$Crc^{BG02738}$ | 13125 | 0.2206 | 0.1467 | 0.0603 | 0.8722 |
| *BG01564* | *CG14430* | *Df(1)Sxl-bt*, $y^{1}$/*Binsinscy* | 3196 | | | | 0.7552 |
| *BG01564* | *CG14430* | *Df(1)HA32/FM7c*, *P*{$ry^{+t7.2}$=*ftz/lacC*}*YH1* | 947 | | | | 0.9392 |
| *BG02415* | *capricious* | *P*{$ry^{+t7.2}$=*PZ*}$caps^{02937}$ $ry^{506}$/*TM3*, $ry^{RK}$ $Sb^{1}$ $Ser^{1}$ | 11579 | 0.3238 | 0.6256 | 0.3043 | 0.7213 |
| *BG02785* | *dacapo* | $cn^{1}$ *P*{$ry^{+t7.2}$=*PZ*}$dap^{04454}$/*CyO*; $ry^{506}$ | 11377 | **0.0112** | 0.3969 | 0.235 | **0.0164** |
| *BG02391* | *neuralized* | $neur^{11}$/*TM6B*, $Tb^{1}$ | 2747 | **0.0019** | 0.8481 | **0.0384** | **0.0205** |
| *BG02572* | *lola* | $cn^{1}$ *P*{$ry^{+t7.2}$=*PZ*}$lola^{00642}$/*CyO*; $ry^{506}$ | 10946 | 0.0643 | 0.9095 | 0.2176 | 0.2148 |
| *BG00372* | *CG1678* | *Df(1)C74*/*FM6* | 6277 | | | | **0.0042** |
| *BG01799* | *CG14998* | $w^{1118}$; *Df(3L)GN50*, *e**/*TM8*, $l(3)DTS4^{1}$ | 3687 | 0.8547 | 0.9176 | 0.8507 | 0.951 |
| *BG01799* | *CG14998* | *Df(3L)GN24*/*TM8*, $l(3)DTS4^{1}$ | 3686 | 0.8732 | 0.9953 | 0.9115 | 0.9081 |
| *BG00320* | *CHES-1-like* | *Df(1)ct4b1*, $y^{1}$/*Binsn* | 3221 | | | | 0.3047 |
| *BG00968* | *mub* | *P*{$ry^{+t7.2}$=*PZ*}$mub^{04093}$ $ry^{506}$/*TM3*, $ry^{RK}$ $Sb^{1}$ $Ser^{1}$ | 11624 | **<0.0001** | 0.4355 | **0.0045** | **0.0006** |
| *BG01628* | *Men* | $Men^{nNC3}$ *Aldox-1*$^{nNC4}$/*TM2* | 4025 | 0.0704 | 0.1983 | 0.6975 | **0.0373** |
| *BG01596* | *erect wing* | $ewg^{2}$ $y^{1}$ *cho* sn**/*FM6*, *l(1)FMa*$^{1}$/*Dp*(1;*Y*)$y^{2}$*61l* | 4750 | | | | **0.0002** |
| *BG00490* | *CG9894* | $y^{1}$ $w^{67c23}$; *P*{$y^{+mDint2}$ $w^{BR.E.BR}$=SUPor-P}$CG9894^{KG00202}$ | 13062 | 0.9864 | 0.8736 | 0.8808 | 0.9302 |
| *BG02244* | *crooked legs* | *P*{$ry^{+t7.2}$=*PZ*}$crol^{04418}$ $cn^{1}$/*CyO*; $ry^{506}$ | 11374 | 0.3943 | 0.7557 | 0.3311 | 0.7351 |
| *BG02356* | *dally* | *P*{$ry^{+t7.2}$=*PZ*}$dally^{06464}$ $ry^{506}$/*TM3*, $ry^{RK}$ $Sb^{1}$ $Ser^{1}$ | 11685 | 0.9777 | 0.784 | 0.8126 | 0.8733 |

*P*(*L*×*S*) is from ANOVA (See Materials and Methods). Effects are expressed as deviations from the contemporaneous control line mean. *P*-values for estimated effects are from Dunnett's *t*-tests comparing the insert line to the control. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

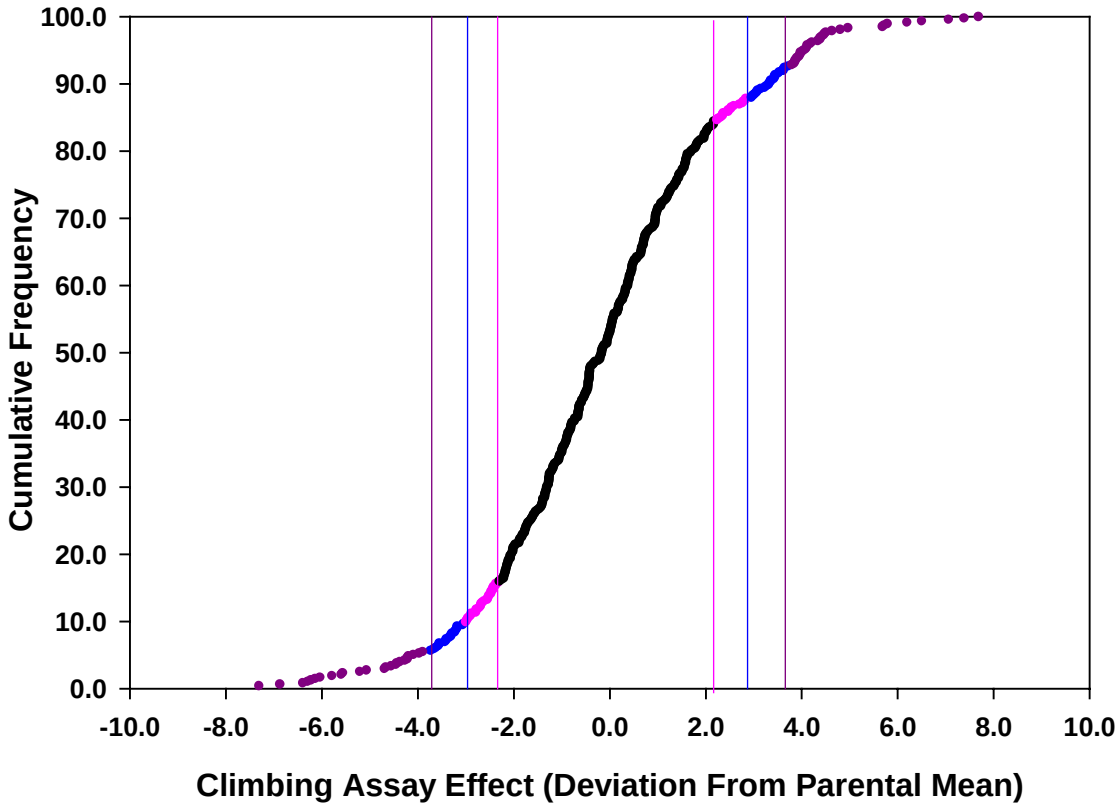



**Figure 1**

**Frequency distribution of mutational effects of 475 *P*-element inserts in the climbing assay, expressed as deviations from the parental mean. Magenta = 99.9% confidence interval threshold; blue = 99% confidence interval threshold; pink = 95% confidence interval threshold. 99.9% CI threshold = –3.858, 3.738; 99% CI threshold = –3.033, 2.913; 95% CI threshold = –2.322, 2.202.**

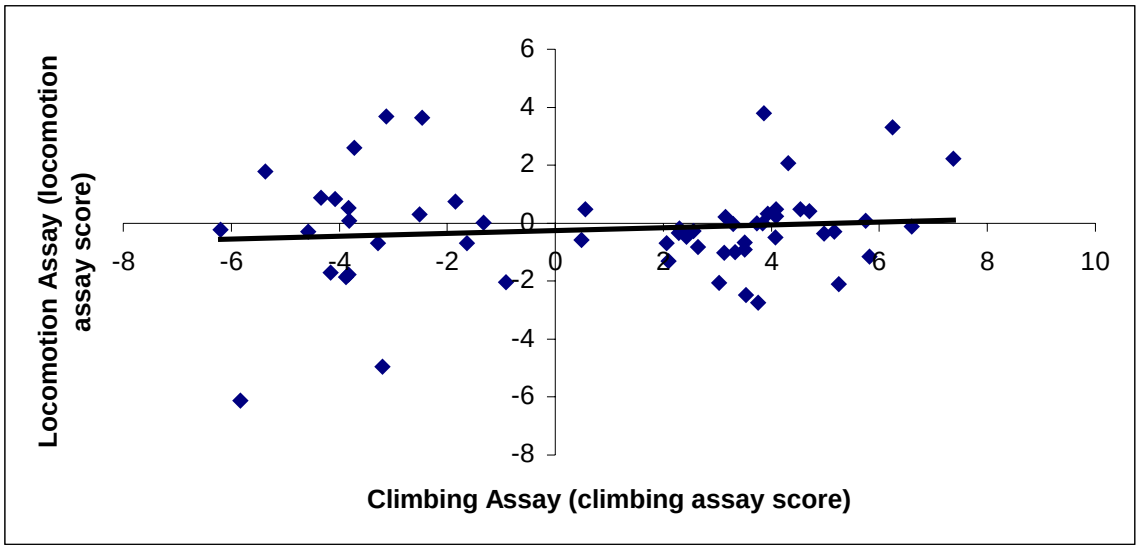
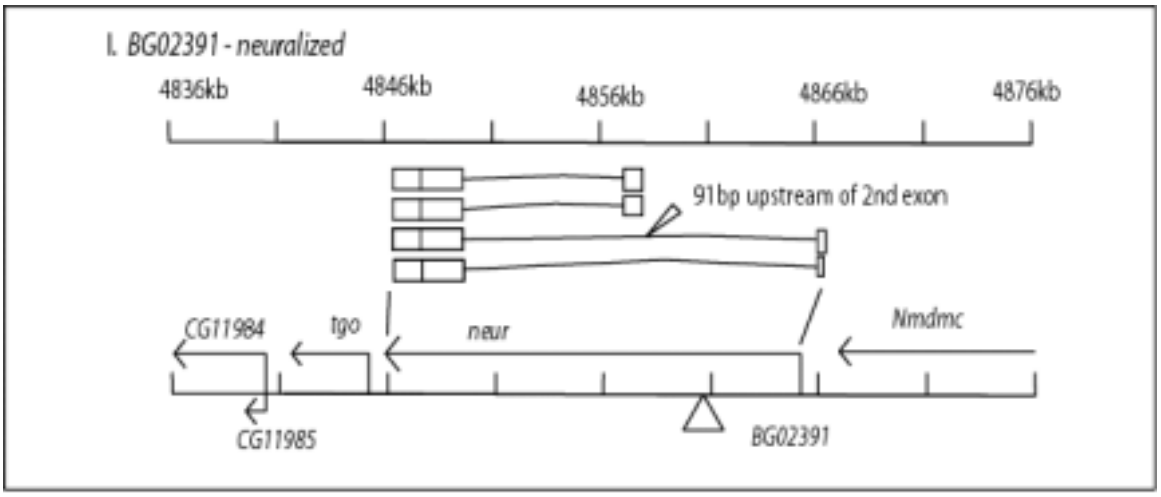
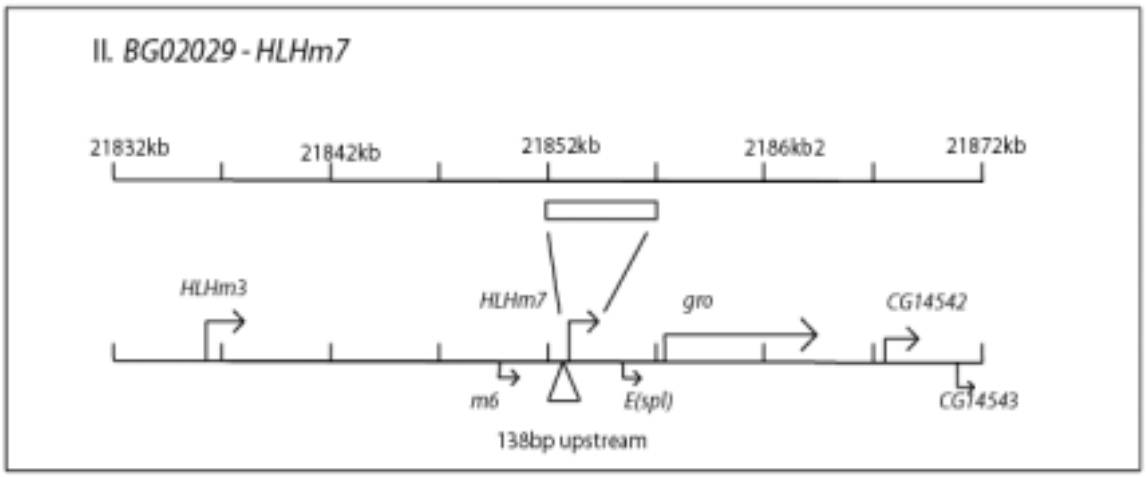
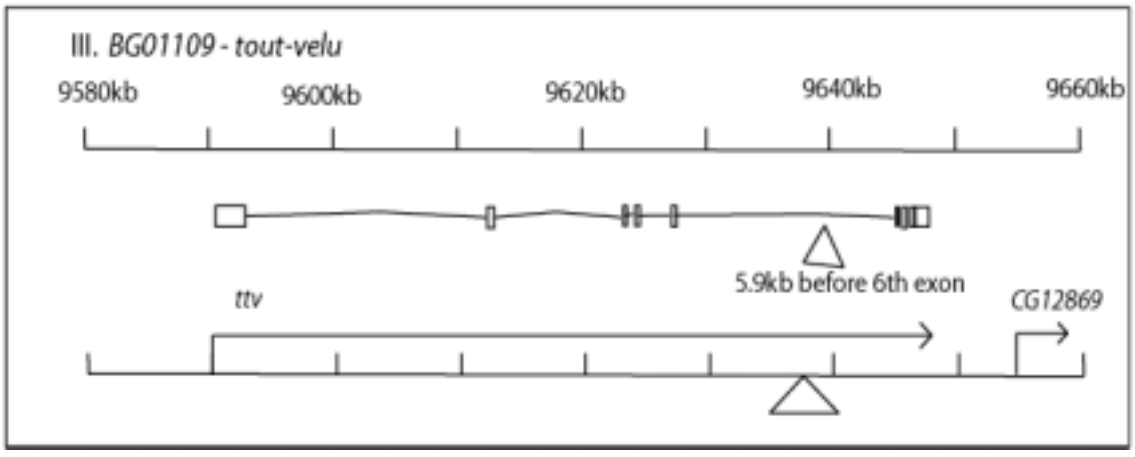


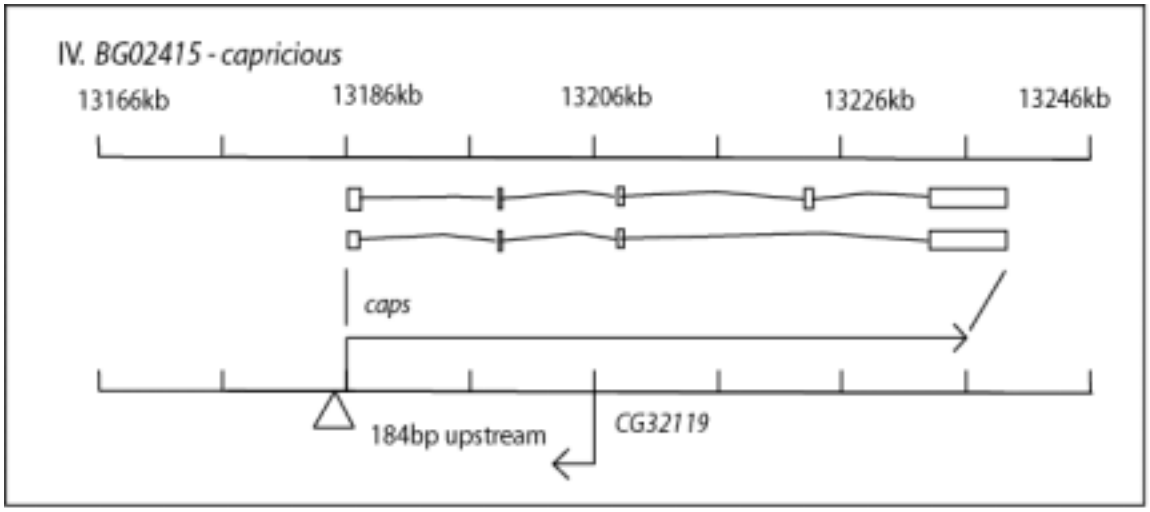

**Figure 2**

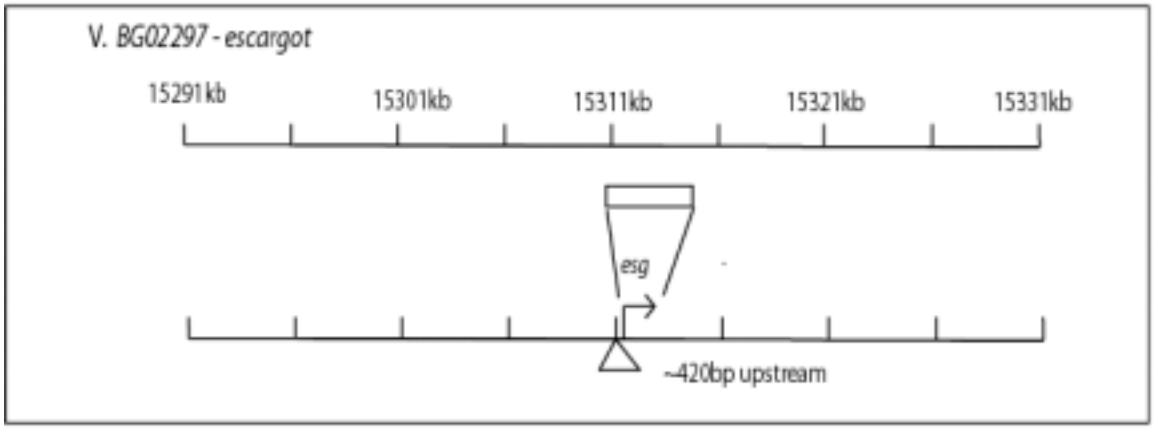
**Correlation between the performance of *P*-element insertion lines in the climbing assay and the locomotion assay. Pearson's product moment correlation coefficient (r) is r = 0.37, which is significantly different from zero (*P* = 0.0189). Units represent assay score differences from the parental line mean.**

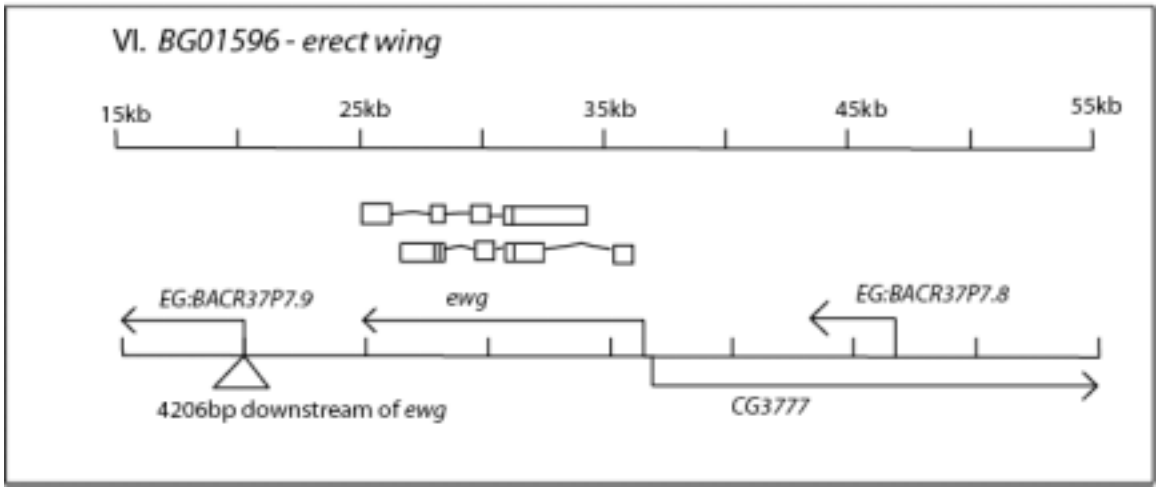

I. BG02391 - neuralized
4836kb
4846kb
4856kb
4866kb
4876kb
91bp upstream of 2nd exon
CG11984
tgo
neur
Nmdmc
CG11985
BG02391

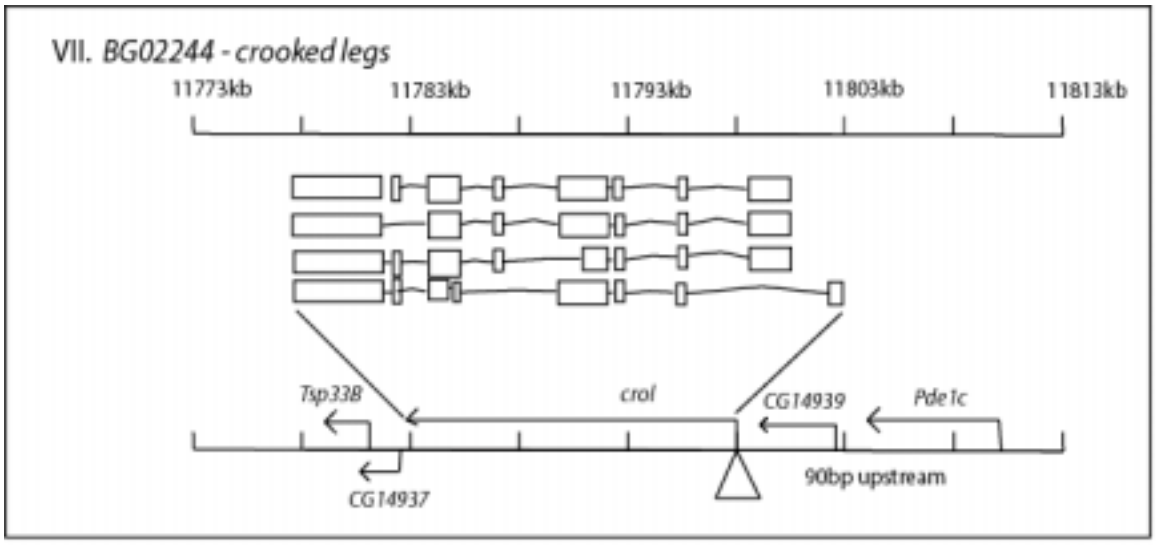

II. BG02029 - HLHm7
21832kb
21842kb
21852kb
2186kb2
21872kb
HLHm3
HLHm7
gro
CG14542
m6
E(spl)
CG14543
138bp upstream

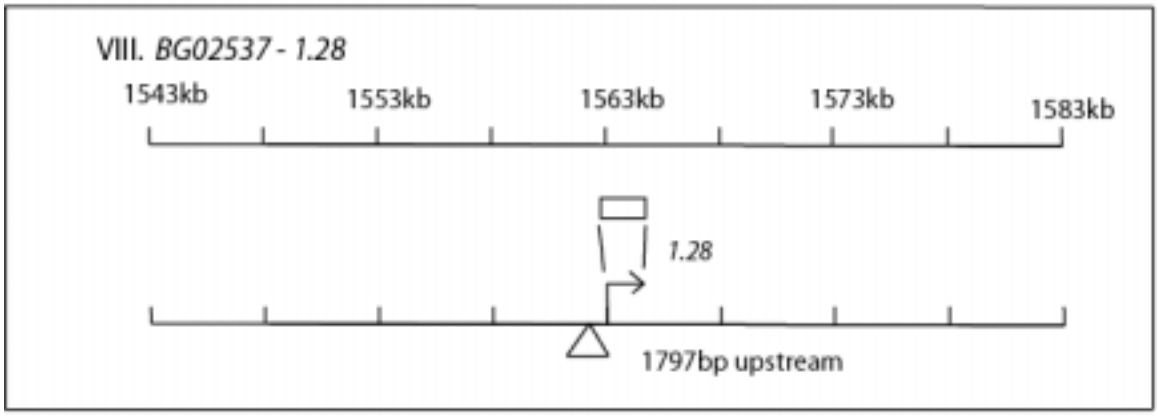

III. BG01109 - tout-velu
9580kb
9600kb
9620kb
9640kb
9660kb
5.9kb before 6th exon
ttv
CG12869

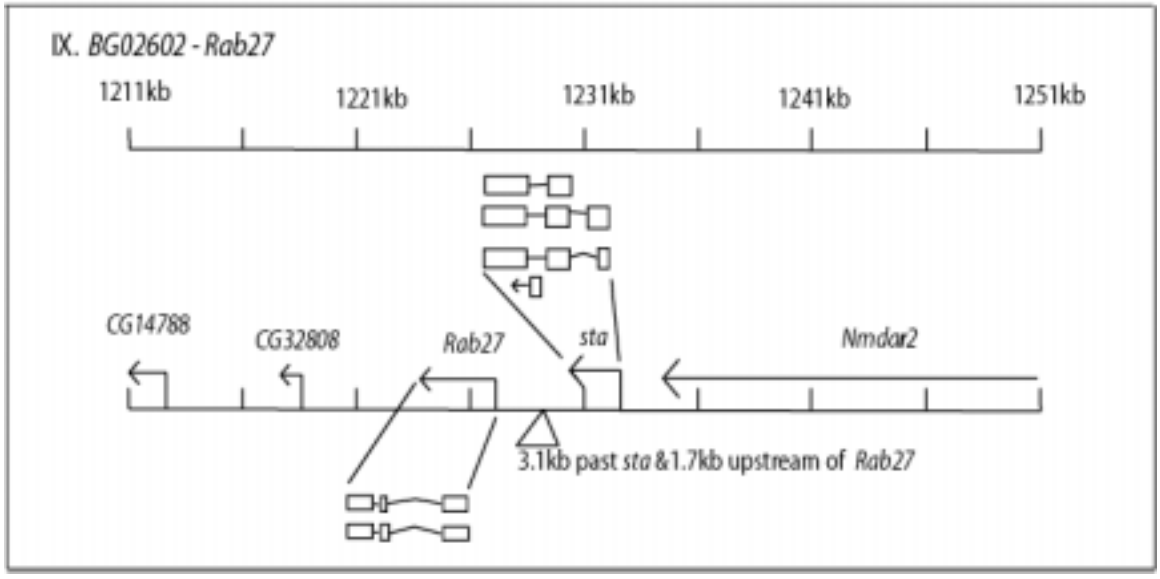

IV. BG02415 - capricious
13166kb
13186kb
13206kb
13226kb
13246kb
caps
184bp upstream
CG32119

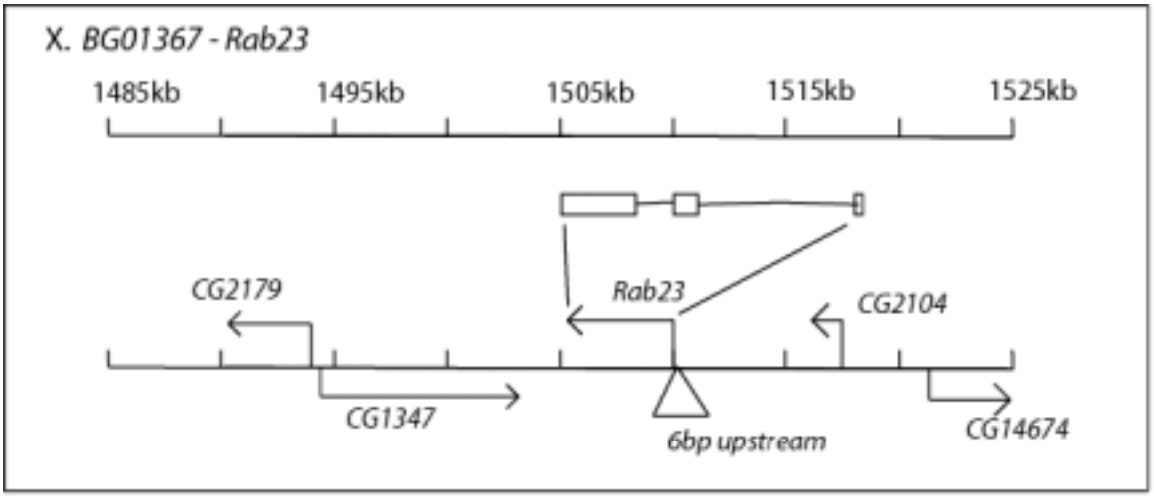

V. BG02297 - escargot
15291kb
15301kb
15311kb
15321kb
15331kb
esg
~420bp upstream

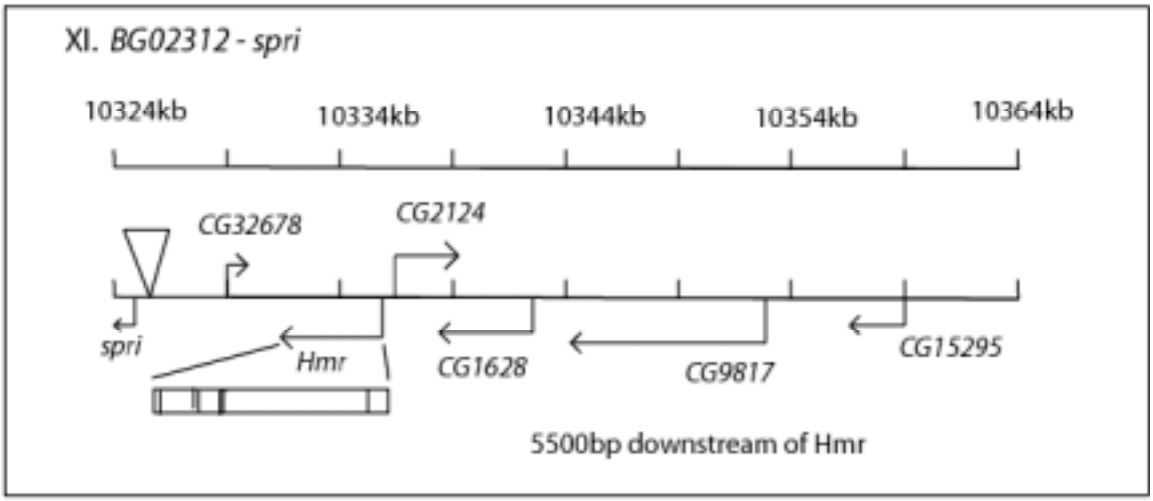

VI. BG01596 - erect wing
15kb
25kb
35kb
45kb
55kb
EG:BACR37P7.9
ewg
EG:BACR37P7.8
4206bp downstream of ewg
CG3777

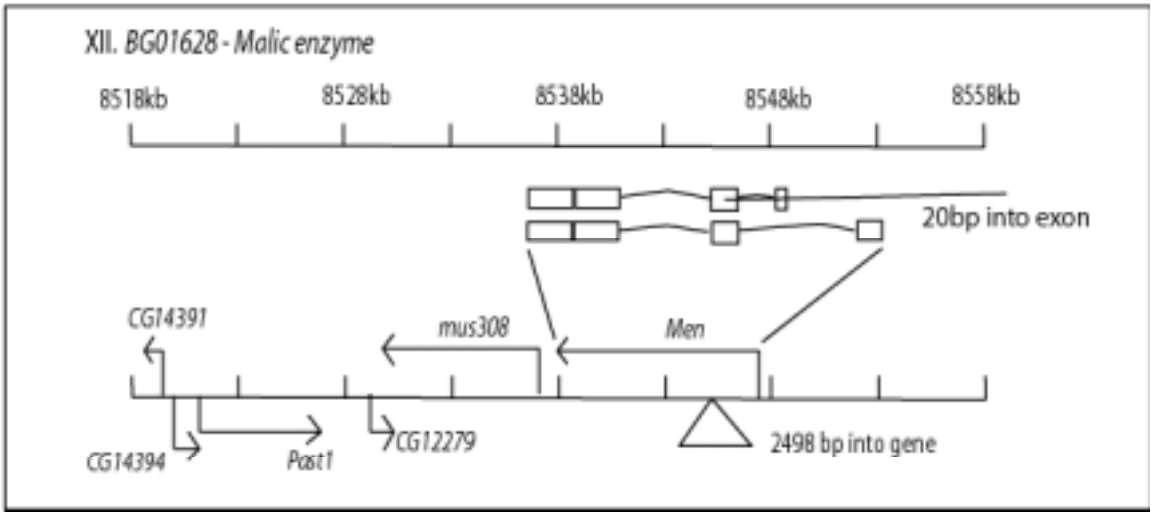

VII. BG02244 - crooked legs
11773kb
11783kb
11793kb
11803kb
11813kb
Tsp33B
croi
CG14939
Pde1c
CG14937
90bp upstream

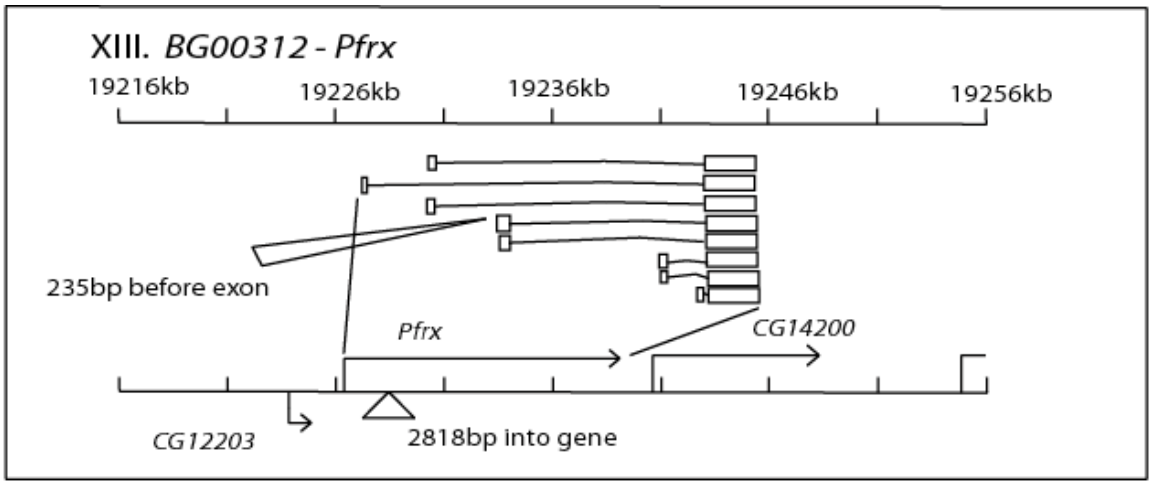

VIII. BG02537 - 1.28
1543kb
1553kb
1563kb
1573kb
1583kb
1.28
1797bp upstream


IX. BG02602 - Rab27
1211kb
1221kb
1231kb
1241kb
1251kb
CG14788
CG32808
Rab27
sta
Nmdar2
3.1kb past sta &1.7kb upstream of Rab27


X. BG01367 - Rab23
1485kb
1495kb
1505kb
1515kb
1525kb
CG2179
Rab23
CG2104
CG1347
6bp upstream
CG14674


XI. BG02312 - spri
10324kb
10334kb
10344kb
10354kb
10364kb
CG32678
CG2124
spri
Hmr
CG1628
CG9817
CG15295
5500bp downstream of Hmr


XII. BG01628 - Malic enzyme
8518kb
8528kb
8538kb
8548kb
8558kb
20bp into exon
CG14391
mus308
Men
CG14394
Past1
CG12279
2498 bp into gene


XIII. BG00312 - Pfrx
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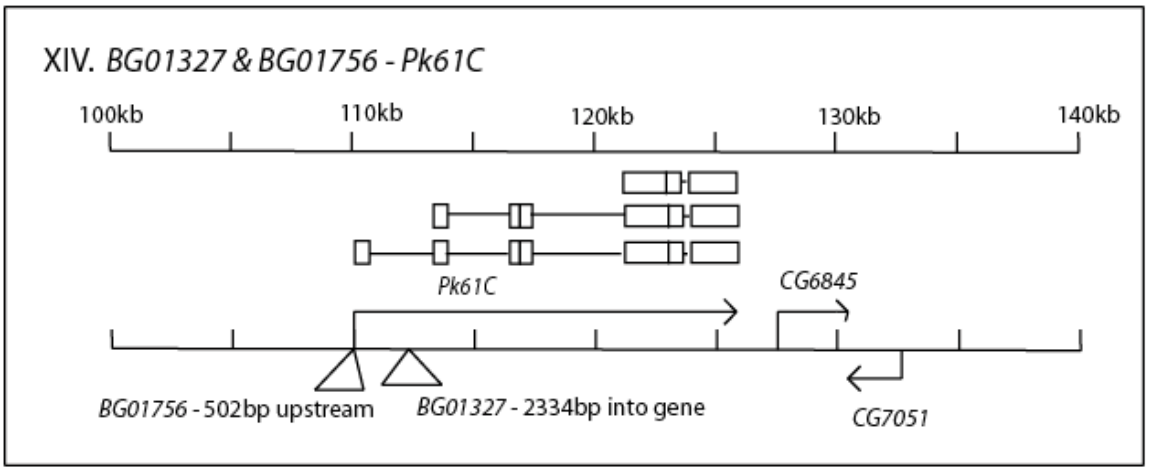
19226kb
19236kb
19246kb
19256kb
235bp before exon
Pfrx
CG14200
CG12203
2818bp into gene


XIV. BG01327 & BG01756 - Pk61C
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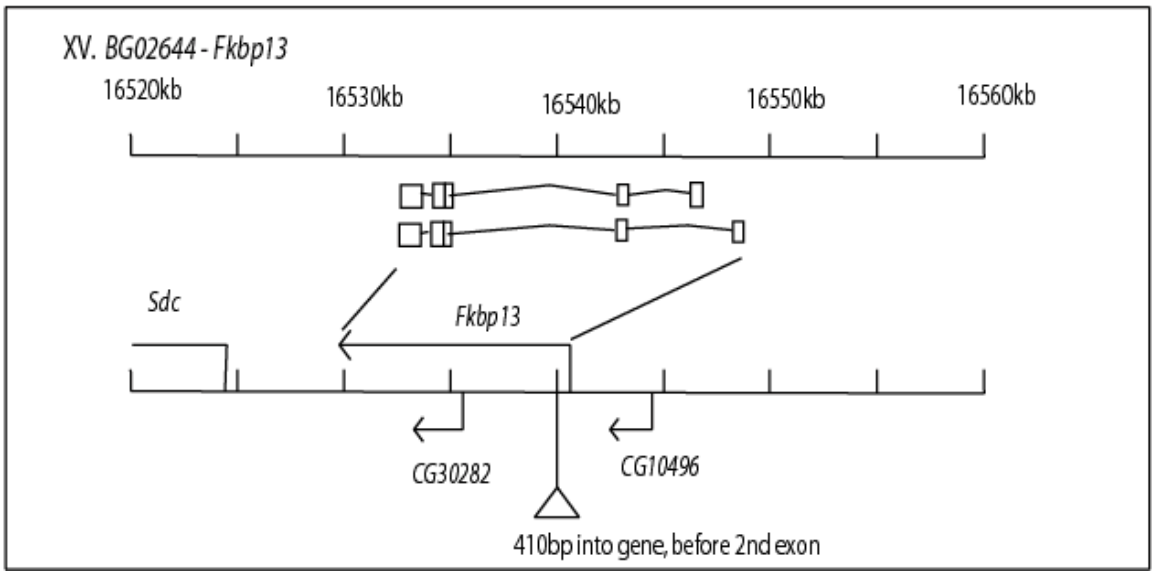
110kb
120kb
130kb
140kb
Pk61C
CG6845
BG01756 - 502bp upstream
BG01327 - 2334bp into gene
CG7051


XV. BG02644 - Fkbp13
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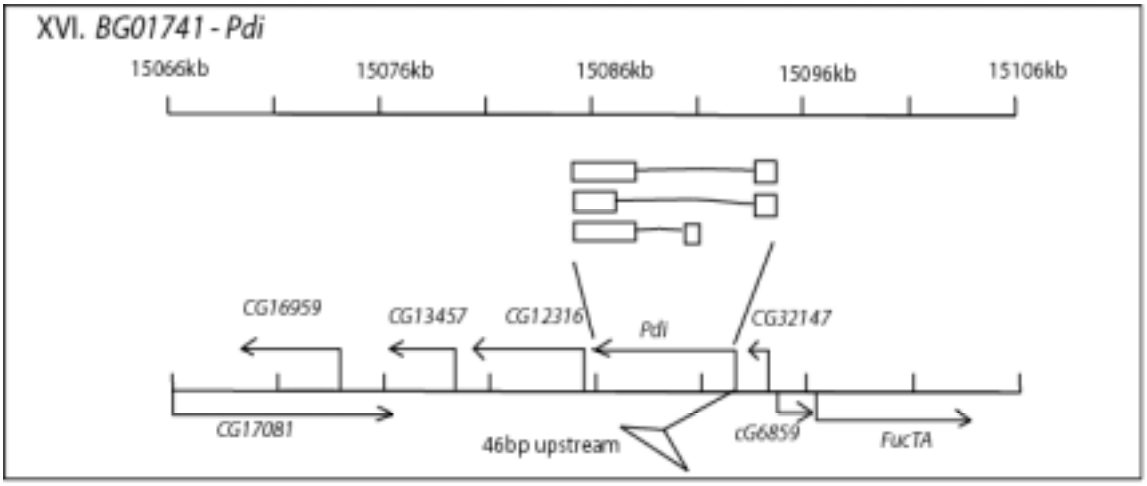
16530kb
16540kb
16550kb
16560kb
Sdc
Fkbp13
CG30282
CG10496
410bp into gene, before 2nd exon


XVI. BG01741 - Pdi
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15086kb
15096kb
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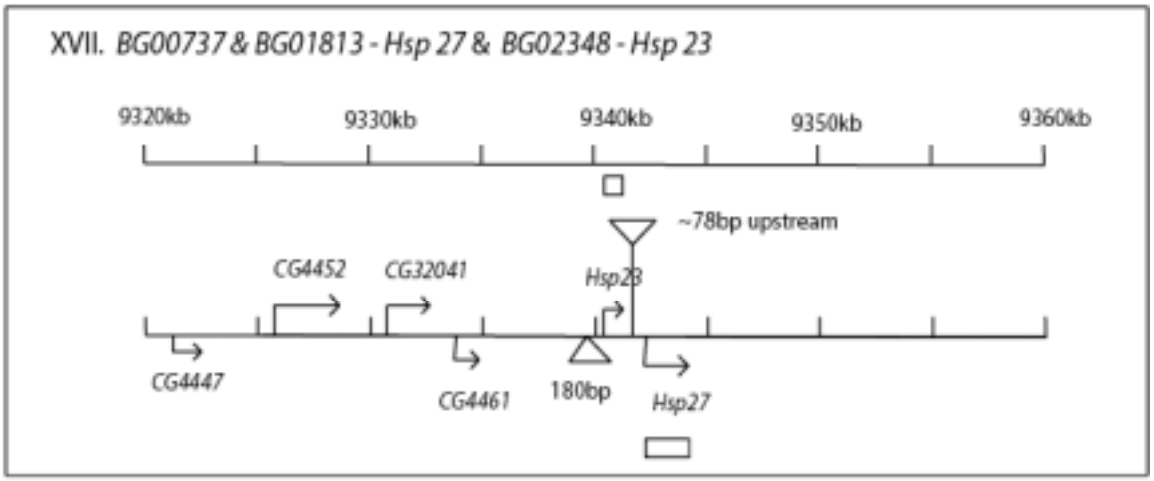
CG16959
CG13457
CG12316
Pdi
CG32147
CG17081
46bp upstream
cG6859
FucTA


XVII. BG00737 & BG01813 - Hsp 27 & BG02348 - Hsp 23
9320kb
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9360kb
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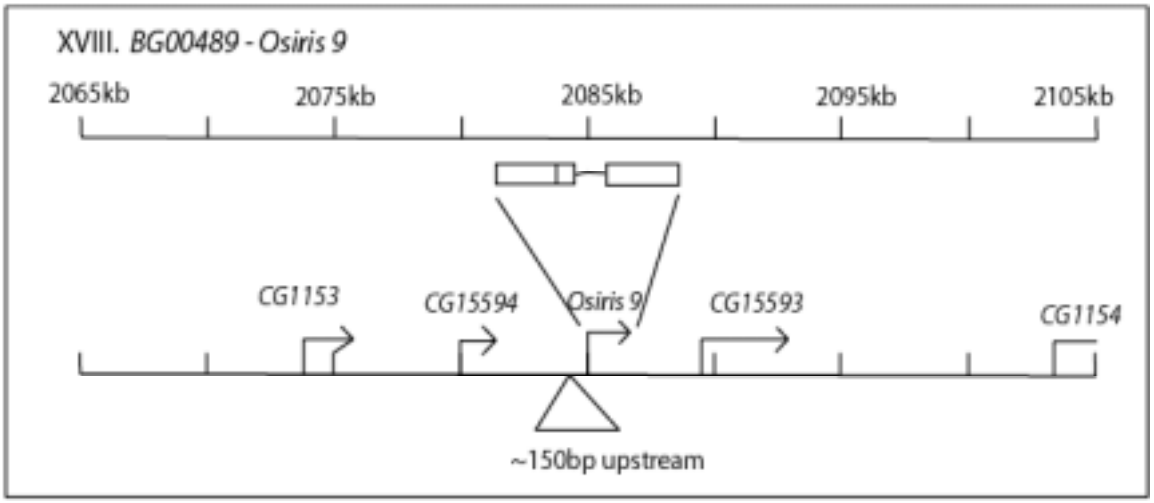
CG32041
Hsp23
CG4447
CG4461
180bp
Hsp27


XVIII. BG00489 - Osiris 9
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2085kb
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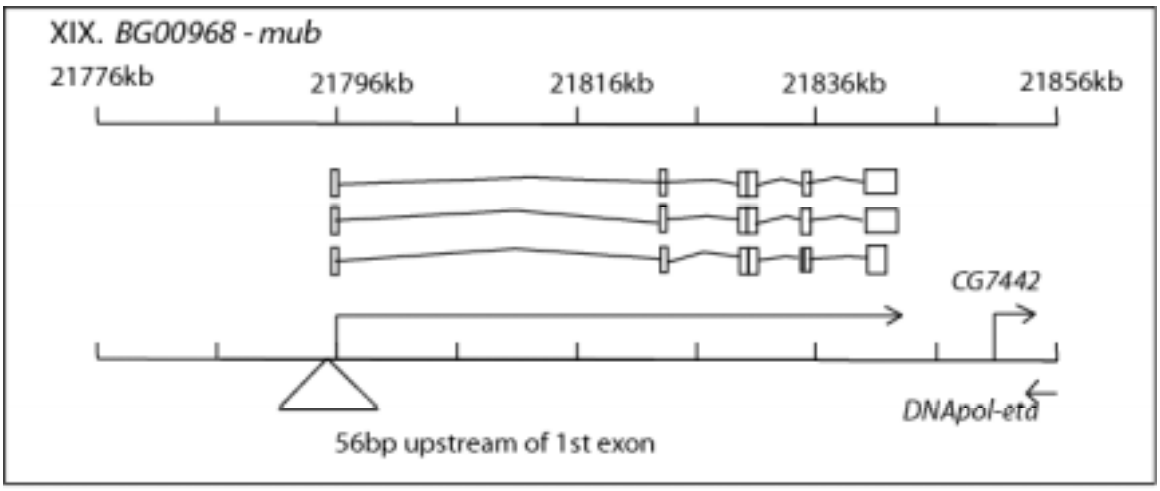
CG1153
CG15594
Osiris 9
CG15593
CG1154
~150bp upstream


XIX. BG00968 - mub
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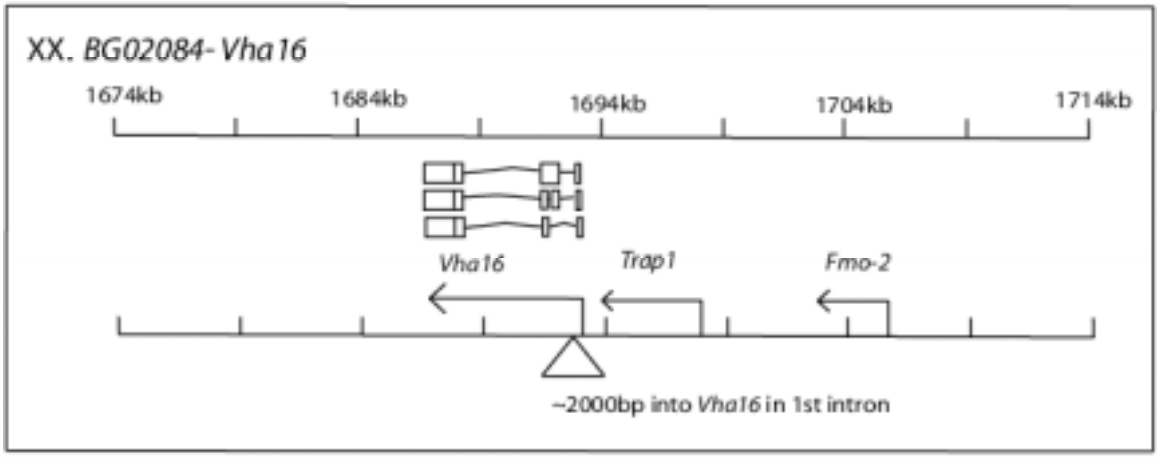
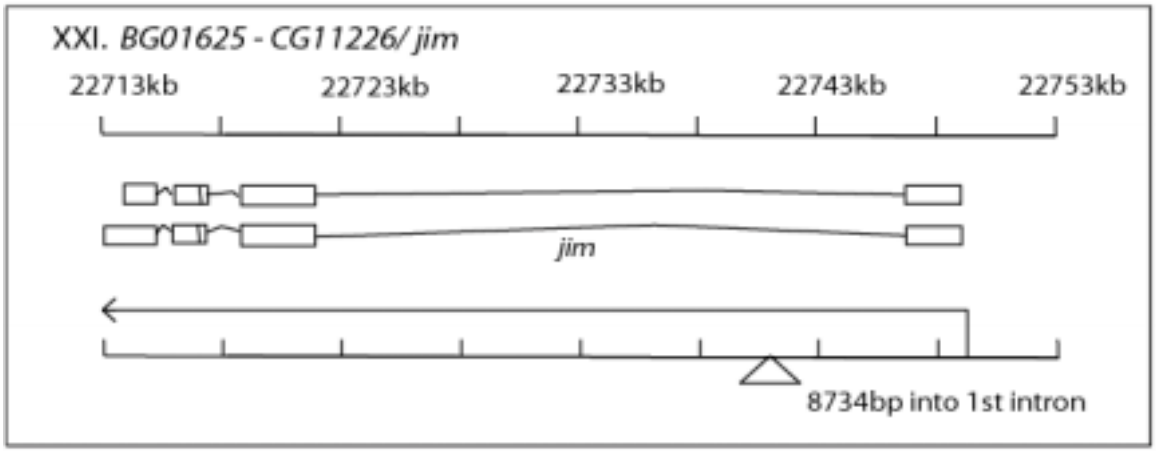
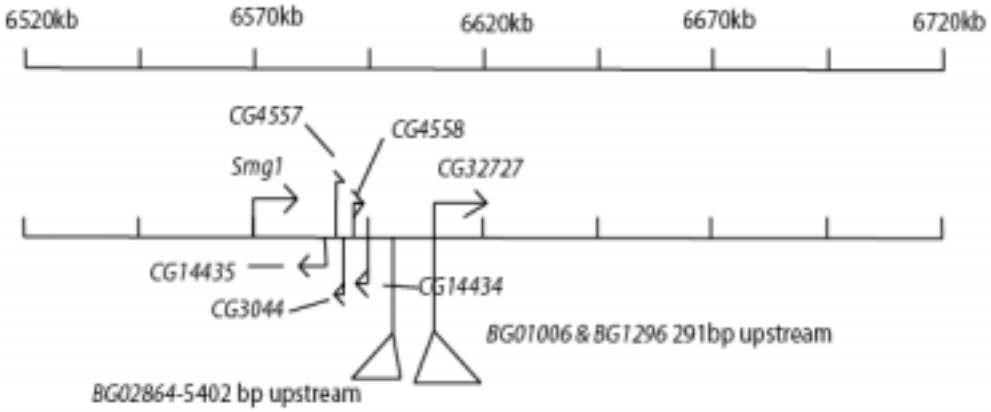
21836kb
21856kb
CG7442
DNApol-eta
56bp upstream of 1st exon

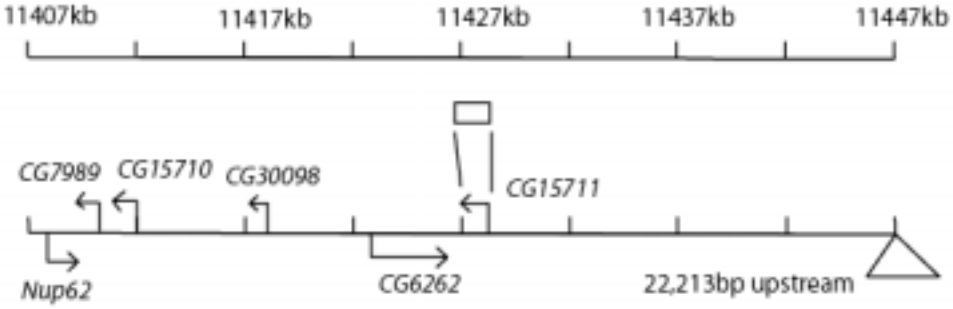

XX. BG02084- Vha16
1674kb
1684kb
1694kb
1704kb
1714kb
Vha16
Trap1
Fmo-2
~2000bp into Vha16 in 1st intron


XXI. BG01625 - CG11226/ jim
22713kb
22723kb
22733kb
22743kb
22753kb
jim
8734bp into 1st intron

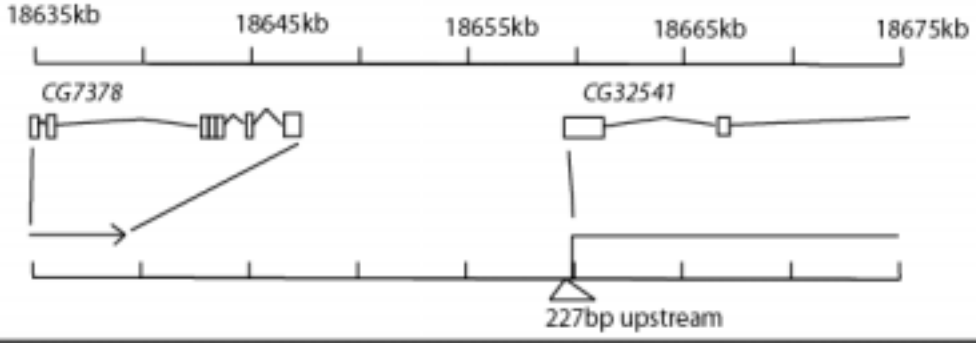
XXII. *BG01006, BG01296, BG02864 - CG4564 or CG32737*

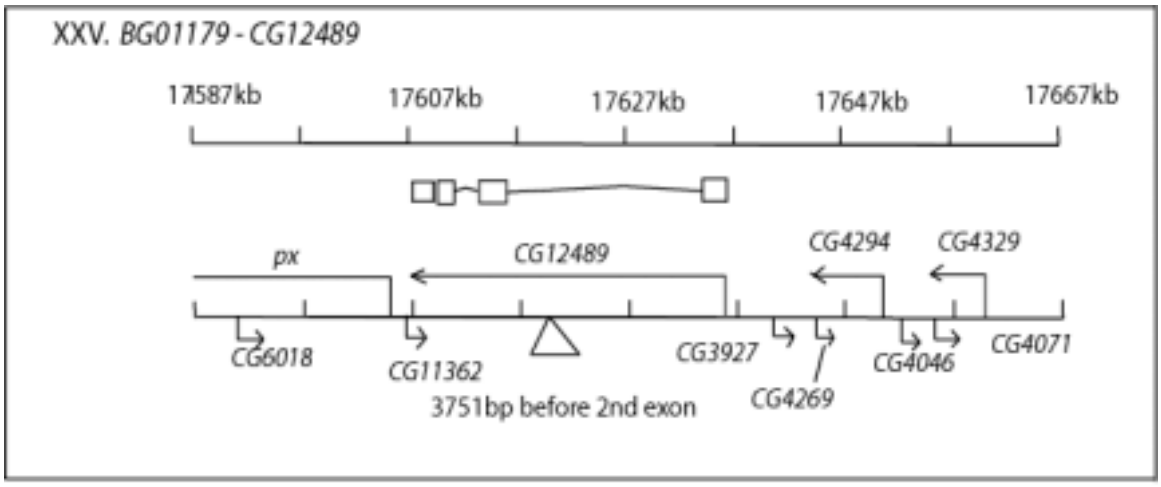


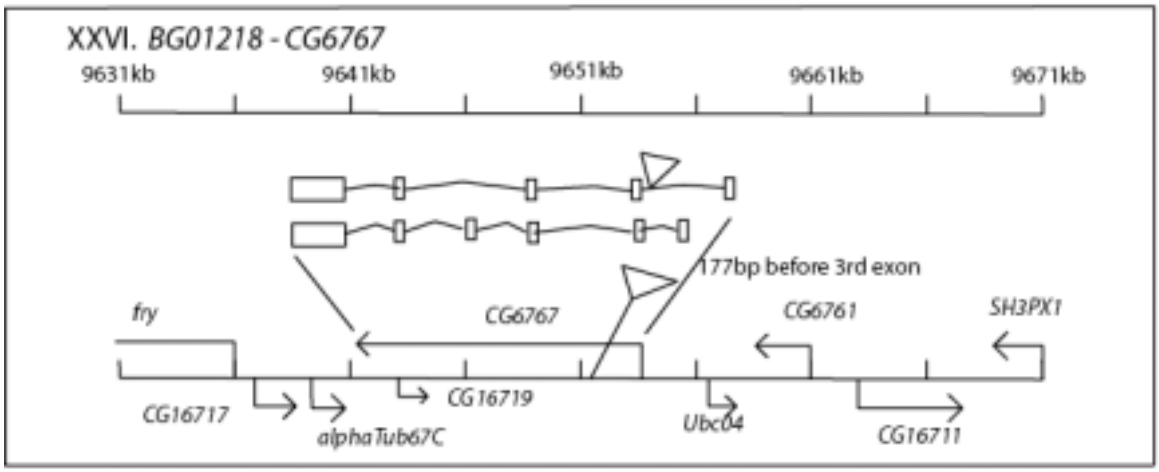

XXIII. *BG02650 - CG15711*

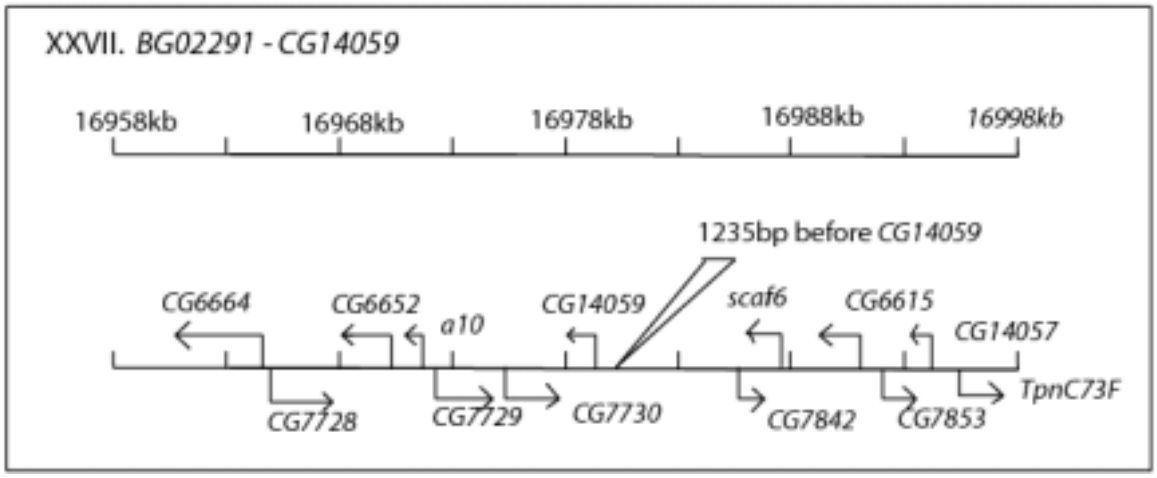



XXIV. *CG00670 - CG7378*




XXV. BG01179 - CG12489
17587kb
17607kb
17627kb
17647kb
17667kb
px
CG12489
CG4294
CG4329
CG6018
CG11362
CG3927
CG4046
CG4071
CG4269
3751bp before 2nd exon


XXVI. BG01218 - CG6767
9631kb
9641kb
9651kb
9661kb
9671kb
177bp before 3rd exon
fry
CG6767
CG6761
SH3PX1
CG16717
alphaTub67C
CG16719
Ubc04
CG16711


XXVII. BG02291 - CG14059
16958kb
16968kb
16978kb
16988kb
16998kb
1235bp before CG14059
CG6664
CG6652
a10
CG14059
scaf6
CG6615
CG14057
CG7728
CG7729
CG7730
CG7842
CG7853
TpnC73F

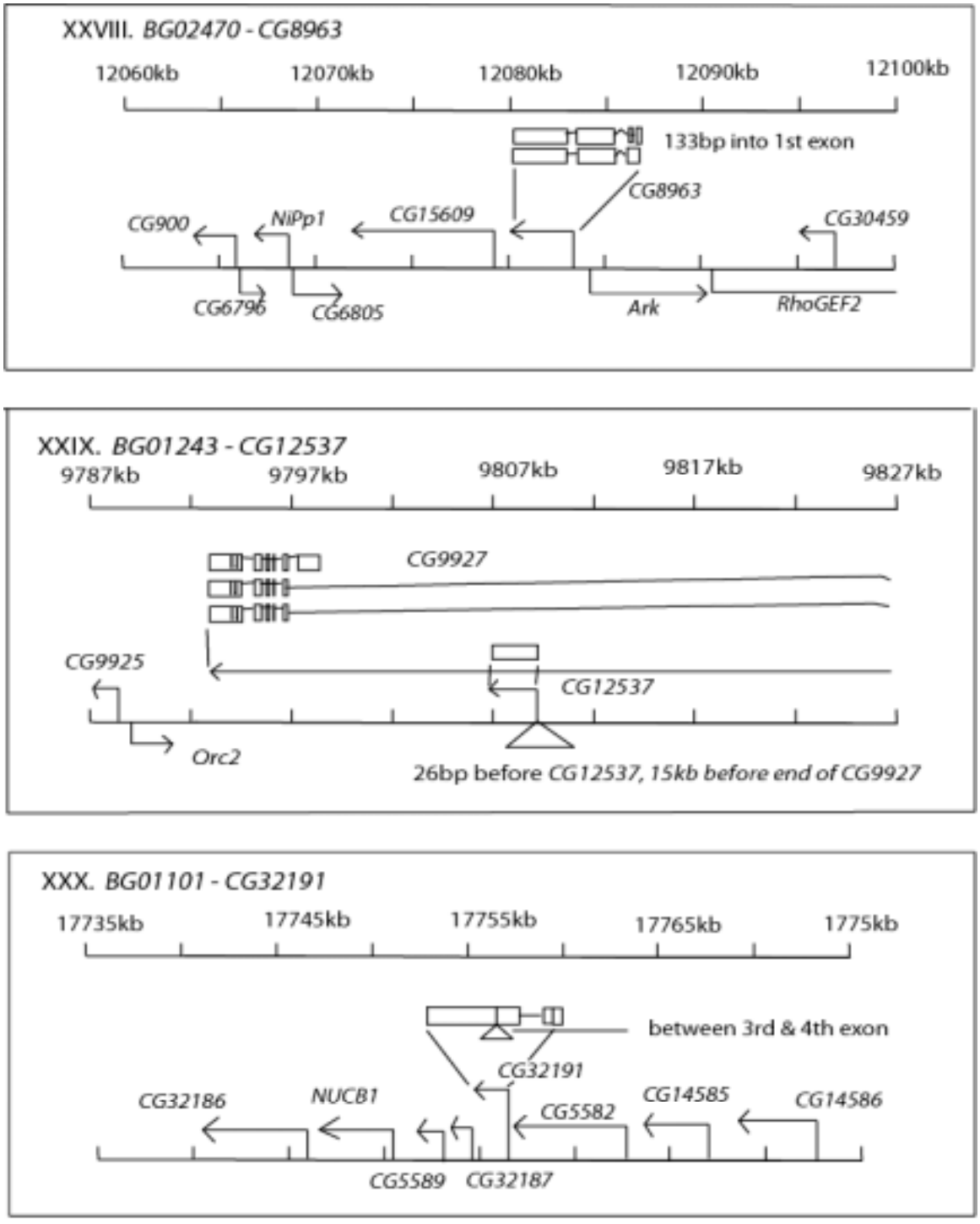
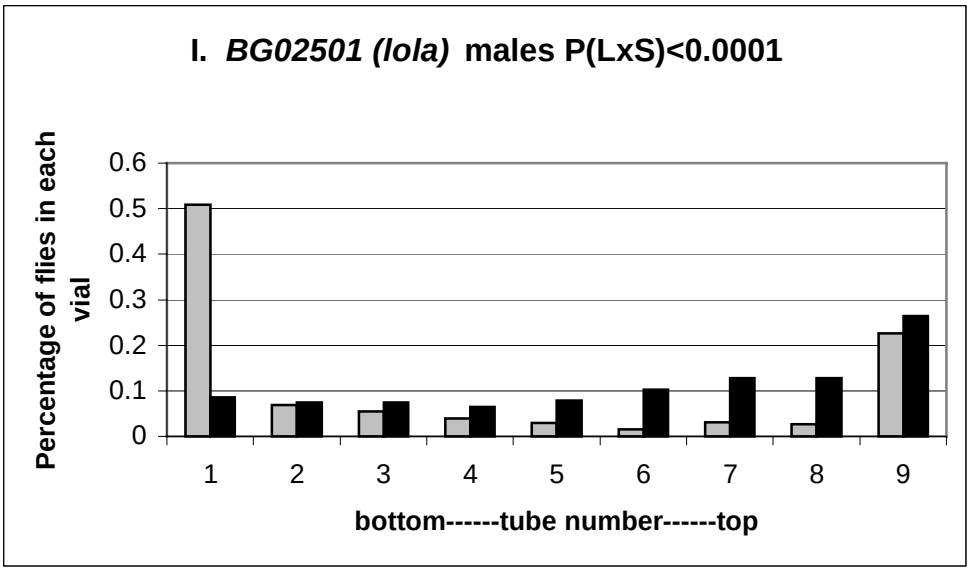
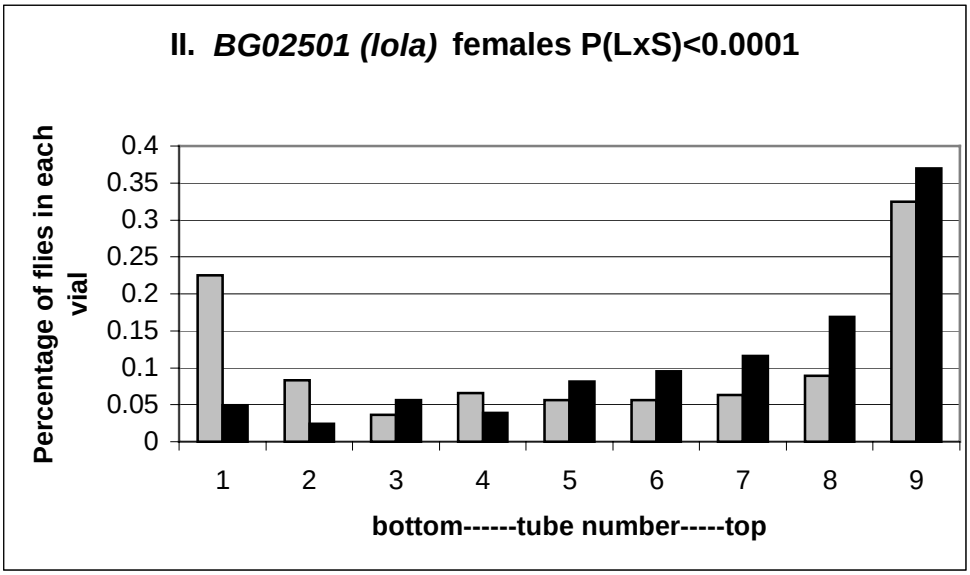
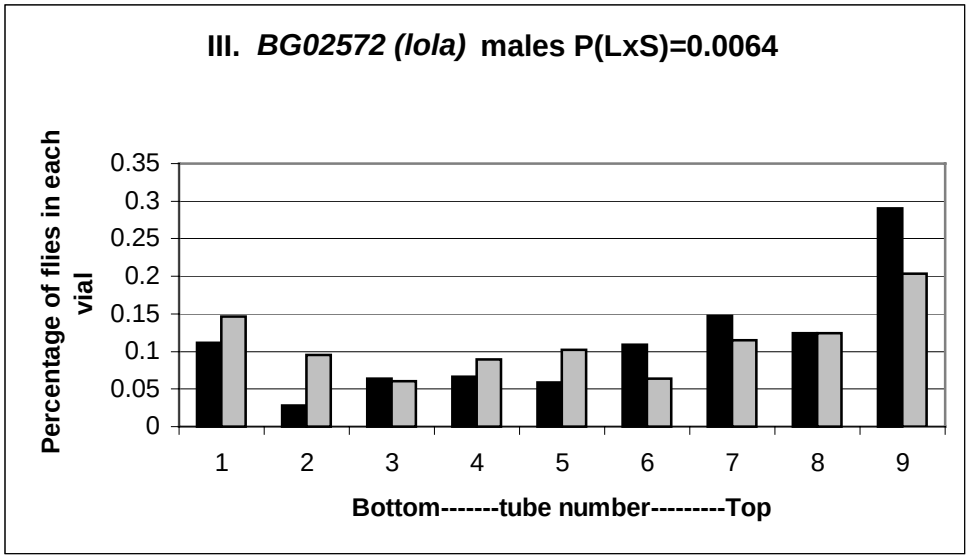


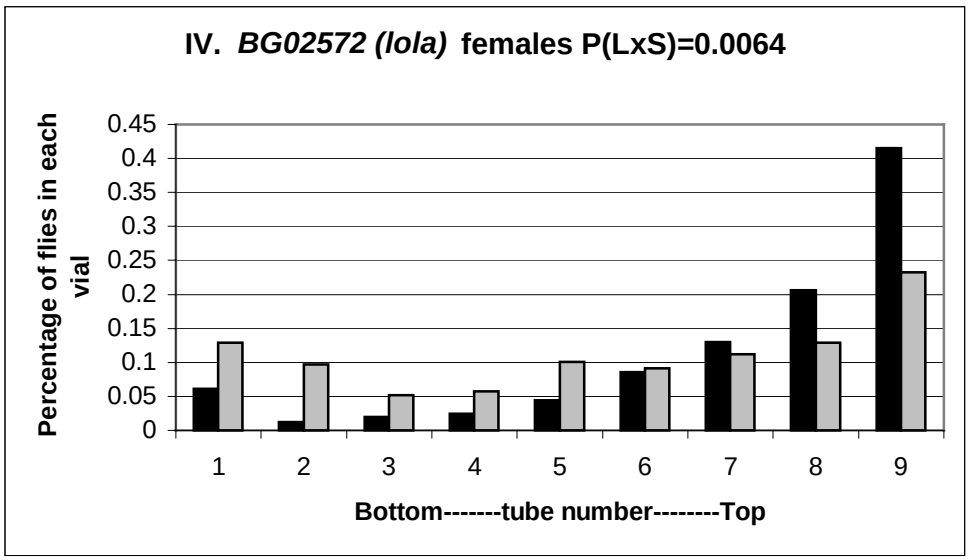

**Figure 3**

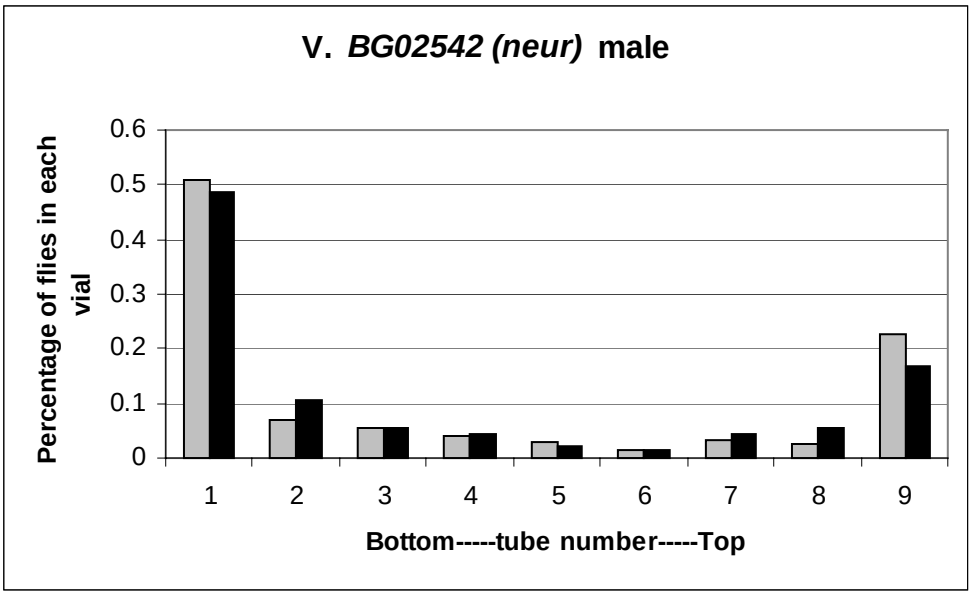
**Schematics of *P*-element insertion sites into the Drosophila genome. Arrowheads indicate *P*-element insertion site. The scale is in kilobases.**

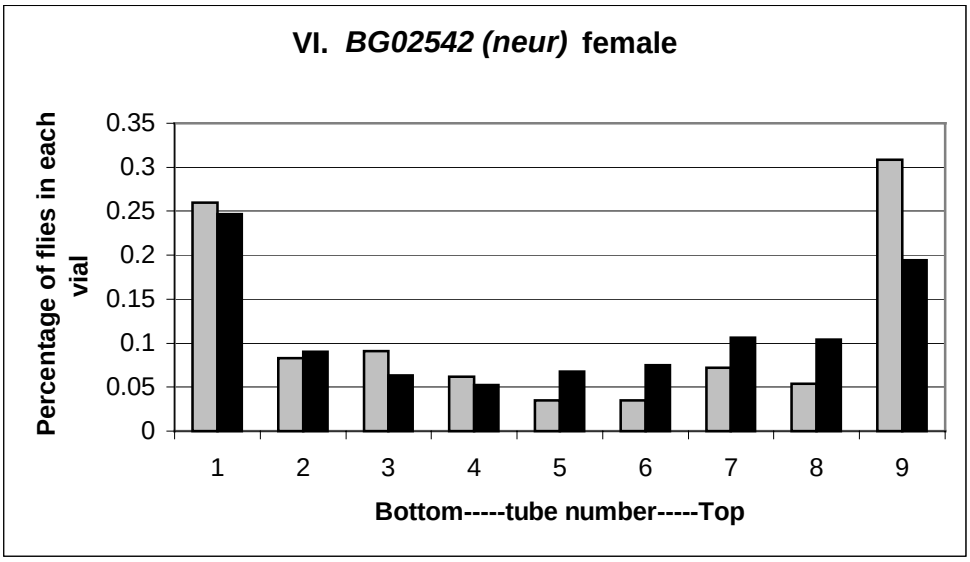

I. BG02501 (lola) males P(LxS)<0.0001
Percentage of flies in each vial
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
bottom------tube number------top

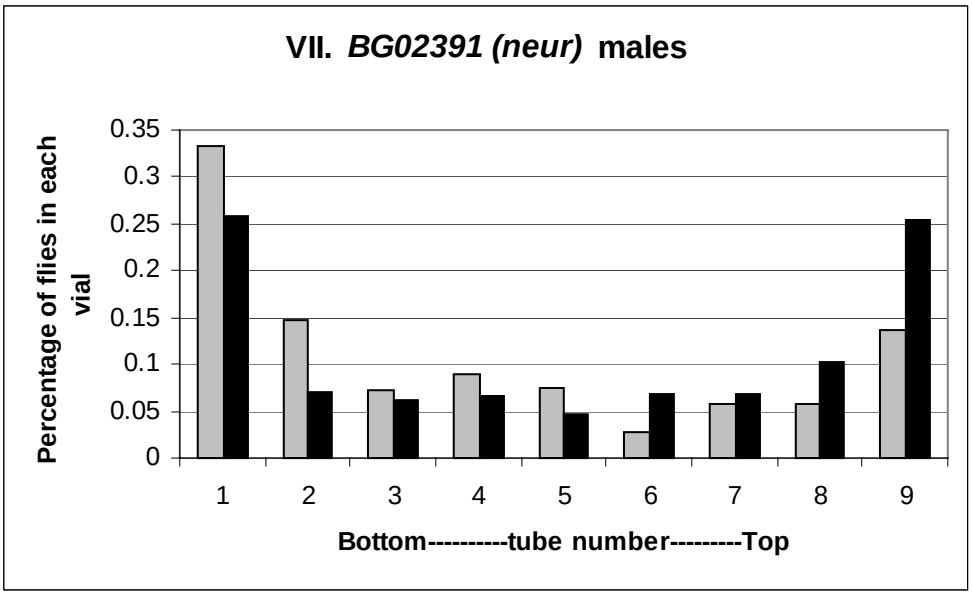

II. BG02501 (lola) females P(LxS)<0.0001
Percentage of flies in each vial
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
bottom------tube number-----top

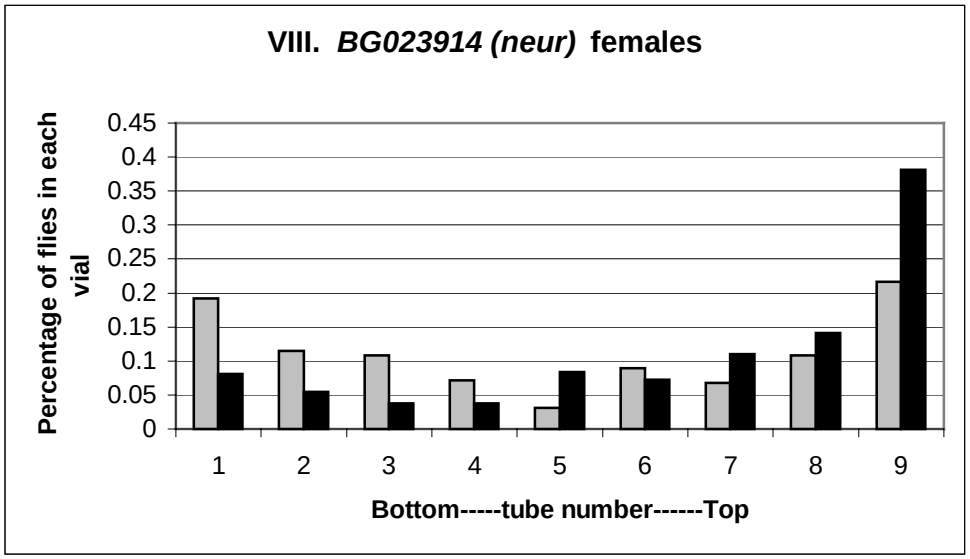

III. BG02572 (lola) males P(LxS)=0.0064
Percentage of flies in each vial
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-------tube number---------Top

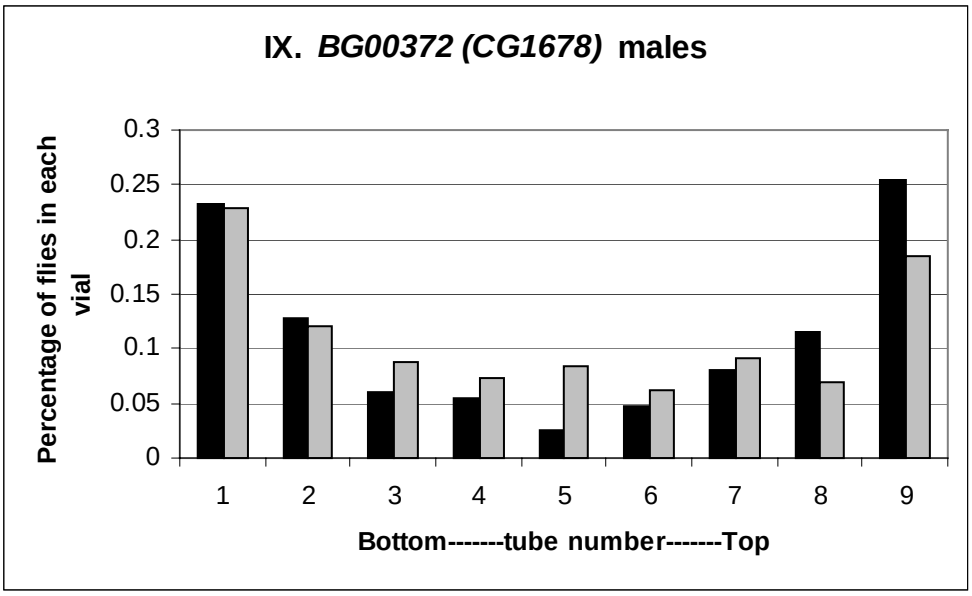

IV. BG02572 (lola) females P(LxS)=0.0064
Percentage of flies in each vial
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-------tube number--------Top

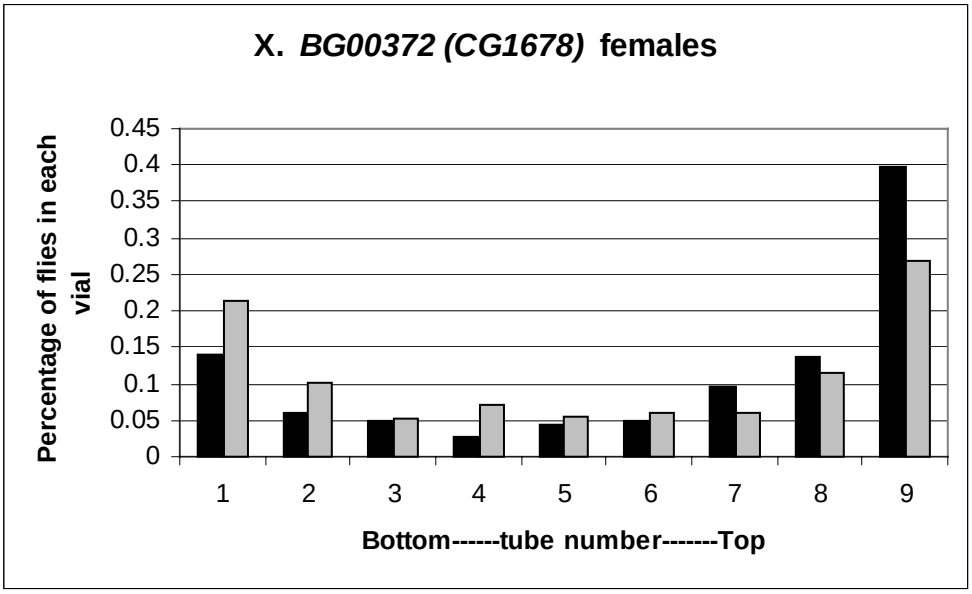

V. BG02542 (neur) male
Percentage of flies in each vial
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
Bottom-----tube number-----Top

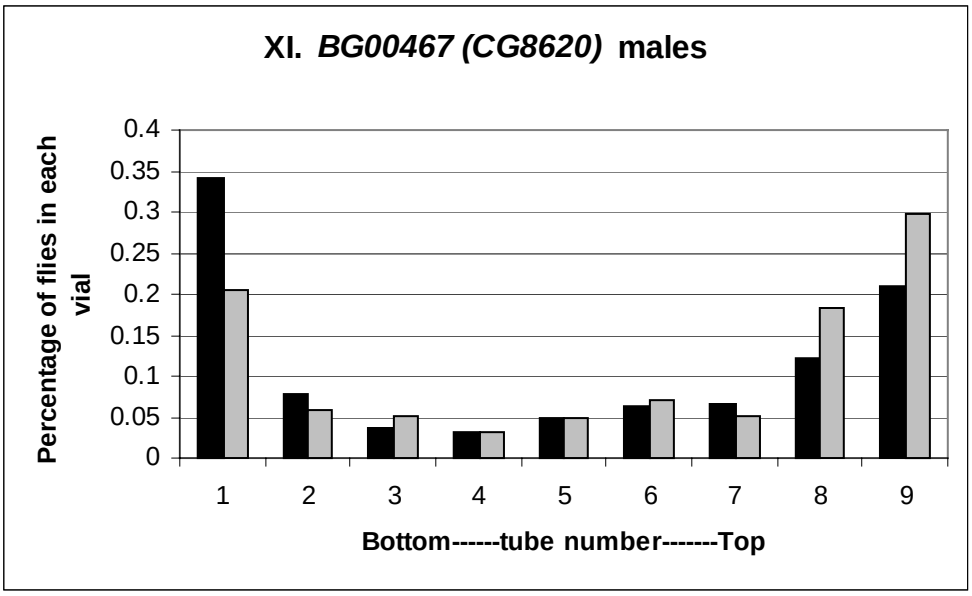

VI. BG02542 (neur) female
Percentage of flies in each vial
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-----tube number-----Top

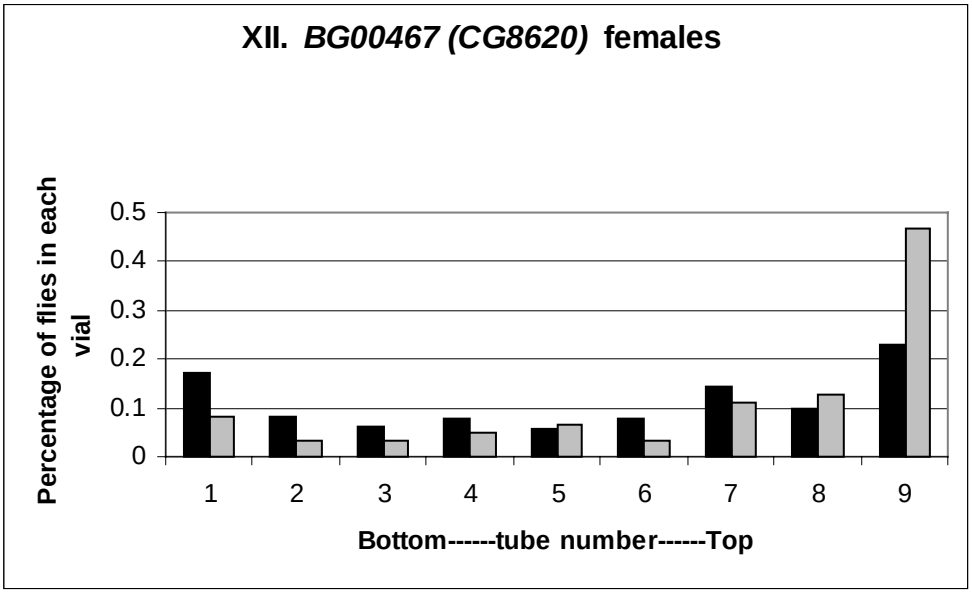

VII. BG02391 (neur) males
Percentage of flies in each vial
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom----------tube number---------Top

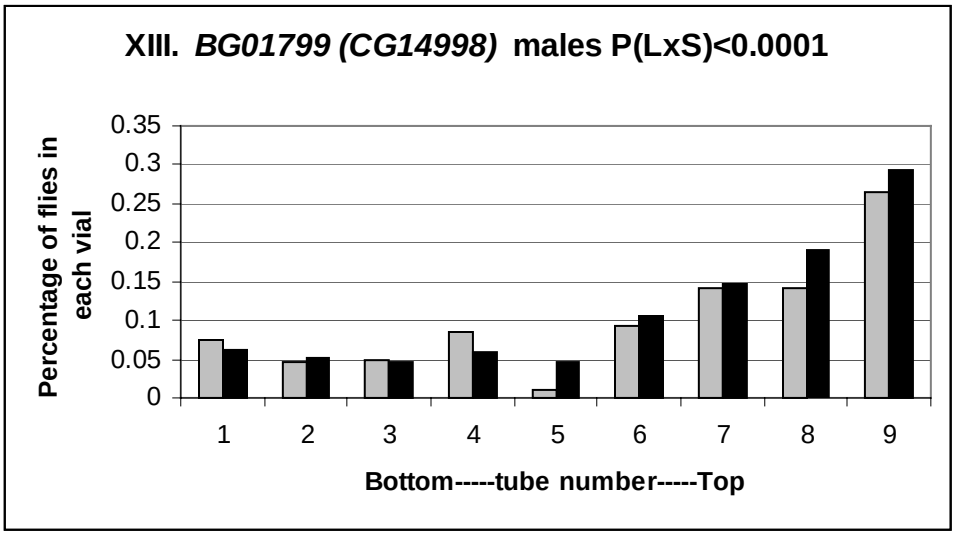

VIII. BG023914 (neur) females
Percentage of flies in each vial
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-----tube number------Top

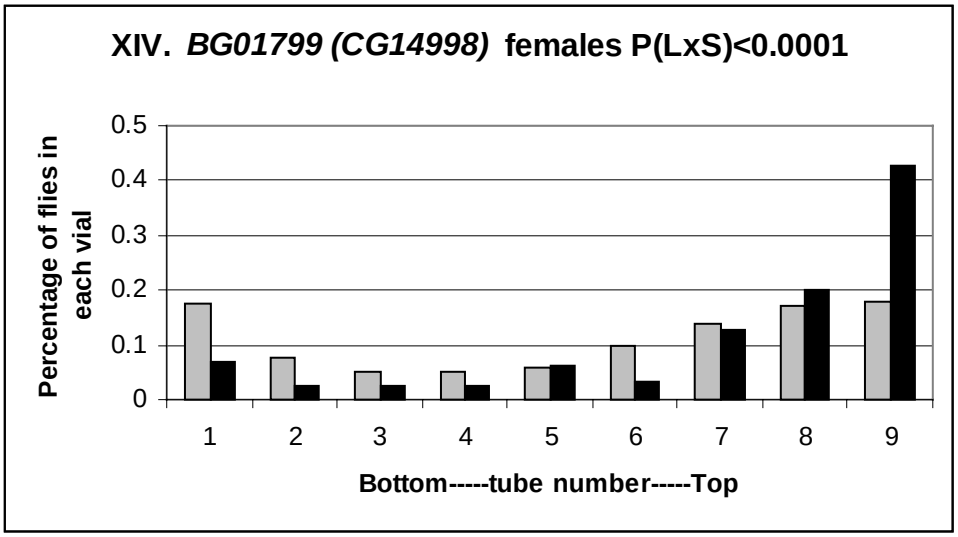

IX. BG00372 (CG1678) males
Percentage of flies in each vial
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-------tube number-------Top

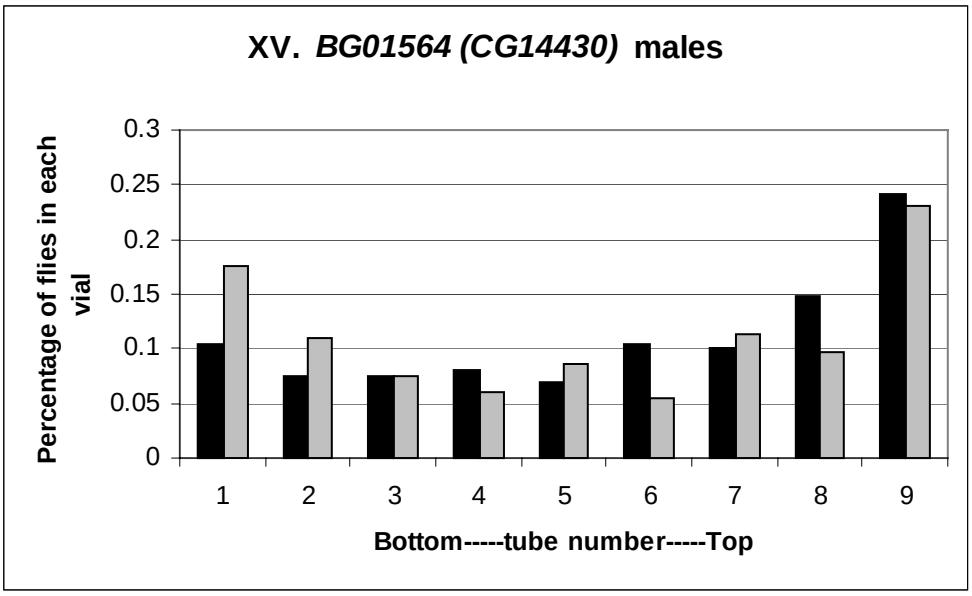

X. BG00372 (CG1678) females
Percentage of flies in each vial
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom------tube number-------Top

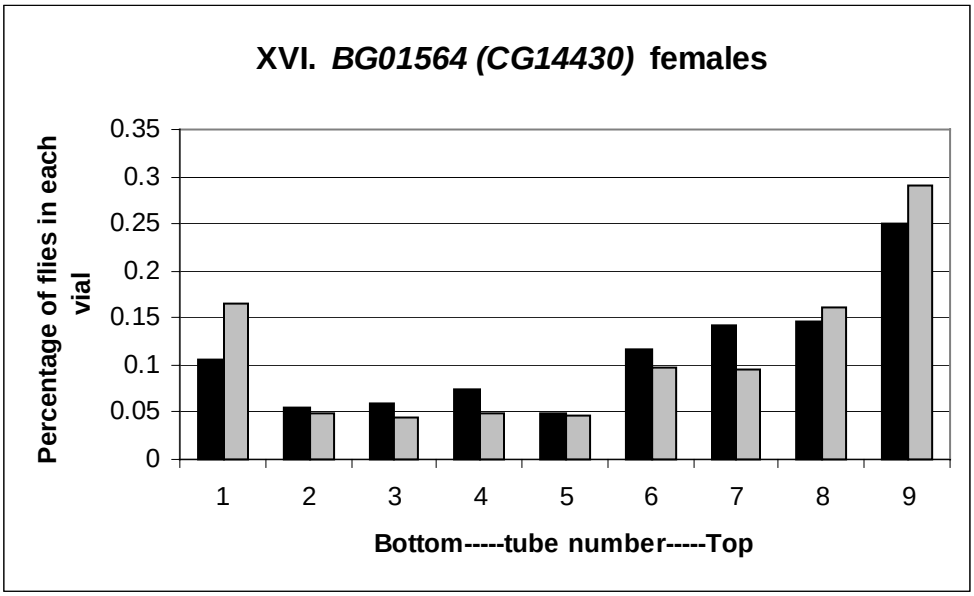

XI. BG00467 (CG8620) males
Percentage of flies in each vial
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom------tube number-------Top

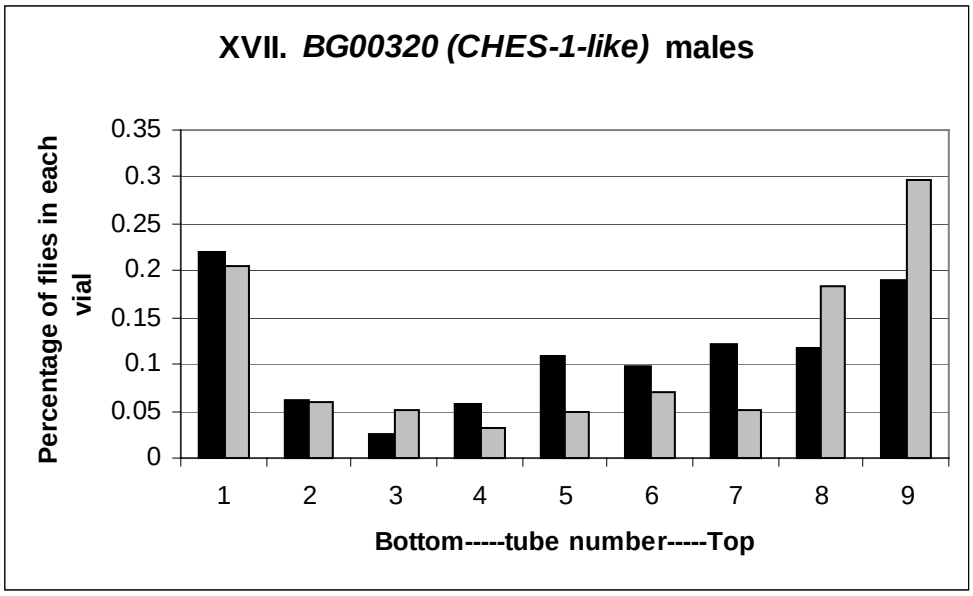

XII. BG00467 (CG8620) females
Percentage of flies in each vial
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
Bottom------tube number------Top

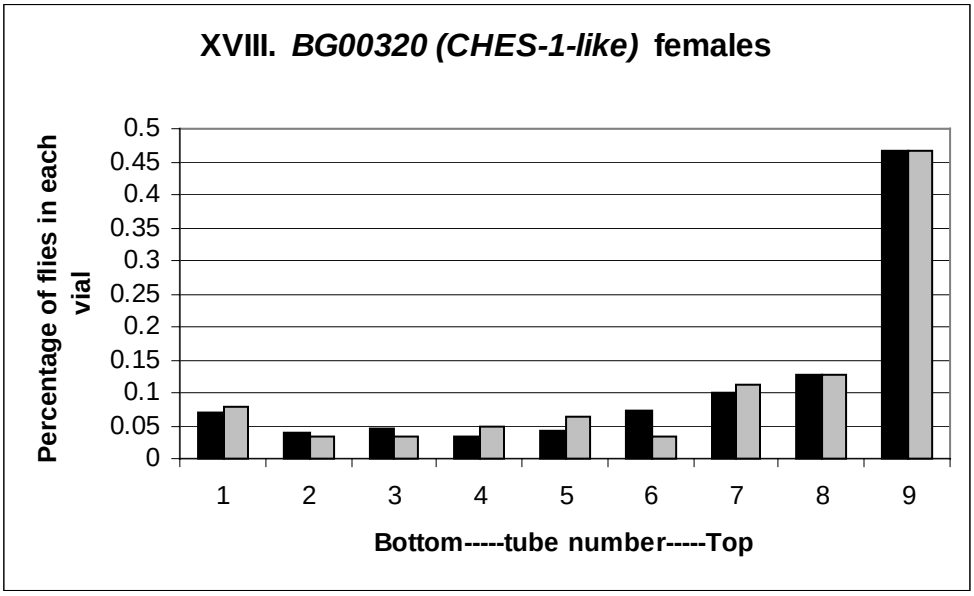

XIII. BG01799 (CG14998) males P(LxS)<0.0001
Percentage of flies in each vial
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-----tube number-----Top

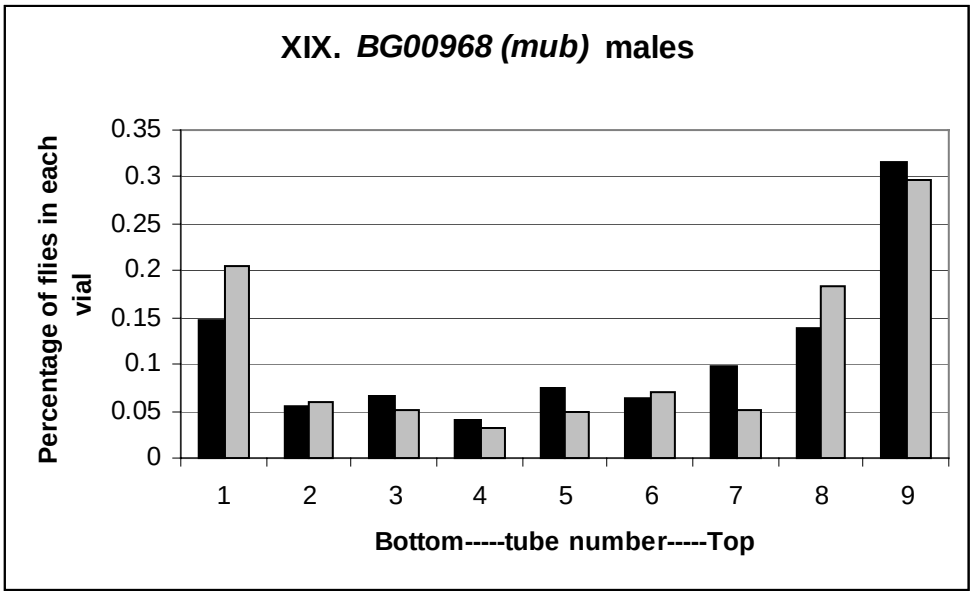

XIV. BG01799 (CG14998) females P(LxS)<0.0001
Percentage of flies in each vial
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
Bottom-----tube number-----Top


XV. BG01564 (CG14430) males
Percentage of flies in each vial
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-----tube number-----Top


XVI. BG01564 (CG14430) females
Percentage of flies in each vial
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-----tube number-----Top


XVII. BG00320 (CHES-1-like) males
Percentage of flies in each vial
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-----tube number-----Top


XVIII. BG00320 (CHES-1-like) females
Percentage of flies in each vial
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1
2
3
4
5
6
7
8
9
Bottom-----tube number-----Top



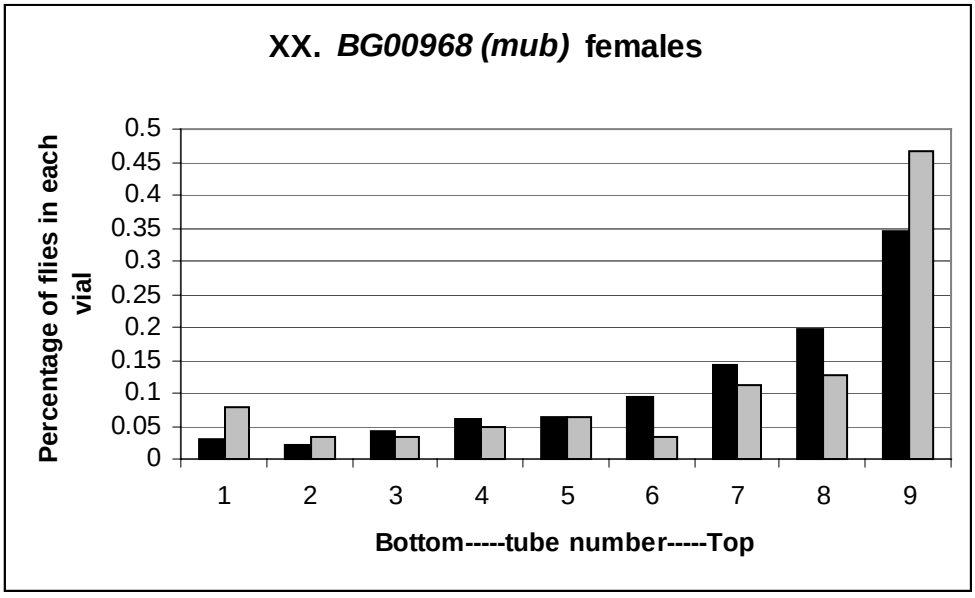
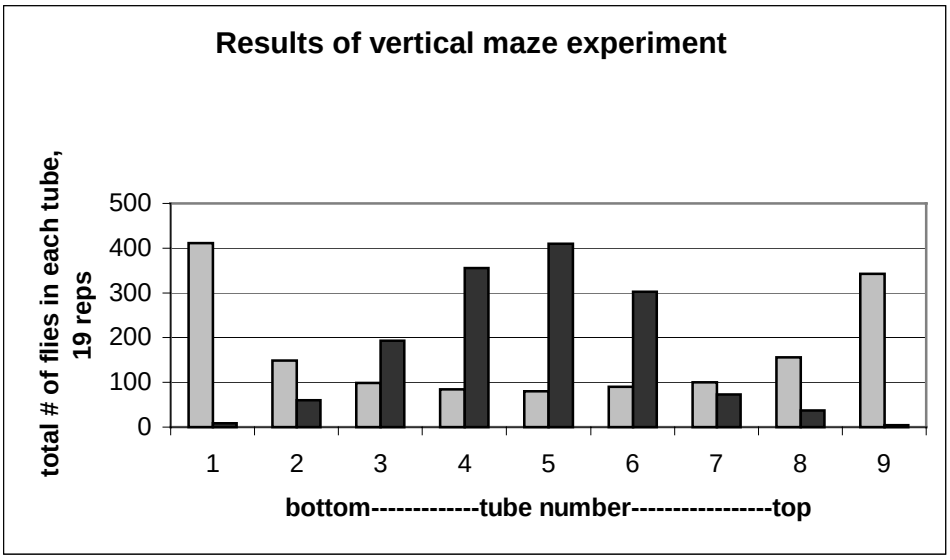




**Figure 4 I-XX**

**Bar graphs show results of maze assays. *P{GTI}* insertion lines are in black, co-isogenic parental lines are gray. Table 6 shows statistical analysis of maze assays. Tube numbers are along the x-axis and the percentage of flies in each vial is denoted along the y-axis.**



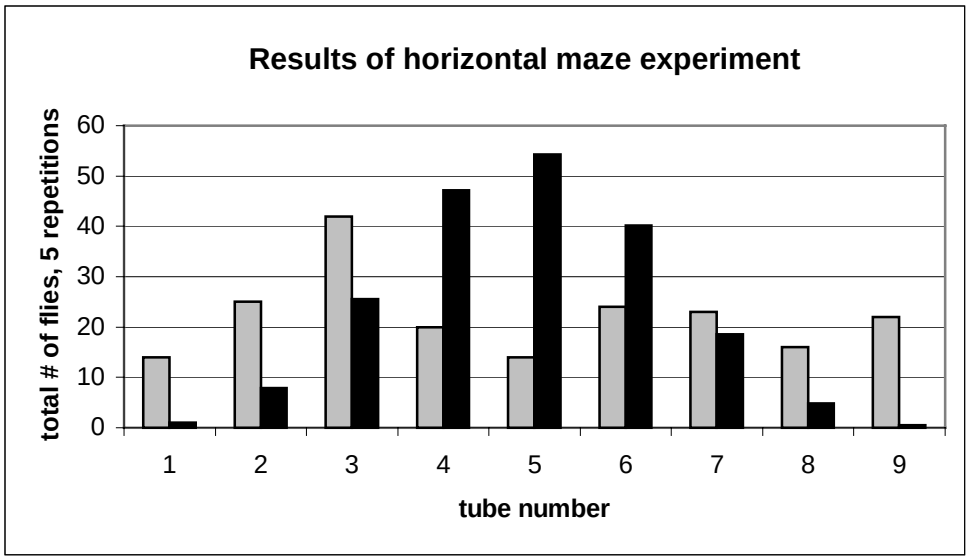




**Figure 5**

**Results of vertical and horizontal maze assays. Vertical maze assays were done during assays of *P*-element insertion lines, totaling 19 replications. 5 replicate with horizontal mazes were done with male co-isogenic B-line flies. Black bars denote expected results; shaded bars denote observed results. Numbers on the y-axis denote total number of flies after five replications.**

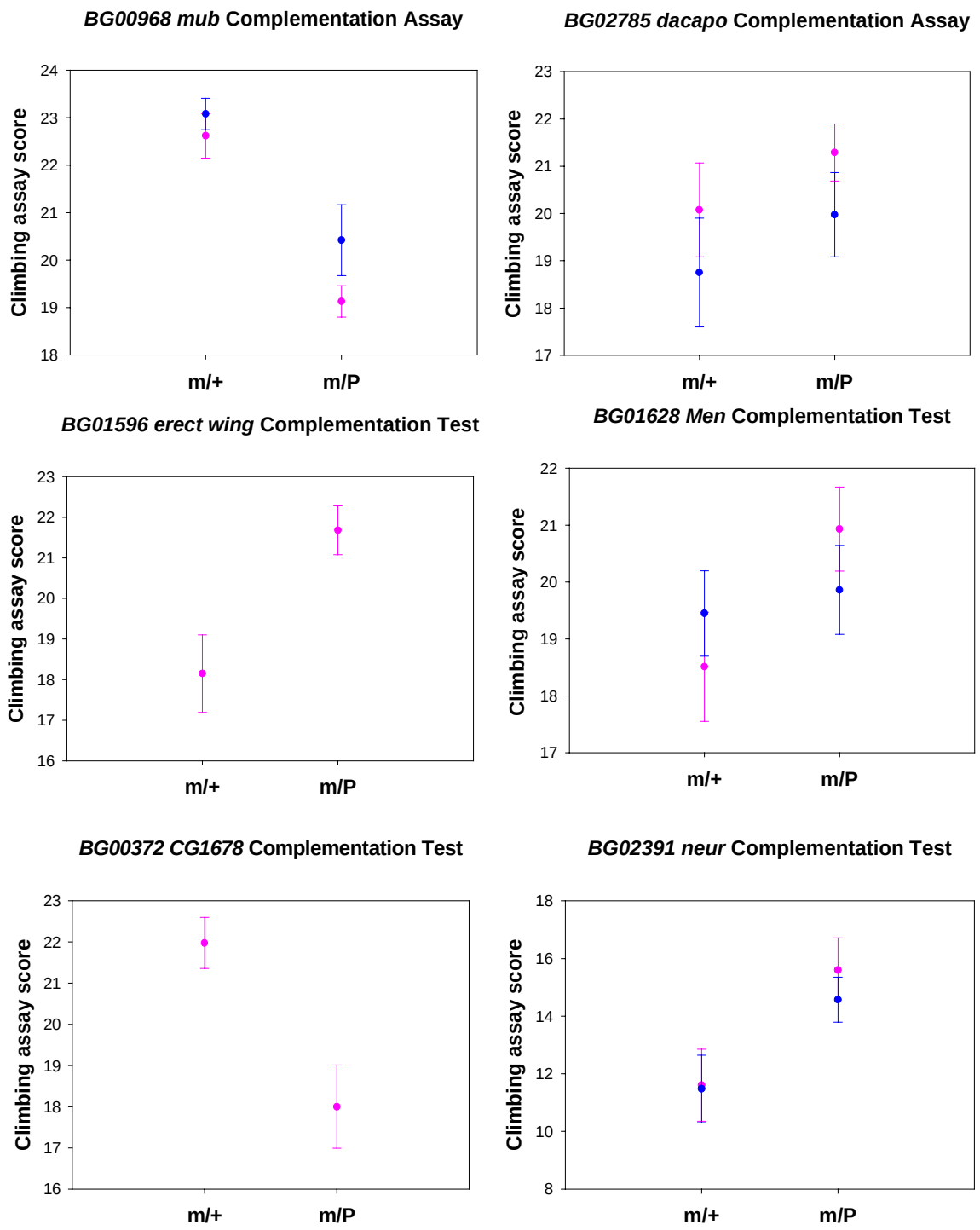



**Figure 6**

**Results of significant complementation tests. +/P denotes parental B-line with the *P*-element and m/P denotes known mutant with the *P*-element. Male scores are in blue, female in pink.**