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Quantitative trait locus mapping of gravitaxis behaviour in *Drosophila melanogaster*

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Summary

Drosophila melanogaster, like other organisms, move and orient themselves in response to the earth's gravitational force. The ability to sense and respond to gravity is essential for an organism to navigate and thrive in its environment. The genes underlying this behaviour in *Drosophila* remain elusive. Using 88 recombinant inbred lines, we have identified four quantitative trait loci (QTLs) that contribute to adult gravitaxis (geotaxis) behaviour in *Drosophila*. Candidate genes of interest were selected from the QTLs of highest significance based on their function in chordotonal organ formation. Quantitative complementation tests with these candidate genes revealed a role for *skittles* in adult gravitaxis behaviour in *D. melanogaster*.

1. Introduction

The force of gravity acts on all living things. Gravity provides organisms with cues as to their position in space, and all organisms move and orient themselves in response to this gravitational force. For example, plants can direct their growth in response to gravity: their roots grow in the same direction as the gravity vector, and their shoots grow in the opposite direction to the gravity vector, towards sunlight. Also, aquatic animals sense gravity to orient themselves in water. This directed movement in response to gravity is known as gravitaxis (geotaxis).

Most animals and plants have specialized organs that sense the gravitational force and signal the organism to respond accordingly. While much is known about the structure of these gravity-sensing organs, less is understood about the genetic and molecular mechanisms underlying the sensation of, and behavioural response to, gravitational force.

Drosophila melanogaster is an excellent model organism to study the genetic basis of behavioural response to gravity. *Drosophila* are easy to maintain, and there are a wide array of tools available for genetic manipulation. Adult fruitflies exhibit walking behaviour in response to gravity: walking up, or away from gravity, is negative gravitaxis, whereas walking down, or towards gravity, is positive gravitaxis. *Drosophila* adults also exhibit negative gravitaxis (walk upward) when startled. In a gravitaxis maze, *Drosophila* are forced to move either up or down at each choice point in order to move through the maze. The number of up or down choices in the gravitaxis maze is used to quantify the response to gravity.

Early studies on the genetic analysis of *Drosophila* gravitaxis are almost 50 years old. Gravitaxis was used by Erlenmeyer-Kimling & Hirsch (1961) to demonstrate that a behavioural phenotype can be analysed genetically. They selected for high and low gravitaxis lines and then analysed the contributions of the various chromosomes to differences in gravitaxis behaviour (Erlenmeyer-Kimling & Hirsch, 1961). Their results showed that these differences in gravitaxis were polygenic and were distributed on the X, second and third chromosomes. Interestingly, the X and second chromosomes contributed to positive gravitaxis, whereas the third chromosomes contributed to negative gravitaxis (Hirsch & Erlenmeyer-Kimling, 1962; Bourguet *et al.*, 2003).

Many years later, a microarray study by Toma *et al.* (2002) identified approximately 250 genes that

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showed expression differences between Hirsch's positive and negative lines. Further investigation revealed that three genes affected gravitaxis behaviour: *Pigment-dispersing factor* (*Pdf*), *Pendulin* (*Pen*) and *cryptochrome* (*cry*). *Pdf* and *cry* also affect the circadian rhythm (Nitabach & Taghert, 2008). Interestingly, Mertens *et al*. (2005) showed that the *Pigment-dispersing factor receptor* (*Pdfr*) also influenced both circadian and gravitaxis behaviours. Finally, Armstrong *et al*. (2006) identified *yuri* as a candidate gene for gravitaxis behaviour through a forward genetic screen. Interestingly, *yuri* is located very close to *Adh*, a gene that mapped close to the difference in gravitaxis between Hirsch's artificially selected lines (Stoltenberg & Hirsch, 1996).

Gravitaxis appears to be a complex behaviour under the regulation of many genes. Quantitative trait locus (QTL) mapping is a common and effective strategy used for the genetic analysis of behavioural traits (Mackay, 2001). QTL mapping uses natural allelic variation to identify genomic regions influencing behaviour (Moehring & Mackay, 2004; Jordan *et al*., 2006; Riedl *et al*., 2007; Bailey *et al*., 2008). Candidate genes within these QTL regions are then assayed using quantitative complementation (Anholt & Mackay, 2004).

We used QTL mapping in *D. melanogaster* to identify significant QTLs for gravitaxis behaviour tested in vertical choice mazes. We screened 88 recombinant inbred (RI) lines that exhibit natural variation in gravitaxis behaviour. Four significant QTLs affecting this phenotype were identified, and we further dissected the QTL region with the highest significance. Candidate genes were selected from this QTL region based on their expression in the chordotonal organ, a structure thought to play a role in gravitaxis in flies. Quantitative complementation allowed us to map the *skittles* gene to this QTL.

2. Materials and methods

(i) *Strains*

All strains and crosses were maintained on a standard yeast–sucrose–agar medium at 25 °C in a 12 h light:12 h dark cycle with lights on at 08·00 h.

(a) *RI lines*

The RI lines used are described in Nuzhdin *et al*. (1997). The RI lines were derived from two unrelated parental lines (Oregon R and 2b), each containing multiple *roo* transposable element insertions distributed throughout the genome. Briefly, the parental lines were crossed and the F_1 progeny were backcrossed to 2b. The progeny of the backcross were then randomly mated for four generations. At the fifth generation, individual pairs were mated, and their progeny underwent 25 generations of full-sib mating. All lines were subsequently maintained by small mass matings of 20 pairs of flies per line per generation. Finally, ten additional generations of mass matings within each line produced the final isogenic RI lines.

Each RI line has a unique combination of the ancestral *roo* transposable element insertions. The presence or absence of these elements in the RI lines indicates the parental origin of the corresponding chromosomal region. The cytological insertion sites of 92 *roo* transposable elements were scored for five individuals per line (Nuzhdin *et al*., 1997). Most lines were homozygous for all markers, but there was some residual heterozygosity for some markers in several lines. Heterozygous markers were considered missing data in subsequent analyses. The recombination frequencies between pairs of *roo* elements were estimated using a Kosambi mapping function (Gurganus *et al*., 1999; Viera *et al*., 2000), allowing these elements to act as genomic markers.

(b) *Quantitative complementation*

Oregon R and the introgressed line 50D-60F were used for quantitative complementation tests of mutations in candidate genes. The genome of 50D-60F is the same as Oregon R, except in the cytological region of 50D-60F, where the genome is the same as 2b.

(c) *Genetic mutants*

The following mutations in candidate genes were obtained from the Bloomington Drosophila Stock Center: $insc^{22}/CyO$, $sktl^{\Delta 20}/CyO$ and $insc^{P49}/CyO$. The *skittles* (*sktl*) gene is nested within an intron of *insc* (Hassan *et al*., 1998). The $insc^{22}$ allele was generated by ethyl methanesulfonate mutagenesis, which created a nucleotide substitution resulting in a premature translational termination codon near the amino-terminus of the coding region and therefore a truncated protein (Buescher *et al*., 1998). This allele affects only *insc* and not *sktl*. $sktl^{\Delta 20}$ is a loss-of-function deletion mutant missing at least part of the open reading frame (Hassan *et al*., 1998). This mutant allele affects only *sktl*. $insc^{P49}$ is an amorphic, loss-of-function deletion mutant that affects both *insc* and *sktl* (Kraut & Campos-Ortega 1996).

(ii) *Gravitaxis maze*

Gravitaxis behaviour was tested using the maze design described in Armstrong *et al*. (2006). Briefly, the maze was assembled from 4·76 mm transparent polypropylene tubing and plastic T-shaped and Y-shaped connectors. This vertical maze design has a single entrance point (in the middle of the maze) and nine exit

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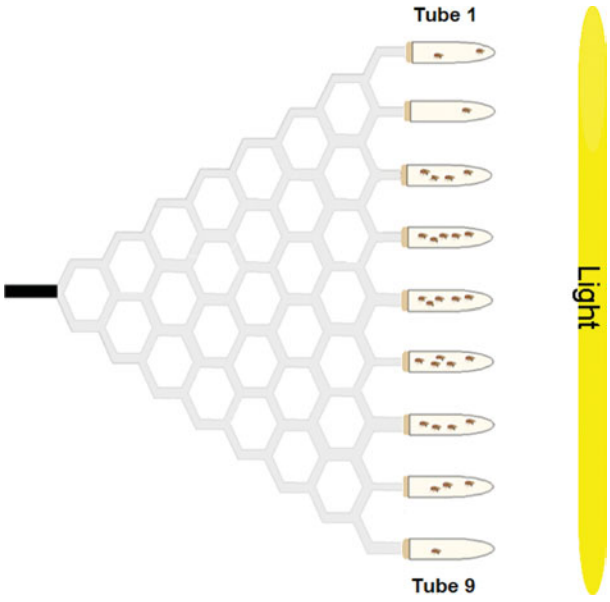

Fig. 1. Choice maze used to assay adult gravitaxis behaviour. *D. melanogaster* flies are loaded into the black entrance tube, and move through eight choice points throughout the maze. At the end of the maze, flies are collected into glass test tubes containing yeast paste. There is a light source at the end of the maze to act as an attractant.

points, offering a total of eight choice points throughout the maze. Flies chose to walk either upwards (geonegative) or downwards (geopositive) at each choice point. The maze was attached to a piece of cardboard (0·79 mm thick) for stability and was covered by a piece of Bristol board.

To place flies in the maze, an 11-cm-long entrance tube was used. This tube was made from a piece of 4·76 mm polypropylene tubing plugged with cotton at one end and covered in black electrical tape. To collect flies from the maze, collecting tubes (15-ml glass test tubes) were attached to each of the nine exit points via plastic tubing that ran through rubber stoppers. These collecting tubes were numbered from 1 to 9, from top to bottom, as was done by Kessler *et al.* (1982). A fly with a score of one made all 'up' choices (high gravitaxis), whereas a fly with a score of nine made all 'down' choices (low gravitaxis). To score the flies in the maze, the total number of flies in each of the nine collecting tubes was counted.

The collecting tubes contained a small amount of yeast paste (distilled water and Baker's yeast) as an attractant. In addition, a 76·2 cm 40 W fluorescent light bulb was positioned vertically at the end of the maze, seven inches away from the collecting tubes. This light source acted as an additional attractant to entice the flies to go through the maze. There was minimal variation in light intensity along the height of the maze. Flies were loaded and run through the maze in red light, so the fluorescent light bulb at the end of the maze was the only source of light in the testing room. A schematic diagram of the maze is shown in Fig. 1.

(iii) *Gravitaxis assay*

Adult male flies (3–7 days old) of a single strain were tested in each gravitaxis maze ($n = 24 \pm 6$ flies per maze). Male flies were used to avoid complicating the effects of female oviposition site preference on maze performance. Young adult (0–72 h old) male flies were collected using CO_2 anaesthesia 2 days prior to the test and were kept on food until the test. Twenty minutes prior to the test, flies were starved on a distilled water–agar substrate (13·89 g agar per litre of dH_2O). To load the flies into the maze, flies were transferred into an entrance tube, and the tube was attached to the maze entrance. Flies were allowed to run through the maze for a total of 3 h. After 1 h, the entrance tube was manually flicked to encourage all flies to leave the tube and enter the maze. After an additional 2 h, the collecting tubes were removed from the maze and plugged with cotton. The number of flies in each collecting tube was counted and a mean exit position was calculated for the strain. Any flies remaining in the mazes were removed.

All experiments were run during the day at room temperature. Up to 40 mazes were tested at the same time. Strain and maze locations were randomized for each test day.

(iv) *QTL screen*

The previously described RI lines were used to identify QTLs that may affect gravitaxis behaviour in adult *D. melanogaster*. Males from 88 different RI lines were screened using the aforementioned gravitaxis assay. All 88 lines were tested each day over five consecutive days. A similar QTL analysis investigating pupation position in *Drosophila* has been performed and is described in Riedl *et al.* (2007).

Briefly, QTL Cartographer (v.1.17) software (Basten *et al.*, 1994; Basten *et al.*, 2003) was used for the QTL analysis of adult gravitaxis behaviour. This software can map QTLs for a particular phenotype to specific genomic regions by plotting a likelihood ratio (LR; the likelihood that a QTL is present at a given genomic location) against the relative genomic positions of the *roo* transposable elements. This ratio reveals the degree of correlation between a certain behaviour and a given genomic position. QTL Cartographer implemented composite interval mapping to calculate the $LR = -2\ln(L_0/L_1)$ and to test the hypothesis that an interval between two neighbouring markers contains a QTL (Basten *et al.*, 1994, 2003; Zeng, 1994; Gurganus *et al.*, 1999). L_0/L_1 is the ratio of the likelihood under the null hypothesis (there is no

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QTL in the given interval) to the likelihood under the alternative hypothesis (there is a QTL in the given interval). Two thresholds of significance (5 and 1%) were used and were calculated by 10 000 random permutations of the data (Churchill & Doerge, 1994; Doerge & Churchill, 1996). For further details, please refer to the QTL Cartographer user's manual (Basten *et al*., 2003).

(v) *Candidate genes*

Subsequent experiments focused on one of the four genomic regions identified by the QTL screen. Candidate genes within this genomic region were identified based on functions related to gravity perception and these genes were tested using quantitative complementation. Quantitative complementation is commonly used to uncover genes underlying significant QTL regions (Gurganus *et al*., 1999; Mackay, 2001).

Briefly, virgin females with heterozygous balanced mutations in candidate genes were crossed with males of two parental strains with opposing gravitaxis phenotypes (Oregon R and 50D-60F). Heterozygous male progeny (parental/mutant and parental/balancer) were tested using the gravitaxis assay. We looked for an interaction where the magnitude of the difference in the gravitaxis phenotype between the parental strains is greater in the presence of the chromosome carrying the mutation compared with the balancer chromosome (a strain-by-chromosome interaction (Riedl *et al*., 2007)), indicating that the candidate gene contributes to adult gravitaxis behaviour (Mackay, 2004). At least three replicates (mazes) per cross were tested over 2 days.

(vi) *Statistical analysis*

Statistical analyses were performed using SigmaStat. There was never a significant day effect and there was never a significant strain-by-day or chromosome-by-day interaction, so in all cases data for the two test days were pooled and analysed using a two-way ANOVA with strain (parental strain, either Oregon R or 50D-60F) and chromosome (mutant chromosome versus balancer chromosome) as sources of variation. A significance value of 0·05 was used.

3. Results

(i) *QTL screen*

The gravitaxis behaviour of adult male flies from 88 RI lines, and from the parental strains Oregon R and 2b, was tested in a large-scale QTL screen. These 88 lines showed behavioural variation in adult gravitaxis, with a mean exit tube position of approximately 4 across all of the lines (Fig. 2). Typically, flies tend to move against the direction of gravity, which may explain the asymmetry in the distribution of RI line scores.

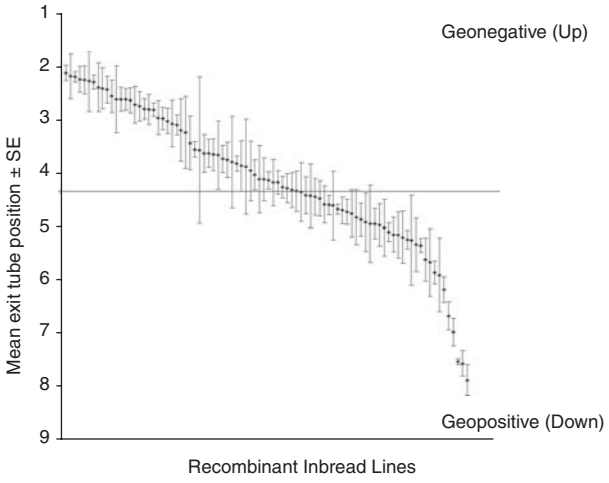

Fig. 2. Gravitaxis behaviour of 88 recombinant *D. melanogaster* inbred lines. These lines showed natural variation in gravitaxis behaviour and a mean exit tube position of approximately 4.

QTL Cartographer identified four significant gravitaxis QTLs on the second and third chromosomes via composite interval mapping (Zeng, 1994; Basten *et al*., 2003). QTL A (LR = 16·24, effect size = 0·46, proportion of variance = 27·4%, second chromosome, cytological region 57C-60E) had an LR above the 1% threshold of significance (LR above 16·03), whereas QTL B (LR = 13·39, effect size = 1·09, proportion of variance = 84·7%, cytological region 61D-65A), QTL C (LR = 13·79, effect size = 0·73, proportion of variance = 65·6%, cytological region 76B-79E) and QTL D (LR = 13·92, effect size = 0·79, proportion of variance = 69·3%, cytological region 85A-85F), all on the third chromosome, had LRs above the 5% threshold of significance (LR above 12·24) (Fig. 3). Subsequent work focused on gravitaxis QTL A, since it had the highest level of significance in the QTL analysis.

(ii) *Oregon R and 50D-60F*

Two lines, Oregon R and the introgressed line 50D-60F, were used as parental strains for quantitative complementation tests with candidate genes. The introgressed region of 50D-60F covers the entire QTL A region. Oregon R had significantly lower gravitaxis behaviour than did the introgressed line 50D-60F ($N = 10$ mazes per strain, Oregon R mean = 4·867 ± 0·3072, 50D-60F mean = 2·698 ± 0·2203, $F_{(1,18)} = 32·92$, $P = 0·0001$). We concluded that these lines differed in their upward versus downward movement in the mazes and were responding to gravitational forces, because when the flies were tested in mazes placed in a horizontal position rather than a vertical position, no strain differences were found (data not shown).

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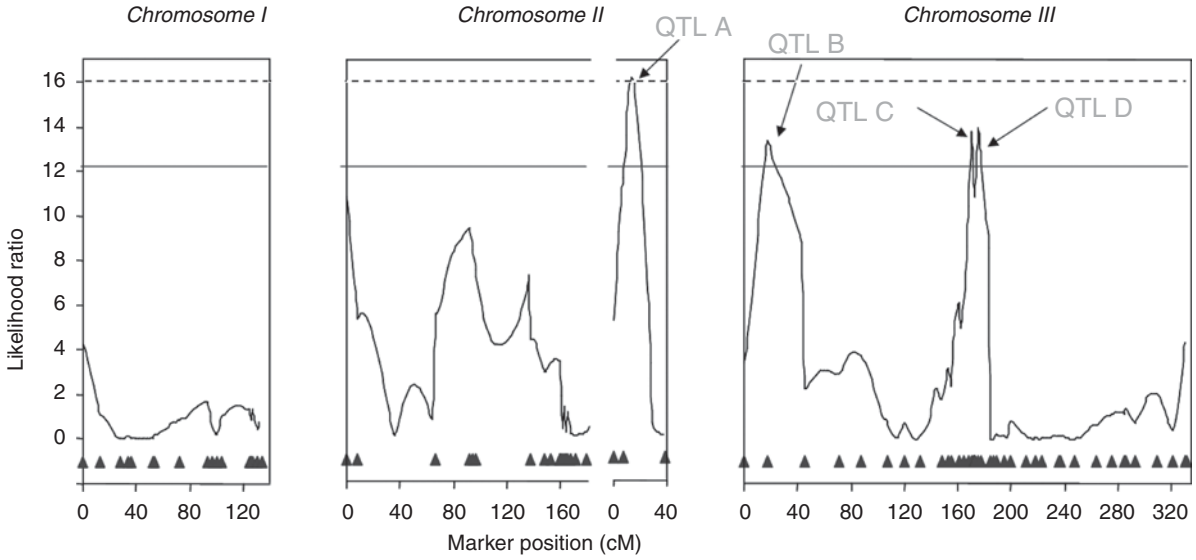

Fig. 3. Results of the QTL screen for gravitaxis behaviour of 88 RI lines of *D. melanogaster*. The genome is divided into the three major chromosomes, and four plots represent the linkage groups. (Since these are RI lines, multiple generations of recombination occurred during their construction, expanding the recombination map (Nuzhdin *et al*., 1997). Because the recombination distance between markers 50F and 57C on chromosome 2 exceeds 50 cM on the expanded map, chromosome 2 is divided into two linkage groups.) The relative marker positions of the *roo* transposable elements are represented by the triangles along the horizontal axis. The likelihood ratio (LR), as calculated by composite interval mapping, is plotted against the recombination map. The LR represents the correlation between the behaviour and variation at each marker position. The solid and dashed lines within the plots indicate the 5 and 1% thresholds of significance, respectively. Four significant QTLs were found: one on chromosome 2 (QTL A) and three on chromosome 3 (QTLs B, C and D).

(iii) *Locomotion*

A correlation analysis was performed to ensure none of the RI lines had locomotor difficulties that might affect their performance in the gravitaxis maze. The analysis revealed an *r*-squared value of 0·02 ($P=0{\cdot}18$), indicating no significant relationship between locomotor ability and maze performance.

(iv) *Candidate genes*

Candidate genes within QTL A were chosen based on their expression in the chordotonal organ, which is a sensory organ in the adult fly that functions in sound or vibration reception (Eberl, 1999; Beckingham *et al*., 2005; Stölting *et al*., 2007). The chordotonal organ is related to vertebrate auditory hair cells (Eberl, 1999) and is thought to play a role in sensing gravitational forces (Eberl, 1999; Beckingham *et al*., 2005). Two candidate genes expressed in this organ were identified within QTL A: *inscuteable* and *skittles*. *inscuteable* (*insc*) encodes a cytoskeletal adaptor protein and is known to be involved in chordotonal organ formation in *D. melanogaster* (Burchard *et al*., 1995; Kania *et al*., 1995; Knirr *et al*., 1997). *insc* is located at 57B3, which falls just outside the region identified in the QTL screen (57C-60E). Candidate genes underlying QTLs are sometimes located just outside the boundaries of the QTL region (Moehring and Mackay, 2004), making *insc* a possible candidate for QTL A.

The other candidate gene, *skittles* (*sktl*), is nested within an intron of *insc* (Hassan *et al*., 1998). *sktl* encodes a 1-phosphatidylinositol-4-phosphate 5-kinase, and is also known to be involved in chordotonal organ development (Kania *et al*., 1995). We determined whether one or both of these genes are responsible for the gravitaxis differences that mapped to QTL A.

Three mutant alleles, $insc^{22}/CyO$, $sktl^{\Delta 20}/CyO$ and $insc^{P49}/CyO$, were tested using quantitative complementation with Oregon R and 50D-60F. Tests with $insc^{P49}$ (affecting both *insc* and *sktl*) revealed a significant strain (parental strain, either Oregon R or 50D-60F) by chromosome (mutant versus balancer chromosome) interaction, where the magnitude of the difference between Oregon R and 50D-60F was significantly greater in the presence of the mutant chromosome than the balancer chromosome (strain $F_{(1,28)}=18{\cdot}716$, $P<0{\cdot}001$, chromosome $F_{(1,28)}=53{\cdot}972$, $P<0{\cdot}001$, strain × chromosome $F_{(1,28)}=20{\cdot}453$, $P<0{\cdot}001$ (Fig. 4*a*)). The results with $insc^{22}$ (affecting only *insc*) revealed no significant strain-by-chromosome interaction (strain $F_{(1,30)}=4{\cdot}286$, $P=0{\cdot}047$, chromosome $F_{(1,30)}=29{\cdot}618$, $P<0{\cdot}001$, strain × chromosome $F_{(1,30)}=0{\cdot}222$, $P=0{\cdot}641$ (Fig. 4*b*)). The $sktl^{\Delta 20}$ (affecting only *sktl*) results revealed a significant strain-by-chromosome interaction (strain $F_{(1,34)}=10{\cdot}389$, $P=0{\cdot}003$, chromosome $F_{(1,34)}=62{\cdot}345$, $P<0{\cdot}001$,

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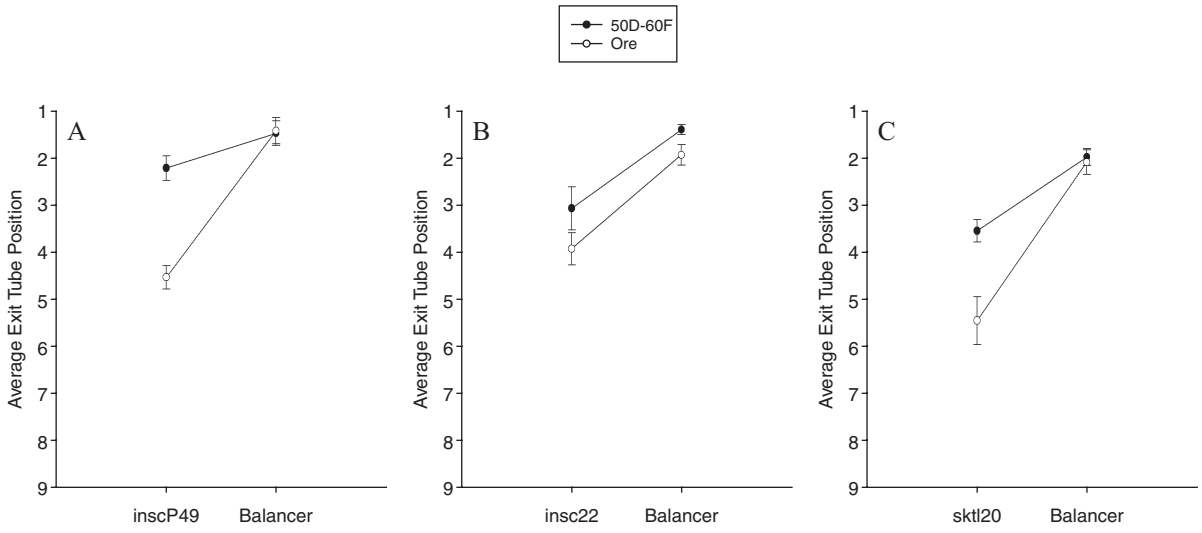

Fig. 4. Quantitative complementation tests of candidate genes with 50D-60F (solid circle) and Ore (open circle). (*a*) $insc^{P49}$ shows a significant strain by chromosome interaction (two-way ANOVA, $P<0{\cdot}001$). (*b*) $insc^{22}$ shows no significant strain by chromosome interaction (two-way ANOVA, $P=0{\cdot}641$). (*c*) $sktl^{\Delta 20}$ shows a significant strain-by-chromosome interaction (two-way ANOVA, $P=0{\cdot}007$). Since $insc^{P49}$ affects both *insc* and *sktl*, these results suggest that *skittles* is involved in adult gravitaxis behaviour in *D. melanogaster*.

strain × chromosome $F_{(1,34)}=8{\cdot}297$, $P=0{\cdot}007$ (Fig. 4*c*)). Taken together, these results suggest that *skittles* is a candidate gene involved in natural variation in adult gravitaxis behaviour in *D. melanogaster*.

4. Discussion

Little is known about the genetic and molecular bases of gravitational response in *D. melanogaster*. Previous studies have demonstrated that natural variation in gravitaxis behaviour is likely a complex trait influenced by multiple genes (Hirsch & Erlenmeyer-Kimling, 1962; McGuire, 1992; McMillan & McGuire, 1992). More recent studies (see below) have begun to identify not only the genes influencing this behaviour, but the physiological and molecular mechanisms by which fruitflies sense and respond to gravity (Toma *et al*., 2002; Beckingham *et al*., 2005; Armstrong *et al*., 2006; Baker *et al*., 2007). In the present study, we found four QTL regions responsible for natural variation in adult gravitaxis behaviour in our *D. melanogaster* RI lines. We also identified *skittles* as a gene important for variation in gravitaxis behaviour in these lines.

(i) *QTL screen*

Previous studies have used microarrays or forward genetics to investigate genes that influence gravitaxis behaviour. The present study is the first to perform a genome-wide QTL screen of *D. melanogaster* gravitaxis behaviour. Further screens using different parental lines will undoubtedly identify more genes that contribute to natural variation in gravitaxis behaviour. We identified four genomic regions that contribute to natural variation in gravitaxis behaviour in our RI lines. While this study focused on only one of the four QTL regions, each of the remaining three regions contains at least one gene influencing gravitaxis. For example, *atonal* is located at cytological position 84F and falls just outside the boundaries of QTL D (85A-85F). *atonal* is an intriguing candidate gene because it disrupts chordotonal neuron differentiation, where *atonal* mutants completely lack the chordotonal sense organs (Jarman *et al*., 1995).

(ii) *Candidate genes*

Most animals have specific organs that sense gravitational forces. For example, the vestibular apparatus of the vertebrate ear contains small particles (otoliths) that move and stimulate mechanosensory hair cells within the ear in response to gravity. The stimulation of these hair cells sends a signal to the brain prompting the animal to orient and balance itself accordingly (Beckingham *et al*., 2005). Similar organs and mechanisms are found in marine invertebrates, but there is no known specific gravity-sensing organ in most arthropods, including *D. melanogaster*.

While specific organs have not yet been identified in *Drosophila*, gravitational response is believed to be mediated by mechanosensory structures, similar to the hair cells in the vertebrate ear. In insects, both bristle type and scolopidial type mechanoreceptors appear to play a role in gravity response (Horn, 1985). Scolopidial mechanoreceptors respond to stretch and are found in chordotonal organs, which are related to vertebrate auditory hair cells (Eberl, 1999; Kernan,

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2007). The Johnston's organ (JO) is a chordotonal type organ analogous to the vertebrate ear and is found in the second antennal segment of *Drosophila* (Eberl & Boekhoff-Falk, 2007; Kernan, 2007). The JO responds to sound, vibration and gravity (Eberl, 1999; Jarman, 2002; Kernan, 2007; Kamikouchi *et al*., 2009).

Three previous studies have implicated the JO in adult gravitaxis behaviour in *Drosophila* (Armstrong *et al*., 2006; Baker *et al*., 2007; Kamikouchi *et al*., 2009). Kamikouchi *et al*. (2009) identified a role for specific neuronal clusters of the JO in adult gravitaxis behaviour. Inactivation of these neuronal clusters abolished the typical negative gravitaxis behaviour seen in wild-type flies. Interestingly, ablation of the second antennal segment (that which contains the JO) did not completely abolish the typical negative gravitaxis behaviour. It is therefore possible that other organs are involved in gravity sensation in *Drosophila*.

Baker *et al*. (2007) showed that disrupting synaptic transmission in neurons projecting from the JO to the brain inhibits gravitaxis maze behaviour in *Drosophila*. Taken together, this information led to the selection of our gravitaxis behaviour candidate genes for QTL A (*insc* and *sktl*) based on their role in chordotonal organ development. Our results show that *sktl* but not *insc* influences adult gravitaxis behaviour. *sktl* encodes a phosphatidylinositol 4-phosphate 5-kinase involved in signal transduction and thus could act in the sensory mechanotransduction pathway that converts gravity sensation into a behavioural response (Hassan *et al*., 1998).

Baker *et al*. (2007) also looked at known expression patterns of lines with aberrant gravitaxis maze behaviours and found that many of these lines have significantly increased expression in certain neural structures, such as the central complex (CC) and the antennal lobes. Synaptic transmission was inactivated in these candidate neural structures using the GAL4-UAS*shi*^{ts} system, and this inactivation altered gravitaxis behaviour, implicating the CC and antennal lobes as neural structures important for gravitaxis behaviour. The CC is a central region of the fly brain known to play a role in the integration of sensory information and motor output. Several substructures of the CC have been identified and manipulated in *Drosophila*, making this an excellent candidate region for the investigation of the neural substrates and circuitry underlying gravitaxis behaviour (Strauss & Heisenberg, 1993).

Interestingly, a recent publication studied graviperception in *Zea mays* (Perera *et al*., 1999). The authors studied the pulvinus (the joint at the base of a leaf that facilitates movement), a part of the plant known to be involved in graviperception and gravity response. They measured phosphatidylinositol 4-phosphate 5-kinase activity in this plant following gravistimulation (plants were placed horizontally and the direction of growth changed to restore the vertical orientation). They found that this kinase is involved in plant orientation in response to the gravity vector (Perera *et al*., 1999). Specifically, the activity levels of phosphatidylinositol 4-phosphate 5-kinase increased during gravistimulation. In the present study we showed that *sktl*, which also encodes a phosphatidylinositol 4-phosphate 5-kinase, affects gravitaxis behaviour. This suggests that the role for phosphatidylinositol 4-phosphate 5-kinase in gravitational response in both *Drosophila* and *Z. mays* may extend to other organisms.

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